

A RE-EXAMINATION OF THE EXCRETION OF NITROGEN BY TERRESTRIAL ISOPODS

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INTRODUCTION

In 1950 Dresel and Moyle published a paper concerning the nitrogenous excretion of amphipods and isopods, upon which most subsequent authors of text-books have relied when discussing protein metabolism and nitrogen excretion in these groups of animals (Prosser & Brown, 1961; Lockwood, 1963). The major findings by Dresel & Moyle pertinent to our discussion were the following:

(1) Isopods are predominantly ammonotelic, releasing 10–30% of their ammonia in gaseous form, the major portion dissolved in faeces and urine.

(2) Terrestrial isopods excrete much less nitrogen than related fresh-water and marine species. The authors concluded that 'in this group, adaptation to terrestrial conditions has been attended by a general suppression of nitrogen metabolism rather than by a transformation of ammonia to other, less toxic products'.

There are two reasons why these conclusions have to be challenged and why it is necessary to re-examine the problem of nitrogen excretion in terrestrial isopods.

The first reason is the recent discovery that gaseous ammonia may constitute an even more important end product of nitrogen metabolism in terrestrial invertebrates than would be expected on the basis of the so-called 'Needham's rule' (Needham, 1938; W. C. Sloan (personal communication)*; Speeg & Campbell, 1968; Hartenstein, 1968).

The second reason is that an inverse relationship seems to exist in isopods between activity and protein metabolism (Wieser, Schweizer & Hartenstein, 1969), so that measuring the end products of the latter during periods of feeding activity—as did Dresel and Moyle—may not give a true picture of the levels of nitrogen turnover in these animals.

MATERIALS AND METHODS

Isopods (*Porcellio scaber* Latr., *Oniscus asellus* L.) were collected in the field and kept in containers at room temperature where they fed on decayed leaves, wood and faecal material. Ceramic pots pushed into moist sand proved to be ideal containers. Experiments were carried out exclusively on non-moulting male specimens at 20° C. in constant darkness.

* Dr Sloan of San Diego State College was the first to carry out a thorough analysis of nitrogen metabolism in isopods. His results, however, have not appeared in print at the time this manuscript went to press.

(1) For the measurement of *gaseous ammonia* the animals were placed in glass-stoppered chambers. On the inside of the lid a small piece of glass wool soaked with 0.1 ml. of N. H₂SO₄ trapped the ammonia released by the animals. The glass wool was removed and replaced by a fresh piece at the time of measurement. This involved taking the animals out of the thermostat and exposing them to light. Ammonium was determined by a modified phenolphthorite reaction with trichloroisocyanuric acid (TICA) as the donor of the hypochlorite radical. A similar method was described by Seely, Petitclerc and Benoiton (1967).

Procedure. The glass wool removed from the reaction chamber was placed in a tube and mixed with (in this order) 1 ml. distilled water, 0.5 ml. phenol reagent (5 g. phenol and 0.025 g. sodium nitroprusside in 100 ml. distilled water), 0.5 ml. of TICA reagent (44 mg. TICA [Schuchardt] in 10 ml. N. NaOH), and incubated for 10 min. at 50° C. The solution was made up to 4 ml. with distilled water and the blue colour was measured at 578 nm. Standard curves were prepared with ammonium sulphate as substrate.

(2) For the determination of *ammonia in faeces* it was necessary to prevent ammonia formation during the assay by deamination reactions or the action of alkali on proteins and other organic nitrogenous substances. Neglect of this possibility leads to erroneously high values for free ammonia. We adopted, and altered slightly, the procedure of Gangolli & Nicholson (1966), precipitating all nitrogenous substances, except ammonia, with mercuric chloride and lead acetate at a pH of 4.1–4.2. The supernatant is then alkalinized and the ammonia driven into an acid trap by aeration, as described by Sobel, Mayer & Gottfried (1944).

Reagents. (a) Precipitating solution: 12.5 g. HgCl₂ in 50 ml. absolute ethanol; 5 g. lead acetate in 20 ml. distilled water. These two solutions are mixed one day before use. The white precipitate formed is dissolved in 2 M acetic acid (about 15–16 ml. required), and the solution made up to 100 ml. with distilled water.

(b) Saturated potassium iodide solution (prepared fresh daily).

(c) Saturated sodium carbonate solution.

(d) Octyl alcohol.

Procedure. The precipitating solution is diluted 1.5:50 with water. A sample of freshly collected faeces (we used 5–30 pellets) is homogenized in 1 ml. of the diluted solution in a Potter–Elvehjem glass homogenizer. After decanting the homogenate the homogenizer is rinsed with 2 ml. of distilled water and the rinsings are added to the homogenate. The sample is then centrifuged at 7000 r.p.m. for 7 min. and 1 ml. of the supernatant is pipetted into an aeration tube. The tube is closed with a rubber stopper into which a capillary of 3 mm. diameter has been inserted so that its tapered end reaches the sample to be aerated. The side arm of the aeration tube is connected with a second tube containing 1 ml. of 0.1 N. H₂SO₄ and the side arm of this tube is connected with an aspirator. After the aeration tube has been closed, 0.4 ml. of the KJ-solution (b), 1 ml. of the Na₂CO₃ solution (c) and one drop of octyl alcohol (d) are pipetted through the capillary into the sample. The upper end of the capillary is quickly connected with the outlet of a wash bottle containing 0.1 N. H₂SO₄ through which air from the outside is drawn, and suction is started. After 30 min. of aeration the ammonia trapped by the acid in the second aeration tube is determined by means of the phenolphthorite reaction as outlined above. In preparing a standard curve the possibility

has to be taken into account that the precipitating solution may bind free ammonia; further, the supernatant of the faeces homogenate might contain substances that combine with ammonia. We therefore homogenized samples of ammonium sulphate, containing 1, 2 and 3 μg . of $\text{NH}_4\text{-N}$, with the diluted precipitating solution and determined the ammonia remaining in the supernatant by following the procedure, described above, for the faecal homogenate. We also added known amounts of ammonia to aliquots of a faecal homogenate and subtracted, after aeration, the faecal ammonia from the ammonia recovered in the acid traps. Total nitrogen of faeces was determined by standard micro Kjeldahl digestion and nesslerization.

RESULTS

1. Levels of ammonia excreted

In spring and summer non-feeding specimens of *P. scaber* and *O. asellus* release gaseous ammonia in a rhythmic fashion, with a pronounced maximum around noon, a minimum before midnight (Wieser, Schweizer & Hartenstein, 1969). Nineteen non-feeding specimens of *P. scaber* from Innsbruck, measured individually over several days in constant darkness and 20° C, released an average of 11 ng. ammonia/mg. body weight \times hr. The rate of ammonia excretion drops strikingly, and the diurnal rhythm is all but abolished, if food is added to the reaction chambers. Specimens of *P. scaber* feeding on old poplar leaves released an average of 2.9 ng. gaseous ammonia/mg. \times hr. at more or less constant rate in the course of a day. In constant darkness the animals feed almost continuously, with a slight maximum of faeces production in the afternoon and a minimum before midnight. In two sets of experiments (between March and April) the effect of trapping the gaseous ammonia fraction released by *P. scaber* on the ammonia content of the faeces was investigated. In the first experiment, lasting 6 days, pairs of specimens produced faeces that contained an average of 6.8 ± 0.7 (S.E.) μg . $\text{NH}_3/10$ mg. dry faeces if gaseous ammonia was *not* trapped; and 4.9 ± 0.8 μg . ammonia if gaseous ammonia *was* trapped. In the second experiment, lasting 5 days, four specimens with and four specimens without, an acid trap produced faeces with an average of 4.2 and 4.3 μg . ammonia/10 mg. dry faeces respectively. It was concluded that trapping the gaseous ammonia released by the animals had no effect, or at most a slight effect, on the ammonia content of the faeces.

In the experiments without an ammonia trap eight specimens of *P. scaber* produced faeces at a rate of from 0.5 to 0.8 pellets per animal per hour which corresponded to 0.46–1.12 ng. ammonia/mg. body weight \times hr. In Fig. 1 the diurnal pattern of the excretion of ammonia by feeding and non-feeding *P. scaber* under constant conditions is plotted on a log scale. The average amount of nitrogen excreted as NH_3 via faeces is 0.84 ng./mg. body weight \times hr., which corresponds to 200 μg ./10 g. \times 24 hr., and thus is very close to the 0.3 mg. N/10 g. \times 24 hr. reported for *P. laevis* by Dresel and Moyle. On this low figure the latter authors had based their conclusion that nitrogen metabolism is suppressed in terrestrial isopods. However, as can be inferred from Fig. 1, feeding specimens of *P. scaber* release about 3.4 times as much ammonia in gaseous form as they do via their faeces; and non-feeding specimens again release about 3.8 times more NH_3 in gaseous form than feeding specimens. Thus faecal ammonia constitutes at most 10% of the total nitrogen released by specimens of *P. scaber*

during periods of temporary starvation. (The fraction of ammonia nitrogen discharged by the animals via faeces is probably even smaller if it is considered that part of the ammonia passing through the gut was already present in the food; see Dunger, 1958.) If this relationship is taken into account it may be assumed that the terrestrial isopods investigated by Dresel and Moyle (*Oniscus asellus*, *Porcellio laevis*, *Armadillidium vulgare*) which discharged an average of 0.3–0.4 mg. N/10 g. × 24 hr. via their faeces actually released 10 times as much or more in gaseous form, mainly during periods of inactivity. The figure thus arrived at, of 3–4 mg. N/10 g. × 24 hr., corresponds well with the figures quoted by Dresel and Moyle for fresh water and marine species of isopods and amphipods. *Asellus aquaticus*, for example, is reported to excrete 1.9–2.9 mg. N/10 g. × 24 hr., and *Gammarus locusta* 4.2–6.2 mg.

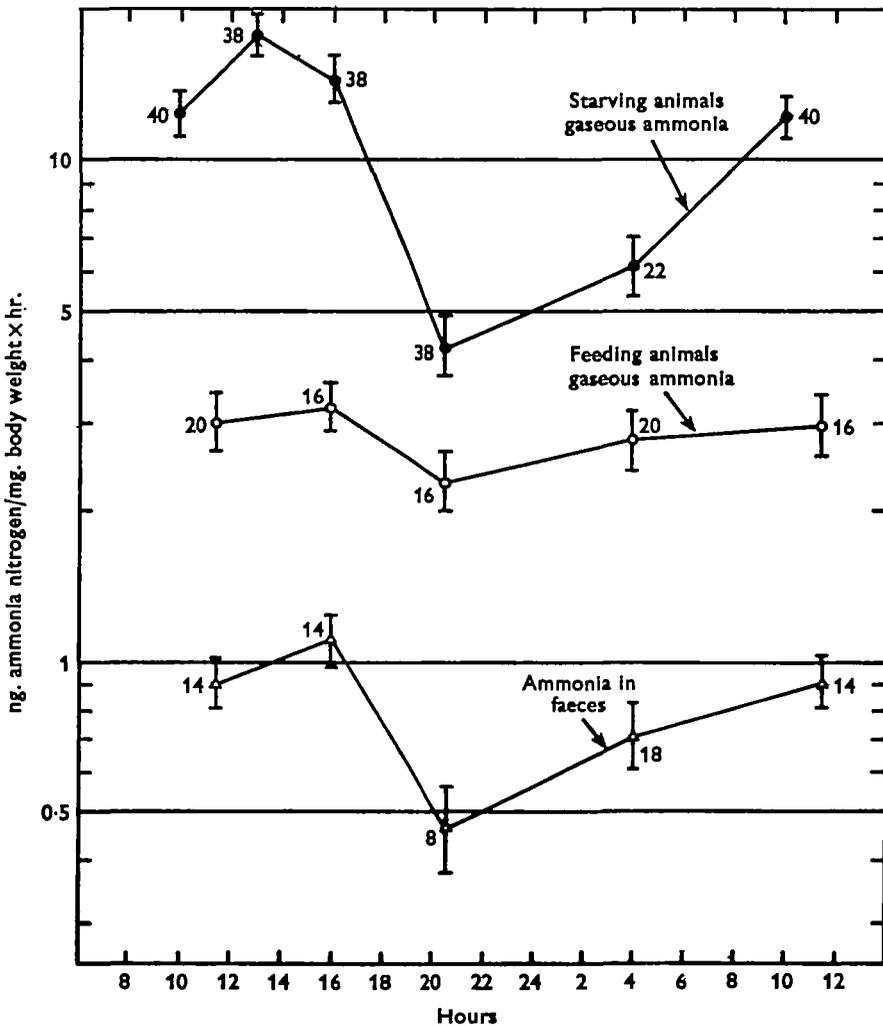


Fig. 1. Semilogarithmic plot of diurnal variations in the excretion of gaseous ammonia and of ammonia dissolved in faeces by feeding and starving *Porcellio scaber*. Symbols represent mean values of several determinations, being indicated next to them. Bars represent \pm one standard error.

2. Seasonal effects

The rhythmical release of gaseous ammonia by terrestrial isopods in spring and summer is replaced by a more burst-like production in autumn and winter. *O. asellus*, in particular, is outstanding in spending days or even weeks without releasing detectable amounts of ammonia, whereupon it will discharge a relatively large volume in the course of several hours. In order to gain an idea about seasonal differences in nitrogen turnover the following figures may serve as an example:

	ng. ammonia nitrogen/ mg. × hr. (average over several days)
<i>O. asellus</i>	
April–May	5·8
September–October	1·15
<i>P. scaber</i>	
May–June	11·0
November	2·1

Thus terrestrial isopods kept under room conditions in Innsbruck in constant darkness at 20° C. release approximately five times more ammonia in spring than in autumn. It should be stressed again that only non-moulting male specimens were used for measurement.

3. Nitrogen content of faeces

The nitrogen content of faeces produced by *P. scaber* after feeding on old poplar leaves was determined on five samples. We obtained the following values: 188, 200, 212, 228, 274 µg. N/10 mg. dry faeces. The mean value of 220 µg. N/10 mg. is in good agreement with the data reported by Dunger (1958) who had found between 1·23 and 2·56% nitrogen in the faeces (air-dried) produced by isopods and diplopods after feeding on various kinds of leaves. The leaves contain about the same amount of nitrogen on a dry-weight basis, which indicates that isopods do not selectively extract nitrogen to any extent from their food. The ammonia content of the faeces, quoted above, of 4–7 µg. ammonia nitrogen/10 mg. dry faeces corresponds to 2–4% of the total nitrogen. This is lower than the figures reported by Dunger (1958) who found approximately 10% of the total nitrogen of faeces to consist of ammonia. This difference may reflect differences in the methods of extracting ammonia from faeces.

4. Effect of temperature

The question as to whether the amounts of gaseous ammonia released by terrestrial isopods convey a quantitative picture of total nitrogen turnover in these animals is answered indirectly by the fair agreement between the figures for ammonia released by *P. scaber* and those for ammonia production in the aquatic ammonotelic species studied by Dresel and Moyle.

A more direct answer would be provided if it could be shown that the release of gaseous ammonia is closely related to another variable of the animals' metabolism. The simplest variable to measure is faeces production—which is also a direct measure of food consumption. If the animal system is subjected to an abrupt change and both

the production of faeces and the release of ammonia are monitored for some time before and after this step function a possible correlation between the two variables would be indicated by their following the same time course. The changes introduced were temperature jumps from 5 to 20 °C and from 20 to 5 °C. The number of faecal pellets produced by eight pairs of *P. scaber* feeding on old poplar leaves were counted before and after each temperature jump for several days. In another series the amount of ammonia released was measured in eight groups of feeding specimens of *P. scaber*. The results, expressed as deviations from the normalized means of all experiments at the first temperature, are set out in Fig. 2. After the temperature jump both variables

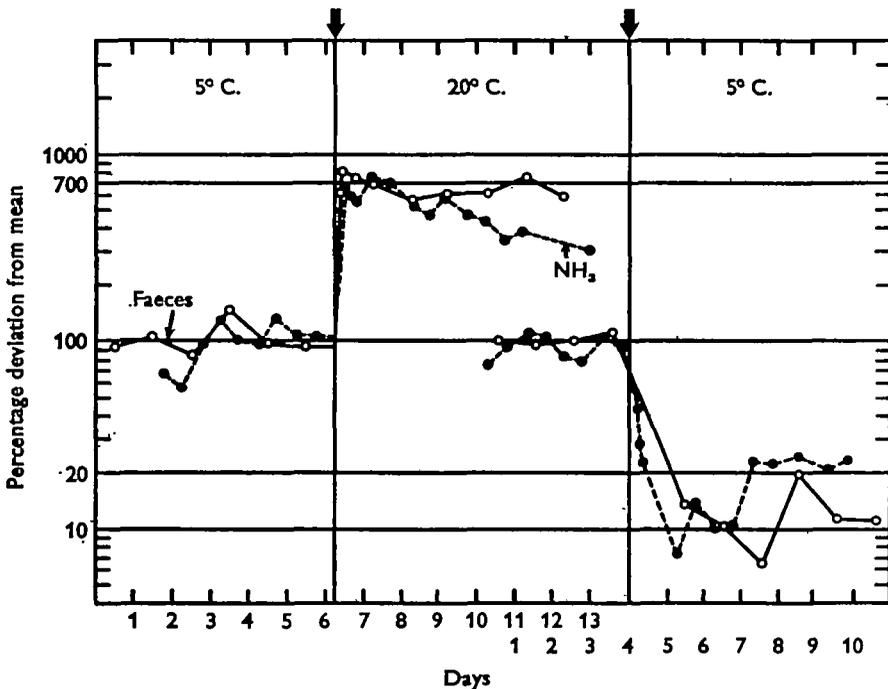


Fig. 2. Release of gaseous ammonia and production of faeces by *Porcellio scaber* before and after two temperature jumps. The curves connect deviations from the normalized means (taken as 100 per cent) at the starting temperature. Immediately after the temperature jump measurements were taken every 2 hr., otherwise one or two per day. Each symbol represents mean values from 6 to 8 experiments. Open circles: faeces; full circles: ammonia.

show parallel increases or decreases lasting for at least 2 days. After this the relative amounts of ammonia released fall below the steady-state level of faeces production if the change is from the low to the high temperature, whereas ammonia rises above faeces production if the change is in the opposite direction. The initial values of Q_{10} following the temperature jumps were approximately 3.7 between 5 and 20 °C., and 4.6 between 20 and 5 °C.

The divergence between faeces production and ammonia excretion that begins to show up approximately 2 days after the change of temperature may reflect differences in the ratio of protein turnover and other parameters of metabolism (as, for example,

energy production). However, this is a problem of its own that will not be pursued here.

It seems that the temperature-jump experiments offer additional evidence that the release of gaseous ammonia by terrestrial isopods is not a spurious phenomenon but reflects important parameters of the metabolism of these animals.

DISCUSSION

The following speculative attempt may be made to correlate quantitatively nitrogen input and output—in the form of ammonia—for *P. scaber*. Under experimental conditions at 20° C., a specimen weighing 70 mg. will consume a daily average of 3.4 mg. dry weight of poplar leaves (Wieser, 1965). Of this the animal will assimilate between 15 and 25% (Gere, 1962; Hartenstein, 1964; Wieser, 1965), corresponding to 510–850 μ g. dry leaf substance. Old leaves contain roughly 2% nitrogen (Dunger, 1958). Our model specimen will therefore assimilate between 10 and 17 μ g. nitrogen per day. In our experiments reported above, non-feeding specimens of *P. scaber* will release—in spring and summer—an average of 11 ng. ammonia per mg. \times hr. which corresponds to 18 μ g. per day for a specimen weighing 70 mg.

Thus it can be said that under experimental conditions and in the course of a normal diurnal cycle during which periods of feeding activity alternate with periods of inactivity, nitrogen output in the form of gaseous ammonia can approximately balance nitrogen input, even if no other nitrogen end-product is excreted by the animals. In nature, food consumption is probably lower by a factor of 3–4 than food consumption measured in the laboratory (Gere, 1962; Wieser, 1965). This would not invalidate our argument as long as nitrogen excretion dropped proportionally. If it did not then the high level of nitrogen excretion in non-feeding isopods would have to be taken as a further example of proteins and amino acids serving as a major source of energy metabolism in crustaceans (Speck & Urich, 1969*a, b*). At any rate, the assumption by Dresel and Moyle that in terrestrial isopods nitrogen metabolism is suppressed does not stand up to scrutiny. Rather it should be said that isopods have adapted protein metabolism to terrestrial conditions by programming the excretion of nitrogen in such a way that it takes place mainly during periods of inactivity when the animals are in their moist retreats. Under these conditions they are in least danger of losing water along with the ammonia excreted through the epithelia of the body wall.

SUMMARY

1. In spring and summer specimens of *Porcellio scaber* feeding on old poplar leaves excrete from 0.46 to 1.12 ng. dissolved ammonia/mg. body weight \times hr. via the faeces, and from 2.3 to 3.25 ng. in gaseous form via the body wall. Non-feeding specimens excrete an average of 11 ng. ammonia/mg. \times hr. in gaseous form. In the course of a normal cycle in which periods of feeding activity alternate with periods of inactivity (temporary starvation), faecal ammonia thus constitutes only 10% of the total ammonia released by the animals.

2. If the gaseous component is taken into account, the rate of nitrogen excretion in terrestrial isopods is comparable with that of fresh water and marine species.

3. *Oniscus asellus* and *P. scaber* excrete approximately five times more ammonia in spring and summer than in autumn and winter.

4. The faeces produced by *P. scaber* contain an average of 220 μg . total nitrogen/10 mg. of dry faeces of which 2-4% consist of ammonia.

5. Following abrupt changes of temperature, from 5 to 20° C. and from 20 to 5° C. the rates of the release of ammonia and of the production of faeces follow similar time courses, increasing with an initial Q_{10} of 3.7 if the change is from the low to the high temperature, decreasing with an initial Q_{10} of 4.6 if the change is in the opposite direction.

6. It is suggested that in terrestrial isopods nitrogen output in the form of gaseous ammonia more or less balances nitrogen input from leaf litter (the animals' main source of food) and that no reduction of protein metabolism takes place in terrestrial species of isopods as compared with aquatic species.

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