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A laboratory system for examining the influence of light on diel activity of stream macro-invertebrates

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Abstract

I describe a laboratory system for investigating the role of light as a proximate cue for diel changes in locomotor activity and vertical location on the substrate of stream macro-invertebrates. The system consisted of computer-controlled halogen lamps positioned over a laboratory stream in which video-recordings were made of *Stenonema modestum* mayfly nymphs located on the undersides of unglazed tile substrates. Locomotor activity of study organisms in response to light changes were quantified during computer-programmed and reproducible light/dark (LD) cycles. The system provided the flexibility to simulate a variety of light environments so that the separate influences of light intensity and light change on diel activities of individuals and populations could be examined, which is difficult under natural light conditions. As a group, nymphs responded similarly to simulated twilight (light decrease from 7.9×10^2 to 6.9×10^{-2} μW cm^{-2} at a constant -1.9×10^{-3} s^{-1} rate of relative light change) and to natural twilight, suggesting that proposed mechanisms of light control of diel activities in nature can be adequately tested in the simulated environment. However, locomotor activity and vertical movements among individual mayflies were highly variable under controlled conditions, suggesting that physiological differences influence their responses to environmental conditions.

Introduction

Many stream invertebrates, such as mayfly nymphs, exhibit strong diel periodicity in their behavior, including locomotor activity and vertical location on substrates (Elliott, 1968; Allan et al., 1986), feeding (Glozier & Culp, 1989; Cowan & Peckarsky, 1994), and drift (Müller, 1966; Waters, 1972). Ultimate causes of many of these adaptive behaviors are selection pressures to avoid predation (reviewed by Dill, 1987). Proximate cues entrain the diel behavioral cycle by informing organisms when it is appropriate to switch between daytime and nighttime behavior.

Light intensity is considered a proximate cue that initiates vertical movements on the substrate and onset of drift in mayflies. However, there is no consistent value for an absolute light-intensity threshold initiating these behaviors (Fig. 1). Relative light change, defined as the rate at which light intensity varies over time, has also been considered a proximate cue (as a stimulus) for the onset of diel vertical migration (DVM) of other aquatic species, such as water fleas (*Daphnia* spp.; Ringelberg, 1964; Buchanan & Haney, 1980), calanoid copepods (*Acartia tonsa;* Stearns & Forward, 1984), and phantom midges (*Chaoborus punctipennis;* Haney et al., 1990). For stream invertebrates, relative light change and light intensity have been proposed as proximate cues for initiating heightened locomotor activity and vertical location changes on the substrate. These behaviors precede entry into the drift (Haney et al., 1983). This proposed mechanism was based solely on drift measurements

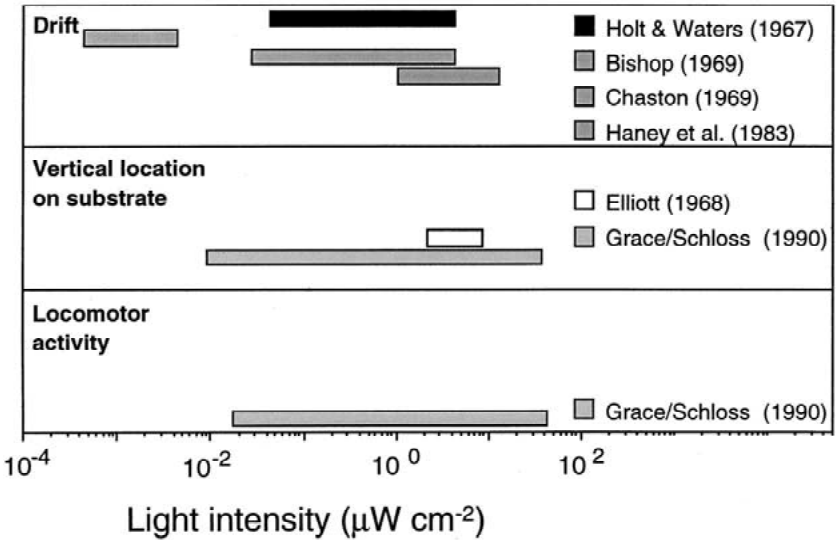

Figure 1. Ranges of light intensities reported in the literature for the onset of nocturnal increases in drift, locomotor activity, and vertical movements from the lower to upper surfaces of rocky substrates of mayfly nymphs. Values were converted to μW cm^{-2} (Wetzel, 1983) for comparison purposes. Genera, location (laboratory or field data), and seasons during which data were recorded are: Holt & Waters (1967) – *Baëtis,* field data, summer; Bishop (1969) – *Ephemerella* and *Stenonema,* laboratory data, winter and spring; Chaston (1969) – *Baëtis, Ephemerella* and *Isoperla*, laboratory and field data, all seasons; Haney et al., (1983) – *Baëtis* and *Leptophlebia,* field data, spring; Elliott (1968) – *Baëtis, Ecdyonurus, Ephemerella, Heptagenia* and *Rhithrogena,* laboratory data, winter, spring and summer; Grace (now Schloss, 1990) – *Stenonema,* laboratory data, all seasons.

and the factors controlling each separate activity have not been tested.

Hypotheses regarding the influence of light on behavior can be most thoroughly tested under controlled light conditions where both light intensity and relative light change can be manipulated. During natural twilight, rapid shifts in the rate of light decrease make it difficult to correlate activity changes with any particular rate of light change (Ringelberg, 1991). Moreover, on overcast days, fluctuations in the rate of light change result in higher variability in the response variables that can mask the response (Daan & Aschoff, 1975; Kavanau & Peters, 1976). Controlled conditions reduce the variability in behavior that is caused by various environmental factors while also providing conditions under which observed responses to light changes can be extrapolated to nature (Buchanan et al., 1982). This paper describes a laboratory system for examining the effects of relative light change and light intensity on the diel behavior of stream invertebrates.

The system makes use of computer-controlled lamps, time-lapse video and digitized image-processing, allowing for the study of behavior of populations and individuals, topics of current interest to ecologists and evolutionary biologists (Peckarsky et al., 1997; Ringelberg, 1999). Modifications in the light response due to changes in such variables as temperature, flow rate, food availability, and predator density, can be assessed using the methods described here. The extent to which these behavioral modifications are adaptive have important implications for the structure and function of stream ecosystems.

Materials and methods

System overview

Light control system

The light system was placed above a laboratory stream completely enclosed in black plastic. Lighting was produced by four overhead tungsten halogen lamps (lamp type FCL, 500 W) housed in a multi-circuit luminaire (Altman Stage Lighting Co., Yonkers, New York, USA) and covered with blue filters (daylight blue gel filter media from GamColor Inc. of New York). Filtered light simulated a natural daytime distribution of wavelengths, an important concern in laboratory investigations of light-mediated behavior in aquatic organisms (Ringelberg, 1964; Daan & Ringelberg, 1969; Buchanan et al., 1982; Forward, 1985; Swift & Forward, 1988).

The halogen lamps were controlled by passing a signal every second from a PC computer to a dimmer (Leprecon LD-360, CAE Inc., Hamburg, Michigan, USA). Whole number signals ranging from 0 to 4095 were passed by a QBASIC program (V3.1, Microsoft Corp., Seattle, Washington, USA) to the dimmer and converted to voltages of 0 to 10 V.

The computer program for controlling the lamps was constructed by first measuring the light intensity put out by the lamps at each whole number signal, and saving these values for later use. It was necessary to associate whole number signals with the corresponding measured light intensities because the relationship between voltage and light intensity was not linear.

The rate of relative change in light intensity (I) has been defined as:

$$RLC = \frac{1}{I} \times \frac{\mathrm{d}I}{\mathrm{d}t}$$

from Ringelberg (1964) (1)

Light intensities for the simulated twilight period were calculated from rearrangement of (1) as:

$$I = I_{\max} \mathrm{e}^{(\mathrm{RLC}\,\Delta t)} \quad (2)$$

where I = the target light intensity at each 1-s time-interval, $I_{\max}$ = the initial light intensity, RLC = the desired rate of relative light change per s (light stimulus), and Δt = total elapsed time in s. Values for RLC (-1.9×10^{-3} s^{-1}) and $I_{\max}$ (7.9×10^{2} μW cm^{-2}) were inserted into [2] to calculate the light intensities for all of the 1-s time steps needed to produce the desired light change curve. These light intensities were then translated into their corresponding whole number signals, using the saved data. The resulting program file of whole number signals was used by the QBASIC program for controlling the lamps.

A variety of twilight conditions, such as clear and cloudy skies, and different seasons and latitudes, can easily be programmed in this manner and simulated with the lamps. Mechanically-based light-control systems including rheostats (Ringelberg, 1964), neutral density filter wheels (Forward, 1985), and resistance-producing salt solutions (Daan & Ringelberg, 1969), are not as versatile as the computer-controlled system.

Laboratory stream

The laboratory stream was constructed of clear acrylic plastic (Fig. 2). Water was recirculated at 5 cm s^{-1} (24 l min^{-1}), and aerated by flowing over upstream barriers that maintained oxygen levels at an average of 8.63 ± 0.39 mg l^{-1} (93 ± 4.0% saturation, n = 192, ± SD). Temperature was maintained at 18.0 ± 2.0 °C with immersion coolers. Light gray-colored unglazed tiles (dimensions 10 × 10 × 0.5 cm) placed in the center of each channel provided substrate for the nymphs. To provide nymphs with space underneath, the tiles were raised 0.5 cm above the streambed by plastic spacers glued to each corner with silicon. Fish odor was added to the water by keeping fish in the water tank throughout the test period (fish density = 10 fish m^{-3}). Fish predators and their odors are considered to enhance diel (Flecker, 1992; McIntosh and Townsend, 1994) and other (Scrimgeour et al., 1994) behavioral responses in mayflies.

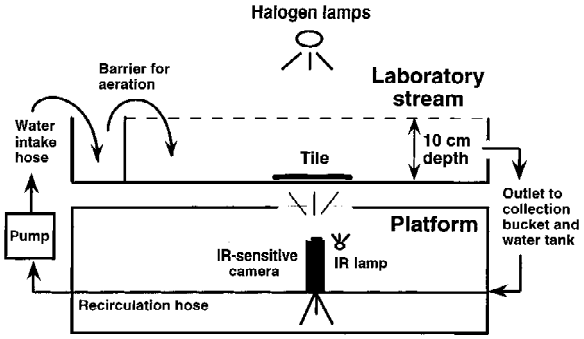

Figure 2. Schematic diagram of the laboratory stream showing the location of the overhead halogen lamps and the video camera in relation to the laboratory stream. Top: one of two adjacent stream channels that were used. Stream channels measured 0.15 m wide × 0.25 m high × 2.4 m long. Water depth was10 cm. Bottom: platform upon which the laboratory stream rested that enclosed the video camera and IR lamp. The platform was made of plywood painted black. Nymphs located on the tile undersides were video-taped through a viewing area cut out of the wood directly beneath the tiles. The entire system was enclosed in a black plastic curtain.

Videotaping was done under infrared illumination (15 wide-angle GaAIAS infrared light-emitting diodes (LEDs) at 940 ± 20 nm) using a black-and-white video camera (Daage Model 65) placed in a viewing area underneath the stream and connected to a time-lapse video recorder (Fig. 2). Insects reportedly are not sensitive to far infrared light (Heise 1992).

Ambient light conditions were monitored using a radiometer (IL-1700, International Light, Inc.) and a silicon photodiode sensor (SED033, International Light, Inc.) with a 2-pi collector corrected for cosine response, placed facing upwards and level with the water surface adjacent to the tiles. Light intensity sampled every second was logged as mean values for each minute.

Activity measurements

Locomotor activity was measured as the distance moved by each nymph between video frames captured every 30 s, a time interval suitable for tracking movements of individuals on rocky substrates (Kohler, 1984). In each video frame, the center of every nymph visible on the lower surface of the tile was recorded as an *x–y* coordinate within the boundary of the tile. The distance moved by each nymph between video frames was calculated as the straight-line distance between its center *x–y* coordinates on every two successive frames. When a nymph left the tile (i.e., was visible on one frame and not on the next), the distance moved was determined as the shortest distance to the edge of the tile. Conversely, when a nymph moved onto the tile underside (i.e., was not visible on one frame and appeared on the next), the distance moved was determined as the shortest distance in from the edge of the tile. This approach may have underestimated the distance moved when nymphs left or returned to the tile undersides, but a preliminary comparison of data collected from the same recording at 1-min, 30-s and 20-s snapshots showed no significant differences in total nymph activity over the time-series, indicating that activity measured this way gave a representative sample. Frames were analyzed using a shareware software package, NIH-Image (Rasband & Bright, 1996).

The 30-s activity measurements were aggregated into 1-min intervals to reduce noise in the data. The response time of nymphs to changes in light was 10 min or longer during prior tests in the laboratory (Grace, 1990), indicating that the timing of activity changes could be determined from 1-min intervals.

Average locomotor activity was calculated for the population from the activity data recorded for individuals. Distances moved by all nymphs were summed for each 1-min interval and divided by the number of nymphs visible during that interval. The average number of nymphs visible was calculated for each 1-min interval by averaging the number of visible nymphs in two consecutive 30-s snapshots.

Implementing the system

Twilight simulation protocol

Evening twilight was simulated by manipulating light intensity through 3 phases (Fig. 3a): (1) a 60-min adaptation period at the brightest light intensity (7.9×10^2 μW cm^{-2}), (2) a period of light decrease at a constant rate of light change (-1.9×10^{-3} s^{-1}), and (3) a 60-min period at the darkest light intensity (6.9×10^{-2} μW cm^{-2}). The beginning and ending light intensities and length of the light decrease period approximated conditions during local evening twilight during the summer (unpubl. data) and provided adequate proximate cues for the mayflies. The brightest light intensity was comparable to incident light intensity at noontime during the summer. The low intensity was darker than values associated with diel activity changes in *Stenonema* during natural twilight (Grace, 1990), thereby ensuring that if minimum light intensity was a factor controlling behavioral periodicity, the minimum light-intensity threshold was attained. The chosen rate of light decrease was stronger than the smallest light stimulus (-1.7×10^{-3} s^{-1}) that elicited a phototactic swimming reaction in *Daphnia* (Ringelberg, 1964; Ringelberg et al., 1991). A stronger light stimulus value was used here to increase the probability of a measurable nymph response, but not so strong as to produce an unnaturally short twilight period. The simulation was begun at 10 AM Eastern Standard Time (EST) to avoid any confounding effects of endogenous rhythms (Elliott, 1968) on the observed responses to controlled light change.

Handling of study animals

Mayfly nymphs (*Stenonema modestum*), excluding last instars, and fish (*Notropis cornutus* and *Rhinichthys cataractae*), were collected from the Oyster River, a 3rd order stream in Durham, NH. The collection site was a 30-m riffle located directly below a dam. The stream bottom consisted of granite bedrock, various-sized boulders and small pebbles. The stream channel was approximately 5 m wide and 6–20 cm deep. Current velocity ranged from 10 to 30 cm s^{-1} during the summer.

Twelve nymphs, collected on the morning of the test, were placed on two tiles at a density equivalent to that on comparable-sized rocks in the river. Periphyton-covered pebbles (2–4 cm diam.), were collected from the river and placed on top of the tiles as a source of food for the nymphs. Video-recordings (over several consecutive evenings) of the upper tile surfaces showed nymphs grazing upon these periphyton-covered pebbles (Grace, 1990), indicating that adequate food was provided.

Natural light comparison data

Measuring the locomotor activity and vertical movements of mayfly nymphs in their natural environment

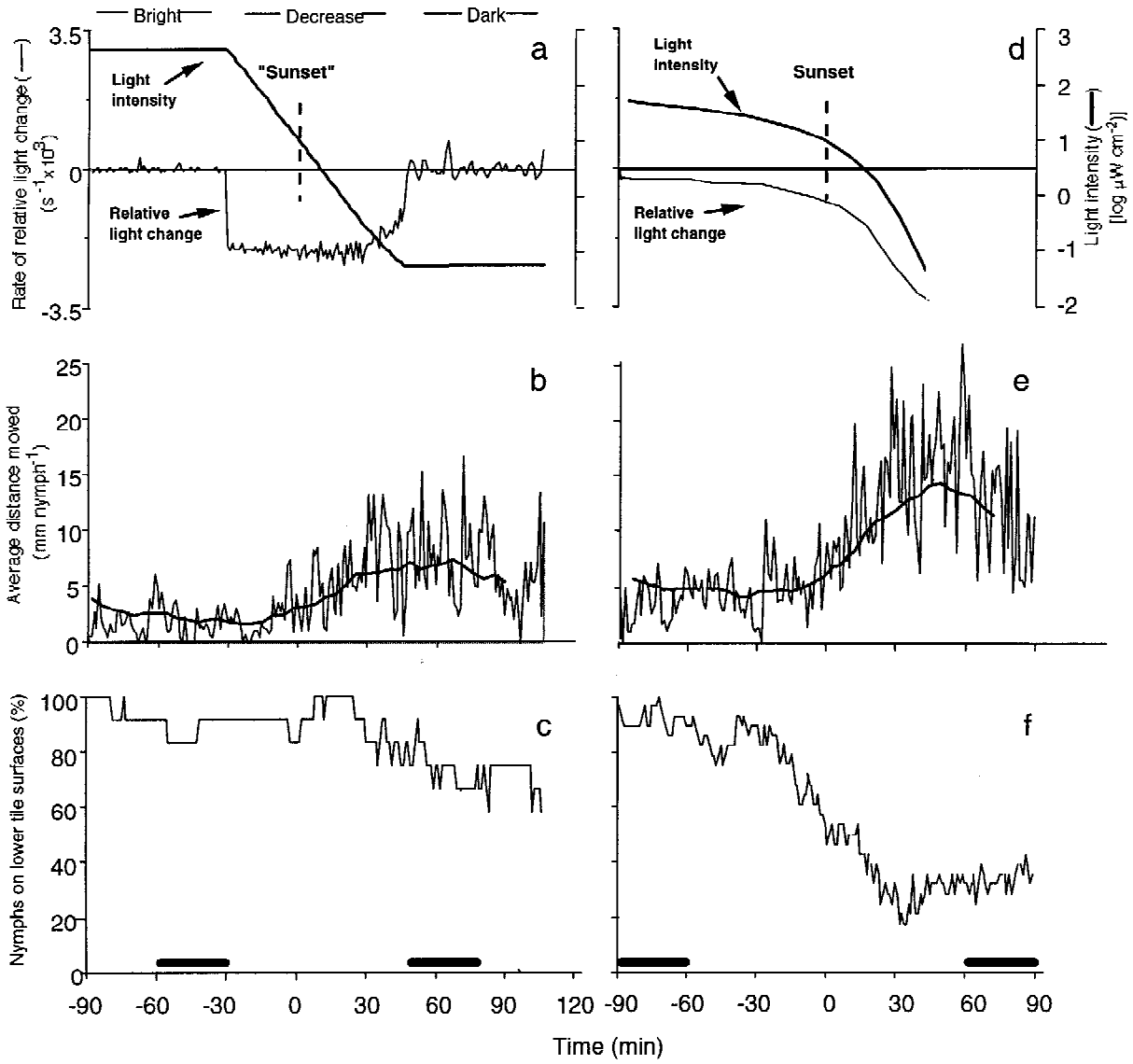

Figure 3. Light intensity, relative light change, locomotor activity and percent of nymphs visible underneath the tiles during simulated (left panels) and natural (right panels) twilight. Nymph locomotor activity is presented as the average distance moved per nymph during each 1-min time interval. Left panels: (a) light intensity and relative light change during simulated twilight. Light intensity (I) is shown on a log scale to demonstrate a linear decline in values at constant rate of light change [RLC = $\frac{dLnI}{dt}$]; (b) actual (—) and smoothed (▬) locomotor activity on tile undersides during (a). Data were smoothed by Exponential Weighted Moving Average (EWMA); (c) percent of total nymphs visible on tile undersides recorded during (a). Right Panels: (d) (e) and (f) same as for (a) (b) and (c) but during natural twilight. For natural twilight, local sunset was marked on the graph as Time 0. For comparison of the light environments between the two sets of observations, Time 0 in the simulation was marked at the light intensity of natural sunset. Light collection ended during natural twilight when light intensity fell below the detection limit of the light sensor. Horizontal bars (panels c, f) mark the 'daytime' and 'nighttime' periods used for comparison. The simulation began at 10 AM EST.

is not currently practical. To quantify these behaviors in a reasonably natural environment in the laboratory, natural conditions were approximated as follows: the laboratory stream was filled with unfiltered Oyster River water, temperature was not regulated (average temperature = 27.0 ± 1.0 °C), and south-facing floor-to-ceiling windows provided ambient light. Locomotor activity and vertical movements of nymphs measured in the laboratory during natural twilight under uncontrolled conditions were used for comparison

with nymph activities under controlled conditions to test if the light response of nymphs was as strong when other environmental variables were kept constant as observed in nature and to assess if responses observed under controlled conditions could then be extrapolated to nature.

Statistical methods

To test for relationships between light and activity changes, Spearman Rank correlation tests were performed between the population activity and the measured light intensity. To test for differences in activity and average number of nymphs located on the lower tile surfaces between the 'daytime' (bright light) and 'nighttime' (dark) periods, population data were compared for 30-min intervals before and after the light changes. For the simulation, the daytime interval was the last 30 min of the bright-adaptation period and the nighttime interval was the first 30 min of the dark period. During natural twilight, the boundaries of twilight were imprecise. A 30-min interval beginning 90 min before sunset and another 30-min interval beginning 60 min after sunset were selected as the daytime and nighttime periods, because these were both well outside of the period of most rapid light decreases.

The amount of locomotor activity during the daytime was different between the two sets of observations (one-way ANOVA: MS = 46.3, $F = 17.5$, $p < 0.0001$, $n = 60$). For comparison purposes, distances moved were reported as percent change in activity from the mean distance moved during the entire record of observation (mean distance moved in natural light = 8.92 mm min^{-1}; in simulated twilight = 4.40 mm min^{-1}). These data met the criteria for normality ($p > 0.05$, Shapiro–Wilk W test), so a 2-factor repeated measures ANOVA was performed to test for activity changes between daytime and nighttime within light treatments and for differences in the change in activity between light treatments. Factors were time (daytime, nighttime) and treatment (simulated twilight, natural twilight).

For comparison of the extent of vertical movements on the substrate by the nymphs, number of nymphs visible on the lower tile surfaces during each 1-min interval was reported as the percent change from the maximum number visible during the record of observation. These data, raw or transformed, did not meet the criteria for normality, so non-parametric Wilcoxon rank tests were performed ($n = 30$, DF = 1) to test for differences in the migration of nymphs away from the lower tile surfaces in response to light changes within and between light treatments. The JMP statistical package (V3.1.5, SAS Institute, Cary NC) was used for all tests.

Table 1. Two-factor repeated measures ANOVA of the effect of light treatment on the locomotor activity of nymphs. Factors were time (daytime, nighttime) and treatment (simulated twilight, natural twilight). Data were percent change in locomotor activity from the treatment mean

Source of variation	MS	F	p
Between subjects			
Treatment	0.2	0.0001	0.99
Error	3406.2		
Within subjects			
Time	407658.0	179.4	0.0001
Time × Treatment	1761.6	0.8	0.38
Error (Time)	2272.6		

Table 2. Wilcoxon rank tests of the effect of light on the vertical movements of nymphs on the substrate. Daytime and nighttime means were compared within each light treatment, and percent change in number of nymphs visible on the substrate before and after the light change was compared between light treatments

Means compared	Score mean	χ^2	$p > \chi^2$
Simulated twilight			
Daytime, nighttime	43.0, 18.0	33.63	< 0.0001
Natural twilight			
Daytime, nighttime	45.5, 15.5	45.17	< 0.0001
Percent change in number			
Simulated, natural twilight	15.5, 45.5	45.40	< 0.0001

Results

Responses of mayfly nymphs to simulated and natural twilight

Changes in locomotor activity and vertical location on the substrate of nymphs during natural and simulated evening twilight were similar (Fig. 3b, c; e, f). In both light conditions, locomotor activity was significantly higher after the light decrease than before (Table 1, time effect). There was no significant difference in

the percent change in activity between light conditions (Table 1, treatment effect). In both light conditions, there were significantly fewer nymphs on the lower tile surfaces after the light decrease than before, but a significantly greater percentage of nymphs left during the period of rapid light decrease during natural twilight than during the simulation (Table 2).

Locomotor activity increased after about 30 min from the onset of the light reduction during the simulation, and increased around sunset during natural twilight (Fig. 3b, 3e). Activity increases were not instantaneous, but took place over periods of 30–45 min, despite large variation in the amount of activity from minute to minute. Spearman Rank correlations between the average distance moved and light intensity (as shown in Fig. 3) were significant for the simulation (Rho = –0.67, $p < 0.0001$, $n = 196$) and for natural twilight (Rho = -0.72, $p < 0.0001$, $n = 133$), indicating that activity changes were correlated with changes in light regardless of other conditions, including differences in temperature and time of day.

The largest number of nymphs left the tile undersides during the periods of rapid light decrease (Fig. 3c, 3f). Spearman Rank correlations between number of nymphs visible on the lower tile surfaces and light intensity were significant for the simulation (Rho = 0.57, $p < 0.0001$, $n = 196$) and for natural twilight (Rho = 0.92, $p < 0.0001$, $n = 133$), indicating that vertical movements between substrate surfaces were correlated with changes in light. However, during the simulation, nymphs continued to leave after decreases in light had ceased, a result that was also consistent with release of negative phototaxis. In both light conditions, overall decline in numbers on the tile undersides were interspersed with short intervals of increasing numbers, indicating that there may be additional, non-light controls, on these movements (Fig. 3c, 3f).

Responses of individuals to simulated twilight

During the simulation, most nymphs were quiet during the bright-adaptation period and became more active within 40 min from the beginning of the light decrease (Fig. 4). During this period, the steady increase in the locomotor activity of the population (Fig. 3b) was caused by additional individuals becoming active rather than by an increase in the activity of each individual, indicating that the population response was not synchronized.

During the period of light decrease, some individuals left the tile undersides for short periods and then returned (Fig. 4A, F, G, I), causing the population numbers to increase and decrease over short intervals during this period (Fig. 3c). Individuals continued to move frequently between the upper and lower tile surfaces throughout the dark period, even though the rapid changes in light had ended. During any 1-min time-interval, as many as 58% of the nymphs were visible on the tile undersides (Fig. 3c). However, inspection of the videotapes showed that 75% left the tile undersides for some part of the light decrease and dark periods, many more movements than were indicated by the population data.

Discussion

Light is generally acknowledged as the most important proximate cue controlling diel behavioral cycles in organisms. Although laboratory systems have been widely used to investigate light intensity and relative light change as proximate cues governing zooplankton diel vertical migration (Ringelberg, 1964; Daan & Ringelberg, 1969; Stearns & Forward, 1984; Forward, 1985; Swift & Forward, 1988), there have been no similar investigations of the light response of stream invertebrates. The methods described here provide a complete system for studying the responses of stream invertebrates to light and alterations of the light response through manipulations of other environmental conditions in streams, such as flow rate, predator type and density, food abundance, and pollutants.

Although it is generally accepted that stream macro-invertebrates are more active under darkened conditions (Elliott, 1968; Waters, 1972; Allan et al., 1986), we know very little about the mechanism of activation. Elliott (1968) proposed that organisms entered the drift after becoming activated and subsequently moving from the darker, lower substrate surfaces to the brighter, upper surfaces. In Elliott's model, an endogenous rhythm controls locomotor activity while negative phototaxis governs vertical location on the substrate. Haney et al., (1983) proposed that organisms initiate evening drift following a photokinetic response (non-directional locomotor activity on the lower substrate surfaces) to relative light change, and a phototactic response (directed vertical movements from the lower to the upper substrate surfaces) to light intensity. Their hypothesis was based on Elliott's model and on observations in the field that drift began

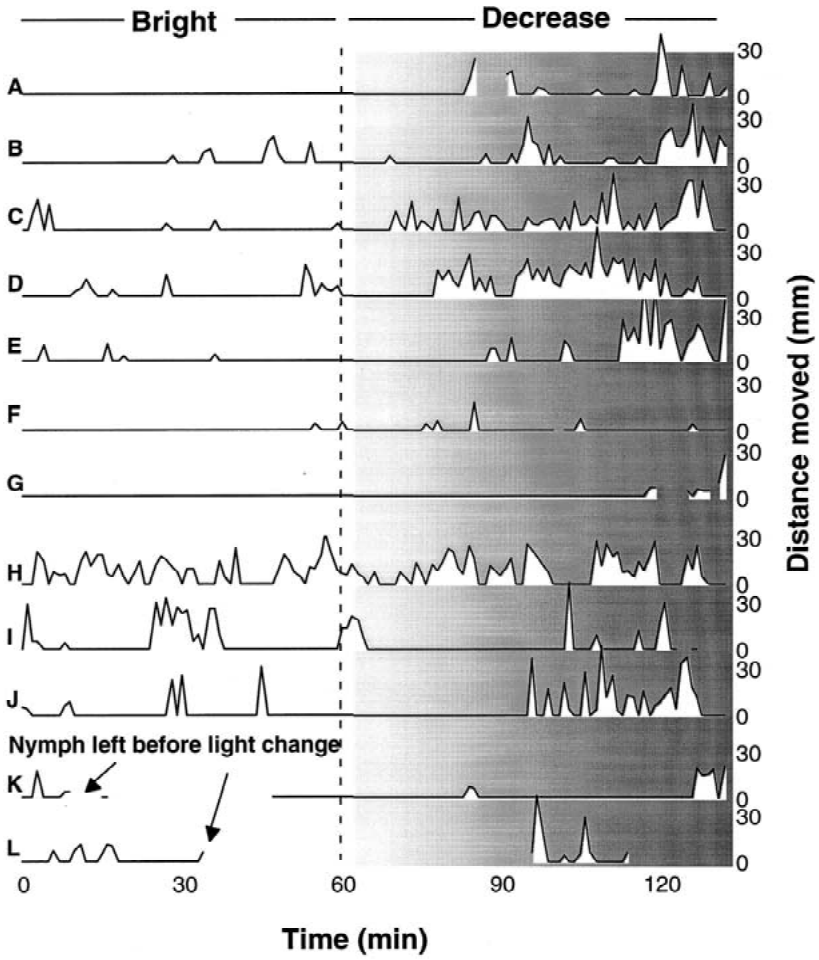

Figure 4. Locomotor activity of individual nymphs recorded during the bright-adaptation and light-decrease phases of the LD cycle illustrated in Figure 3a. Breaks in the data along the x-axis represent times when a nymph was not visible underneath a tile. Data for all nymphs were averaged to create the time-series shown in Figure 3b. The onset of the light decrease is marked by the vertical dotted line. Data are shown only during bright-adaptation and light decrease as individual nymphs could not be identified for tracking once several nymphs left the tile undersides.

during the post-sunset period of most rapid relative changes in light intensity, but was delayed by several minutes in a covered section of a stream compared to an open section. The results of my study, although preliminary, indicate that both locomotor activity and vertical location on the substrate are controlled by changes in light.

The higher overall activity during natural twilight was probably caused by warmer temperatures. However, in both light conditions, there were similar percent rises in locomotor activity in response to light decrease, suggesting that light change (light stimulus) initiated responses of the same magnitude even though the actual minimal and maximal activity of the nymphs were probably temperature related. The higher percentage of nymphs that left the tile undersides in natural twilight than during simulated twilight could also be due to temperature differences, or may be due to a stronger nymph response to differences in the quality of the light stimuli during natural and simulated twilight. During natural twilight, relative change in light became larger and the most rapid decreases in light intensity took place after sunset. Nymphs may have responded to either the larger light stimuli during natural twilight or to the acceleration in the rate of light decrease as twilight progressed. There is evidence from studies of *Daphnia* (Van Gool & Ringelberg, 1997; Ringelberg, 1999) that the acceleration in the rate of light decrease during natural twilight enhances the light response so that the complete mechanism for

control of DVM cannot be described without including acceleration in the model. Much less is known about the light response of stream macro-invertebrates.

Causes of variation in population responses to proximate cues are not well understood, but include: (1) responses of individuals to a spatially variable environment (Peckarsky et al., 1997); and (2) differences in the responses of individuals to a homogeneous environment due to either variation in physiological state (Ringelberg et al., 1991) or genetic differences among individuals (Spaak & Ringelberg, 1997). During the simulation, there was considerable variability among individuals in the timing and extent of their activities despite the controlled conditions of food, temperature, and light changes. These differences could not be due to patchiness in the environment but were more likely the result of physiological or genetic differences among individuals. Variation among individuals raises questions about relationships between activity, responsiveness to light cues, and individual fitness. Such short-term behaviors may be important in avoiding predators or in continually assessing conditions without leaving the relative safety of the undersides of substrates. In heterogeneous ecosystems such as streams, variability in individual responses to environmental conditions may be necessary for the continued success of the population. The laboratory system described here could be used to test proposed mechanisms of control as well as the factors that regulate individual responses to light while also providing the means to examine the importance of individuals to the observed population response.

Field assessments of the role of light in the diel activities of mayfly nymphs inhabiting the undersides of rocky substrates is not currently practical. Light changes at a single rate were used here to illustrate the usefulness of a controllable light environment in studying the response of nymphs to light stimuli. By combining light control with varying physical and biological factors such as temperature, flow rate, predators, and food availability, important insights can be gained into the mechanisms by which stream macroinvertebrates have successfully maintained their populations under the highly variable conditions of streams.

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