THE DIURNAL RHYTHM OF ACTIVITY OF MAYFLY NYMPHS

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Moon (1940) has shown that nymphs of *Ecdyonurus* and *Baetis* move more during the night than the day under natural conditions, and he conjectured some indirect relationship between the stage of development and activity. This investigation has been undertaken as an attempt to discover the range of the diurnal periodicity of movement and the conditioning factors.

METHOD

Diurnal rhythm has been investigated in the movements of three species of mayfly nymphs: *Ecdyonurus torrentis* Kimmins, *Heptagenia lateralis* (Curtis), and *Baetis rhodani* (Pictet).

The simplest method of investigating activity has been used; in all but one experiment the animals were observed for periods of 10 min. at hourly intervals over the 24 hr.; each 10 min. period was divided into 300 sub-periods of 2 sec., and the number of animals seen to move in each sub-period was recorded. For reference purposes the movement of one animal in a 2 sec. period is termed an activity unit. In order to eliminate the systematic effect of any errors in observation due to fatigue of the observer (e.g. in the late evening) each 24 hr. series of observations was begun at a different time of day; and from one series to another the 10 min. period was chosen so as to cover a different part of the hour (e.g. from 10 to 20 min. past the hour, from half past to 20 min. to the hour, etc.), so that as far as possible there should be no period of the 24 hr. at which the animals had not been observed. Each experiment, involving a 24 hr. period of observation, was repeated five times under each set of conditions, unless otherwise stated, and each graph represents the activity during each hour calculated as the average of these five series.

Five animals were placed in a $6\frac{1}{2} \times 4\frac{1}{2}$ in. dish containing stones and water from the stream. The nymphs in two dishes only were observed in each 24 hr. period to allow some relaxation for the observer. Two sources of light were used, daylight and light from a 70 c.p. lamp giving an intensity of 200 f.c. measured by an 'Avo' light meter.

In order that the activity could be measured during the night the nymphs were painted on the thorax with a spot of white paint which was coated with a spot of

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luminous paint. The effect of the luminous paint on the nymphs was tested by comparing activity counts of control groups in very dim light, counts of the same insects before and after painting, and, to check any effect on other insects, counts of nymphs placed in dishes with small luminous stones. These experiments showed that the luminous paint did not appear to affect the activity of the nymphs.

During experiments carried out in the dark the stopwatch and record sheets were kept in a light-tight box containing a dim light, with a sunken slit in the top through which the stopwatch could be seen. The end of the box had been removed and was covered with a black cloth which allowed the entry of the hand, without the light being shown outside. Temperatures were taken in a control dish separated from the observation dish by a screen; readings of temperature during the day showed that there was no difference between the two dishes. The whole apparatus was placed on a table in front of an open window in a room in which there was no artificial lighting or heating except for the source of light aforementioned. In the later experiments a metronome was used instead of a stopwatch so that the attention of the observer was in no way distracted from the nymphs.

Except where otherwise stated, the nymphs were brought direct from the stream, and the experiments carried out immediately on arrival at the laboratory.

(a) *Natural daylight and darkness*

The first counts of activity of *Ecdyonurus torrentis* were carried out at half hourly intervals over 24 hr. in order that detailed observations could be made. Thereafter counts were only taken every hour over the 24 hr. periods, and comparison of the results showed this small number of readings to be adequate.

Five series of 24 hr. readings were taken in February, May and November; the animals being, respectively, May–March Stage XV, March–June Stage XV, and June–May Stage X nymphs (Harker, 1952). Fig. 1 shows the results of the activity counts of these five series.

Peaks of activity occurred at definite intervals, appearing consistently at 03.00–04.00, 05.00–07.00, 11.00–12.00, 16.00–17.00, 20.00–21.00, and 23.00–01.00 hr. The average length of each period of activity was 2 hr. Activity reached its peak rapidly, usually within an hour, and fell off slowly over a period of 2 or even 3 hr. The total activity recorded during the 10 min. periods over 24 hr. was 164 units in May, as compared with 112 and 116 units in February and November respectively.

These results indicate that the degree of activity varies according to the stage or brood of the nymphs. The variation in temperature in the 3 months suggests a connexion between temperature and activity. The fact that active periods were more frequent between 19.00 and 07.00 hr. than between 07.00 and 19.00 hr. suggests that variation in natural daylight is connected with the rhythm of activity. The times of the active periods were constant throughout the year, which further suggests that the connexion between light and the rhythm is not a direct one.

Further experiments were carried out in an attempt to correlate the rhythm with light variations, and with temperature variations which follow those of light.

(b) Continuous light or darkness

Observations were made in continuous light of animals taken directly from the stream, and of animals which had been kept in continuous light for 3 months. As animals kept in continuous darkness for long periods are difficult to feed, observations were made on nymphs only recently subjected to continuous darkness. The nymphs for these experiments were kept in a room in which the temperature was kept as close as possible to the outdoor temperature.

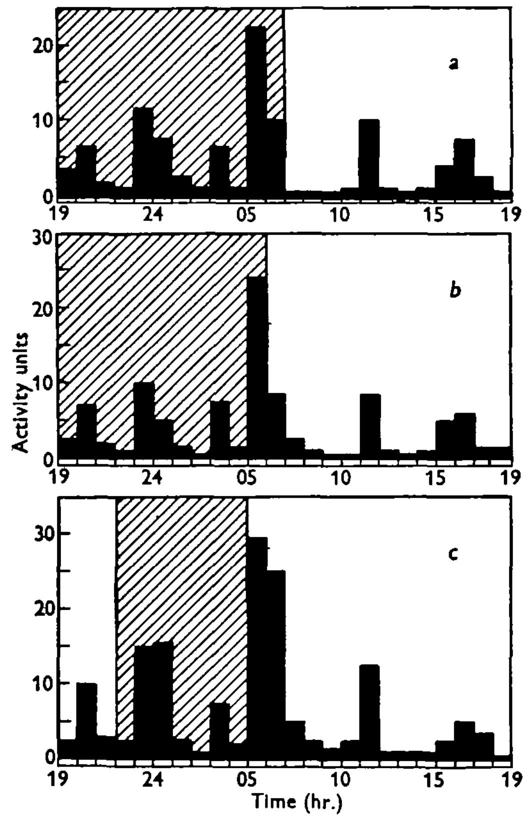

Fig. 1. The results of five series of activity counts of *Ecdyonurus torrentis* in: *a*, February; *b*, November; *c*, May. Hatched areas represent dark periods.

The results of five series of activity counts of nymphs under each of these conditions are given in Fig. 2*a*, *b*. It will be seen that in continuous light there was no change in the diurnal rhythm as seen in natural daylight and darkness. The total activity was increased from 129 to 189 units when the animals were first placed in continuous light, but this increased activity was reduced to a normal level of 119 units after 2 months. Continuous darkness did not alter the diurnal rhythm. The total activity was reduced to 109 units, the decreased activity taking place during the period of natural daylight.

(c) *Artificial reversal of light and darkness*

Animals taken from the stream were transferred to conditions of 'reversed' illumination (light from 19.00 to 07.00 hr., and darkness from 07.00 to 19.00 hr.). The dark period was probably darker than is normally experienced.

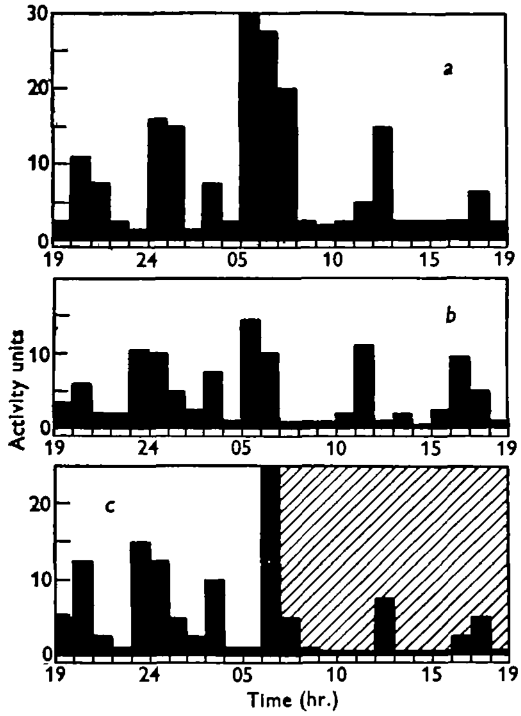

Fig. 2. The results of five series of activity counts of nymphs of *Ecdyonurus torrentis*: *a*, in continuous light; *b*, after being exposed to continuous light for 1 month; *c*, in 'reversed' light and darkness.

Results are shown in Fig. 2*c*. Activity increased during the light period to 93 units compared with 65 units under natural conditions. There was also a fall in activity in the dark period to 33 units compared with a 'natural' 51 units. After 2 months the activity had returned to normal.

The rhythm of activity was in no way altered by conditions of 'reversed' illumination.

(d) *Effect of constant temperature*

Fig. 3 shows the activity of nymphs which had been kept at a constant temperature of 20 ± 1° C. for 3 weeks. The actual observations were not made in the constant temperature room (C.T.R.), but under the conditions described in section (*a*). To check the effect of removing the nymphs from the constant temperature room observations were made over limited periods during 5 days in the C.T.R.

The nymphs removed from the C.T.R. showed no change in their rhythm of activity, but their total activity was higher than under normal conditions, being 138 units. The nymphs in the C.T.R. (the rhythm of activity of these nymphs is shown in Fig. 3*b*) likewise showed no change in their rhythm of activity, but their total activity was even higher than that of the previous set.

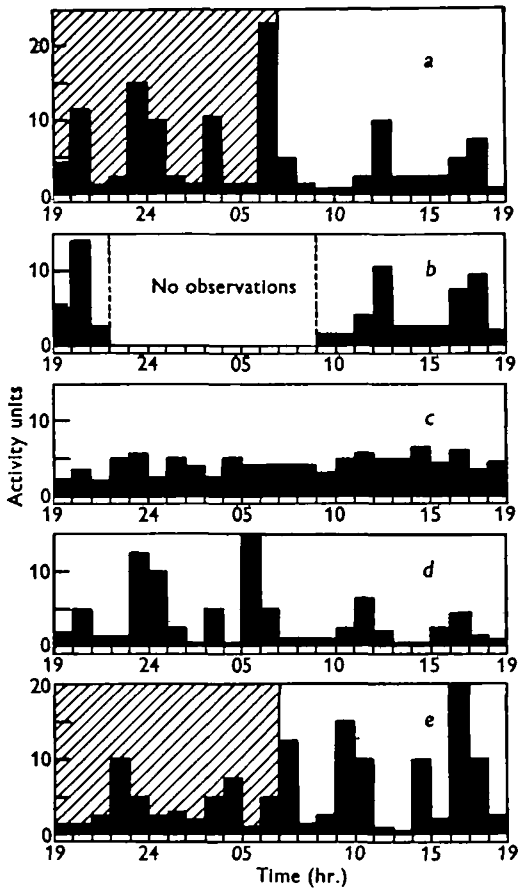

Fig. 3. The results of five series of activity counts of *Ecdyonurus torrentis*: *a*, after being kept at 20° C. for 3 weeks (readings not taken at 20° C.); *b*, after being kept at 20° C. for 1 week (readings taken at 20° C.); *c*, after being kept in continuous light from oviposition; *d*, the same nymphs after exposure to natural daylight and darkness for 1 day and then being left in continuous light for 1 week. *e*, after being kept in continuous light from oviposition, being exposed to darkness from 10 a.m. to 4.0 p.m. G.M.T. for 1 day, and then left in natural daylight and darkness.

(*e*) *Effect of continuous light from oviposition*

A number of eggs were taken immediately after oviposition and kept in continuous light until 7 months after hatching. Five groups of fifteen nymphs were observed at intervals over a period of some weeks, and, as can be seen in Fig. 3*c*, it was found that the rhythm had either been broken or had failed to develop. There was no rhythm in the activity at all, and the total activity had dropped considerably.

The exposure of these nymphs to natural daylight and darkness for 1 day was sufficient to impress a rhythm upon them. Fig. 3*d* shows the activity a week after the nymphs had been transferred to continuous light again. The total activity is still low.

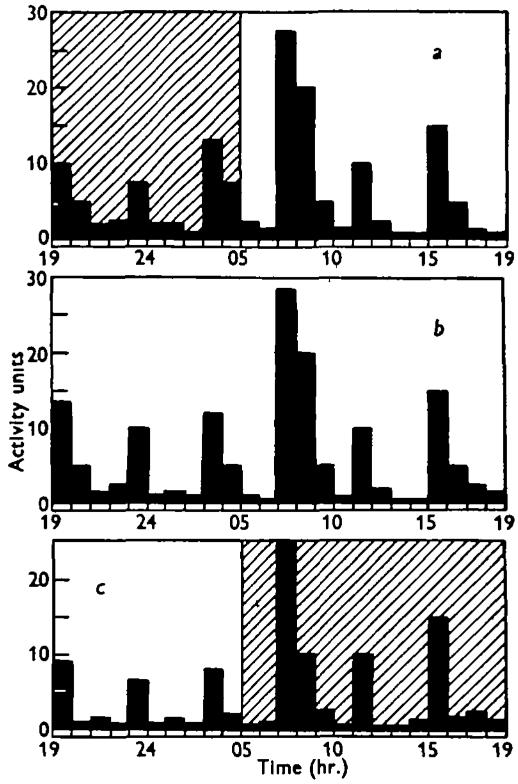

Fig. 4. The results of five series of activity counts of nymphs of *Heptagenia lateralis*: *a*, in conditions of natural daylight and darkness; *b*, in continuous light; *c*, in 'reversed' light and darkness.

Another group of nymphs, bred in continuous light, was exposed to darkness from 10.00 to 16.00 hr. for 1 day, and then transferred to the same conditions of natural daylight and darkness as a control group of nymphs taken directly from the stream. Fig. 3*e* shows that the experimental group took up a rhythm of activity, but not the same rhythm as the stream group. The peaks of activity for the former group are closer together between 10.00 and 16.00 hr., when they were first exposed to darkness, than in the normal group. This experiment further indicates that the original rhythm of light and darkness to which the eggs or nymphs are exposed impresses the rhythm of activity.

(*f*) *Rhythm in ecologically related species*

Observations of activity of *Heptagenia lateralis* and *Baetis rhodani* showed that both of these species had a type of rhythm similar to that of *Ecdyonurus torrentis*.

Fig. 4 gives the results of five experiments on *Heptagenia lateralis* in natural daylight and darkness. The main peaks of activity occur at 03.00, 07.00, 10.00, 14.00, 16.00, 18.00 and 23.00 hr.; again the intervals between the active periods are less in the early morning than for the rest of the 24 hr. The highest activity is regularly shown at 07.00 hr., and the second highest at 15.00 hr.

It is interesting to note that the rhythm of activity is in the same form as that of *Ecdyonurus torrentis*. Specimens were collected from another stream and observed through a series of 24 hr. periods. Fig. 6*b* shows that these had the same rhythm of activity as the previous specimens, although the peaks of activity occurred at slightly different times of day.

Baetis rhodani (Fig. 5) showed a rhythm similar in form, but not as marked, as the other two species. The peaks of activity in this species occurred at the same time as peaks in *Ecdyonurus torrentis*.

Experiments were carried out under 'reversed' conditions of illumination with both *Heptagenia lateralis* and *Baetis rhodani*, and again the same effects were shown as in *Ecdyonurus torrentis*. The total activity was higher during the light period, but the rhythm remained unaltered (Figs. 4*c* and 5*c*). Nymphs in continuous light kept their rhythm (Figs. 4*b* and 5*b*), but the general level of activity was increased.

DISCUSSION

The greater part of the work published on diurnal rhythms of other insects has indicated that the daily rhythm in light intensity is the most important factor conditioning the distribution of activity. Temperature, too, has been shown to affect the rhythm; and it has been suggested that in fact activity cycles are controlled not by one, but by a number of factors acting simultaneously.

These experiments carried out on mayfly nymphs suggest that neither light nor temperature has a direct effect on the diurnal rhythm once that rhythm has been established. The evidence from the nymphs which had been subjected to continuous light from the egg stage suggests that the rhythm is imposed very early in life. There is, however, no indication of the way in which the diurnal rhythm of activity is related to the natural rhythm of daylight and darkness. On the one hand the three broods of *E. torrentis* from the same stream showed the same rhythm, although, being hatched at different times of the year, they could have been exposed to different conditions of natural light and darkness. On the other hand, the rhythms of the nymphs from two different streams did not coincide, although it might be supposed that the difference in the light cycle between the two streams would not be greater than the difference within the one stream over the period from March to July when the three broods emerge.

It is suggested that the reaction to the light-darkness rhythm is in some measure inherent. As the chances of the mayfly fauna of one stream mating with another are limited by the short adult life and the occurrence of swarming, there may be a tendency for each species in a stream to develop its own rhythm within its specific range.

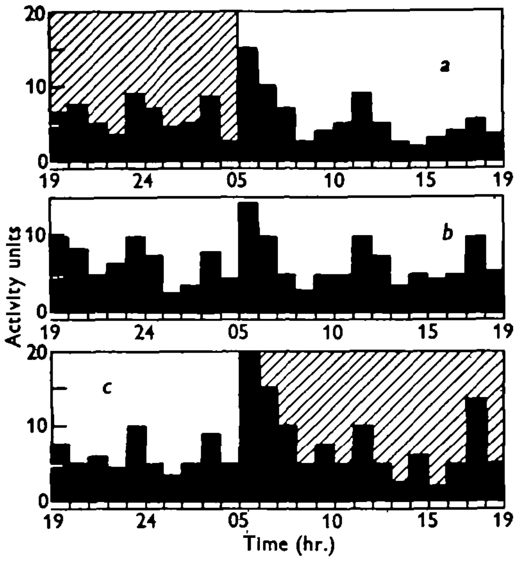

Fig. 5. The results of five series of activity counts of nymphs of *Baetis rhodani*: *a*, in conditions of natural daylight and darkness; *b*, in continuous light; *c*, in 'reversed' light and darkness.

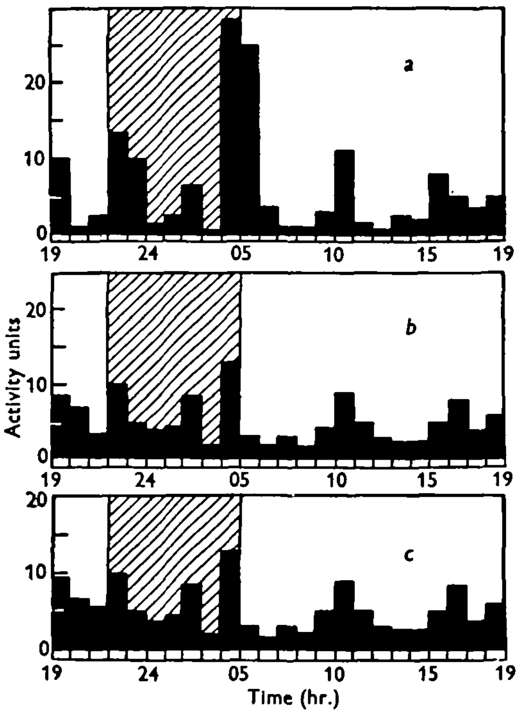

Fig. 6. The results of five series of activity counts of nymphs of: *a*, *Ecdyonurus torrentis*; *b*, *Heptagenia lateralis*; *c*, *Baetis rhodani*. All taken from another stream in May.

SUMMARY

1. The activity of *Ecdyonurus torrentis* Kimm., *Heptagenia lateralis* (Curt.) and *Baetis rhodani* (Pict.) has been measured over a series of 24 hr. periods by recording their movements over 10 min. periods every hour.

2. Diurnal rhythms have been found to occur under conditions of natural daylight and darkness in all three species, but the periods of activity occur at different times, according to the species.

3. These rhythms are maintained under conditions of continuous light or darkness and under conditions of 'reversed' illumination.

4. In *Ecdyonurus torrentis* the rhythm is not seen in nymphs bred from eggs in continuous light, but appears abruptly, in almost fully developed form, after exposure of the nymphs to natural daylight and darkness for a period of no more than 24 hr.

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