Oecologia (Berl.) 3, 390—400 (1969)

Patterns in the Release of Gaseous Ammonia by Terrestrial Isopods

W. Wieser and G. Schweizer*
Institut für Zoologie, Lehrkanzel für Tierphysiologie, Universität Innsbruck, Austria

R. Hartenstein
Department of Zoology, State University College of Forestry, Syracuse, New York

Received July 7, 1969

Summary. In the fall and in early spring *P. scaber* and *O. asellus* released gaseous ammonia in the form of more or less regularly spaced bursts. In the spring about twice as much ammonia was released by *O. asellus* than in the fall. In late spring and summer, however, both species released ammonia in a rhythmic fashion, with a maximum at noon and early in the afternoon, and a minimum early at night. Sometimes a second maximum occurred late at night.

In *O. asellus* the addition of a moist substrate to the reaction chamber shifted the maximum of the release of ammonia from noon to late night and early morning.

Fed specimens of *P. scaber* released only about one-third as much NH_3 as fasting animals and — at least in constant darkness — with a period of much reduced amplitude.

It is concluded that the rhythmical release of ammonia is inversely related to the pattern of locomotory activity of these animals. This would implicate mechanisms that regulate either the production or the release of ammonia in such a way that the maximum occurs at a time when the animals' production of energy is at a minimum and when they are protected against loss of water by sitting in their moist retreats.

The fact that many terrestrial invertebrates release nitrogen in the form of gaseous ammonia has recently gained prominence. The evolution of this gas has been shown to occur im isopods (Dresel and Moyle, 1950), snails (Speeg and Campbell, 1968), and insects (Blight, 1969). Evidence is increasing with regard to isopods that volatile ammonia may in fact represent the dominant endproduct of protein metabolism (Sloan, 1967; Hartenstein, 1968). Since ammonia is easily measured and its evolution can be monitored conutinously (e.g. titrimetrically with pH-stat recording) an excellent opportunity is provided for a quantitative appraisal of protein metabolism in a group of animals with a wide ecological range.

* The work at Innsbruck was supported by the "Fonds zur Förderung der wissenschaftlichen Forschung" of Austria.

Two of us (W. W., R. H.) had begun independent studies on the pattern of ammonia excretion from terrestrial isopods under various environmental conditions. In this paper we report some of the patterns that were observed, and discuss several similarities and apparent differences between two geographically distinct populations of isopods.

Methods and Materials

Isopods were collected in the field and kept in containers at room temperature or at 15° C, where they fed on decayed leaves, wood, and fecal material. Ceramic pots pushed into moist sand proved to be ideal containers. Experiments were carried out at 20° C in constant darkness (Innsbruck), or at 23° C under natural diurnal photofluctuations (Syracuse).

The evolution of ammonia was measured differently at the two institutions. Specimens of *Oniscus asellus* in Syracuse were placed separately in 15 ml Warburg flasks attached to a Gilson differential respirometer. Air was flushed through the flasks at a rate of 30 to 60 cc/min after first passing through a gas washing bottle containing 300 ml 5 N H_2SO_4 and a second gas washing bottle with distilled water. After flushing the chambers, the air was passed from a capillary tube through 4 ml 0,01 N HCl. The ammonia content of the air was presumably quantitatively trapped. This was determined initially by placing from 0 to 0.6 μM ammonia as ammonium sulfate into the side arms and 0.5 ml saturated potassium carbonate in the main chamber, mixing the contents at time zero, and checking the recovery with a sensitive, nitroprusside technique (Brown *et al.*, 1957) after two hours of flushing. The average recovery was 97% with a range of 96 to 98%.

In Innsbruck single male specimens of *Porcellio scaber*, *O. asellus* and *Tracheoniscus rathkei* were placed in glass-stoppered chambers. Glasswool, soaked with 0.1 ml of 0.1N H_2SO_4, was placed inside the lid to trap excretory ammonia. The glass wool was removed and replaced by a fresh piece at the time of measurement. During this process the animals were removed from the thermostat and were exposed to light. Ammonia was determined by a modified phenol-hypochlorite reaction in which trichloroisocyanuric acid (TICA) was used. A similar method was described by Seely *et al.* (1967). The reagents included 44 mg of TICA (Suchardt) in 10 ml 1 N NaOH (stable at 4° C for approximately three days) and 5 g phenol and 0.025 g sodium nitroprusside in 100 ml of distilled water. The procedure used was as follows: The ammonia trap (glass wool) was placed in a tube and mixed consecutively with 1 ml water, 0.5 ml phenol reagent, and 0.5 ml TICA reagent. Following an incubation period of ten minutes at 50° C, the solution was made up to 4 ml with water and the blue color was measured at 578 nm in a Spectronic 20 photometer. The reaction is not specific for ammonia. However other volatile bases excreted by these animals are quantitatively insignificant (Dresel and Moyle, 1950). Standard curves were prepared with ammonium sulfate as substrate.

Results

1. Rhythms and Bursts

The relationship between time of day and production of ammonia was first studied in fasting males of *P. scaber* from Innsbruck at various times between March and July 1968. During this period the animals displayed conspicuous rhythmicity, with a maximum evolution of NH_3

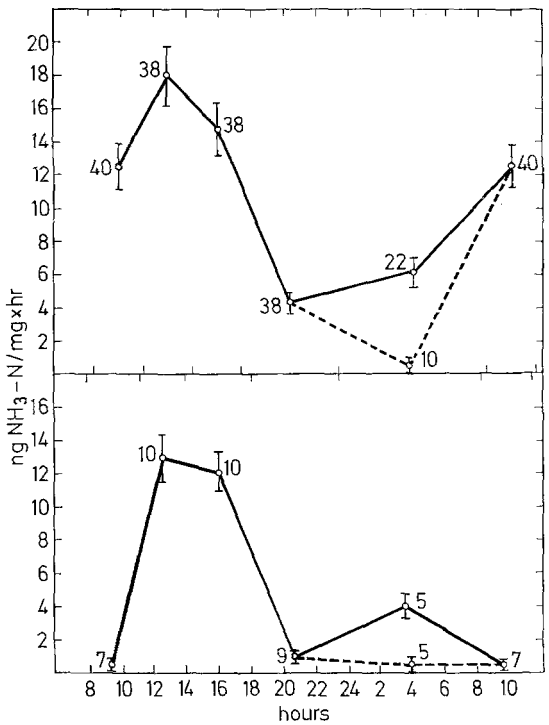

Fig. 1. Release of ammonia as related to time of day in *Porcellio scaber*. Means, standard errors and numbers of measurements are indicated. The times at which the release of ammonia was measured are indicated on the middle abscissa. Dashed curve links main curve with a group of specimens that had produced less than 1 ng of ammonia between midnight and morning

at noon and in the afternoon, and a minimum in the evening and early night. Some animals released no ammonia between midnight and morning while others exhibited a second maximum in this interim. Moreover, a significant distinction could be made between males tested from the end of March to the beginning of April and those tested from May to July. The earlier group released significantly less ammonia in the morning and evening and this in turn resulted in a mid-day peak that appeared pronounced relative to that exhibited by the later group. These findings are summarized in Fig. 1.

Similar tests were conducted in autumn with *P. scaber*, *O. asellus*, and *T. rathkei* in Innsbruck, and *O. asellus* in Syracuse. The results, however, were strikingly different from those described above, in that the NH_3 was given off in the form of seemingly erratic bursts. The case has been studied best for *O. asellus* in Innsbruck and Syracuse (Tables 1, 2). Some specimens produced only a single burst in 24 days. Other specimens

Table 1. *Release of ammonia (ng/mg×hr) by fasting Oniscus asellus from Innsbruck in September and October 1968*

Day after beginning of experiment	1		2				3			4		5	
Period of measurement (hour)	8—12	12—24	24—9	9—12	12—18	18—8	8—12	12—18	18—9	9—12	12—18	19—9	10—18
Animal no.													
1	0.9	0.1	0	0	0	0	0	0	0	0	0	0.1	0
2	0.5	0	0	0	0	0	0	0	0	0	0	0	0.4
3	0.7	0	0	0	0	1.4	0.8	0.2	0	0	0	0.1	*2.8*
4	0.5	0	*7.2*	1.6	0	*3.6*	*4.9*	0.5	*4.0*	1.3	0	*3.2*	0.4
5	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1.5	0	0	0	0	0	*13.8*	*14.0*	1.5	*12.7*	*26.0*	*3.3*	1.4
7	2.0	0	0	0	0	0	0	0	0	0	*24.0*	0.6	*2.8*
8	1.3	0	0	0	0	0	0	0	0	0	0	1.7	0.9
$\bar{x}$	0.9	0	0.9	0.2	0	0.6	2.4	1.8	0.7	1.7	6.2	1.1	1.1

Period of measurement	8(9)—12	12—18	18—8(9)
$\bar{x}$ all values	*1.33*	*1.80*	*0.84*
n	32	40	32
$\bar{x}$ values > 0	*3.3*	*6.6*	*2.4*
n	13	12	11

Table 2. *Release of ammonia (ng/mg×hr)*[a] *by fasting Oniscus*

Fall specimens weight (mg)	Day after beginning experiment										
	2	3	4	5	6	7	8	9	10	11	12
44	8.3	0	16.4	3.6	0	0	0	16.0	1.5	0	5.2
		6.7	6.6	0	0	0	0	16.0			
50	0	0	10.0	0	0	5.4	0	0	4.1	2.3	8.5
		7.6	4.1	0	0	0	0	0			
44	0	0	0	0.1	0	0	0	0	0	0.3	0
		0	33.8	0	0	0	0	0			
42	0	6.6	0.5	0	18.0	0	0	7.5	0	0	10.0
		7.7	3.7	0	0	0	0	0			
34	0	0	0	13.2	0	0	0	2.5	4.2	7.2	14.6
		0	4.1	0	0	0	0	0			

Spring specimens weight (mg)	Day after beginning experiment										
	1	2	3	4	5	6	7	8	9	10	11
55	0	14.6	41.2	14.8	15.0	14.0	5.6	1.8	9.0	11.5	1.7
102	0	3.2	37.4	19.0	41.6	10.0	5.9	20.4	6.3	11.4	12.2
41	0	8.4	24.3	3.6	21.2	3.5	7.5	7.5	5.2	7.0	1.7
34	4.2	1.2	23.5	0.2	7.0	4.6	1.6	1.6	2.5	14.6	0
40	9.0	21.8	3.1	3.5	30.0	10.8	4.5	4.0	3.2	12.2	9.0
38	12.1	4.9	16.0	6.9	2.7	12.5	10.8	9.8	3.4	15.0	17.5
49	1.0	10.0	1.9	2.1	1.4	8.9	1.4	1.3	2.2	1.7	4.0

[a] Based on initial weight. No correction was made for loss in weight with time.
[b] Where two figures are given, the first represents ammonia released between 7^{30} and 17^{30} hours, and the second figure between 17^{30} and 7^{30} hours.

exhibited more regularly-spaced bursts, with a number of "silent" days between. The Innsbruck data suggest — where measurements were made in the morning, at noon, and in the evening on dark-adapted animals, at 20°C — that ammonia is released maximally in the afternoon and minimally at night. On the other hand, an arhythmic pattern emerged in Syracuse where the animals were subjected to diurnal fluctuations in light, and measurements were made twice during a 24-hour period. A given individual released approximately equal amounts of ammonia during approximately eleven hours of light and thirteen hours of darkness over a 24-hour period, or different amounts of ammonia during the daytime and at night, or all of the ammonia at night on some days and during the daytime on other days. That these results were not merely

asellus from Syracuse in late Fall 1968 and early Spring 1969[b]

13	14	15	16	17	18	19	20	21	22	23	24	25	26
0	0	0	5.2	0	0	0	0	4.0	0	11.5	0	0	0
0	0	2.3	12.1	0	0	0	0	1.1	0	13.5	4.1		
0	0		0	0	3.2	0	0	0		2.9			
6.0	6.0		8.3	0	27.5	0	7.5	20.0		18.0			
13.8	2.1	2.1	10.0	4.2	9.7	9.9	9.2						

12	13	14	15	16	17	18	19	20	21	22	23	24	25
10.3	29.5	15.6	25.5	2.2	30.7	44.2	12.7	51.0	26.7	18.9	44.2	46.6	28.6
0.8	0.8	19.2	7.0	4.3	6.4	13.4	16.0	3.5	8.4				
2.6	0	21.5	11.3	7.8	34.5	28.5	35.2	61.0	61.0	11.8	17.3	9.2	
1.8	0	10.3	13.2	14.0	8.2	12.8	12.0	13.6	27.4	34.8	9.3	23.0	
3.2	0	0	25.5	8.6	17.4	9.2	8.7	16.0	35.0	40.0	4.7	7.0	45.0
0	0	44.0	12.2	13.0	11.4	38.0	79.0	36.5	45.5	32.0	36.5	42.0	
0	0	0	0	0	2.4	1.6	1.4	4.0	0	4.2	13.6	21.0	23.7

apparent and due to the restriction of two sampling periods over a 24-hour duration was shown subsequently through experiments in which single specimens were continuously monitored through pH stat-recording with a Radiometer TTT 1.

In March and in the beginning of April *O. asellus* produced more ammoniathan in autumn, both in Syracuse and in Innsbruck, but still in a burst-like fashion. In Syracuse, for example, six specimens examined during the fall released a mean of 4.9 ng NH_3/mg tissue x hour over a period of 15 days, in contrast to 9.4 ng NH_3/mg tissue x hour for 15 days from 14 specimens during the spring.

However, when *O. asellus* of Innsbruck was tested again in May, a replica of the rhythmical pattern of ammonia evolution shown by *P. scaber* one year earlier was obtained, although less amonia was released (Fig. 2). Under the conditions in Innsbruck both species released ammonia minimally early at night and maximally between noon and afternoon.

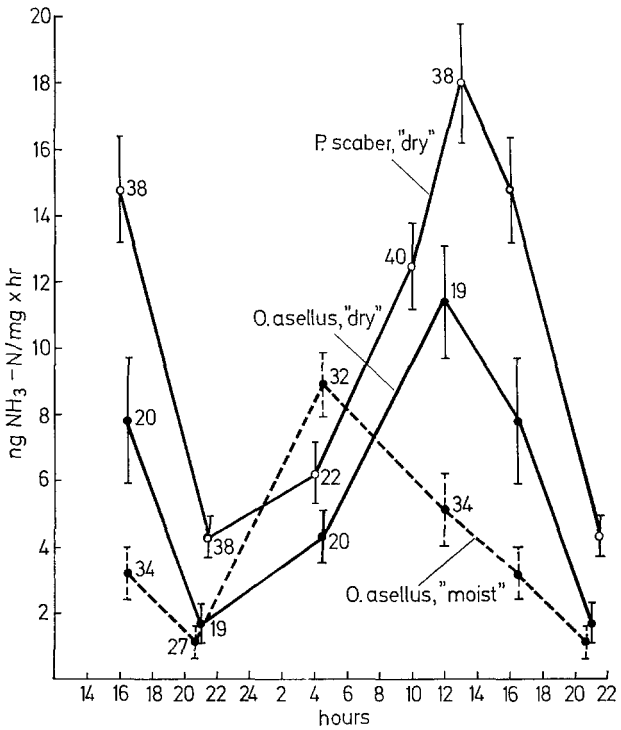

Fig. 2. Release of ammonia as related to time of day in 2 species of isopods and at 2 levels of humidity in the reaction chambers. "Moist" conditions are represented by experiments in which a piece of moist glass wool was put at the bottom of the reaction chamber. Means, standard errors and n's are indicated

2. Effect of Humidity

In the Innsbruck experiments considered so far, the animals were kept in chambers with a strip of moist filter paper attached to the walls to compensate for the slight drying effect of the sulfuric acid. Since *O. asellus* is a very hygrophilic species we investigated the effect of increased humidity by placing moist glass wool at the bottom of the chamber to humidify the ventral sides of the animals. The ammonia content of this glass wool was measured simultaneously with that of the ammonia trap at the top of the chamber. The increased humidity shifted the maximum of the release of ammonia to late night and early morning, but the minimum remained early at night (Fig. 2).

3. Effect of Feeding

The specimens considered so far were not allowed to feed during the experiments. In experiments conducted with animals feeding on decayed

leaves the pattern of NH_3 discharge changed drastically. The amount of ammonia given off during a 24-hour period dropped to about 30% of the fasting value and the daily periodicity all but disappeared (Table 3).

Table 3. *Mean values and standard errors of ammonia production (ng/mg × hr) of fasting and feeding Porcellio scaber during different times of day*

	Period of measurement			
	a. m. 24—8	8—12	p. m. 12—18	18—24
Fasting	6.2 ± 0.9	12.5 ± 1.35	16.4 ± 1.3	4.3 ± 0.6
n	22	40	76	38
Feeding	2.8 ± 0.37	3.0 ± 0.41	3.25 ± 0.31	2.3 ± 0.37
n	20	16	16	8

Discussion

The most striking aspect of our findings is the fact that in the isopods investigated the rhythmical release of ammonia is inversely related to their pattern of locomotory activity (Fig. 3). Both *O. asellus* and *P. scaber*, the former species with or without a moist substrate, released minimal amounts of NH_3 at the hours before midnight. This period coincides with a time when these species are most active in the field (Brereton, 1957) and under experimental light/dark conditions (Cloudsley-Thompson, 1956). It is not clear why the addition of a moist substrate shifted the maximum release of ammonia from noon to late night and early morning in *O. asellus*, but it is significant that this species has a second minimum of locomotory activity at this time (Brereton, 1957; Cloudsley-Thompson, 1956). It is conceivable that at high humidities isopods are relatively more active in daylight and thus produce less NH_3 at this time than specimens with exclusively nocturnal habits. At a relatively high humidity the late night and early morning hours may represent a period of restricted activity relative to the noon and afternoon hours.

The feeding experiments further support the contention that activity and discharge of ammonia are inversely related. The addition of leaves to dark reaction chambers at constant temperature stimulated nearly continuous feeding activity as evidenced by the steady production of fecal pellets (Wieser and Schweizer, in preparation). Under these conditions nitrogen production was reduced from an average of 10 ng NH_3-N/mg/hr in fasting specimens of *P. scaber* to approximately 3 ng and the rhythmicity all but disappeared.

The three patterns of activity combined in Fig. 3 give the impression that the release of ammonia during periods of feeding reflect the sum of

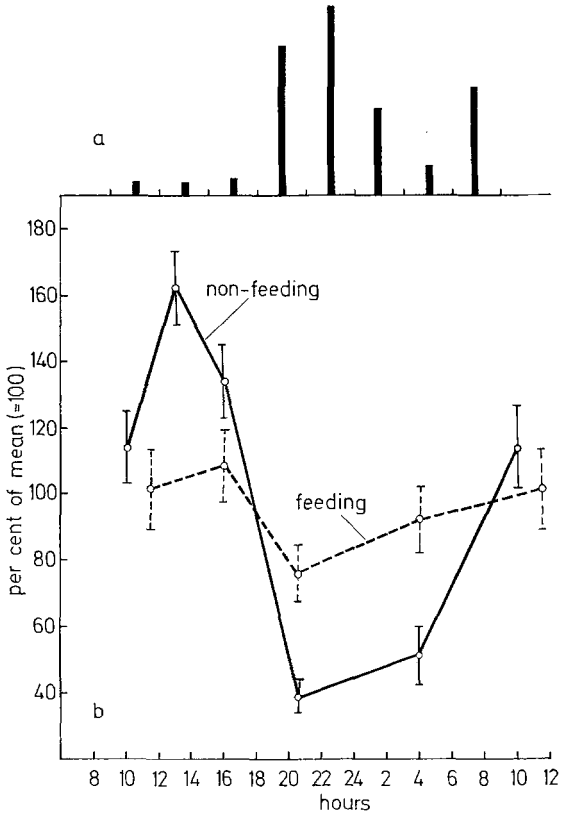

Fig. 3. a Activity levels of *P. scaber* at different times of day, calculated from Cloudsley-Thompson (1956, Fig. 1). b Levels of ammonia production at different times of day in feeding and non-feeding specimens of *P. scaber*, expressed as per cent deviation from mean

two rhythmical patterns with a phase difference of approximately 90 degrees: the locomotory rhythm with a maximum at night, and the rhythm of N-production with a maximum during the day. The persistence of the locomotory rhythm during constant darkness and at constant temperature implicates an endogenous clock, as suggested by Cloudsley-Thompson (1952). On the other hand, the manner in which the rhythmical pattern underlying the release of ammonia in fasting animals relates to the pattern exhibited when food is available remains to be established. This problem will be pursued further.

There are two basic mechanisms that may be invoked to explain the inverse relationship between locomotory activity and the release of ammonia. First, the metabolic processes involved in N-turnover are separated in time from the processes involved in the maintenance of

locomotory activity. Second, N-metabolism per se is not separated in time from locomotory activity, but the permeability of membranes is regulated in a way that gaseous NH_3 is discharged only during sedentary periods. Both mechanisms could be interpreted as being of adaptive significance.

The most pertinent indication of the possible existence of the second mechanism is the observation by Lindquist (1968) of an inverse relationship between locomotory activity and water loss in *P. scaber* and *Armadillidium vulgare*. Lindquist suggested that the permeability of membranes in isopods, presumably of the pleopods, is hormonally regulated. By employing this mechanism the animals would be insured against an excessive loss of water during periods of major activity. It would be an interesting fact if the permeability of membranes to NH_3 was coupled with permeability to water. In this way, the discharge of ammonia would be maximal at periods of inactivity when the animals are likely to sit in their moist retreats.

There is substantial evidence regarding the first mechanism that nitrogen turnover is low in fed, feeding or generally more active animals, and relatively high in fasting and starved animals. This suggests that nitrogen turnover and energy production (usually expressed as oxygen consumption) are inversely related. In the present study specimens of *P. scaber*, fasted for four to five days, released about three times more nitrogen per hour than fed animals (Table 3). In a related study, it was noted that the oxygen consumption after three days of fasting was about 80% of that exhibited by fed, quiescent specimens, and only 30% of that of moving animals (Wieser, 1962). That protein serves as a predominant source of energy in starving crustaceans also has been suggested by Neiland and Scheer (1953), and Jungfreis (1968). It is questionable, however, whether the inverse relationship between nitrogen turnover and locomotory activity during one photoperiod may be interpreted simply as the expression of switches between different substrates for energy production. Rather, we are inclined to think that the regulation of maintenance metabolism in these animals, involving the turnover of proteins and amino acids, occurs when it interferes least with processes of high energy production during locomotory activity. One could look at the hepatopancreas, the central metabolic organ in isopods, as capable of executing different metabolic functions at different times of the day in order to minimize interference between them.

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Dr. R. Hartenstein
Dept. of Zoology
State University
College of Forestry
Syracuse, New York

Prof. Dr. W. Wieser
Dr. G. Schweizer
Institut für Zoologie
Lehrkanzel für Tierphysiologie
der Universität
A-6020 Innsbruck
Peter-Mayr-Str. 1a