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The daily activity patterns of mayfly nymphs (Ephemeroptera)

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(With 10 figures in the text)

Nymphs of *Baëtis rhodani* (Pictet), *Ephemerella ignita* (Poda), *Ecdyonurus venosus* (Fabricius), *Rhithrogena semicolorata* (Curtis), and *Heptagenia lateralis* (Curtis) showed a similar endogenous rhythm of activity under conditions of natural illumination, continuous light and continuous darkness; and were most active at night when they moved from the lower to the upper surface of the stone. The nymphs were positively thigmotactic and negatively phototactic in flowing water, but these taxes and the endogenous activity rhythm of all species except *Heptagenia lateralis* changed markedly when the flow of water ceased.

Contents

Introduction

There are many studies on the daily activity patterns of insects, especially in relation to diel rhythms (Harker, 1961; Corbet, 1966). Few of these studies deal with aquatic insects and only one with mayfly nymphs (Harker, 1953). The purpose of the present study was to provide more information on the daily activity patterns of mayfly nymphs. Nymphs of *Baëtis rhodani* (Pictet), *Ephemerella ignita* (Poda), *Ecdyonurus venosus* (Fabricius), *Rhithrogena semicolorata* (Curtis), and *Heptagenia lateralis* (Curtis) were used in the experiments and emphasis has been placed on *Baëtis rhodani.* As all the nymphs came from a stream, their activity patterns were usually recorded in flowing water. Activity records were also taken in still water so that the results could be compared with those of Harker (1953), who collected nymphs from flowing water and recorded their activity in still water. All activity records were analysed statistically and simple statistical methods were also used in comparisons of mean activity.

In contrast to the single study on activity patterns of mayfly nymphs, several studies describe a diel periodicity in the movements of mayfly nymphs in streams (references in Elliott, 1967*a*). Most of this work deals with nymphs which are transported downstream in the "invertebrate drift" and there may be a close relationship between the rate of drifting and the activity of nymphs in the benthos (Müller, 1966; Elliott, 1967*b*). Nymphs of *Baëtis rhodani* and *Ephemerella ignita* drift downstream in large numbers and the results of the present study enable a comparison to be made between the diel periodicity in drift rate and the activity pattern of the nymphs.

Material and methods

All the nymphs were obtained from the Wilfin Beck, a small stony stream in the Lake District. The nymphs were observed on a large stone in a stream tank with a glass bottom (Fig. 1). Ten nymphs were used for each experiment, and normally both nymphs and stone were changed

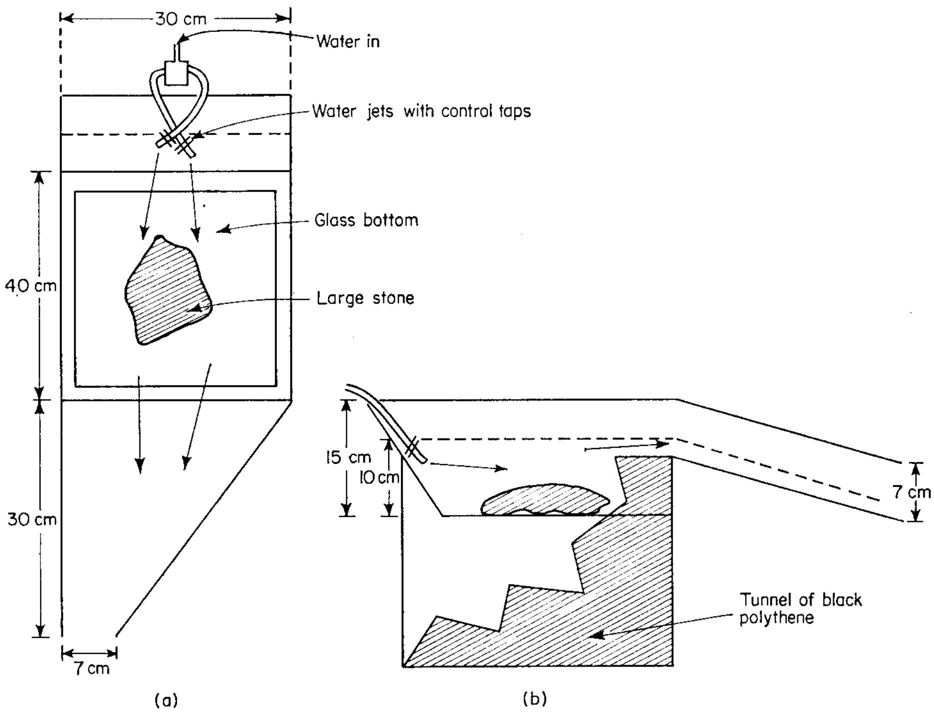

FIG. 1. Diagram of stream tank. (a) Plan view; (b) lateral view. Arrows show direction of flow through tank.

between experiments. The upper surface of the stone was the same in the tank as in the stream and the area of both upper and lower surfaces was about 225 sq. cm. Water was supplied by gravity feed from a large reservoir tank and entered the stream tank through two tubes below the water surface (Fig. 1). The flow through each tube was controlled by a clip and the rate of flow through the tank was 7·5 l. per min. The tank sat at the top end of a vertical tunnel through which observations were made of the nymphs under the stone (light intensity less than 0·5 lx). Preliminary observations showed that it took a maximum of 24 h for the nymphs to settle down to a fairly constant level of activity. Therefore the nymphs were left in the tank for a day before the start of an experiment.

In each experiment, the movements of 10 nymphs were recorded for periods of 10 min at half-hour intervals to give 48 counts per day. Each count is expressed as number of activity units per 10 min and an activity unit is defined as the movement of a nymph for 10 sec or less. Movements were only recorded when they caused a change in the position of a nymph. Readings of water temperature and oxygen concentration were taken with a Mackereth (1964) oxygen meter. Light intensity was measured at the water surface (1·5–2 cm above the upper surface of the stone) and readings were taken at half-hour intervals with an "Eel" or "Megatron" light meter. Daylight was used for most experiments but illumination could be controlled by using artificial light (intensity 500 to 0·5 lx) or a tank cover (light intensity less than 0·5 lx). A dull red light was used for all the observations in the dark. Comparative counts in dim light (intensity less than 3 lx) with and without the red light were not significantly different and therefore the red light did not appear to affect the activity of the nymphs. This conclusion was checked at night by using nymphs which had been marked with luminous paint (method of Harker, 1953).

Statistical methods

The Poisson series was used as a statistical model in the analysis of the activity records. If a series of events (e.g. movements of nymphs in unit time) occur randomly in a continuum of time, the events should be distributed according to the Poisson series. Use of the Poisson series also involves the following conditions:

(1) The probability of an event happening on any one occasion is very small; but when the number of occasions is sufficiently large, the event is observed not too infrequently.

(2) The samples must be small relative to the population.

(3) The events must be separate discrete units, i.e. independent and discontinuous.

The last condition could possibly not be fulfilled if (*a*) one nymph walked into another and caused it to move, (*b*) a nymph kept moving over several activity units. As only 10 nymphs were used in each experiment, the chance of a collision was very small and only one reading had to be discarded for this reason. Preliminary observations indicated that a single movement of position did not last longer than 10 sec and hence this time interval was chosen for an activity unit. The other two conditions were clearly fulfilled in each experiment.

The χ^2 test (Fisher, 1954) was used to test for randomness (i.e. agreement with the Poisson series). If s^2 = variance of the counts, N = number of counts, and $\bar{x}$ = mean number of activity units per count, then:

$$\chi^2 = \frac{s^2(N-1)}{\bar{x}}$$

If the χ^2 value lay between the 95% and 5% points of the χ^2 distribution, then the hypothesis of randomness was not disproved. When N exceeds 31, $\sqrt{2\chi^2}$ is distributed normally and values of d are given (d is a normal variable with zero mean and unit standard deviation). As the product $N\bar{x}$ was usually larger than 30 for each Poisson distribution, values of d were also used to compare the means of different Poisson distributions. The result was significant at the 5% level if the absolute value of d was greater than 1·96.

Results

*Activity of **Baëtis rhodani** in natural daylight and darkness*

Activity records were taken in July 1966, January 1967 and March 1967, and were over four separate 24-hour periods in each month (experiments A, B, C, D). There were 24-hour intervals between the four experiments in a month and different nymphs were used in each experiment. Although the modal lengths (front of labrum to tip of abdomen) were the same for the four experiments in each month, the nymphs in the January experiments were slightly smaller than those in the July and March experiments (Table I). Water temperature remained fairly constant during each monthly series, but was higher in July than in the other two months (Table I). Oxygen concentration was high in all experiments.

TABLE I

***Baëtis rhodani**. Modal lengths and size range of nymphs (front of labrum to tip of abdomen), range of water temperature, and oxygen concentration in each series of experiments*

	July 1966	January 1967	March 1967
Modal length (mm)	7·5	5·5	7·5
Size range (mm)	5·5 to 11·0	4·0 to 8·5	5·0 to 10·5
Water temperature (°C)	11·72 to 11·97	4·85 to 5·13	5·50 to 6·14
Oxygen concentration (mg per l.)	10·10 to 9·3	15·81 to 14·40	16·90 to 14·82

In Figs 2 to 4 the activity of the nymphs over 24 hours is compared with changes in light intensity and the position of the nymphs on the stone. Each point is the mean of the readings from experiments A, B, C, and D, and all times are in G.M.T. The nocturnal activity was clearly greater than the diurnal activity in all experiments. Although the marked changes in activity at dusk and dawn occurred at the same light intensities for the four experiments in each month, they did not occur at the same light intensities in all three months. The high nocturnal activity started at a higher light intensity in July (*c.* 20 lx) than in January and March (*c.* 5 lx), and finished at different light intensities in July (*c.* 60 lx), January (*c.* 2 lx), and March (*c.* 40 lx). During the period of high nocturnal activity there was a movement of nymphs onto the upper surface of the stone. The increase and decrease in numbers on the upper surface did not parallel the sudden changes in activity at dusk and dawn, but lasted about 3–4 hours at the beginning and end of the night (Figs 2 to 4). All ten nymphs moved onto the upper surface in three experiments in March (Fig. 4), whereas the maximum numbers on the upper surface were six and seven in July and January respectively (Figs 2 and 3). The nymphs on the upper surface appeared to be grazing on algae, and gut analyses later confirmed this impression.

When the 48 counts of activity in each experiment were tested for randomness, the χ^2 values were all well above the 5% point of the χ^2 distribution (d varied from 11·61 to 15·82). The contagious distribution of the counts was due to the marked difference between diurnal and nocturnal activity. The sudden changes in activity at dusk and dawn separated day counts from night counts, and the two groups of counts were tested separately for randomness (Table II). All the χ^2 values for the day counts lay within the 95% and 5% points of the χ^2 distribution. Randomness was also not disproved for the combined day counts of experiments A, B, C, D in each month. This conclusion was checked by the additive χ^2 of the four experiments and by applying the χ^2 test for goodness-of-fit. The night counts for January and March also showed no significant departure from randomness when experiments A, B, C, and D were tested separately or combined, but in July

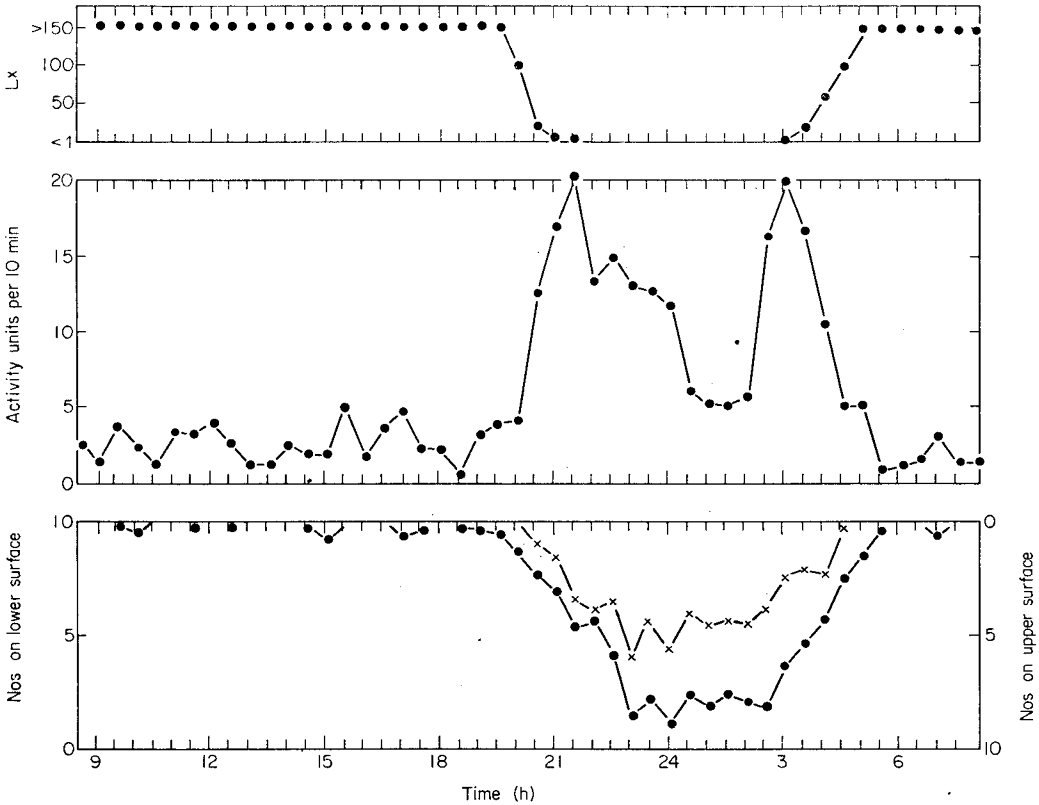

FIG. 2. *Baëtis rhodani;* July 1966. Daily activity pattern of 10 nymphs compared with changes in light intensity and position of nymphs on stone (● — ●, numbers on lower surface; × — ×, numbers on upper surface). The number of nymphs on the edge of the stone is the difference between the numbers on the upper and lower surfaces. All readings are the means of experiments A, B, C, D, and all times in G.M.T.

the χ^2 values were well above the 5% point for all except experiment C. When the night counts for July were divided into early, middle and late night, the χ^2 values for each group of counts were all well within the 95% and 5% points (Table II).

When randomness was not disproved for a combination of experiments A, B, C, D; the mean of the combined counts was clearly the best estimate of the mean activity of the population. These estimated means are given in Table III together with their 95% confidence limits. As all these means were from Poisson distributions, they were compared

TABLE II

Baëtis rhodani. *Total activity, mean activity, and χ^2 values for day and night counts in July 1966, January and March 1967; for early, middle and late night counts in July. Separate figures are given for experiments A, B, C, D, and the combined counts of the four experiments*

	JULY Experiments					JANUARY Experiments					MARCH Experiments				
	A	B	C	D	combined	A	B	C	D	combined	A	B	C	D	combined
DAY															
Total activity units	98	95	82	72	347	37	33	35	21	126	40	37	44	43	164
Mean activity per count	3·06	2·97	2·56	2·25	2·71	1·95	1·74	1·84	1·11	1·66	1·74	1·61	1·91	1·87	1·78
Degrees of freedom (no. of counts −1)	31	31	31	31	127	18	18	18	18	75	22	22	22	22	91
χ^2	45·14	39·34	28·94	35·57	155·95	20·95	27·35	24·2	16·13	95·69	17·47	14·55	18·83	18·5	70·83
d	1·71	1·06	−0·20	0·62	1·75					1·63					−1·61
NIGHT															
Total activity units	217	199	194	198	808	512	457	461	452	1882	409	403	407	421	1640
Mean activity per count	13.56	12·44	12·13	12·38	12·63	17·66	15·76	16·46	15·59	16·37	16·36	16·12	16·28	16·84	16·4
Degrees of freedom (no. of counts −1)	15	15	15	15	63	28	28	27	28	114	24	24	24	24	99
χ^2	59·92	35·33	20·43	29·3	148·85	38·85	31·14	37·24	29·21	141·46	15·59	22·74	16·1	18·49	73·66
d					6·07					1·75					−1·90
JULY NIGHT	Early night					Middle night					Late night				
Total activity units	127	108	105	124	464	17	26	27	20	90	73	65	62	54	254
Total activity per count	15·88	13·5	13·13	15·5	14·5	4·25	6·5	6·75	5	5·63	18·25	16·25	15·5	13·5	15·88
Degrees of freedom	7	7	7	7	31	3	3	3	3	15	3	3	3	3	15
χ^2	12·49	9·63	4·37	2·84	32·9	4·41	1·69	0·11	0·4	8·76	6·4	5·59	1·36	1·26	18·29

by calculating values of *d*. Apart from the middle night period of July, the mean activity at night was very similar in all three months. Therefore the differences in water temperature and modal size of the nymphs did not appear to affect the level of activity at night. The

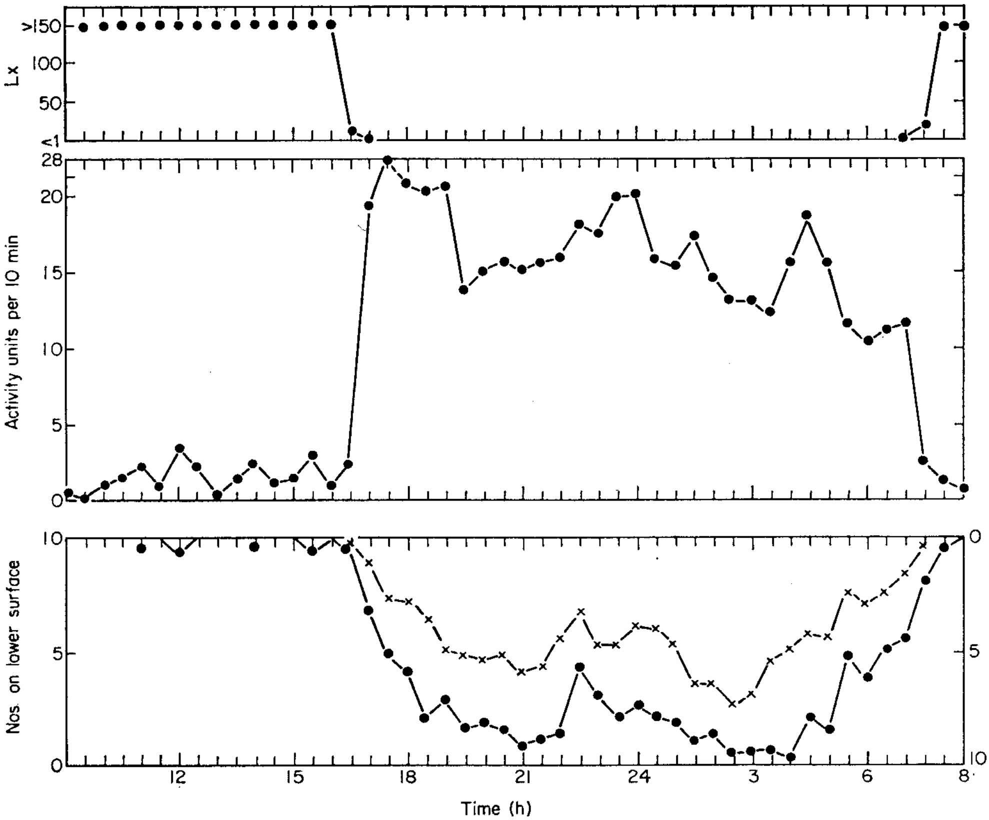

FIG. 3. *Baëtis rhodani;* January 1967. Conventions as in Fig. 2.

TABLE III

Baëtis rhodani. *Estimates of the mean activity of the population (mean number of activity units per 10 min±95% confidence limits)*

	Night Mean activity	Day Mean activity
July 1966 early	14·50±1·32	
middle	5·63±1·16	2·71±0·28
late	15·88±1·95	
January 1967	16·37±0·74	1·66±0·28
March 1967	16·40±0·79	1·78±0·27

mean activity during the day was very similar in January and March, but was significantly higher in July than in the other two months.

In January and March, the nymphs of experiment D were left in the tank for 20 days and records of activity were taken after 5, 10, 15, and 20 days (Table IV). Randomness was not disproved for the day and night counts of each activity record and the mean activity did not significantly change during the 20 days (values of d all well below 1·96).

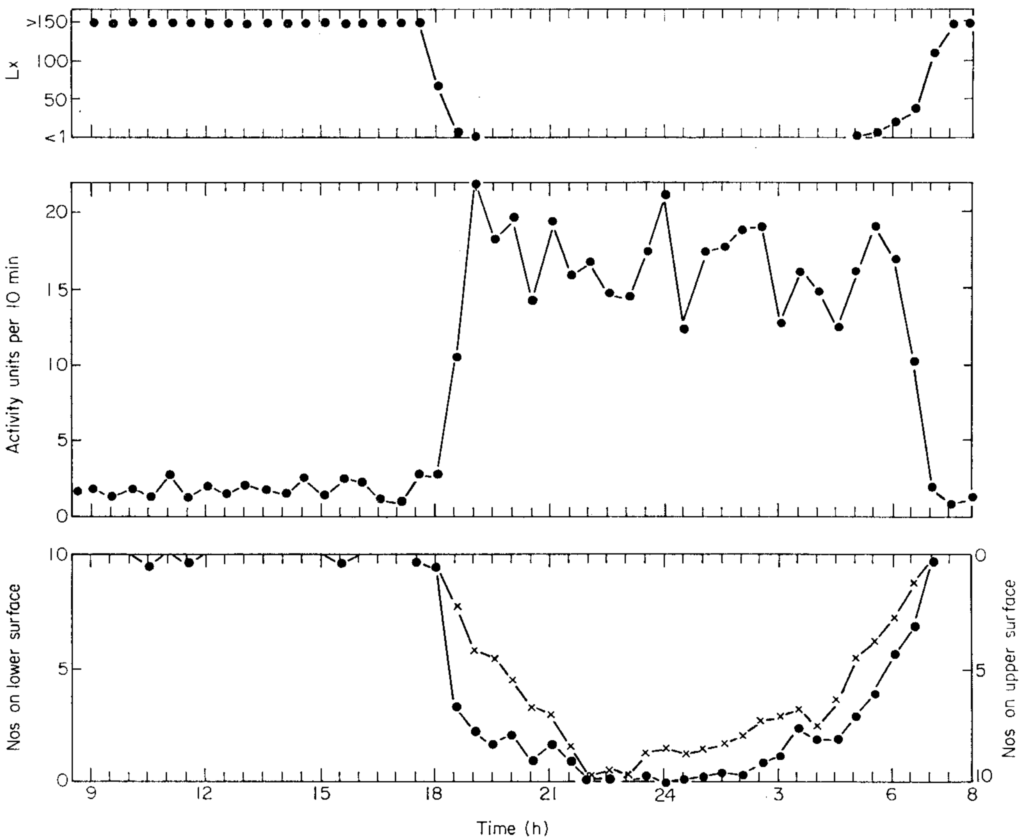

FIG. 4. *Baëtis rhodani;* March 1967. Conventions as in Fig. 2.

Therefore, under conditions of natural daylight and darkness, the mean activity for day or night was constant not only for different groups of nymphs taken from the population at about the same time, but also for the same nymphs over at least 20 days. The counts of activity were always randomly distributed around a low mean during the day and a high mean at night, except in July when the high level of activity was divided by a period of low activity in the middle of the night. Although the high mean at night was similar in all three months, the total activity depended on the length of the night and varied considerably from a maximum in January to a minimum in July (Table II).

Activity of ***Baëtis rhodani*** *in continuous light*

In July 1966, January 1967 and March 1967, activity records were taken over 24 hours of continuous light (intensity 500 lx). Although the activity pattern in continuous light was the same as that obtained under natural light conditions, the light did prevent any nymphs moving onto the upper surface of the stone at night. In a further experiment in March 1966, the mean activity did not significantly change over nine days of continuous light, but increased significantly ($d > 6$) on the first night after the light had been turned off (Table V). This increase in nocturnal activity was accompanied by a rapid movement of all nymphs onto the upper surface of the stone.

TABLE IV

Baëtis rhodani. *Activity of 10 nymphs over 20 days in January 1967 and from 14 March to 2 April 1967. Records of activity were taken over 24 h on days 2, 5, 10, 15, and 20*

	Day number	2	5	10	15	20
January						
	Total activity units	21	26	25	31	29
Day counts	Mean activity per count	1·11	1·37	1·32	1·48	1·38
	No. of counts	19	19	19	21	21
	χ^2	16·13	21·64	22·36	23·67	26·18
	Total activity units	452	478	474	418	435
Night counts	Mean activity per count	15·59	16·48	16·35	15·48	16·11
	No. of counts	29	29	29	27	27
	χ^2	29·21	32·36	36·49	32·12	30·78
March and April						
	Total activity units	43	45	41	47	42
Day counts	Mean activity per count	1·87	1·96	1·78	1·88	1·68
	No. of counts	23	23	23	25	25
	χ^2	18·50	21·47	19·64	24·13	26·12
	Total activity units	421	416	378	365	371
Night counts	Mean activity per count	16·84	16·64	15·12	15·87	16·13
	No. of counts	25	25	25	23	23
	χ^2	18·49	26·18	22·64	21·39	15·78

The reaction of the nymphs to light was further investigated in four experiments in March 1966 and 1967. In each experiment, 100 nymphs were observed on four large stones which were illuminated from above at night and from below during the day with the tank covered. The nymphs on the illuminated surface of the stones were counted at different light intensities (Fig. 5). At nearly all light intensities, maximum numbers on the upper surface of the stones at night were slightly less than the numbers under the stones during the day. The nymphs did not react to light intensities of 1 lx or less (or to a dull red light). A few nymphs did react at 3 lx, with more nymphs moving away from the light at night. Most of the nymphs showed a strong negative phototaxis at a light intensity of 5 lx, but about 15% of the nymphs did not react until the light intensity was greater than 25 lx. When

TABLE V

Baëtis rhodani. *Activity of 10 nymphs over 12 days in March 1966. Activity was recorded on days 2, 10, and 12 with continuous light (500 lx) from day 3 to day 11. Range of water temperature was 5·40 to 6·02°C and modal length of nymphs was 7·5 mm (range was 6·5 to 10·3 mm)*

	Day number	2	10	12
Days counts	Total activity units	46	43	44
	Mean activity per count	2·00	1·87	1·91
	No. of counts	23	23	23
	χ^2	26·05	29·21	31·16
Night counts	Total activity units	467	477	688
	Mean activity per count	18·68	19·08	27·52
	No. of counts	25	25	25
	χ^2	29·18	26·87	35·82

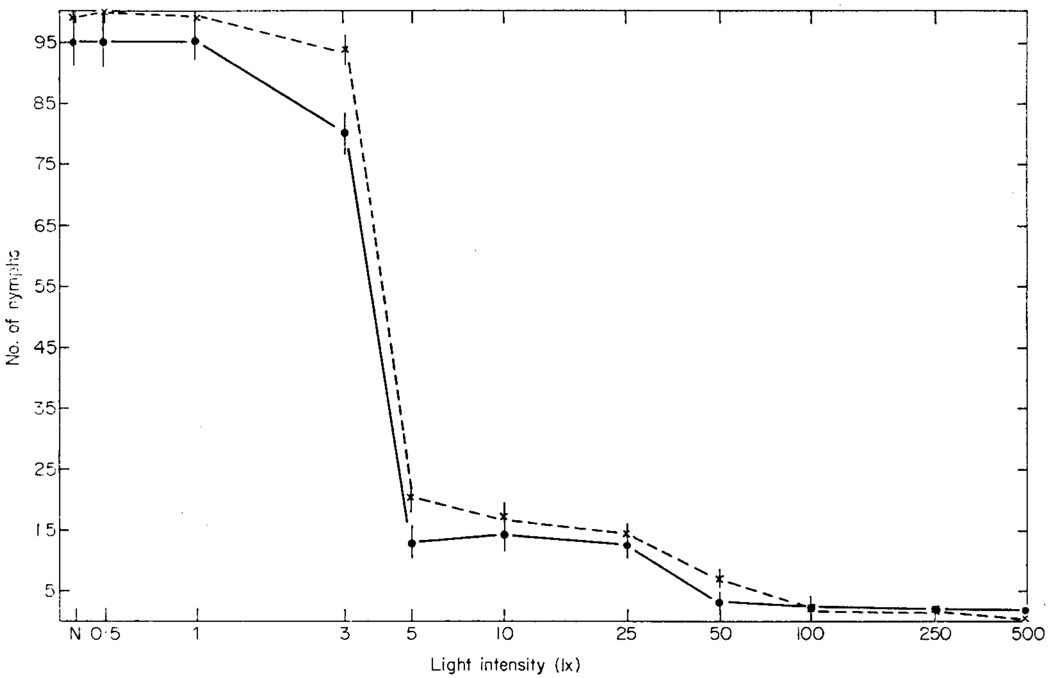

FIG. 5. *Baëtis rhodani.* Reaction of nymphs to different light intensities. Night experiments: maximum number of nymphs on upper surface of four stones at different light intensities above the stones. Day experiments: number of nymphs on lower surface of four stones at different light intensities under the stones. Each point is the mean of four experiments (100 nymphs in each experiment), and vertical line is the range. N was the reading under natural light conditions. Light intensity is on a log scale. ● — ●, Night experiments; × - - - ×, day experiments.

the latter nymphs were separated from the rest, both groups remained consistent in their response to light. A third group of nymphs (about 2%) did not show a negative phototaxis at any light intensity and an occasional nymph did move onto the upper surface of a stone in daylight. The nymphs in these three groups were all of similar size and there were no apparent morphological differences which would explain the differences in behaviour. Although several experiments under natural light conditions coincided with moonlit nights, there was no change in the nocturnal activity of the nymphs. The intensity of moonlight was not measured accurately but was less than 0·5 lx. Lees (1955) notes that the intensity of direct moonlight is usually within the range 0·01 to 0·05 ft candles and this is well below the threshold at which the nymphs showed a negative phototaxis.

TABLE VI

Baëtis rhodani. *Activity of 10 nymphs over 12 days in April 1966. Activity was recorded on days 2, 4, 6, and 12 with the nymphs on a slate from day 3 to day 12 and a continuous light (500 lx) from day 6 to day 12. Range of water temperature was 5·98 to 6·43°C and modal length of nymphs was 8 mm (range 6·5 to 11 mm)*

	Day number	2	4	6	12
	Total activity units	43	196	200	185
Day counts	Mean activity per count	1·72	7·84	8·00	7·40
	No. of counts	25	25	25	25
	χ^2	26·62	34·81	32·68	34·32
	Total activity units	364	376	280	252
Night counts	Mean activity per count	15·83	16·35	12·17	10·96
	No. of counts	23	23	23	23
	χ^2	30·08	28·96	32·61	31·09

As about 98% of the nymphs showed a strong negative phototaxis, experiments were repeated with the nymphs on a flat slate instead of a stone. The nymphs were thus confined to the upper surface of a slate and could not move away from the light. There was a significant increase in mean activity during the day ($d>9$) but not at night (Table VI). A continuous light (intensity 500 lx) did not affect this higher day activity but significantly decreased ($d>3$) the mean activity at night. There was no change in mean activity over seven days of continuous light. Although the difference between nocturnal and diurnal means decreased in these experiments, the mean activity was still higher at night than during the day and the individual counts of activity were always randomly distributed around their respective means (χ^2 values in Table VI all lie within the 95% and 5% points of the χ^2 distribution).

Activity of ***Baëtis rhodani*** *in continuous darkness*

Activity records were taken over 24 hours of continuous darkness in July 1966, January and March 1967, and the activity pattern was the same as that obtained under natural light conditions. In a further experiment in March 1966 the activity pattern did not change over eight days of continuous darkness (Fig. 6). Means of both day and night counts did not significantly change throughout the experiment and there was no significant difference between these means and those obtained in the March experiments of 1967. Under natural light conditions (day 2 in Fig. 6), the nymphs moved onto the upper surface of the stone at night with maximum numbers at midnight. In continuous darkness (days 4, 6, 8, 10 in Fig. 6), some nymphs were always on the upper surface of the stone during the "day" period and the majority of the nymphs were on the upper surface for a longer time at

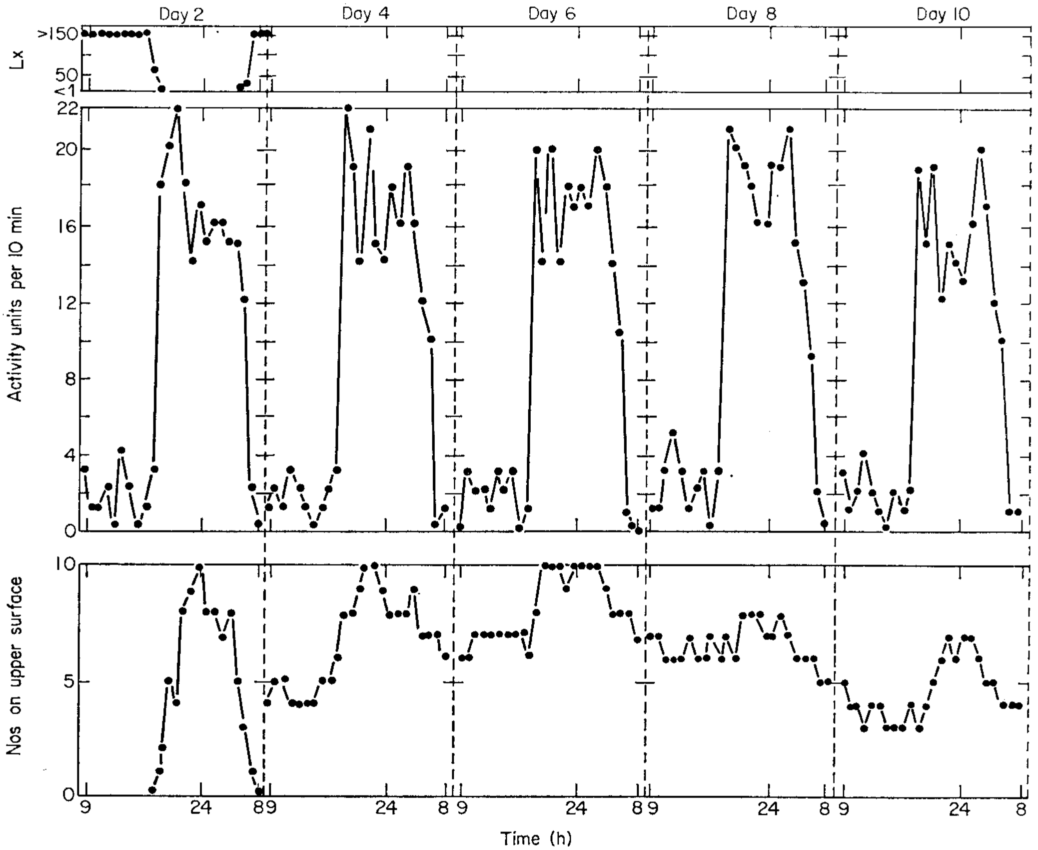

FIG. 6. *Baëtis rhodani.* Activity of 10 nymphs over 10 days in March 1966. Activity was recorded on days 2, 4, 6, 8, 10 with continuous darkness (less than 0·5 lx) from day 3 to day 10. Changes in light intensity are given for day 2 and the number of nymphs on the upper surface of the stone is given for each activity count. Range of water temperature was 5·22 to 5·91 °C and modal length of nymphs was 7·5 mm (range 6·5 to 11 mm).

"night" than on the natural night. These changes were presumably due to the absence of light and hence a strong negative phototaxis no longer operated during the day. The numbers on the upper surface of the stone were at a maximum on day 6 and gradually decreased from day 6 onwards. There was no apparent reason for this decrease which was not due to a shortage of algae on the stone.

*Activity of **Baëtis rhodani** at different rates of flow*

Flow was turbulent in the stream tank and water velocity could only be measured at least 3 cm from the surface of the stone. Therefore rates of flow were always expressed as litres of water passing through the tank in a minute and the equivalent water velocities varied from 5 to 10 cm per sec at 7·5 l. per min (normal rate of flow) and from 11 to 17 cm per sec at 20 l. per min. In July 1966 activity was recorded at seven different rates of flow in each of four experiments. There were ten nymphs in an experiment and 20 counts of diurnal activity for each flow rate. The means of these counts (Table VII) did not differ

TABLE VII

***Baëtis rhodani.** Mean activity of 10 nymphs at different rates of flow in July 1966. The means in experiments A, B, C, D, were obtained from 20 counts of activity during the day. χ^2 values for the 20 counts were nearly all well within the 95% and 5% points of the χ^2 distribution (exceptions were all at 0 l. per min with χ^2 values of 62·95, 53·22, 48·86, and 50·44 for experiments A, B, C, and D respectively)*

Rate of flow (l. per min)	Mean activity per 10 min count			
	Experiment A	Experiment B	Experiment C	Experiment D
20	2·91	2·46	2·35	2·67
15	3·05	2·98	2·44	2·69
7·5	2·38	2·64	2·84	2·32
4	2·67	2·96	2·94	2·88
2	4·96	5·12	5·28	4·86
1	5·26	5·31	4·92	5·08
0	7·45	7·20	7·85	7·20

significantly for the four experiments at each flow rate nor for flow rates between 4 and 20 l. per min. At 2 l. per min the mean activity significantly increased ($d > 3$) in all experiments but was not significantly different from the mean activity at 1 l. per min. The nymphs all showed a strong negative phototaxis at flow rates above 2 l. per min, but at the lower flow rates one or two nymphs moved onto the upper surface of the stone. Counts of activity were randomly distributed around their respective means at all flow rates from 1 to 20 l. per min, but at 0 l. per min the counts followed a contagious distribution.

The records of activity in still water were continued over 24 hours in experiments A, B, C, D and the mean readings show the typical pattern in the four experiments (Fig. 7). Water temperature remained fairly constant during these experiments and the percentage saturation of oxygen was always over 82·5%. The χ^2 values for both day and night counts were well above the 5% point of the χ^2 distribution in all experiments and the contagious distribution of the counts was due to alternating periods of high and low activity throughout the 24 hours. Nymphs were often seen swimming in the tank during periods of high activity, whereas they always showed a strong positive thigmotaxis in flowing water. The negative phototaxis also weakened in still water and many of the nymphs appeared on the upper surface of the stone during periods of high diurnal activity (Fig. 7). Therefore the activity pattern and behaviour of the nymphs changed very little over different rates of flow but altered markedly when the flow of water ceased.

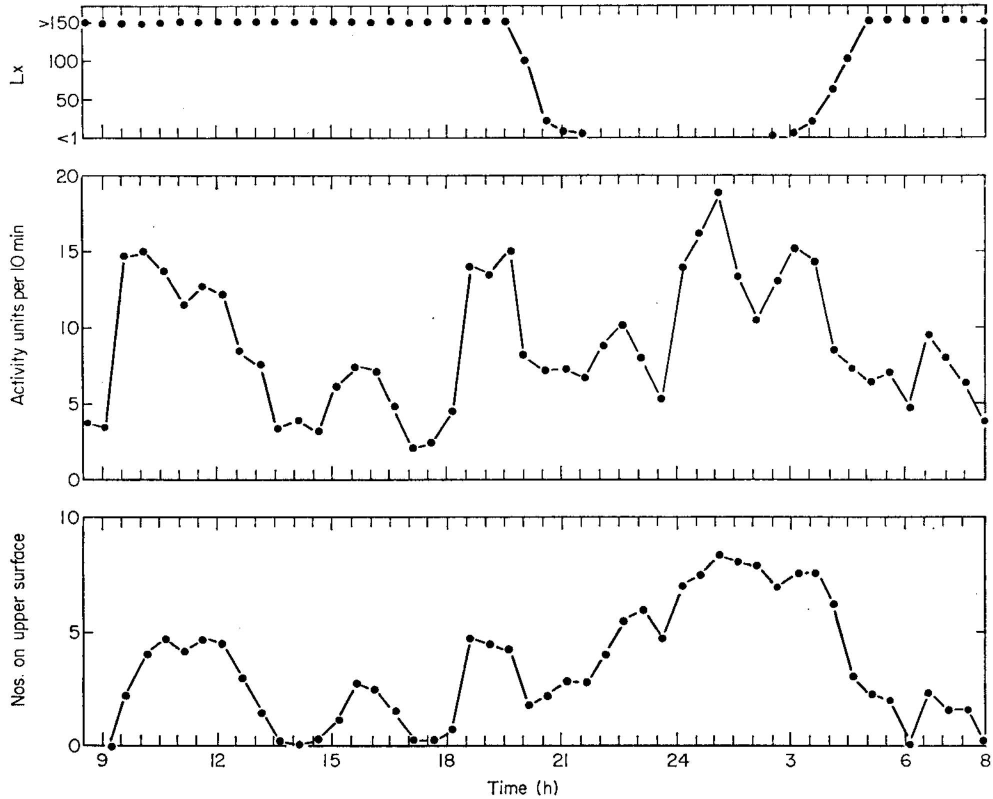

FIG. 7. *Baëtis rhodani.* Daily activity pattern of 10 nymphs in still water compared with changes in light intensity and number of nymphs on upper surface of stone. All readings are the means of experiments A, B, C, D in July 1966. Modal length of nymphs was 7·5 mm (range 5 to 11 mm), and ranges of water temperature and oxygen concentration were 11·81 to 12·03°C and 10·12 to 8·82 mg per l.

Activity of ***Ephemerella ignita, Rhithrogena semicolorata, Ecdyonurus venosus*** *and* ***Heptagenia lateralis***

Activity was recorded in three months for nymphs of *Rhithrogena semicolorata* (Fig. 8) and in one month for nymphs of *Ephemerella ignita, Ecdyonurus venosus* and *Heptagenia lateralis* (Fig. 9). The readings were over two separate 24-hour periods for each species in each month and were taken simultaneously with the readings for *Baëtis rhodani.* Therefore the ranges of water temperature and oxygen concentration were the same as those given in Table I. The activity patterns of all four species were very similar to those of *Baëtis rhodani* with the same light intensities for the start and finish of the periods of high nocturnal activity. Nymphs of all four species moved onto the upper surface of the stone at night and maximum numbers were recorded in March (Figs 8 and 9). Nearly all χ^2 values for day and night counts were well within the 95% and 5% points of the χ^2 distribution. The night counts for *Rhithrogena semicolorata* were the exceptions and showed a tendency towards a regular distribution (χ^2 values on 95% point) when experiments A and B were combined. A 24-hour period of continuous light or darkness did not alter the activity pattern of the four species.

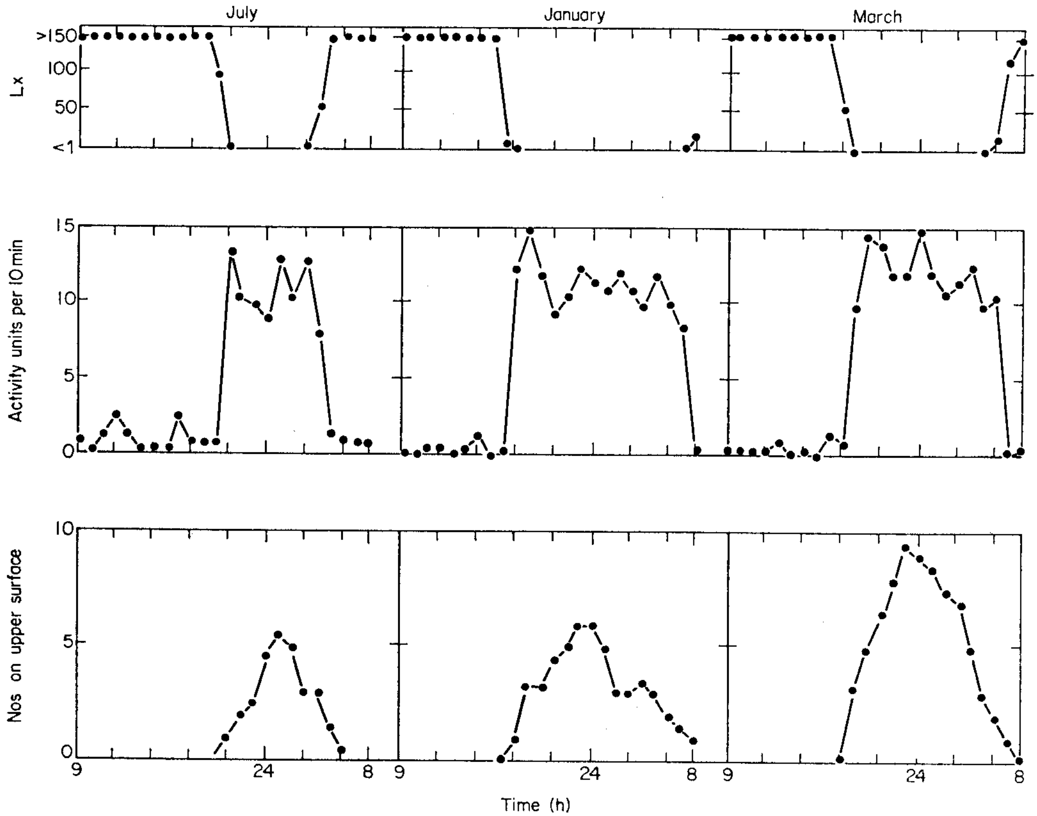

FIG. 8. *Rhithrogena semicolorata;* July 1966, January and March 1967. Daily activity pattern of 10 nymphs compared with changes in light intensity and number of nymphs on upper surface of stone. All readings are the means of experiments A and B in each month. Modal lengths of nymphs were 8·0, 6·0 and 7·0 mm in July, January and March respectively.

Although all species followed a similar pattern of activity over 24 hours, the mean activity varied considerably from species to species (Table VIII). Day and night means have been compared separately (Table IX) and a significant difference (5% level) between means is indicated by D (day means) and N (night means). Nymphs of *Ephemerella ignita* had the highest mean activity of the five species, and both day and night means were significantly higher than all others except the day mean of *Baëtis rhodani* in July and the night mean of *Heptagenia lateralis*. *Baëtis rhodani* had the next highest mean activity and the nocturnal means were significantly higher than those of *Rhithrogena semicolorata* and *Ecdyonurus venosus* in all months, whilst the diurnal means were significantly higher than those of *Rhithrogena semicolorata* in all months and those of *Ecdyonurus venosus* and *Heptagenia lateralis* in July. The mean activity of *Heptagenia lateralis* was very similar to that of *Baëtis rhodani* with no significant difference between the means of the two species in all except two comparisons. Although there was no significant difference between the mean activity of *Heptagenia lateralis* and *Ecdyonurus venosus* during the day, the mean

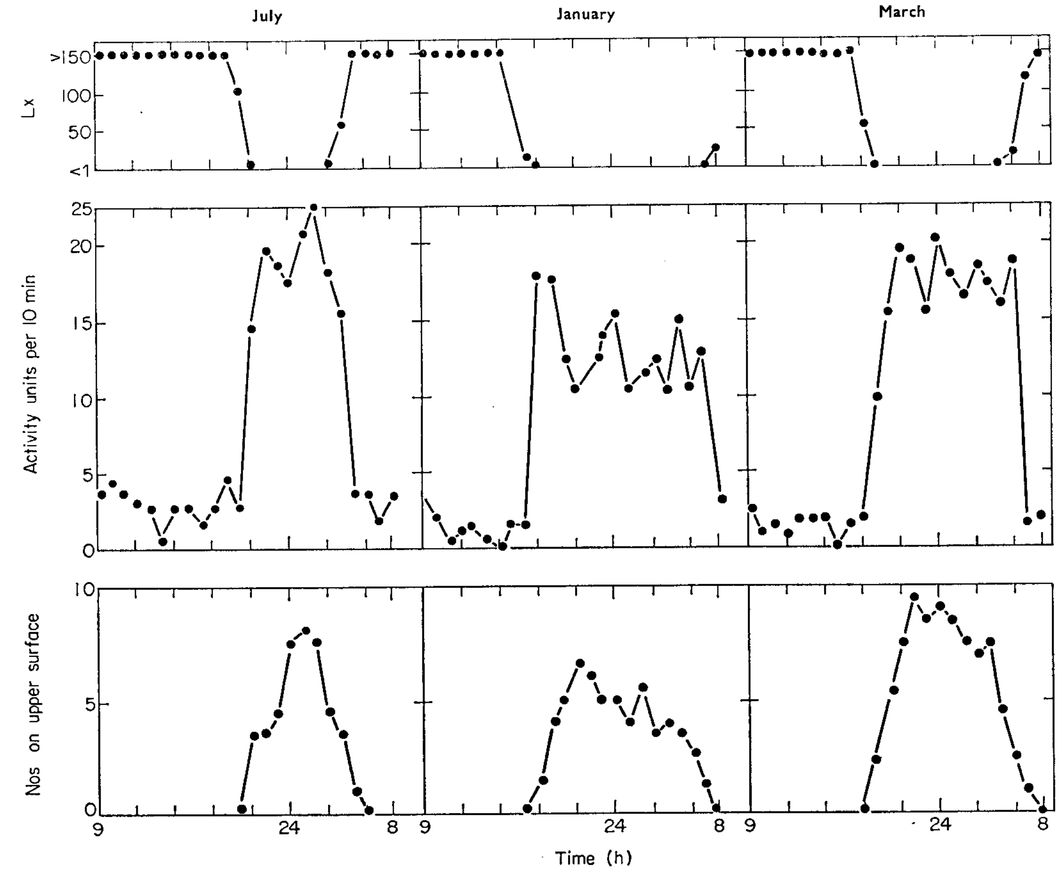

FIG. 9. *Ephemerella ignita* in July 1966, *Ecdyonurus venosus* in January 1967, and *Heptagenia lateralis* in March 1967. Daily activity pattern of 10 nymphs compared with changes in light intensity and number of nymphs on upper surface of stone. All readings are the means of experiments A and B in each month. Modal lengths of nymphs were 6·0 mm for *E. ignita*, 7·0 mm for *E. venosus* and for *H. lateralis*.

activity of *Ecdyonurus venosus* at night was significantly lower than the mean activity of all species except *Rhithrogena semicolorata*. Nymphs of *Rhithrogena semicolorata* had the lowest mean activity and the means were never significantly higher than those of other species.

The reaction of 100 nymphs to different light intensities (0·5 to 500 lx) also varied from species to species. Experimental conditions were the same as those described for *Baëtis rhodani* (Fig. 5). Nymphs of *Ephemerella ignita* and *Heptagenia lateralis* showed the same response as nymphs of *Baëtis rhodani*; i.e. most nymphs showed a strong negative phototaxis at light intensities from 5 to 500 lx, but about 15% of the nymphs did not react to intensities below 25 lx. The threshold for a negative phototaxis was higher for nymphs of

TABLE VIII

Rhithrogena semicolorata, Ephemerella ignita, Ecdyonurus venosus** and **Heptagenia lateralis. *Estimates of the mean activity of the population (mean number of activity units per 10 min ±95% confidence limits)*

	July 1966		January 1967		March 1967	
	Day	Night	Day	Night	Day	Night
Rhithrogena	1·16 ±0·37	10·94 ±1·62	0·40 ±0·28	11·29 ±1·24	0·58 ±0·31	12·08 ±1·39
Ephemerella	2·88 ±0·59	18·81 ±2·14				
Ecdyonurus			1·50 ±0·54	13·11 ±1·35		
Heptagenia					1·58 ±0·50	17·13 ±1·65

TABLE IX

*Comparisons of mean activity of **Baëtis rhodani, Rhithrogena semicolorata, Ephemerella ignita, Ecdyonurus venosus** and **Heptagenia lateralis***

		Baëtis rhodani			*Rhithrogena*			*Ephem:*	*Ecdy:*	*Hept:*
		July (late night)	Jan.	March	July	Jan.	March	July	Jan.	March
Baëtis rhodani	July (early night)	0	0	0	N	N	N	N	N	N
	July (late night)		0	0	N	N	N	N	N	0
	January	D		0	N	N	N	N	N	0
	March	D	0		N	N	N	N	N	0
Rhithrogena semicolorata	July	D	D	D		0	0	N	N	N
	January	D	D	D	D		0	N	N	N
	March	D	D	D	D	0		N	0	N
Ephemerella ignita	July	0	D	D	D	D	D		N	0
Ecdyonurus venosus	January	D	0	0	0	D	D	D		N
Heptagenia lateralis	March	D	0	0	0	D	D	D	0	

0 indicates no significant difference (5% level) between means, D indicates a significant difference between the means of the day counts, and N indicates a significant difference between the means of the night counts.

Ecdyonurus venosus and *Rhithrogena semicolorata*. About 99% of the nymphs did not show a negative phototaxis at light intensities below 25 lx, about 10% did not react to intensities below 50 lx, and about 4% did not react to any light intensity.

In still water, the activity pattern of *Ephermerella ignita*, *Rhithrogena semicolorata*, and *Ecdyonurus venosus* changed to a pattern which was very similar to that of *Baëtis* *rhodani* in still water (Fig. 7). There were alternating periods of high and low activity throughout the 24 hours, both day and night counts followed a contagious distribution, and many of the nymphs no longer showed a negative phototaxis or a positive thigmotaxis. Nymphs of *Heptagenia lateralis* were the exceptions and did not show any change in activity or behaviour when the flow of water ceased.

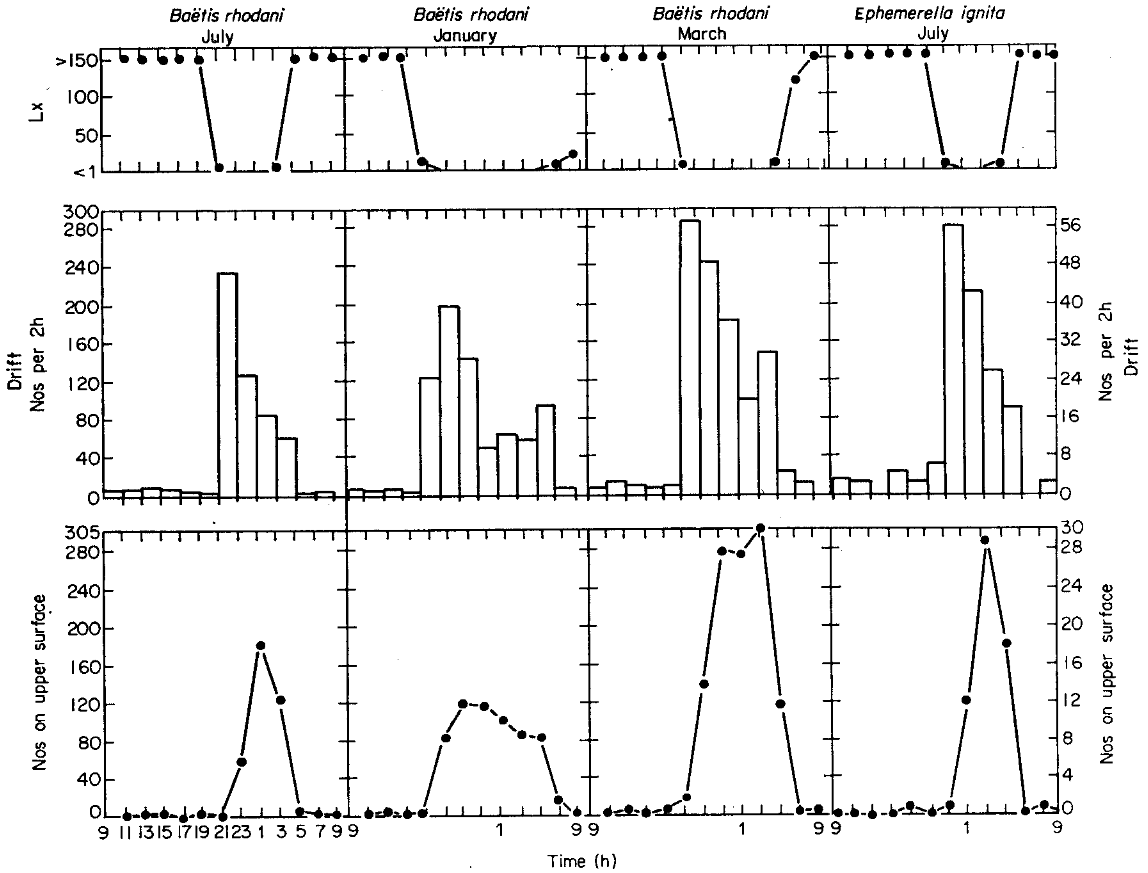

Fig. 10. *Baëtis rhodani* in July, January, March; and *Ephemerella ignita* in July. Daily variation in drift rate (number of nymphs taken in drift samples over 2-h period) compared with changes in light intensity (lx) and number of nymphs on upper surface of stones (counts made every 2 h over 10 m stretch of stream).

Comparison of activity pattern and drift rate

Nymphs of *Baëtis rhodani* and *Ephemerella ignita* were numerous in drift samples from the Wilfin Beck and the diel periodicities in drift rate were very similar to those recorded for the same species in a Dartmoor stream (Elliott, 1967*a*) and in the River Duddon (Elliott & Minshall, 1968). Examples of diel periodicities in both drift rate and number of nymphs on the upper surface of stones are given for the same months as the activity experiments (Fig. 10). The diel periodicity in numbers on the upper surface of stones was very similar to that recorded in the activity experiments and maximum numbers were recorded in March. As the drift samples in each month were from equal volumes of water, they were tested for agreement with a Poisson series. The day samples contained very few nymphs but these drifting nymphs were randomly distributed in the water (χ^2 values between 95% and 5% points of the χ^2 distribution), and therefore drift rate and activity followed a similar pattern during the day. Drift rate increased at night and the samples followed a contagious distribution (χ^2 values well above 5% point), which was chiefly due to the very high drift rate in the early hours of the night. Therefore drift rate and activity showed a similar nocturnal periodicity but the variance in nocturnal drift rate was far greater than the variance in nocturnal activity.

Discussion

A "diel periodicity" is defined as the recurrent temporal pattern of an activity with a 24-hour period (Corbet, 1966). Two diel periodicities are recognized in the results of the present study; (1) in the movement of nymphs onto the upper surface of the stone, (2) in the activity of the nymphs.

Although the two periodicities occurred together, they will be considered separately and the relative importance of exogenous and endogenous components (defined in Corbet, 1966) will be ascertained.

The first periodicity was nocturnal in flowing water and was chiefly due to the negatively phototactic behaviour of the nymphs. This periodicity was thus controlled by diel fluctuations in light intensity (an exogenous component), and its non-persistence in continuous light or darkness indicates that no endogenous components were involved. The negatively phototactic behaviour of mayfly nymphs is well known, but a small number of nymphs in the present study were not negatively phototactic at any light intensity and therefore did not show the first periodicity. Although Scherer (1962) gives a definite "Licht-Barriere" of 40 lx for nymphs of *Baëtis rhodani*, it is difficult to state an absolute light intensity at which each species is negatively phototactic because nymphs of the same species react at different light intensities.

The second periodicity was also nocturnal in flowing water and followed normal diel fluctuations in light intensity. As the periodicity persisted in continuous darkness, it may be controlled by an endogenous rhythm with the decrease in light intensity at dusk acting as an external time-cue ("Zeitgeber" of Aschoff, 1954). The nocturnal periodicity also persisted in continuous light, but the amplitude (defined in Harker, 1964) of the periodicity was reduced when the nymphs were unable to avoid the light. Apart from light intensity, the other obvious exogenous factors of water temperature and oxygen concentration remained fairly constant during each monthly series. Therefore the nocturnal periodicity was controlled either by subtle geophysical variables (Brown, 1962) or by an endogenous rhythm. The latter conclusion is supported by the very consistent activity patterns of each species at different times of the year, except for the bimodal activity pattern of *Baëtis rhodani* in July.

Neither of the nocturnal periodicities persisted at low flow rates or in still water. As many of the nymphs no longer showed a negative phototaxis in still water, the first periodicity disappeared completely. The non-persistence of the second periodicity implies that the endogenous rhythm of activity was affected by the exogenous factor of water velocity when the latter was considerably reduced or ceased. Of the five species, only *Heptagenia lateralis* had a similar activity pattern in still and flowing water, and this is the only species which is common on lake shores as well as in streams. The daily activity patterns of the other four species changed in still water to a pattern which was very similar to that shown by nymphs of *Ecdyonurus torrentis* Kimmins, *Heptagenia lateralis*, and *Baëtis rhodani* in the earlier experiments of Harker (1953). As her experiments were confined to still water, the nymphs did not show a negative phototaxis or a nocturnal periodicity in activity. Although the activity patterns in still water were very different from those seen in flowing water, and also presumably in the stream, Harker did conclude that the nymphs possessed an inherent (endogenous) rhythm of activity. This conclusion implies that the endogenous activity rhythm in flowing water does not break down when the flow of water ceases, but changes suddenly to a new rhythm which is also endogenous.

Corbet (1966) notes that few workers have integrated field and laboratory studies when they have attempted to resolve a field periodicity into its different components. Therefore the well documented field periodicity in the downstream drifting of the nymphs was compared with the two periodicities shown by the nymphs in the stream tank. The movement of nymphs onto the upper surface of the stone followed a similar nocturnal periodicity in both stream tank and stream, whereas the nocturnal periodicities in drift rate and activity followed a different pattern during the night. Therefore the endogenous rhythm of activity of nymphs in the benthos is undoubtedly an important factor but is only one of a complex of factors which determine the nocturnal periodicity in drift rate. All the other factors are not yet known but probably include the number of nymphs on the upper surface of the stones (exogenously controlled by light intensity), the water velocity to which the nymphs are exposed, and the competition between nymphs for food and space (discussed in Elliott, 1967*a*,*b*).

Summary

The daily activity patterns of *Baëtis rhodani* (Pictet), *Ephemerella ignita* (Poda), *Ecdyonurus venosus* (Fabricius), *Rhithrogena semicolorata* (Curtis), and *Heptagenia lateralis* (Curtis) were studied in a stream tank. Movements of nymphs over the surface of a large stone were recorded for 10-minute periods every half-hour (48 counts per day).

Nymphs of all species showed a constant pattern of activity under conditions of natural illumination and fairly constant water temperature, oxygen concentration and rate of flow. Activity was greatest at night and the counts followed a random distribution around a low mean during the day and a high mean at night, except for *Baëtis rhodani* in July when a period of low activity occurred in the middle of the night. The nymphs were on the lower surface of the stone during the day, and there was a gradual movement of most nymphs onto the upper surface at night. The high nocturnal activity started at light intensities between 5 and 20 lx, and finished at light intensities between 2 and 60 lx.

The mean activity was highest in *Ephemerella ignita*, followed by *Baëtis rhodani*, *Heptagenia lateralis*, *Ecdyonurus venosus* and finally *Rhithrogena semicolorata* with the lowest mean activity.

Continuous light (intensity 500 lx) or darkness (light intensity below 0·5 lx) did not change the activity pattern from that seen in natural illumination, but very few nymphs moved onto the upper surface of the stone in continuous light, and some nymphs were

always on the upper surface of the stone in continuous darkness. When nymphs were confined to the upper surface of a slate, the mean activity increased during the day and decreased in continuous light at night.

In flowing water, all nymphs showed a strong positive thigmotaxis and most nymphs showed a strong negative phototaxis. The latter taxis was not shown by *Baëtis rhodani*, *Ephemerella ignita*, and *Heptagenia lateralis* at light intensities below 5 lx (25 lx for about 15% of the nymphs), and by *Ecdyonurus venosus* and *Rhithrogena semicolorata* at light intensities below 25 lx (50 lx for about 10% of the nymphs).

The daily activity patterns of all species except *Heptagenia lateralis* changed markedly when the flow of water ceased. In still water there were alternating periods of high and low activity throughout the 24 hours, both day and night counts followed a contagious distribution, and many of the nymphs no longer showed a positive thigmotaxis or a negative phototaxis.

Nymphs of *Baëtis rhodani* and *Ephemerella ignita* showed a nocturnal periodicity in both drift rate (a field periodicity) and activity, but the variance in nocturnal drift rate was far greater than the variance in nocturnal activity.

It is concluded that the mayfly nymphs showed two diel periodicities in flowing water; (1) in their numbers on the upper surface of the stone, (2) in their activity. Both periodicities were nocturnal, but the first was controlled by light intensity (an exogenous component) and the second by an endogenous rhythm.

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REFERENCES

Aschoff, J. (1954). Zeitgeber der tierischen Tagesperiodik. *Naturwissenschaften* **41**: 49–56.

Brown, F. A. (1962). Extrinsic rhythmicality: a reference frame for biological rhythms under so-called constant conditions. *Ann. N.Y. Acad. Sci.* **98**: 775–787.

Corbet, P. S. (1966). The role of rhythms in insect behaviour. *Symp. R. ent. Soc. Lond.* No. 3: 13–28.

Elliott, J. M. (1967*a*). Invertebrate drift in a Dartmoor stream. *Arch. Hydrobiol.* **63**: 202–237.

Elliott, J. M. (1967*b*). The life histories and drifting of the Plecoptera and Ephemeroptera in a Dartmoor stream. *J. Anim. Ecol.* **36**: 343–362.

Elliott, J. M. & Minshall G. W. (1968). A comparative study of invertebrate drift in the river Duddon (English Lake District). *Oikos* **19**: 39–52.

Fisher, R. A. (1954). *Statistical methods for research workers.* (12th ed.). London: Oliver & Boyd.

Harker, J. E. (1953). The diurnal rhythm of activity of mayfly nymphs. *J. exp. Biol.* **30**: 525–533.

Harker, J. E. (1961). Diurnal rhythms. *A. Rev. Ent.* **6**: 131–146.

Harker, J. E. (1964). *The physiology of diurnal rhythms:* 114. Cambridge: University Press.

Lees, A. D. (1955). *The physiology of diapause in arthropods:* 151. Cambridge: University Press.

Mackereth, F. J. H. (1964). An improved galvanic cell for determination of oxygen concentrations in fluids. *J. scient. Instrum.* **41**: 38–41.

Müller, K. (1966). Die Tagesperiodik von Fliesswasserorganismen. *Z. Morph. Ökol. Tiere* **56**: 93–142.

Scherer, E. (1962). Phototaxtisches Verhalten von Fliesswasser—Insektenlarven. *Naturwissenschaften* **49**: 477.