

# Clouds, shadows or twilight? Mayfly nymphs recognise the difference

ANNETTE L. SCHLOSS\* AND JAMES F. HANEY<sup>†</sup>

\*Complex Systems Research Center, Institute for the Study of Earth, Oceans and Space, University of New Hampshire, Durham, NH, U.S.A.

<sup>†</sup>Department of Zoology, University of New Hampshire, Durham, NH, U.S.A.

## SUMMARY

1. We examined the relative changes in light intensity that initiate night-time locomotor activity changes in nymphs of the mayfly, *Stenonema modestum* (Heptageniidae). Tests were carried out in a laboratory stream to examine the hypothesis that nymphs increase their locomotion in response to the large and sustained reductions in relative light intensity that take place during twilight but not to short-term daytime light fluctuations or a minimum light intensity threshold. Ambient light intensity was reduced over a range of values representative of evening twilight. Light was reduced over the same range of intensities either continuously or in discrete intervals while at the same time nymph activity on unglazed tile substrata was video recorded.

2. Nymphs increased their locomotor activity during darkness in response to large, sustained relative light decreases, but not in response to short-term, interrupted periods of light decrease. Nymphs did not recognise darkness unless an adequate light stimulus, such as large and sustained relative decrease in light intensity, had taken place.

3. We show that nymphs perceive light change over time and respond only after a lengthy period of accumulation of light stimulus. The response is much lengthier than reported for other aquatic organisms and is highly adaptive to heterogeneous stream environments.

*Keywords:* benthic activity, diel cycles, light change, mayflies, streams

## Introduction

Diel changes in the activities of stream macroinvertebrates, in particular mayflies, take place during morning and evening twilight, strongly implicating the twilight light environment as a factor initiating these changes (Müller, 1966; Waters, 1972). Evening twilight, the focus of this study, is characterised by large and sustained decreases in light intensity followed by an extended period of darkness beginning at nightfall. One or both of these aspects of evening twilight have been considered as external

cues for the timing of diel changes in the activities of mayfly nymphs, namely locomotor activity, vertical movements on rocky substrata and drift (Holt & Waters, 1967; Elliott, 1968; Bishop, 1969; Chaston, 1969; Haney *et al.*, 1983). Yet, in streams, large fluctuations in light intensity and low light levels are not confined to twilight. On any given day, the daytime light environment varies in time and space, a consequence of canopy cover (Davies-Colley & Payne, 1998) and the passing of overhead clouds (Casey, 1987). Ambient light intensity varies considerably among rocky substrata, so that organisms within even the smallest localised patches may be exposed daily to a range of light environments.

There is no doubt that mayfly nymphs are capable of responding to light changes throughout the 24-h period. Departures from typical diel drift patterns

---

Correspondence: Annette L. Schloss, Complex Systems Research Center, Institute for the Study of Earth, Oceans and Space, 446 Morse Hall, University of New Hampshire, Durham, NH 03824, U.S.A. E-mail: annette.schloss@unh.edu

have been observed during natural events such as solar eclipse (Cadwallader & Eden, 1977) or a full moon (Bishop & Hynes, 1969). In laboratory studies, nymphs reduced their locomotor activity and vertical movements on the substratum under continuous illumination (Chaston, 1968; Elliott, 1968; Bishop, 1969) and entered the drift when the light environment was artificially darkened (Holt & Waters, 1967; Bishop, 1969). We have observed increased daytime locomotor activity in *Stenonema modestum* (Banks) nymphs in a laboratory stream following prolonged darkening of the sky during thunderstorms (A.L. Schloss, unpublished data).

The expression of diel behaviours in mayflies depends on exposure to fish or their odours (McIntosh & Townsend, 1994; Huhta *et al.*, 1999). However, once initiated, diel behavioural cycles are controlled by light. It is critically important that nymphs recognise the difference between transient daytime light changes and twilight because they must move to exposed locations to feed while at the same time avoiding multiple predators (Culp, Glozier & Scrimgeour, 1991; Cowan & Peckarsky, 1994; Huhta *et al.*, 1999). Although there is little evidence that nymphs respond to the daily occurrences of short-term light fluctuations, observations do suggest that they monitor light continuously and respond to cues appropriate to their lifestyle.

We have proposed that the large and sustained relative changes in light intensity during twilight provide a light stimulus for initiating diel changes in the locomotor activity of mayfly nymphs (Schloss & Haney, 2002). Our model predicts that nymphs time their evening locomotor activity based on the rate and duration of relative light decrease. In other words, nymphs perceive how fast or slowly the light is decreasing and they respond once an adequate light stimulus has accumulated over time. Mayflies respond earlier when relative light changes (RLC) are more rapid. This stimulus–response system is recognised as a mechanism for regulating diel vertical migration (DVM) in other aquatic species, including water fleas (*Daphnia* spp.; Ringelberg, 1964, 1999), phantom midges (*Chaoborus punctipennis* Say; Haney *et al.*, 1990), marine copepods (*Acartia tonsa* Dana; Stearns & Forward, 1984) and crab larvae (*Rhithropanopeus harrisi* Gould; Forward, 1985). Plankton respond to light stimulus by changing swimming speed and direction, whereas mayflies alter their rate

of locomotion and may also use light stimulus to initiate vertical movements on the substratum (Schloss & Haney, 2002) and for timing evening drift (Haney *et al.*, 1983).

The stimulus–response mechanism described by our model can account for both diel activity changes in mayflies and their insensitivity to daytime light fluctuations. The model predicts that a combination of large plus sustained decreases in light intensity during twilight is necessary to provide an adequate light stimulus that initiates the locomotor activity response. Consequently, short-term daytime light fluctuations caused by shadows and clouds may not provide an adequate light stimulus. Unusual events, such as solar eclipse or large gatherings of storm clouds, may or may not provide an adequate light stimulus, depending on the strength and duration of RLC during each event. The adequacy of a light stimulus event depends on whether or not the animals can accumulate the amount of light stimulus necessary to trigger a response. We have measured delays between the onset of light stimulus and responses in *S. modestum* of several minutes to an hour or more (Schloss & Haney, 2002), suggesting that many transient daytime light fluctuations are not adequate for building the necessary accumulation. Implicit in the model is that the animals' response to light change is independent of ambient light intensity; thereby, the model assumes that lower ambient light intensity is not a reliable predictor of impending darkness. By separating the effects of light intensity and RLC on the locomotor activity response, the model also accounts for the ability of animals to reside in a very heterogeneous light environment, but still use light cues to time their diel activities.

We set out to further examine the locomotor response of *S. modestum* nymphs to light by testing the following null hypotheses: (i) locomotor response is the same in sustained and fluctuating light change, that is the net accumulation of light stimulus is the same regardless of the number of discrete light change episodes; (ii) rate of RLC does not influence the magnitude of the locomotor response, that is the strength of the light stimulus does not alter the rate of locomotion; and (iii) ambient light intensity does not affect the locomotor response, in other words the response is independent of absolute light intensity. Tests were carried out in the laboratory using a computer-manipulated light environment so that the

effects of both strength and duration of light stimulus and ambient light intensity could be examined under well-defined light conditions. Controlled light changes were used in place of natural light to separate each of these three effects on the locomotor activity response of the nymphs.

## Methods

### Laboratory setup

Tests were carried out in a clear acrylic laboratory stream located in the Anadromous Fish and Aquatic Insect Research (AFAIR) Laboratory at the University of New Hampshire in Durham, NH, U.S.A., during July and August 1996. *Stenonema modestum* nymphs, excluding last instars, were collected on the morning of each test from the nearby Oyster River, a third order stream. Nymphs were videotaped during light-change scenarios from the undersides of unglazed tiles placed in two stream channels (tile dimensions  $10 \times 10 \times 0.5$  cm, raised 0.5 cm above the streambed). Channels were filled to a depth of 10 cm with well water that was circulated from a common tank at a flow rate of  $5 \text{ cm s}^{-1}$ . This was within the range of velocities at the collection site. The water was continuously filtered (150- $\mu\text{m}$  net), aerated over upstream barriers ( $\text{O}_2$  saturation =  $93 \pm 4\%$  SD) and maintained at  $18 \pm 2.0$  °C SD. Fish odour was added to the water to enhance behavioural responses in the nymphs by keeping fish collected from the river in the tank throughout the test period [two *Luxilus cornutus* Mitchill (formerly *Notropis*) and *Rhinichthys cataractae* Valenciennes, at a density of  $10 \text{ fish m}^{-3}$ ]. Periphyton-covered pebbles (2–4 cm diameter) were placed on top of the tiles as a source of food for the nymphs. Six nymphs were placed on each tile for a total of 12 nymphs per test. This was representative of natural densities found in the Oyster River.

The entire stream was enclosed in black plastic to block out all natural light. Ambient light was generated by four computer-controlled 500 W halogen lamps covered with blue filters that simulated a natural daytime distribution of wavelengths (GamColor, New York, NY, U.S.A., daylight blue gel filter media). The undersides of the tiles were illuminated by an array of wide-angle GaAlAs infrared-emitting LEDs ( $940 \pm 20$  nm). Nymphs reportedly are not sensitive to far infrared light

(Heise, 1992). Ambient light intensity was continuously monitored and recorded once every minute with an International Light IL-1700 radiometer (Newbury, MA, U.S.A.), SED033 probe with 2-pi collector corrected for cosine response, placed at the water level adjacent to the tiles and facing upwards. Schloss (2002) describes the light control system in detail. For each test, animals were held at the starting light intensity for a minimum of 6 h. Videotaping began 60-min before the start of each light-change scenario and continued for 60-min following the onset of darkness. Light reductions began at 18.00 hours Eastern Standard Time (EST).

### Experimental design

*Overview.* Our approach was to design a series of tests to distinguish between: (i) different strengths of light stimulus ( $S$ ); and (ii) the effects of sustained and interrupted light decreases, while applying the same total light stimulus during each test. Light stimulus was provided by reducing light intensity at a particular rate of light change (RLC) over a range of light intensities between 600 and  $0.07 \mu\text{W cm}^{-2}$ . The high and low values are typical of light intensities measured during the summertime at the collection site near noontime and 1-h post-sunset. In our earlier study, nymphs exhibited diel changes in their behaviour when light intensity was reduced over this range of light intensities (Schloss & Haney, 2002).

*Stimulus strength.* To determine the effect of stimulus strength on the light response, tests were carried out at three different values of RLC:  $-1.7$ ,  $-2.5$  and  $-3.6 \times 10^{-3} \text{ s}^{-1}$ . These values are within the range of RLC values that occur in the period of rapid light changes during natural twilight. The low value is equal to the minimum RLC that has been shown to induce positively phototactic swimming behaviour in the water flea *Daphnia*, and thus, the onset of DVM (Ringelberg, 1964). Relative changes in light intensity ( $\text{s}^{-1}$ ) were determined according to Ringelberg (1964) as:

$$\text{RLC} = \frac{1}{I} \frac{dI}{dt} \quad (1)$$

where  $I$  is the light intensity in  $\mu\text{W cm}^{-2}$  and  $dt$  is the time interval in s.

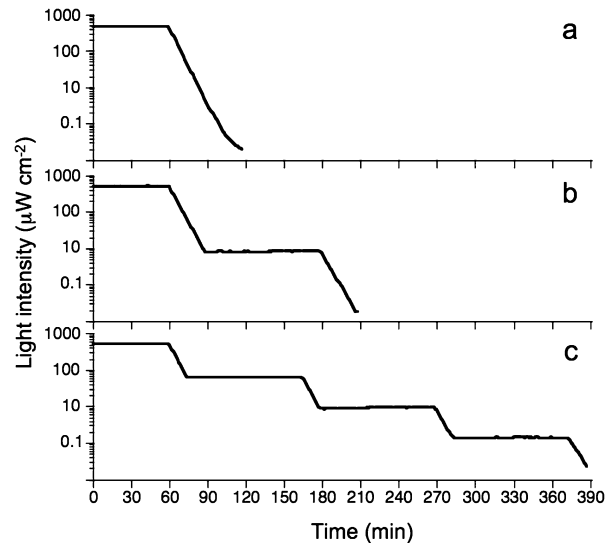
*Periods of sustained light stimulus.* To determine the effect of continuous and discrete episodes of light stimulus ( $S$ ) on the animals' light response, tests were carried out in which light intensity was reduced over the experimental range of light intensities either continuously or over increasingly shorter time intervals. Time intervals were based on our empirical model for the length of time necessary at each value of RLC to accumulate an adequate light stimulus, determined as:

$$\ln(LP) = -1.3156 - 0.7762 \times \ln(|RLC|) \quad (2)$$

where RLC is measured in  $s^{-1}$  and LP is the delay in the response in min, also known as the 'latent period' (Ringelberg, 1964). As used here, the term latent period represents the amount of time required to build up an excitatory state in the animal. The excitatory state results in a measurable reaction. In this study, the excitatory state was caused by decrease in light intensity (e.g. light stimulus) and the measured reaction was an increase in locomotor activity. The delay in building up the excitatory state may be related to physiological limitations such as the latency in membrane conductance leading to an electric potential that has been described for invertebrate photoreceptors (Devoe, 1985) or may be a result of neuromodulation of membrane conductance, a current topic of interest in invertebrate behavioural research (Birmingham & Tauck, 2003).

The excitatory state is built up in proportion to the stimulus strength, but also leaks away (or decays) at a constant rate (Ringelberg, 1964). This is analogous to the storing-up and leakage of charge in an electrical circuit (Järvilehto, 1979). Therefore, the length of the LP depends on both the build-up and decay rates of light stimulus (Ringelberg, 1964). The rate of decay, also known as the disintegration constant, was calculated for *S. modestum* as  $3.3 \times 10^{-5} s^{-1}$  (Schloss & Haney, 2002).

Light intensity was reduced over time intervals that were longer than ( $S > LP$ ), equal in length ( $S = LP$ ) or shorter than ( $S < LP$ ) the latent periods (Fig. 1). For the  $S > LP$  tests, light intensity was decreased at each value of RLC without interruption over the full range of light intensities (Fig. 1a, Table 1). During all other tests, periods of light reduction were broken up by 90-min periods of no light change, thereby partitioning the same total amount of light stimulus into



**Fig. 1** Graphic representation of continuous and discrete episodes of light stimulus ( $S$ ) during the tests. Light stimulus was generated by decreasing light intensity at a particular rate (RLC) during each test. Panels represent ambient light intensity versus time for which (a)  $S$  was sustained over the full experimental range of light intensities ( $S > LP$ ); (b)  $S$  was sustained over two discrete episodes each equal in length to one latent period ( $S = LP$ ); and (c)  $S$  was sustained over four discrete episodes each equal in length to one-half latent period ( $S < LP$ ). For simulations (b) and (c), 90-min periods of no light change were interspersed with  $S$  until light intensity was reduced to the minimum value. The graphic represents the amount of time necessary to complete the reduction in light intensity at relative light change (RLC) =  $-2.5 \times 10^{-3} s^{-1}$ . Time for other values of RLC used in this study are listed in Table 1.

**Table 1** Number of minutes light stimulus ( $S$ ) was sustained at each value of relative light change (RLC) during tests of the light response of *Stenonema modestum*

Model (predicted) values		Test values		
RLC ( $s^{-1} \times 10^{-3}$ )	LP (min)	$S < LP$ (min)	$S = LP^*$ (min)	$S > LP$ (min)
-1.7	38	21	41	83
-2.5	28	15	29	58
-3.6	21	10	21	41

The predicted minimum number of minutes necessary to initiate a response (i.e. latent period, LP) is listed for each value of RLC. The equation for calculating the latent periods is contained in the *Methods* section of the text.

\*Tests are within 4% of the modelled LP times.

discrete episodes (Fig. 1b,c, Table 1). The  $S > LP$  tests were comparable to those we used to simulate light changes during evening twilight and to determine the

LP (Schloss & Haney, 2002), and were considered as the reference condition.

*Ambient light intensity.* To determine the effect of ambient light intensity on the light response, tests were carried out in which animals were held at a reduced light intensity of  $8.0 \mu\text{W cm}^{-2}$ . This value is typical of light intensities measured at the nymph collection site near the time of sunset during the summertime. For these tests, light stimulus was applied in one discrete episode equal to the length of the LP ( $S = \text{LP}$ ).

#### Measurements of activity

Locomotor activity of each nymph was measured from the time-lapse video frames (recording speed =  $1 \text{ frame s}^{-1}$ , time compression = 1 : 72) as the distance the nymph moved during consecutive 1-min time intervals. The mean locomotor activity was calculated for each tile (there was one tile in each of two stream channels) by summing the individual distances in each 1-min interval and dividing by the number of nymphs that were counted on the lower tile surface during the interval. These estimates represent only the activity of nymphs that were visible on the lower tile surfaces and contain no information about the activity of nymphs that were out of the view of the camera. Previous videotapes of the upper tile surfaces showed that during the evening, nymphs moved about on the upper tile surfaces and spent time grazing (A.L. Schloss, unpublished data). Measurements of activity are fully described in Schloss (2002).

Values of locomotor activity for each 60-min daylight period were grouped into a daytime measure. Values for each 60-min dark period were grouped into a night-time measure. For tests in which light stimulus was partitioned into smaller discrete episodes, values from the first 60 min of each 90-min period of no light change were grouped into intermediate measures, so that those periods could be directly compared with the daytime and night-time measures.

#### Statistical analysis

*Stenonema modestum.* exhibited diel activity patterns in both locomotor activity and vertical location on the substratum. Because these two activities may have

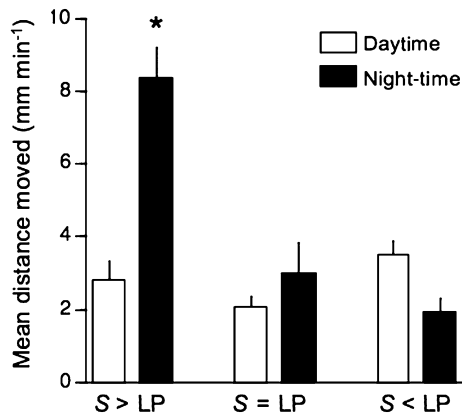
been correlated, Spearman rank correlations were performed. Correlations were both positive and negative. In 16 of the 24 tests (67%), correspondence between the two variables was either not significant or was less than 30%. From these statistics, we concluded that the amount of locomotor activity and the number of nymphs under the rocks were not coupled.

Changes in nymph locomotor activity in response to light change were examined using repeated measures ANOVA. The experimental factors were RLC (three strengths:  $-1.7$ ,  $-2.5$  and  $-3.6 \times 10^{-3} \text{ s}^{-1}$ ) and the partitions of sustained light stimulus (three levels:  $S > \text{LP}$ ,  $S = \text{LP}$  and  $S < \text{LP}$ ). For the ambient light intensity tests, the experimental factor was the starting light intensity (two levels: high and low). For all tests, the repeated variable was time (two levels: daytime and either night-time or intermediate period between discrete episodes of  $S$ ). A total of 12 tests were carried out in random order, with two replicates per test. Nine tests were carried out using nymphs held at the brightest light intensity (three strengths of  $\text{RLC} \times$  three partitions of  $S$ ) and three tests were carried out using nymphs held at the reduced light intensity (three strengths of  $\text{RLC} \times$  one partition of  $S$ ).

There were differences in the daytime locomotor activity among tests that made it difficult to directly compare results (see Schloss & Haney, 2002). To overcome this difficulty, locomotor activity was normalised to the minimum and maximum value for all the tests combined and then transformed using the square root transformation of Zar (1999). All data used in the repeated measures ANOVA tests met the Hyunh-Feldt conditions regarding compound symmetry of the covariance matrices, as described by Potvin, Lechowicz & Tardif (1990). Statistical tests were performed using SAS (V5.0, SAS Institute, Cary, NC, U.S.A.).

#### Results

The change from daytime to night-time locomotor activity in *S. modestum* depended on both the accumulation of light stimulus and exposure to continuous light change. Nymphs responded differently when the same total amount of light stimulus was applied continuously or was partitioned into discrete intervals (Fig. 2). Nymphs significantly increased their night-time locomotor activity during



**Fig. 2** Mean locomotor activity of *Stenonema modestum* during daytime and night-time periods following application of light stimulus ( $S$ ) at all values of RLC over the full experimental range of light intensities either continuously ( $S > LP$ ) or partitioned into progressively shorter discrete episodes ( $S = LP$ ,  $S < LP$ ). Bars show actual mean distances moved by the nymphs ( $\text{mm} \pm \text{SE}$ ,  $n = 6$  tests). Paired bars were compared with repeated measures ANOVA; significant differences at  $P < 0.05$  are marked (\*). Statistics were performed on normalised, transformed data (see *Methods*).

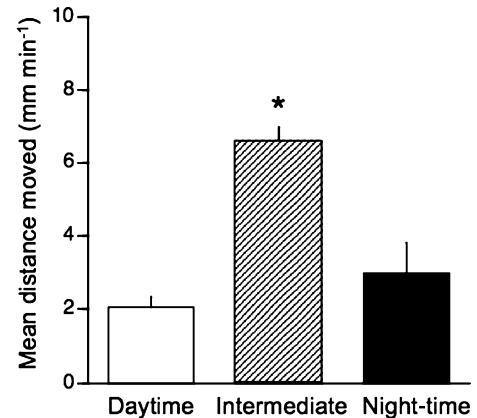
the tests when light stimulus was sustained over the entire experimental range of light intensities, i.e. where  $S > LP$  (Fig. 2). When light stimulus was partitioned into discrete episodes that were just adequate, i.e. where  $S = LP$ , night-time locomotor activity was higher, but not significantly higher, than daytime activity (Fig. 2). Further partitioning of light stimulus into shorter intervals, i.e. where  $S < LP$ , brought about no increase between daytime and night-time levels of activity (Fig. 2). These results, summarised by the repeated measures ANOVA (Table 2, significant Time  $\times$  LP term), support our hypothesis that the accumulation over time of light stimulus is the proximate cue for diel changes in locomotor activity in these mayflies.

The stimulus-response model did not predict the lack of a response to a 'just adequate' light stimulus. According to the model, animals should have significantly increased their locomotor activity as soon as an adequate light stimulus had accumulated. During the  $S = LP$  tests, nymphs were exposed to two separate episodes of 'just adequate' light stimulus (Fig. 1b). They responded to the first episode by increasing their locomotor activity significantly above the daytime level during the intermediate period of no light change (Fig. 3). Nymphs did not increase their

**Table 2** Repeated measures ANOVA comparison of mean locomotor activity during the 60-min daytime and night-time periods following application of light stimulus over the full experimental range of light intensities

	d.f.	MS	F	P
Between subjects				
RLC	2	0.006	1.07	0.38
LP	2	0.014	2.63	0.13
RLC $\times$ LP	4	0.015	2.88	0.09
Error	9	0.005		
Within subjects				
Time	1	0.008	3.60	0.09
Time $\times$ RLC	2	0.001	0.54	0.60
Time $\times$ LP	2	0.016	7.52	0.01
Time $\times$ RLC $\times$ LP	4	0.004	2.12	0.16
Error (time)	9	0.002		

Factors were relative light change (RLC) and discrete episodes of light stimulus partitioned into portions of the latent periods (LP).



**Fig. 3** Mean locomotor activity of *Stenonema modestum* during daytime and the periods following light stimulus ( $S$ ) at all values of RLC sustained over the length of one latent period ( $S = LP$ ). Significant differences at  $P < 0.05$  between daytime activity and each period of no light change by repeated measures ANOVA are marked (\*). Statistics were performed on normalised, transformed data (see *Methods*).

locomotor activity any higher in response to the second light stimulus nor did their initial increase in activity persist into the night-time (Fig. 3). We cannot determine from our tests if the additional light stimulus had an inhibitory effect on locomotor activity or if the 'just adequate' stimulus was capable only of triggering a short-lived response.

Strength of the light stimulus did not affect the locomotor activity response (Table 2, non-significant RLC and Time  $\times$  RLC terms). Nymphs responded the same across the three test values of RLC by either

increasing their night-time locomotor activity when the stimulus was adequate (i.e.  $S \geq LP$ ) or not responding when stimulus was inadequate (i.e.  $S < LP$ ). These results support our hypothesis that animals accumulate light stimulus at a rate proportional to the rate at which light decreases and thereby do not distinguish between slow and rapid changes in light intensity in their reaction to falling light levels.

Ambient light intensity did not alter the change from daytime to night-time locomotor activity. Nymphs held at reduced light intensity responded similarly to a 'just adequate' light stimulus as nymphs held at high light intensity (Fig. 4). Their reactions were indistinguishable even though ambient light intensity was the same at the end of light stimulus during the high light tests as at the start of light stimulus in the reduced light tests. These results, summarised by the repeated measures ANOVA (Table 3, significant Time term, all non-significant *Ambient* terms), support our hypothesis that the locomotor activity response is independent of light intensity. Additionally, because nymphs did not increase their night-time locomotor activity during all the tests (Fig. 2), we determined that they neither anticipated the onset of darkness based on low light conditions nor utilised a minimum light intensity threshold as a cue to initiate diel changes in locomotor activity. The daytime activity of nymphs held at reduced light intensity was considerably higher than that of nymphs held at high light intensity (Fig. 4),

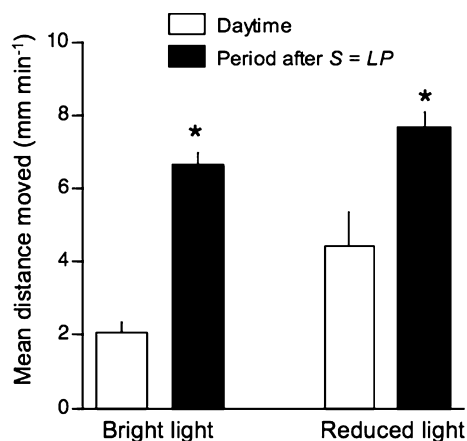


Fig. 4 Mean locomotor activity of *Stenonema modestum* held at bright and reduced light intensity during daytime and periods immediately following light stimulus ( $S$ ) sustained over the length of one latent period ( $S = LP$ ). Bars and statistics are as described in Fig. 2.

Table 3 Repeated measures ANOVA comparison of mean locomotor activity during the 60-min daytime and 60-min periods following application of light stimulus for the length of one latent period ( $S = LP$ )

	d.f.	MS	F	P
Between subjects				
RLC	2	0.004	0.70	0.53
Ambient	1	0.008	1.47	0.27
RLC × Ambient	2	0.010	2.11	0.20
Error	6	0.006		
Within subjects				
Time	1	0.04	16.6	<0.01
Time × RLC	2	0.007	3.35	0.10
Time × Ambient	1	0.002	1.07	0.34
Time × RLC × Ambient	2	0.000	0.04	0.96
Error (time)	6	0.002		

Factors were relative light change (RLC) and starting light intensity (Ambient).

suggesting that light intensity may play a role in the magnitude of but not the change in nymph activity.

## Discussion

Our study clearly demonstrates that mayfly nymphs distinguish among light change events, responding only to those representative of twilight and not to transient or inconsequential events. They measure light change rather than absolute light intensity and respond only when light reductions at dusk (and presumably, light increases at dawn) are sustained over a relatively long time period. Thus, changes in light-controlled activities such as locomotion, vertical location on the substratum and drift take place on a diel basis and not as a consequence of the short-term daytime light fluctuations that routinely occur in streams.

The response times to light stimulus we measured for *S. modestum* contrast sharply with those reported for *Daphnia*, a pelagic organism for which locomotor responses to relative change in light intensity have been well documented (Ringelberg, 1964, 1999). *Daphnia* use RLC to regulate the onset, swimming speed and direction of DVMs in lakes. As with diel behaviours in mayflies (Flecker, 1992; McIntosh & Townsend, 1994; Huhta *et al.*, 1999), DVM is considered an avoidance response to visually hunting fish predators (Zaret & Suffern, 1976; Gliwicz, 1986; Lampert, 1993). *Daphnia* respond to light stimulus after LP of several seconds to a few minutes (Ringel-

berg, 1964). These are considerably shorter than the several minutes to an hour or more we previously measured in *S. modestum* (Schloss & Haney, 2002) and verified in this study. The rate of decay or leakage of light stimulus in *Daphnia* is also more rapid than in *S. modestum*, by about two orders of magnitude ( $2-7 \times 10^{-3} \text{ s}^{-1}$  for *D. magna* Straus and *D. longispina* Müller, Ringelberg, 1964; J. Ringelberg, personal communication versus  $3.3 \times 10^{-5} \text{ s}^{-1}$  for *S. modestum*, Schloss & Haney, 2002). These differences suggest that both accumulation and leakage of light stimulus are important to the animals' light response. We examined environmental conditions, role of locomotion in daily activities and life histories of both organisms to understand adaptive strategies that may account for their widely differing responses to external light cues.

*Daphnia* maintain their vertical position in the water column by swimming continuously in a 'hop-and-sink' motion (O'Keefe, Brewer & Dodson, 1998). They use downwelling light to maintain depth during the daytime and for orienting directional swimming up and down (Ringelberg, 1999). Because there may be a considerable distance, 2–20 m, between their daytime light refugium and predators located in the upper waters (Gliwicz, 1986; Lampert, 1989), *Daphnia* can move up and down some distance without greatly increasing their risk of predation. Small vertical movements, such as the *Daphnia* hop-and-sink, result in predictable changes in light intensity encountered by the animal that limit the vertical distance travelled at inappropriate times (Daan & Ringelberg, 1969). To complete upward migrations, *Daphnia* must increase their swimming speed (Van Gool & Ringelberg, 2003), which is accomplished during twilight as a locomotor response to the exponential increases in the relative rate of light change. Van Gool & Ringelberg (1997, 2003) labelled these exponential changes, which are a consequence of the sun's movement during twilight, as the 'acceleration' in RLC. *Daphnia* complete their upward migration only during light change events at twilight, producing the observed diel cycles.

In contrast, mayfly nymphs may enter the drift (Waters, 1972) or move about intermittently on rocky substrata either in search of food (Kohler & McPeck, 1989; Wilzbach, 1990) or to avoid encounters with benthic-foraging predators (Peckarsky, 1980; Culp *et al.*, 1991). Constant locomotion is not a necessity for maintaining position on the substratum (Wilzbach, 1990), nor desirable, because nymphs that leave

cryptic hiding places, unlike *Daphnia*, have a high likelihood of immediate exposure to one or more fish or invertebrate predators (McIntosh & Townsend, 1994; Scrimgeour & Culp, 1994; Peckarsky, 1996).

Reproductive strategies are very different in mayflies and *Daphnia*. In temperate zones, parthenogenic daphnids reproduce throughout the season, whereas reproductive success of mayfly populations depends on large numbers of nymphs emerging in synchrony (Brittain, 1982; Dahl & Peckarsky, 2003). Mayflies must therefore be more conservative in their activities so that immature nymphs develop successfully during the several months spent in the aquatic phase. Nymphs cannot afford to risk increased exposure by responding to transient daytime light events or responding too early during twilight. The faster response of *Daphnia* to light stimulus reflects both their ability to sustain higher mortality and their lower risk associated with moving about. Consequently, *Daphnia* do not require lengthy accumulation of light stimulus to prevent inappropriate vertical movements during transient light events or to initiate DVM during twilight.

Lengthy accumulation of light stimulus over time in *S. modestum* may seem needlessly complex for maintaining diel cycles, as there are other equally dependable daily and seasonal aspects of twilight. Light to dark ratio and duration of daylight are both dependable, but are not known to elicit diel activity changes (Corkum, 1978; Ciborowski, 1979). The sky grows dark every evening, yet minimum light intensity is not reliable due to temporal and spatial variability in the stream light environment (Davies-Colley & Payne, 1998) or because of large changes in light intensity that may be encountered by an animal moving between the top and bottom of a stone (A.L. Schloss, unpublished data). Not surprisingly, light intensity reported at the onset of drift or locomotor activity varies widely within and among studies (see comparison Fig. 1, Schloss, 2002), suggesting that a fixed minimum light intensity threshold is unlikely to be a useful external cue. However, no studies have measured light intensity in the microhabitats on and under substrata at the time when animals become activated, and it is not known what intensities animals are exposed to. Haney *et al.* (1983) demonstrated that a combination of surpassing a RLC threshold and attaining a minimum light intensity threshold can predict the timing of stream drift. Both thresholds may be exceeded during the daytime either by



movements of the animals in and around substrata or during daytime light fluctuations (Ringelberg *et al.*, 1991), indicating that animals may have entered the drift in darkened conditions after accumulation of an adequate light stimulus. Except in rare instances such as solar eclipse, sustained light change takes place only during twilight and thereby is the most unambiguous and reliable predictor of the onset of darkness.

Responsiveness to falling light levels during evening twilight and lack of responsiveness to transient light fluctuations suggest that mayflies wait for darkened conditions before initiating movements. But, in several field observations, nymphs became active, initiated movements on the substratum or entered the drift roughly 40–90 min prior to darkness (Chaston, 1968; Haney *et al.*, 1983; Kohler, 1983; Casey, 1987; Wilzbach, 1990). Nymphs forage throughout the night (Culp *et al.*, 1991; Cowan & Peckarsky, 1994) and sometimes during the day (Allan, Flecker & McClintock, 1986; Wilzbach, 1990), so it is not immediately apparent what advantage might be inferred on individuals that come out or enter the drift before dark. The transition period between day and night may be a time of relative safety from predation. Adaptation of fish visual pigments may not quite keep up with the rapid rate of light change (Lythgoe, 1979), while at the same time fluctuating patterns of different spatial frequencies underwater make targets increasingly difficult for fish to fixate on (McFarland & Loew, 1983). The foraging efficiency of trout on drifting organisms is reduced in shade (Wilzbach, Cummins & Hall, 1986) and declines precipitously in darkened conditions (Metcalf, Valdimarsson & Fraser, 1997; Young, Rader & Belish, 1997). These factors may combine to disrupt the visual acuity of day-active fish predators and thereby decrease their efficiency during the transition period. Although high predation on invertebrates at dusk has been reported (Allan, 1981; Miyasaka & Nakano, 1999; Ovidio *et al.*, 2002), data were collected over time intervals (e.g. 2–4 h) longer than the transition period. High-resolution data are needed to determine the foraging efficiency of fish predators during the period of rapid light changes during twilight.

We have shown that mayflies utilise the rapid changes in light intensity during twilight to time diel changes in locomotor activity. Activation is thought to precede vertical movements on the substratum

(Elliott, 1968; Haney *et al.*, 1983; Schloss & Haney, 2002). Both activities may be precursors to entering the drift. Nymphs may use a small window of opportunity when visual predators are less efficient and nocturnal predators are not yet active to stake out profitable foraging sites or abandon risky substrata co-occupied by night-active predators such as stoneflies. In our study, nymphs did not react to darkness in the absence of an adequate light stimulus cue, indicating that they do not use light intensity as a cue to time diel changes in locomotor activity. Nymphs recognised light events as either transient or twilight by way of lengthy accumulation of light stimulus, a response that protects them from movements at inappropriate times. We have not determined the limitations of the response by testing how quickly accumulation dissipates between transient light events nor have we tested if the acceleration in RLC enhances the locomotor activity responses in mayflies. Absence of accelerations in our tests may explain the weak response to a 'just adequate' light stimulus. The light response we reported here for *S. modestum* may be specialised for a particular type of mayfly, in this case a cryptic species not abundant in the drift. Other species that behave differently during the day and drift profusely during the night, such as *Baetis*, may respond differently to light cues. We have shown how light responses can be tailored to different habitats such as streams and lakes and yet serve similar purposes towards success of populations.

### Acknowledgment

We wish to thank J. Ringelberg for his inspiring and insightful comments on the manuscript.

### References

- Allan J.D. (1981) Determinants of diet of brook trout (*Salvelinus fontinalis*) in a mountain stream. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 184–192.
- Allan J.D., Flecker A.S. & McClintock N.L. (1986) Diel epibenthic activity of mayfly nymphs, and its non-concordance with behavioral drift. *Limnology and Oceanography*, **31**, 1057–1065.
- Birmingham J.T. & Tauck D.L. (2003) Neuromodulation in invertebrate sensory systems: from biophysics to behavior. *Journal of Experimental Biology*, **206**, 3541–3546.

- Bishop J.E. (1969) Light control of aquatic insect activity and drift. *Ecology*, **50**, 371–380.
- Bishop J.E. & Hynes H.B.N. (1969) Downstream drift of the invertebrate fauna in a stream ecosystem. *Archive für Hydrobiologie*, **66**, 56–90.
- Brittain J.E. (1982) Biology of mayflies. *Annual Review of Entomology*, **27**, 119–147.
- Cadwallader R.L. & Eden A.K. (1977) Effect of a total solar eclipse on invertebrate drift in Snobs Creek Victoria. *Australian Journal of Marine and Freshwater Research*, **28**, 799–806.
- Casey R.J. (1987) Diel periodicity in density of Ephemeroptera nymphs on a stream substrata and the relationship with drift and selected abiotic factors. *Canadian Journal of Zoology*, **65**, 2945–2952.
- Chaston I. (1968) Endogenous activity as a factor in invertebrate drift. *Archive für Hydrobiologie*, **64**, 324–334.
- Chaston I. (1969) The light threshold controlling the periodicity of invertebrate drift. *Journal of Animal Ecology*, **38**, 171–180.
- Ciborowski J.J.H. (1979) The effects of extended photoperiods on the drift of the mayfly *Ephemerella subvaria* McDunnough (Ephemeroptera, Ephemerellidae). *Hydrobiologia*, **62**, 209–214.
- Corkum L.D. (1978) Is benthic activity of stream invertebrates related to behavioral drift? *Canadian Journal of Zoology*, **56**, 2457–2459.
- Cowan C.A. & Peckarsky B.L. (1994) Diel feeding and positioning periodicity of a grazing mayfly in a trout stream and a fishless stream. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 450–459.
- Culp J.M., Glozier N.E. & Scrimgeour G.J. (1991) Reduction of predation risk under the cover of darkness: avoidance responses of mayfly larvae to a benthic fish. *Oecologia*, **86**, 163–169.
- Daan N. & Ringelberg J. (1969) Further studies on the positive and negative phototactic reaction of *Daphnia magna* Straus. *Netherlands Journal of Zoology*, **19**, 525–540.
- Dahl J. & Peckarsky B.J. (2003) Developmental responses to predation risk in morphologically defended mayflies. *Oecologia*, **137**, 188–194.
- Davies-Colley R.J. & Payne G.W. (1998) Measuring stream shade. *Journal of the North American Benthological Society*, **17**, 250–260.
- Devoe R.D. (1985) The eye: electrical activity. In: *Comprehensive Insect Physiology and Biochemistry and Pharmacology*, Vol. 6, *Nervous System: Sensory* (Eds G.A. Kerkut & L.I. Gilbert), pp. 277–354. Pergamon Press Publishers, New York.
- Elliott J.M. (1968) The daily activity patterns of mayfly nymphs. (Ephemeroptera). *Journal of Zoology*, **155**, 201–221.
- Flecker A.S. (1992) Fish predation and the evolution of invertebrate drift periodicity: evidence from neotropical streams. *Ecology*, **73**, 438–448.
- Forward R.B. (1985) Behavioral responses of larvae of the crab *Rhithropanopeus harrisi* (Brachyura: Xanthidae) during diel vertical migration. *Marine Biology*, **90**, 9–18.
- Gliwicz Z.M. (1986) Predation and the evolution of vertical migration behavior in zooplankton. *Nature*, **320**, 746–748.
- Haney J.F., Beaulieu T.R., Berry R.P., Mason D.P., Miner C.R., McLean E.S., Price K.L., Trout M.A., Vinton R.A. & Weiss S.J. (1983) Light intensity and relative light change as factors regulating stream drift. *Archive für Hydrobiologie*, **97**, 73–88.
- Haney J.F., Craggy A., Kimball K. & Weeks F. (1990) Light control of evening vertical migrations by *Chaoborus punctipennis* larvae. *Limnology and Oceanography*, **35**, 1068–1078.
- Heise B. (1992) Sensitivity of mayfly nymphs to red light: implications for behavioral ecology. *Freshwater Biology*, **28**, 331–336.
- Holt C.S. & Waters T.F. (1967) Effect of light intensity on the drift of stream invertebrates. *Ecology*, **48**, 225–234.
- Huhta A., Muotka T., Juntunen A. & Yrjonen M. (1999) Behavioral interactions in stream food webs: the case of drift-feeding fish, predatory invertebrates and grazing mayflies. *Journal of Animal Ecology*, **68**, 917–927.
- Järvilehto M. (1979) Receptor potentials in invertebrate visual cells. In: *Vision in Invertebrates, VII/6A, Handbook of Sensory Physiology* (Ed. H. Autrum), pp. 315–356. Springer Publishers, New York.
- Kohler S.L. (1983) Positioning on substrates, positioning changes, and diel drift periodicities in mayflies. *Canadian Journal of Zoology*, **61**, 1362–1368.
- Kohler S.L. & McPeck M.A. (1989) Predation risk and the foraging behavior of competing stream insects. *Ecology*, **70**, 1811–1825.
- Lampert W. (1989) The adaptive significance of diel vertical migration in zooplankton. *Functional Ecology*, **3**, 21–27.
- Lampert W. (1993) Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. *Archiv für Hydrobiologie/ Beihefte Ergebnisse Limnologie*, **39**, 79–88.
- Lythgoe J.N. (1979) *The Ecology of Vision*. Clarendon Press, Oxford.
- McFarland W.N. & Loew E.R. (1983) Wave produced changes in underwater light and their relations to vision. In: *Predators and Prey in Fishes (Developments in Environmental Biology in Fishes)* (Ed. D.L.G. Noakes), pp. 11–22. Springer Publishers, New York.

- McIntosh A.R. & Townsend C.R. (1994) Interpopulation variation in mayfly antipredator tactics: differential effects of contrasting predatory fish. *Ecology*, **75**, 2078–2090.
- Metcalfe N.B., Valdimarsson S.K. & Fraser N.H.C. (1997) Habitat profitability and choice in a sit-and-wait predator: juvenile salmon prefer slower currents on darker nights. *Journal of Animal Ecology*, **66**, 866–875.
- Miyasaka H. & Nakano S. (1999) Effects of drift- and benthic-foraging fish on the drift dispersal of three species of mayfly nymphs in a Japanese stream. *Oecologia*, **118**, 99–106.
- Müller K. (1966) Die tagesperiodik von Fließwasserorganismen. *Zeitschrift Morphologie Oekologische Tiere*, **56**, 93–142.
- O'Keefe T.C., Brewer M.C. & Dodson S.I. (1998) Swimming behavior of *Daphnia*; its role in determining predation risk. *Journal of Plankton Research*, **20**, 973–984.
- Ovidio M., Baras E., Goffaux D., Giroux F. & Philippart J.C. (2002) Seasonal variations of activity pattern of brown trout (*Salmo trutta*) in a small stream, as determined by radio-telemetry. *Hydrobiologia*, **470**, 195–202.
- Peckarsky B.L. (1980) Predator–prey interactions between stoneflies and mayflies: behavioral observations. *Ecology*, **61**, 932–943.
- Peckarsky B.L. (1996) Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. *Ecology*, **77**, 1888–1905.
- Potvin C., Lechowicz M.J. & Tardif S. (1990) The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. *Ecology*, **71**, 1389–1400.
- Ringelberg J. (1964) The positively phototactic reaction of *Daphnia magna* Straus. *Netherlands Journal of Sea Research*, **2**, 319–406.
- Ringelberg J. (1999) The photobehavior of *Daphnia* spp. as a model to explain diel vertical migration in zooplankton. *Biological Reviews*, **74**, 397–423.
- Ringelberg J., Flik B.J.G., Lindenaar D. & Royackers K. (1991) Diel vertical migration of *Daphnia hyalina* (sensu latiori) in Lake Maarsseveen: part 1. Aspects of seasonal and daily timing. *Archive für Hydrobiologie*, **121**, 129–145.
- Schloss A.L. (2002) A laboratory system for examining the influence of light on diel activity of stream macroinvertebrates. *Hydrobiologia*, **479**, 181–190.
- Schloss A.L. & Haney J.F. (2002) Direct observations of the activity responses of mayfly nymphs to relative light change and light intensity. *Archive für Hydrobiologie*, **151**, 353–374.
- Scrimgeour G.J. & Culp J.M. (1994) Foraging and evading predators: the effect of predator species on a behavioural trade-off by a lotic mayfly. *Oikos*, **69**, 71–79.
- Stearns D.E. & Forward R.B. (1984) Copepod photobehavior in a simulated natural light environment and its relation to nocturnal vertical migration. *Marine Biology*, **82**, 91–100.
- Van Gool E. & Ringelberg J. (1997) The effect of accelerations in light increase on the phototactic downward swimming of *Daphnia* and the relevance to diel vertical migration. *Journal of Plankton Research*, **19**, 2041–2050.
- Van Gool E. & Ringelberg J. (2003) What goes down must come up: symmetry in light-induced migration behaviour of *Daphnia*. *Hydrobiologia*, **491**, 301–307.
- Waters T.F. (1972) The drift of stream insects. *Annual Review of Entomology*, **17**, 253–272.
- Wilzbach M.A. (1990) Nonconcordance of drift and benthic activity in *Baetis*. *Limnology and Oceanography*, **35**, 945–952.
- Wilzbach M.A., Cummins K.W. & Hall J.D. (1986) Influence of habitat manipulations on interactions between cutthroat trout and invertebrate drift. *Ecology*, **67**, 898–911.
- Young M.K., Rader R.B. & Belish T.A. (1997) Influence of macroinvertebrate drift and light on the activity and movement of Colorado River cutthroat trout. *Transactions of the American Fisheries Society*, **126**, 428–437.
- Zar J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice-Hall, Upper Saddle River, NJ.
- Zaret T.M. & Suffern J.S. (1976) Vertical migration in zooplankton as a predator avoidance mechanism. *Limnology and Oceanography*, **21**, 804–813.

(Manuscript accepted 8 March 2006)