THE MECHANISMS OF HUMIDITY REACTIONS OF TERRESTRIAL ISOPODS

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(With One Text-figure and Seven Graphs)

INTRODUCTION

RECENT work by Gunn (1937) and Miller (1938) on land isopods clearly shows that these animals are very sensitive to humidity gradients, aggregating in the areas of highest humidity. The present series of experiments were carried out mainly on *Porcellio scaber* Latr., to analyse the mechanisms of reactions whereby the animals are retained in the damper zones. Attempts were also made to see whether the behaviour pattern, shown by Ullyott (1936) to be characteristic of the phobotactic reactions of the planarian *Dendrocoelum lacteum*, is also exhibited in the humidity reactions of the woodlouse. Further comparative experiments on *Oniscus asellus* Lin. and *Armadillidium vulgare* Latr. were undertaken to find whether there are any differences in the hygrokinetic responses correlated with the natural habitats of the three species, and how far the differences in behaviour were influenced by the rate of evaporation of water from the woodlice.

METHODS

The apparatus initially used was a rectangular dish 3 in. deep, covered by a sheet of glass with a perforation in the centre for the insertion of the animal. A platform of perforated zinc 2½ in. above the glass floor was supported on glass rods. The required humidities were obtained by placing dishes with sulphuric acid of known specific gravity underneath the zinc floor on either side of the dish. Edney paper hygrometers were used for measurement of humidity. The range of the gradient obtained was large, namely, from 20 to 90% R.H. However, this apparatus was found to be unsuitable for work with *Porcellio scaber*, which is strongly thigmokinetic and hence tends to keep very close to the sides of the dish when moving, pressing one of the antennae against the glass wall. Moreover, this animal showed a tendency to remain motionless at the corners of the dish. It was interesting to note that when *P. scaber* was placed in constant humidities in this type of apparatus, its thigmokinetic response increased with the rise of relative humidity. There appeared to be a balance between thigmokinetic and hygrokinetic behaviour, and further experiments were undertaken to elucidate this point (see p. 118).

The second type of apparatus tried was the 'alternative chamber' described by Gunn & Kennedy (1936). This, in spite of being free from corners, again proved unsuitable, since the animals instead of moving about freely on the zinc platform tended to keep to the sides of the glass dish.

A tubular apparatus had therefore to be chosen, since it presents an equal surface of stimulation throughout its whole length to the strongly thigmokinetic animals. A modification of Gunn's circular humidity gradient apparatus was tried, and proved to be suitable. The apparatus used (Fig. 1) consists of a wide glass tube 1½ in. in diameter and 58½ in. long, bent into an oval. A circular tube would have been preferable, but this could not be obtained owing to difficulty of construction. However, the rounded corners of this apparatus did not appear to impede the progress of the animals or to alter their behaviour in any way. Externally the tube is divided into inches by thin strips of adhesive paper, and the inches are marked in white ink. At the two ends of the apparatus, and in the middle of each tube connecting the two ends are glass funnels A_1, A_2, A_3, A_4, designed to hold Edney paper hygrometers. Holes are bored in the hygrometers to ensure that the readings taken in the funnels are identical with those in the tube. The funnels A_1, A_2, A_3, A_4 are covered by small glass plates. Tubes B_1 and B_2 hold rubber stoppers, through which thermometers are inserted. The animal is inserted through a small opening at C. Below A_1 and A_2 are tubes D_1 and D_2, half an inch in diameter, which can be inserted into dishes containing water or sulphuric acid of known specific gravity. The dishes containing sulphuric acid are covered to prevent alteration in specific gravity due to absorption of moisture.

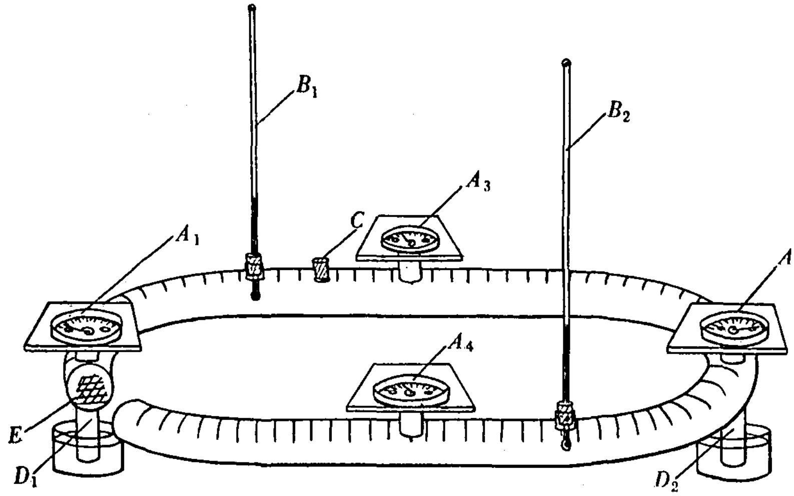


Fig. 1. Tubular humidity gradient apparatus for testing locomotory reactions of thigmokinetic animals. A_1, A_2, A_3, A_4, funnels with hygrometers; B_1, B_2, thermometers; C, opening for insertion of the animal; D_1, D_2, tubes dipping into sulphuric acid; E, perforated zinc platform.

The apparatus in this condition was used for experiments in which a humidity gradient was required. When constant humidity was needed, a further modification was introduced. A_3 was connected to a long glass-bead tower, which in turn was connected with two U tubes arranged in series, filled with glass beads. Sulphuric acid of known concentration was introduced into the tower and the U tubes. A_1 was connected to an air pump. A very slow current of air was then drawn through the whole apparatus. Tubes D_1 and D_2 were made air-tight by placing their ends into dishes containing mercury.

All the experiments were carried out in an underground dark cellar at Birkbeck College, University of London. The annual range of temperature was between 13 and 20° C. Experiments were carried out at between 14 and 18° C., the variation in temperature at the time of any one experiment not exceeding 1° C. Diffuse light of intensity of 2 Ferranti foot-candles was used throughout the experiments.

Woodlice were isolated and starved for 3 days before the beginning of each experiment in dishes in which R.H. was maintained between 90 and 95%, and probably reached saturation under stones contained in them. To minimize the Weber-Fechner effect, i.e. the fact that a response depends not on the actual quantity of stimulus, but on the proportion which the increase bears to the preceding stimulus, the animals, when placed in the glass tube apparatus, were initially introduced into about 90% R.H., and left for 10 min. to recover from the effects of handling. A gentle current of air bubbled through sulphuric acid of known specific gravity was then drawn through the apparatus for 5 min. Preliminary experiments had shown that such a slow rate of air flow did not influence the direction of movement of *P. scaber*. The apparatus was then made air-tight, and the speed and the number of turnings performed by the animal were recorded every 30 sec. for a period of 1 hr. The number of 'rests', or periods of complete inactivity over a period of 30 sec., was also noted. Although the absolute speed could be recorded by noting the number of inch divisions traversed per 30 sec., it was impossible to determine the absolute number of turnings (i.e. klinokinesis, according to Gunn and the rate of change of direction, or r.c.d., according to Ullyott), as the shape of the apparatus did not allow for turning in all the possible directions. The animal moved about freely and could exhibit turnings of three different types: (*a*) it could swing itself completely round and then proceed in the original direction, i.e. undergo an angular deviation of 360°; such movements in *P. scaber* were observed at low humidities only, and not once at relative humidities over 85%; (*b*) the animal could swing around and proceed in the opposite direction, such turning involving an angular deviation of 180°; (*c*) the animal could turn 90°, and then swing back and proceed in the original direction. For the sake of convenience a turning of 90° was taken as a unit of measurement. Although the absolute number of angular deviations could not be recorded, the relative number at different humidity conditions could be obtained, and for this reason they are of value.

EXPERIMENTAL DATA

(1) *Reactions of* Porcellio scaber *to air currents* (100 *and* 50% R.H., $t = 16$–$17°$ C.)

It was important to find out whether *P. scaber* orientated itself to air currents, since these were used at the beginning of each 'constant humidity' experiment.

Six woodlice were introduced into a glass tube 12 in. long and ½ in. in diameter, and a gentle current of air was drawn through it by means of an air pump. The number of animals facing the current and of those turned away from it was recorded every 15 min. for a period of 2 hr. in the case of 100% R.H., and for a period of 1 hr. in the case of 50% R.H. The same animals were not kept for more than 1 hr. at 50% R.H., as they were in danger of being desiccated.

In both humidities forty readings of six animals in each case were recorded. A new set of six animals was used for each experiment. In the majority of cases woodlice were seen to aggregate at either end of the tube. The animals 'resting' or moving at right angles to the current were neglected.

At 100% R.H., $t = 16$–$17°$ C., 108 animals faced the current, 110 faced away from it. At 50% R.H., $t = 16$–$17°$ C., 96 animals faced the current and 101 faced away. From these results it was concluded that woodlice do not orientate themselves to slow air currents.

(2) *Thigmokinetic behaviour of* Porcellio scaber

It was observed that the thigmokinetic response characteristic of woodlice varied with relative humidity. A series of simple experiments was undertaken in this connexion. The animals were introduced into the 'alternative chamber' glass dish; constant humidity was maintained and altered between one set of experiments and the next by means of different concentrations of sulphuric acid. Temperature variation in the experiments ranged between 14 and 17° C., and within a single degree for any one experiment. Diffuse light of intensity of 2 Ferranti foot-candles was used.

Since the animals became progressively less active with the rise of relative humidity, the thigmokinetic response appeared to be closely associated with the hygrokinetic one. To distinguish between the two, the percentage of the time the woodlouse spent next to the wall of the glass dish while it was *still moving* was taken as the final indicator of the thigmokinetic behaviour. One woodlouse at a time was introduced and readings were taken every 15 sec. for a period of half an hour. To minimize the handling effect, readings were not taken in the first 10 min.

Twenty animals were taken through relative humidities of 20–25, 50–55, 70–75 and 90–95%. When the animals came to rest they invariably did so touching the wall of the vessel, with antennae folded ventrally and closely pressed against the solid surface. If the animals were placed in a rectangular dish, they usually came to rest in one of its corners. When the animal walked near the glass side, one of its antennae always touched the wall, even if the rest of the body was 1 mm. or so away from it. If one of the antennae be removed, the animal still keeps to the side, with the remaining antenna pressed against the glass surface. This seems to indicate that some of the organs of touch are localized in the antennae.

From Table 1 it can be assumed that the thigmokinetic response is dependent on the degree of humidity, and its intensity rises with the rise in relative humidity, increasing steeply on the approach to saturation.

Table 1. *Thigmokinesis of twenty* Porcellio scaber *in different relative humidities*

$t=14$–$16°$ C.	20–25 % R.H.	50–55 % R.H.	70–75 % R.H.	90–95 % R.H.
% of 30 min. spent in moving next to the wall (thigmokinesis)	81·6	79·5	85·9	92·6

(3) *Behaviour of* Porcellio scaber *under constant humidities*

Thirty *P. scaber* were kept at 90–95 % R.H. One at a time was introduced into the glass tube apparatus and allowed 15 min. before a gentle current of air was passed through for 5 min. The air current was bubbled through sulphuric acid of known specific gravity, and thus a known degree of relative humidity was obtained (see Methods, p. 116). Three sets, of ten animals in each, were experimented on. Sets I and III were taken through relative humidities of 0–10, 20–25, 40–45, 58–63, 80–85, 90–95 and 98–100 %. Set II was taken through relative humidities of 0–10, 40–45, 58–63, 68–73, 85–90 and 98–100 %. Readings were taken every 30 sec. by a stop-clock, and four factors were noted in the behaviour of each animal:

(*a*) *activity*, as shown by the actual number of inches passed through by an animal in a given period of time;

(*b*) *speed*, which differs from activity in that it shows the actual velocity while the animal is moving, disregarding the periods of rest;

(*c*) *the number of turnings* (klinokinesis) in a given time;

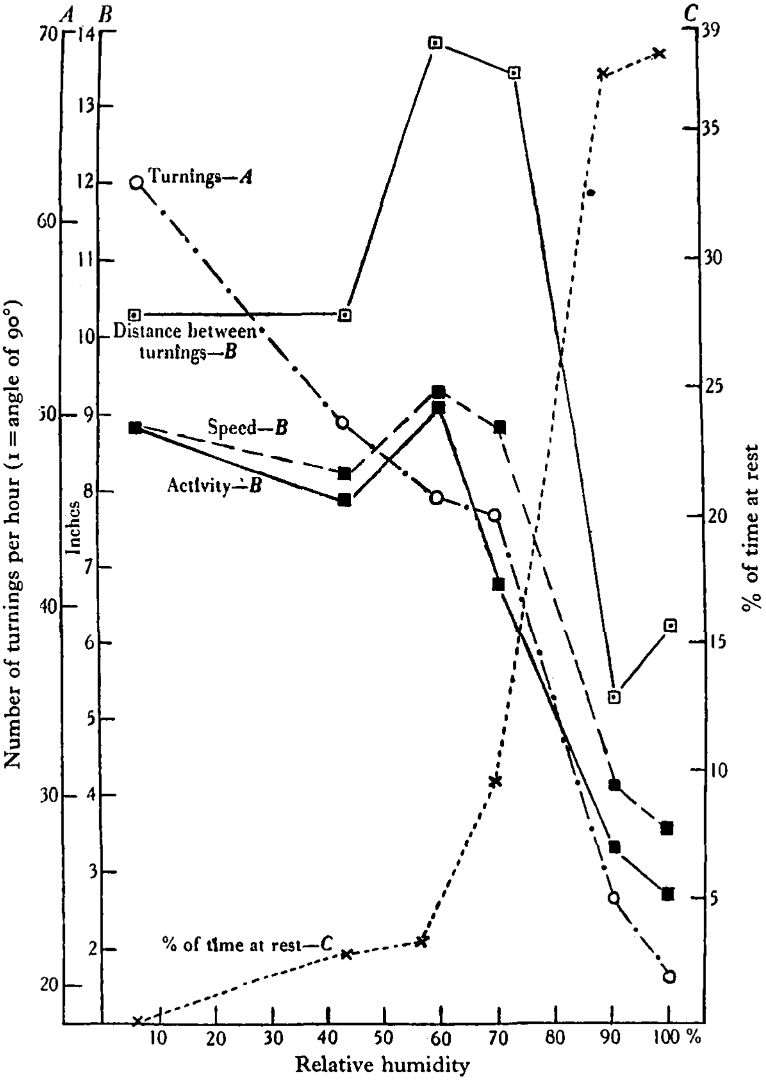
(*d*) *the number of rests*, i.e. periods of complete inactivity for 30 sec. Clearly (*a*) and (*d*) are two different methods of expressing the same facts.

The results obtained from these experiments are shown in Graphs 1 and 2.

As can be seen in Graph 1 the number of turns, speed and activity decrease with the rise of relative humidity, while correspondingly there is a rise in the number of 'rests' Whereas the fall of the number of turns with the rise of relative humidity is continuous, the maximum speed and activity were obtained at 58–63 % R.H. It is interesting to note that Gunn (1937) has observed that the intensity of reaction 'was greatest between 35 and 65 % R.H. approximately, and that at 55 % R.H. there was a definite reaction to a difference of 6 % R.H.' He further notes that the intensity of the reaction diminishes in the higher and lower humidities.

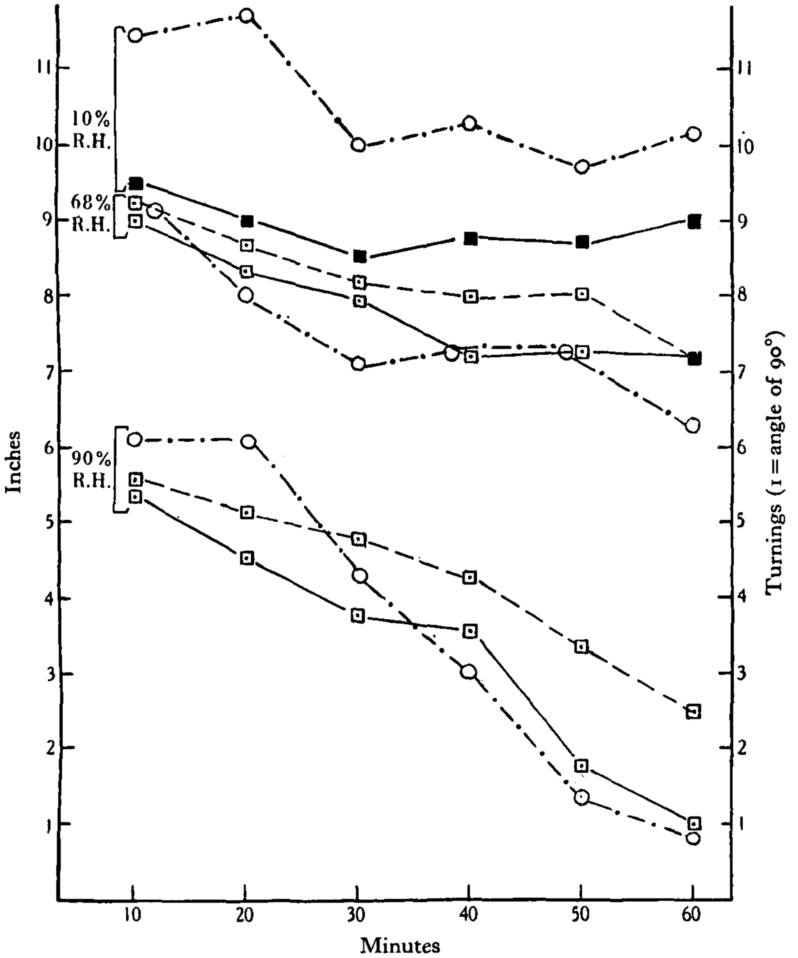
Speed and activity are almost identical up to 68 % R.H., being greatest at 58–63 % R.H. (average speed 9·38 in., average activity 9·34 in. per min. for a period of 1 hr., when sets I, II and III are taken into account. When the averages of twenty animals (sets I and III) are taken into account, the greatest speed and activity are obtained at 20–25 % R.H. (speed 9·98 in., activity 9·88 in. per min.).

Appreciable difference between speed and activity was first observed at 68–73 % R.H. (speed = 8·88 in., activity = 6·82 in. per min. from sets I, II and III). This showed that although speed was maintained at almost the same level as at lower relative humidities, activity was already decreasing. Speed is, therefore, not an indicator of hygrokinesis, but is only one of its factors. Above 68–73 % R.H. both speed and activity fell off with the rise of humidities. However, as stated above, there was a steady fall in the number of turnings (1 turning = 90°) with the rise of relative humidity. Whereas at 0–10% R.H. the average number of turnings was 61·95 per hour, at 98–100% R.H. it was 20·33.



Graph 1. Behaviour of the woodlouse *Porcellio scaber* in constant humidities, calculated from the average of 30 specimens. The number of turnings is read from the ordinate *A*. The average distance between turnings, the activity and the speed per minute from the ordinate *B*. The percentage of time at 'rest' is read from ordinate *C*.

Attempts were made to correlate the variables by dividing the total distance travelled by the animal in 1 hr. by the number of turnings per hour, i.e. to estimate the average distance between turnings. The figures obtained are those shown in Table 2 and Graph 1. It is thus seen that not only the total number of turns but the distance between turns decreases with the rise of humidity. This apparent discrepancy is due to a large number of 'rests' and the low activity of the animals at high humidities. The smaller distance between turnings, together with decreased activity will tend to restrict the woodlice to the regions of higher or 'optimum' humidity. The significance of this result is discussed later (see p. 134).



■——■ Activity and speed coinciding. □——□ Activity. □- - -□ Speed. O-·-O Turnings.

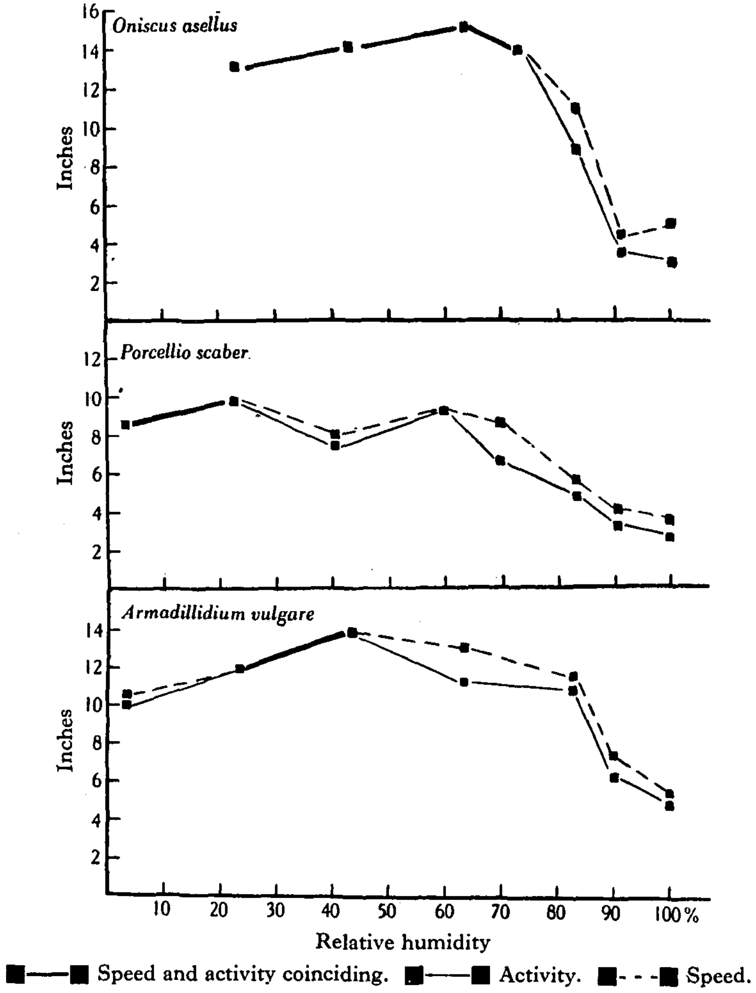
Graph 2. The behaviour of *Porcellio scaber* (average of 30 specimens) throughout the 1 hr. experiments at 10% R.H., 68% R.H. and 90% R.H. The average activity and speed per minute, and the number of turnings are recorded for every 10 min.

Table 2. *Distance travelled between turnings obtained from the averages of thirty* Porcellio scaber

0–10% R.H.	40–45% R.H.	58–63% R.H.	68–73% R.H.	90–95% R.H.	98–100% R.H.
10·3 in.	10·4 in.	13·9 in.	13·5 in.	5·3 in.	6·2 in.

(4) *Comparison of behaviour of* Oniscus asellus *Lin.*, Porcellio scaber *Latr. and* Armadillidium vulgare *Latr. under similar constant humidities*

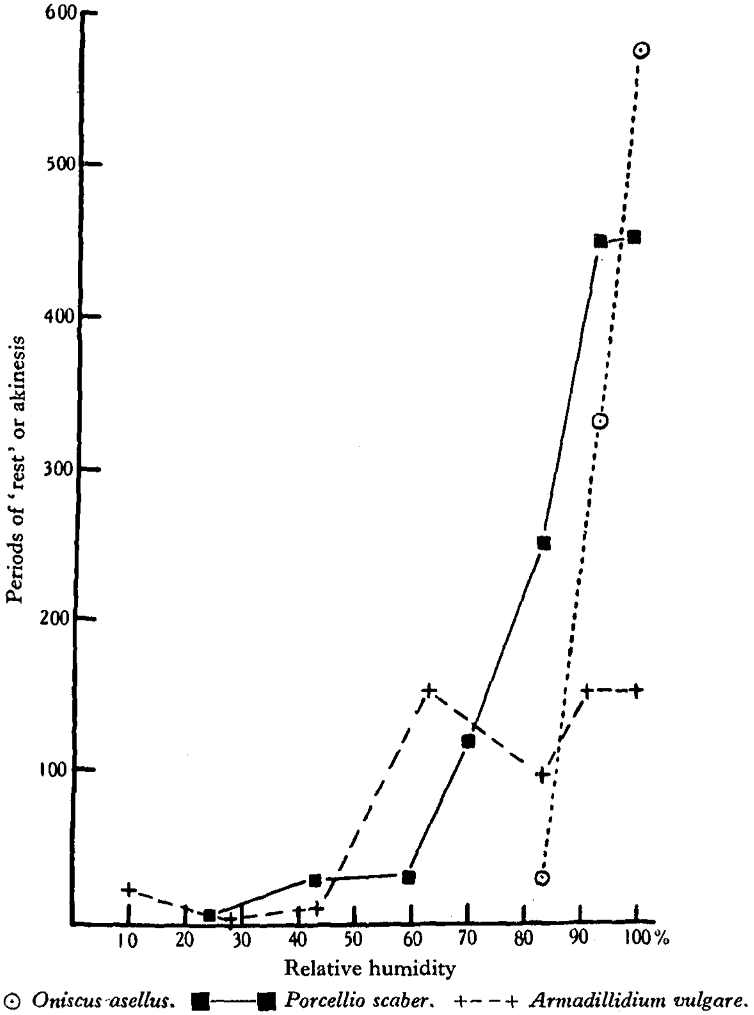
The methods used in observations on *Oniscus asellus* and *Armadillidium vulgare* were identical to those employed in constant humidity experiments on *Porcellio scaber*. It was of interest to see whether there were any differences in behaviour which could be correlated with the differences in their natural habitats. Both *P. scaber* and *Oniscus asellus* are restricted to damp situations under stones, humus, bark of decaying logs and pieces of wood, but although the habitats of the two species overlap to a great extent, *Porcellio scaber* is never found in contact with actually wet undersurfaces of stones or wood, whereas *Oniscus asellus* frequently is.



Graph 3. Average activity and speed per minute of *Oniscus*, *Porcellio* and *Armadillidium* in relation to constant humidities.

Armadillidium vulgare, which may be considered to be one of terrestrial isopods best adapted to life on land, is restricted to drier calcareous soils.

Ten specimens of *Oniscus* were observed in relative humidities of 20–25, 40–45, 60–65, 70–75, 80–85, 90–95, 98–100 %, and ten specimens of *Armadillidium vulgare*



Graph 4. The number of periods of 'rest' (30 sec. of complete inactivity) in relation to constant humidities, in *Oniscus*, *Porcellio* and *Armadillidium*.

in relative humidities of 0–10, 25–30, 40–45, 60–65, 80–85, 90–95 and 98–100 %, at temperatures between 14 and 18° C. The animals chosen for all experiments were as nearly as possible of the same size, and varied between 1·25 and 1·65 cm.

In all essentials the behaviour of the three species was the same, namely, with increase of relative humidity there was a decrease in activity and speed, i.e. hygrokinesis was clear in all cases (Graph 3), e.g.

% R.H.	Speed per minute (in.)		
	Oniscus asellus	*Porcellio scaber*	*Armadillidium vulgare*
20–25	13·24	9·98	12·04
60–65	15·30	9·38	13·04
90–95	4·5	4·16	7·6

The number of 'rests', i.e. 30 sec. of complete inactivity, increased consistently with the rise of relative humidity in the cases of *Oniscus* and *Porcellio*, but remained at approximately the same level from 60 to 100% R.H. in *Armadillidium* (Graph 4), e.g.

'Rests'

% R.H.	10 *Oniscus asellus*	10 *Porcellio scaber*	10 *Armadillidium vulgare*
25	0	4	0
60–65	2	40	155
80–85	38	258	97
90–95	343	448	157
98–100	574	457	159

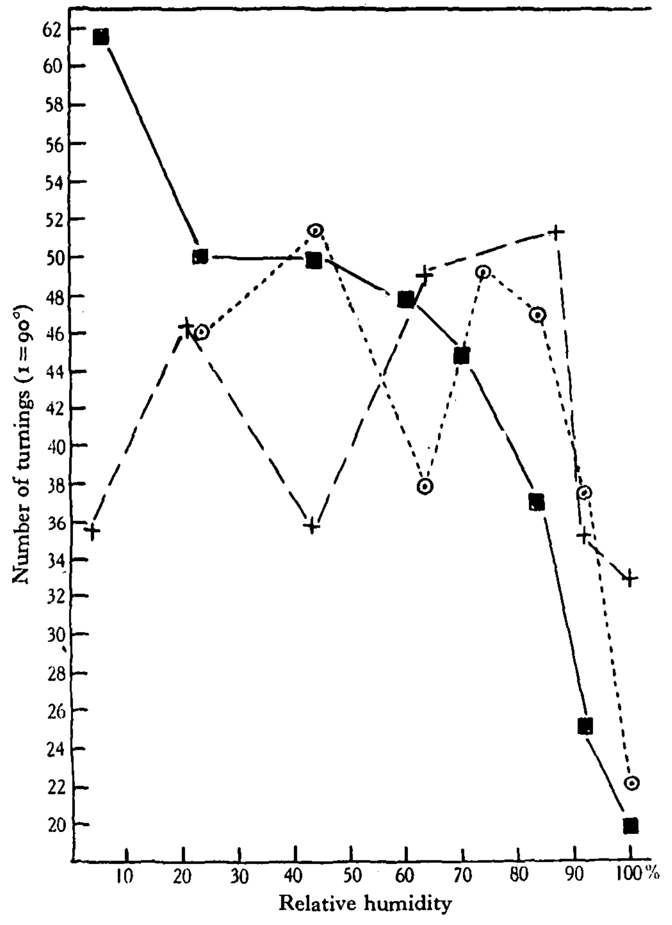
The smaller and more constant number of 'rests' of *A. vulgare* may be an expression of its lesser sensitivity to humidity. This supposition is borne out by the subsequent experiments which show the greater resistance of this species to desiccation (see p. 128). In this connexion it is interesting to note that although *A. vulgare* can roll itself into a sphere, at no time was 'rolling up' observed at low humidities; on the contrary, when the isopod was left in the apparatus for several hours it remained active until it became moribund on desiccation.

The total number of turnings per hour of *Porcellio* and *Oniscus* decreased with the rise of relative humidity, but *Armadillidium* did not show any appreciable corresponding decrease (Graph 5). However, the distance travelled between turnings, obtained by the ratio of total distance/number of turns decreased very rapidly with the rise in humidity in *Oniscus*, and to a lesser extent in *Armadillidium*, e.g.

% R.H.	Average distance between turns (in.)		
	Oniscus asellus	*Porcellio scaber*	*Armadillidium vulgare*
25	26·5	10·9	16·7
45	21·1	10·4	22·3
60–65	25·9	13·9	14·5
90–95	7·9	5·3	10·8
98–100	6·6	6·2	9·4

See also Graph 6.

It thus seems that the mechanism by which isopods are retained in areas of higher humidities, namely, hygrokinesis and increase in the frequency of turns, are operative in all the three species, but are best developed in *Oniscus asellus*. This indicates the greatest sensitivity of this species to humidity, and is probably associated with its most rapid loss of water by evaporation (see experiments on desiccation, p. 128). The lesser sensitivity to humidity of *Armadillidium vulgare*, probably correlated with its greater resistance to desiccation, was indicated by the smaller number of periods of inactivity or 'rests'.



Graph 5. The average number of turnings (angle of 90° taken as unit) per hour in *Oniscus*, *Porcellio* and *Armadillidium*, in relation to constant humidities.

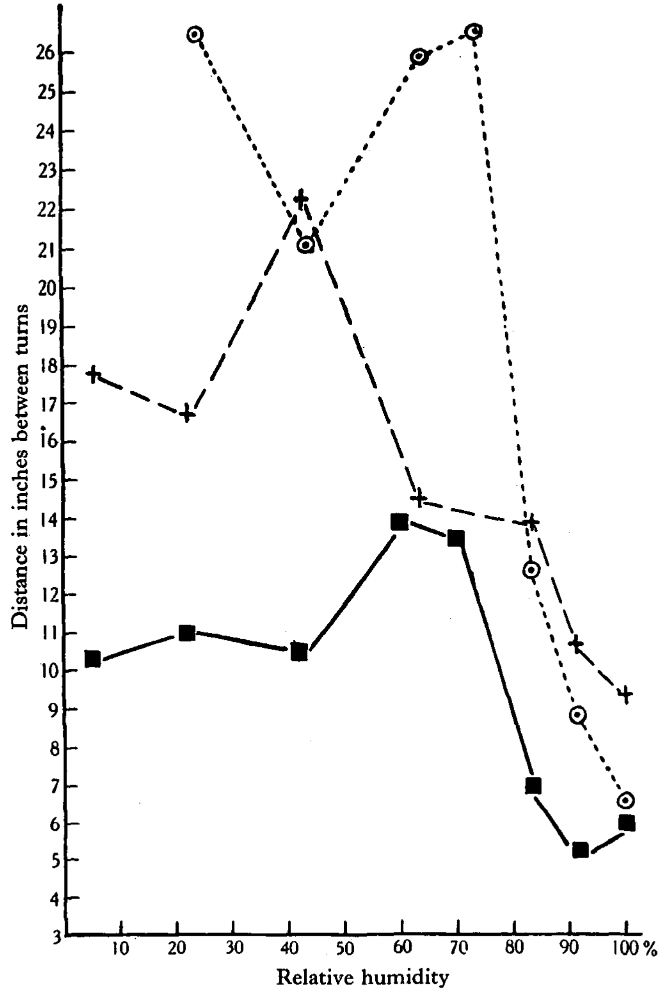
It was interesting to note that the specimens of *Oniscus asellus* and *Armadillidium vulgare* were consistently more active than those of *Porcellio scaber*. Moreover, much less individual variation was seen in the first two species than in the last. Abbott (1918) in experiments on photic responses also noted that the behaviour of *Oniscus* was much more stereotyped than that of *Porcellio*.

(5) *Behaviour of* Porcellio scaber *in humidity gradients*

The method of obtaining humidity gradients has already been described (see Methods, p. 116).

Altogether fifty-five *Porcellio scaber* were experimented with:

10 in 75–95 % R.H. (t = 15–18° C.),
10 in 65–85 % R.H. (t = 14–15° C.),
10 in 55–75 % R.H. (t = 14–15° C.),
18 in 20–73 % R.H. (t = 14–18° C.),
7 in 42–60 % R.H. (t = 14–15° C.).



⊙- - -⊙ *Oniscus asellus.* ■——■ *Porcellio scaber.* +– – + *Armadillidium vulgare.*

Graph 6. The average distance between turnings in relation to constant humidities, in *Oniscus*, *Porcellio* and *Armadillidium*.

In each case one animal at a time was introduced into the glass tube apparatus for a period of 1 hr. Readings were not taken for the first 10 min. to allow for recovery from handling. The results of the experiments are given in Table 3. The number of turns, speed and the number of 'rests' were recorded every 30 sec., and the behaviour of the animal in the damp half of the apparatus was compared with that in the dry half. In each case the following points were calculated:

(1) Percentage of total time spent in damp half of the apparatus.
Percentage of total time spent in dry half of the apparatus.
(2) Percentage of total time spent at 'rest' in the damp half.
Percentage of total time spent at 'rest' in the dry half.
(3) Average speed per minute in damp half.
Average speed per minute in dry half.
(4) Total number of turns in damp half.
Total number of turns in dry half.
(5) Average distance travelled between turns in the damp half.
Average distance travelled between turns in the dry half.
(6) Average time interval between turns in the damp half.
Average time interval between turns in the dry half.

Table 3. *Behaviour of* Porcellio scaber *in humidity gradients*

	% of total time spent in		% of total time spent at rest in		Average speed (in.) per minute in		Total no. of turns (1 = 90°) in		Average distance (in.) between turnings in		Average no. of minutes between turnings in	
	Damp half	Dry half	Damp half	Dry half	Damp half	Dry half	Damp half	Dry half	Damp half	Dry half	Damp half	Dry half
75–95 % R.H. t = 15–18° C. 10 *P. scaber*	87·17	12·83	50·42	0	4·48	7·48	143	41	5·99	13·65	1·32	1·61
65–85 % R.H. t = 14–15° C. 10 *P. scaber*	73·75	26·25	34·67	0	5·52	8·00	183	106	8·55	18·89	1·42	2·21
55–75 % R.H. t = 14–15° C. 10 *P. scaber*	70·83	29·17	26·03	6·25	5·70	6·56	186	80	6·59	11·89	1·38	2·21
20–73 % R.H. t = 14–18° C. 18 *P. scaber*	74·49	25·51	29·03	0·18	5·38	6·52	347	255	7·73	6·05	1·39	0·99
42–60% R.H. t = 14–15° C. 7 *P. scaber*	67·71	32·29	23·57	0	6·68	7·60	113	164	10·54	5·46	1·51	0·73

Examination of Table 3 shows that in all the humidity gradients provided, the animals spent the greater part of the hour in the damper half of the apparatus. Speed was consistently higher in the dry half and the number of 'rests' greater in the damp half.

In the gradients of 75–95, 65–85 and 55–75 % R.H. the average distance between turnings and the time interval between turnings are shorter in the damper half of the apparatus. Such behaviour, associated with hygrokinesis (indicated by the larger number of 'rests' and lower speed in higher humidities), would tend to restrict the isopods to these conditions. These results are consistent with those obtained in experiments with constant humidities. No such correlation, however, was obtained when the animals were subjected to gradients of 20–73 and 42–60 % R.H., for although speed was again higher in the dry half, the greater part of time spent in the damp half and the periods of 'rest' almost entirely restricted to the damp half, the average distance, and the average time interval, between turnings, were greater in the drier part of the apparatus, and such results are inconsistent with any which were previously obtained. No satisfactory explanation for this difference in behaviour can be given, but it may be suggested that the normal behaviour pattern was altered by the continual physiological instability caused by loss of water by evaporation at low humidities. It is also probable that behaviour alters with the steepness of gradient, for whereas in four sets of experiments the gradient was approximately that of 20 %, it was 53 % between 20 and 73 % R.H.

(6) *Loss of water by evaporation*

It was interesting to see how far the differences in behaviour of woodlice in different humidities could be associated with the rate of loss of water by evaporation, and the following series of simple experiments were undertaken in this connexion.

(*a*) *Duration of life of* Oniscus asellus, Porcellio scaber *and* Armadillidium vulgare *under different relative humidities.*

Twenty specimens of each species, of approximately the same size (1·25–1·65 cm.) previously starved in 100 % R.H. for 3 days, were placed in desiccators at 0, 25, 50, 75, 85, 93 and 100 % R.H. (*t* = 15–18° C.). To prevent conservation of water due to possible formation of groups, each isopod was placed in a separate desiccator and the number of hours of its survival was noted (see Table 4). It was found that the resistance to desiccation determined by death-point was consistently greatest in *Armadillidium vulgare* and greater in *Porcellio scaber* than in *Oniscus asellus*. The figures in Table 4 are probably purely relative, and do not represent the exact survival under natural conditions, since in the desiccators the whole ventral side of the animal, as well as the dorsal, is exposed to uniform humidity. Normally the ventral surface of the animals at rest is closely pressed against a solid surface, and undoubtedly a microclimate of higher humidity is thus retained, at least for some time.

Table 4. *Duration of life of* Oniscus asellus, Porcellio scaber *and* Armadillidium vulgare *at different relative humidities* (*t* = 14–18° C.). *Number of hours of survival based on the averages of twenty animals in each case*

R.H. %	*Oniscus asellus*		*Porcellio scaber*		*Armadillidium vulgare*	
	Average	Limits	Average	Limits	Average	Limits
0	4·3	3–5	5·27	4–6	7·15	6–10
25	6·85	5–7·5	8·17	6·5–10	9·92	6–16
50	6·2	5–8	10·17	6–16	30·15	16–44
75	16·25	12–20	25·25	18–44	59·15	36–87
85	25·5	11–28	29·0	18–43	65·65	35–96
93	33·17	16·5–75	39·17	20–67	114·6	49–240
100	—	32 hr. to over a month	—	47 hr. to over a month	—	76 hr. to over a month

(*b*) *Estimation of water loss by evaporation of* Oniscus asellus *during the* 1 *hr. experiments.*

(i) As before, twenty specimens of *Oniscus asellus*, starved for 3 days at 100% R.H., were weighed and placed in desiccators at 0, 50 and 75 % R.H. for a period of 1 hr., after which the animals were reweighed. The following figures, taken from the average of twenty animals, represent the percentage loss of water by evaporation per hour:

0% R.H., t = 16–18° C.	50% R.H., t = 15–18° C.	75 % R.H., t = 18° C.
9·9%	6·3%	3·6%

After each experiment the animals were returned to 100% R.H., left for 24 hr. and reweighed. With the exception of four cases out of sixty, the animals gained in weight. This gain varied widely and could not be correlated either with the original weight of the animal or its previous percentage loss by evaporation.

(ii) As a check to the above experiments another set was undertaken. Ten or twenty specimens of *Oniscus* were placed in 95, 75, 50 and 20 % R.H. for 1 hr., one woodlouse to a desiccator. Each animal was then weighed and placed in a weighed specimen tube containing anhydrous copper sulphate, from which it was separated by a strip of perforated zinc. The tubes were placed in a drying oven for 24 hr.—1 hr. at 100° C. to stop the action of enzymes, and 23 hr. at 60° C. After 24 hr. the animals and the specimen tubes were reweighed. The water content of *O. asellus* after 1 hr. at different relative humidities (t = 15–16° C.) was calculated and is shown in the following figures:

Relative humidity %	95	75	50	20
Water content %	63	59·6	57·3	57·2
No. of specimens	20	10	20	10

Since the loss of weight is mainly due to evaporation of water on drying, it can be concluded that at the end of 1 hr. the animals kept at 95 % R.H. have a much higher water content than those kept at lower humidities; moreover, the evaporation of water in *Oniscus* exposed to dry conditions appears to be rapid. It must be remembered that *O. asellus* may be regarded as an isopod little adapted to a terrestrial habit, and is found in habitats where humidity must be nearly always at saturation point.

(iii) The rate of loss of water in *O. asellus* during one hour was then calculated at 0 and 50% R.H. (t = 14–17° C.). One animal at a time was placed in a small box of perforated zinc attached to the arm of a balance by a fine wire. The zinc box was suspended in a small vessel at 0 and 50% R.H. This method gave a set of continuous readings, but its chief disadvantage was the disturbance of the initial humidity on the introduction of the zinc box. The rate of loss of weight is shown in Table 5.

Table 5. *Ten* Oniscus asellus *weighed for* 1 *hr. at* 0 *and* 50% R.H.

Minutes	5	10	15	20	25	30	40	50	60
% loss of weight at 0% R.H.	0·4	1·1	1·6	2·3	2·8	3·6	4·4	5·7	6·4
% loss of weight at 50% R.H.	0·3	0·7	1·3	1·6	2·0	2·4	3·0	3·9	5·6

From all the above results it may be concluded that the loss of water by evaporation in *O. asellus* is proportional to time and to the percentage of relative humidity. It may be suggested that the differences in the activity of woodlice are due to the continuous alteration in their water content, the loss of which does not appear to be regulated.

(7) *The effect of loss of water by evaporation on phototaxis of* Oniscus asellus

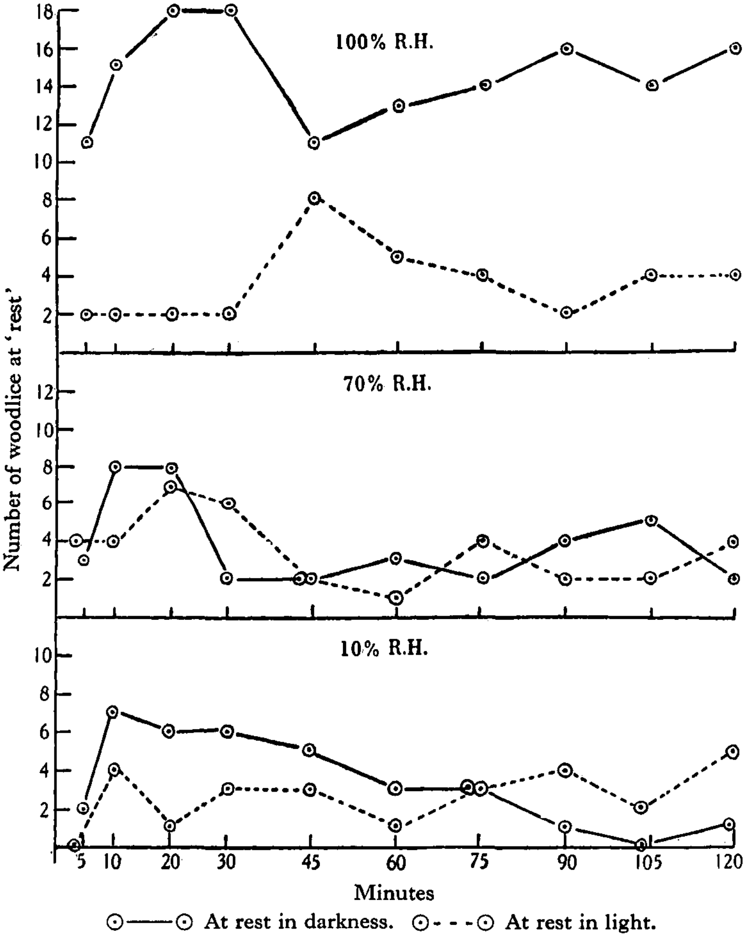
The present series of experiments are here included, as they indicate that there is a balance between phototaxis and humidity reactions of *Oniscus asellus.*

Abbott (1918) in phototactic experiments on *Porcellio scaber* and *Oniscus asellus* found that the two species are sensitive to a range of light from 100 to 0·01 c.m., the response being the same for all light intensities. He also states that the reaction of *Oniscus* was essentially the same whether the animal had been previously kept in maximum or minimum moisture, but notes that *Porcellio* was less negative after living in a dry habitat. The same tendency to reversal of phototactic reaction in *Porcellio*, and a definite reversal from negative to positive phototaxis after a period of desiccation in *Armadillidium* has been described by Henke (1930).

In the present set of preliminary experiments on phototaxis of twenty specimens of *Oniscus asellus* under constant humidity conditions of 100, 93, 75, 70, 60, 50, 40, 25 and 10% R.H. at temperature 14–15° C. and at light intensity of 45 Ferranti foot-candles, it was noted that the animals show definite negative phototaxis above 75% R.H. Below 75% R.H. the initial negative phototaxis seems to disappear, and the animals appear indiscriminately on the illuminated and shaded halves of the apparatus. Below 40% R.H. there is an indication that a reversal from negative to positive phototaxis occurs, as the number of the woodlice at rest in the light half of the apparatus is equal or greater than in the dark half (see Graph 7). These experiments, although in their initial stages, suggest that a reversal from negative to positive phototaxis, probably associated with water loss by evaporation, occurs in *O. asellus.*

The effect of humidity gradients on the phototaxis of *O. asellus* was next observed. It is known that when land isopods are introduced into an 'alternative chamber', with low humidity at one end and high humidity at the other, they aggregate in the damp half of the apparatus (Gunn, 1937). Preliminary and control experiments have also shown that in saturated air *O. asellus* invariably comes to rest in the shaded half of the apparatus. However, when the animals are placed in the alternative chamber with the range of 50–90% R.H. ($t = 18°$ C.), the drier half being screened with black paper and the damper half exposed to diffuse light of intensity of 45 Ferranti foot-candles, the isopods at first wander about the apparatus indiscriminately. The behaviour of forty specimens of *Oniscus*, previously kept in darkness at 100% R.H. for 3 days, was noted under such conditions. One animal was introduced into the apparatus at a time to prevent the possible conservation of water due to the formation of aggregations (Allee, 1926). Each animal was weighed before and after each experiment.

Six out of the forty animals came to rest in the dark, dry half of the apparatus and eventually died of desiccation; five settled in the dark for a short period of time and then continued to move until they finally came to rest in the light damp half;



Graph 7. Tendency to reversal from positive to negative phototaxis, at low humidities, in *Oniscus asellus* (20 specimens). The numbers of animals at rest in the shaded and illuminated (diffuse light of 45 Ferranti foot-candles) halves of the apparatus at 10, 70 and 100% R.H. are recorded.

Eight specimens moved continuously in both halves of the apparatus, settling finally in the light damp half;

Twenty-one others, after much initial activity, came to rest in the dark dry half, but then moved to the light damp half and came to rest there. 15 min. at rest in the light half was taken to indicate the final position of each animal.

The initial and final weights of the woodlice, the percentage loss of weight by evaporation, and the time taken to come to rest in the light damp half were recorded. The average loss of weight was 6·2% of the original body weight (limits 2·5 and

10·2 %). No correlation was observed between the weight of the woodlice and the percentage loss of water. The time taken to come to rest in the light varied between 20 min. and 3 hr. 20 min.:

Twenty-six out of thirty-four settled in the light half in between 50 min. and 2 hr.;

Four in under 50 min.; and

Four in over 2 hr.

It thus seems that the initial negative phototaxis is stronger than the humidity reaction of *Oniscus*. However, the increased activity of woodlice, due to the loss of water by evaporation and the masking or the reversal of the negative photic reaction, combine to retain the animals in the regions of greater humidity.

DISCUSSION

The three species of isopods studied, namely, *Oniscus asellus* Lin., *Porcellio scaber* Latr. and *Armadillidium vulgare* Latr., are widely distributed and common in Europe and North America. The animals are restricted to damp situations in which the microclimatic humidity conditions approach those of saturation. They are members of a very old stock in which there is but little morphological plasticity. Their stereotyped behaviour, controlled to a great extent by external stimuli, may be associated with the primitive scalariform, vermian type of nervous system. The brain of *Porcellio*, characterized by only slightly developed sight centres, and absence of antennal glomeruli and corpora pedunculata, is considered to be one of the most primitive of Arthropod brains (Hanström, 1928).

One of the most important adaptations of isopods to land, which, however, is not found in all species, is the development of tracheae on the pleopods, and hence of aerial respiration. The endopodites of woodlice are damp with a distinct film of water, and retain their 'gill-like' character. These are the only respiratory organs in *Oniscus*, which is capable of picking up drops of water by the movements of its telson and uropods, and conveying them to the respiratory surfaces through minute channels (Verhoeff, 1920). In *Porcellio* and *Armadillidium* while the exopodites are invaginated to form small branching air tubes, the endopodites are 'gill-like', and the isopods seek conditions for both methods of respiration.

Miller (1938) has found that the optimum relative humidity for terrestrial isopods is close to 100 %, and that there is an indication that survival is inversely proportional to the saturation deficiency. In the present set of experiments it was seen that the resistance to desiccation was consistently greatest in *Armadillidium*, and greater in *Porcellio* than in *Oniscus*. However, all the three species survived for indefinitely long periods only in desiccators at 100 % R.H. From experiments on *O. asellus* it could be concluded that the peril of desiccation is a very real one to woodlice, for not only the respiratory organs are affected, but there appears to be no control of loss of water through the cuticle, the loss of weight due to evaporation being proportional both to relative humidity and the time. It was noted that although *Armadillidium vulgare* is able to roll up into a ball, at no stage in the desiccation experiments was it seen to do so. It may be that rolling up is a response to rapidly changing conditions, rather than to a continuous stimulus of constant low relative humidity.

Allee (1926) has suggested that the formation of aggregations by woodlice is of survival value, since the loss of water of a group is much lower than that of an isolated animal. The difficulty of keeping isolated woodlice in laboratory conditions was continually noted. No doubt the animals not only live in a microclimate, but produce a microclimate of their own, for while the dorsal surface of the body is exposed, the damp pleopods of the ventral surface are normally closely pressed against the substratum, and under such circumstances the air in contact with the lower surface must be at saturation point. The isopods which survive for any length of time at 90% R.H. in laboratory conditions, provided they are in contact with a solid surface, die of desiccation when the whole body is exposed to this humidity.

The survival of isopods on land has undoubtedly been favoured by the development and combination of hygrokinetic, thigmokinetic and negatively phototactic behaviour. It was seen that the degree of thigmokinetic response of *Porcellio scaber* rises with the relative humidity of the air. The correlation which exists between hygrokinesis and thigmokinesis is significant and can also be considered of survival value, since it prevents the adherence of animals to solid surfaces in dry conditions, and hence their desiccation.

The effect of water loss on behaviour of woodlice was clearly shown by *Oniscus asellus*. According to modern terminology (Fraenkel & Gunn, 1940) this species shows low hygrokinesis and is negatively phototactic. When these woodlice were introduced into a humidity gradient, the drier end of which was shaded, and the damper exposed to diffuse light, the initial negative phototaxis was stronger than the reaction to humidity, but with the loss of water by evaporation, there was a masking or reversal of the reaction, which together with increased activity, brought the animals to the regions of 'optimum' humidity. Such an alteration in the reactions is of general interest, since it clearly indicates that the behaviour of an organism is not fixed, but consists of variables depending on the balance between the external and internal environments.

From the foregoing it is clear that the humidity reactions are of primary importance to terrestrial isopods. The behaviour of *Oniscus asellus*, *Armadillidium vulgare* and *Porcellio scaber* in constant humidities, and of the last-named species in humidity gradients, was observed, and it appears that there are two mechanisms whereby the animals are retained in the damper zones:

(1) *Hygrokinesis*, or the decrease of activity with the rise of relative humidity, was seen in all the three species. Gunn (1937) has demonstrated hygrokinesis in *Porcellio scaber*, but further details of this mechanism were obtained, as it was found that it is expressed not only by the number of minutes during which the animals are moving, or are at complete rest at any constant humidity, but also by the variations in the speed of the movements in different humidities. At high humidities activity and speed are consistently low, and the animals tend to become completely akinetic. This is true of *Oniscus* and *Porcellio*, in the case of *Armadillidium*, on the other hand, although the speed again decreases with the rise of humidity,

the number of 'rests', i.e. of periods of akinesis, remains relatively low and constant above 65 % R.H. In conditions below 85 % R.H. ($t = 14$–18° C.) all the three species remained active to the end of the 1 hr. experiments.

Hygrokinesis clearly results in bringing the animals to the regions of 'optimal', i.e. high humidity. Such a response is considered to be a primitive one (Mast, 1938), and is widely distributed throughout the animal kingdom.

(2) In addition, there appears to be a more complicated mechanism whereby the animals are retained in the 'optimal' zone, for when the total number of turnings is divided into the distance passed by the animals in the 1 hr. experiments, it becomes clear that the distance between turnings decreases with the rise of humidity and increases with its fall. This change in the behaviour pattern may be associated with the loss of water by evaporation from the animals, and together with the hygrokinetic effect, which in *Oniscus* and *Porcellio* results in complete inactivity at 90–100 % R.H., is effective in retaining the isopods in moist air. The decrease of distance between turnings with the rise of humidity has been observed in all the three species. In humidity gradient experiments on *P. scaber* this mechanism was again exhibited in gradients of high, but not in those of low, humidity.

It is interesting to note that Gunn & Pielou (1940) describe a similar mechanism designated as 'virtual inactivity' in the humidity reactions of *Tenebrio molitor*, which in contrast to the isopods aggregate at the drier end of a humidity gradient.

Although the mechanisms of these reactions in *Oniscus*, *Porcellio* and *Armadillidium* are the same, some differences in their intensity have been detected. The greater activity, at high humidities, of *Armadillidium*, as compared to that of *Porcellio* and *Oniscus*, may be an expression of its lesser sensitiveness to alterations in humidity and of its greater resistance to desiccation. The mechanisms are most clearly expressed in *O. asellus*, which, of the three species, must be considered the least adapted to land.

When a comparison is made of the mechanisms of the reactions of isopods with photophobotaxis described by Ullyott (1936) in the planarian *Dendrocoelum lacteum*, two main differences (among others) emerge, viz..

(1) No kinetic effect was shown in *D. lacteum*, whereas hygrokinesis is clear in the isopods.

(2) Sensory adaptation is the basic factor determining the photic responses of *D. lacteum*. The meaning of sensory adaptation is that the effect of a continuous stimulus is not uniform, but that the intensity of the reaction produced by it decreases with the time and gradually dies down (Hecht, 1919). No such adaptation was satisfactorily demonstrated in the isopods, and it was particularly difficult to approach this question, since at low humidities woodlice are continually losing water by evaporation, and hence not maintaining a balanced physiological state.

As to the humidity receptors, none are known in woodlice (Gunn, 1937). It can be suggested that the rapid loss of water by evaporation and the consequent concentration of body fluids would in itself have an effect on the reactions of the animals. Any sense organs connected with this mechanism would be proprioceptive. If this suggestion is correct and there are no specialized receptors, no sensory adaptation need take place, and this is borne out by experimental results. This, however, would not account for immediate reactions. Gunn (1937) has suggested that the receptors probably lie in the thoracic region. It has been continually noted that the relatively delicate thoracic appendages are the first to show the effects of desiccation, and it may be that they function as the 'hygrometers' of the body.

In conclusion, it may be said that the terrestrial isopods are the only group of Crustacea which are able to live on land throughout the whole of their life cycle. However, they are just able to survive the land conditions, not so much by morphological adaptations, as by evolving a series of patterns of behaviour, which restrict them to moist dark habitats. In other words, they are able to survive on land by avoiding the typical land conditions.

SUMMARY

1. The humidity reactions of *Oniscus asellus*, *Porcellio scaber* and *Armadillidium vulgare* have been analysed and compared.
2. The mechanism whereby the three species collect in moist air is twofold, consisting of (*a*) hygrokinesis, or decrease in activity and speed in moist air, and (*b*) of more frequent turnings in space, retaining them in the areas of greater humidity.
3. These mechanisms are most clearly expressed in *Oniscus asellus* and least in *Armadillidium vulgare*. This sequence may be correlated with the resistance to desiccation of the three species, which is greatest in *Armadillidium vulgare*, and greater in *Porcellio scaber* than in *Oniscus asellus*.
4. It is suggested that the humidity reactions of isopods are controlled by water loss by evaporation from the whole body.
5. A correlation between hygrokinesis and thigmokinesis was observed in *Porcellio scaber*.
6. There appears to be a reversal from negative to positive phototaxis in *Oniscus asellus*, correlated with the water loss by evaporation.
7. The humidity reactions, low thigmokinesis and negative phototaxis combine to retain the isopods in damp, dark habitats.

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