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Water vapour absorption in the penicillate millipede *Polyxenus lagurus* (Diplopoda: Penicillata: Polyxenida): microcalorimetric analysis of uptake kinetics

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Summary

The aberrant millipedes of the order Polyxenida are minute animals that inhabit xeric microclimates of bark and rock faces. The lichens and algae that provide their main food substrates tolerate extensive dehydration, effectively eliminating a liquid water source during periods of drought. In this study, we used microcalorimetry to test whether *Polyxenus lagurus* (L.) exploits active water vapour absorption (WVA) for water replenishment. Individual animals were pre-desiccated to 10–20% mass-loss and heat fluxes then monitored using a TAM 2277 microcalorimeter. The calorimetric cell was exposed to an air stream increasing progressively in humidity from 84% to 96%. WVA was distinguishable as large exothermic fluxes seen in ⩾86% RH. Owing to very small and opposing heat fluxes from metabolism and passive water loss, the measured flux provided a good measure of water uptake. WVA showed an uptake threshold of 85% RH and linear sorption kinetics until >94% RH, when uptake became asymptotic. Uptake was rapid, and would allow recovery from 20% dehydration (by mass) in little over 5 h. The uptake flux scales ∝ mass$^{0.61}$, suggesting an area-limited mechanism. *Polyxenus* possesses a cryptonephric system, analogous to that of tenebrionid beetle larvae. Measurements of water absorption and desorption from faecal pellets voided in different humidities gave an estimated rectal humidity of 85.5%. The close congruence between this value and the WVA threshold provides evidence for a cryptonephric uptake mechanism derived independently from that of tenebrionids. *Polyxenus* represents the first documented example of WVA in the myriapod classes.

Key words: water vapour absorption (WVA), *Polyxenus lagurus*, heat flux, calorimetry.

Introduction

Water vapour absorption or WVA refers to the ability of several groups of arthropods and one described plant species to absorb water vapour from relative humidities below the equilibrium water activity of the tissues (Machin, 1979a; Machin, 1983; Machin et al., 1982; Rudolph and Knülle, 1982; Knülle, 1984; O'Donnell and Machin, 1988). Since thermodynamics dictates that water will always move down a vapour pressure gradient, WVA processes depend on the regional lowering of vapour pressure by a vapour-absorbing surface. The minimum vapour pressure generated by a particular system at a given temperature will, in turn, determine the minimum relative humidity from which water vapour can be recovered, referred to by Machin as the uptake or 'pump' threshold (Machin, 1979a). Net water uptake by the organism will be possible at some humidity above the threshold where uptake across the absorbing epithelium exceeds the total transpiratory water losses across the remaining body surface. This has been variously defined in the literature as the critical equilibrium humidity (CEH) or critical equilibrium activity (CEA) (Knülle and Wharton, 1964; Wharton and Devine, 1968). In terms of maintaining long-term water balance, the CEA and the sorption kinetics as a function of RH are the two most important biological parameters, determining the rate at which a given water deficit can be replenished in a given temperature and humidity regime.

Although WVA has been documented for only a rather small number of arthropod species their taxonomic diversity, combined with clear physiological differences among the uptake mechanisms (Hadley, 1994), clearly shows that it has evolved independently several times. Examples among the Insecta include tenebrionid and anobiid beetle larvae (Ramsay, 1964; Knülle and Spadafora, 1970; Machin, 1975; Machin, 1976; Serdyukova, 1989), flea larvae (Siphonaptera) (Rudolph and Knülle, 1982; Bernotat-Danielowski and Knülle, 1986), Psocoptera and Phthiraptera (Rudolph, 1982; Rudolph, 1983), desert burrowing cockroaches *Arenivaga* spp. (Edney, 1966; O'Donnell, 1977; O'Donnell, 1981a; O'Donnell, 1981b;

O'Donnell, 1982a; O'Donnell, 1982b), and Thysanura (Noble-Nesbitt, 1969; Noble-Nesbitt, 1970a; Noble-Nesbitt, 1970b; Noble-Nesbitt, 1975; Neuhaus et al., 1978; Gaede, 1989). WVA is also described in the Acari among the Chelicerata (Lees, 1946; Rudolph and Knülle, 1974; Rudolph and Knülle, 1978; Arlian and Veselica, 1981; Knülle and Rudolph, 1983; Kraiss-Gothe et al., 1989; Sigal et al., 1991; Gaede and Knülle, 1997; Gaede and Knülle, 2000; Yoder and Benoit, 2003), and in the oniscidean isopods among the Crustacea (Wright and Machin, 1990; Wright and Machin, 1993a; Wright and Machin, 1994b; Wright and O'Donnell, 1992). A capacity for WVA has not been described in the non-acarine arachnids or in any of the myriapod classes.

Polyxenus lagurus (L. 1758) is the most common European millipede in the aberrant basal subclass Penicillata Latreille 1831 (formerly Pselaphognatha). This taxon comprises a single order Polyxenida Verhoeff 1934, with a worldwide distribution and 160 described species (Nguyen Duy-Jacquemin and Geoffroy, 2003). Most of these belong to the family Polyxenidae and the cosmopolitan genus *Polyxenus*. Like congenerics, *Polyxenus lagurus* is minute, measuring 3–4 mm in length, and the body is covered with fans of strongly sculpted trichomes (Eisenbeis and Wichard, 1987). Caudally, *Polyxenus* spp. bear two dense brushes of long, detachable spines with recurved tips that are released if the animal is attacked. They form a remarkably effective defence mechanism, hooking onto the setae of ants and spiders and then hooking into one another as the assailant attempts to groom (Eisner et al., 1996). In a short period, the animal is entangled and helpless.

Polyxenus spp. are mesic–xeric in habit, contrasting markedly with most other Diplopoda. Their typical habitats are generally described as litter and bark (Schömann, 1956; Seifert, 1960; Eisenbeis and Wichard, 1987; David, 1995), although the present authors have most commonly collected *P. lagurus* from rocks and old walls. They are diurnally active, feeding on algal films and lichens (Schömann, 1956; Eisenbeis and Wichard, 1987), often in warm and dry conditions and direct sunlight. While most millipedes have high integumental permeability, with standardized fluxes in the range of 0.1–2.5 μg h^{-1} cm^{-2} Pa^{-1} (Appel, 1988; Hopkin and Read, 1992; Meyer and Eisenbeis, 1985), *P. lagurus* is remarkably impermeable, with an integumental conductance comparable to that of the most xeric insects. Eisenbeis and Wichard (Eisenbeis and Wichard, 1987) measured losses for *P. lagurus* of about 0.012 μg h^{-1} cm^{-2} Pa^{-1} in relative humidities of 0 and 33% RH, similar to the permeability of *Tenebrio molitor* (L.) pupae (Holdgate and Seal, 1956) and to values described for most mesic–xeric arachnids (see Hadley, 1994). Even the large desert millipede *Orthoporus ornatus* (Girard) has a permeability about 5 times greater (0.06 μg h^{-1} cm^{-2} Pa^{-1}) (Crawford, 1972) than that of *P. lagurus*.

In spite of its low water-loss rates, the small size of *P. lagurus* will impose whole-animal conductances or mass-specific water loss rates far greater than those experienced by larger mesic–xeric arthropods in the 100 mg to 10 g size range. Maintenance of long-term water balance in this species will consequently require an effective avenue of water uptake. This is made all the more critical by the ability of rock-encrusting lichens and algae to withstand near-complete water losses (Beckett, 1995; Kranner et al., 2003), reducing or eliminating the availability of pre-formed water to the epilithic arthropod fauna during periods of drought. Whether *P. lagurus* depends primarily on the existing water in food, dew, metabolic water or WVA is unknown. The ability of *P. lagurus* to rehydrate rapidly in 98% RH has been reported (Eisenbeis and Wichard, 1987). Although they did not clarify whether liquid water might have been available, or whether an active process was involved, Eisenbeis and Wichard concluded that this represented water vapour uptake. To explore the possibility that *P. lagurus* is able to absorb water vapour from sub-haemolymph water activities, we used microcalorimetry to monitor water exchange of this species in flowing air at precisely controlled relative humidities. This method has been used with success in the study of WVA in *Tenebrio* larvae (Hansen et al., 2004) and provides superior sensitivity to gravimetric procedures.

Materials and methods

Polyxenus lagurus L. were collected from old walls in Roskilde, Denmark, and transported to the lab at Roskilde University. This species comprises both bisexual and parthenogenetic (thelytokous) populations, though these are not reliably distinguishable by external morphology (Enghoff, 1976). The local abundance of animals allowed new specimens to be collected from the field for each period of study. Prior to calorimetry, animals were weighed on a digital microbalance (Mettler-Toledo AT 261, Columbus, OH, USA) with a resolution of 10 μg and then dried over P_2O_5 in a desiccator to between 10 and 20% mass-loss. This typically took 36–48 h. Since the millipedes are able to climb almost any surface, they were contained within small polyethylene tubes with foam plugs during desiccation.

Calorimetric measurements used an isothermal Thermal Activity Monitor (TAM) 2277 (Thermometric, Järfälla, Sweden). All experiments were run with individual animals using an air-flow rate of 100 ml h^{-1} and temperature of 25°C. Thermistors connecting to the animal cell (Cell 1) recorded the total heat flux due to metabolism and water exchange. Full details are given elsewhere (Hansen et al., 2004). In a subset of trials, the air-stream from Cell 1 was passed into a second calorimeter cell containing 700 μl water. The humidity of the air-stream exiting Cell 1 determines the amount of water that must be evaporated to saturate the air exiting Cell 2, and hence the endothermic heat signal in the second channel, provided that the air stream reaches saturation during the passage. The latter was confirmed in controls with saturated solutions of, respectively, NaCl (a_w=0.753) and KNO_3 (a_w=0.936) (Greenspan, 1977) in Cell 1 and in trials with variable air flow rates. These control experiments concurrently suggested that a possible error due to a vapour pressure deficit in the air leaving

Cell 2 was small compared to other experimental limitations. The heat signal from Cell 2 (μW) thus allows an accurate determination of the equilibrium humidity in Cell 1. The vapour density of this air stream (g l^{-1}) is readily calculated knowing the latent heat of vapourization of water at 25°C (2443 J g^{-1}) (Atkins and de Paula, 2002):

$$VD_{cell\ 1} = (HF_{cell\ 2} \times 3600) / (AF \times 2443) ,$$

where $VD_{cell\ 1}$ is vapour density in Cell 1 (g l^{-1}); $HF_{cell\ 2}$ is heat flux attributable to Cell 2 (W); 3600 = number of seconds h^{-1}; AF is air flow (l h^{-1}). Dividing the result by the vapour density of saturated air at 25°C (0.02213 g l^{-1}) then yields the relative humidity of the air stream (%).

This value will be primarily determined by the RH of the initial air stream, but also by the addition or removal of water vapour from Cell 1 by the animal. By subtracting the heat signal measured in Cell 2 from a blank using an empty cell, the net humidity change (%) resulting from the animal is calculated. In order to convert a deviation in the measured signal (μW) into a measure of water exchange, the integration of the signal over time is simply divided by the latent heat of vapourization of water at 25°C:

$$\text{Water exchange} = \text{Integral} \times 3600 / 2443 ,$$

where water exchange is in μg and Integral is μW h. Similarly, in order to convert a measured heat signal (in μW) from Channel 2 into a measure of water flux (μg h^{-1}), a blank run is subtracted from the signal and the corrected value divided by the latent heat of vapourization:

$$\text{Water flux} = \text{Heat flux} \times 3600 / 2443 .$$

Preliminary calorimetric runs showed two distinct features. First, the baseline-corrected heat signal in humidities between 70% and 85% was extremely small and negative, in the order of 0.2–0.4 μW. Second, a much larger negative signal indicating water vapour absorption was routinely seen in relative humidities above 86%. To study the sorption kinetics, we used a protocol starting at 84% and then increasing the humidity stepwise by increments of 2% every 4 h. Owing to the increasing equilibration times imposed by adsorption and desorption of water from the calorimetric cell at the highest humidities, experimental runs were not extended above 96% RH. In order to differentiate the positive (endothermic) water loss and negative (exothermic) metabolism components of the signal in non-absorbing animals, we also recorded the metabolic heat signal independently using hydrated animals in still air at 100% RH. This was done by loading an animal into the calorimetric cell and adding 2 small (ca. 1 μl) water droplets to the vertical sides of the cell to saturate the humidity. The cell was then sealed and lowered into the TAM 2277. The heat signal equilibrated within 1 h. The disparity between this signal and the signal in flowing air at sub-saturated humidities represents the humidity-dependent contribution of evaporative heat flux to the net signal. We were also able to estimate this value independently using the permeability calculated from the initial desiccation of animals in 0% RH.

Results

Calorimetric runs were performed on a total of 24 animals. An example plot showing the heat signal recorded during the step-wise increases in RH over time (*t*) is shown in Fig. 1. The red and black traces in Fig. 1A illustrate two separate runs with a millipede in Cell 1 and the green trace is a blank experiment with an empty calorimetric cell. The hydrated masses were, respectively, 0.49 mg and 0.50 mg and both animals were dried to 0.40 mg prior to the calorimetric measurements. The figure also shows the RH step-profile. The heat flow between the peaks, when the adsorption is equilibrated, quantifies WVA. Exothermic heat-flows are given with negative signs. For example, the red trace shows no absorption (i.e. the signal is equal to the blank) for RH<90%. At this humidity, however, (at *t*~16 h) a sharp decrease signifies the onset of WVA. Analogously, the black curve shows onset of WVA at *t*~7 h (RH=86%) which ceases at *t*~18 h when RH has reached 92%. Animals were sometimes observed to terminate WVA, only to start again within a few hours, showing that both the initiation and termination of WVA are clearly under voluntary control.

Fig. 1B shows data from Cell 2 during the latter period of these same experiments. These data directly reflect water exchange in the animal. Thus, if the experimental animal loses water, thereby enriching the air flow with water vapour, the endothermic heat flow will be lower than the blank. Conversely, water uptake by the animal in Cell 1 will enlarge the heat flow in Cell 2 above that of the blank. Inspection of the results shows that the (numeric) differences between experiment and blank discussed for Fig. 1A are mirrored in Fig. 1B. For example, at *t*=17 h the red and black traces are 6 μW below the blank in Fig. 1A and 6 μW above the blank in Fig. 1B, and at *t*=20 h the discrepancy between the blank and the red trace is 8 μW in both panels. This relationship between Cells 1 and 2 was found to be consistent, and it indicates that the exothermic shift in heat flow with respect to the blank in Cell 1, HF_{WVA}, solely reflects the condensation of water during WVA.

In principle, the baseline-corrected heat signal in non-absorbing animals represents the sum of the exothermic heat flux due to metabolism (HF_{met}) and the endothermic flux due to net (transpiratory and respiratory) water losses (HF_{wl}). In 84% RH, the net signal (HF_{met}+HF_{wl}), estimated for 10 non-absorbing animals, was about 0.2–0.4 μW, which is comparable to the detection level in perfusion experiments. The net value for inactive animals is only about 5% of HF_{WVA} in 88% RH, and about 1% of the signal in 94%. We conclude that the accordance of the numeric values of HF_{WVA} in Cell 1 and Cell 2 and the insignificant size of the sum (HF_{met}+HF_{wl}) collectively suggest that the rate of WVA is reflected directly in HF_{WVA} taken from Cell 1. Since the measurements in Cell 2 are more experimentally demanding, and have lower resolution due to the large background (cf. Fig. 1B), we base the following analyses on HF_{WVA} values from Cell 1.

In humidities between 86% and 92%, HF_{WVA} measured in Cell 1 was typically very uniform throughout the duration of WVA. By contrast, in 94% and 96% RH the signal showed a

small asymptotic increase, typically stabilizing within 3–4 h at a value 10–20% above the initial reading. This can be attributed to the depletion of the chamber RH by WVA, which in turn lowers the rate of WVA until steady-state equilibrium is attained. Since the amount of water removed from the air stream by the animal is quantified by HF_{WVA}, the steady-state humidity in the chamber can be readily calculated by mass conservation considerations. Thus, the height of the steps in Fig. 1B shows that a change in RH of one unit corresponds to a heat flow of 16 μW (the same number can be derived theoretically from the equations in the Materials and methods section). It follows that the ambient humidity at steady-state WVA, $RH_{s–s}$, can be written $RH_{s–s}=RH–HF_{WVA}/16$. Applying this latter equation to our data shows that the reduction in ambient humidity due to WVA ranges from 0.1% RH for the lower to 0.7% RH for the higher uptake rates.

Mean uptake fluxes (± s.e.m.), expressed both as the negative heat signal (HF_{WVA}; μW) and the calculated water uptake (in μg h^{-1}), are plotted as a function of ambient RH in Fig. 2; summary data for WVA are shown in Table 1. The humidity is specified by the steady-state values, $RH_{s–s}$, defined above. Thus, in Fig. 2, each data point represents the average $RH_{s–s}$ ± s.e.m. for each RH step during WVA. Several animals initiated WVA in 86% RH, but with one exception it terminated again after a short period, possibly because WVA reduced the water activity of the calorimetric cell below the uptake threshold. The sorption curve extrapolates to an estimated uptake threshold of 85% RH (a_w=0.85). Because of the extremely low passive loss at this humidity, the disparity between the threshold activity and CEA is negligible. The sorption curve shows an asymptotic profile with the uptake flux saturating as the humidity increases above 94%. Below this humidity, the sorption curve is linear with a slope of 1.94 μg h^{-1} RH^{-1}. Standardized uptake fluxes in Table 1 are calculated using the linear portion of the sorption curve and express uptake as a

Table 1. *Uptake flux measures for the experimental animals*

Uptake flux	Mean ± s.e.m.	*N*
Standardized (μg h^{-1} Pa^{-1})	0.061±0.0026	63
Mass-specific (% h^{-1} Pa^{-1})	0.014±0.0006	63
Mass-specific in 95.3% RH (% h^{-1})	3.7±0.3	4

Sample sizes vary according to the number of individual animals that absorbed in a given humidity.

Vapour pressure gradients for uptake are calculated assuming an uptake threshold of 0.85 or 2690 Pa at 25°C. The standardized uptake flux is calculated using only data from the linear part of the sorption curve (RH<94%).

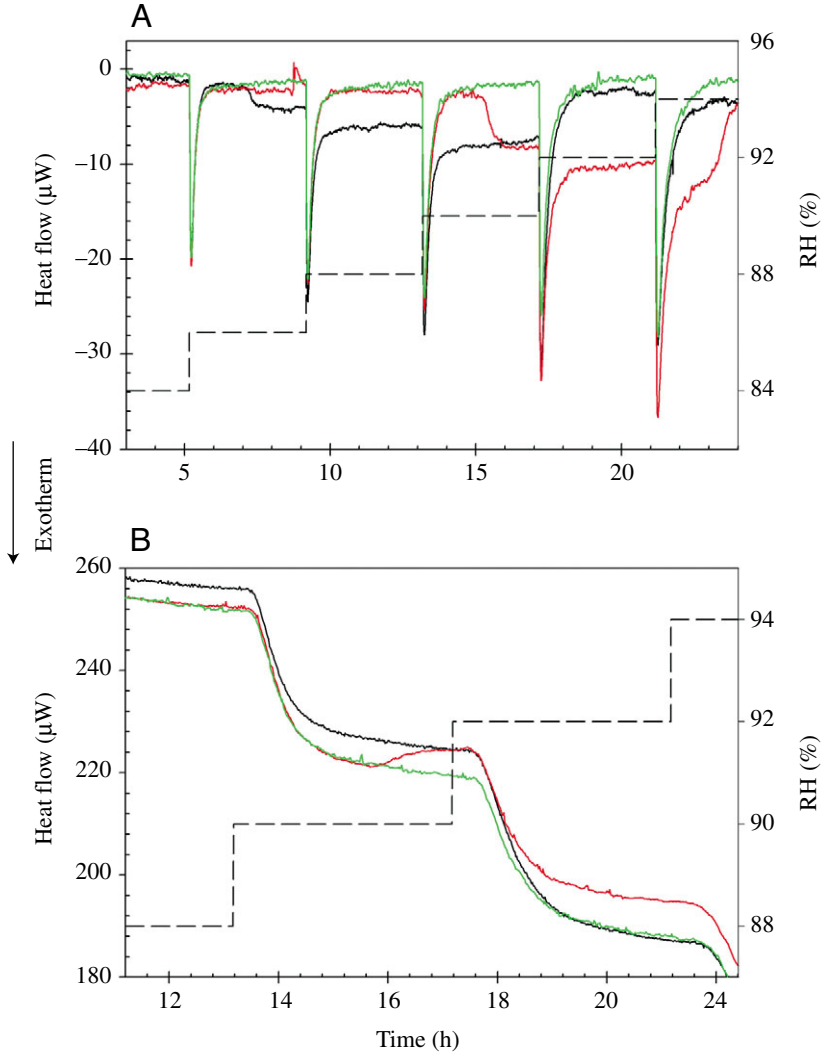

Fig. 1. (A) Sample plot of a recording trace in which the relative humidity was increased stepwise from 84% to 94%. The left ordinate shows the measured heat signal (μW) and the right ordinate shows the relative humidity (RH), plotted as the broken line. Exothermic heat flows are negative. The black and red plots show data for two different animals; the green plot is a blank recorded with an empty calorimetric cell. The (negative) peaks at each RH increment represent the exothermic heat of water adsorption to the walls of the calorimetric vessel. Clear deviations from the blank in the equilibrated trace show exothermic heat flow attributable to WVA. The abrupt decrease in heat flow in the red trace at 23 h shows that the animal terminated WVA at this time and the black trace shows a similar termination of WVA at ca. 18 h. Further explanation is provided in the text. (B) The heat signals recorded in the second (water) channel for these same animals during the later period of data collection. Depression of the RH in Cell 1 during WVA results in a corresponding increase in evaporation from Cell 2 and augmented endothermic (positive) heat flow compared to the blank. The red trace shows the onset of WVA in this animal at ~16 h. The black trace shows an animal that is initially absorbing, but ceases WVA at ~18 h. Fuller discussion is given in the text.

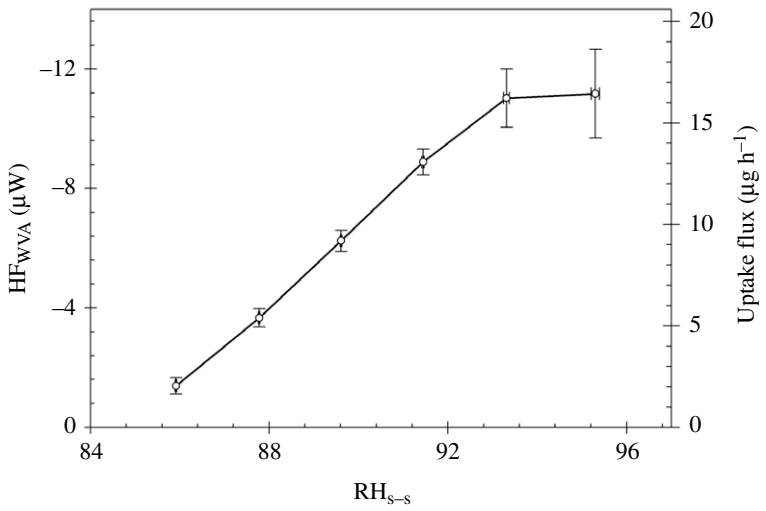

Fig. 2. Mean WVA uptake fluxes (± s.e.m.; vertical error bars), expressed both as the exothermic heat signal (HF_{WVA}; μW) and the uptake flux (μg h^{-1}), plotted as a function of the steady-state relative humidity in the calorimetric cell (RH_{s-s} in %). RH_{s-s} values are calculated as indicated in the text and the data points represent the means for each RH-step (± s.e.m.; horizontal error bars). Water vapour uptake in *P. lagurus* increases linearly with relative humidity up to 92% RH, but shows asymptotic kinetics above this value. *N*=4-16.

function of the vapour pressure gradient between the ambient RH and the threshold (85%). The corresponding vapour pressure gradient was calculated assuming a saturation vapour pressure at 25°C of 3169 Pa; thus 1% RH represents 31.69 Pa.

The data shown in Fig. 2 are not standardized for animal mass, so variation in uptake flux and/or uptake threshold as a function of size will contribute variability to the data. Animal masses for our trials varied approximately twofold, from 0.35 to 0.72 mg. In order to analyze the effects of body mass on uptake flux, the uptake fluxes measured in different RH conditions were standardized for the vapour pressure gradient between the uptake site and ambient air (μg h^{-1} Pa^{-1}), using the calculated threshold activity of 0.85. A log–log comparison of standardized uptake flux against body mass then permits the calculation of intraspecific scaling parameters for WVA in *Polyxenus* (see Wright and Machin, 1993a). The resulting least-squares regression generates a slope of 0.605±0.144 (± s.e.m.) (r^2=0.33; *N*=38), thus indicating that standardized uptake flux scales in proportion to body mass $(M_b)^{0.605}$. This is considered further in the Discussion.

Table 2. *Mean water loss and related measures for 17 experimental animals*

	Mean ± s.e.m.
Standardized loss flux (ng h^{-1} cm^{-2} Pa^{-1})	7.40±1.17
Permeability (cm $s^{-1}\times10^4$)	2.89±0.457
Mass-specific loss in dry air (% h^{-1} a_w^{-1})	0.330±0.052
Mean flux in 84% RH (μg h^{-1})	0.221±0.036

For the standardized loss flux, the surface area was estimated as $12M_b^{0.67}$ (Edney, 1977). The mean animal mass (M_b) was 0.45 mg.

The metabolic heat flux measured for test animals in closed calorimetric cells at 100% RH had a mean value of 0.35±0.22 μW (*N*=4), which was not significantly different from zero. In subsaturated humidities, evaporative heat flux (HF_{wl}) will also contribute to the net heat flow measured. This can be estimated for a given humidity based on the gravimetrically determined water loss rates of whole animals, shown in Table 2. These data give an estimated mean water loss for a 0.45 mg animal in 84% RH of 0.22 μg h^{-1}. Multiplying this value by the latent heat of evaporation of water (see above) gives an HF_{wl} value of 0.15 μW. The negligible contribution of both metabolic heat flux and HF_{wl} to the net measured heat signal, combined with the fact that they represent opposing heat signals, confirms the validity of quantifying WVA directly from the data in Cell 1.

As discussed above, the water recovery measured directly in Cell 2 generates the same (numerical) heat flow as the HF_{WVA} observed in Cell 1 during WVA. This shows that a possible increase in metabolic heat output, HF_{met}, during WVA is below the experimental resolution in Cell 2, which is about 1 μW. This resolution corresponds to about a threefold increase above the standard metabolic rate (SMR). It is likely that the actual increase in HF_{met} during WVA is considerably smaller, based on measurements for *Tenebrio molitor* (Hansen et al., 2006) and the low energetic cost of WVA predicted from the Gibbs equation (Lees, 1948; Ramsay, 1964; Edney, 1977; O'Donnell and Machin, 1988). The very low metabolic cost of WVA, compared to the large HF_{WVA}, does not represent a violation of fundamental laws of thermodynamics since the HF_{WVA} is due to the latent heat of condensation. If water was moving down an activity gradient solely in the liquid phase, the heat released would be smaller than the metabolic heat consumed in generating that gradient, in accordance with the 2nd law of thermodynamics.

Discussion

This paper demonstrates clearly that *Polyxenus lagurus* is capable of active water vapour absorption with an uptake threshold of 0.85 at 25°C. This confirms the suggested utilization of WVA in this species (Eisenbeis and Wichard, 1987) and makes it the only documented species capable of WVA in the Diplopoda or any of the myriapod classes.

Water vapour uptake is rapid, plateauing at approximately 17 μg h^{-1} in 96% RH for a 0.45 mg animal. The linear portion of the sorption curve between 86% and 92% RH represents a standardized uptake flux of 0.061 μg h^{-1} Pa^{-1}. This is quite close to the value of 0.104 μg h^{-1} Pa^{-1} estimated for a 0.45 mg animal using the following allometric equation for a range of vapour-absorbing arthropods:

$$J_{WVA} = 0.18M_b^{0.692}$$

(derived from Wright and Machin, 1993a), where J_{WVA} is the

standardized uptake flux ($\mu g\ h^{-1}\ Pa^{-1}$) and M_b is the animal mass (mg).

The exponent for interspecific scaling of standardized uptake flux is close to the value of 0.67 that would be predicted for geometric scaling if uptake flux were area-limited. Intraspecific scaling of standardized uptake flux has only been studied in the larvae of the tenebrionid beetles *Tenebrio molitor* and *Onymacris marginipennis* (Breme) (Coutchié and Machin, 1984), with both species showing similar exponents (0.355 and 0.418, respectively), lower than our estimate for *Polyxenus*. However, measurements of rectal length and circumference for both species show a strongly allometric scaling of rectal surface area with mass, and the calculated exponents (0.370 and 0.379) provide a reasonable match to the scaling of uptake flux, again indicating an area-limited process. The intraspecific scaling of uptake flux against body mass in *Polyxenus*, determined from the present study, has an exponent of 0.605±0.144. Although the modest sample size and small mass range of the animals limits the precision of this estimate, it indicates that uptake flux in *Polyxenus* conforms to the predicted geometric scaling for an area-dependent function, as seen for interspecific scaling.

The most physiologically and ecologically significant consequence of the 0.692 exponent for interspecific scaling of WVA is that mass-specific uptake fluxes decrease in larger species. In small arthropods, WVA provides very effective mass-specific water recovery (O'Donnell and Machin, 1988). Our calculated uptake rates for *P. lagurus* would allow replenishment of 3.7% mass loss per hour, or 20% in 5.4 h, by a representative 0.45 mg animal in ⩾94% RH. Combined with the remarkably low integumental permeability, measured here as 7.2±1.3 ng $h^{-1}\ cm^{-2}\ Pa^{-1}$, this renders the species extremely well adapted to the highly exposed and frequently xeric microclimate of bark and rock surfaces.

The site and mechanism of WVA in *P. lagurus* are not known, but the structure of the rectum indicates a likely absorption site and colligative process. Like the tenebrionid beetles, this species possesses a cryptonephric system (Schlüter and Seifert, 1985). The medial region of the 2 Malpighian tubules forms a 'thick meandering segment', lying closely apposed to the rectal wall and ensheathed by a perinephric membrane. The cryotonephric system is well established as the site for WVA in *Tenebrio* (Ramsay, 1964; Machin, 1979a; Machin, 1979b; Tupy and Machin, 1985). In such a system, the presence of an osmotically impermeable perinephric membrane, combined with the generation of high osmolalities within the tubule lumina, provides a mechanism for the unidirectional movement of water from the rectal lumen into the perinephric space surrounding the tubules (Grimstone et al., 1968). In both *Polyxenus* and the tenebrionids, such a system likely evolved for purposes of dehydrating the faeces, and by generating depressed water activities it was elegantly pre-adapted for WVA. There are, to our knowledge, no anatomical studies on hindgut structure in other Polyxenida to indicate whether a cryptonephric system is present in other genera. The remaining millipede orders apparently lack this specialization, with the Malpighian tubules lying free in the haemolymph (Seifert, 1979; Hopkin and Read, 1992).

Physiological studies on specific ion concentrations and water activities generated by the Malpighian tubules in *Polyxenus* have not been done, but *Glomeris marginata* tubules transport sodium, not potassium, as the primary cation driving fluid transport (Farquharson, 1974). This is potentially significant since the saturation water activity for potassium chloride is 0.85 (Winston and Bates, 1960), making potassium a potentially problematic primary cation for a species with an uptake threshold of 0.85. Machin and O'Donnell, however (Machin and O'Donnell, 1991), showed that *Onymacris marginipennis*, with a WVA threshold of 0.841 (Coutchié and Machin, 1984), still transports potassium as the primary cation in the cryptonephric portion of the Malpighian tubules. Measured potassium concentrations in the tubule lumina *in vitro* reached 3350 mmol l^{-1}, sufficient to generate a water activity of 0.89 when combined with peak measured concentrations for Na^+ and Cl^-. In view of some osmotic dilution of the preparation from bathing saline in the rectal lumen, a space that is naturally air-filled, the authors determined that the active accumulation of KCl by the cryptonephric tubules probably generates lower activities, consistent with the WVA threshold, *in vivo*. Since the measured threshold activity of 0.841 is below the saturation activity for KCl, they concluded that KCl supersaturation occurs in the tubule lumina. In view of the slightly higher threshold activity determined here for *Polyxenus*, we might therefore reasonably expect either K^+ or Na^+ to be the major cation transported by the Malpighian tubules.

Corroborative evidence for a rectal uptake site was sought by examining water exchanges from faecal pellets in hydrated animals. The amount of water absorbed or evaporated from voided faecal pellets in different humidities provides a sensitive means of determining the rectal water activity (Wright and Machin, 1993b). Calorimetric runs examining water exchanges from faecal pellets were conducted with fifteen freshly collected *Polyxenus* using various protocols ranging from 0 to 96% RH. The amount of water that must be evaporated or condensed to bring the faecal pellet into equilibrium with a given RH will increase as the ambient water activity deviates further from the faecal pellet (rectal) activity. The mass of water exchanged varies linearly with the reciprocal of the water activity deficit or vapour pressure deficit (Machin, 1975; Machin, 1979a; Machin, 1979b).

Heat fluxes during the release of faecal pellets in relative humidities below 84% were easily distinguished as sharp endothermic spikes, lasting about 10 min in duration. Similar endothermic spikes were not seen in any higher humidity. Nine clear exothermic spikes were seen in non-absorbing animals in 88% and 90% RH, but were not reliably distinguishable in higher humidities, mainly because most animals initiated WVA and this made their identity ambiguous. One clear peak in 92% RH almost certainly arose from the water desorption from more than one voided pellet, based on its duration, and was excluded owing to this uncertainty. The integrated heat

signal for each pellet was used to calculate the mass of water exchanged as described before. The results for all pellets are plotted in Fig. 3. Although variation in faecal pellet size will add variance to the data, the equilibrium vapour pressure deficit where no net water exchange occurs is independent of pellet size and well defined in the figure at 1/0.00218, equivalent to a water activity of 0.855. This indicates that the cryptonephric system functions to depress the rectal water activity to conserve water from the faeces, and the congruence of the activity values determined from faecal water exchange and the WVA sorption curve provides good experimental support for a rectal uptake site for WVA. This conclusion is further supported by the fact that faecal pellet spikes were not reliably distinguishable in the near-threshold humidities (84% and 86% RH), and were not resolved for some animals that nevertheless released faecal pellets into the calorimetric cell. If low rectal water activities are maintained for faecal dehydration, the initiation of WVA in above-threshold humidity would simply entail opening of the anal valves. This is consistent with the voluntary control over the onset and termination of WVA, and the simultaneous abrupt changes in water flux.

The saturation kinetics of the WVA sorption curve in *P. lagurus* shows that one or more components of the uptake mechanism become rate-limiting at high RH. This could involve the rate at which water can be delivered to the absorbing epithelium, or the rate at which absorbed water can be cleared from the cryptonephric system. While the diffusional supply of water vapour into the rectum may limit uptake rates in lower humidities and dictate the slope of the linear part of the sorption curve, it is unlikely to change significantly as a function of RH. We believe instead that the rate-limiting step lies within some component of the cryptonephric system. Studies of the cryptonephric system in tenebrionids (Machin, 1979b; O'Donnell and Machin, 1991; Machin and O'Donnell, 1991) show that osmolalities increase in a radial direction from the rectal lumen *via* the perinephric space to the Malpighian tubule lumina in vapour-absorbing animals. During faecal dehydration, the radial gradient disappears, probably because the smaller water fluxes impose negligible dilution of the perinephric fluid (Machin, 1979b). In either absorption mode, the osmolality within the cryptonephric system falls steadily from posterior to anterior. This is consistent with the anterior movement of absorbed water into the haemolymph, with only a small fraction moving across the Malpighian tubule epithelia and into the tubule lumina. How water moves against the osmotic gradient between the perinephric space and the haemolymph is not known, but presents a likely rate-limiting step for net water recovery by any cryptonephric system. Possible rate-limiting parameters (e.g. osmotic water movements, tubule ion transport, or diffusion down a hydrostatic pressure gradient) are all temperature-sensitive and their relative importance may be clarified by studying the effects of temperature on the sorption kinetics.

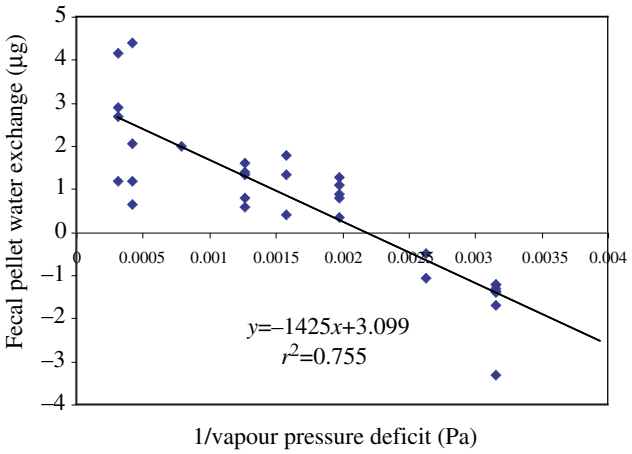

Fig. 3. Graph showing the linear relationship between faecal pellet water exchange (µg) and the reciprocal of the vapour pressure deficit (kPa^{-1}). The least-squares regression line (r^2=0.755; N=31) is shown. The x intercept, at which faecal pellets show no net water exchange, is 0.00218, equivalent to a water activity of 0.855.

List of symbols and abbreviations

AF	air flow
a_w	water activity (RH/100)
CEA	critical equilibrium activity
CEH	critical equilibrium humidity
$HF_{cell\ 2}$	heat flux measured in cell 2
HF_{met}	heat flux attributable to metabolism
HF_{wl}	heat flux attributable to water loss
HF_{WVA}	heat flux attributable to water vapour absorption
J_{WVA}	water vapour absorption flux
M_b	body mass
RH	relative humidity
RH_{ss}	steady-state relative humidity
s.e.m.	standard error of the mean
SMR	standard metabolic rate
$VD_{cell\ 1}$	vapour density in cell 1
WVA	water vapour absorption

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