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TOTAL AMMONIA CONCENTRATION AND pH OF HAEMOLYMPH, PLEON FLUID AND MAXILLARY URINE IN *PORCELLIO SCABER* LATTREILLE (ISOPODA, ONISCIDEA): RELATIONSHIPS TO AMBIENT HUMIDITY AND WATER VAPOUR UPTAKE

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Summary

Ammonia levels and pH were studied in body fluids of *Porcellio scaber* with special reference to ambient humidity and the timing of pleon fluid secretion and pleopodal ventilation. In high humidity (approximately 96.7%), concentrations of total ammonia (NH_3+NH_4^+) in haemolymph display pronounced episodic changes, ranging from less than 1mmol l^{-1} to more than 50mmol l^{-1}. Elevated concentrations (>5mmol l^{-1}) occur in discrete bouts lasting from 40min to more than 2h. Concomitant changes in total ammonia levels are seen in pleon fluid and maxillary urine. Overall, mean ammonia concentrations in pleon fluid, but not maxillary urine, are significantly higher than in the haemolymph, which may indicate active secretion of NH_4^+ in the pleon. Elevated ammonia concentrations are not observed in reduced ambient humidities (85% and 30%). Evidence supports a physiological coupling between pleon NH_3 volatilization and active water vapour absorption. Pleon fluid and haemolymph pH values are similar, discounting a role of alkalization in NH_3 volatilization. Published rates of ammonia excretion are compatible with intermittent volatilization from pleon fluid and with measured NH_3 levels during elevations in ammonia concentration.

Introduction

There is a substantial body of evidence showing that aquatic Crustacea are primarily ammonotelic (Greenaway, 1991; Kormanik and Cameron, 1981; Regnault, 1987). In the terrestrial isopods (Isopoda, Oniscidea) this pattern is retained, ammonia being eliminated largely in gaseous form (Hartenstein, 1968; Wieser *et al.* 1969; Wieser and Schweizer, 1970). Urea is undetectable in *Oniscus asellus* L., and there is no evidence for a functional urea cycle (Hartenstein, 1968). *De novo* synthesis of uric acid also appears unlikely (Hartenstein, 1968), and uric acid deposits in the cuticle probably result from excess purine intake in the diet. Elimination of these deposits at moulting accounts for

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less than 1.5% of nitrogen excretion. Similarly, uric acid levels in the gut account for less than 3% of total nitrogenous excretion. It seems likely, therefore, that terrestrial isopods excrete about 95% of their total nitrogenous waste as NH_3. Of this, less than 10% is attributable to faecal losses (Hartenstein, 1968; Wieser and Schweizer, 1970).

The mechanism of ammonia volatilization is unclear. Hartenstein (1968) reported 1.20mg of volatilizable ammonia per 100g fresh mass of tissue in *O. asellus*, equivalent to tissue concentrations of $0.8 mmol\,l^{-1}$. This would provide a significant partial pressure gradient for volatilization across the general integument. Hoese (1981) has proposed that aqueous ammonia is excreted by the paired maxillary glands. Maxillary urine is conveyed to the pleoventral cavity *via* the hydrophilic, infolded pleurae comprising the 'Wasserleitungssystem' (WS). Alkalization of the urine or pleon fluids would increase the proportion of NH_3 relative to NH_4^+, augmenting the partial pressure gradient for volatilization (Wieser, 1972*a*,*b*; Hoese, 1981). However, measurements of pH are required to test this proposal.

The pleoventral cavity has also been implicated in active absorption of water vapour from subsaturated atmospheres (Wright and Machin, 1990; J. C. Wright and J. Machin, in preparation). Osmolalities of pleon fluid are as high as $8.2 osmol kg^{-1}$, sufficient to provide the requisite vapour pressure lowering for atmospheric water vapour absorption (Wright and O'Donnell, 1992). The predominant osmolytes are Na^+ and Cl^-, and the composition of the fluid differs from that of the urine or haemolymph. The site of salt secretion into the pleon fluid has not been identified, though the epithelial ultrastructure of the pleopodal endopods indicates a role in ion transport (Kümmel, 1984).

Although field studies of water vapour absorption are lacking, it appears likely that terrestrial isopods become dehydrated during nocturnal foraging and employ vapour absorption diurnally, when they reside in humid microclimates (Brereton, 1957; Den Boer, 1961; Sutton *et al.* 1984). Furthermore, water vapour absorption has never been observed during locomotory activity (Wright and Machin, 1990; J. C. Wright and J. Machin, in preparation) and animals routinely forage in humidities below absorption thresholds (Cloudsley-Thompson, 1974; Den Boer, 1961; Paris, 1963; Warburg *et al.* 1984). Ammonia volatilization is also discontinuous, with peak rates occurring diurnally when activity is minimal (Wieser *et al.* 1969; Kirby and Harbaugh, 1974). These workers suggested that diurnal volatilization serves to minimize concomitant water loss. On the basis of these studies, it appears that the processes of water vapour absorption (WVA) and NH_3 volatilization may be coincident. Given the suggestive evidence for involvement of the pleon in both WVA and ammonia volatilization, the two processes might therefore be physiologically linked.

This paper examines possible mechanisms of ammonia volatilization by analysis of ammonia levels and pH in maxillary urine, pleon fluid and haemolymph.

Materials and methods

Porcellio scaber Latr. were collected locally and maintained in a laboratory culture provided with leaves and bark. Culture humidity was maintained above 95% relative

humidity (RH) with moistened cotton wool. Study animals were taken either directly from culture or after 1–5h of desiccation in the laboratory at 30–40% RH.

The methods for sampling pleon fluid and urine with micropipettes have been described previously (Wright and O'Donnell, 1992). Fluids were collected in micropipettes pulled on a vertical pipette puller, then broken back to tip diameters of about 50 μm. Fluid volumes were determined by expelling fluid from the micropipette into a 200 or 400nl Drummond microcapillary. Volumes in partially filled microcapillaries were determined from the length of the fluid column measured by an eyepiece micrometer in a dissecting microscope.

Total ammonia concentrations were determined using a commercial kit (Sigma, St Louis, MO) accurate to within 10%. The assay is based on the reductive amination of 2-oxoglutarate, using glutamate dehydrogenase (GDH) and nicotinamide adenine dinucleotide (NADH), as follows:

$$\text{2-oxoglutarate} + NH_3 + \text{NADH} \xrightarrow{\text{GDH}} \text{glutamate} + \text{NAD}.$$

The decrease in absorbance at 340nm, resulting from oxidation of NADH, is proportional to ammonia concentration. Sensitivity of the assay was determined using the supplied control solution diluted to concentrations comparable to those of the experimental fluids. Absorbances were measured using 750 μl quartz glass cuvettes and a Perkin-Elmer λ-3 spectrophotometer (with a photometric reproducibility of 0.002Å). For initial sample volumes of 200nl, the empirically determined detection limit was a concentration of 1–2mmol l^{-1}; for the smallest samples of 50nl, the limit was approximately 5mmol l^{-1}.

Ammonia levels were measured in samples of haemolymph, pleon fluid and maxillary urine. In order to assess possible associations between water vapour absorption and ammonia levels, animals were maintained in different humidities during sampling. High-humidity chambers (approximately 96.7% RH), providing an atmosphere compatible with WVA, were humidified with water. Chambers were also maintained at humidities below the threshold for WVA (88.9% RH; J. C. Wright and J. Machin, in preparation), either by leaving them open to the ambient laboratory humidity (30–40% RH), or by using saturated KCl to provide 85% RH. In some experiments, relationships between ammonia levels in different fluids were examined by collecting paired samples of haemolymph and either urine or pleon fluid within a 5-min period.

pH was determined for haemolymph, urine and pleon fluid. For measurements of haemolymph pH, a pH-selective and reference microelectrode were inserted through the thin arthrodial membranes at the base of the legs. The pH of urine and pleon fluid was determined by positioning the microelectrodes so as to contact the respective fluid films. The pH microelectrodes were fabricated from 1mm unfilamented capillary glass and silanized by exposure to dimethyldichlorosilane vapour at 200°C. The tip was filled with a cocktail based on the proton ionophore tridodecylamine (Hydrogen ionophore I, cocktail A, Fluka Chemical Corp., Ronkonkoma, NY) and the pipette was then backfilled with a solution of 0.1mol l^{-1} NaCl and 0.1mol l^{-1} sodium acetate, adjusted to pH6. A chlorided silver wire inserted into the backfilling solution was connected to the high-impedance input stage (>10^{15} Ω) of an electrometer, and the electrical ground of the

amplifier was connected through a second silver wire to a reference microelectrode filled with 3moll^{-1} KCl. The pH microelectrodes were calibrated using NaCl solutions mimicking the ionic strength of haemolymph (350mmoll^{-1}) or pleon fluid (1moll^{-1}). Calibration solutions were buffered with 20mmoll^{-1} Hepes and adjusted to a range of pH values bracketing the expected range of experimental values. Slopes of the pH microelectrodes were 55–60mV per pH unit. Before and after each test measurement, voltages were recorded in calibration solutions. The pH of fluid samples was then determined by interpolation.

Results

Pre-desiccated isopods transferred to high humidities generally responded by secreting a large volume of readily sampled fluid into the pleoventral chamber. Within a few minutes, such animals initiated a metachronal ventilatory beating of the pleopods with a typical frequency of 0.2–0.3Hz. This sequence of events is identical to that associated with hyperosmotic fluid production and water vapour absorption (Wright and O'Donnell, 1992). The period between initial secretion of pleon fluid and the onset of ventilation is referred to as 'pre-ventilation'. When exposed to humidities below 90% RH, or when fully hydrated, animals did not ventilate and maintained only small volumes of fluid beneath the pleopods. Ammonia assays required pleon fluid samples of at least 50nl, and were feasible only for ventilating and pre-ventilating animals. Haemolymph samples (100–400 nl) were collected from both ventilating and non-ventilating animals; the latter category subsumes pre-ventilating animals, which were not reliably distinguishable without accompanying sampling of pleon fluid. Maxillary urination was observed irregularly, though sometimes for prolonged periods (2–3h), and was invariably associated with pleopodal ventilation. As with haemolymph, large fluid volumes (>100nl) could usually be collected.

Sample distributions of total ammonia in pleon fluid, haemolymph and urine are illustrated in Fig. 1. These data refer exclusively to pre-desiccated animals in humid sampling chambers (approximately 96.7% RH). The most striking feature of the data is the periodic occurrence of greatly elevated ammonia concentrations. Pleon fluid, in particular, shows a broad distribution of concentrations reaching over 100mmoll^{-1} in both ventilating and pre-ventilating animals. In contrast, haemolymph ammonia levels for both ventilating and non-ventilating animals are strongly skewed towards concentrations below 10mmoll^{-1}, although a few measurements of higher concentrations were recorded for both categories. Urine ammonia levels are similarly skewed towards low concentrations.

Statistical comparisons between mean ammonia levels for the different fluids were based on unpaired Student's *t*-tests; data sets were normalized by log-transformation before comparison. Mean pleon fluid ammonia concentrations are significantly higher than mean haemolymph and urine levels for both ventilating and pre-ventilating animals ($P<0.05$). No significant differences are evident between haemolymph ammonia levels in ventilating *versus* non-ventilating animals, or between either haemolymph category and

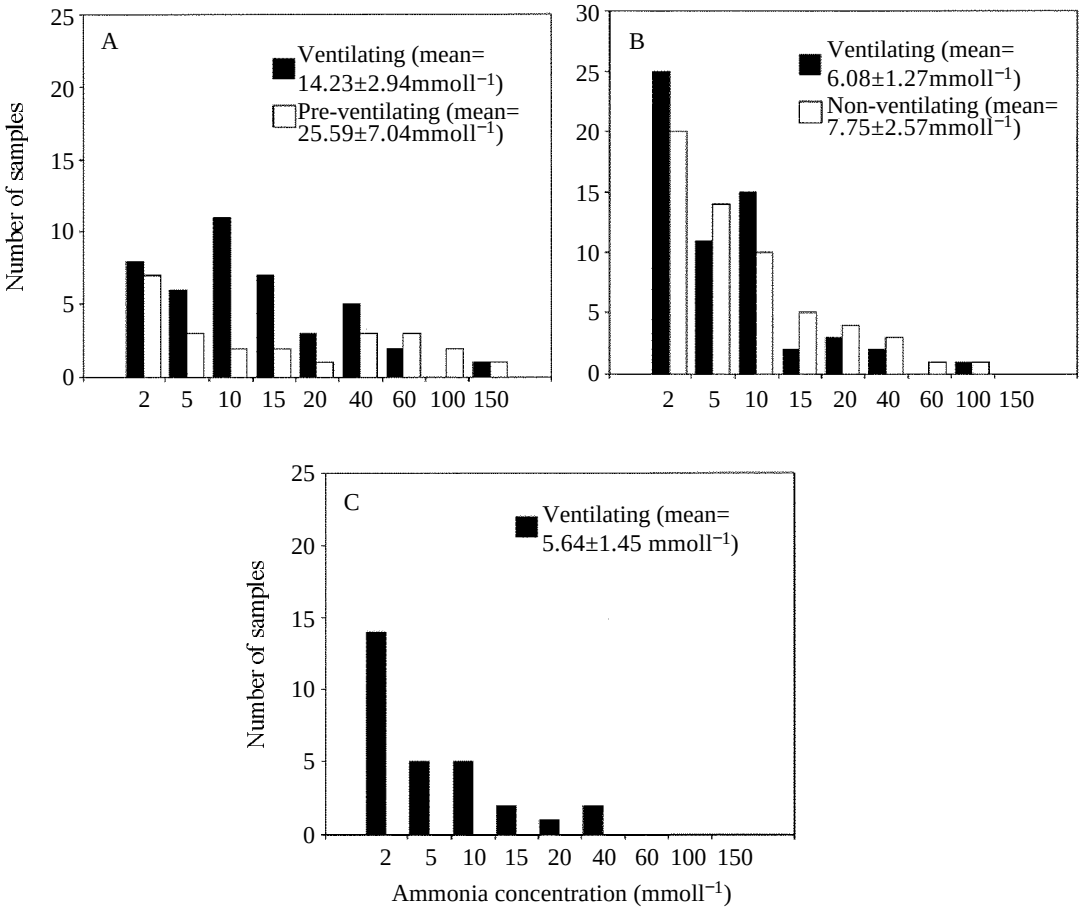

Fig. 1. Sample distributions of ammonia concentrations in pleon fluid (A), haemolymph (B) and maxillary urine (C) from ventilating, pre-ventilating and non-ventilating animals. Mean concentrations and standard errors are listed. Concentrations in haemolymph and urine are strongly skewed towards low concentrations but reveal low incidences of greatly elevated concentrations (10–100mmoll^{-1}). Pleon fluid concentrations show higher incidences of elevated concentrations, particularly when sampled prior to ventilation. 'Non-ventilating' in B includes the category 'pre-ventilating', which could not always be reliably distinguished when sampling haemolymph.

urine. The higher concentrations of ammonia in the pleon fluid suggest a likely role in NH_3 volatilization; no corresponding role is implicated for maxillary urine.

Coincidence between elevated ammonia levels in haemolymph and the other fluids was studied by product-moment correlation analysis of paired samples collected within a 5-min interval. The association between ammonia levels in haemolymph and pleon fluid displays considerable scatter – temporal changes in ammonia levels can be rapid and samples could not be recovered simultaneously – but is positive and highly significant (r=0.865, P<0.005, 72 d.f.). It thus appears that animals release ammonia into the haemolymph intermittently and accumulate it in the pleon fluid, from which it could be

volatilized by pleopodal ventilation. Since mean ammonia levels in pleon fluid are significantly higher than in haemolymph for ventilating animals, excretion may involve active movement of ammonia into the pleon fluid. However, no significant difference was evident between ammonia levels in haemolymph and pleon fluid for the smaller number of paired samples (Student's *t*-test; $P>0.1$, 35 d.f.).

Correlation analysis also reveals a significant positive association between paired haemolymph and urine samples ($r=0.650$, $P<0.01$, 16 d.f.). Since, however, there is no significant difference between ammonia levels in haemolymph and urine, the observed correlation probably reflects passive equilibrium of urine and haemolymph ammonia levels.

Time courses of changes in ammonia levels in individual animals could not be obtained for pleon fluid owing to the difficulties of rapid sampling, but were possible for haemolymph. Three examples are illustrated in Fig. 2. The periodic elevation of ammonia levels during pleopodal ventilation occurs in discrete bouts lasting from approximately 40min to over 2h ($N=5$). The initiation and termination of such bouts are relatively abrupt and intervening ammonia levels fluctuate without obvious long-term increases or decreases in concentration. Such periodic elevations in ammonia concentration often coincided with the initial secretion of pleon fluid ('pre-ventilation') but could occur at any time during prolonged periods of ventilation. They indicate rapid mobilization of ammonia from a sequestered source.

Sample distributions of haemolymph ammonia levels monitored from animals in two humidities (30–40%, 85% RH) below the threshold for water vapour absorption are illustrated in Fig. 3. No significantly elevated concentrations were measured, in contrast to the high-humidity data in Fig. 1. Animals remained quiescent with their pleopods tightly apposed to the ventral surface. Mean ammonia concentrations in the low- and high-humidity data sets were compared using unpaired *t*-tests after normalizing the sample distributions by log-transformation. Haemolymph ammonia levels from animals in high humidities (96.7%) are significantly greater than for animals maintained in sub-threshold humidities ($P<0.001$), whilst ammonia concentrations for the two sub-threshold humidities did not differ significantly ($P>0.1$). The increase in mean ammonia levels in above-threshold humidities supports the possibility of a physiological coupling between ammonia excretion and vapour absorption.

Measurements of fluid pH do not indicate any enhancement of NH_3 volatilization by alkalization. Means and standard errors of fluid pH values are shown in Fig. 4. Both haemolymph and pleon fluid pH values varied between approximately 7.5 and 7.6 whether in ventilating, pre-ventilating or non-ventilating animals. The two pH measurements obtained for urine were 7.07 and 7.76. There is thus no indication of significant alkalization in either of the external fluids.

Prior to adopting this *in vivo* technique for pH determination, we made preliminary measurements of fluid pH in isolated droplets expelled under mineral oil, as for previous measurements of Na^+, K^+ and Cl^- concentrations (Wright and O'Donnell, 1992). However, owing to solubilization of CO_2 into the oil, and consequent alkalization of the fluid sample, these pH estimates were probably exaggerated. Mean values ranged from 7.8 to 8.5, with haemolymph being significantly more alkaline than both pleon fluid and

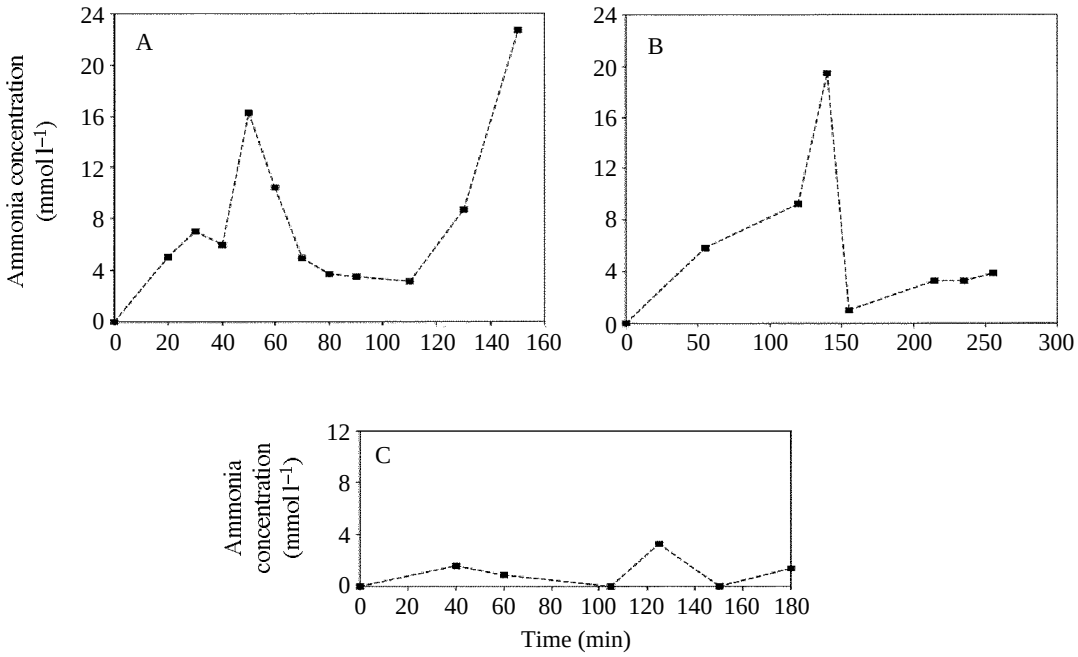

Fig. 2. Examples of time courses of changes in ammonia levels in the haemolymph of individual animals. All animals were sampled during pleopodal ventilation in 96.7% RH; the time scale on the abscissa refers to the elapsed time following transfer to high humidity. Examples A and B illustrate different patterns of periodic ammonia elevation, with the animal in C maintaining low ammonia levels.

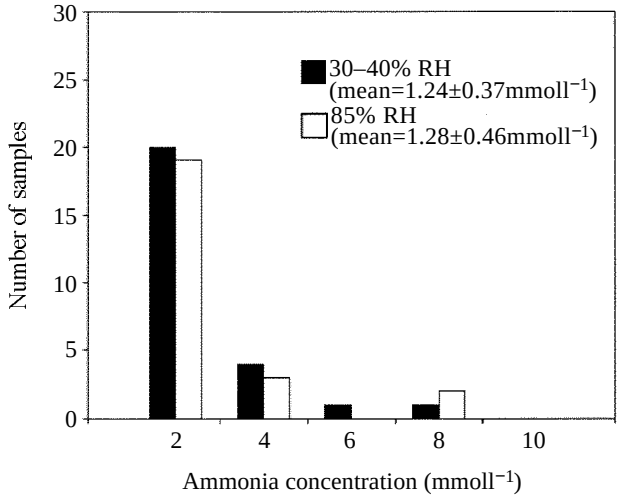

Fig. 3. Sample distributions of ammonia concentrations in haemolymph from animals in humidities below the threshold for water vapour absorption. No ventilatory activity was observed in these humidities. The periodic occurrence of elevated concentrations seen in 96.7% RH is no longer evident, few samples exceeding 2mmol l^{-1}.

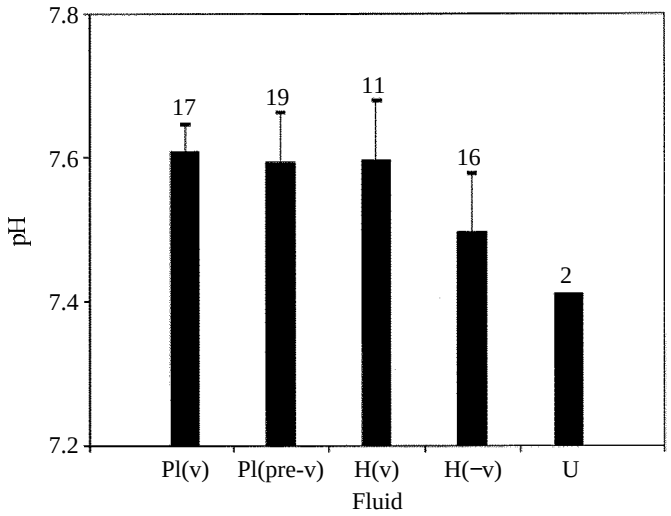

Fig. 4. Histogram showing mean + S.E. pH values and *N* values for the different body fluids. Significant alkalization of pleon fluid (Pl) and urine (U), which would serve to increase P_{NH_3} with respect to the haemolymph (H), is not suggested. v, ventilating; pre-v, pre-ventilating; −v, non-ventilating.

urine in ventilating animals ($P<0.02$). Since urine and pleon fluid will already have sustained a certain alkalization from CO_2 volatilization when measured *in vivo*, the combined data from both measurement techniques indicate that these fluids may actually be more acidic than haemolymph when initially secreted.

On three occasions, we observed secretion of a viscous, amber-coloured fluid into the WS. Following apparent resorption in the rectum, a brownish, crystalline deposit remained in the WS that was later flushed by the normal colourless, watery urine. Unlike the latter, 'brown urine' was markedly acidic in both samples analysed (pH4.95, 6.88), and values are likely to be overestimates owing to CO_2 loss. It clearly represents a different excretory product from the usual maxillary urine, but its occurrence was too infrequent to permit more detailed characterization.

Discussion

This paper demonstrates that pleon fluid, haemolymph and maxillary urine of *P. scaber* all display pronounced fluctuations in total ammonia concentration when animals are maintained in high ambient humidities. Sample distributions of haemolymph and urine ammonia levels are strongly skewed towards low concentrations whilst pleon fluid levels, although still skewed, are more evenly distributed over a range of higher concentrations up to 150mmol l^{-1}. High ammonia levels (>5mmol l^{-1}) in both urine and pleon fluid are associated with elevated haemolymph levels, suggesting intermittent release of ammonia into the haemolymph and its subsequent movement into the external fluids. Time course data show that elevated ammonia concentrations occur in discrete bouts lasting for extended periods.

Ammonia levels in urine and haemolymph do not differ significantly. There is thus no indication of active concentration of ammonia by the maxillary glands, nor any suggestion that maxillary excretion is more frequent when haemolymph ammonia levels are high. By contrast, ammonia levels in the pleon fluid of ventilating animals are significantly higher than in the haemolymph, suggesting a specific role of the pleon fluid in ammonia excretion. However, since this finding was not vindicated by the smaller number of paired samples, the possibility that ammonia is actively concentrated in the pleon fluid must remain tentative.

Two types of pleon fluid secretion have been demonstrated (Wright and O'Donnell, 1992). The first is a scant iso-osmotic secretion, which apparently maintains a permanent thin film over the respiratory pleopodal endopods (Verhoeff, 1920). In the present study, sensitivity of the ammonia assay precluded analysis of the small quantities of isosmotic fluid (<50nl) collectable by micropipettes. The second mode of secretion is the copious, hyperosmotic 'uptake fluid', in which Na^+ and Cl^- are the predominant osmolytes. Uptake fluid is secreted into the pleoventral chamber during active water vapour absorption. Use of pre-desiccated animals and humid sampling chambers (approximately 96.7% RH) promotes initiation of WVA, characterized by the secretion of a large volume of uptake fluid prior to pleopodal ventilation and the onset of absorption (Wright and O'Donnell, 1992). Ventilation provides a convenient marker for WVA because gravimetric studies (Wright and Machin, 1990) indicate an invariable association between the two processes. The only other activity during which animals routinely employ pleopodal ventilation is maxillary urination. This is distinguishable from WVA by the higher ventilation cycle frequencies and the synchronous pulses of urine flow in the WS.

Animals maintained in sub-threshold humidities produced only small volumes of pleon fluid and never initiated ventilatory activity. Although such pleon fluid volumes were too small for ammonia assays, haemolymph samples from the same animals never showed the intermittent high concentrations associated with ventilatory and pre-ventilatory activity in high humidity.

On the basis of these findings, it seems probable that a physiological coupling exists between ammonia excretion and WVA. Such a coupling is indicated by the associations of both processes with pleon fluid secretion and pleopodal ventilation, as well as the non-occurrence of elevated haemolymph ammonia levels when animals are maintained in ambient humidities below the vapour absorption threshold. The copious pleon fluid ('uptake fluid') would thus serve in colligative vapour condensation and simultaneous ammonia volatilization. By replenishing humid, ambient air and dispelling ammonia, ventilation would serve adaptive functions in both processes. The active secretion and resorption of Na^+ and/or Cl^- implicated in absorption might also permit coupling of Na^+ influx to NH_4^+ efflux. A plausible location for Na^+/Cl^- transporting activity is the deeply infolded, mitochondria-rich basal membranes of the pleopodal endopods (Kümmel, 1984). Whether these serve in fluid secretion or resorption, the hyperosmotic Na^+ and Cl^- concentrations in uptake fluid provide a strong gradient for apical Na^+ influx. This influx could drive ammonia excretion directly *via* Na^+/NH_4^+ exchange (Evans and Cameron,

1986; Hunter and Kirschner, 1986; Greenaway, 1991) or indirectly *via* Na^+/H^+ exchange, which would favour diffusion trapping of NH_3 in the pleon fluid (Towle, 1990).

Wieser (1972*a*,*b*) and Wieser and Schweizer (1970, 1972) have previously proposed that the pleopodal epithelia serve as the primary site for ammonia excretion, departing from more conventional views of maxillary excretion (Verhoeff, 1920; Edney, 1968; Hoese, 1981; Wieser, 1984). Proponents of both theories have suggested that fluid pH is increased in the pleon, thereby increasing the concentration of aqueous NH_3. The present study finds no evidence for a role of alkalization in ammonia excretion. Pleon fluid is of similar pH to haemolymph, despite some probable loss of CO_2 on exposure to air. High concentrations of ammonia in the pleon fluid, combined with pleopodal ventilation, presumably provide a sufficient partial pressure gradient of NH_3 to generate necessary rates of volatilization.

The concentration of total ammonia in the pleon fluid required to explain reported rates of volatilization can be estimated by a simple calculation. Wieser (1972*a*) determined mean rates of ammonia volatilization for male and female *P. scaber* as 9.1 and $6.0\,\mu g\,day^{-1}$ (0.38 and $0.25\,\mu g\,h^{-1}$) respectively. We assume this is mostly volatilized from the pleon fluid. From an estimate of the effective permeability of a free water surface in unstirred air, we can determine the partial pressure of dissolved ammonia (P_{NH_3}) required to generate this flux, given a P_{NH_3} for the external air of zero. Gravimetric estimates of the boundary layer permeabilities for 10–100 μl water droplets in an excess volume of unstirred air give values (actually 'standardized fluxes') of approximately $20\,\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ (J. C. Wright, unpublished data). Permeabilities to outward flux of ammonia and water will be similar in view of their very similar diffusion coefficients in air ($0.2–0.25 cm^2\,s^{-1}$ at 20°C and atmospheric pressure; Reid *et al.* 1977). We can thus divide this permeability estimate into the measured rates of volatilization to yield the partial pressure gradient: $0.38\ (\mu g\,h^{-1})/20\ (\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}) = 0.019 Pa cm^2$.

When the pleopods are lifted during ventilation, the fluid surface area for volatilization is approximately $0.05 cm^2$ (J. C. Wright and J. Machin, in preparation). The partial pressure required is thus 0.019/0.05=0.38Pa. Multiplying this partial pressure by the solubility coefficient for ammonia in water at 20°C ($0.52 mmol\,l^{-1}\,Pa^{-1}$; Reid *et al*. 1977) yields the molar concentration of NH_3 (Henry's law): 0.38 (Pa) × 0.52 ($mmol\,l^{-1}\,Pa^{-1}$) = $0.20 mmol\,l^{-1}$.

The mean pH of pleon fluid sampled during pleopodal ventilation was 7.61. Since the pK of ammonia is 9.25, it follows from the Henderson–Hasselbalch equation that $0.20 mmol\,l^{-1}$ NH_3 represents $8.93 mmol\,l^{-1}$ total ammonia at pH7.61. This represents the required concentration of ammonia in the pleon fluid to generate measured rates of volatilization, if excretion were continuous. The intermittent pattern of ammonia volatilization will clearly require periodic concentrations to be considerably higher. Nevertheless, even a fivefold increase would be quite compatible with the pleon fluid total ammonia levels reported here.

By a similar procedure, it is possible to estimate the total ammonia concentration in the haemolymph that would be required for passive diffusional excretion across the entire body wall. The cutaneous permeability to outward water flux in *P. scaber* is approximately $0.7\,\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ (J. C. Wright and J. Machin, in preparation). Again,

similar physical constants for ammonia and water, including aqueous diffusion coefficients, molecular radii, lipid solubilities and partition coefficients (Reid *et al.* 1977; Nicholls, 1979; Evans and Cameron, 1986; Weast, 1990), suggest that permeabilities of the cuticle to both molecular species will be similar. For a net ammonia flux of $0.38\,\mu g h^{-1}$ and net surface area of $2.0 cm^2$ (as above), the requisite partial pressure gradient will thus be: $0.38\ (\mu g h^{-1})/[0.7\ (\mu g h^{-1}\,cm^{-2}\,Pa^{-1}) \times 2.0\ (cm^2)] = 0.27 Pa$.

Multiplying by the solubility coefficient of NH_3 in water gives a molar concentration of $0.141 mmol\,l^{-1}$. The mean 'non-ventilating' haemolymph pH recorded in this study was 7.50, equivalent to an NH_3/NH_4^+ ratio of 0.0178. For an aqueous NH_3 concentration of $0.141 mmol\,l^{-1}$, the concentration of total ammonia is thus 0.141 + [0.141/0.0178] = $8.07 mmol\,l^{-1}$.

Animals would, therefore, require substantially elevated haemolymph ammonia levels to sustain excretion by passive volatilization. A concentration of $8.07 mmol\,l^{-1}$ is significantly higher than mean levels maintained between bouts of assumed volatilization (1.22 and $1.24 mmol\,l^{-1}$ for animals in sub-threshold humidities). Such results suggest that limited long-term tolerance of elevated ammonia levels might have shifted selection in favour of intermittent pleon excretion as an alternative to passive volatilization. For *P. scaber* this may represent only a modest adaptive benefit. However, passive elimination across the integument will require increasing haemolymph concentrations for species with lower cuticle permeabilities. *Venezillo arizonicus*, for example, has a cuticle permeability of only $0.11\,\mu g h^{-1} cm^{-2} Pa^{-1}$ (Warburg, 1965) and would require a haemolymph ammonia concentration of about $51.6 mmol\,l^{-1}$ for continuous passive elimination, assuming a similar rate of ammonia excretion to *P. scaber*. The proposed coupling of ammonia volatilization to the production of hyperosmotic fluids during WVA may have been of considerable preadaptive significance in permitting oniscideans to radiate into xeric habitats that have excluded other Crustacea.

Localized volatilization of NH_3 from pleon fluid, even without any coupling to WVA, avoids the compromise of requiring either a sustained high internal P_{NH_3} or a high cuticle permeability. Perhaps the most significant adaptive consequence of coupling ammonia volatilization to WVA would be the possibility of ammonotelic excretion without concomitant water loss. The tendency for any gas to diffuse into or out of a liquid is defined by its invasion rate, the product of the solubility and diffusion coefficients (Withers, 1992). For water and NH_3 volatilizing into air, the invasion rates are similar (3.00 and $2.71 cm^2\,s^{-1}\,kPa^{-1}$ respectively). A droplet of an aqueous ammonia solution will therefore lose water and NH_3 at similar rates for given partial pressure gradients. An isopod could thus conserve water, whilst releasing NH_3, simply by exploiting a near-saturated environment, offering no partial pressure gradient for water exchange but favouring outward NH_3 diffusion. Many workers have suggested that confining excretion to the day, when animals inhabit humid microclimates, serves in water conservation (Wieser, 1972*b*, 1984; Wieser *et al.* 1969; Warburg *et al.* 1984). However, confining excretory bouts to periods of saturated RH, in order to eliminate simultaneous water loss, would greatly restrict the time available for ammonia release, and might also result in high gaseous concentrations in densely populated diurnal retreats. By contrast, coupling of NH_3 excretion to WVA would provide a unique example of ammonotelic excretion

actually involving simultaneous net water uptake in an extended range of humidities down to the critical equilibrium humidity for vapour absorption (91.3% for *P. scaber*; J. C. Wright and J. Machin, in preparation).

The sub-order Oniscidea, infra-order Ligiamorpha, has been divided into three sections – Diplocheta, Synocheta and Crinocheta (Holdich *et al.* 1984) – the last of which includes such familiar genera as *Porcellio, Oniscus* and *Armadillidium*. The intermittent patterns of ammonia excretion seen in the Crinocheta (Wieser, 1984) can be attributed to the periodic nature of water vapour absorption. It would be interesting to know whether the predominantly mesofaunal Synocheta, which apparently lack the capacity for WVA (J. C. Wright and J. Machin, in preparation), show different patterns of ammonia production. Information for the Diplocheta is restricted to *Ligia beaudiana* (Wieser, 1972*b*). This intertidal species shows a similar intermittent pattern of ammonia volatilization to the terrestrial woodlice, although most excretion takes place during contact with liquid water. Pronounced diurnal maxima of ammonia release have also been demonstrated for the marine isopod families Valvifera and Flabellifera (Kirby and Harbaugh, 1974), although it is unclear whether these also involved the short-term 'bursts' noted by Wieser. Notwithstanding diel patterns resulting from variations in protein metabolism, ammonia excretion in other Crustacea is a continuous process (Regnault, 1987; Kormanik and Cameron, 1981).

The maximum concentrations of haemolymph ammonia for *P. scaber* reported in the present study are at least two orders of magnitude greater than intracellular levels tolerated in vertebrates (Lehninger, 1982; Meijer *et al.* 1990). Ammonia toxicity involves inhibition of the Krebs cycle through competitive depletion of α-ketoglutarate, disruptions of chloride channels and hence neuronal hyperpolarisation, as well as perturbations of the metabolism of neurotransmitters, notably glutamate and aspartate. Whether oniscideans transport aqueous ammonia *via* a carrier molecule, or possess efficient mechanisms for maintaining low intracellular ammonia levels, is not known. Hartenstein (1968) and Wieser and Schweizer (1972) reported ammonia concentrations up to $17\,\mathrm{mmol\,l^{-1}}$ in the somatic tissues ('body wall') of *P. scaber* and *O. asellus*, although their samples are likely to have included some haemolymph. Nevertheless, such data suggest that the cells may be physiologically adapted to tolerate high ammonia levels, at least in the short term. High extracellular ammonia levels have been reported in several other Crustacea (Greenaway, 1991). A mean haemolymph concentration of $20.0\,\mathrm{mmol\,l^{-1}}$ has been measured for *Uca pugilator* (Green *et al.* 1959) and concentrations as high as $271\,\mathrm{mmol\,l^{-1}}$ and $131\,\mathrm{mmol\,l^{-1}}$, respectively, have been determined for the oplophorid shrimps *Notostomus gibbosus* and *N. elegans* (Sanders and Childress, 1988). During dehydration, the gecarcinid *Cardiosoma carnifex* tolerates sustained haemolymph ammonia concentrations of $7\,\mathrm{mmol\,l^{-1}}$ (Wood *et al.* 1986). Extreme ammonia tolerance may therefore be a generic trait.

Intermittent ammonia excretion requires a non-toxic storage product in which ammonia can be sequestered following deamination and transamination. The most widespread storage compounds, in both vertebrates and invertebrates, appear to be glutamate and glutamine, which have both been detected in significant concentrations in the somatic tissues of *P. scaber* (Wieser and Schweizer, 1972). Glutamine is also present

in high concentrations (>500 μmol l^{-1}) in the haemolymph of terrestrial oniscideans (Sevilla and Lagarrigue, 1974), although the haemocyte/plasma ratios are unknown. Wieser (1972*c*) detected significant concentrations of glutaminase in the somatic tissues, perhaps indicating that ammonia is stored at the site of production and periodically released into the haemolymph. Evidence for ammonia detoxification pathways in the crabs *Carcinus* and *Cancer* indicates glutamine as the major storage product and glutaminase activity suggests specific release of ammonia at the gills (King *et al.* 1985). Further work is required to elucidate these pathways in terrestrial isopods so as to determine specific sites of ammonia storage, the molecular form of haemolymph ammonia, and the regulatory steps in the ammonia storage and release pathways.

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