DEFAECATION IN RELATION TO THE SPONTANEOUS ACTIVITY CYCLES OF *ARENICOLA MARINA* L.

By G. P. Wells
Department of Zoology, University College, London

(Text-figs. 1–6)

Four years ago, I showed that the behaviour of lugworms in sand could be studied by recording the water movements through their burrows, and that their activities are patterned in time, to a large extent, by inherent rhythms (Wells, 1949*a*, *b*). The following note describes a new series of experiments, in consequence of which the conclusions of the earlier papers are extended and modified in detail.

The work was done at Plymouth in July, August and September 1952. The expenses were in part defrayed by a grant from the Central Research Fund of the University of London.

Methods

Three types of apparatus were used (Fig. 1).

Type I, suitable only for the smallest worms, consists of a glass U-tube (internal diameter 25 mm.) with arms of unequal length and a short side-tube (internal diameter 5 mm.) on the longer arm. Capillary C (internal diameter about 1 mm.) is connected by rubber tubing to the side-tube. The apparatus is immersed in a tank of sea water to the level L and partly filled with muddy sand (a mixture of clean sand with a black muddy clay from Millbrook) to the height shown by stippling in the drawing. Sea water flows continually into the tank and the level L is held constant by an overflow. If a healthy worm of suitable size is allowed to enter the sand, it makes a burrow with one end in each limb of the U-tube. Such water currents as it sets up must pass through capillary C, and, because of the slight resistance of the capillary, will cause changes of water-level in the long limb of the U-tube. These changes are recorded by the following means on a slowly moving kymograph (a Casella thermo-barograph clock turning once a day). A Palmer gimbal lever (amplification ×6) is connected by means of a thread and a blob of sealing-wax type adhesive to a circular cover-glass as used for microscope preparations (G). The cover-glass has a film of vaseline on the upper surface but is clean on the lower; it clings to the water surface, and is a great improvement on the paraffin floats used in my earlier work.

Types II and III differ from each other only in dimensions. Each consists of two rectangular glass plates held 22–24 mm. apart by a plasticene rim and centre-piece (cross-hatched in the drawing). The space between the plates is

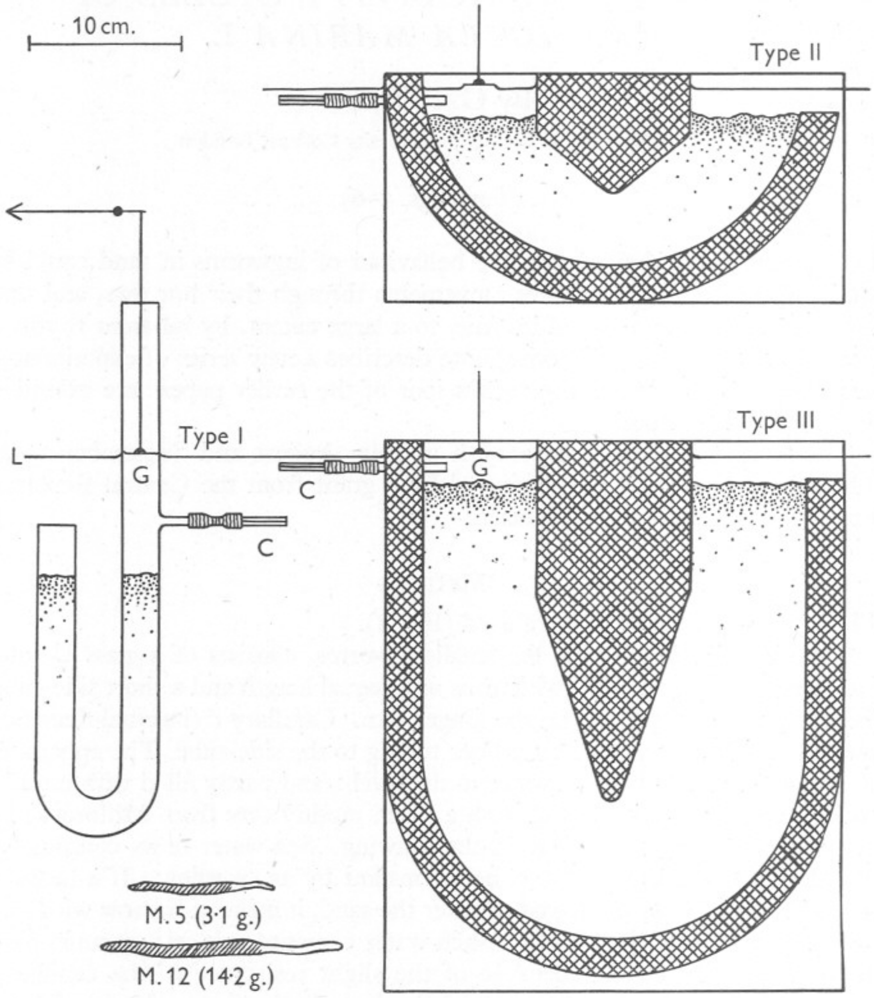

Fig. 1. Drawings of the three types of apparatus used. Outlines of two of the worms are included, to the same scale, in the lower left-hand corner.

filled with muddy sand to a maximum depth of about 100 mm. in Type II and 300 mm. in Type III. The worms burrow in the sand, and their water currents are recorded by the system already described.

The experiments were set up in groups of four. Fourteen individuals were used, the first two for 3 days only, while the apparatus was being tested and adjusted, and the remaining twelve for from 7 to 18 days. The temperature ranged from 16·1 to 17·9° C.

The worms are generally invisible in the sand, and defaecation is almost the only activity that can be directly observed.[1] The following steps were taken to correlate the timing of defaecation with the records of water movements. (i) Two or three times a day the experiments were examined, the presence or absence of faeces was noted, and any faeces seen were destroyed with a glass rod. (ii) On several occasions the experiments were kept under continuous observation for 4–6 hr., and the time of each defaecation was marked on the tracing and noted down to the nearest minute.

Results

The worms often showed the characteristic behaviour pattern described in the following citations:

> Whenever the worms were feeding from a gradually subsiding cone and piling up castings, as they do in the field, a characteristic cyclical pattern was traced. This was marked by conspicuous diphasic excursions at intervals of about 40 min. Defaecation occurs at the summit of the first phase. By comparison with records got from worms in glass tubes, it is inferred that the first phase consists of tailward locomotion to the sand surface, and the second to headward irrigation accompanied by gentle creeping back to the feeding point. (Wells, 1949*b*, p. 477.)
>
> The pattern just described invariably accompanied feeding and defaecation. (Wells, 1949*b*, p. 471.)

These statements must be modified in two respects in the light of the new results. Firstly, the interval given as 'about 40 min.' varies considerably, one of the determining factors being the size of the worm; and secondly, defaecation may occur when the pattern is not discernible on the tracings.

Three examples of the typical pattern, taken from the new series of experiments, are seen in Fig. 2. The position of the base level (i.e. the point at which the lever settles when the capillary is removed) shows that the pattern is traced against a background of headward irrigation. At each of the conspicuous 'needles' on the tracings, the writing-point first moves towards the zero mark, as the worm ceases to irrigate and creeps backward to the surface of the sand. Then there is a very sudden further movement of the lever in the same direction (generally crossing the zero line) as a faecal cylinder shoots out. The act of expulsion takes about half a second, and the almost violent movement of the lever suggests that a powerful, piston-like swelling runs backward along the worm's body at this moment.[2] Immediately

[1] One occasionally sees the tail-tip appear at the caudal end of the burrow, move around a little as if clearing the opening, and then disappear again.

[2] Once during the earlier series of experiments I noted that the ejection of a cylinder was accompanied by a 'violent down-suck of water into the head shaft'. The fact that each 'needle' consists of two distinct parts is clearly shown in Fig. 3 of Wells 1949*b*.

after defaecation, the lever moves towards its original position as the worm creeps down to the bottom of its burrow and begins to irrigate once more. The second phase of the diphasic cycle, consisting of unusually vigorous headward irrigation, follows at once; but the extent to which this second peak stands out from the general irrigation background is variable. Sometimes, as towards the end of Fig. 2C, it is barely discernible, and it may not emerge at all.

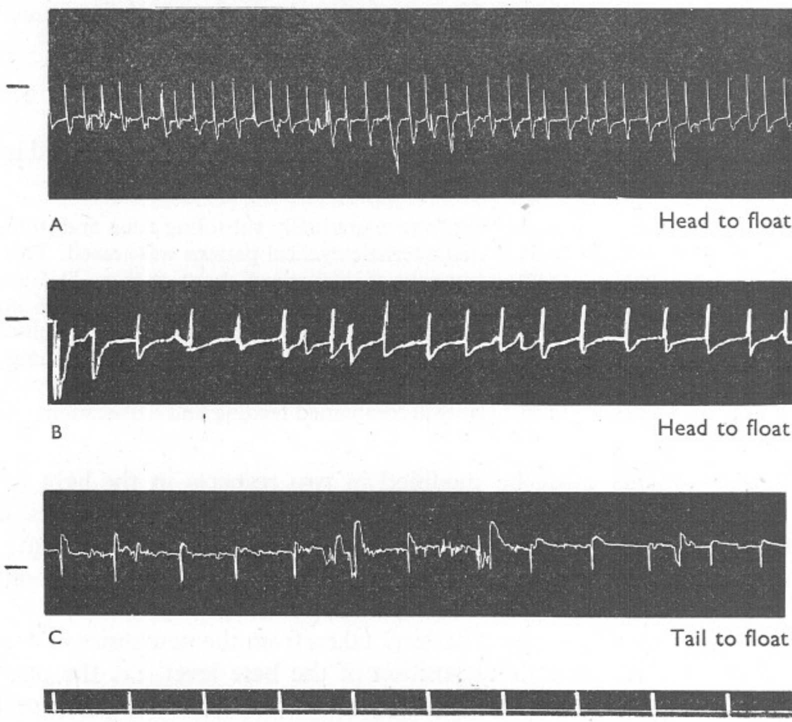

Fig. 2. Ten-hour records of frequently-defaecating worms.

A: worm M5 (3·1 g.). Apparatus Type I. Regular series of defaecations at average interval of 15 min. 20 sec.

B: worm M14 (10·1 g.). Apparatus Type III. Defaecations at average interval of 34 min. 20 sec.; occasional irregularities of the background irrigation between them.

C: worm M13 (12·1 g.). Apparatus Type III. Defaecations at intervals of about 45 min., with a rather irregular period in the middle of the record.

In all tracings: read from left to right; time signal marks once an hour; the mark to the left of each tracing is the zero-flow base level. The orientation of the worm relative to the float is printed under each extract. Wider capillaries were used for the larger worms, so a given lever excursion does not always correspond to the same flow rate.

The effect of size on the timing of the pattern is evident in Fig. 2. The experiments of 1948/9 were done on 'large' worms, unfortunately not weighed. The worms of the 1952 series ranged in weight from 2·6 to 18·6 g.

When defaecating regularly, the larger worms did so at intervals of 'about 40 min.', but the smaller gave much shorter intervals (15–20 min. for worms weighing about 3 g.).

Other types of record may be traced. In the 1948/9 experiments, the pattern of Fig. 2 appeared, accompanied by regular feeding and defaecation, during 45% of the total recorded time. The remaining records were grouped as follows: (i) cycles rather similar to those of Fig. 2 but without defaecation; (ii) periods of complete quiescence, which might last for many hours on end; (iii) periods of unexplained activity, the tracings of which were described as 'confused'. The records of the 1952 series include all of these types, and they also show a gradation of intermediate stages between the typical pattern of Fig. 2 and tracings which must be put in the 'unexplained' or 'confused' category. On several occasions, the worms defaecated frequently and quite regularly while 'confused' records were being written. The new results are reviewed in detail in the following paragraphs. Taken together, they suggest that the worms tend to settle into the pattern of Fig. 2 when conditions are favourable, and that limitation of space in the apparatus promotes 'confusion'.

Disregarding the preliminary tests, the 1952 material includes three sets of four worms each, those of the first set being the smallest and those of the third the largest.

Set I. Worms M3–6. (2·6–3·2 *g*)

These worms were studied in Type I apparatus. They burrowed vigorously and swiftly into the sand when placed on its surface, and their behaviour was recorded for 7 days. Extracts from the records are printed in Figs. 2A, 3 and 6C, D. The individual worms performed as follows.

M3 gave a few long spells of regular defaecation with needles like those of the first half of Fig. 3B—i.e. lacking a prominent second phase—and a few short spells (2–3 hr.) resembling Fig. 3A. Most of its tracings, however, were confused, with the defaecations few and irregularly scattered; there were several spells with pauses followed by defaecation, much as in Fig. 3C.

M4 was a consistently frequent defaecator. There were occasional short spells of irregularity, but most of its tracings were regular, like the examples in Figs. 3A and 6D. The second phase however, was not always distinct from the background irrigation.

M5 defaecated steadily for 5 days with only short spells of irregularity, its cycles being like those of Figs. 2A and 6C on some days but without a distinct second phase on others.[1] On the last 2 days the irregular periods increased in duration while the defaecation spells shortened correspondingly and finally

[1] On the 5th day, when extract 2A was taken, the worm moved nearly 10 c.c. of sand from one end of the tube to the other in 24 hr. As it defaecated about 100 times, this means a transport of 0·1 c.c. per defaecation. Weight of worm 3·1 g.; approximate length 9 cm. of which 2 cm. was tail.

ceased; and various confused patterns, including records resembling Fig. 3C, were traced.

M6 defaecated fairly frequently on most days, but gave confused records, including numerous spells of the type shown in Fig. 3C.

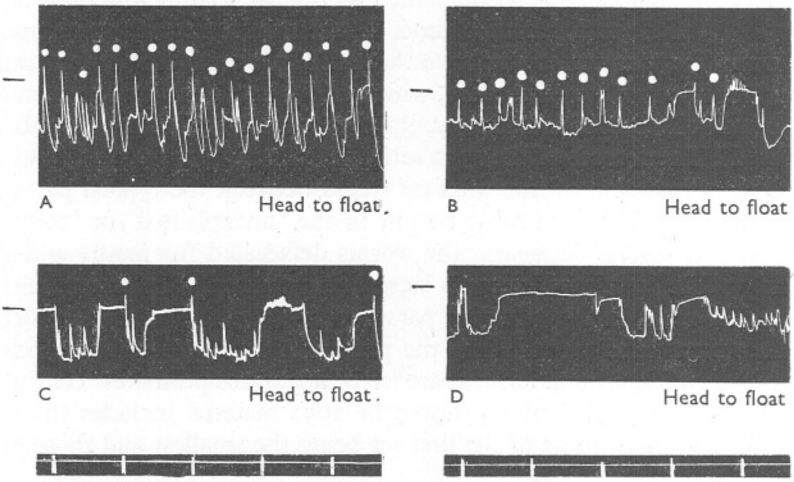

Fig. 3. Five-hour records traced by small worms in apparatus Type I. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M4 (2·6 g.). Intervals between defaecations 15, 16, 16, 15, 15, 16, 16, 18, 16, 17, 15, 18, 17, 18, 17, 18, 15 min. The record shows a pattern like that of Fig. 2A but against a more fluctuating background. There is a close correspondence between defaecation and the first peak of the diphasic excursion except that the 3rd defaecation is atypically placed; it occurs on a sharp needle sandwiched between two blunter ones.

B: worm M3 (3·0 g.). Intervals between defaecations 19, 15, 19, 14, 23, 17, 16, 16, 26, 38, 16 min. The defaecations occur on well-marked needles but there is no prominent second phase; the whole record becomes confused towards the end and defaecation ceases.

C: worm M6 (3·2 g.). Intervals between defaecations 58, 158 min. The record shows fluctuating activity alternating with periods of almost or quite complete quiescence; defaecation sometimes occurs as the worm 'wakes up' after a pause.

D: worm M3 (3·0 g.). A confused tracing with no defaecations.

Although these worms could burrow rapidly in the Type I apparatus, and often showed regularly spaced defaecations associated with typical diphasic excursions on the irrigation tracing, the results of Set II suggest that the rigid walls of the U-tubes may have been somewhat restrictive even for the small worms of Set I.

Set II. Worms M7–10 (7·0–8·0 g.)

This experiment lasted for a week. The worms were first put up in Type I apparatus, and transferred to Type II after 2 days (M7, 8) or 5 days (M9, 10).

When put into Type I, the worms began at once to make strenuous burrowing movements, but they had obvious difficulty in entering the sand.

This was doubtless because burrowing is done in the main by thrusting the sand aside, and only a small worm has room enough to do this in the U-tube. The worms of Set II could work their anterior segments into the sand, but they generally remained with the tail, or the tail and several branchiate segments, exposed at the surface. Only M 10 was seen to get itself com-

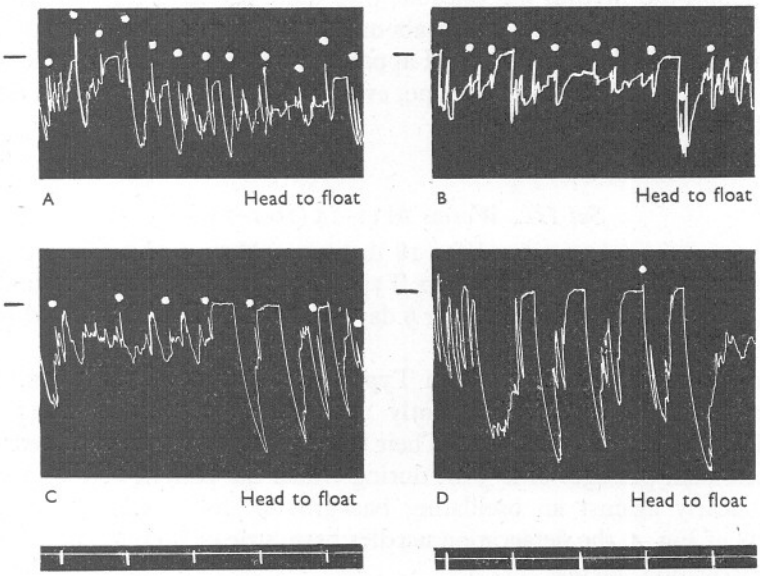

Fig. 4. Five-hour records traced by large worms in apparatus Type II. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M 7 (7·3 g.). Intervals between defaecations 26, 23, 22, 23, 24, 25, 19, 30, 31, 24, 23 min. Base level drops suddenly about $1\frac{1}{2}$ hr. after start of extract, due to a change in rate of the sea-water supply to the tank in which the apparatus stood. Several of the defaecations are associated with diphasic excursions of the typical form, but many are not.

B: worm M 14 (10·1 g.). Intervals between defaecations 29, 18, 19, 19, 19, 35, 17, 36, 27, 23 min. A confused tracing, the defaecation needles bearing no relation to the general irrigation pattern.

C: worm M 12 (14·2 g.). Intervals between defaecations 58, 43, 37, 41, 56, 36 min. There is again no relation between the defaecation needles and the general irrigation pattern.

D: worm M 7 (7·3 g.). A confused record, with one defaecation only. The pattern resembles that of Fig. 3 C.

pletely into the sand; this happened on two occasions, but each time its tail soon appeared again. Nevertheless, they all defaecated repeatedly and must therefore have fed. The tracings were of small amplitude, probably because part of the irrigation water returned along the limb of the U-tube where the worms were lying, and they were very confused in pattern.

When transferred to Type II, the worms burrowed rapidly out of sight. Only M7 defaecated frequently under the new conditions, and its records were never of the simple cyclic pattern of Fig. 2. Extracts are given in Figs. 4A, D and 6B. The other worms defaecated rather seldom and gave confused tracings, e.g. Fig. 6A. Short spells of the curious rapidly-swinging rhythm seen between the reversals in Fig. 6A were shown by all these three worms, and the rhythm was once kept up quite regularly for about 6 hr. by M9. The worms of Set II were about 15 cm. long at full elongation, and the space available in the Type II apparatus was insufficient for the setting up of a burrow of the normal shape, even though they were able to descend rapidly into the sand.

Set III. Worms M11–14 (10·1–18·6 g.)

This experiment continued for 18 days, but M11 died on the 12th day. The worms were first put up in Type II apparatus, and transferred to Type III after 4 days (M11, 12) or 9 days (M13, 14). They burrowed rapidly down in both types.

The behaviour of the worms in Type II is illustrated by Fig. 4B, C. All of the worms defaecated frequently under these conditions, M13 being especially regular and consistent. Their tracings, however, are confused; there are occasional passages of 2–3 hr. during which the pattern of Fig. 2 is seen fairly clearly against an oscillating background, but generally, as in the extracts of Fig. 4, the defaecation needles have little or no relation to the rest of the irrigation tracing.

The behaviour in Type III is shown in Figs. 2B, C and 5. The case of M11 is exceptional. This worm became quiet after burrowing down in Type III and registered practically no water currents for 3 days; then it gave a rhythmic pattern without defaecation for about 12 hr. (Fig. 5C); then it suddenly became very active, tracing a graph like the usual recovery after a period of oxygen lack (as Wells, 1949*a*, fig. 6) followed by some hours of vigorous but confused activity; then its irrigation became less and less vigorous, until it died on the 12th day. The remaining worms often gave confused spells, sometimes with occasional defaecations. They also traced many long periods in which the defaecations came regularly and were associated with diphasic excursions of the usual kind (Fig. 2B, C), though this was sometimes written against a rather fluctuating background (Fig. 5B). A comparison of the records before and after the transfer leaves little room for doubt that the more spacious conditions of Type III favour the appearance of the regular pattern of Fig. 2.

Three rest periods, of 1–1½ days' duration, during which there was little or no irrigation, were shown by M12; these alternated with active periods. The pattern traced by M13 in Fig. 5A was not seen in any other record. It was presumably caused by a regular periodic reversal of the direction of travel of the irrigation waves along the worm's body.

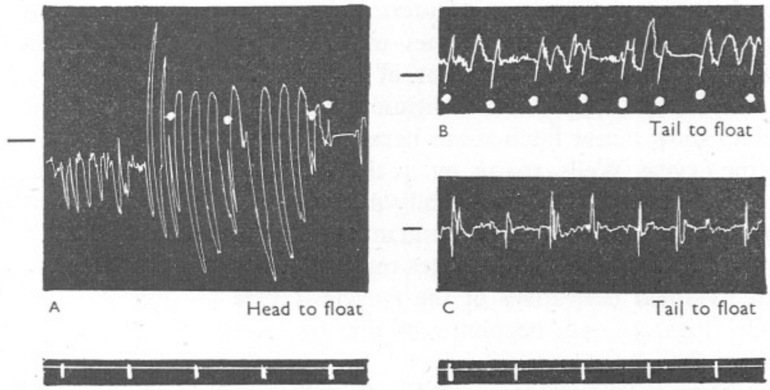

Fig. 5. Five-hour records traced by large worms in apparatus Type III. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M13 (12·8 g.). Intervals between defaecations 52, 74, 18 min. The record shows an unique irrigation pattern, with periodic reversal of the direction of flow.

B: worm M14 (10·1 g.). Intervals between defaecations 43, 39, 42, 36, 33, 37, 46 min. The defaecations occur on diphasic excursions of the typical form, against a fluctuating background.

C: worm M11 (18·6 g.). The record shows periodic outbursts of irrigation without defaecation.

Discussion

The relation between the irrigation patterns and defaecation may be summarized as follows.

Fasting worms in glass tubes often settle down, for many hours at a stretch, to a pattern consisting of periods of rest alternating with outbursts of activity (the *i–d* cycles, in the terminology of Wells & Albrecht, 1951). Each outburst typically consists of three phases—backward locomotion, headward irrigation with headward locomotion, tailward irrigation—but their form is variable in detail; the third phase, in particular, is often dropped out altogether. They sometimes appear with almost clockwork regularity, but their timing can vary, either after a period of oxygen lack or without obvious external cause. They apparently depend on a spontaneously discharging pacemaker whose activity can be controlled according to the circumstances, much as the beat of a vertebrate heart is spontaneous although its pattern and frequency can be regulated from without (Wells, 1949*a*; Wells & Albrecht, 1951).

Worms in sand, feeding and defaecating regularly, often show essentially similar cycles, except that they now appear against a continuous but often rather fluctuating background of headward irrigation (Fig. 2). Sometimes the worms in sand show a similar pattern when they are not passing food through the gut; and then, as in glass tubes, they generally rest between the outbursts (Figs. 5C, 6B).

Other spontaneous components may enter into the lugworm's behaviour. A second characteristic pattern of intermittent rhythmic activity originates in the oesophageal wall; it determines movements of the anterior end and proboscis accompanied by inhibition of the irrigation current (the *f* cycle of Wells & Albrecht, 1951). The irrigation records got from worms in sand sometimes show minor fluctuations between the *i–d* outbursts which may be due to the *f* cycle (Wells, 1949*b*, fig. 4; this paper, Fig. 2C). Additional types of periodic behaviour are occasionally indicated (Fig. 5A), but many of the tracings are confused, both in sand and in glass tubes, and it is often impossible to decide whether particular passages represent the appearance of something new, or modified derivatives of the *f* cycle (centre portion of Fig. 6A) or *i–d* cycle (Figs. 3C, 4D, beginning of Fig. 6A; compare Wells & Albrecht, 1951, Fig. 4).

Defaecation is frequently coupled with great regularity to the *i–d* cycles, with one defaecation on the first phase of each outburst (Fig. 2). In favourable circumstances, and given plenty of space, the worms tend to settle into this routine for many hours on end, and there is little doubt that they spend much of their time in this way in the field. However, as we have seen, it is not a necessary condition for the appearance of the *i–d* cycles that the worms should feed and defaecate; neither is it a necessary condition for defaecation that a regular irrigation pattern of this type should be traced.

The advantages of basing the organization in time of the lugworm's activities on spontaneous pacemaker systems, rather than on *ad hoc* reflexes to its various needs as they arise, were discussed elsewhere (Wells, 1949*a*, *b*). The *i–d* cycle is well suited to serve as a means of integration when the worm is living in an established burrow of the typical form. It brings the worm alternately to the place of feeding and the place of defaecation; it can be modified according to the composition of the water and the worm's respiratory needs; under low-tide conditions, it can be adapted for aerial respiration. Some method of coupling defaecation to the *i–d* cycle is therefore to be expected. On the other hand, the coupling should not be too rigid, for circumstances might arise in which the *i–d* pattern would be inappropriate. The worms of Set II fed and defaecated regularly in the Type I apparatus though unable to burrow wholly into the sand; this indicates a flexibility of behaviour which may sometimes be of service under natural conditions.

The mechanism of coupling appears to involve more than one factor. It is suggestive that defaecation often occurs as the worms 'wake up' suddenly and begin to irrigate in a headward direction after a period of rest (Figs. 3C, 4D). Similarly, in a typical *i–d* outburst, it is at the momentary pause at the top of the first phase, just before the onset of headward irrigation, that defaecation takes place (Fig. 2). The headward irrigation cannot be regarded as a conse-

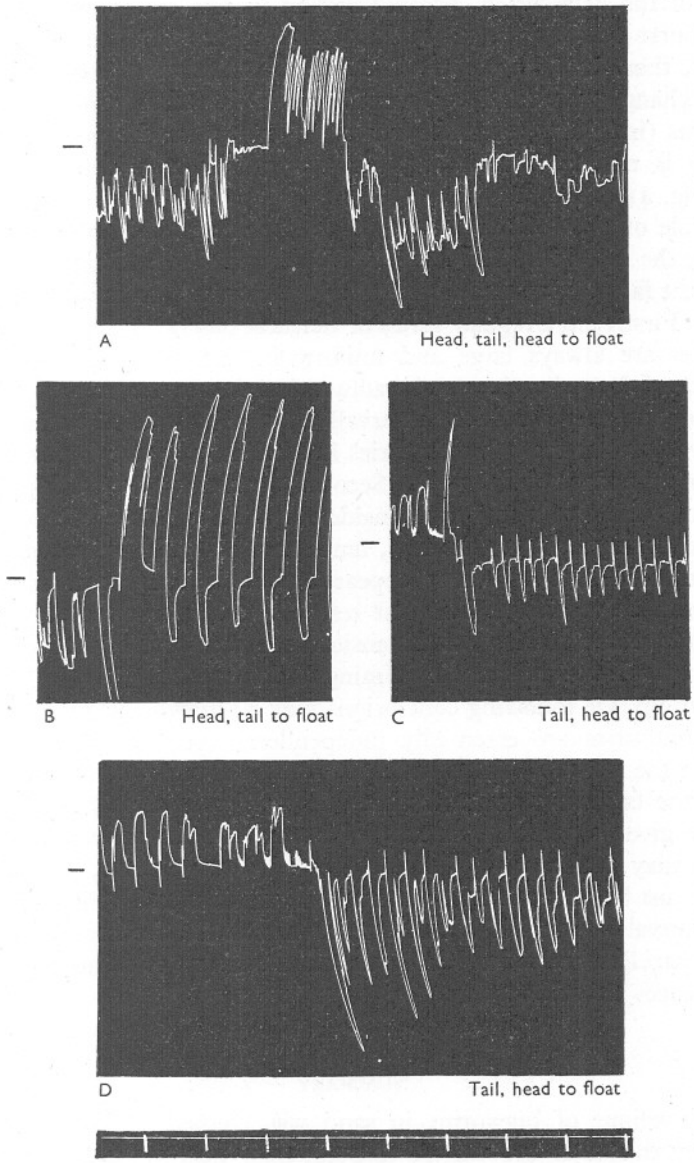

Fig. 6. Five- and nine-hour records to show reversal of the worms in their burrows.

A: worm M8 (8·0 g.). Apparatus Type II. A very confused tracing, with no defaecations. The worm reverses itself twice, with about $1\frac{1}{4}$ hr. between reversals.

B: worm M7 (7·3 g.). Apparatus Type II. Reversal is followed by a regularly spaced series of irrigation outbursts without defaecation.

C: worm M5 (3·1 g.). Apparatus Type I. Reversal is followed by a regular series of defaecations with diphasic excursions of the usual form.

D: worm M4 (2·6 g.). Apparatus Type I. Defaecation occurs four times at the beginning, and frequently after reversal.

quence of the defaecation, because resting worms can 'wake up', and the *i–d* outburst can assume its typical form, in the absence of defaecation. Possibly, therefore, the onset of headward irrigation is preceded by some internal change which acts as a stimulus for defaecation, provided that other conditions (including a certain loading of faeces in the gut) are satisfied. But this is not the whole story, for the worms may defaecate at other times (Fig. 4B, C).

The role of rectal distension could perhaps be studied by collecting and weighing the individual faecal cylinders. Rough observations on the dimensions of the faeces were made during the present work, from which two points emerge. Firstly, in a regular series of defaecations (as in Fig. 2 or Fig. 3A), the faeces are always large and uniform in size. This suggests that the frequency of the *i–d* cycles can be adjusted to fit in with the rate of feeding, and the slight variations in the intervals between successive outbursts which appear even in the most regular series in sand may be due to this cause (see details in legends to Figs. 3–5). Secondly, when faeces are less regularly produced, their size may vary considerably. Thus, three of the cylinders produced during Fig. 3B (the first, tenth and eleventh) were conspicuously smaller than the rest, and these appeared after the longest intervals (27, 26 and 38 min. respectively). The last result is rather unexpected, and shows that factors other than rectal distension and a particular inflexion of the irrigation graph can influence the timing of defaecation.

To sum up, the following conclusions may be drawn. (i) Defaecation and the *i–d* outbursts are essentially independent, cyclically repeated events. (ii) When the worm is living under favourable conditions in an established burrow, the familiar pattern in which defaecation is closely coupled to the *i–d* cycle gives a workable means of integrating its activities. (iii) The coupling may perhaps be brought about by two factors, a tendency to defaecate just before the onset of headward irrigation and a 'fine adjustment' of the intervals between *i–d* outbursts to match the feeding rate. (iv) The factors controlling irrigation behaviour and the timing of defaecation in other circumstances are still obscure.

Summary

The behaviour of lugworms in sand was studied by recording kymographically the water movements through their burrows and observing the timing of defaecation. The worms ranged in weight from 2·6 to 18·6 g. Three types of apparatus were used, differing in the amount of space allowed to the worms.

The worms often traced a characteristic pattern described elsewhere, consisting of regularly spaced diphasic excursions of the irrigation graph with a defaecation on the apex of each first phase. The interval between

excursions was about 15–20 min. for worms weighing about 3 g. and increased with size to about 40 min.

The worms sometimes traced similar periodic bursts of irrigation activity without defaecation.

Many of the tracings were confused in pattern. The worms often defaecated—sometimes sporadically, sometimes at regular intervals—in the absence of the characteristic diphasic excursions. Restriction of a worm to a small space appears to favour the production of confused tracings.

The relation between the irrigation patterns and the timing of defaecation is discussed.

REFERENCES

WELLS, G. P., 1949*a*. Respiratory movements of *Arenicola marina* L.: intermittent irrigation of the tube, and intermittent aerial respiration. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 447–64.

—— 1949*b*. The behaviour of *Arenicola marina* L. in sand, and the role of spontaneous activity cycles. *Journ. Mar. Biol. Assoc.*, Vol. 28, pp. 465–78.

WELLS, G. P. & ALBRECHT, E. B., 1951. The integration of activity cycles in the behaviour of *Arenicola marina* L. *Journ. exp. Biol.*, Vol. 28, pp. 41–50.