THE WATER RELATIONS OF EARTHWORMS

II. RESISTANCE TO DESICCATION AND IMMERSION, AND BEHAVIOUR WHEN SUBMERGED AND WHEN ALLOWED A CHOICE OF ENVIRONMENT

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I. INTRODUCTION

In this paper the reactions of whole worms of six species to various water conditions are described, and an attempt is made to correlate the information obtained with the ecological distribution of the species.

II. ECOLOGY OF THE SPECIES USED

Allolobophora caliginosa Savigny, 1826

A. caliginosa is found particularly in cultivated soil, its optimal conditions being in pastures, meadows, and foliated forests (Černosvitov, MS.; Kollmannsperger 1936; Černosvitov & Evans, 1947; Guild, 1948). It has been recorded from clay soil, from humid soil on river banks, under stones and from purely limnic localities (Friend, 1910; Černosvitov & Evans, 1947; Černosvitov, MS.), but is more numerous on light loams than elsewhere, and is absent from very acid peaty soil (Guild, 1948, 1951).

Allolobophora chlorotica Savigny, 1826

This species is found in a great variety of habitats. Although it is a hydrophilous species it can withstand considerable fluctuations in humidity. *A. chlorotica* is frequently found by ponds, lakes and rivers, and less frequently in streams and in subterranean water (Friend, 1891, 1897, 1909, 1911, 1926; Davies, 1950, and personal communication). It is also found in Lake Windermere up to 20 m. from the shore at a depth of about 2 m. (Černosvitov, 1945). In addition, this species also occurs in semi-fixed dunes in Anglesey, where the water table falls to at least a metre during summer (Dr T. B. Reynoldson, personal communication).

Although never in great numbers, *A. chlorotica* is frequently found in gardens, arable fields and meadows, especially amongst the roots of plants (Evans & Guild, 1947). Populations are greatest on alluvium, clay and gravelly sand (Kollmannsperger, 1936; Guild, 1948, 1951).

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Allolobophora terrestris Savigny, 1826 forma *longa* Ude, 1885

A. terrestris f. *longa* is one of the commonest British earthworms. It is a typical pasture form, and is found in cultivated soils. Although it is a terrestrial animal, it is occasionally found in limnic localities, and is particularly abundant in the moist soil on the banks of rivers and lakes (Černosvitov and Evans, 1947; Guild, 1948).

Dendrobaena subrubicunda Eisen, 1874

This species is found in places rich in decaying organic matter: in compost heaps, leaf-mould, and in rich garden soil (Friend, 1910, 1910–11, 1911, 1926; Černosvitov, MS.; Černosvitov & Evans, 1947). Although *D. subrubicunda* should be regarded as a terrestrial species it is occasionally found in limnic habitats.

Lumbricus rubellus Hoffmeister, 1843

This species is a stenotope humus form requiring a certain humidity, and is therefore absent from very moist localities and dry steppes (Kollmannsperger, 1934, 1936). It occurs in places rich in humus, under moss, gardens, pastures and river banks (Friend, 1910, 1910–11, 1926; Moszyński, 1928; Černosvitov & Evans, 1947). It is most numerous on light loams and is tolerant of acid soils, where it often becomes the dominant species (Guild, 1948, 1951). It is rarely amphibious (Černosvitov & Evans, 1947).

Lumbricus terrestris Linnaeus, 1785

L. terrestris is a purely terrestrial, deeply burrowing species. It is tolerant of a wide range of pasture types, altitude and soil acidity. According to Černosvitov & Evans (1947) *L. terrestris* shows a preference for clayey soil, but Guild (1948) found that it is most numerous on light loam.

III. THE RESISTANCE OF EARTHWORMS TO DESICCATION

Water rapidly evaporates from earthworms. In natural environments fatal desiccation is avoided either by diapause or by a retreat to the subsoil. Some species undergo a facultative diapause, rolling up into a tight ball, and remaining inactive in a spherical earthen cell, when conditions become too dry or otherwise unfavourable. Others have an obligatory diapause which is not dependent on external factors, but which occurs during the summer when adverse conditions are most likely to prevail. Still others retreat to the subsoil. Nevertheless, earthworms can withstand a considerable degree of desiccation (Schmidt, 1918; Hall, 1922; Jackson, 1926).

In my experiments *Lumbricus terrestris* and *Allolobophora chlorotica* were used. The water content of each species was found so that the effect of water loss on the concentration of the coelomic fluid could be estimated.

(1) *Water content*

The worms were kept on moist filter-paper for 3 or 4 days before the experiments, to clear their guts of earth. They were slit open on filter-paper and dehydrated individually in a vacuum desiccator at 100° C. with phosphorus pentoxide as the dehydrating agent. The water content of the filter-paper was found in the same way, and the results for the water content of the worms were corrected accordingly.

A period of 24–29 hr. was necessary for constant weight to be reached. Determinations were made for seven *L. terrestris* (fresh weights from 3·230 to 6·240 g.) and six *A. chlorotica* (fresh weights from 0·261 to 0·363 g.). The water contents, expressed as a percentage of the body weight, were: *L. terrestris* from 81·9 to 87·7, mean 84·8 (S.D. 1·597); *A. chlorotica* from 74·7 to 82·2, mean 80 (S.D. 2·4860). This difference between the means is highly significant. Application of the '*t*' test shows that there is a probability of only 1 in 500 of this difference occurring by chance.

(2) *Survival after partial desiccation*

Again, the worms were kept on filter-paper before the experiments to clear the gut of earth. They were weighed individually and then covered with wire gauze or nylon net and placed in desiccators with anhydrous calcium chloride as the dehydrating agent. After desiccation to various degrees they were re-weighed and placed on moist filter-paper and left to recover. The worms were deemed to have recovered if they lived for at least 2 days after the experiment.

About 20–24 hr. were required for *L. terrestris* to lose 50 % of its body weight, and 3 hr. for the same loss in *A. chlorotica*. The temperature ranged between 15 and 18° C. during the experiments.

The results are shown in Table 1. The greatest loss which *L. terrestris* can survive is about 60 % of body weight, i.e. 70 % of the water content of the body. Several of the *A. chlorotica* made a temporary recovery, i.e. they regained the power of movement but died within a day. The limit for true recovery in this species is about 60 % loss of body weight, which is 75 % of the water content of the body.

My results differ from those obtained by Jackson (1926) for *L. terrestris*, and by Hall (1922) for *A. chlorotica*. In Jackson's experiments the limit of tolerance of loss of body weight was between 43 and 50 % as compared with 57–59·7 % in mine, the time taken for desiccation being very much shorter, 5–9 hr. instead of more than 20 hr., which may be responsible for the different results.

Hall found that one specimen of *A. chlorotica* survived after a loss of 69·6 % of its body weight, and that all worms desiccated until up to 26 % of their body weight had been lost survived. Losses of more than 26 % were followed by varying degrees of survival. The limit in my experiments (between 57·9 and 60 %) represents a greater loss than in Hall's experiments unless his exception is taken into account. In Hall's experiments desiccation was carried out at 27–30° C. Although no information is available for *A. chlorotica* Wolf (1938*b*, 1941) found the heat-death temperature for

L. terrestris to be 28° C. (exposure time 500 min.), and it seems probable that the high temperatures affected Hall's results.

Table 1. *Recovery of earthworms after desiccation*

No.	% body wt. lost	Recovery	No.	% body wt. lost	Recovery
		L. terrestris			
1	30·3	+	21	55·0	+
2	31·1	+	22	56·4	+
3	34·2	+	23	57·0	+
4	36·7	+	24	59·7	−
5	38·3	+	25	59·8	−
6	39·6	+	26	59·9	−
7	40·4	+	27	60·6	+
8	40·6	+	28	60·8	−
9	42·1	+	29	63·1	−
10	43·1	−	30	63·9	−
11	45·1	+	31	64·0	−
12	47·3	+	32	66·2	−
13	47·5	+	33	68·4	−
14	48·4	+	34	70·2	−
15	48·5	+	35	71·6	−
16	48·7	+	36	72·4	−
17	50·8	+	37	74·4	−
18	52·4	+	38	83·0	−
19	53·5	+	39	85·0	−
20	54·7	−			
		A. chlorotica			
1	28·0	+	12	49·6	+
2	31·0	+	13	52·5	+
3	32·4	+	14	56·2	Temporary
4	34·2	+	15	56·8	+
5	42·2	+	16	57·6	+
6	44·1	+	17	57·9	+
7	44·8	+	18	60·0	Temporary
8	45·3	−	19	61·0	Temporary
9	45·7	+	20	69·2	−
10	45·9	Temporary	21	69·2	−
11	49·0	Temporary	22	72·5	−

+ = full recovery, − = no recovery.

IV. THE RESISTANCE OF EARTHWORMS TO IMMERSION

The ability of earthworms to survive in water has long been known. In 1874 Perrier reported the survival of *L. terrestris* in frequently changed tap water for more than 4 months. Since then similar results have been reported for several species (Colosi, 1925; Nagano, 1934). I made a comparative study of the resistance of six species to immersion.

(1) *Length of survival in soil under water*

A layer of soil 1–1½ in. deep was placed in tanks and covered with 3–4 in. of tap water, care being taken to ensure that no air was left in the soil. Woodland soil was used for *L. rubellus*, and clayey loam for the other species. The water was aerated, and the level maintained by the addition of glass-distilled water. One or two worms were placed in each tank. All species, except *A. caliginosa*, were able to live for long periods under these conditions (see Table 2). As only two specimens of *A. caliginosa* were used, and as there appears to be an initial danger period to be survived (Maluf, 1939, and results for other species), the results for *A. caliginosa* cannot be regarded as conclusive. It will be noted that in all cases some part of the worm's body was normally above the surface of the soil. Only during a spell of very cold weather when the temperature fell to 9° C. did the worms go completely below the soil surface. Colosi (1925) observed that *A. caliginosa* and *Dendrobaena subrubicunda* lived for nearly 8 months under similar conditions, and that the anterior end was kept in the soil, leaving the posterior part in the water. The significance of this is at present unknown, but it may be necessary for respiration. According to Merker (1926, 1928), water filtered through soil loses much of its oxygen. It is possible that in the tanks the soil remained deficient in oxygen despite aeration of the water above.

Table 2. *The survival of earthworms in soil under water*

Species	Soil	Worm no.	Length of survival	Notes on behaviour	Temperature range (° C.)
A. caliginosa	Clayey loam	1 2	< 7 days < 7 days	Lay on soil surface	20
A. chlorotica	Clayey loam	1 2 3	7 days 31 weeks 48 weeks	Anterior or posterior end on surface or whole worm under surface litter	20 9–18 9–20
A. terrestris f. *longa*	Clayey loam	1 2	14 days 50 weeks	Part of body always above soil surface	18–20 8·5–20
D. subrubicunda	Clayey loam	1 2	50 weeks 50 weeks	Just beneath soil surface horizontally with anterior ends projecting	9–20
L. rubellus	Woodland	1	1½ days	Whole worm quiescent on surface.	18
		2	39 weeks	One end on soil surface	9–19·5

(2) *Length of survival immersed in aerated tap water*

In this experiment the tanks each contained five or six worms, and were kept under similar conditions. Unfortunately the tanks were not uniform, which, together with the different sizes of the worms, meant that the weight of worm per standard volume of water and the surface area of the water was not the same in each case. As, however, the water was well aerated these discrepancies were probably not significant.

The results are seen in Table 3. All species are capable of living for many weeks in aerated water without food, and no significant difference is apparent between them. The length of survival may depend on ability to survive starvation rather than submergence.

Table 3. *Length of survival in aerated tap water*

Species	No. of worms	Survival times in days			Volume of water (ml.)	Surface area of water (cm.²)	Gram worm/ 1000 ml. water	Temperature range (° C.)
		Min.	Mean	Max.				
A. caliginosa	5	100	100	100	1000	141·1	2·57	11–19
A. chlorotica	6	134	137	141	1232	154	1·51	8–17·5
A. terrestris f. *longa*	6	93	99	124	2304	256	6·51	8–17·5
D. subrubicunda	5	63	72	77	1000	141·1	0·61	11–19
L. rubellus	6	3	78	195	1425	150	3·20	8–17·5
L. terrestris	6	88	137	166	2262	282·8	9·39	8–17·5
L. terrestris	5	35	92	111	2262	282·8	7·83	8–17·5

V. THE BEHAVIOUR OF EARTHWORMS WHEN SUBMERGED

Observations on the activity of submerged earthworms were made, to find out how far the worms can be regarded as viable fresh-water animals under natural conditions. Particular attention was paid to *A. chlorotica* because of its frequent occurrence in limnic habitats.

If specimens of *A. chlorotica* collected from a garden are able to feed and reproduce under water the occurrence of this species in Lake Windermere can be explained by the accidental introduction of worms or cocoons into the lake, and it is then unnecessary to postulate that the Lake Windermere individuals constitute a separate race.

Worms in glass tubes

Straight pieces of glass tubing of a diameter roughly equal to that of the worm were placed in the tanks containing some of the worms living in aerated tap water. The dimensions of the tubes were as follows:

Length	Diameter	Species
18 cm.	1 cm.	*L. terrestris* (tank with 5 worms)
11·5 cm.	6 mm.	*L. rubellus*
9 cm.	5 mm.	*A. chlorotica*

One tube per worm.

All *L. terrestris* specimens entered the tubes during the night, and then spent most of their time in them with either the anterior or posterior end, or a part of both projecting. At intervals, periods of a few days were spent out of the tubes. *L. rubellus* did not usually use the tubes, although occasionally one was found inside. *A. chlorotica* used the tubes but often, as the diameter of the tubes was too great, two folded worms shared a short length. They were transferred to a larger tank and given tubes 12 cm. long and 2 mm. in diameter. They entered these without folding, but kept either the posterior or anterior end projecting.

Worms in soil

Vivaria made to the design described by Wells (1949) were used in these and some following experiments. Each vivarium consisted of two pieces of glass separated by a strip of rubber coated with vacuum grease and kept together by means of six 'G' clamps or 'bulldog' clips (see fig. 2). The size of the glass and the cross-section of the rubber strip was varied.

A single specimen of *A. chlorotica* was added to a small vivarium of $3\frac{1}{4} \times 4\frac{1}{4}$ in. glass plates and with rubber strips $\frac{1}{8}$ sq.in. in cross-section, set up with a layer of clay soil covered with water. This was repeated three times. In each case the worm burrowed straight down, and during the night constructed a U-shaped burrow, from one arm of which the posterior end was projected. There was a tendency for the arm of the burrow housing the posterior end of the worm to be constructed immediately below the aeration jet; when the latter was moved, so the worm moved.

Specimens of *A. chlorotica* from Lake Windermere in a large tank of water-covered soil with plants were observed to burrow incompletely into the soil so that the posterior end projected in the same way.

When placed in a similar vivarium *L. rubellus* showed a different type of behaviour. It did not burrow into the soil, but contrived to keep as much of its body as possible suspended between the glass plates in the small air space above the water.

Like *L. rubellus*, *L. terrestris* did not construct burrows when placed in a larger vivarium (15×15 in. plates and $\frac{3}{8}$ sq.in. cross-section rubber), with all the soil below water. One of the three worms used burrowed extensively during the first night, but thereafter remained on the surface of the soil. One of the other two worms burrowed when first placed in the vivarium, but returned to the surface again within 15 min. and made no further attempts to burrow. Numerous attempts to climb out of the vivarium were made by all the worms, particularly at first.

Thus *A. chlorotica* was the only one of the three species which was observed to construct a U-shaped burrow when the earth was covered by water.

Irrigation of the burrow by Allolobophora chlorotica

It was thought possible that *A. chlorotica* might irrigate its burrow (or glass tube) as do many truly aquatic annelids. The worms were watched carefully but no movements could be detected, even when the tank was placed on graph paper. The use of a carmine suspension did not indicate any movement when the worms were in glass tubes or natural burrows. It is possible, however, that such movements only come into play when the water lacks oxygen, or has excess carbon dioxide, or when there is a combination of both of these factors. To test this possibility each of the conditions was produced experimentally.

A high concentration of carbon dioxide caused the worms to withdraw completely into their tubes or burrows, and to remain quiescent. This occurred when the whole of the water was replaced by carbon dioxide saturated water, or when a little was introduced in the region of the exposed anterior or posterior end. A slight increase in the carbon dioxide content of the water produced no response.

When the tap water was replaced by cooled boiled water (oxygen lack) the worms either left their tubes or showed no response.

A combination of oxygen lack and carbon dioxide excess, produced by bubbling carbon dioxide for 15 min. through cooled boiled water, covered with a layer of liquid paraffin, did not elicit a clearly marked response. Complete replacement of the original tap water by water prepared as above resulted in the worms withdrawing their exposed ends for a few minutes. A little of the water, introduced in the region of the exposed parts of the worms, produced no response. Nor could any currents be detected by carmine suspension.

Thus no circulation of water through the tube occurs under any of the above circumstances.

Feeding

Five *A. chlorotica* specimens were placed in aerated tap water to allow the gut to become empty. They were examined at intervals, but even after a month the gut still retained some earth. In two worms the whole of the anterior part of the gut as far back as the clitellum was quite empty, while the rest of the intestine contained a small amount of earth throughout its length. These were placed in a tank with soil, leaf-mould, and bits of shredded cabbage leaf and carrot, and covered with water 3–4 in. deep. Although the worms did not diminish in size as those left in water without food, no positive evidence of feeding could be obtained, the gut seeming to be in exactly the same condition at each examination, even after 4 months. Indirect evidence for feeding is that worms in water with some soil survive for much longer periods than those without soil. (See also evidence given in the paragraph on the development of cocoons, below.)

Cocoon production

To see if worms can reproduce when submerged, six mature worms were placed in a tank containing water and some autoclaved soil. Food was washed before adding it to the tank to avoid the accidental introduction of cocoons. No reproduction was observed during a year.

Development of cocoons

Eighteen cocoons of *A. chlorotica*, collected from culture pots, were placed in a dish of aerated tap water.

All but three cocoons produced young worms, one producing two. On hatching the young worms were transferred to a shallow dish containing tap water, some soil and small pieces of organic matter. These worms produced castings and one was observed making a cast. The alimentary canal, empty on hatching, soon became full of earth. Three worms were weighed on hatching, and placed in a dish with water and soil. They were then weighed at intervals. The results, which are given in

Fig. 1, show conclusively that the worms grow under these conditions, and therefore are able to feed under water.

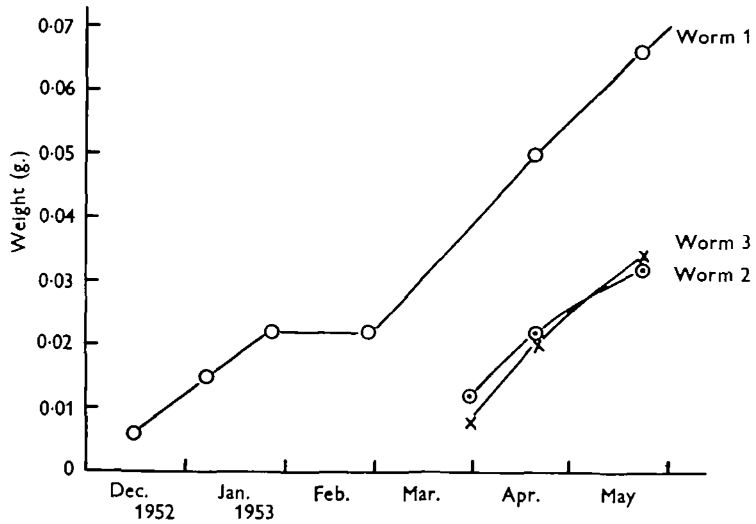

Fig. 1. Increase in weight of young *A. chlorotica*.

VI. THE BEHAVIOUR OF EARTHWORMS WHEN ALLOWED A CHOICE OF ENVIRONMENT

All the species investigated are able to live in water when forced to do so, but only some of them occur in water under natural conditions. The following experiments were designed to study behaviour as a possible cause of differences in distribution.

Worms in soil

These experiments were made in glass-sided vivaria as described above. Some tap water was put into the apparatus, and soil was added. The amounts of soil and water were adjusted so that the final result was water-saturated air-free soil under aerated water on one side, and water-saturated air-free soil topped by moist air-containing soil on the other (Fig. 2). The positions of the worms were recorded daily by drawings. They moved little during the day, so only one observation every 24 hr. was made.

The following factors were varied during the experiments:

(*a*) *The distance between the glass plates.* This should be wide enough to allow the worms to move freely, and narrow enough to ensure that they can be seen. The finding of *A. chlorotica* was particularly difficult owing to their small size and grey colour, and in several of the experiments on this species many of the worms escaped observation.

(*b*) *The nature of the soil.* This was varied because chemical factors might alter the results. The following soils were used, giving a wide variation in organic content: leaf-mould, clay, equal parts by volume of leaf-mould and clay, and washed fine silver sand. The leaf-mould was soil enriched by decayed oak and hawthorn leaves.

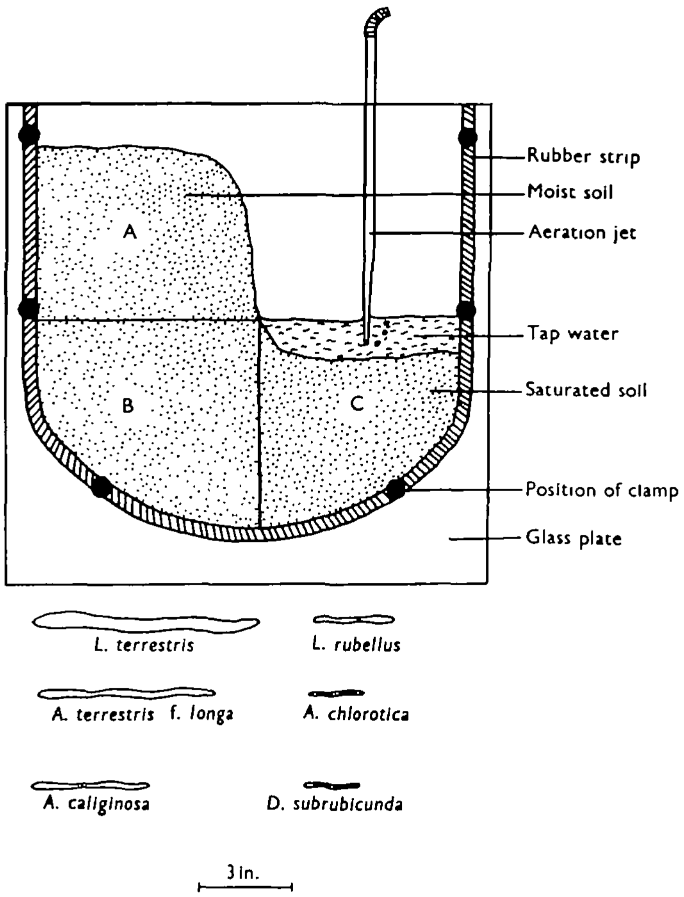

Fig. 2. Diagram of glass-sided vivarium showing the size of the worms relative to the apparatus.

The results are shown in Table 4. For the purpose of making the analysis shown in the table the soil was divided into the following zones: A, soil above water-level; B, soil below water-level but with no free water surface above; C, soil below water-level with a free water surface above (Fig. 2). The category A–B was made to accommodate *A. caliginosa* and *A. terrestris* f. *longa*. In the *A. terrestris* f. *longa* and the *L. terrestris* vivaria subsidence took place leaving a gap between A and B. Worms of the species *A. terrestris* f. *longa* were frequently stretched across this air space with one end of their bodies in A and the other in B. Although there was no subsidence in the *A. caliginosa* vivarium the worms tended to congregate on the boundary between A and B, when in clay.

It will be seen from the table that, whereas *D. subrubicunda* and *L. rubellus* were entirely restricted to zone A, *A. caliginosa*, *A. chlorotica* and *A. terrestris* f. *longa* were found also in the water-saturated zones B and C, at least in certain soils. *L. terrestris* appears to avoid water strongly, for although a few specimens were found in zone C, all save two in this situation were attempting to escape up the side. In this species the diameter of the worms was only 1 mm. less than the distance between the plates, and their size relative to the apparatus was much greater than that for other species. Five specimens of *L. terrestris* meant considerable crowding, and might have resulted in the worms' occupying wetter parts than they would otherwise have done.

Table 4. *Analysis of results of choice experiments in vivaria*

Species	Soil	Distance between plates (in.)	% in A	% in B	% in C	% in A–B	% in water	% unaccounted for	Value of 100 (total worm-days)	No. of worms
A. caliginosa	Clay	1/4	30	30	0	40	0	0	20	5
	Leaf-mould +clay	3/8	95·7	0	0	0	0	4·3	70	5
A. chlorotica (garden specimens), series I and II	Clay I	3/8	49·4	11·3	8·5	0	0	30	176	8
	Clay II	1/8	66·6	13·3	3·3	0	0	16·8	60	5
	Leaf-mould +clay I	1/4	71·4	14·3	0	0	0	14·3	28	5→3
	Leaf-mould +clay II	1/8	97	3	0	0	0	0	34	4→3
	Sand I	1/4	77	0	3	0	0	20	70	5
	Sand II	1/8	88·6	0	0	0	11·4	0	53	5→4
A. terrestris f. *longa*	Clay	3/8	37	13	12·5	30	0	7·5	120	5
D. subrubicunda	Leaf-mould	1/4	86	0	0	0	0	14	100	5
L. rubellus	Leaf-mould	1/4	90·4	0	0	0	0	9·6	73	4→2
	Leaf-mould +clay	1/4	88·9	0	0	0	0	11·1	63	5→4
	Sand	1/4	87·5	0	0	0	12·5	0	64	5→4
	Clay	1/4	89·1	0	0	0	0	10·9	55	5
(acclimatized)	Leaf-mould	1/4	87·5	0	0	0	12·5	0	56	5→3
L. terrestris	Clay	3/8	76·6	2·6	0	0	11·7	9	77	4

Some specimens of *A. chlorotica* and *A. terrestris* f. *longa* in zone C constructed burrows which they occupied for several days, always keeping their posterior ends out in the water above the soil, often near the aeration jet.

However, even in these species which showed the greatest tolerance of water-saturated soil, the majority of the worms were seen in the air-containing zone.

Worms on moist blotting-paper

These experiments were designed to eliminate the complicating effects of soil. The bottom of a dish was covered with blotting-paper, water was added and the dish tilted so that half the bottom was covered by water and half by moist blotting-paper. The whole was covered by an acetate sheeting box and a black cloth (Fig. 3).

The species used were *A. chlorotica*—garden and Lake Windermere specimens—and *L. terrestris*. The latter were less crowded in these experiments than in those using soil. Five worms, which were placed at water-level, were used in each experiment, and observations were made each morning and evening. The results are shown in Table 5.

The results for the Windermere specimens of *A. chlorotica* were unexpected. Approximately the same number of worm-half-days were spent out of the water, in the water, and half in the water. It had been anticipated that the worms would enter and remain in the water. Most of the garden specimens of *A. chlorotica* left

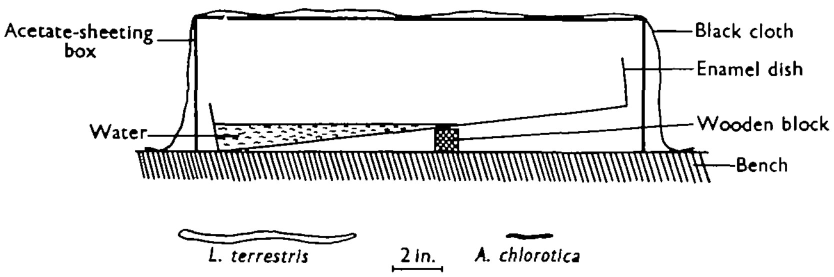

Fig. 3. Diagram to show arrangement of the covered enamel dishes. The size of the worms is shown relative to the apparatus.

Table 5. *Results of choice experiments in enamel dishes*

	In water	Out of water	Half in water
L. terrestris (tap water)	7	24	16
L. terrestris (filtered lake water)	1	24	20
A. chlorotica (from garden)	1	5	2
A. chlorotica (from Lake Windermere)	13	12	10
L. terrestris (not acclimatized)	11	17	21
L. terrestris (acclimatized)	26	6	9
A. chlorotica (from garden)	0	5	0
A. chlorotica (from garden, acclimatized)	41	0	6

Units are worm-half-days.

the dish and escaped from the apparatus after a short time. Those remaining appeared to avoid being submerged. It may be recalled that *A. chlorotica* avoided water in sand where no humus was present (Table 4).

An almost equal number of worm-half-days were recorded for *L. terrestris* out of the water, and half in the water. There was no strong avoidance of water under these conditions, as is shown by the number of records in, and half in the water.

Wolf (1938*a*), working with *L. terrestris*, found that while most worms avoided entering tap water, they were indifferent to natural water. The possibility that London tap water also elicited an avoiding reaction was tested by making a control experiment with filtered lake water. The results were not significantly different. These experiments confirm that both species show an aversion to water in the absence of soil.

Acclimatization

Experiments were made with *L. rubellus*, *A. chlorotica* (garden) and *L. terrestris* to find out whether worms which had been kept in tap water before being put into the apparatus showed different behaviour from those which had not been acclimatized.

Five individuals of *L. rubellus* were kept in aerated tap water for 20 days before being placed in a vivarium set up with leaf-mould. All the worms, excepting two which died, behaved exactly like the unacclimatized worms (Table 4).

The experiments with *A. chlorotica* (garden) and *L. terrestris* were made using the type of apparatus of Fig. 3. Five worms of each species were kept in aerated tap water for 8 days before being placed at water level. The non-acclimatized worms were kept in pots of moist soil for a similar period. Windermere specimens of *A. chlorotica* were kept in a large tank of water in the soil. As may be seen from Table 5, acclimatization has a marked effect on these two species, the majority of the worms entering the water.

VII. DISCUSSION

A. chlorotica and *L. terrestris* are able to withstand considerable water losses, and although there is no marked difference between them, *A. chlorotica* is able to survive a slightly greater loss (75 % of the body water) than *L. terrestris* (70 %). The reverse might have been expected as *L. terrestris* is found in drier habitats than is usual for *A. chlorotica*, although it is occasionally found in very dry areas. The ability of *A. chlorotica* to survive considerable desiccation is a factor in its ability to withstand large fluctuations in the humidity of its habitat, for although the worms undergo a facultative diapause they do so in the top 6 in. of soil, not in the moist subsoil (Dr W. J. McL. Guild, personal communication), and loss of water is inevitable. The cilia of *L. terrestris* are more resistant to hypertonic media than those of *A. chlorotica* (Roots, 1956). Thus there is no correspondence between the ability of the cilia to function in hypertonic media and that of the whole worm to survive desiccation, indeed there may well be an inverse correlation.

The spreading of earthworms into new areas, by their own activity, is limited. Their maximum rate of locomotion is about 0·4 m./min., and active wandering over the surface is limited in most species (Stephenson, 1930). Earthworms are in most cases carried to new areas accidentally and small forms are more easily transferred than large ones. The distribution of a species thus depends mainly on its size and on its ability to establish itself in the new region. In the case of accidental transference to a wet or limnic locality a species which shows some tolerance of water will burrow before it can be caught by predators or killed by ultra-violet light (see below). This accounts for the frequent occurrence of *A. chlorotica* in limnic localities. It is a small worm which has been shown experimentally to have a tolerance of water and to construct burrows under water readily. Although *A. terrestris* f. *longa* shows about the same degree of tolerance of water it is only occasionally found in limnic localities. It is, however, a much larger species and therefore less likely to be carried accidentally. *A. caliginosa*, a fairly small worm, shows some tolerance of water and is sometimes found in limnic localities. Occasional records, in limnic localities, of species showing a strong avoidance of water in the behaviour experiments, e.g. *D. subrubicunda*, may be due to survival followed by acclimatization. The two terrestrial species investigated, *L. terrestris* and *L. rubellus*, both show marked avoidance of water, and although there is evidence that the former at least can be acclimatized by immersion they do not burrow when forced to live under water.

In his paper recording the occurrence of *A. chlorotica* in Lake Windermere Černosvitov (1945) remarked '...it remains to be proved by experiment whether *A. chlorotica* of Lake Windermere constitute a biological race'. No differences in the behaviour of garden and Lake Windermere specimens have been detected. Garden specimens show some tolerance of water and make burrows under water, and those from Lake Windermere do not remain exclusively in water when given a choice. Individuals from both habitats project their posterior ends from their underwater burrows. The cocoons of garden specimens of *A. chlorotica* develop under water, and produce young worms which feed and grow under water. It has already been shown that there is no physiological difference between the nephridiostome cilia of the two groups (Roots, 1956). Thus, although cocoon production under water has not been observed, it seems unnecessary to postulate that the Lake Windermere individuals constitute a 'biological race' in order to explain the occurrence of the species in that lake.

Although earthworms are able to live in water, many are found on the surface of the soil and in puddles either dead or dying after heavy rain, and this has never been satisfactorily explained. Merker (1925, 1926, 1928) and Merker & Bräunig (1927) have shown that the cause of death is ultra-violet light, but the problem of why the worms leave their burrows remains. I found that the majority of the worms investigated (including those occurring in limnic localities) avoided water when allowed a choice. Although other factors, such as oxygen lack, may contribute, this water-avoiding reaction alone would explain the worms' behaviour after heavy rain.

VIII. SUMMARY

1. The water content of *Lumbricus terrestris*, after keeping on moist filter-paper for 3 or 4 days, is 84·8 % of its body weight. That of *Allolobophora chlorotica* is 80 % of its body weight. Both species can survive a loss of 60 % of the body weight, but not much more.

2. Earthworms of the species *A. chlorotica*, *A. terrestris* f. *longa*, *Dendrobaena subrubicunda*, *L. rubellus* and *L. terrestris* are all able to survive from 31 to 50 weeks in soil totally submerged beneath aerated water. The same species, and *A. caliginosa* can survive for 72–137 days in aerated tap water without food.

3. Garden specimens of *A. chlorotica* make U-shaped burrows in soil beneath water. They do not irrigate either the burrows or glass tubes. Egg-cocoons of *A. chlorotica*, taken from culture pots of soil, will hatch under water and the young worms will feed and grow though totally immersed.

4. *D. subrubicunda*, *L. rubellus and L. terrestris* avoid immersion in water when allowed a choice between water-saturated and moist but air-filled soil. When *A. caliginosa*, *A. chlorotica* and *A. terrestris* f. *longa* are allowed a similar choice, a small proportion of the population is always found in the water-filled soil.

5. Enforced submergence before being allowed a choice of environment does not affect the behaviour of *L. rubellus*, i.e. water is still avoided. Similar treatment of *A. chlorotica* and *L. terrestris* results in a more frequent choice of water.

6. It is unnecessary to postulate that the individuals of *A. chlorotica* occurring in Lake Windermere constitute a 'biological race'.

7. The avoiding reaction of earthworms to immersion may explain why they leave the soil after heavy rain.

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