HUMIDITY REACTIONS OF THREE AQUATIC AMPHIPODS, *GAMMARUS DUEBENI, G. OCEANICUS* AND *PONTOPOREIA AFFINIS* IN THE AIR

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It has been previously shown that the aquatic isopod *Asellus aquaticus*—when taken out of water—is able to select the higher of two different humidities of the air (Lagerspetz & Lehtonen, 1961). In that connexion, it was suggested that some aquatic amphipods, when exposed to air, would probably also exhibit behavioural reactions to differences in atmospheric humidities.

One of the Finnish species of the genus *Gammarus*, *G. duebeni* Lillj., is an inhabitant of rock pools on exposed rocky shores in the Finnish archipelago. Its ecology has been studied by Dahl (1944), Segerstråle (1946), Forsman (1951), and by Kinne (1959), who in a series of papers has also presented various aspects of the ecological physiology of this species. Dahl (1944, p. 13), Segerstråle (1946, p. 18), and Forsman (1951, p. 229) have also paid attention to the nocturnal movements on land of *G. duebeni*. The animals are very active at night and have been observed to leave the pools voluntarily and creep rapidly on land, even up the face of vertical rocks. Thus the animals may find new pools and escape from the drying ones, the habit of moving on land being of importance for the dispersal and survival of the species. The differences in the humidity of the air might represent a possible type of sensory clue for orientation on dry land. The reactions of *G. duebeni* to differences in the humidity of the air were thus chosen for study. For purposes of comparison, the humidity reactions of two other aquatic amphipods, *G. oceanicus* Segerstråle and *Pontoporeia affinis* Lindström were also studied.

The terrestrial amphipods of the family *Talitridae, Talitrus saltator, Talorchestia deshayesii*, and *Orchestia gammarellus* have been shown earlier to exhibit behavioural humidity reactions (Williamson, 1951).

EXPERIMENTS

(a) *Group experiments*

The specimens of *Gammarus duebeni* were collected from rock pools on the rocky islands of Storsundsharun, Segelskär, and Brännskär, in the vicinity of the Tvärminne Zoological Station, S.W. Finland. The specimens of *G. oceanicus* were collected from the *Fucus* vegetation and the specimens of *Pontoporeia affinis* from the ooze of sea bottom (depth 3 m.) close to the Zoological Station. The work was done in July 1962.

The animals were kept at room temperature (17–19° C.) in their native brackish water. All experiments were performed at 17–19° C.

The alternative-chamber method used is based on that of Gunn & Kennedy (1936) and has been described by Perttunen (1953, 1961) and by Lagerspetz & Lehtonen (1961). The chemicals used in order to obtain different relative humidities in the two halves of the alternative chamber were de-ionized water, a saturated solution of KH_2PO_4, and dried silica gel with a moisture indicator. The theoretical values for the relative humidity of the air in equilibrium with these chemicals were 100, 97 and 0 %,
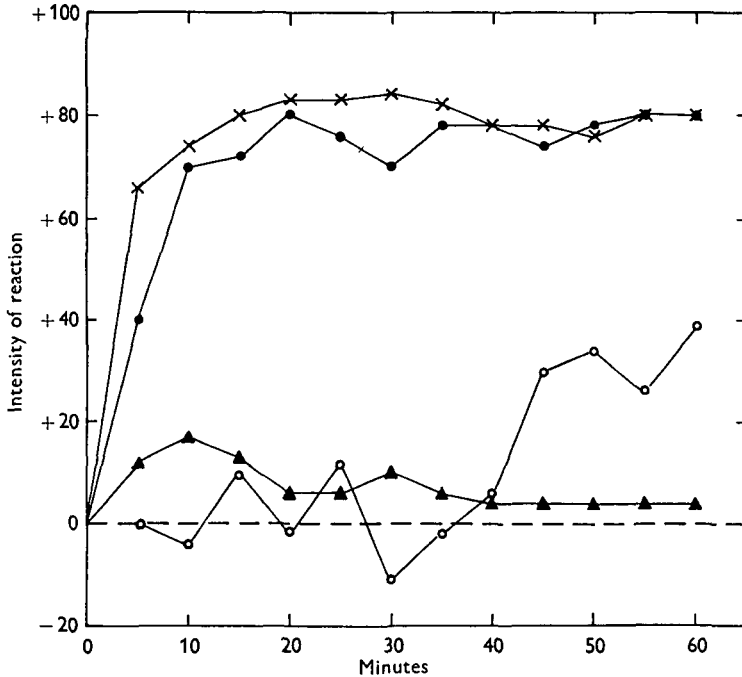

Fig. 1. Intensity of the humidity reaction of the amphipods in group experiments. Time in minutes from the beginning of the experiment. Closed circles: *G. duebeni* (0–100 % R.H.), open circles: *G. duebeni* (97–100 % R.H.), crosses: *G. oceanicus* (0–100 % R.H.), triangles: *P. affinis* (0–100 % R.H.).

respectively (cf. Perttunen, 1953). The position of the animals in the alternative chamber was recorded at intervals of 5 min. for a period of 1 hr. Between the recordings the apparatus was enclosed in dark cardboard cylinders with covers. The usual precautions of turning the apparatus 180° between the experiments, of preparing the apparatus 1 hr. before the experiment, etc., were taken. The surface of each specimen was carefully dried with filter paper before it was placed in the apparatus. Five experiments, each with ten fresh specimens, were carried out for each species, using the theoretical alternatives of 0 and 100 % R.H. In addition one series of five experiments, each with ten specimens of *Gammarus duebeni*, was carried out using the alternatives of 97 and 100 % R.H.

The results of the group experiments are shown in Fig. 1, where the intensity of the reaction [100 (number of records on the more humid side minus number of

records on the drier side) per total number of records] is plotted against time, starting from the time when the animals were placed in the apparatus. For statistical analysis one-half of the number of records of animals at the mid-line of the alternative chamber was added to the numbers of both the moist and dry side records. The statistical analysis of the results is presented in Table 1.

Table 1. *Results of the group experiments on the humidity behaviour of amphipods in an alternative chamber*

Species (humidity alternatives)	Number of records			χ^2	P
	Dry side	Mid-line	Humid side		
G. duebeni (0–100%)	59	44	497	320	<0·0005
G. duebeni (97–100%)	237	54	309	8·64	<0·01
G. oceanicus (0–100%)	36	57	507	348	<0·0005
P. affinis (0–100%)	235	85	280	3·76	N.S.

It appears that *G. duebeni* and *G. oceanicus* are found more often in the humid side of the alternative chamber, when a great difference in R.H. of the air prevails between the sides of the chamber. The graphs (Fig. 1) show that the humidity reaction of *G. oceanicus* develops more rapidly to its full intensity and is more stable than that of *G. duebeni*. The specimens of *P. affinis*, in the experimental conditions, are distributed at random in the alternative chamber. The specimens of *G. duebeni* also react to a small difference in the relative humidity of the air (97–100%) after the animals have been out of water for about 40 min. This has not been tested with the other species.

(b) *Experiments with single individuals*

The tracks of ten specimens of each species, placed one at a time in the alternative chamber with silica gel and water, were recorded separately during two successive periods of 5 min. The time spent in each half of the chamber and the number of stops were also recorded. The length of the tracks was measured with the aid of a map-measurer. From these data the speed of the animals on both sides was calculated. The number of times the animal had crossed the mid-line, the number of times it had turned around at the mid-line and the number of random turnings per 10 min. in the dry and in the moist atmosphere were also calculated. The mean results, together with their standard errors, are presented in Table 2.

The specimens of *G. duebeni* and *G. oceanicus* spent about 87% of the time in the more humid side of the apparatus. The specimens of *P. affinis* did not show any preference for either side. The results of the group experiments are thus confirmed. As shown by the results of the track-length measurements, the motility of *G. duebeni* was significantly higher on the drier side of the alternative chamber. A similar tendency can also be observed in *G. oceanicus*. Hence the orthokinetic type of orientation (Fraenkel & Gunn, 1940) seems to be one of the reaction mechanisms in this case. In addition, the frequency of random turnings of the specimens of *G. duebeni* is significantly higher on the dry side of the apparatus. This shows the importance of the klinokinetic orientation mechanism in the humidity behaviour of these animals.

The lesser motility of *G. oceanicus*, when exposed to air, makes the analysis of the humidity reaction in that species more difficult. When placed in the apparatus these animals rapidly oriented themselves and, after finding an area of high humidity, became inactive. In contrast to this, the specimens of *G. duebeni* continued to move at intervals. *P. affinis* was about as inactive as *G. oceanicus*, but did not show any signs of humidity discrimination.

Table 2. *Results of the experiments on the humidity behaviour of single amphipods in the alternative chamber*

	G. duebeni		G. oceanicus		P. affinis	
	Dry side	Humid side	Dry side	Humid side	Dry side	Humid side
Mean time spent (sec.)	78±16	522±16	77±60	523±60	301±84	299±84
Mean track length (cm.)	86·5±16·0	177·9±25·3	7·1±3·7	22·2±5·9	7·3±3·7	17·0±6·0
Mean speed (mm./sec.)	14·8±2·6	3·4±0·5	6·9±3·2	0·4±0·1	3·2±2·1	0·6 ±0·2
t		4·63		2·04		1·25
P		<0·001		~0·05		N.S.
Mean frequency of random turnings (per min.)	24·3±11·3	0·6±0·1	21·2±12·5	0·5 ±0·2	6·5±6·0	4·7±4·0
t		2·10		1·68		0·25
P		0·05		N.S.		N.S.

Table 3. *Weight and survival time of amphipods in air saturated with water vapour (19° C.)*

	Mean initial weight (mg.)	Survival time for 50% of animals (hr.)	Maximum survival time (hr.)
G. duebeni	59	172	228
G. oceanicus	101	48	120
P. affinis	11	40	70

The specimens of *G. duebeni* approached the mid-line of the apparatus seventy-eight times when coming from the humid side. In thirty-six of these cases at mid-line the animal turned around about 180° and returned to the humid side. In none of the forty-six cases in which the animals approached the mid-line from the dry side did the animal return to the dry but always crossed the mid-line towards the humid side. This type of reaction has often been interpreted as a klinotactic reaction. However, if the animal in question is strongly thigmotactic, like *G. duebeni*, a random turning when coming to the dry air, combined with thigmotaxis, causes a 180° turning at the mid-line. This was also pointed out by Williamson (1951). Thus there is no evidence for the occurrence of the klinotactic type of humidity orientation in the amphipods.

(c) Survival in air

Twenty individuals of each species were placed in small open plastic vessels, five in each. These vessels were kept in glass jars, the bottoms of which were covered with water, to which strips of wet filter paper extended. The jars were closed with tightly fitting lids. Thus the atmosphere inside the jars was saturated with water vapour. The number of survivors was recorded at intervals of 1–20 hr. The animals which

ailed to react to repeated tactile stimulation were considered as dead. These animals were transferred to their native brackish water but no case of a subsequent recovery was observed. The mean weight of the animals and the results of this experiment are shown in Table 3.

All species studied were able to withstand at least a short stay in air of high relative humidity. In spite of the large difference in weight the survival times for *G. oceanicus* and *P. affinis* are not markedly different. On the other hand, *G. duebeni* differs from these species, because of its much better ability to live in humid air.

DISCUSSION

Two of the three species of aquatic amphipods studied, *Gammarus duebeni* and *G. oceanicus*, seem to be able to avoid the lower of two atmospheric relative humidities presented to them in experimental conditions. This reaction is based on orthokinetic and klinokinetic orientation mechanisms (Fraenkel & Gunn, 1940). The same types of orientation also occur in the humidity reactions of terrestrial amphipods (Williamson, 1951). The more rapid and more stable humidity reaction in *G. oceanicus* is apparently a result of the lesser motility of this species, when exposed to air. Even when the humidity difference is rather small (97–100 %), *G. duebeni* avoids the lower humidity after a stay of 40 min. in the gradient.

The ability to avoid low humidities, when exposed to air, has a survival value to *G. duebeni*, which is an inhabitant of small, often temporary, pools. In the practically tideless Baltic *G. oceanicus* is seldom exposed to air. This, however, certainly often occurs in the other coastal areas, where this species is usually found between the tide marks (Spooner, 1951; Segerstråle, 1959; Steen, 1951). It is an interesting fact that the capacity for behavioural humidity reactions has also been retained in the Baltic population of this species, although it does not seem to offer any direct selective advantage for the population living in the Baltic. *Pontoporeia affinis* occurs in the Baltic even at small depths, but in other parts of its distibutional range it is confined to the bottom of such depths, so that it is never exposed to air by low tides (Segerstråle, 1959). This is in agreement with the present observations, which indicate that specimens of *P. affinis* do not react to differences in the humidity of the air.

The site of the humidity receptors in amphipods has not been discovered (Williamson, 1951). The salinity receptors of the antennae and antennulae of amphipods probably do not function as receptors for humidity differences (Lagerspetz & Mattila, 1961).

Besides the humidity reactions the long time of survival of *G. duebeni* in air saturated with water vapour, as well as the great motility and the relatively high speed of these animals on dry land, show that this species is relatively well adapted to occasional periods of terrestrial life. *G. oceanicus*, like *P. affinis*, survives only a short stay in humid air, which *G. oceanicus*, however, is able to distinguish from drier alternatives. Its lesser motility on dry land also indicates that this species is adapted rather to wait passively for suitable conditions to return than to seek actively for water (Steen, 1951, p. 235). *P. affinis* again is an aquatic species which in nature is never exposed to air and consequently has not acquired evolutionary adaptations advantageous for terrestrial life.

SUMMARY

1. Specimens of the Baltic population of the aquatic amphipods *Gammarus duebeni* and *G. oceanicus* are able to avoid the lower of two relative humidities of the air when out of water.

2. This humidity reaction is based on the orthokinetic and the klinokinetic types of orientation.

3. *G. duebeni* is an inhabitant of seashore rock pools and has been reported to move on land between the pools.

4. Although *G. oceanicus* is seldom exposed to air in nature in the practically tideless Baltic area, it lives between tide-marks in other parts of its distributional range.

5. The third amphipod studied, *Pontoporeia affinis*, does not discriminate between high and low humidities of the air in an alternative chamber. It is always confined in nature to the sea bottom below the low tide level.

6. Of the three studied species, individuals of *G. duebeni* move rapidly on dry land and survive a markedly longer time in humid air than the amphipods belonging to the two other species.

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