

## Diel Activity Patterns in Nymphs of an Australian Mayfly *Atalophlebioides* sp. (Ephemeroptera: Leptophlebiidae)

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### Abstract

Activity of nymphs of *Atalophlebioides* sp. on the surface of a stone in a laboratory stream was greatest at night, with peak activity occurring at about 2400 h. Most nymphs remained on the lower surface of the stone during the day, but there was gradual movement on to the upper surface at night. Although nymphs showed a nocturnal maximum in both drift rates (field observation) and activity, peaks of activity did not correspond with peaks in drift rates.

Nymphs at high density (15 per stone) showed a significantly greater level of activity during light hours (1200-1800 h) and the first two hours of darkness compared with nymphs at lower densities (five and two per stone) but there was no significant difference in activity between the two lower densities for the period 1200-2400 h. Feeding behaviour was observed at all three densities, but activities in the form of rapid darting (duration < 1 s) and a 'swimming' response occurred at the higher density. It is postulated that a relationship exists between the swimming response (as a result of competition for space) and the insect's propensity to drift.

### Introduction

Activity patterns in insect larvae in streams are of interest because of the importance of the larvae as food for predators, and in the investigation of stream colonization. Nevertheless, very few studies have been undertaken. Lehmann (1965), Thorne (1969), Elliott (1970) and Gallep (1974) investigated diel activity patterns of caddis larvae and Harker (1953) and Elliott (1968) studied similar activity patterns in various species of mayfly nymphs.

Müller (1966) and Elliott (1967) both proposed a possible relationship between the rate of drifting and activity of insects in the stream benthos. Elliott (1968) demonstrated that although the movement of nymphs onto the upper surface of stones followed similar nocturnal periodicities in both stream tank and stream, the nocturnal periodicities of drift rate and activity followed different patterns. The present study considers the diel activity patterns and the effect of density variation on this activity in the mayfly *Atalophlebioides* sp. The majority of observations were carried out in the laboratory in either an artificial stream or still-water tank, although some field observations were made. As numbers of *Atalophlebioides* sp. nymphs drift downstream (Bailey 1981), a comparison could be made between diel periodicities of activity patterns and drift rates.

## Materials and Methods

### *Diel Activity Patterns in Atalophlebioides sp.*

The apparatus consisted of a gravity-fed artificial stream (Fig. 1). Creek water was pumped from a large 50-litre constant-temperature bath into a reservoir which in turn supplied the artificial stream. The water entered through a tube below the water surface. Flow through the tube was controlled by a series of clips and the rate of flow through the stream tank was adjusted to be  $5.0 \text{ l min}^{-1}$ . Illumination was supplied from a bank of tungsten filament bulbs controlled by a rheostat and measured, at the same height as the water surface but outside the aquarium, by a digital photometer (J16 digital photometer with J6501 probe, Tektronix, Beaverton, Oregon, U.S.A.). Measurements of water temperature and oxygen concentration were taken at half-hourly intervals (oxygen meter, model 548P, Yellow Springs Instruments Co. Inc., U.S.A.). The apparatus was allowed to stabilize at about  $7^\circ\text{C}$  for 2 days before the experiments began.

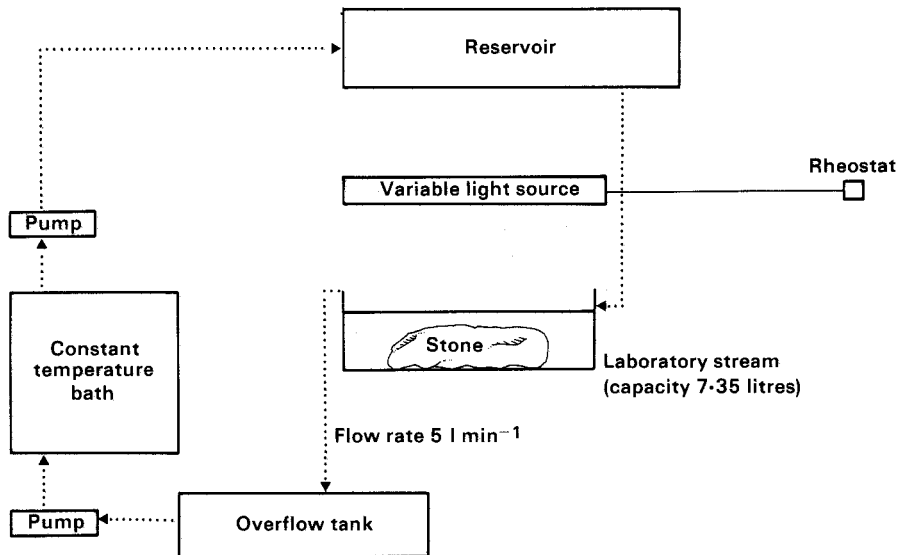


Fig. 1. Diagrammatic representation of the apparatus used to create a laboratory stream. Not to scale.

*Atalophlebioides sp.*\* nymphs were collected from Condor Creek, A.C.T. (Bailey 1981), and carefully washed from the undersurface of rocks into insulated containers. At the same time 10 rocks were selected at random on the criterion that their areas were between 200 and 225  $\text{cm}^2$ . The rocks were carefully rinsed to remove macroinvertebrates but to not dislodge algae or similar organisms. The nymphs were then brought back to the laboratory as soon as possible but always within 90 min.

One rock was picked at random and placed into the stream tank. Mayfly nymphs were examined and head width (between inner lateral ridge of eye) and body length (from front of labrum to tip of abdomen) were measured. Five nymphs of equal size were then introduced into the stream tank. The illumination and water and air temperatures were arranged to resemble, as closely as possible, the natural conditions found at Condor Creek (Table 1 and Fig. 2). 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 stream tank for a day before the start of an experiment.

In each experiment, the movements of five nymphs were recorded for a period of 5 min at half-hourly intervals for the first 12 h and then at hourly intervals for the remaining 12 h to give a total of 36 counts in the 24-h period. Each count is expressed as the movement of a nymph for 10 s or less. Movements of nymphs were only recorded when they resulted in an actual change of position. Any movements that resulted from one nymph walking into another were disregarded also (Elliott 1968).

\*The specimens of *Atalophlebioides sp.* nymphs used during the study have been lodged in the Department of Entomology, South Australian Museum, Adelaide, S.A. 5000.

Observations during darkness (<0.10 lux) were conducted with a torch masked with red cellophane paper. This produced illumination of less than 0.3 lux. Comparative counts in dim light (<2.0 lux), with and without the red light, were not significantly different ( $\chi^2 = 30.1$ ,  $P < 0.01$ ) and therefore the red light did not appear to affect the activity of the nymphs. Similar results were reported by Harker (1953) and Elliott (1968) who checked activity at night, with and without red light, by using nymphs which had been marked with luminous paint.

Activity records were taken on 28–29 August, 31 August–1 September and 3–4 September (experiments A, B and C). There were 24-h intervals between each experiment and different nymphs and stones were used in each experiment.

In order to compare laboratory results with field observations, hourly drift samples of *Atalophlebioides* sp. nymphs were taken on 5–6 September 1978 from Condor Creek, A.C.T. (see Bailey 1981). In addition, observations were made every 2 h, using a water telescope and masked torch, to estimate the number of *Atalophlebioides* sp. nymphs on the surface of rocks along a 20-m length of stream downstream from the nets.

**Table 1. Modal lengths and size range of *Atalophlebioides* sp. nymphs and range of air and water temperatures and oxygen concentration in each experiment**

Experiment	Modal length <sup>A</sup> (mm)	Size range <sup>A</sup> (mm)	Air temperature (°C)	Water temperature (°C)	Oxygen concentration (mg/l)
A	7.99	6.18–9.71	5.0–12.0	6.2–8.3	16.4–15.0
B	7.90	6.18–10.71	5.0–11.5	6.0–7.8	17.2–15.4
C	7.5	5.88–11.21	5.8–11.8	6.0–8.0	16.8–15.2

<sup>A</sup>Length from front of labrum to tip of abdomen.

#### *Changes in Diel Activity Patterns of Atalophlebioides sp. as a result of Density Alterations*

*Atalophlebioides* sp. nymphs and stream rocks were collected from Condor Creek as outlined above. Three rocks were chosen randomly from the collection and each was placed in a separate glass dish (21.5 cm diam.) containing fresh creek water to a depth of 7 cm. The three dishes were maintained at 7°C in a constant-temperature bath, and were placed by a window but out of direct sunlight. Two air stones, each 3 by 1 by 1 cm, provided additional aeration for each dish. Either 2, 5 or 15 nymphs, of about equal size, were introduced into each of the dishes. The nymphs were allowed 3 h to settle before the first set of observations was made at 1200 h.

The type and level of nymph activity, water temperature, oxygen concentration and illumination were taken as outlined above (Table 2). Readings were recorded over a 12-h period from 1200 to 2400 h on 9 September 1978. The experiment was repeated on the two following days using freshly collected nymphs, creek water and rocks.

**Table 2. Modal length and size range of *Atalophlebioides* sp. nymphs, water temperature, and range of air temperature and oxygen concentration at three different density levels of nymphs**

Density (No. per stone)	Modal length <sup>A</sup> (mm)	Size range <sup>A</sup> (mm)	Air temperature (°C)	Water temperature (°C)	Oxygen concentration (mg/l)
2	8.0	6.1–9.4	9.8–14.2	7.0±0.5	9.0–8.1
5	7.6	5.0–8.9	9.8–14.2	7.0±0.5	9.2–7.6
15	7.8	5.8–8.7	9.8–14.2	7.0±0.5	9.0–7.0

<sup>A</sup> Length from front of labrum to tip of abdomen.

## Results

### *Diel Activity Patterns in Atalophlebioides sp.*

Modal lengths (front of labrum to tip of abdomen) of nymphs, air and water

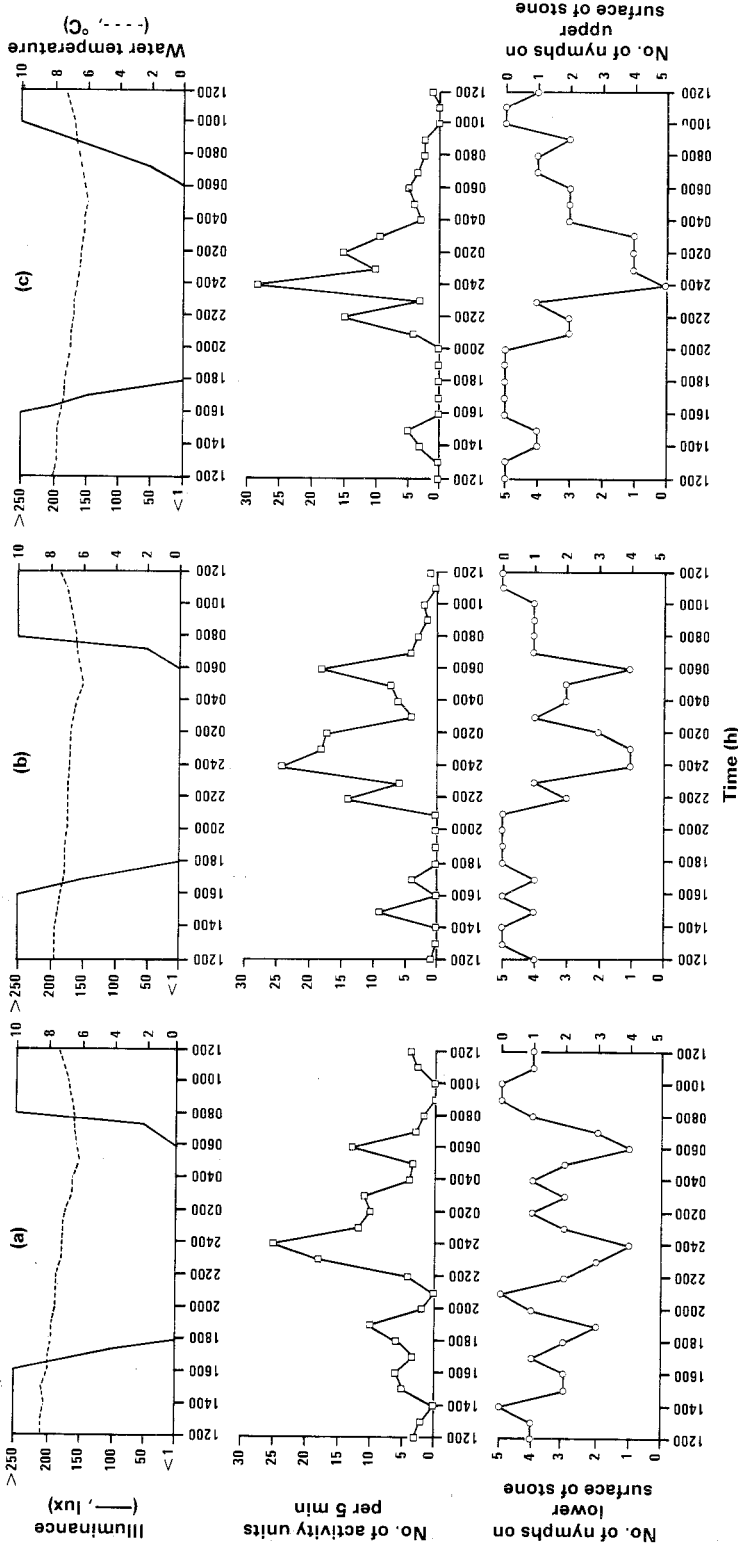


Fig. 2. Changes in light intensity and corresponding changes in activity and position on stone of five *Atalophlebitoides* sp. nymphs recorded over 24 h in a laboratory stream. Experiments were conducted on 28-29 August (expt A), 30 August-1 September (expt B) and 3-4 September 1978 (expt C).

temperatures, and oxygen concentration for all three experiments are shown in Table 1.

Figs 2a–2c show the activity of the nymphs over 24 h compared with changes in light intensity, water temperature and position of the nymphs on the stone. All times are Eastern Standard Time (EST).

Statistical analysis of variation of activity between day and night was carried out using the Mann–Whitney *U*-test. The results are presented in the following tabulation:

Experiment	<i>U</i> -statistic	<i>P</i>
A	261	<0.05
B	302	<0.05
C	288	<0.05

The nocturnal activity was clearly greater than the diurnal activity in all three experiments, with a pronounced change of activity level occurring 3 h after artificial sunset and at dawn. Table 3 shows the significant peaks of activity occurring during the night compared with mean hourly activity for the night.

**Table 3. Significant peaks of activity of *Atalophlebioides* sp. occurring during the night compared with mean hourly activity for the three experimental periods**

During the night, illumination was less than 0.10 lux

Time of activity	Experiment	$\chi^2$	<i>P</i>
2200	C	4.7	<0.05
2300	A	9.0	<0.01
2400	A, B, C	>27.0	<0.001
0100	B	10.6	<0.01
0200	B, C	9.0 <sup>A</sup> , 4.7 <sup>B</sup>	<0.01 <sup>A</sup> , <0.05 <sup>B</sup>
0600	B	7.6	<0.01

<sup>A</sup> Experiment B. <sup>B</sup> Experiment C.

Fig. 2 shows that during the period of high nocturnal activity there was movement of nymphs onto the upper surfaces of the stone. All five nymphs moved onto the upper surface once only, at 2400 h in experiment C, whereas all five nymphs were to be found on the lower surface at various times in all three experiments. While on the upper surface the nymphs appeared to be grazing on algae, and gut analysis later confirmed this.

The variance of the activity units for all three experiments were found to be homogeneous following the application of Bartlett's test of homogeneity of variance and a mean activity value was calculated for each hour. The 12 nocturnal hourly activity values were divided into three groups of 4 h, each corresponding with early, middle and late night period. The Kruskal Wallis analysis of variance with tie correction was used to analyse the three groups (Table 4). The total activity values for the early, middle and late periods were compared by  $\chi^2$ -test. The middle period was found to be significantly different from the early ( $\chi^2 = 13.135$ ,  $P < 0.010$ ) and later ( $\chi^2 = 5.65$ ,  $P < 0.05$ ) periods. No significant difference was found between early and late activity.

As the apparatus did not permit observations to be carried out on nymphs on the lower surface of the stone, the total level of activity for all nymphs over 24 h is not known.

Fig. 3c shows the hourly drift of *Atalophlebioides* sp. in Condor Creek during 5–6 September 1978, compared with the mean periodicities of nymph activity (Fig. 3b) as measured in the laboratory, and movement onto the upper surface of the stones in both laboratory and creek (Fig. 3a). The movement of nymphs onto the upper surfaces of

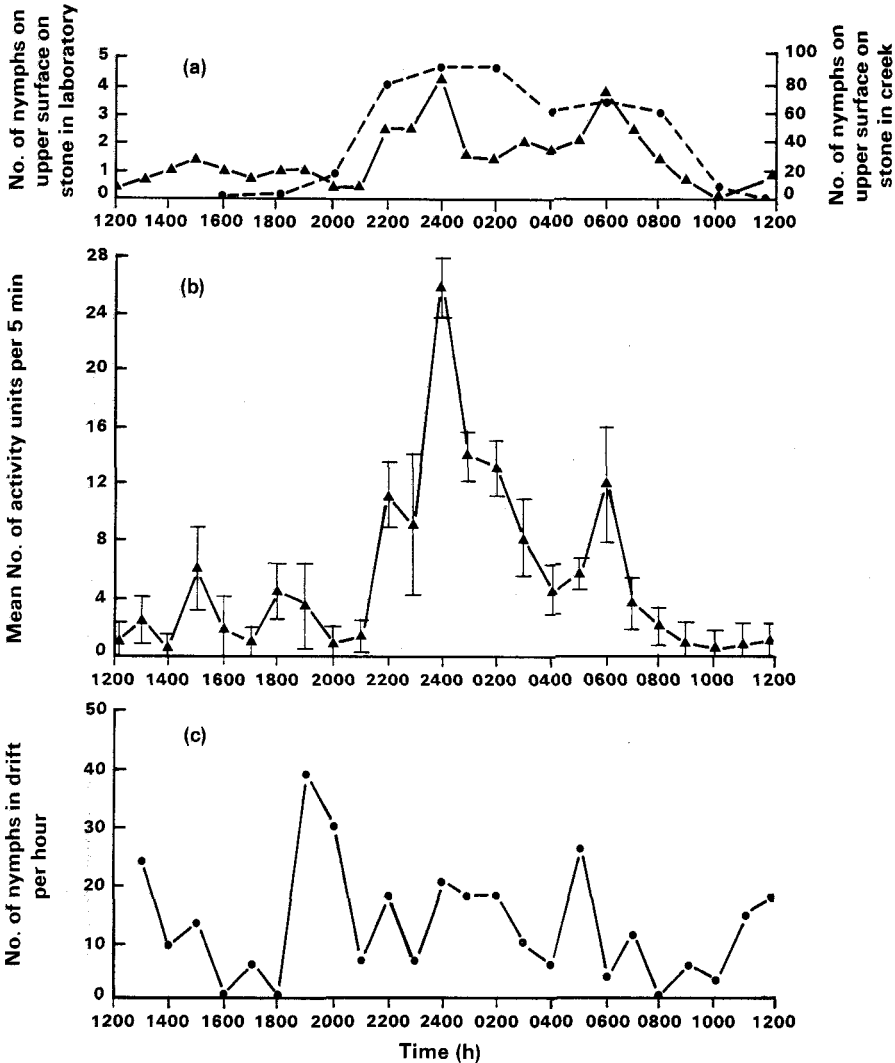


Fig. 3. Hourly variation in the number of individuals of *Atalophlebioides* sp. on the upper surface of a stone in a laboratory stream ( $\blacktriangle$ ) and the number on the upper surfaces of stones along a 20-m length of Condor Creek, A.C.T. ( $\bullet$ ) (a); in the mean number of activity units as measured in the laboratory stream (b); and in the drift numbers as sampled over 5–6 September 1978 in Condor Creek, A.C.T. (c).

the stones in the laboratory and creek showed a similar pattern although fluctuations in numbers in the creek were not so pronounced as those observed in the laboratory. The maximum level of activity, as measured in the laboratory stream, corresponded with the maximum number of nymphs on the upper surface of the rocks in both the laboratory experiment and field. Maximum drift numbers in September did not correspond with any observed increase in activity measured in the laboratory stream.

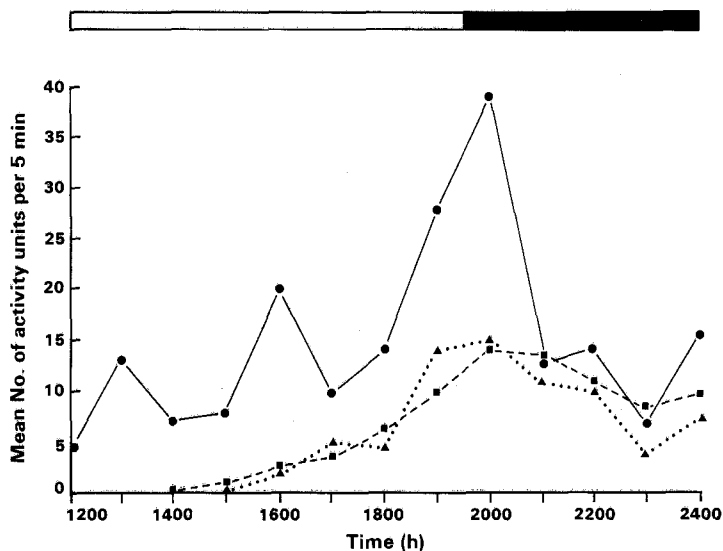
There was a degree of correlation between the initial movement of nymphs onto the upper surface of rocks measured in both the laboratory and field and the increase in drifting individuals. No statistical and thus significant analysis was carried out on the above comparison of field and laboratory observations due to lack of data.

**Table 4.** Four mean hourly activity records of *Atalophlebioides* sp. during early, middle and late periods during the night  
Early, 1800–2000 h; middle, 2300–0200 h; late, 0300–0600 h

Period	Mean hourly activity values				<i>H</i> -statistic	d.f.	<i>P</i>
Early	3.3	1	1.3	11.3	6.6153	2	<0.05
Middle	9	26	14	13.6			
Late	8	4.6	5.3	12.3			

#### *Changes in Diel Activity Patterns of Atalophlebioides* sp. as a Result of Density Alterations

The levels of activity over 12 h for all three experiments were tested for homogeneity of variance with Bartlett's test. As the variances were found to be homogeneous ( $P > 0.75$ ) the three experiments were combined to provide mean values. Fig. 4 shows



**Fig. 4.** Comparison of changes in number of mean activity units per 5 min of *Atalophlebioides* sp. nymphs at three different density levels (15 (●), 5 (■) and 2 (▲) nymphs per stone) over a 12-h period (1200–2400 h). Dark region of horizontal bar indicates period of darkness (<0.5 lux).

the mean activity units per animal for the three different densities over 12 h. The nymphs under the high density ( $n = 15$ ) were active on the upper surface of the stone over the 12 h observed. The level of activity each hour, up to and including 2000 h, was significantly different from the two lower densities ( $\chi^2 = 21.2$ ,  $P < 0.01$ ). After 2000 h no significant difference was found between the level of activity at each density. Over the entire 12 h no significant difference was observed between the activity levels at

densities of two and five nymphs per stone. At these two densities the main behavioural activity seemed to be grazing on the algae on the upper surface of the rock; gut analysis later confirmed this.

At the highest density ( $n = 15$ ) less time was spent in feeding activity but two additional activities were observed. The first was a rapid, dart of short duration ( $< 1 \cdot 0$ s) across the stone, usually from the lower surface to either the side or upper surface of the stone. The second activity was a 'swimming' response. Nymphs would suddenly launch themselves from the stone, quite often from a lower surface, and swim to the water surface with a very characteristic 'jerky' motion of the body. On reaching the surface the nymph would remain motionless with all six legs and anal cerci spread out in the lateral plane (Fig. 5*b*), and then slowly sink to the bottom, whereupon the

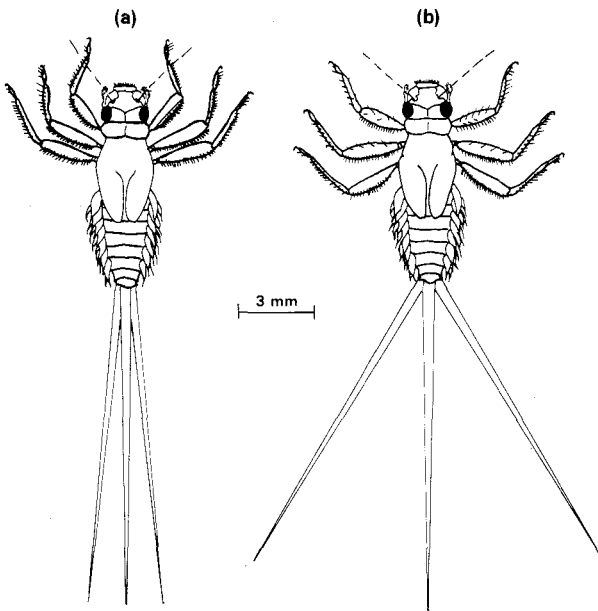


Fig. 5. Positioning of legs and anal cerci of *Atalophlebioides* sp. nymphs during swimming (a) and passive (b) phase of the swimming behaviour. See text for description.

whole process could be repeated. If, on floating down towards the bottom, the legs or anal cerci came into contact with the rock, the nymph very rapidly orientated itself towards the rock and clung to the side momentarily and then moved rapidly to the lower surface. Figs 5*a* and 5*b* show the relative positions of legs and anal cerci during the swimming and floating phase, respectively, of the swimming response. The swimming response was observed mainly during the light hours but occasionally at night (see Table 5). McLay (1968) also observed a swimming response in mayfly nymphs while observing *Deleatidium* sp.

## Discussion

A diel periodicity is defined as the recurrent temporal pattern of activity with a 24-h period (Corbert 1966). In the experiments carried out on *Atalophlebioides* sp. two periodicities are recognized: (i) the movement of nymphs onto the upper surface of the rock, and (ii) the activity of the nymph. The first periodicity was nocturnal in flowing water and is similar to that shown by Elliott (1968). The negatively phototactic behaviour of mayfly nymphs is well known (Harker 1958; Elliott 1968) but, in the

present study, it was observed that a number of the nymphs did not show an immediate negative response to increase in light intensity (see Fig. 2). Other nymphs did not show any photonegative behaviour, that is they were present on the upper rock surface in all light intensities. Harker (1953) found no negative phototactic response in mayfly nymphs confined to still water. The secondary periodicity was also nocturnal in flowing water and followed normal diel fluctuations in light intensity. However, the occurrence of significant peaks of activity (see Table 3) throughout the night period in the absence of light variations is consistent with the possibility that they are controlled by an endogenous rhythm. Other possible exogenous cue factors—changes in water temperature or, as Brown (1962) put it, some unknown geophysical variable—may be involved in timing of nocturnal activity. Obviously, further experimentation is required to identify such relationships in regards to *Atalophlebioides*.

**Table 5.** Numbers of *Atalophlebioides* sp. nymphs on the upper surface of a stone, active, or swimming at three different densities from 1200 to 2400 h

Illumination between 1200 and 2000 h, > 5 lux; between 2000 and 2400 h, < 5 lux

Time (h)	No. on upper stone surface at density (No. per stone):			No. active at density (No. per stone):			No. 'swimming' at density (No. per stone):		
	15	5	2	15	5	2	15	5	2
	1200	4	0	0	3	0	0	1	0
1300	4	0	0	4	0	0	2	0	0
1400	5	1	0	5	1	0	2	0	0
1500	3	1	1	3	1	1	1	0	0
1600	8	2	1	8	1	1	0	0	0
1700	6	3	1	5	2	1	3	0	0
1800	7	2	1	6	2	1	1	0	0
1900	10	3	2	9	3	2	1	0	0
2000	13	4	2	13	4	2	0	0	0
2100	10	4	2	7	4	2	1	0	0
2200	11	5	1	7	4	1	0	0	0
2300	10	4	2	6	4	2	0	0	0
2400	12	4	2	7	3	2	1	0	0

Corbet (1966) and Elliott (1968) both note that very few workers have integrated field and laboratory studies when they have attempted to resolve a periodicity observed in the field into its different components. Although the experiment, mentioned earlier, of comparing laboratory and field observations was not considered to be significant due to lack of data, it is interesting that while the movement of nymphs onto the upper surface of rocks followed a similar diel periodicity in both the laboratory and field (Fig. 3a), the nocturnal periodicities of drift (Fig. 3c), as measured in the stream, and activity, as measured in the laboratory, followed a different pattern. And thus it would appear that the drift of *Atalophlebioides* sp. does not result from normal diel activity, but requires additional environmental stimuli (e.g. density of individuals in benthos) that promote further behavioural activity.

The importance of mayfly density has been examined, mainly in relation to downstream drift (Waters 1965; Dimond 1967; Lehmkuhl and Anderson 1972; Hildebrand 1974; Kovalak 1978). In the present study the effect of increased density (as measured by number of individuals per stone) on normal diel activity patterns can

be seen. The normal photonegative response which is observed in the lower densities is absent, with a number of individuals moving on the upper surface (see Table 5) throughout the daytime period.

Kovalak (1978) studied the diel changes of benthos density on natural and artificial rocks and concluded that the nocturnal increase in density on the top of the rocks resulted from movements from areas of low oxygen concentration to those of higher concentration.

In the present study the water was still, although well aerated, and this may have promoted physiological stress on the mayfly nymphs. The constant, high level of activity, however, in the tank with 15 nymphs would suggest that space may be of significant importance. In the field, mayfly larvae must remain flattened against a stone to prevent them being washed downstream. This would suggest a minimum area of stone surface per nymph. If this critical area is reduced by encroachment by other individuals, collision and loss of attachment can occur. Thus the physical interference would be proportional to numbers of individuals (interspecific) present. McLay (1968) suggested a similar mechanism for density having an indirect effect on the composition of the drift. He supported his argument by including this type of interaction under Birch's (1957) second meaning of competition: interference when there is no shortage of a resource.

From the present study it is postulated that if competition for space is taking place, those animals that are dislodged or stimulated by contact with others may respond with swimming behaviour. This swimming up would cause the nymph to be carried along with the current until it reached a suitable substrate. The passive position adopted by the nymphs possibly prevents structural damage. The spread posture does, however, ensure that the maximum number of sensory appendages are spaced over the widest possible area. In addition, as the nymph is normally thrown against the substrate by the current (Madsen 1968, 1969) rather than actively searching out favourable substrate, the open posture will assist in obtaining immediate purchase on the rock and prevent the nymph from being washed away again. Also the spread position adopted would give a greater surface area, aiding movement downstream, that is drift.

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