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The use of an artificial light system to assess the influence of relative light change on diel activity cycles of nymphs of the mayfly, Stenonema modestum, in the presence and absence of predators

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THE USE OF AN ARTIFICIAL LIGHT SYSTEM TO ASSESS THE INFLUENCE OF RELATIVE LIGHT CHANGE ON DIEL ACTIVITY CYCLES OF NYMPHS OF THE MAYFLY, *STENONEMA MODESTUM*, IN THE PRESENCE AND ABSENCE OF PREDATORS

BY

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DISSERTATION

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This dissertation has been examined and approved.

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11/27/97
Date

DEDICATION

To Jeff, Alyssia, and Emily, my patient and supportive family.

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I am very fortunate to have had the help and support of many people. John Canfield, a gifted electrical engineer and a marvelous person, for building the circuit board that lets the computer talk to the lights, and for spending many hours getting the system working. Many thanks to my committee; the UNH Threesome -- Drs. James Haney, Larry Harris and Winsor Watson III, who have been my advisors seemingly from the beginning of time; Dr. Barbara Peckarsky, for her enthusiasm; and Dr. Joop Ringelberg, without whose pioneering work and insights, I would never have even imagined the concept of relative light change. I also wish to acknowledge the support of Dr. Charles Vörösmarty and everyone at Complex Systems Research Center who cheered me on over the years. Many thanks to Dr. Bill Stark from Mississippi College for confirming my stonefly identifications.

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ABSTRACT

THE USE OF AN ARTIFICIAL LIGHT SYSTEM TO ASSESS THE INFLUENCE OF RELATIVE LIGHT CHANGE ON DIEL ACTIVITY CYCLES OF NYMPHS OF THE MAYFLY, *STENONEMA MODESTUM*, IN THE PRESENCE AND ABSENCE OF PREDATORS

By

Annette L. Schloss
University of New Hampshire, December, 1997

A mechanism by which light controls diel changes in locomotor activity and surface location of mayfly nymphs (*Stenonema modestum* Banks), named the Stimulus-based Timing and Activity-Rate (STAR) Model, was tested. Nymph movements were video-recorded in time-lapse from underneath unglazed artificial substrates in a laboratory stream. Light/dark cycles were simulated using computer-controlled halogen lamps. Light increases and decreases were generated to maintain constant rates of relative light change throughout simulated twilight periods. Nymph locomotor activity and position on the substrate were measured in response to rate of light change. Experiments tested whether adaptation light intensity (10^{-4} or 10^{-6} W cm^{-2}), time of day (AM or PM), length of the period of light change, or predators, altered nymph responses to light change.

Timing of both heightened nocturnal locomotor activity and leaving the substrate were significantly correlated ($R^2 = .93$; $p < 0.001$ and $R^2 = .71$; $p < 0.004$, respectively) with rate of relative light decrease. Rate of change in light was also correlated with the difference between daytime and nighttime locomotor activity ($R^2 = .38$, $p < 0.02$). The onset of nocturnal locomotor activity was advanced when nymphs were adapted to a low daytime light intensity. Lowered daytime light did not change the time mayflies left the undersides of the substrate. There was no difference in the locomotor activity response

between AM and PM experiments, but significantly greater numbers of nymphs left the substrate undersides during simulated twilight in the PM experiments ($p < 0.009$, $F_{1,14} = 9.3$). The difference between daytime and nighttime locomotor activity diminished during shortened periods of light decrease. When the time intervals over which light was reduced became smaller than the latency period of the response, there was no nocturnal increase in locomotor activity. Nymphs left the substrate undersides regardless of the length of time over which light was reduced. Locomotor activity was greater in the presence of fish odor (*Notropis cornutus* and *Rhinichthys cataractae*) than in water not containing predators. Locomotor activity was reduced during the daytime in the presence of *Paragnetina media* stoneflies. Synergistic effects between fish and stoneflies resulted in differences in the timing and locomotor activity of both stoneflies and mayflies.

INTRODUCTION

Proximate and Ultimate Factors that Influence Diel Behavioral Cycles

Light and predation are important proximate and ultimate factors influencing diel periodicity in the behavior of stream invertebrates, in particular mayfly nymphs. Periodicity in nymph behavior may be manifest as changes in the amount of locomotor activity (Elliott 1968, Allan *et al.* 1986, Grace 1990), preference for a particular substrate surface (Elliott 1968, Graesser and Lake 1984, Casey 1987, Glozier and Culp 1989, Grace 1990, Peckarsky and Cowan 1995, McIntosh and Peckarsky 1996), feeding at a particular time of day (Meier and Bartholomae 1980, Grace 1990, Scrimgeour *et al.* 1991, Cowan and Peckarsky 1994) and/or changes in the numbers drifting in the water column (reviews by Waters 1972, Müller 1974, Brittain and Eikeland 1988).

Behavioral patterns, such as diel periodicity, may evolve as a result of the feeding habits of predators. Mayfly nymphs are under a variety of predation pressures from fish and other invertebrates. Many species of fish are visual predators that feed primarily from the drift during daylight. These include trout, (e.g., *Oncorhynchus, Salvelinus, Salmo spp.*) (Allan 1981, McNicol *et al.* 1985), and darters (*Percidae)* (Cordes and Page 1979). Other common lotic fish feed from the benthos primarily at night, including sculpins (*Cottus bairdi*) and some longnose dace (*Rhinichthys cataractae*) (Beers and Culp 1989, Culp 1989, Culp *et al.* 1991, Hoekstra and Janssen 1985), while others such as the speckled dace (*Rhinichthys osculus*), reportedly feed during the twilight periods (Angradi *et al.* 1991). Stoneflies are important invertebrate predators that feed primarily at night (Malmqvist and Sjöström 1980, Walde and Davies 1985, Soluk and Collins 1988, Peckarsky and Cowan 1995).

Recent studies have suggested that diel drift periodicity protects mayfly nymphs from visual fish predators and that the behavior has probably evolved as a defense against discovery by drift-feeding fishes (Allan 1978, Allan *et al.* 1986). Mayflies in streams containing visual-feeding fish exhibit diel behaviors, but mayflies in naturally fishless streams often do not (Malmqvist 1988, Flecker 1992, Cowan and Peckarsky 1994, Douglas *et al.* 1994, McIntosh and Townsend 1994, McIntosh and Peckarsky 1996). Less is known about the influence of benthic-foraging fishes on the evolution of diel periodicity. To date, there is no evidence that mayfly nymphs have evolved diel periodicity to evade stonefly predators (Peckarsky and Cowan 1995), an indication that avoidance of daytime foragers has been the most important ultimate cause of diel behavior in mayflies. Stoneflies are also vulnerable to predation by fish and must time their foraging activities to best avoid detection by fish (Allan 1981, Moore and Gregory 1988, Feltmate and Williams 1989a, 1991), suggesting that their activities may also have evolved to best avoid visual fish predators.

Light is universally accepted as a proximate cue that regulates day/night activity cycles (Bünning 1973, Moore-Ede *et al.* 1982, reviewed by Page 1985, Rapp 1987). Light has been shown to control mayfly locomotor activity, location on the substrate, and drift (Elliott 1968, Chaston 1969, Kohler 1985, Glozier and Culp 1989). Manipulations of light intensity have demonstrated that drift can easily be turned off and on (Holt and Waters 1967, reviewed by Brittain and Eikeland 1988) or the timing of the onset of evening drift during twilight can be altered, by artificially darkening or illuminating a section of a natural stream (Haney *et al.* 1983).

Other proximate cues, such as the immediate risk of predation or food abundance, may alter the likelihood that an individual will exhibit a particular behavior. Diel cycles are maintained within the organism through an internal clock, or endogenous rhythm (reviewed for insects by Page 1985). Evidence that locomotor activity and drift may be regulated by an endogenous clock comes from observations of nymphs kept in continuous darkness in

which they maintained several 24-hr cycles of activity (Elliot 1968) and drift (Müller 1965, Chaston 1968). Individual differences within a population are observed as variability in the timing of a behavioral change or plasticity in behavior, suggesting that endogenous rhythms are not fixed. Many genera of mayflies are grazers (Merritt and Cummins 1984) that feed primarily at night on the daily crop of algae located on stone tops. Mayflies show plasticity in location on the substrate and feeding by altering the amount of time spent grazing and on the upper stone surfaces in the presence of fish (Kohler and McPeek 1989, Culp *et al.* 1991, Cowan and Peckarsky 1994, McIntosh and Townsend 1994), in the presence of high and low food resources (Kohler 1985, Kohler and McPeek 1989, Peckarsky 1996), and in numbers in the drift in response to different risks of predation by drift-feeding fishes (Malmqvist 1988, Flecker 1992, Douglas *et al.* 1994, Forrester 1994, Peckarsky 1996). Individuals may drift in response to encounters with actively foraging stoneflies (Peckarsky 1980, Malmqvist and Sjöström 1987, Peckarsky 1996). Such behavioral changes are most likely related to tradeoffs between obtaining food and avoiding predators (Dill 1987, Lima and Dill 1990, Scrimgeour and Culp 1994a, 1994b).

Proximate and ultimate causes of diel periodicity in behavior have been investigated in other aquatic environments, such as lakes and marine systems. Diel vertical migration of zooplankton is a prominent example, occurring in lakes containing planktivorous fish (Gliwicz 1986, Haney 1988), but not necessarily in fishless lakes (Gliwicz 1986). There are numerous examples of rapid induction of vertical migrations of prey species in freshwater and marine environments after the introduction of predators or water that had previously contained predators (Bollens and Frost 1991, Forward and Rittschof 1993, Neill 1990, Ringelberg 1991a, 1991b). Large differences in vertical depth in the water column between the leading and trailing edges of migrating populations demonstrate that there are differences in the response of individuals within a single lake population to daily light cues (Haney *et al.* 1990, Ringelberg *et al.* 1991a, 1991b). It is not yet known whether the same individuals are typically on the leading or trailing edge, suggesting a

genetic basis for the difference, or if particular conditions determine an individual's location during the migration on any given day, suggesting an interaction between environmental cues and the individual's response to light (Ringelberg *et al.* 1991a).

Diel cycles are also common in the behavior of many terrestrial species (reviews by Daan and Aschoff 1975, Lima and Dill 1990). The change in illumination during twilight has been shown to be the environmental cue by which fireflies and glowworms time the daily onset of luminescent activity (Dreisig 1975), and by which mosquitoes (Jones 1982), pond bats (Voûte *et al.* 1974), and several species of nocturnal moths (Dreisig 1980) time the onset of evening flight, but these behaviors have not been studied in the context of both light and predation. Light control of the diel activity patterns in several species of birds has also been documented (Daan and Aschoff 1974), but also not in the context of predator-prey interactions. Nocturnal foraging in some terrestrial species, such as deer mice (Clarke 1983), kangaroo rats (Lockard and Owings 1974), and fruit bats (Morrison 1978), has been shown to be reduced during periods of bright moonlight, presumably a strategy to avoid predators (Clarke 198, Kotler 1984), suggesting similar light controls on foraging behavior in nocturnal organisms in terrestrial and aquatic systems.

Development of the STAR light control model of diel activity cycles of mayfly nymphs

The mechanisms by which mayflies become active and move to the upper substrate surfaces to feed or enter the drift are not well understood (reviews by Waters 1972, Brittain and Eikeland 1988). Falling light levels at evening twilight have long been regarded as proximate cues for diel changes in the locomotor activity of terrestrial organisms (Daan and Aschoff 1975), suggesting that properties of light that are unique to twilight provide the necessary external cue for timing of diel changes in activity. Two important aspects of the twilight period are a large change in absolute light intensity and large relative changes in

light intensity. During evening twilight, the rate of relative light change during a given time interval is a measure of how quickly the illumination decreases over that time-interval (Ringelberg 1964). Changes in absolute light intensity are largest before sunset, but the rates of light change are smallest; whereas after sunset, changes in absolute light intensity are small, but the rates of light change are large. Relative light change as a measure of the rate at which light is varying in time is therefore independent of absolute light intensity (Ringelberg 1964, Haney *et al.* 1983, Ringelberg 1991b, Ringelberg *et al.* 1991a).

My Master's Degree research combined direct observation of the behavior of nymphs of a locally abundant riffle-dwelling mayfly, *Stenonema modestum*, in a laboratory stream under natural light conditions together with continuous measurements of light, in order to assess how changes in light during twilight act as a proximate cue for the initiation of diel changes in locomotor activity and migrations away from the lower substrate surfaces (Grace 1990). Aspects of the light environment that were considered as the mechanisms of control of behavioral changes were the rate of relative light change as the control of the onset of evening locomotor activity, and light intensity as the control of the moment nymphs began to leave the substrate undersides. A particular rate of relative light change has been related to the onset of vertical migration in the water flea, *Daphnia* (Ringelberg 1964) and in the phantom midge, *Chaoborus* (Haney *et al.* 1990), suggesting that there is a minimum, or threshold, rate of light change that can trigger an activity response in those aquatic organisms. I assumed these two particular mechanisms of control based on the model proposed by Haney *et al.* (1983). Their model predicts that the diel increase in locomotor activity is a photokinetic response to the surpassing of a threshold rate of relative light change during evening twilight, and that the vertical movement to the upper substrate surfaces is a phototactic response to a minimum level of illumination (their model and other models regarding light control of diel behaviors in mayflies are described in Chapter Two).

I collected data over consecutive 24-hr periods during several months in 1987-88. Results from the study did not strongly support the predicted rate of light change and light

intensity thresholds, because variability in the light environment associated with diel changes in activity and position on the substrate was large from day to day and mean values changed seasonally (Fig. 1), indicating that there were no fixed thresholds.

An unexpected finding of the research was that increases in daytime locomotor activity underneath the substrate corresponded to periods of light decrease (cloud events). This result was surprising because changes in locomotor activity in response to light were assumed to only occur during twilight, the time period when the diel changes in behavior typically begin. Both the length of time over which light was decreasing and the magnitude of the rate of light decrease (and not the absolute change in light intensity) contributed to the likelihood of a response and to the amount of activity change (Fig. 2). For example, a 40-minute cloud event in which the rate of light decrease was reasonably weak did not elicit an observable change in locomotor activity (Fig. 2, hatched area), nor did a 10-min cloud event in which the rate of light decrease was reasonably strong (Fig. 2, checkered area). The cloud event that did elicit a significant change in locomotor activity was both long-lasting (~ 40 min) and the rate of light decrease was reasonably strong (Fig. 2, solid area). These and other data (Grace 1990), suggest that impressive changes in activity, positioning on the substrate, and numbers of individuals in the water column typically occur around sunset and sunrise, because those are the periods when there are both large and sustained relative changes in light, two aspects of light that appear to strongly influence diel activities.

None of the previously proposed light control models of diel activity changes in stream invertebrates included relative light change as a regulator of locomotor activity during periods outside of twilight (the more prominent light control models are discussed in Chapts. 1, 2). Therefore, I propose a light control model of the diel activity patterns of stream invertebrates that can predict both the timing of diel changes in locomotor activity and vertical movements on the substrate, and also the difference between daytime and nighttime locomotor activity, as a consequence of relative light change. The basic premise of this Stimulus-based Timing and Activity-Rate Model (STAR) is that both the rate of light

change, or the strength of the light stimulus, and the time interval over which light changes occur, or the duration of the light stimulus, determine the timing of heightened locomotor activity and leaving the substrate undersides, as well as the magnitude and time-course of the initial peak in nocturnal locomotor activity. I define light stimulus here as the rate of relative light change, described by Ringelberg (1964). Specific predictions of the model are outlined below.

Predictions of the STAR Model

A. Predictions regarding diel changes in locomotor activity in relation to light stimulus.

1. *Timing*. Based on the strength-duration relationship between light stimulus (the rate of light decrease) and the timing of the onset of diel vertical migration that has been developed for *Daphnia magna* (Ringelberg 1964), the model predicts that the timing of the initial change between daytime and nighttime locomotor activity levels can be defined by a strength-duration curve. This type of a relationship is common in physiological excitable systems (cf. Grinnell 1977), because in such systems, a response occurs after a buildup of the appropriate stimulus. The time-interval over which the stimulus builds is known as the latent period. The length of the latent period is dependent on the strength of the stimulus, such that, in the case of a stimulus-based timing and activity response, the latent period between the beginning of the light stimulus and the onset of the activity change should be shorter at larger rates of light change (strong light stimuli) and longer at smaller rates of light change (weak light stimuli).

2. *Magnitude (difference between daytime and nighttime levels of locomotor activity).* Based on my own observations that the level of locomotor activity in *S. modestum* nymphs increased during cloud-related daytime periods of light decrease (Grace 1990, Fig. 2), and observations by Haney *et al.* (1990) that the vertical displacement of a population of migrating chaoborid larvae was proportional to the rate of relative change in light intensity, the model predicts that changes in the amount of locomotor activity shall be a direct consequence of light stimulus. Therefore, the amount of locomotor activity beneath the substrate should increase during periods of decreasing light, and decrease during periods of increasing light, by an amount proportional to the rate of relative light change. During those periods when relative light changes are smaller than the minimum rate or time-duration capable of eliciting a response, locomotor activity should oscillate around a daytime or nighttime mean, the level of which is seasonally dependent (Elliott 1968, Grace 1990).

3. *Time-course.* Based on observations that there are seasonal differences in the height and width of nocturnal activity peaks (Chaston 1968, 1969, Grace 1990), the model predicts that the duration of the initial peak in heightened locomotor activity should be longer when stimulus strength is weak, and shorter when stimulus strength is strong; conversely, the height of the peak should be smaller when stimulus strength is weak, and larger when stimulus strength is strong. Seasonally, stronger stimuli occur during fall and winter when the length of twilight is reduced, and weaker stimuli occur during spring and summer when the length of twilight is increased, because of differences in the angle of the sun in relation to the ground (cf. Daan and Aschoff 1975 and Dreisig 1980, for detailed representations of the course of daily light intensity seasonally and at different latitudes). During the tests of the Haney *et al.* model, seasonal differences were apparent in the time-course of the initial peak

of nocturnal locomotor activity of *Stenonema* recorded over several 24-hr periods during various months in the laboratory (Grace 1990). Peaks were sharper and considerably abbreviated during the fall compared to summer, when nocturnal locomotor activity endured throughout most of the nighttime period. The model seeks to explain these differences in terms of a response to light stimulus.

4. *Influence of the endogenous clock.* Circadian clocks govern the daily biological rhythms of most organisms (Brady 1975, reviewed for insects by Page 1985). The circadian clock keeps time and regulates physiological processes on a cyclical basis. As a result of such regulation there is a sensitive period at a particular time of day during which the organism is in a heightened excitatory state, and this period occurs prior to the timing of the change in activity (Brady 1975). Because circadian clocks are so common in insects (review by Page 1985), and some species of mayflies have been shown to possess an endogenous circadian rhythm in one or more of their activities (Müller 1965, Chaston 1968, Elliot 1968), it is assumed that there is an endogenous component in the diel activity cycles examined here. Because the increase in locomotor activity takes place during evening twilight, the sensitive period is proposed to occur in the evening. Greater responsiveness to light at that time should translate into an earlier onset of heightened locomotor activity and larger changes in the magnitude and extent of the initial peak of heightened locomotor activity at any given rate of light change, than at other times of the day.

B. Predictions regarding vertical movements on the substrate in relation to light stimulus.

The timing when nymphs begin to leave the substrate undersides in relation to light stimulus should also be defined by a strength-duration curve, as is the timing of diel

vertical migration of *Daphnia magna* (Ringelberg 1964). Evidence suggests that these movements are strictly phototactic (Elliot 1968, Grace 1990), and not governed by an internal rhythm; therefore, an influence of the endogenous clock on vertical movements between the substrates is not expected.

C. Predictions regarding the influence of adaptation light intensity on the response to light stimulus.

Based on observations that the timing of the activities of nocturnal organisms is advanced in lowered illumination (Edwards 1962, Dreisig 1975, 1980, Haney *et al.* 1983, Baldwin 1993), the model predicts that adaptation light intensity will influence the timing of heightened locomotor activity and migrations away from the lower substrate surfaces. Terrestrial examples include nocturnal moths that reacted more quickly to artificial light decrease when adapted to reduced light intensity than when adapted to high light intensity (Dreisig 1980), and other nocturnal moths that advance their flight times during cloudy skies, so that nocturnal moths may even fly during the day (Edwards 1962). In aquatic systems, invertebrate drift has been shown to begin earlier in artificially darkened sections of a stream than in unmanipulated sections (Haney *et al.* 1983, Baldwin 1993), indicating that the diel activity of stream insects is influenced by sky conditions.

D. Predictions regarding alterations in the light response in the presence of predators.

1. *Day-active foragers.* Because the diel activity pattern is assumed to have evolved to reduce the risk of predation from visual predators (Allan 1978, Allan *et al.* 1986), the amount of nocturnal locomotor activity under the rock should be greater in presence of day-active fish or fish odors than in the absence of fish or fish odors. The timing of movements away from the substrate undersides

should take place later, because nymphs are predicted to move to the substrate uppersides at lower light intensities, to avoid exposure in light bright enough that visual foragers are still active. The latter prediction is based on drift studies in which aperiodic or weakly periodic drift in mayfly populations from naturally fishless streams became nocturnal in the presence of fish (Douglas *et al.* 1994, McIntosh and Townsend 1994), indicating that the presence of fish can modify the timing of diel activities in mayflies.

2. *Night-active foragers.* In the presence of night-active fish and invertebrate predators, the timing of heightened locomotor activity and movements to the upper substrate surfaces should not be altered, because there is no evidence that the timing of these activities evolved as avoidance strategies for night-active predators (Peckarsky and Cowan 1995). This prediction assumes that mayflies can distinguish between the odors of day-active and night-active fish, an assumption that has not yet been tested. The change in locomotor activity following the period of light decrease should be directly related to the activity of the predators, because mayflies have been shown to actively avoid predator encounters with stoneflies (Peckarsky 1980, Malmqvist and Sjöström 1987, Peckarsky 1996), and benthic-foraging fish (Scrimgeour and Culp 1994).

Chapter Overview

The chapters that follow describe a mechanistic study of the proximate cue, light stimulus, that has been proposed to be responsible for the observed diel behavioral cycles in stream invertebrates, using nymphs of the mayfly, *Stenonema modestum*, as the test species. The objective of the study was to test the predictions of the STAR model in a controlled laboratory environment that included artificial manipulations of the 24-hr light/dark cycle. Chapter One describes the various components of the system used in the model tests, including the computer-controlled artificial light system, the laboratory stream and the video-recording system. Validation that the expected changes in locomotor activity and vertical movements on the substrate actually take place in an artificial light environment is presented, thus providing the groundwork for the detailed tests of the STAR model in Chapter Two. Mathematical relationships between rate of light decrease and three of the activity response variables; timing of heightened locomotor activity, the difference between the "daytime" and "nighttime" levels of locomotor activity, and the timing of leaving the tile undersides, are presented in Chapter Two. Tests of STAR predictions regarding the influence of adaptation light intensity (cloudiness) and time of day (the endogenous clock) are discussed. Because both rate of light change and the length of time over which the light change takes place are important in eliciting a response (Fig. 2), a series of experiments for which light was decreased over abbreviated time intervals was performed in order to assess how rate of light change and duration of the light change interact to produce a response. Results from those tests are also presented in Chapter Two. The influence of some predator combinations; no predators, fish odor, stoneflies, and fish odor + stoneflies, on the light response of *S. modestum* nymphs are reported in Chapter Three. Those experiments were carried out using artificial light/dark cycles for which the light changes occurred at the same rate. Therefore, direct comparisons of locomotor activity and position on the substrate could be made between predator treatments. Chapter Four presents a preliminary

comparison of the light response of *Stenonema* in natural light/dark cycles with the light response in artificial light/dark cycles. Objects of the comparison were the mathematical relationships developed in Chapter Two and data collected during the tests of the Haney *et al.* model. The first step towards developing a comprehensive light-control model of diel changes in behavior was to correlate the timing and magnitude of the activity change with particular rates of light change (Chapter Two). The rate of light change during natural twilight is not constant, and the next step will be to predict activity changes under natural-light conditions. In Chapter Four, the feasibility of translating the light-stimulus/activity relationships developed at constant rates of light change into relationships applicable to light changes at variable rates is demonstrated. The closing discussion section considers the adaptive significance of a stimulus-based timing and activity response, and possible physiological pathways by which the response may function.

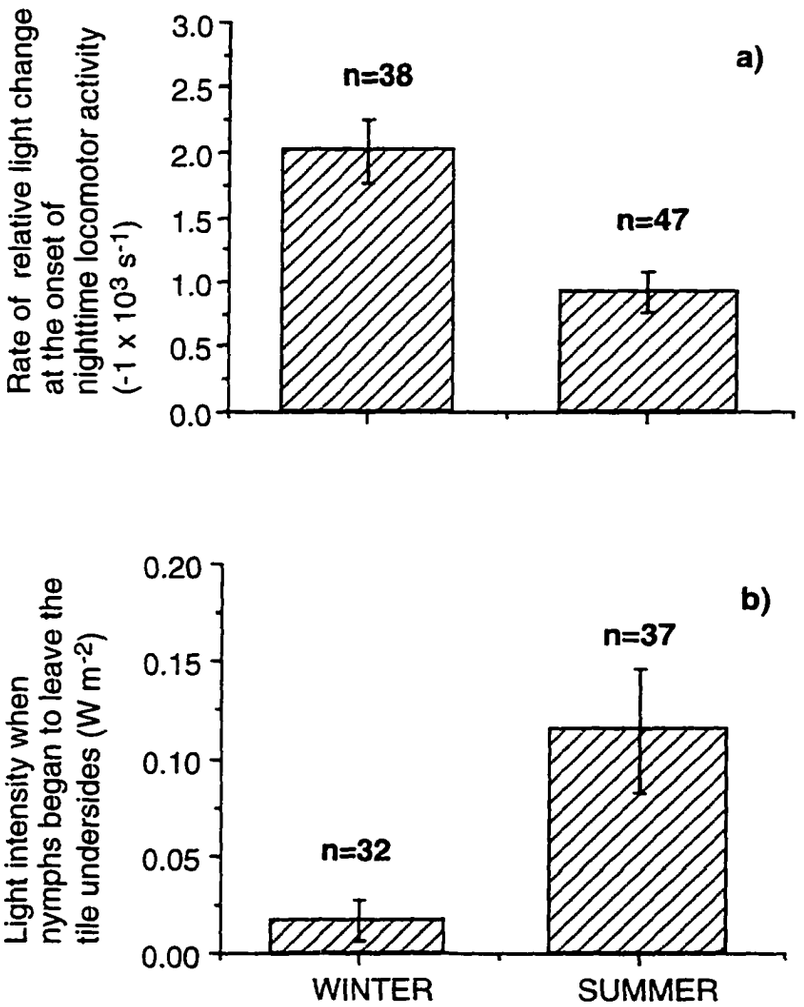

Figure 1. Results from tests of the mechanisms by which *Stenonema* nymphs use light cues to initiate diel behavioral changes recorded as part of the study to test the Haney *et al.* (1983) model: (a) rate of light change at the moment nymphs became more active during evening twilight, and (b) the light intensity at the moment when nymphs began to move away from the tile undersides in the laboratory stream. The data recorded during evening twilight for three consecutive days during the months of May, Jun., Jul., Aug., and Sep. 1987-88 were included in the summer estimates, and the months of Oct., Nov., Dec., and Feb. 1987 were included in the winter estimates (From Grace 1990). Data are mean ± SD.

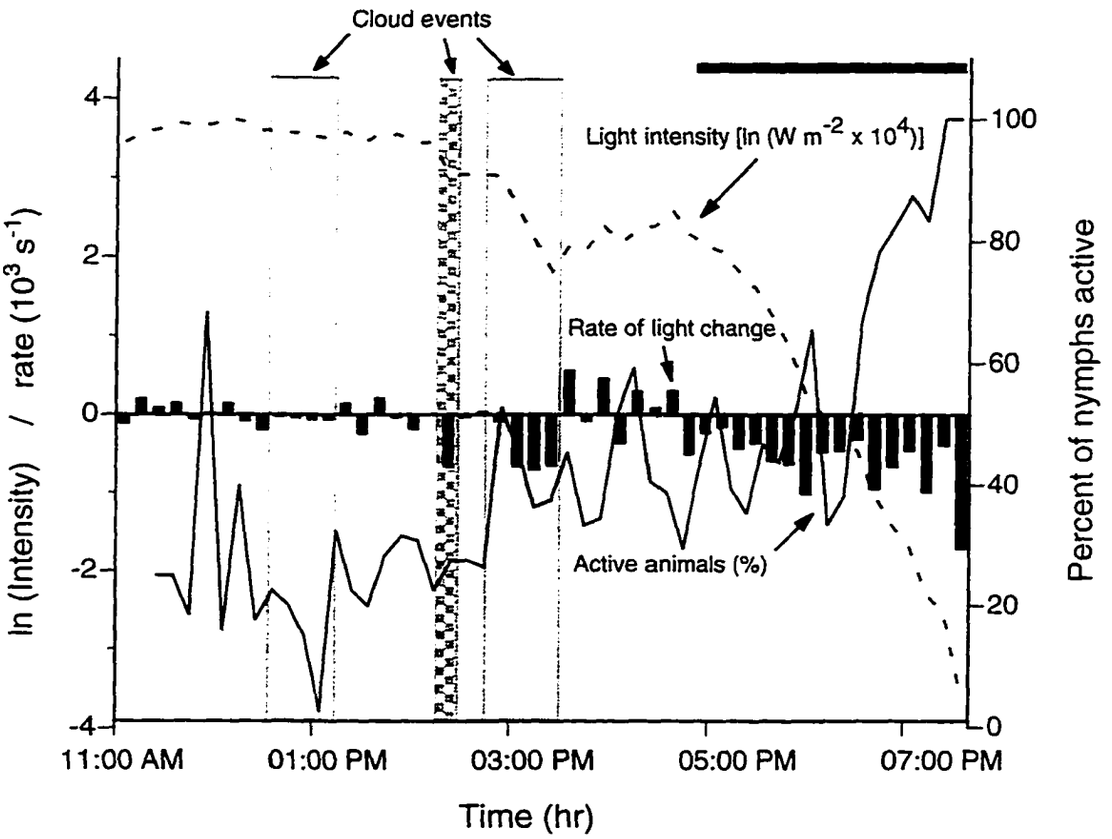

Figure 2. Comparison of locomotor activity (solid line) and the rate of light change (black bars) during the daytime and twilight period on May 7, 1988 recorded during tests of the model of Haney *et al.* (1983). Shaded areas represent some cloud events in the daytime period during which light decreased. The cloud event near 3:00 PM (solid area) elicited a significant ($p < 0.05$, ANOVA) increase in overall locomotor activity from an average of 23 ± 2.4, % ± SD, to 40 ± 3.3, % ± SD of nymphs active, whereas the two other hi-lighted cloud events (hatched area and checkered area), did not. Each bar (solid black) represents the rate of light change during a 10-min interval; bars above the zero line represent periods when the light was increasing and bars below the zero line represent periods when the light was decreasing (light intensity is shown by the broken line). Horizontal bar at top delineates the twilight period. Time is reported in Eastern Standard Time (EST). Data are from Fig. 22, Grace 1990.

CHAPTER I

THE USE OF AN ARTIFICIAL LIGHT SYSTEM TO ASSESS THE INFLUENCE OF RELATIVE LIGHT CHANGE ON DIEL ACTIVITY CYCLES OF NYMPHS OF THE MAYFLY, *STENONEMA MODESTUM*: PART 1. A TEST OF THE METHOD

Introduction

Mayfly nymphs exhibit diel periodicity in one or more behaviors, including locomotor activity (Elliott 1968, Kohler 1985, Grace 1990, McIntosh and Townsend 1994), preference for an exposed or unexposed substrate surface (Elliott 1968, Allan *et al.*, 1986, Grace 1990, Kohler 1983, McIntosh and Townsend 1994, McIntosh and Peckarsky 1996), feeding (Elliott 1968, Casey 1987, Glozier and Culp 1989, Wilzbach 1990, Cowan and Peckarsky 1994), and/or drift (Müller 1966, Elliott 1968, Waters 1972, reviewed by Brittain and Eikeland 1988). Selection pressures to avoid predation are the ultimate causes of many of these behaviors, which are considered to have adaptive significance (Sih 1980, reviewed by Dill 1987). There is strong evidence that the threat of predation from day-active visual-foraging fish has been a major influence in shaping mayfly diel periodicity (Allan *et al.* 1986, Flecker 1992, Malmqvist 1992, Forrester 1994, McIntosh and Townsend 1994, McIntosh and Peckarsky 1996).

This study investigates the proximate mechanisms or cues used by mayfly nymphs to recognize when it is appropriate to switch between daytime and nighttime behavior and thereby entrain the diel cycle on a daily basis. There is some evidence that diel rhythms in mayfly locomotor activity and drift are endogenous, as they persist in continuous darkness

(Müller 1965, Elliott 1968). It is well accepted that circadian rhythms are regulated by light-sensitive mechanisms and that the endogenous natural oscillation can be entrained to a periodic light signal with a 24 hr period (Bunning 1973, Moore-Ede *et al.* 1982, for insects reviewed by Page 1985, Rapp 1987).

Falling light levels at evening twilight have long been regarded as proximate cues for diel changes in the locomotor activity of terrestrial organisms, including birds (Daan and Aschoff 1975, Daan 1976), small mammals (Voûte *et al.* 1974, Daan and Aschoff 1975), primates (Kavanau and Peters 1976), and insects such as nocturnal moths (Persson 1971, Dreisig 1980), mosquitoes (Nielsen and Nielsen 1962), chafer beetles (Evans and Gyrisco 1958), glowworms and fireflies (Dreisig 1975). Onsets of evening activity changes have been variously related to absolute light intensity (Dreisig 1980), seasonally variable light intensity (Nielsen and Nielsen 1962, Persson 1971, Daan and Aschoff 1975, Dreisig 1975), and light intensity together with the surpassing of a threshold, or minimum, rate of relative decrease in light intensity (Voûte *et al.* 1974). At evening twilight, the rate of relative light decrease is a measure of how quickly the illumination level is falling over time. Properties of the 24-hour light/dark (LD) cycle that have been considered as proximate cues regulating the amount of dispersion or precision surrounding the onset of diel activity changes include the range of light intensities between daytime and nighttime, daylength or ratio of light to dark period, and duration of twilight (Daan and Aschoff 1975, Dreisig 1975, 1980). These properties vary with season and latitude and are thought to be factors that determine the strength of the light cue. Stronger cues are associated with higher precision and weaker cues with higher dispersion of individual onsets around the group mean activity change (Daan and Aschoff 1975).

Attempts to identify the mechanism by which light controls substrate location preference and drift in mayflies indicated that both are responses to light intensity (Holt and Waters 1967, Elliott 1968, Bishop 1969, Chaston 1969, Haney *et al.* 1983, Glozier and Culp 1989, Grace 1990), but there is no accepted value for an absolute light-intensity

threshold required to trigger these responses (Fig. 1). Laboratory and field manipulations of light intensity (Haney *et al.* 1983), light to dark ratio (Chaston 1968, Elliott 1968; Bishop 1969, Corkum 1978) and duration of daylight (Ciborowski 1979) also have not conclusively defined any single light parameter as the controlling mechanism, similar to the findings for terrestrial species.

In lakes and marine systems, diel vertical migration of zooplankton (DVM) is also an evolved predator-avoidance behavior timed to the 24-hr light/dark cycle (Neill 1990, Bollens and Frost 1991, Ringelberg 1991a, b, Forward and Rittschof 1993, but see Bayly (1986) for other views on the adaptive significance of DVM). Zooplankton swimming velocity and mayfly locomotor activity, and zooplankton vertical swimming direction and mayfly vertical position on the substrate, appear to have some similar adaptive consequences for each group. For example, vertical position in either the open water column in a lake or on the unexposed or exposed substrate surface in a stream incur differential risks of either being seen by a visual predator or detected by a non-visual predator. Food availability increases higher up in the water column in lakes and on exposed substrate tops in streams, linking food availability with greater risk of predation and with the necessity of locomotor movement in the direction of the food source, for both groups. Aspects of DVM, including the onset, swimming velocity and vertical swimming direction, have recently been related to relative decreases and increases in light intensity for two aquatic genera in the field, the water flea, *Daphnia* (Ringelberg 1991a) and the phantom midge, *Chaoborus* (Haney *et al.* 1990). Because diel behaviors in both stream and lake organisms are adaptive and have evolved around the same predictable environmental variable, the 24 hr light/dark cycle, it is reasonable to test relative light change as an external mechanism controlling those diel behaviors in mayflies which are analogous to diel behaviors in zooplankton.

Relative change in light intensity is defined as the rate at which the amount of light intensity varies over some time interval, usually measured in seconds or minutes. In

general, relative changes in light intensity are largest after the sun has sunk below the horizon (Nielsen 1963, Dreisig 1980, Grace 1990, cf. Ringelberg *et al.* 1991a), whereas the changes in absolute light intensity are largest *before* the sun has sunk below the horizon (cf. Grace 1990, Ringelberg *et al.* 1991a). Sustained periods of large relative decreases in light intensity are unique to evening twilight (cf. Haney *et al.* 1983, Grace 1990, Ringelberg *et al.* 1991a), suggesting that activity changes that occur during twilight may be stimulated by the strength and/or duration of these large relative light decreases. Relative light change is a particularly attractive stimulus or response cue in aquatic systems because there are large variations in absolute light intensity within the water column in a lake or along a reach of stream, whereas the relative change in light is unaffected by the level of light intensity and is therefore consistent everywhere within the system (cf. Ringelberg 1964, Haney *et al.* 1983).

Hypotheses regarding the influence of light on behavior can only be tested thoroughly under controlled light conditions. Natural light is not suitable for several reasons, mostly because relative light change cannot be controlled or manipulated. During twilight, rapid changes in the rate at which the light decreases make it difficult to correlate activity changes with any particular rate of light change (Grace 1990). Furthermore, relative light changes fluctuate when the sky is cloudy, resulting in higher variability in the response variables (Grace 1990).

I describe a method by which the effects of light, including relative light change and light intensity, on mayfly nymph behavior, can be tested in the laboratory. The method makes use of computer-controlled lamps, video-tape and image-processing technology. The system was designed to test the effects of relative decreases in light on the timing and magnitude of heightened locomotor activity, and substrate preference of nymphs of the riffle-dwelling mayfly, *Stenonema modestum*. This chapter describes the feasibility of using the system to test the response of *Stenonema* nymphs to changes in light by describing a representative light manipulation experiment. I chose *S. modestum* because

nymphs maintain diel cycles of both locomotor activity and location on the substrate (Haney and Grace 1988, Grace 1990), and can be found in the evening drift (Bishop 1969, Bishop and Hynes 1969, Kohler 1983, Krueger and Cook 1984, Forrester 1992), although they are not highly abundant in the drift. Using examples from field studies reported in the literature, I show why it is important to study behavior intensively at short time intervals during twilight before we can begin to understand the mechanism by which light controls diel behaviors.

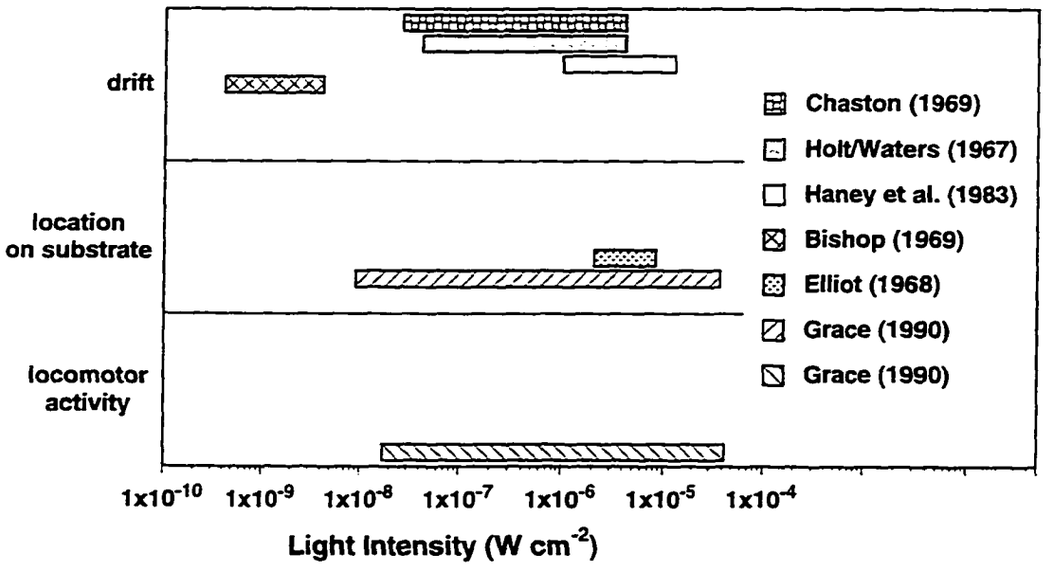

Figure 1. The range of light intensities associated with the onset of nocturnal increases in various mayfly activities as reported in the literature. Values reported in other units were converted to W cm^{-2} for comparison purposes (conversions used were taken from Wetzel 1983).

Methods

Overview

Light-manipulation experiments were carried out in the Anadromous Fish and Aquatic Invertebrate Research Laboratory at the University of New Hampshire, in Durham. Two channels of a clear acrylic laboratory stream were used, each measuring 0.15 m wide x 0.25 m high x 2.4 m long (Fig. 2). A tank located at the lower end of the stream was filled with well water that was recirculated at a rate of 5 cm-sec^{-1} (24 l min^{-1}) and aerated by flowing over upstream barriers (Fig. 2). Oxygen was measured at 8.63 mg l^{-1} ± 0.39 SD (n = 280), an average saturation of 93% ± 4.0 SD. Water depth in the channels was 10 cm. Water temperature was maintained at 18 ± 2.0 °C by the use of immersion coolers. Fish odor was added to the water from two common shiners (*Notropis cornutus*) and two longnose dace (*Rhinichthys cataractae*) kept in the tank throughout the experimental period (fish density = 2.5 fish m^{-2}). Measurements of the light responses of mayflies in the laboratory while in the presence of fish should be more applicable to the field than measurements in the absence of fish, because the majority of natural streams contain fish (Berra 1981) and fish are important in shaping mayfly activity patterns (Flecker 1992, Douglas *et al.* 1994, McIntosh and Townsend 1994). Fish water was used in the experiments to better approximate the natural conditions under which diel behaviors in mayflies have been consistently observed.

Substrate for the nymphs was one unglazed tile (dimensions 10 x 10 x 0.5 cm) placed in the center of each of the two channels. Tiles were raised 0.5 cm above the stream bottom by plastic spacers glued to each corner with silicon. The tile undersides were videotaped continuously by a black-and-white Daage Video Camera (Model 65) placed in the viewing area underneath the stream and connected to a time-lapse video recorder (Gyyr, Fig. 2). Recordings were made at a rate of one frame per second at a time compression of

1:72. The camera is sensitive into the far infrared range (> 750 nm), allowing videotaping in the dark under the infrared illumination provided by an array of wide-angle GaAIAS infrared emitting diodes (LEDs, average power of 20 mW at peak wavelength ± 50%, 940 ± 20 nm). Insects reportedly are not sensitive to infrared light (Heise, 1992).

The stream was completely enclosed in black plastic to ensure that light came only from the halogen lamps located directly above the tiles (Fig. 2; see description of the light source below.). Light intensity was manipulated between 7.9×10^{-4} W cm^{-2} and 1.3×10^{-7} W cm^{-2}. The high value is comparable to local noontime incident light intensity in July and the low value occurs about 30 minutes after the period of largest light changes during local twilight (unpubl. data). The low value was chosen because it was lower than values associated with changes in *Stenonema* locomotor activity and vertical movements between the substrates (Grace 1990), but also high enough to be measurable with the light meter and maintained at a steady intensity by the lamps for long periods of time. The low value is below light intensities at which nymphs became active and left the tile undersides under natural light conditions in an earlier study (Grace 1990). This ensures that if minimum light intensity is a factor controlling behavioral periodicity, the minimum light-intensity threshold would be attained.

Handling of Experimental Animals

Stenonema modestum nymphs (excluding last instars), were collected from the Oyster River, a permanent 3rd order stream in Durham, NH. The fish were collected from the same reach. The collection site is a 30 m riffle directly below a dam. Natural substrates consist of granite bedrock and various-sized boulders and small pebbles. The river channel is approximately 5 m wide and 6-20 cm deep. Current velocity is highly variable during the summer months, depending on daily rainfall, and ranges typically from < 10 to > 30 cm sec^{-1}. A survey of benthic invertebrates in the Oyster River (Hooker *et al.*, 1996) showed

that the density of Ephemeroptera exceeded 2000 individuals m^2, of which *S. modestum* were the most abundant. Reaches such as this are very typical of coastal New England rivers.

Six nymphs were placed on each tile at a density equivalent to that on comparable-sized rocks in the river. To avoid unknown effects of light history on their behavior, nymphs were collected every day and used once. Periphyton was provided at natural levels on pebbles (2-4 cm diam.) obtained from the Oyster River and placed on top of the tiles. In earlier experiments, video-recordings of the upper tile surfaces showed nymphs continuously grazing upon these periphyton-covered pebbles (Grace 1990), indicating that the food was adequate. The level of food was not controlled. Activity of several species of mayflies kept on natural or artificial substrates in the laboratory has been shown to be strongly regulated by light regardless of whether supplemental food was provided (Bishop 1969, Ciborowski 1979, Grace 1990) or not (Chaston 1968, 1969, Elliott 1968). There is evidence that mayflies do not alter their diel patterns of vertical movements in relation to food abundance (Glozier and Culp 1989).

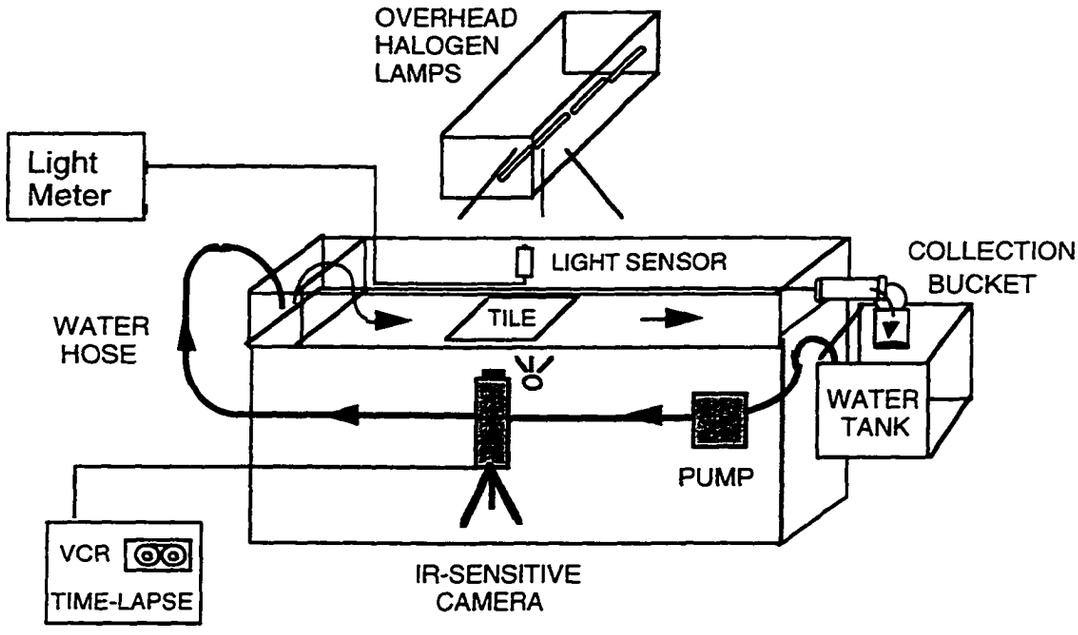

Figure 2. Schematic diagram of the laboratory stream showing the location of the overhead halogen lamps in relation to the tile substrates and the light sensor. The diagram shows one of the two stream channels used. One unglazed tile (dimensions 10 x 10 x 0.5 cm) was placed underneath the lamps in each of the two stream channels as illustrated.

Light Control and Light Monitoring Systems

Illumination was provided by four FCL 500 W tungsten halogen lamps (horizontal beam angle = 50°; field angle = 70°; vertical beam angle = 65° of field angle) housed in a 4-cell ground cyc compartmented multi-circuit luminaire (Altman Stage Lighting Co.) and covered with blue filters that simulate daytime distribution of wavelengths. Drop-off of light intensity with distance from the lamps was measured (Fig. 3). Drop-off was not considered a problem because the substrates provided for the mayflies were placed directly beneath the lamps where light was the brightest, and the distance from the tiles to the area where the light became considerably reduced was about 60 cm (Fig. 3), a distance considered longer than that generally traveled by *S. modestum* nymphs in bright light in this stream (Grace 1990). Light values across the channels were within < 1% of each other.

The lamps were powered by passing a signal once every second from a Zenith 80/86 PC computer to a Leprecon LD-360 dimmer pack (CAE, Inc.). Signals were generated through the QBASIC (Microsoft) programming language and ranged by whole numbers from 4095 (maximum voltage, approximately 10 volts) to 0 (no voltage). The lamps were calibrated by measuring the light intensity associated with each whole number signal and the calibration data were stored on disk. The light intensity values needed to generate the desired light curve, in this case a constant rate of change over the entire light-change period, were determined as:

$$I = I_{max} * e^{(S*\Delta t)} \qquad (1)$$

where I = the target light intensity, I_{max} = the beginning light intensity, S = the desired rate of light change per second (light stimulus), and Δt = total elapsed time in s (derived from Ringelberg 1964). The whole number signal most closely associated with

each target light value was obtained from the stored calibration data and the whole number signals were saved on disk. It was necessary to perform these calculations because the relationship between voltage (represented by the whole numbers) and light-intensity was not linear throughout the entire range. The resulting file of whole number signals was then used by the QBASIC program to control the lamps. To account for differences in actual lamp output due to aging and use, I calibrated the lamps once a week and regenerated the whole number file when necessary.

Ambient light conditions were monitored using an International Light radiometer (Model IL-1700) and sensor (SED033) with a 2-pi collector corrected for cosine response. The light sensor was placed facing upwards 10-cm above the water surface adjacent to the tiles in one channel of the laboratory stream (Fig. 2). Light intensity was sampled every second and mean values for every minute were saved on disk.

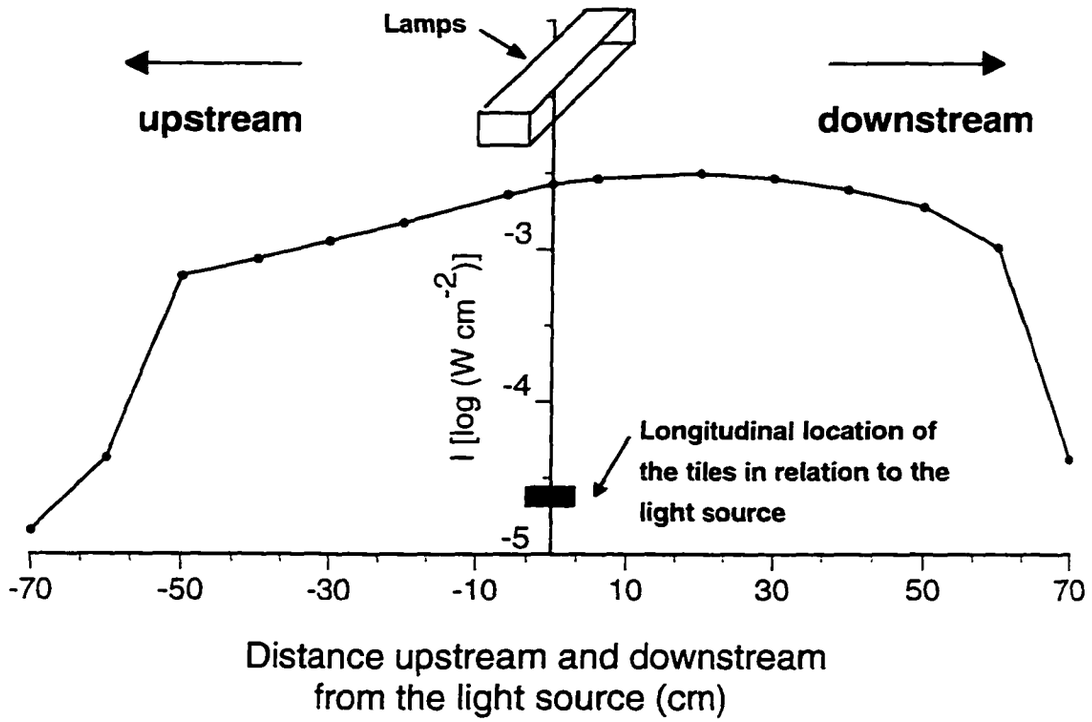

Figure 3. Longitudinal distribution of light intensity (I) upstream and downstream from the four 500-W halogen lamp light source. The tiles measured 10 cm across and were placed on the stream bed directly underneath the lamps as shown in Fig. 2. The shaded area represents the location of each tile in relation to the longitudinal distribution of light in the stream.

Experimental Protocol

An artificial light/dark cycle was generated by manipulating light through four phases (Fig. 4): (1) an adaptation period at the brightest light intensity (BRITE) of at least 60 min, (2) a period of light decrease (DECRS) at a constant rate of light change, (3) a 60-min dim-light adaptation period (DARK), and (4) a period of light increase (INCRS) at the same, but opposite rate of light change as used to decrease the light. The length of the DECRS and INCRS phases were 76 min each.

Constant rates of light change were used to examine the effects of a particular rate of light change on the timing and magnitude of heightened nymph activity. This was the first step in studying the response of nymphs to the changing relative decreases in light typical of natural twilight. The applied rate for the initial test described here was $\pm$ 1.9 x 10^{-3} sec^{-1}. This value is larger than the 1.7 x 10^{-3} sec^{-1} "Ringelberg stimulus value" estimated by Ringelberg (1964) and Ringelberg *et al.* (1991a) as the strength of the light stimulus below which no phototactic swimming reaction took place in *Daphnia*, and used as a target threshold for diel changes in the activity of mayflies by other researchers (Haney *et al.* 1990, Grace 1990, Baldwin 1993). I used a larger value to increase the probability of a strong nymph response without also applying an unnaturally large rate of light change. The time required to complete the light decrease at this particular rate, 76 min, is a reasonable representation of the temporal duration of twilight in New Hampshire.

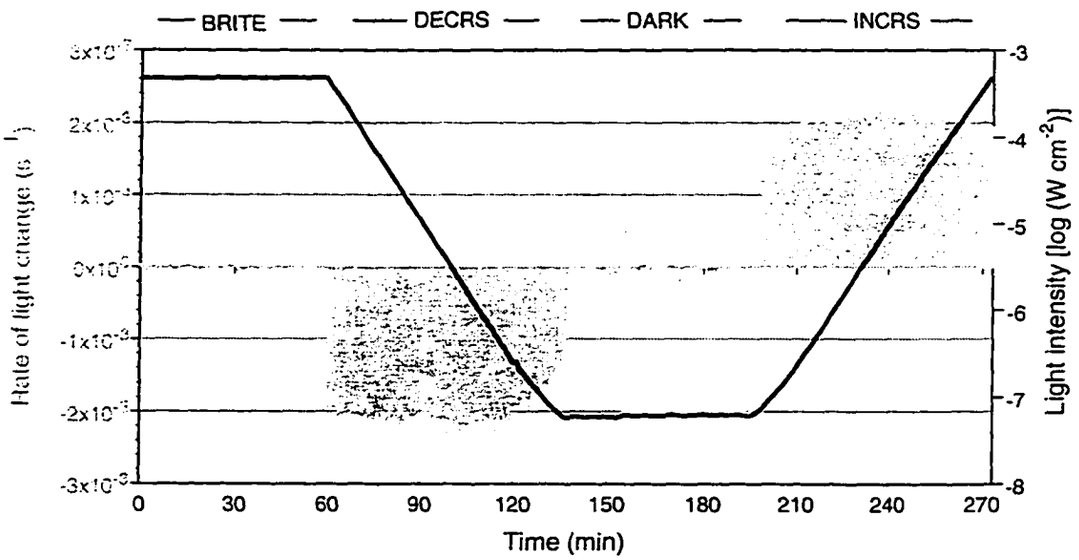

Figure 4. Light intensity and rate of light change (light stimulus) for one experiment. From left to right, the time-series represents the 60-min bright-adaptation phase (BRITE), the light decrease phase (DECRS), the 60-min dim-adaptation phase (DARK) and the light increase phase (INCRS). Light was decreased and increased at a constant rate of $\pm$ 1.9 x 10^{-3} sec $^{-1}$. The time required to complete the light reduction (DECRS) and light increase (INCRS) phases was 76 minutes each.

Data Analysis

There are no universally accepted methods for quantifying activity of organisms in time and space. Locomotor activity of mayfly nymphs has been measured by direct observation (Elliott 1968, Allan *et al.* 1986) or by time-lapse recording (Wiley and Kohler 1981, Soluk and Collins 1988b, Grace 1990, Wilzbach 1990, McIntosh and Townsend 1994). Activity has been estimated as number of animals moving within some minimal time interval, such as 10 s (Elliott 1968, Allan *et al.* 1986), or 30 s (Wilzbach *et al.* 1990), and then aggregated into larger time intervals, generally of 10 min. Activity has also been estimated as distance moved during a particular time interval, either as number of body lengths (Wiley and Kohler 1981, Soluk and Collins 1988b) or movements between patches of a common size (McIntosh and Townsend 1994).

I measured locomotor activity as the distance a nymph moved during 30 s intervals using computer-aided image-processing software (NIH-Image 1996, v1.60; the macro-language code is written out in Appendix B). Video frames were captured every 30 s and saved on disk. I chose the 30 s time interval because it was the longest interval over which the movements of individual mayflies on the tiles could be readily distinguished and allowed nymphs to be tracked by hand. In each frame, the position of every nymph was recorded as an x-y coordinate. The distance moved by each nymph was then calculated as the straight-line distance between 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. Although this approach would tend to underestimate the distance moved when nymphs left or returned to the tile undersides, a preliminary comparison of data collected from 1-min, 30 s and 20 s snapshots showed no appreciable difference in total nymph

activity over the time-series, indicating that the activity measured at 30 s intervals was representative of the true nymph activity.

Time-series of the average nymph activity were constructed for each experiment by combining the individual 30 s data into 1-minute snapshots and then calculating the 1-minute averages as:

$$\frac{\sum_{i=1}^{n} (d_j)}{n_j} \qquad (2)$$

where d_j = total distance in mm moved by each nymph during the 1-min interval j, and n_j = number of nymphs visible during time interval j. Data were collected at 30 s intervals only to simplify the tracking of individuals. Because the response time of the nymphs to changes in light was expected to be > 10 min (Grace 1990), 1-minute intervals were considered as sufficient to detect activity changes and also adequately measure light changes.

Results

Response of *Stenonema* to an Artificial Light/Dark Cycle

Reduction in artificial light at a constant rate from a high noontime intensity to darkness was accompanied by the expected changes in nymph locomotor activity and location on the substrate (Fig. 5). Activity was lowest during the BRITE adaptation phase, then began to increase well before dark, within 15-30 minutes of the onset of the light reduction (DECRS phase), supporting the hypothesis that the mechanism of control is not simply the onset of darkness. The activity increase was not instantaneous, but continued over a period of about 45 min. Nymphs began to leave the lower tile surfaces within 40-50 min of the onset of light decrease and continued to leave through the first half of the DARK phase. The number of nymphs visible on the lower tile surfaces ranged between a high of 12 to a low of 6 (Fig. 5). Overall, activity was higher during the DARK phase than during the BRITE phase (Table I), and the heightened activity persisted during the first half of the light increase (INCRS) phase (Fig. 5). Activity decreased about 30 min following the onset of the light increase to an average of 2.7 mm nymph^{-1} min^{-1} ± 0.3 SE (n = 45, Fig. 5).

TABLE I. Mean activity during each of the four light phases of the artificial light/dark cycle. Different letters represent values significantly different from each other (α = 0.05, Tukey-Kramer (HSD) multiple comparisons test[a]).

Light phase	Activity (mm nymph^{-1} min^{-1} $\bar{X}$ ± 1 SE)	n
BRITE	1.8 ± 0.2 a	60
DECRS	4.4 ± 0.4 b	76
DARK	6.9 ± 0.5 c	60
INCRS	4.3 ± 0.5 b	76

[a]Tukey-Kramer test performed on normalized data.

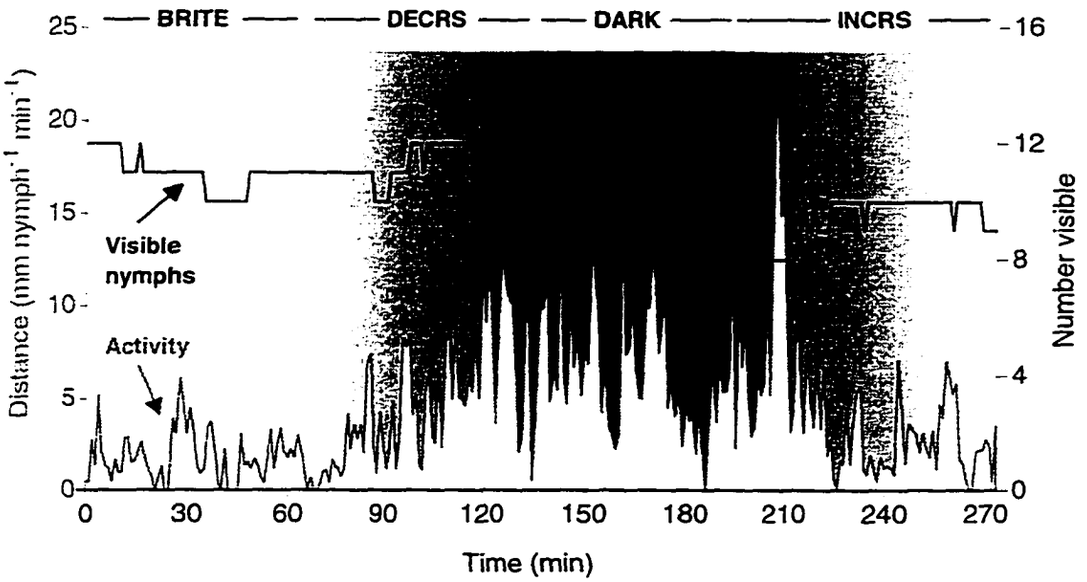

Figure 5. Locomotor activity (white area) and number of nymphs visible on the tile undersides (—) during the light manipulation plotted in Figure 4. Locomotor activity for each 1-min time interval represents the average distance moved per nymph. Activity is defined as: [(total distance (mm) moved by all nymphs) / number of nymphs visible during the time interval]. From left to right the time-series is as in Fig. 4. Data were recorded on July 16, 1995. Light reduction began at 10 AM Eastern Standard Time, and the applied rate of light change was $\pm$ 1.9 x 10^{-3} sec $^{-1}$. The time required to complete the light reduction (DECRS) and light increase (INCRS) phases was 76 minutes each.

Differences in activity between the four light phases were tested for significance. Because the variability appeared to be larger once locomotor activity increased, the O'Brien test for equal variances was performed. Variances were not equal ($p < 0.02$, $F_{3,270} = 3.3$; due to a smaller variance around the BRITE-adaptation mean compared to the other three light phases). The Welch ANOVA test for unequal variances was used to test for differences in the average locomotor activity between light phases. There was a significant difference in the mean locomotor activity between the four light phases ($p < 0.0001$, $F_{3,140} = 44.5$). Variances were normalized and means compared using the Tukey-Kramer (HSD) multiple comparisons test (Table I). Significant differences in activity between light phases that were adjacent in time indicate that the artificial light/dark cycle strongly influenced the amount of nymph activity (Table I).

Response of *Stenonema* to Natural Twilight.

I compared the results of the artificial light test to an earlier recording of *Stenonema* taken in natural light (Fig. 6). The increase in activity and reduction of nymphs on the tile undersides over a period of 30 - 60 min while light was decreasing were consistent in both situations. Locomotor activity, averaged over the first and last hours shown (an hour beginning 30 min before sunset and an hour beginning 30 min after sunset, Fig. 6), was used as an estimate of activity in natural light. The pre-sunset average distance moved per nymph was 4.0 mm $min^{-1} \pm 0.2$ SE ($n = 60$), and post-sunset, the distance moved per nymph rose to an average of 14.4 ± 0.7, mm $min^{-1} \pm$ SE ($n = 60$).

Response to the light change was larger in natural light than in artificial light (activity increased by an average of 10.4 mm $nymph^{-1}$ min^{-1} in natural light and 5.1 mm $nymph^{-1}$ min^{-1} in artificial light). The larger response in natural light supports the hypothesis that the change in activity is proportional to the strength of the light stimulus, because the stimulus (defined as the rate of relative light change) was bigger, and therefore stronger, in natural light than in the artificial light test (Figs. 4, 6). The larger response in

natural light also supports the presence of an endogenous clock, because the larger response in natural light corresponded with the time when nymphs would be in a higher excitatory state and expected to react more strongly to light stimulus (Brady 1975), whereas the smaller response in artificial light took place in the morning, when nymphs would be in a lower excitatory state.

The overall amount of activity prior to the light decrease was significantly higher in natural light than in the artificial light test (pre-sunset vs. BRITE period; $p < 0.0001$, $F_{1,118} = 54.7$ by ANOVA). This difference between the activity during the natural and artificial light tests may be due to several factors, one of which was that temperature was not controlled during the natural-light test and averaged 27°C, compared to 18°C in the artificial light test. Despite differences in temperature, year (1988 vs. 1995), time of day (evening twilight vs. morning), size of substrate (5 x 5 cm vs. 10 x 10 cm), and source of the water (Oyster River water vs. well water plus fish odor), the time-course of activity changes in relation to falling light levels are so similar, that it is likely that light has a major influence on mayfly activity.

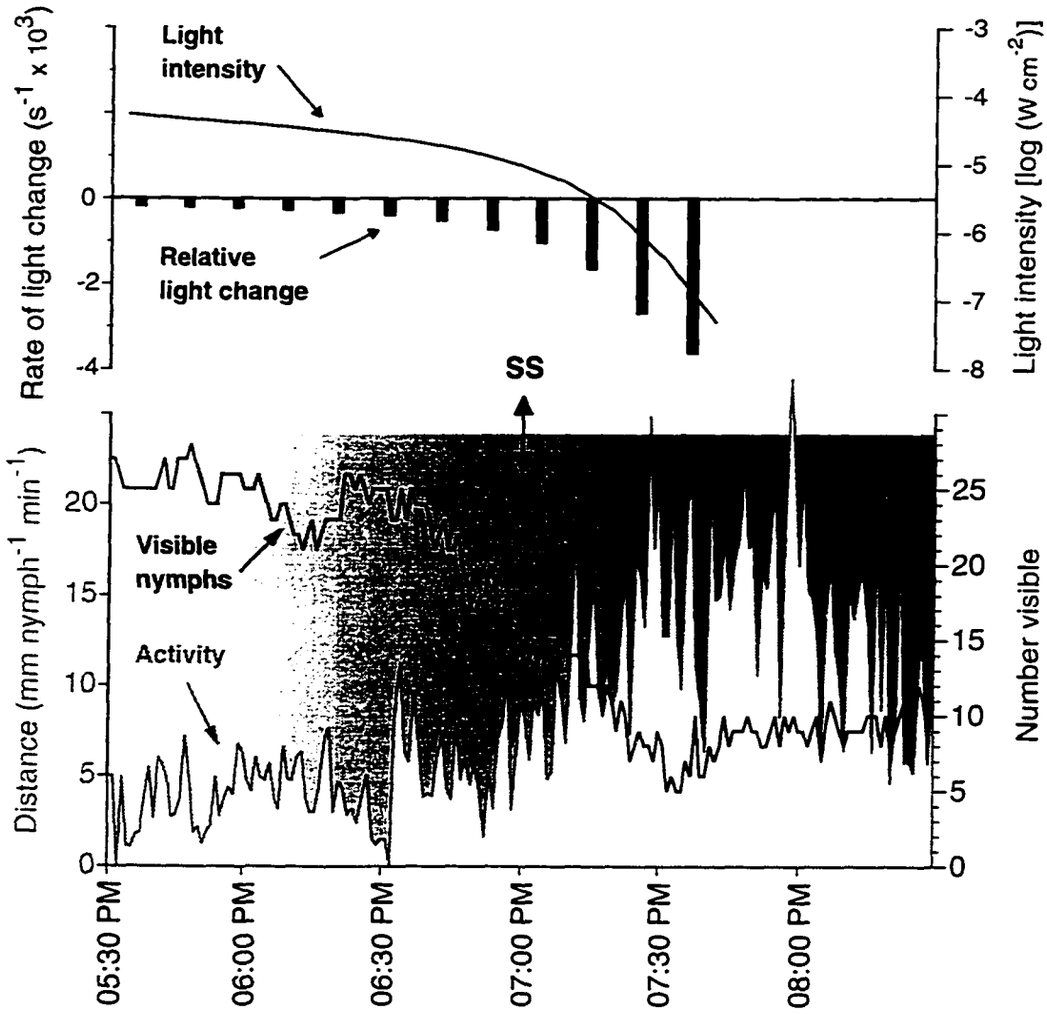

Figure 6. Time-series of locomotor activity (white area) and number of nymphs visible on the tile undersides (___) in the laboratory stream during natural twilight. Data were recorded on Aug. 4, 1988 (cf. Grace 1990). Light was measured at 10 min intervals, but the nymph data were re-analyzed at 1-min intervals for comparison with the data recorded in artificial light. Top panel) light intensity and relative light change beginning 90 min before sunset, Eastern Standard Time (Old Farmer's Almanac, 1988). Bottom panel) locomotor activity and number of nymphs visible on the underside of the tiles. Sunset is marked by the arrow.

Responses of Individuals to the Artificial Light/Dark Cycle

As an initial step to understanding individual responses to light, I plotted the activity of each nymph during the BRITE and the DECRS phases. Individual behavior appeared to fall into three distinct types based on the activity during the BRITE phase (Fig. 7).

A nymph was classified as "non day-active" when the average activity was ≤ 6 mm min^{-1} (Fig. 7, 1-7); as "day-active" when average activity was > 6 mm min^{-1} (Fig. 7, 8-10); and as "other" for various reasons, the most common being either the nymph was not visible on the lower tile surface at all during the BRITE phase, or the nymph was visible during some portion of the BRITE phase, but left before the application of the light decrease (Fig. 7, 11-12). I chose 6 mm as the cutoff distance because that was the average body length of the test nymphs ($\pm$ 0.5 mm, 1 SD) and movement of at least one body-length has often been used as a measure of mayfly activity.

Most of the non day-active nymphs appeared to respond to the light decrease by increasing their activity above the daytime level (Fig. 7, nos. 1-5). Activity of some day-active nymphs oscillated between activity and no activity (Fig. 7, nos. 9,10), suggesting that in the absence of light-cues there may be some rhythmicity in the level of activity. Some nymphs did not appear to respond to the light decrease (Fig. 7, no. 8), or did not respond strongly (Fig. 7, nos. 6,7).

Sizes of nymphs in the non day-active category were compared to the day-active and "other" categories to test if size was a factor in the amount of daytime activity expressed by the nymphs. The mean length of the non day-active nymphs was 6.4 mm $\pm$ 0.3 SE ($n = 7$) and the day-active and "other" nymphs combined was 6.5 mm $\pm$ 0.5 SE ($n = 5$). The difference was not significant by ANOVA ($p = 0.6$, $F_{1,10} = 0.4$).

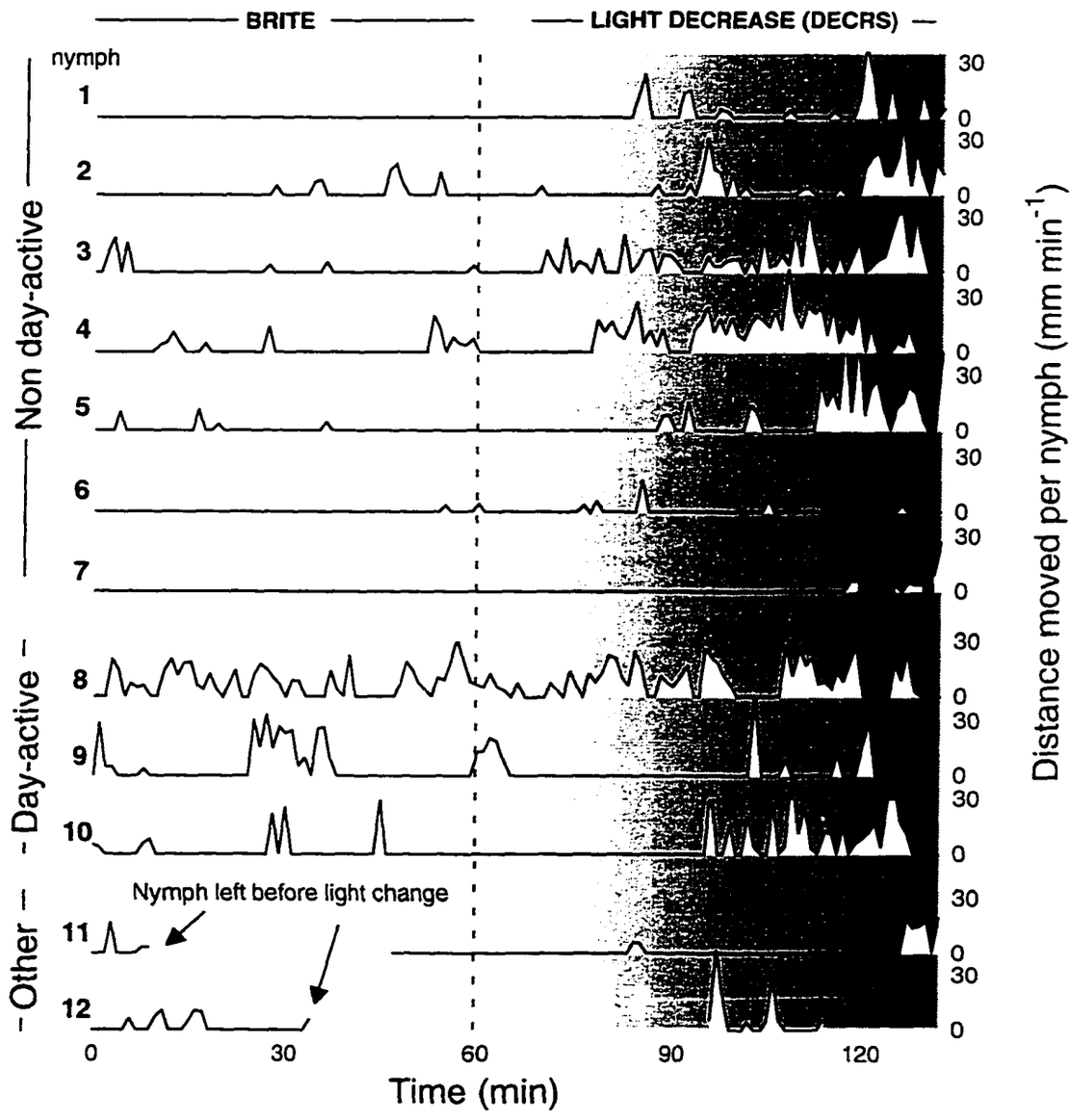

Figure 7. Time-series of the activity of the twelve individual nymphs by type (see text for description of types) measured as distance moved during 1-min time intervals. Breaks in the data along the x-axis represent times when a nymph was not visible underneath the tile. Data for all nymphs, non day-active (n=7), day-active (n=3), and "other" (n=2), were combined to create the time-series shown in Figure 5. From left to right this time-series represents the bright-adaptation (BRITE) and the light decrease (DECRS) phases of the artificial light/dark cycle. Once nymphs began to move frequently between the under and upper tile surfaces in response to falling light levels, it was not possible to distinguish if an individual that had left was the same individual that later returned. Therefore, traces of individuals are limited to the BRITE and DECRS phases. The onset of the light decrease is marked by the vertical dotted line.

Discussion

It has been observed that many species of stream invertebrates exhibit diel periodicity in some of their behaviors, be it substrate preference (Elliott 1968, Kohler 1983), feeding (Casey 1987, Glozier and Culp 1989, Wilzbach 1990, Cowan and Peckarsky 1994), locomotor activity or drift (reviewed by Brittain and Eikeland 1988). These diel cycles are ecologically important as they often represent evolutionary trade-offs between obtaining food and avoiding predators (Dill 1987, Kohler and McPeek 1989, Culp *et al.* 1991, Scrimgeour and Culp 1994a, b).

Light is generally acknowledged as the most important proximal environmental factor that controls diel cycles. Data presented here suggest that activity and positioning changes occur just after sunset, so are stimulated by some aspect of light that is unique to twilight. I propose that it is the sustained, large relative light decreases that are the most significant aspect of the twilight stimulus, as has been suggested for other aquatic species (Ringelberg 1964, 1991b, Buchanan and Haney 1980, Stearns and Forward 1984, Haney *et al.* 1990).

There are a few studies in which nymph behavior has been systematically observed in the field without disturbance, and these studies are supportive of the idea that the twilight period is the critical time when diel changes in behavior commence (Kohler 1983, Allan *et al.* 1986, Casey 1987, Wilzbach 1990). Most studies report hourly observations, but some consistencies are apparent. The largest changes in benthic density on stone tops and drift of *Baëtis* in a Maryland stream (Wilzbach 1990), in activity of *Baëtis* and *Cinygmula* nymphs on stone tops in a Colorado stream (Allan *et al.* 1986), and in drift of *Baëtis, Paraleptophlebia* and *Ephemerella spp.* in a Michigan stream (Kohler 1983) all occurred between the two hourly observations bounding sunset. My own study in the Oyster River recorded the appearance of *Stenonema* nymphs on the uppersides of artificial substrates in

conjunction with recordings in the laboratory of nymphs underneath similar substrates. Position changes from the lower to upper surfaces in the two systems began around sunset (Grace 1990). Only Casey (1987) reported diel changes in location on the substrate of several species of mayflies that began more than a half-hour post-sunset in an Alberta stream. If relative changes in light are an important cue, then Casey's data may have been confounded by overcast skies, as he reported > 50% cloud cover on each collection date. The majority of these studies suggest that twilight is a critical time in the onset of diel changes in behavior. Haney *et al.* (1983) measured drift in a New Hampshire stream at 5-minute intervals and demonstrated that the onset of evening drift took place during the period of most rapid relative changes in light intensity near sunset. Additional observations at shorter time intervals during the twilight period would more clearly define the moment when behavioral changes occur and lead to a better understanding of the relationship between light changes at twilight and diel changes in behavior.

If diel cycles were fixed, there would be no further purpose in studying them beyond a determination of where and for whom they exist. Because animal behavior is not fixed, but plastic, there arises opportunity for a whole array of behavioral possibilities (Kohler and McPeek 1989, Culp *et al.* 1991, Culp and Scrimgeour 1993, McIntosh and Peckarsky 1994, Peckarsky 1996) not attainable in a population that acts in complete synchrony. The advantages of plasticity are obvious, for as environmental conditions change, animals that can react favorably have the best chance of survival. The different behaviors recorded for individual nymphs from the same population support the presence of plasticity in mayfly behavior. Daan and Ringelberg (1969) also reported differences in the swimming behavior of *Daphnia magna* in the absence of light cues. Daphnids were described as either rhythmic or non-rhythmic based on the amount of vertical displacement in constant light. Clones of daphnids have been observed to be both rhythmic and non-rhythmic, indicating that the presence or absence of rhythmicity is not fixed within an

individual (Ringelberg, pers. comm.). In mayflies, it is not presently known how differences in daytime activity or responsiveness to light cues affect individual fitness.

Well-designed laboratory experiments can be reveal patterns relevant to the natural environment. Assessing the role of light in the diel activity of mayfly nymphs and other lotic invertebrates in the field under natural light conditions is not currently practical. To fully understand proximate mechanisms behind diel behaviors, we must first understand how individuals recognize an environmental cue. My results indicate that light is important in regulating diel periodicity of mayfly nymph behavior, and show an encouraging consistency to nymph behavior in natural situations. Additional studies describe linear relationships between the rate of light change and the timing and magnitude of the heightened locomotor activity (Chapter Two) and the modification of the light response under different predator treatments (Chapter Three).

CHAPTER II

THE USE OF AN ARTIFICIAL LIGHT SYSTEM TO ASSESS THE INFLUENCE OF RELATIVE LIGHT CHANGE IN DIEL ACTIVITY CYCLES OF NYMPHS OF THE MAYFLY, *STENONEMA MODESTUM*: PART 2. TEST OF A MODEL

Introduction

Diel periodicity in locomotor activity, vertical location on the substrate and drift of stream invertebrates, in particular, mayfly nymphs, is well documented (Elliott 1968, reviews by Waters 1972, Brittain and Eikeland 1988). The 24-hr light/dark cycle is recognized as a strong environmental driver of diel behavioral cycles, most of which result from complex couplings between a photoreceptor organ and a circadian oscillator (reviewed for insects by Pener 1985). Light plays a crucial role in regulating the physiological processes that lead to cyclic behaviors such as locomotion and feeding (Beck 1980, Jones 1982, Powers and Barlow 1985, Lee *et al.* 1996, Myers *et al.* 1996).

Circadian behavioral cycles in nocturnal animals are known to be connected with twilight (Nielsen and Nielsen 1962, Daan and Aschoff 1975, Daan 1976). The onset of nocturnal activity in birds, mammals and moths has been related to illumination level; variations in both the timing and in the amount of dispersion around the mean onset have been attributed to the ratio of light to darkness (reviewed by Page 1985 for insects), the

level of illumination during the light and dark periods, and the duration of twilight (Aschoff 1969, Daan and Aschoff 1975, Dreisig 1980). Regulation of the diel activity cycles of stream insects has not been thoroughly tested and it is not clear which aspects of light that stream insects use as their cue to initiate changes in behavior.

Early researchers of mayfly drift periodicity tested for a minimum absolute light-intensity threshold that signaled when it was appropriate to enter the drift. For many reasons, including seasonal differences between studies and widespread heterogeneity in the light environment between streams and within the same stream, there has been no consensus on a threshold value required to initiate evening drift (Holt and Waters 1967, Elliott 1968, Bishop 1969, Chaston 1969, Haney *et al.* 1983). Elliott (1968) hypothesized that drift was preceded by diel changes in two other behaviors; in particular, an endogenous cycle of locomotor activity combined with release of negative-phototaxis at a minimum light intensity. Elliott's model was modified and successfully field-tested by Haney *et al.* (1983), who proposed a photokinetic-phototactic (PK-PT) model. Their model predicts the timing of evening drift based on two mayfly responses to the light environment during evening twilight: (1) diel increase in locomotor activity (the photokinetic activity) following the surpassing of a threshold rate of relative light change and (2) the subsequent vertical movement to the upper substrate surfaces (the phototactic activity) at a minimum light intensity threshold. Relative light change is defined as the rate at which light intensity changes over time. The term is most often used in the context of changes in light intensity that occur during twilight. At evening twilight, changes in absolute light intensity are largest before sunset, but the rates of relative light change are smallest; whereas after sunset, changes in absolute light intensity are small, but the rates of relative light change are large. Relative light changes are therefore a measure of the rate at which light is changing and are independent of absolute light intensity (Ringelberg 1964, Haney *et al.* 1983, Ringelberg 1991b, Ringelberg *et al.* 1991a).

The rate of relative light change used as the threshold value by Haney *et al.* was the same value previously determined as a releasing stimulus for the onset of diel vertical migration (DVM) in the water flea, *Daphnia* (Ringelberg 1964). Their data, collected during manipulations of light intensity in two sections of a stream, indicated that the length of time drift was delayed following the threshold rate of light change was linearly related to the light intensity at the time the threshold occurred. Thus, drift began only during the most rapid light decreases during twilight (i.e., only after the threshold rate of light decrease had occurred), and began earlier in the darkened section of the stream than in the unmanipulated section. Despite the predictive power of the PK-PT model, there was no direct evidence that the sequence of events proposed in the PK-PT model actually took place in the benthos.

Tests of the predictions of the PK-PT model made in previous studies in the laboratory with the riffle-dwelling mayfly, *Stenonema modestum* indicated a strong influence of relative light change on locomotor activity (Grace 1990). Changes in locomotor activity at times other than twilight occurred when large increases in cloud cover darkened the sky over periods of twenty minutes or longer. The data suggested that relative light changes regulated the amount of locomotor activity rather than merely triggering an all-or-nothing response at twilight by the surpassing of a threshold rate of light change. Vertical movements to the upper substrate surfaces appeared to be independent of heightened evening locomotor activity, and so the temporal PK-PT sequence of events was not supported. Based on those observations, I propose a new light-control model for diel changes in mayfly locomotor activity and vertical movements between the substrates.

The Stimulus-based Timing and Activity-Rate Model (STAR) seeks to explain some components of nocturnal locomotor activity in mayfly nymphs: timing, magnitude and time-course of the initial peak of heightened locomotor activity. Differences in the expression of each component represent tradeoffs between minimizing energy costs,

minimizing predation risks and taking advantage of maximum food availability (Allan *et al.* 1986, Kohler 1985, Kohler and McPeek 1989, Soluk 1993, Scrimgeour and Culp 1994a, 1994b, Palmer 1995, McIntosh and Peckarsky 1996). For example, nymphs that begin to move about later may reduce the risk of predation but also may reduce the opportunity of obtaining food than nymphs that begin to move about earlier.

The basis of the STAR model is that an adequate light stimulus produces changes in locomotor activity. The definition of light stimulus as used here is the rate of relative light change described by Ringelberg (1964). An adequate light stimulus is one that is large enough, or above the threshold necessary to evoke a response in the organism (Ringelberg 1964). The timing and magnitude of nocturnal locomotor activity are determined during periods of light decrease by the combined effects of stimulus strength (measured as the rate at which light change takes place) and length of time over which the light decrease takes place. (Daytime locomotor activity is similarly determined during periods of light increase). The difference between the levels of daytime and nighttime activity should be proportional to the strength of the stimulus (e.g., the difference between daytime and nighttime locomotor activity should be larger when the rate of light decrease is larger and vice-versa), whereas the duration, or time-course, of the initial peak of heightened nocturnal activity should be *inversely* related to stimulus strength (e.g., the duration of the initial peak should be shorter when the rate of light decrease is larger and vice-versa). These predictions are based on observations of seasonal changes in the nocturnal locomotor activity of mayflies (Holt and Waters 1967, Chaston 1968, 1969, Elliott 1968, Grace 1990) that might correspond with the length of the twilight period and the strength of the stimulus during the twilight period. For example, in New Hampshire, the extended twilight periods of summer (weaker stimuli) correspond with long-lasting heightened nocturnal activity whereas the shorter twilight periods of fall (stronger stimuli) correspond with an initial sharp activity peak that decays more quickly (Grace 1990).

The model assumes that animals do not respond immediately to light stimulus, and predicts that there should be a latent period between the time when adequate decreases in light begin and the onset of nocturnal locomotor activity (Ringelberg 1964, Dreisig 1975, 1980). The length of the latent period should also be proportional to the strength of the light stimulus (Ringelberg 1964).

Although relative light change is considered to be the most important control of diel changes in locomotor activity, absolute light intensity has been shown to influence the timing of diel behaviors (Daan and Ringelberg 1969, Dreisig 1975, 1980, Haney *et al.* 1983). Nocturnal moths reacted more quickly to light decrease when adapted to reduced light intensity than when adapted to high light intensity (Dreisig 1980). Cloudiness, and thus lowered light intensity, is known to advance the flight times of some species of moths, so that typically nocturnal moths may even fly on cloudy days (Edwards 1962). Invertebrate drift has also been shown to begin earlier in artificially darkened sections of a stream than in unmanipulated sections, locally (Haney *et al.* 1983), and in a subarctic stream (Baldwin 1993). These observations suggest that ambient light intensity, which changes depending on sky conditions, affects the timing of the onset of nocturnal activities in aquatic as well as in terrestrial organisms.

An important assumption of STAR is that mayflies respond to light stimulus regardless of when it may occur in the 24 hr period. Striking variations in locomotor activity are not usually observed outside of the twilight periods only because relative changes in light are not normally of sufficient strength or duration to elicit such differences. This is not in conflict with the known endogenous component of the expressed locomotor activity cycle (as shown by diel activity cycles in mayflies kept in continuous darkness by Müller 1965 and Elliott 1968), but indicates that animals will be in a higher excitatory state near natural twilight (Brady 1975). The higher excitatory state should be expressed by a shorter latent period and greater increase in heightened locomotor activity in the evening than at other times of the day.

Diel vertical movements between substrates appear to be phototactic in some mayfly species (Elliott 1968, Casey 1987, Glozier and Culp 1989, McIntosh and Peckarsky 1996), including *Stenonema* (Grace 1990). These vertical movements are likely a response to the surpassing of a minimum threshold rate of relative light change similar to that initiating the phototactic swimming response of *Daphnia* (Ringelberg 1964). With decreasing light the probability of an individual leaving the underside of the substrate is predicted to be proportional to the strength of the light stimulus. This prediction implies that vertical movements will be more synchronous at larger rates of light change and more disperse at smaller rates.

This chapter reports results of tests of the STAR model on *Stenonema* mayflies using the laboratory stream and system for generating artificial light/dark cycles described in Chapter One. These tests represent a first step toward determining the proximate cues that control diel behaviors of mayfly nymphs. Understanding the cues that lead to particular behaviors will lend insight into the mechanism of control, and help to assess how environmental conditions, such as predator assemblages and changes in the patterns of cloud cover (Houghton *et al.*, 1995), may alter the response. Because information gained from studying stream invertebrates may be appropriate to diel cycles of aquatic organisms in general, such insights may be crucial to long-term management and protection of aquatic resources.

Methods

Detailed descriptions of the laboratory stream, artificial light system, and methods used to measure locomotor activity are given in Chapter One. Analyses pertaining to tests of the STAR model along with brief overviews of the methods are presented here.

Overview

Mayfly nymphs (*Stenonema modestum* Banks) were taken daily, avoiding last instars, from the Oyster River, in Durham, NH, during the summer of 1995, and from both the Oyster River and a nearby stream, the Bellamy River, in Madbury, NH, in 1996. Collection was expanded in 1996 to include the Bellamy River because large numbers of nymphs were needed and the population in the Oyster River was relatively small. Earlier tests of the photokinetic-phototactic activity (PK-PT) model of Haney *et al.* (1983) included nymphs from both rivers and there were no observable differences in behaviors between the two populations (Grace 1990). Both collection sites are riffles directly below dams in permanent 3rd order streams. The Oyster River channel is approximately 5 m wide and 6-20 cm deep, whereas the Bellamy River channel is about 8 m wide and 2-10 cm deep. Current velocities in both rivers are highly variable and reliant on daily rainfall during the summer months, typically ranging from < 10 to > 30 cm sec^{-1}.

Experiments were carried out in two channels of a clear acrylic laboratory stream (dimensions 0.15 m wide x 2.4 m long). The channels were filled to a depth of 10 cm with well water (18 ± 2.0 °C ± SD; O_2 saturation = 93 ± 4, % ± SD) that was continuously filtered (150-µm net) and recirculated from a tank located at the lower end of the stream at a flow rate of 5 cm sec^{-1}. Two shiners (*Notropis cornutus*) and two longnose dace (*Rhinichthys cataractae*) taken from the Oyster River were kept in the tank throughout the

experimental period and provided fish odor to the water (fish density = 2.5 fish m^{-2}). Two-thirds of the volume of water in the tank was replaced with fresh water every week. Fish water was used in all tests of the model because the diel behaviors are considered to be an evolved response to day-active visual fish predators (Allan *et al.* 1986, Flecker 1992); therefore the model results should be more readily applicable to the field if fish were present during the tests than if fish were absent. Also, fish water has been shown to enhance the phototactic swimming response of *Daphnia* (Ringelberg 1991a), and in a population of *Baëtis* mayflies taken from a fish stream, to increase the numbers of drifting *Baëtis* without altering the timing of the onset of drift (McIntosh and Peckarsky 1996). Responses to light that give rise to diel changes in behavior may therefore be closely tied to extant predation. The fish were fed natural assemblages of Oyster River benthos each afternoon during the times when no experiments were underway.

The entire stream was enclosed in black plastic to block out all natural light. Four 500 W halogen lamps controlled by computer were used to generate artificial light/dark cycles. Downwelling light intensity was measured continuously (International Light IL-1700 radiometer, SED033 probe with 2-pi collector corrected for cosine response) from a location at the water level adjacent to the tiles. Illumination from two arrays of wide-angle, narrow-wavelength GaAIAS infrared emitters (average power of 20 mW at peak wavelength ± 50%, 940 ± 20 nm) allowed videotaping during the darkened periods of the light/dark cycles.

Six nymphs were placed on an unglazed tile (10 x 10 x 0.5 cm, raised 0.5 cm above the streambed) located in each of the two stream channels. Nymphs were acclimated at the highest light intensity for a minimum of 1 hour. Time-lapse videos were recorded from the tile undersides (recording speed = 1 frame s^{-1}, time compression = 1:72). Locomotor activity was measured as the distance moved by each nymph between consecutive video-frames captured at 30 s intervals. Movements of individual nymphs were tracked by hand using the NIH-Image software package (NIH-Image v1.60, 1996).

Data collected at 30 s intervals were combined to produce total distances moved by each nymph over whole minutes. Data from individuals were pooled and 1-minute time-series of the average nymph activity underneath the tiles and the number of nymphs visible were constructed. Because the response time of the nymphs to changes in light was expected to be > 10 min (Grace 1990), 1-minute intervals were considered as sufficient to detect activity changes and also to adequately measure light changes. For this work, over 400 hours of videotape were processed, and over 3 gigabytes of computer disk were needed for image storage.

Experimental Design

Ambient light intensity was manipulated between 7.9×10^{-4} W cm^{-2} and 1.2×10^{-7} W cm^{-2}, an approximately 4 log-unit range in light intensity. The high value is comparable to noontime incident light intensity in July in New Hampshire and the low value occurs about 30 min after the period of largest relative light changes during local twilight (unpubl. data). The low value was chosen because it was lower than values associated with changes in *Stenonema* locomotor activity and vertical movements between the substrates (Grace 1990), but also high enough to be measurable with the light meter and maintained at a steady intensity by the lamps for long periods of time.

For each experiment, light was manipulated in sequence through four phases (illustrated in Chapter One): (1) an adaptation period at the brightest light intensity (BRITE) of at least 60 min, (2) a period of light decrease (DECRS) at a constant rate of light change, (3) a 60-min dim-light adaptation period (DARK), and (4) a period of light increase (INCRS) at the same, but opposite rate of light change used to decrease the light. The lengths of the DECRS and INCRS phases were dependent upon the strength of the light stimulus, i.e., the rate of light change applied (Fig. 1).

Characteristic nymph responses to the light/dark cycle were predicted based on observed diel cycles in locomotor activity and vertical position on the substrate in *S.*

modestum (Grace 1990) and other mayfly species (Chaston 1968, Elliott 1968, Glozier and Culp 1989). Locomotor activity was expected to start out at a low level during the BRITE-adaptation phase, increase during the DECRS phase, remain elevated during the DARK phase and decrease again during the INCRS phase. Numbers of nymphs visible beneath the tile surfaces were expected to be highest during the BRITE-adaptation phase, decline during the DECRS phase, remain low during the DARK phase, and increase during the INCRS phase. Combined, these typical responses are referred to as the *response curve* .

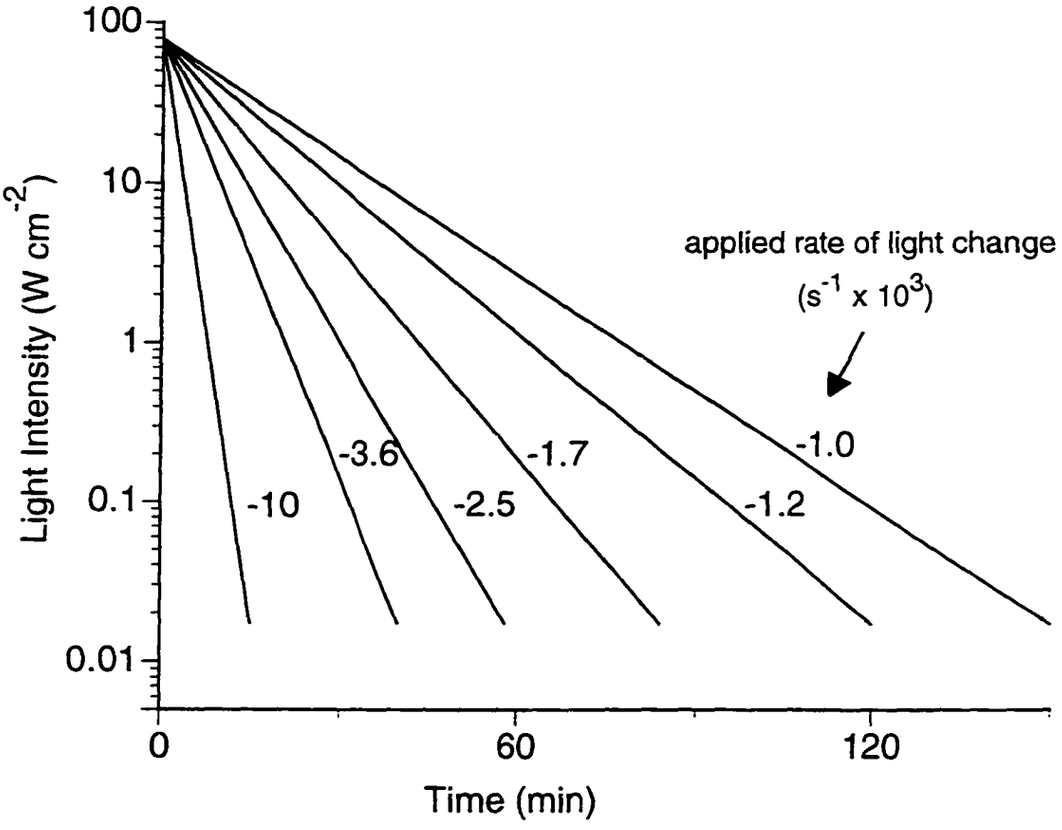

Figure 1. Graphical representation of the amount of time required to decrease ambient light intensity between the experimental high and low values (7.9×10^{-4} W cm^{-2} and 1.3×10^{-7} W cm^{-2}) at some of the rates of light change used to test the predictions of the STAR model. The number of minutes necessary to decrease the light between the high and low values is smaller at larger rates of light change, and longer at smaller rates; thus the light stimulus (S) is stronger at larger rates of light change and weaker at smaller rates of light change. The negative signs represent light decrease (positive signs would represent light increase). A complete list of rates used to test the model is located in Table A.1. in the Appendix.

Tests of the STAR Model

Predictions of the STAR model were tested with a series of artificial light/dark cycles following the experimental design outlined above. A set of experiments was conducted to establish a baseline relationship between rate of light change and the light response of the nymphs (Table Ia). In these experiments, the BRITE-adaptation light intensity was maintained at the ambient noontime value, and all light/dark cycles were started in the AM (Table A.1 in the Appendix).

Treatments to test for effects of the endogenous clock and cloud cover on the light response were performed at four rates of light change (Table A.1 in the Appendix). In the experiments that tested for alterations in the light response due to the influence of the endogenous clock, the light reduction (DECRS phase) was begun at one of two times of day, AM or PM (Table Ia). The effect of cloud cover on the light response was simulated by adapting the nymphs at a reduced light intensity (Table Ia). Light was manipulated between this lowered BRITE-adaptation intensity and the same low value of light intensity used to develop the baseline relationships and in all subsequent experiments. Treatments were combined to make a complete 2 x 2 factorial design. The four rates chosen for the treatments represent certain conditions within the typical range of conditions that occur during twilight at most locations except at high latitudes: (1) a sub-threshold [$\pm$ 1.4 x 10^{-3} s^{-1}] rate of relative light change, (2) the Ringelberg (1964) stimulus value [$\pm$ 1.7 x 10^{-3} s^{-1}] for the onset of phototactic swimming in *Daphnia*, (3) a mid-range value [$\pm$ 2.5 x 10^{-3} s^{-1}], and (4) a large value [$\pm$ 3.6 x 10^{-3} s^{-1}] close to the maximum rate of relative light change recorded at local twilight. These four values were considered sufficient to characterize differences in the stimulus-based activity responses between the baseline and treatments.

A set of experiments to test the effect of short and discontinuous periods of light stimulus (such as occur during cloud events), on locomotor activity were carried out for three rates of light change (Table Ia). The rates were the same as used for the time-of-day

and adaptation light-intensity treatments, excluding the sub-threshold value (Table A.2. in the Appendix). For each rate of light change tested, experiments were conducted in which the DECRS phase was divided into one, two, or four equally long periods (steps) separated by 90-min periods of no light change (Table A.2 in the Appendix). The difference between these "step" experiments and the experiments already described was in how the light decrease (and light increase) was accomplished. The light decrease was continuous over the entire range of light values (approximately 4 log units) in the 1-step, the baseline, and the time-of-day and adaptation light-intensity treatment experiments. The light decrease was continuous over half the range of light values (~ 2 log units) in the 2-step experiments and over one-quarter of the range of light values (~ 1 log unit) in the 4-step experiments. Each partial light decrease during the 2-step and 4-step experiments was followed by 90 minutes of no light change. The total change in absolute light intensity from the beginning to the end of all light-decrease steps was therefore the same (~ 4 log units) in all experiments; the only difference being that in the step experiments, the light decrease was interrupted by periods of no light change. The subsequent phases of the light increase were applied in a similar manner so that the ending light intensity value was equal to the beginning BRITE-adaptation light intensity in all experiments.

TABLE Ia. Predictions of the STAR-model regarding locomotor activity and description of experiments that tested each prediction. (Terms in *italics* are defined in Table Ib.)

Test	Variable tested	STAR-predicted response[a]	Expected departure from baseline	Description of experiments
Baseline relationships	*Rate of relative light change, or light stimulus (S)*	- *latent period* ∝ *light stimulus* - *magnitude of response* ∝ *light stimulus*.	N/A N/A	Series of *artificial light/dark cycles* at sixteen *rates of light change* (listed in Table A.1. in the Appendix). The light decrease (*DECRS phase*) commenced at 10AM EST. The change in absolute light intensity was ~10^{-4} W cm^{-2}.
Treatments	Endogenous clock	- *latent period* Δ^b in PM. - *magnitude of response* Δ in PM.	Shorter Larger	Series of *artificial light/dark cycles* at four of the above sixteen *rates of light change* (listed in Table A.1. in the Appendix). Treatment was time of day. The light decrease (*DECRS phase*) commenced at either 10AM or 6PM EST. The change in absolute light intensity was ~10^{-4} W cm^{-2}.
	Adaptation light-intensity (cloud cover)	- *latent period* Δ in reduced light. - *magnitude of response* ∝ *light stimulus*.	Shorter No change	Series of *artificial light/dark cycles* at a subset of four of the above sixteen *rates of light change* (listed in Table A.1. in the Appendix). Treatment was *BRITE*-adaptation light intensity level. Levels were noontime-ambient (approx. 10^{-4} W cm^{-2}) and reduced (approx. 10^{-6} W cm^{-2}).
Periods of short and discontinuous light decrease *(steps)*	Length of time over which *light stimulus* is applied; light changes applied over discrete periods disconnected in time.	- if length of time is >= response time of nymphs: - *latent period* ∝ *light stimulus*. - *magnitude of response* ∝ to length of each *step* - if length of time is < response time of nymphs: - *latent period* becomes infinite. - *magnitude of response* not controlled by *light stimulus*.	 No change Smaller N/A Variable	Series of *artificial light/dark cycles* at three of the above sixteen *rates of light change* in which the light decrease was distributed over one, two, or four equal *steps* (Table A.2. in the Appendix). Multiple light-decrease steps were interspersed with 90-min periods of no light change. The length of each step was dependent on the applied rate of relative light change (cf. Fig. 1). The light decrease (*DECRS phase*) commenced at 6PM EST. At the completion of all DECRS steps, the change in absolute light intensity was ~10^{-4} W cm^{-2}.

[a] Response = a change in locomotor activity due to the application of a light stimulus.
[b] Δ = an expected change from the baseline in the activity response due to the applied treatment.

Table Ib. List of terms defined in testing the STAR model.

Name	Definition and Comments
Light environment	
Artificial light/dark cycle	Simulated 24-hr light/dark cycle comprised of four light phases that represent: daytime (BRITE), evening twilight (DECRS), nighttime (DARK), and morning twilight (INCRS).
Phases of the artificial light/dark cycle	
BRITE	Adaptation period at the maximum light intensity.
DECRS	Period of light decrease.
DARK	Adaptation period at the minimum light intensity.
INCRS	Period of light increase.
Steps	Shortened periods of light decrease (DECRS) or increase (INCRS) interrupted by periods of no light change (labeled as "LOW" when between light-decrease phases and as "HI" when between light-increase phases).
LOW	
HI	
Light change terms	
Rate of light change, or Relative light change, or Light stimulus (S)	The first derivative of the light intensity vs. time curve, estimated from the equation: $S = [\ln(I_{j+1}) - \ln(I_j)] / dt$ where, S = the rate of relative light change per s, I = light intensity in W cm^{-2} at time period j or $j+1$, t = length of time-intervals in s (from Ringelberg 1964).
Strength of *S*, the light stimulus	A measurement of the magnitude of the rate at which light changes over time. Stronger stimuli are associated with larger rates of light change, and weaker stimuli are associated with smaller rates of light change (see Fig. 1).
Response variables	
Locomotor activity	Average distance (mm) moved per nymph per min.
Response curve	Activity response to each of the light phases measured as the average distance moved per nymph per light phase. Used as repeated measures in the analysis.
Latent period	The delay (min) between the beginning of the light decrease and the onset of the change in locomotor activity. A delay occurs because the animal must accumulate a large enough light stimulus (provided by the rate of relative light decrease) to evoke a reaction (from Ringelberg 1964).
Magnitude of the change in activity	The difference in locomotor activity between the "daytime" and "nighttime" periods.
Data smoothing technique	
Exponential Weighted Moving Average (EWMA) transformation	Smoothing of a time series by application of a weight (r) to the point of interest and all preceding points. The weight is largest for the point of interest and decreases exponentially with each point further back in time. (From SAS v.5, SAS Institute Inc., 1989-95).
r parameter	Value of weight applied to the point of interest, ($0<r\leq1$). Data are smoothed more when smaller weights are used, as smaller weights are less sensitive to short-term data fluctuations than are larger weights.

Data Analysis

Smoothing Technique for Improving the Resolution of Activity Patterns from Time-Series Data.

Because time-series data can be highly variable, (examples, see Cobelas *et al.* 1995, Prairie *et al.* 1995) and it is difficult to determine precisely the timing of a change in activity or to identify patterns, it was desirable to smooth the time-series data. Smoothing techniques of a single time-series are typically some variation of a moving average (Box and Jenkins 1976, Chatfield 1997). In the data presented here, activity at each point in time was assumed to be dependent on the activity at the points before it, with diminishing influence. For these reasons, the exponential weighted moving average technique (EWMA, SAS/QC SAS Institute, 1989) was chosen for smoothing the time series. EWMA-transformed time-series of individual nymph activity were used in estimating the length of the latent periods between the beginning of the DECRS phase and the onset of heightened locomotor activity. EWMA-transforms of the average nymph activity produced from the pooled data were used for all time-series plots of locomotor activity and for visual comparisons of activity patterns within and between experiments. All other analyses were made from the raw data.

Each point in an EWMA time-series represents the weighted average of the point of interest plus all previous points. The weight of each point decreases exponentially going backward in time starting at the point of interest. The weight r ($0 < r \leq 1$) assigned to the point of interest is a parameter of the EWMA. Small values of r are less sensitive to short fluctuations, and larger values of r are more sensitive to short fluctuations (Fig. 2). For example, if $r = 1$, the EWMA-transformation returns the original data, because the point of interest carries all of the weight. The recommended value for r is 0.2 (SAS Institute, 1989).

The choice of r was important as it could bias the estimates of the response variables. As an example, estimates of the timing of the onset of heightened locomotor activity, measured as the length of the latent period between the beginning of light decrease and the moment when nymph activity increased above the BRITE-adaptation mean, were compared between four values of r and the default value, $r = 0.2$ (Fig. 3). When r was large ($r \geq 0.5$), relatively long latent periods were estimated at strong rates of light decrease (shown by 's' Fig. 3c, d), indicating that data were still too noisy to reliably detect an activity change. Relatively long latent periods were estimated at weak rates of light decrease when $r = 0.9$ (shown by 'w' Fig. 3d), but not when $r = 0.5$ (Fig. 3c). When r was small ($r = 0.05$), latent periods were also relatively long (Fig. 3a). This was particularly problematic when weak light stimuli were tested, because the response of the nymphs was dampened so much that the smoothed data revealed no activity change. In between the two extremes (when $r = 0.1$ or 0.2), the estimated latent periods were within a few minutes of each other and were more consistently within the mid-range of estimates for all values of r (Fig. 3b). As it was not possible to know which estimate of the latent period was the correct value, the raw data and the EWMA-smoothed data were visually compared and a decision was made to use the recommended weight ($r = 0.2$), as it appeared to best estimate the timing without excessive smoothing of the data. The removal of the noise allowed for visual comparisons of locomotor activity between experiments without altering the general shape of the curves (Fig. 2). The EWMA-transformation was also appealing because the transform did not alter the mean activity values (Table II), so that reasonable opinions about the magnitude and the time-course of the initial peak of heightened locomotor activity could be made from visual inspection of the time-series. EWMA-transformations and statistical analyses were made with SAS (SAS Version 5, or JMP Version 3.1.5, SAS Institute Inc., 1989-95).

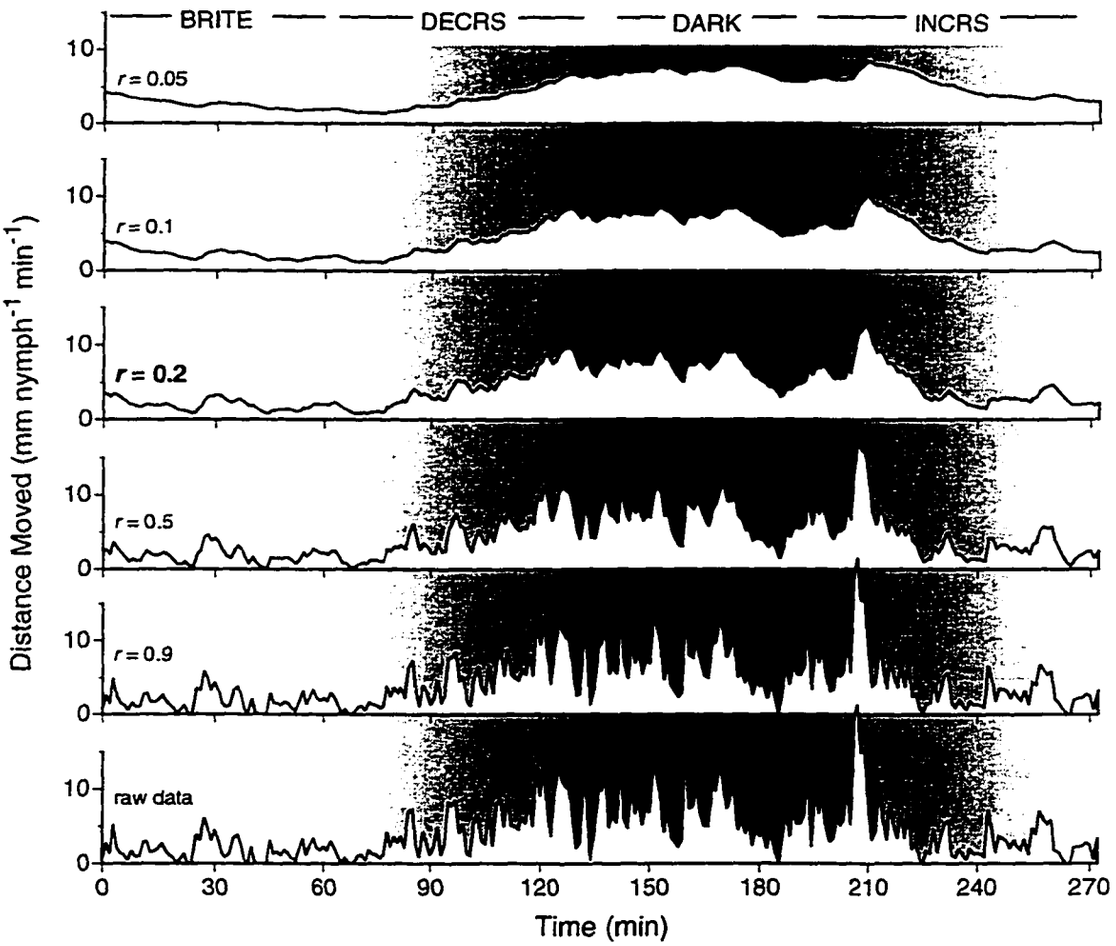

Figure 2. Representative time-series of average locomotor activity demonstrating the effect of EWMA-smoothing at five values of *r*, the parameter used as a weight that determines the amount of smoothing (terms are defined in Table Ib). From left to right, the time-series represents the 60-min BRITE-adaptation phase, the DECRS phase, the 60-min DARK phase, and the INCRS phase of the artificial light/dark cycle. Shading represents the light environment. The applied rate of light change was $\pm$ 1.9 x 10^{-3} s^{-1}. The time required to complete the light reduction and light increase (DECRS and INCRS phases) was 76 minutes each. Data are same as shown in Table 2 and in Chapter One, Figures 5 and 7.

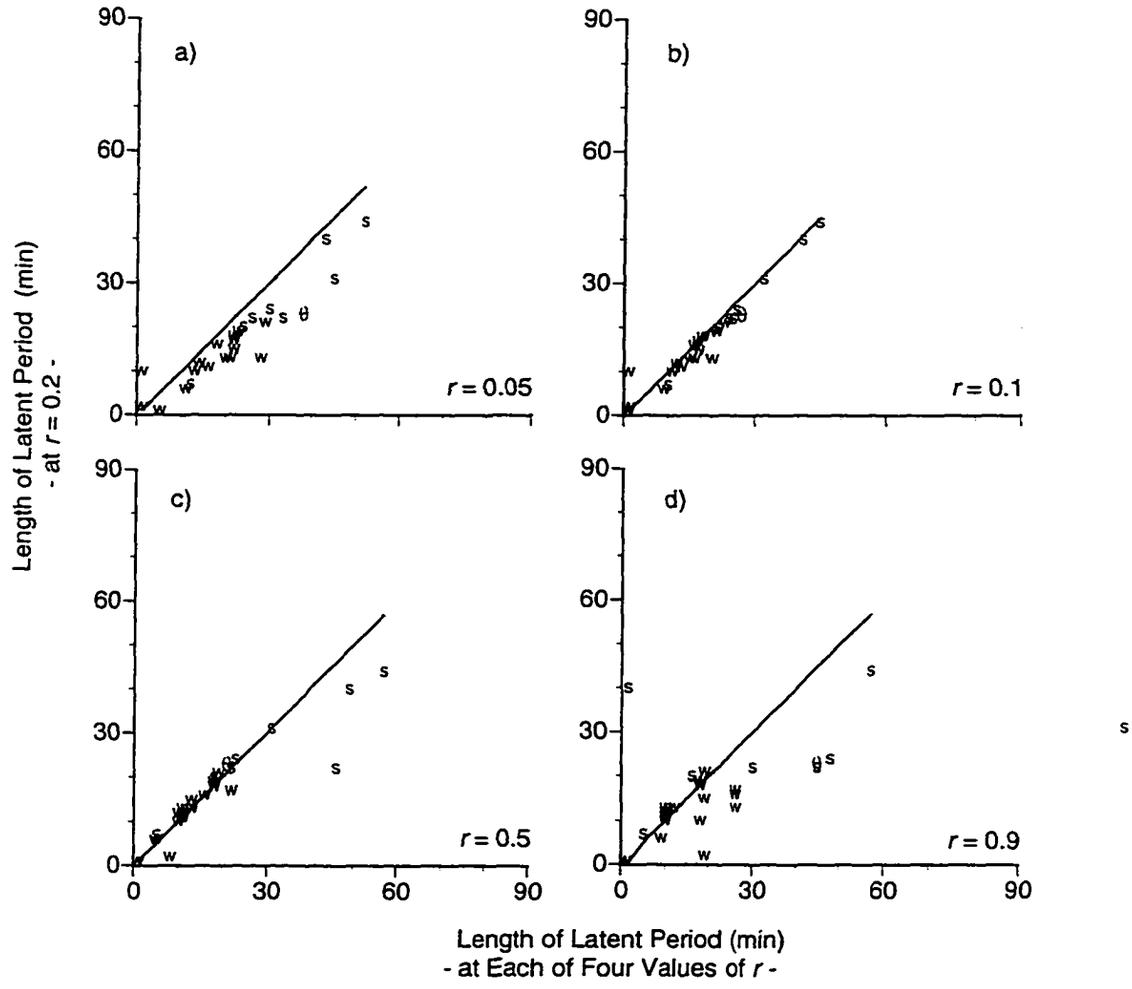

Figure 3. Comparisons of estimates of the latent period (the length of time between the beginning of the light decrease and the moment when nymphs began to increase their activity) determined from Exponential Weighted Moving Average (EWMA) time-series for the default parameter weight r = 0.2, and each of four other values of r (see text and Table 1b for explanations of r). Data points represent the individual experiments used to test the STAR model (n=28, including all tests except those of discontinuous periods of light change; see Table A.1. in the Appendix). Experiments for which the rate of light decrease was $\leq -1.7 \times 10^{-3}$ s^{-1} are marked with (w); those for which the rate was $> -1.9 \times 10^{-3}$ s^{-1} are marked with (s); and the experiment depicted in Figs. 2 and 4, for which the rate of light decrease was -1.9×10^{-3} s^{-1}, is marked with (θ). The 1:1 lines are drawn on each plot for comparison purposes.

TABLE II. Mean distance moved per nymph (mm min^{-1}) during selected phases of an artificial light/dark cycle. Comparisons are between raw data and five EWMA-transformations (*r* values). Data are the same shown in Fig. 2 and in Chapter One, Figs. 5 and 7, for which the applied rate of light decrease was -1.9×10^{-3} s^{-1}.

r value	BRITE Phase	DARK Phase	Entire time-series
0.05	2.6	7.0	4.5
0.1	2.2	7.1	4.5
0.2	2.0	6.9	4.4
0.5	1.9	6.8	4.4
0.9	1.9	6.8	4.4
raw data	1.9	6.8	4.4

Analysis of the Activity Response Variables

Response curve (activity changes during the artificial light/dark cycle). Mean values of the locomotor activity measured during each of the four light phases were used together in a repeated-measures analysis of variance to compare activity within and between treatments. Mauchly's criterion test for the compound symmetry of the variance-covariance matrix was non-significant ($p > 0.05$) for all analyses, indicating that the probabilities associated with ordinary *F* tests were correct, and the univariate mode of the repeated measures tests (ANOVAR) are reported as recommended by Potvin *et al.* (1990). Before the time-series data could be compared, it was necessary to make sure that patterns were not confounded by lengthy periods in which there were no nymphs visible on the tile undersides and consequently zero locomotor activity. Because no experiments fell into this category, all were included in the analysis.

Timing (latent period). Preliminary results suggested that nymphs that were inactive during the BRITE-adaptation phase responded more strongly to the light decrease than did nymphs that were active during the BRITE-adaptation phase (Chapter One). Therefore, estimates of latent periods were made from time-series data for non day-active

nymphs only (Fig. 4). Nymphs were classified as non day-active when the average distance moved during the BRITE-adaptation phase was ≤ 6 mm min^{-1} (the average body length of the mayflies, see Chapter One). The length of the latent period was calculated from the EWMA-transformed time-series as the amount of time that passed between the beginning of the DECRS phase and the moment at which the individual's locomotor activity rose above the BRITE-adaptation mean (Fig. 4). Increases in activity were considered spurious if the average increase was not sustained above the BRITE-adaptation mean for a minimum period of 10 minutes (Fig. 4, example: nymph 6). Ten minutes has been shown to be the shortest amount of time necessary to elicit a response in *S. modestum* under cloud conditions in natural light (Grace 1990), and was considered to be a conservative estimate of the timing of the actual change in activity.

Other researchers have classified animals by their predominant activity for purposes of detecting a change, including Belanger and Orchard (1988) who grouped freshwater leeches (*Macrobdella decora*) into three types; as still (i.e., not moving), movers and swimmers, before application of an activity-producing hormone; and Daan and Ringelberg (1969), who described water fleas (*Daphnia magna*), as either rhythmic or non-rhythmic based upon the amount of vertical displacement in constant light prior to a light-manipulation. In both cases, the response of the animals to the treatment was dependent upon their initial activity. These studies suggest that at any given time, differences in individuals may be common, and must be considered when studying response variables. By describing the activity change in terms of the starting activity, a clearer understanding was made of the change in activity following the treatment in both studies.

Nymphs not used in the estimates of the latent period were those classified as day-active (average activity > 6 mm min^{-1}), or "other" (either the nymph was not visible on the lower tile surface at all during the BRITE period, or the nymph was visible for some portion of the BRITE-adaptation period, but left before the application of light decrease). Examples from each category are illustrated in Chapter One. Non day-active nymphs

comprised 47% (n = 197) of the total nymphs used in all tests of the STAR model (n = 420). Of the rest, 32% (n = 134) were day-active and 21% (n = 89) were classified as "other". Of those, 9% (n = 38) never moved to the tile undersides and 12% (n = 51) left the tile undersides before any light change. Despite these differences in individuals, the averaged time-series of locomotor activity produced from the pooled data of all nymphs showed the expected changes to the light/dark cycle at all rates of light change tested.

Magnitude of the change in activity. Locomotor activity increased in response to light decrease. Estimates of the magnitude of the activity change were made for each experiment by subtracting the mean activity during the BRITE phase from the mean activity during DARK the phase. A least-squares regression of the rate of light change on the resultant differences was performed for the baseline experiments and for each treatment.

Vertical movements between substrate surfaces. There was little change in the numbers of nymphs visible during the BRITE-adaptation periods, making it straightforward to estimate the moment when nymphs began leaving the tile undersides. Using the technique outlined by Haney *et al.* (1983), the onset of leaving was recorded as the mid-point between the first two of three points having decreasing numbers of nymphs below the BRITE-adaptation average.

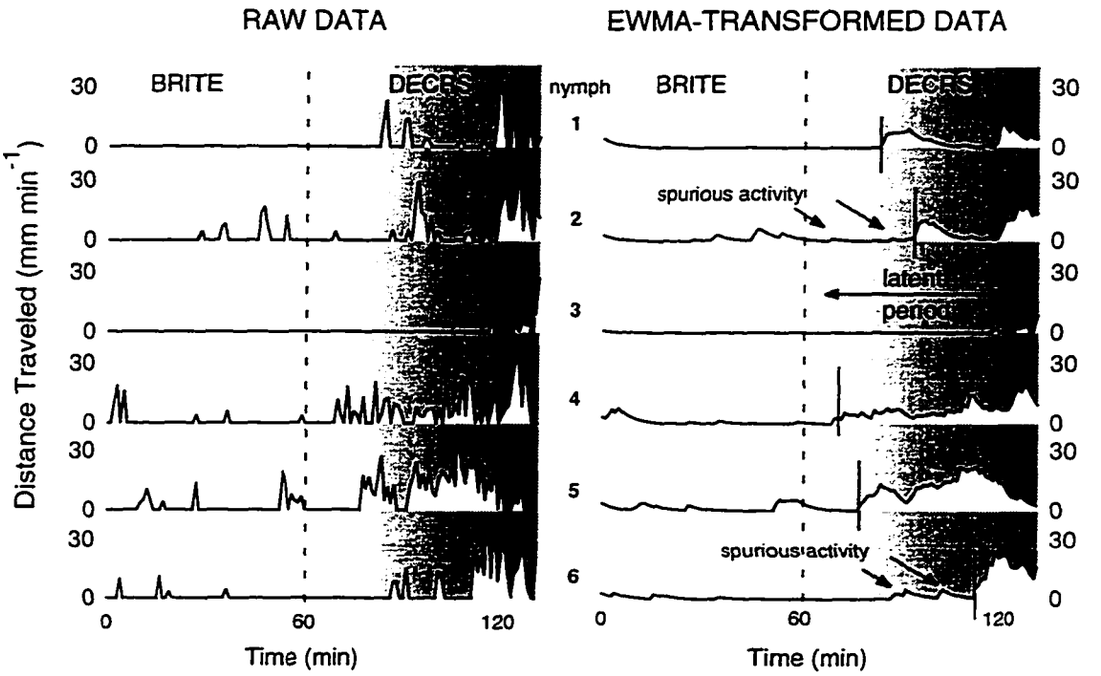

Figure 4. Time-series of individual non day-active nymphs during the BRITE-adaptation and DECRS phases of an artificial light/dark cycle. Right panels). Examples of latent periods (the length of time between the beginning of the light decrease and the moment when nymphs began to increase their activity) estimated from EWMA-transformed time-series of the non day-active nymphs. Short vertical lines mark the onset of increased activity for each nymph. Heightened activity of < 10 min duration was not considered a response to light change (marked as "spurious activity"). Left panels). Raw data used in generating each EWMA-transformed time-series. The onset of the light decrease (DECRS phase) is marked by the vertical dotted line. Shading represents the light environment during the two light phases. The average latent period for this experiment was estimated as 34 min ± 3.2 SE (n = 6). Light was decreased at a constant rate of -1.9×10^{-3} sec $^{-1}$ over a period of 76 minutes. Time-series of the average locomotor activity generated from the pooled data for all nymphs is shown in Fig. 2, and in Chapter One, Figs. 5 and 7.

Results

Relationship of Relative Light Change to the Timing, Magnitude and Time-Course of the Initial Peak of Heightened Locomotor Activity

Timing (Latent Period)

The latent period, estimated as the length of time between the beginning of the light decrease and the onset of heightened locomotor activity (of the non day-active nymphs), was significantly correlated with rate of light decrease (Fig. 5). The correlation curve traces a typical strength-duration relationship of physiological excitable systems (Grinnell 1977), suggesting that the locomotor activity response is the result of the buildup of light stimulus over time. It was therefore possible to estimate the rheobase, or the minimum rate of light decrease that was capable of eliciting an activity response (Fig 5). Rates of light decrease smaller than the rheobase are incapable of eliciting a response, and are therefore considered as "inadequate stimuli", or sub-threshold. The buildup of the excitatory state in such cases is equal to or lower than the rate of decline of the excitatory state; therefore there is no net accumulation of stimulus over time and no change in locomotor activity.

The rheobase was calculated as $1.0 \times 10^{-4} s^{-1}$ from equation (1):

$$\ln (S/S-R) = c \times t \qquad (1)$$

where S is the applied rate of light decrease per second, R is the minimum rate of light decrease per second capable of eliciting an activity response (the rheobase), c is the rate of decline or the disintegration constant of the excitatory state, and t is the measured length of the latent period for each rate tested, in seconds. Data used for t and S in the equation were the measured latent periods and their corresponding rates of light decrease, respectively ($n = 14$, data points from Fig. 5). The rheobase was determined by iteration; expected values were substituted in the equation until the best linear fit to the data was

obtained (Ringelberg 1964, pers. comm.). The resulting relationship ($R^2 = .94$, $p < 0.0001$, $n = 14$), was used to estimate the value of the disintegration constant, c (3.3×10^{-5} s^{-1}). This relationship between the onset of locomotor activity and relative light change in *Stenonema* is similar to the relationship between the phototactic swimming response and relative light change previously determined in *Daphnia* (Ringelberg 1964).

To test whether non day-active nymphs behaved differently than the population as a whole, latent periods were estimated from the averaged time-series that had been produced from the pooled locomotor activity of all nymphs and compared to the baseline relationship estimated from the non day-active nymphs (Fig. 6). The shape of the curves were the same but intercepts were significantly different ($p < 0.008$, $F_{1,1} = 15.3$, ANCOVA). Consequently, the estimates of the amount of time between the beginning of the light decrease and the onset of heightened locomotor activity were about 7 minutes shorter for the group average than for the non day-active nymphs. This is not consistent with a spreading out of the population's response over a broad time period that would tend to mask the detectable response until later, rather than earlier. However, because the day-active nymphs were already active prior to the light stimulus, the earlier onset may have been caused by their reacting sooner to the light stimulus than did the non day-active nymphs (Ringelberg pers. comm.). Similar differences in the onsets of upward swimming of daphnids classified as rhythmic and non-rhythmic have been reported (Daan and Ringelberg 1969), suggesting differences in individual responses to light stimulus may be important in shaping the observed response of the population as a whole.

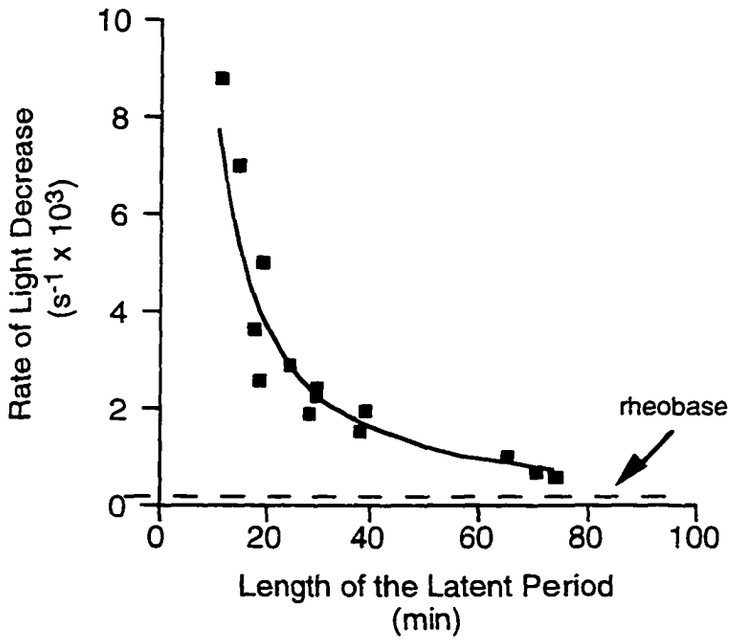

Figure 5. Relationship between the rate of light decrease (S) and the length of time to the onset of heightened locomotor activity (the latent period). The equation of the line is: ln[latent period (min)] = -1.32 - 0.776 x ln |S (s^{-1})|, (R^2=.93; p< 0.001; n=14). Each data point represents the mean estimate from the non day-active nymphs at each rate of light decrease. Dotted line represents the rheobase value below which no response to a light stimulus occurs (see text for explanation of the method of calculating the rheobase). This relationship represents the baseline from which comparisons with the various treatments are made. The line was fitted using log-log transformed data.

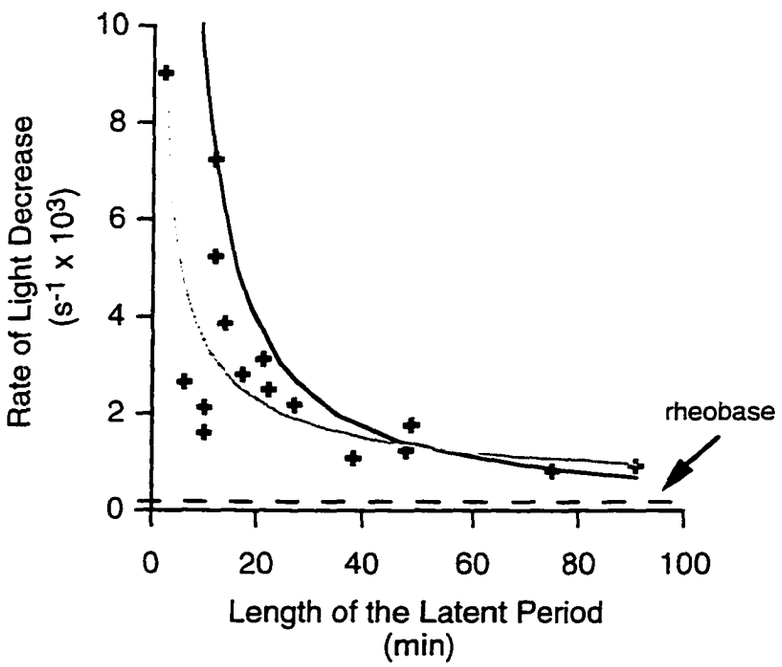

Figure 6. Comparison of the baseline relationship (shown in Fig. 5) between rate of light decrease (S) and length of the latent period estimated from non day-active nymphs (redrawn here with the black line), and the relationship produced using data from the averaged locomotor-activity time-series (+, gray line). Slopes of the lines were equal, but differences in intercepts resulted in an average 7 minute difference in the estimates of the length of time between the beginning of the light decrease and the onset of heightened locomotor activity between the non day-active nymphs and the "population" (by ANCOVA). The lines were fitted using log-log transformed data.

Magnitude of the Change in Locomotor Activity and Time-Course of the Initial Peak in Heightened Locomotor Activity

As predicted, the magnitude of the changes in locomotor activity as a result of the decrease in light were greater at larger rates of light change (stronger stimuli) than at smaller rates of light change (weaker stimuli, examples, Fig. 7). Initial peaks of heightened locomotor activity appeared to be sharper when stronger stimuli (e.g., $S \geq -4.8 \times 10^{-3}\ s^{-1}$) were applied than when weaker stimuli (e.g., $S \leq -2.5 \times 10^{-3}\ s^{-1}$) were applied, also as predicted (Fig. 7, left panels). At most of the applied rates of light change, locomotor activity reached a maximum within the DECRS period, and then decayed to a lower, but still elevated, level throughout the entire DARK phase (Fig. 7). The initial peaks decayed faster at stronger stimuli than at weaker stimuli , supporting the STAR prediction that duration of the initial activity peak is inversely proportional to the rate of light decrease.

At very weak stimuli (e.g., $-1.2 \times 10^{-3}\ s^{-1}$), secondary peaks were common in the INCRS phase that were as large as the original activity peak (Fig. 7, left panels). At larger stimuli, secondary peaks began earlier and were lower than the initial peak, with some secondary peaks that began well within the DARK phase (e.g., $S \geq -3.6 \times 10^{-3}\ s^{-1}$; Fig. 7, left panels), suggesting that although the duration of the highest activity was short-lived, there were more complex changes in activity during the dark adaptation period (when there were no light changes) than predicted by the STAR model.

The change in locomotor activity following the completion of the DECRS phase was examined as a function of the rate at which the light decreased. Despite the variability in the amount of activity during each minute over the time-series (Fig. 7, left panels), the magnitude of the change in locomotor activity increased as a function of increasing rate of light change as predicted by the STAR model (Fig. 7, right panels; Fig. 8).

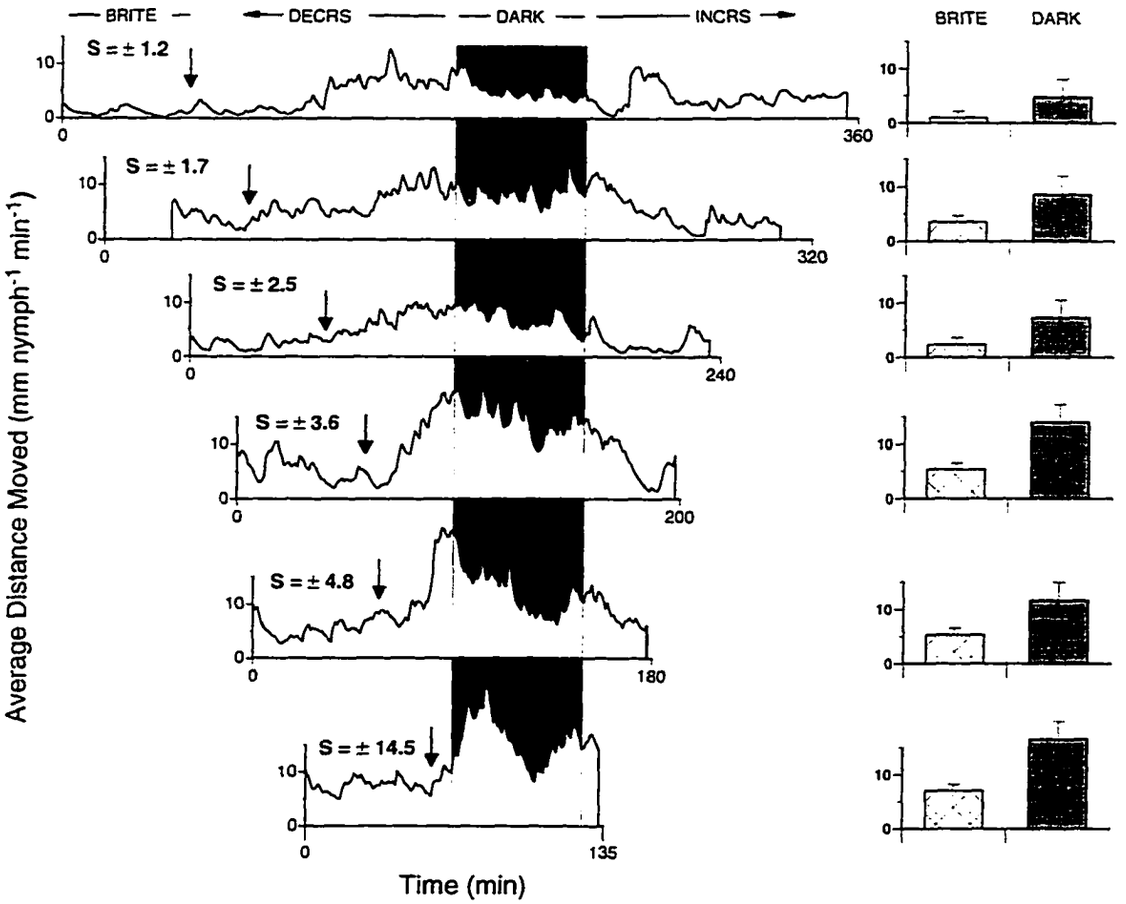

Figure 7. Sample time-series illustrating the differences in locomotor activity during artificial light/dark cycles at six rates of light change ($S = s^{-1} \times 10^3$), increasing in strength from top to bottom. Left panels). EWMA-transformed time-series of average locomotor activity during each artificial light/dark cycle. Arrows mark the beginning of the light decrease (DECRS) phases, shaded area represents the DARK phases. $Time_0$ = 0900 EST. Right panels). Mean locomotor activity during the phases before (BRITE) and after (DARK) the light decrease. Bars are mean ± SD.

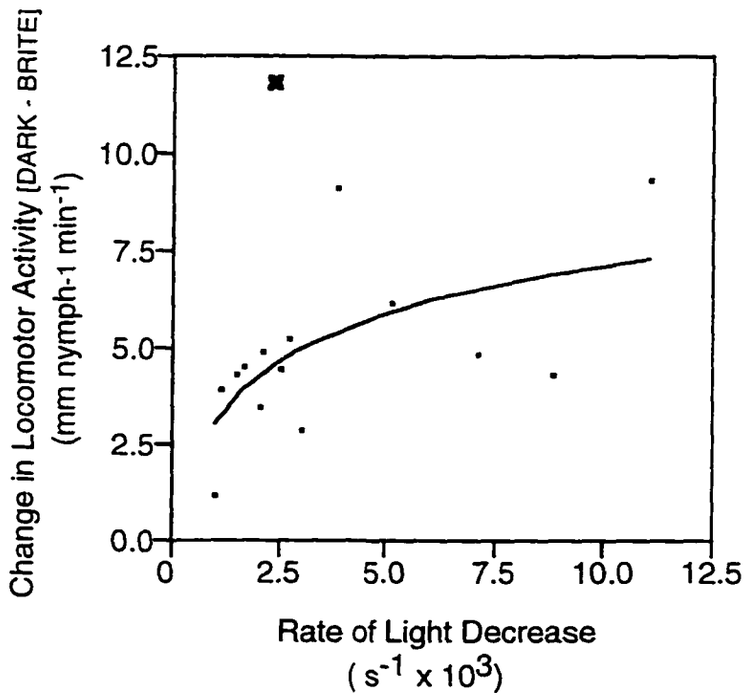

Figure 8. The relationship between rate of light decrease (S) and the magnitude of the change in locomotor activity from before and after the light decrease. Each data point represents the difference between the mean activity during the DARK and BRITE light phases. The equation of the line is:
Change in locomotor activity (mm nymph^{-1} min^{-1}) = 15.386 + 1.79 x ln |S in s^{-1}|,
(R^2 = .38, $p < 0.02$, n = 14). Data point marked by (**x**) was excluded from the determination of the regression line, because the standardized residual was > 3 SD from zero (Neter et al. 1990).

Effects of the Time of Day and Adaptation Light-Intensity on the Activity Response to Relative Light Change

Response Curve

Activity responses to the light/dark cycles were examined for each treatment (Fig. 9). The main influence of time of day was a larger activity change during the DECRS and DARK phases in the PM experiments than in the AM experiments (Fig. 9). Adaptation light intensity appeared to have a strong influence on locomotor activity, both in the AM and PM experiments, as the characteristic responses to the various phases of the light/dark cycle were observed in the ambient-light adaptation treatments (Fig. 9 a, c), but not in the reduced-light adaptation treatments (Fig. 9 b, d).

Significant differences between treatments were not detected by repeated measures tests of locomotor activity across the four light phases (Table III). A weak effect of time of day (DAY) was detected, and may have been a result of the overall higher locomotor activity during the PM than during the AM experiments (Fig. 9). The significant effect of Time within treatments indicates that nymphs responded to the light changes in all treatments. There was a strong interaction between Time x BRITE-adaptation illumination level (Table III), that indicated that the light level at which nymphs were adapted was related to differences in activity during particular light phases. Two observations may explain where these differences occurred: first, for some of the BRITE-reduced experiments, the length of the measured latent period was within 1-2 min of the length of the DECRS phase, consequently there were no significant differences in locomotor activity between the BRITE and DECRS phases (Fig. 9, b, d); and secondly, during many of the BRITE-reduced experiments, locomotor activity did not decline during the INCRS phase as expected, but remained elevated, most notably in the BRITE-reduced/PM experiments (Fig. 9, d). There were no significant interactions between Time x Time of Day indicating that

time of day did not strongly affect the shape of the response curves at either level of adaptation light intensity.

TABLE III. Analysis of variance with repeated measures[a] for treatment[b] effects of time of day (Day) and adaptation light intensity (BRITE) on the activity response of *S. modestum* nymphs.

	Source of variation	MS	F	df	P
Between treatments	Day	112.1	4.1	1	0.07
	BRITE-level	0.6	0.02	1	0.9
	Day x BRITE	6.8	0.3	1	0.6
	Error	27.5		12	
Within treatments	Time	91.5	22.4	3	0.0001
	Time x Day	6.8	1.6	3	0.2
	Time x BRITE	30.1	7.4	3	0.0006
	Time x Day x BRITE	2.6	0.6	3	0.6
	Error (Time)	4.1		36	

[a]Repeated measures (time) = average locomotor activity during the BRITE, DECRS, DARK, INCRS phases.

[b]Treatments = combinations of time of day (AM vs. PM start-times) and BRITE-adaptation light intensity (ambient noontime vs. reduced), see Table Ia for description of treatment experiments.

Overall, the locomotor activity response during various phases of the artificial light/dark cycle was altered by the different treatments; in some cases the response was contrary to that expected (e.g., heightened locomotor activity during the INCRS phase in the BRITE-reduced/PM experiments). The stimulus-activity response was triggered in all combinations of treatments, supporting the hypothesis that relative light change is the most important control of locomotor activity in this mayfly species.

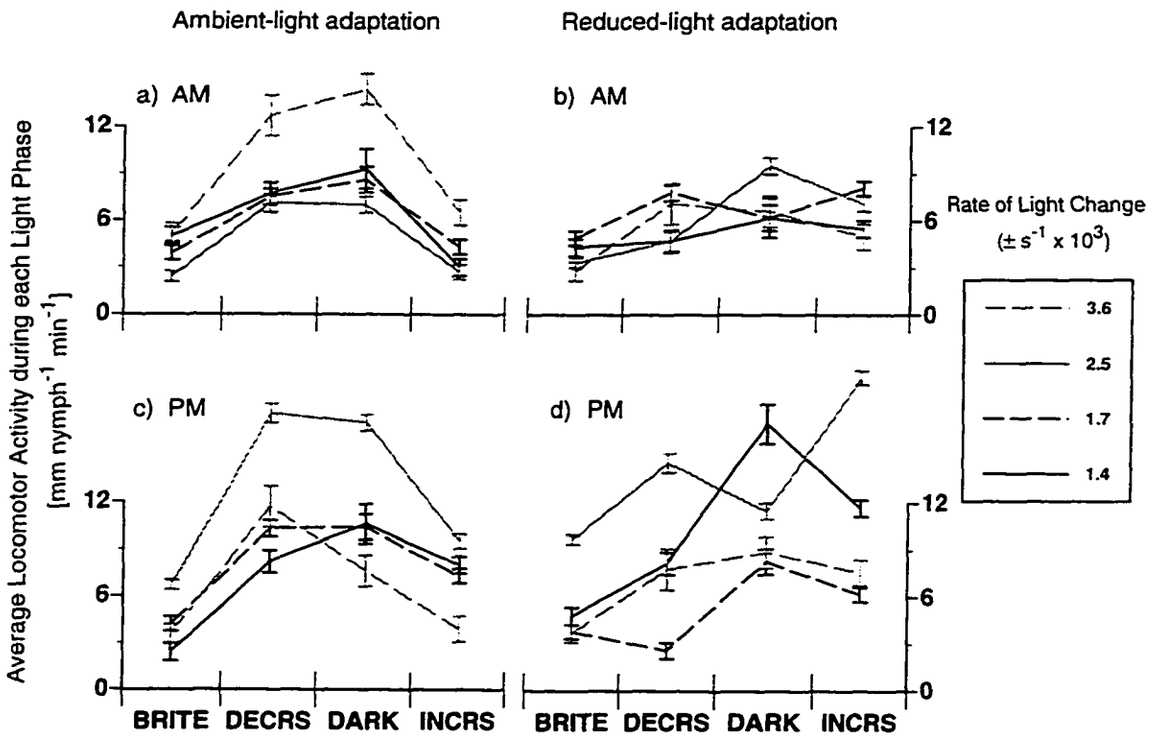

Figure 9. Response curves of locomotor activity during artificial light/dark cycles by time of day and light-adaptation treatments: (a) bright-adaptation/AM, (b) reduced-light adaptation/AM, (c) bright-adaptation/PM, and (d) reduced-light adaptation/PM.

Timing (Latent Period)

Least-squares regressions were made between rate of light decrease and the timing of the increase in locomotor activity (measured as the length of the latent period between the beginning of the light decrease and the onset of heightened locomotor activity) for each treatment. Correlations were significant for all treatments ($p < 0.05$) except for BRITE-reduced/PM. Tests for covariance of slopes and intercepts (ANCOVA) between the baseline regression (see Fig. 5) and those treatment regressions that were significant did not detect differences in the slopes ($p > 0.05$), but the intercept of the BRITE-reduced/AM treatment was significantly different from the baseline ($p < 0.002$, $F_{1,1} = 14.6$). This indicates that the latent periods were different in length when the adaptation light intensity was reduced, as expected. (Because the regression was not significant for the BRITE-reduced/PM treatment, an estimate was made by subtracting the individual latent periods at each rate from the calculated baseline latent period at the same rate [c.f. Fig. 5], and averaging the results.) The length of the latent period at any particular rate of light change was significantly shorter for nymphs in the BRITE-reduced treatments, by an average of 16 and 11 minutes for the AM and PM experiments, respectively. There was no shortening of the latent period in the BRITE-noontime ambient/PM treatments, contrary to predicted effects of the endogenous clock.

Although reduced light-adaptation modified the timing of the activity change, there was no particular value of light intensity associated with the onset of heightened locomotor activity. The average light intensity at the onset of heightened locomotor activity was significantly lower ($p < 0.0001$, $F_{1,13} = 45.7$, ANOVA) in the BRITE-reduced treatments than in the BRITE-ambient treatments ($8.4 \pm 2.3 \times 10^{-7}$ vs. $3.3 \pm 1.3 \times 10^{-5}$ W cm^{-2}, respectively). Light intensities at which the BRITE-noontime adapted nymphs initiated changes in locomotor activity were higher than the light intensity at which the BRITE-reduced nymphs were originally adapted. Even at the reduced adaptation light intensity,

locomotor activity did not increase until after the beginning of the DECRS period, indicating that although low light intensity enhanced the stimulus-activity response, low light intensity did not initiate the increase in locomotor activity.

Magnitude of the Change in Activity

The mean locomotor activity during the BRITE and DARK phases and the difference in activity between them were compared within and among treatments (Fig. 10). Locomotor activity was significantly higher during the DARK periods than during the BRITE periods within all treatments ($p < 0.0001$, $F_{1,11} = 51.77$, ANOVAR), indicating that in all cases there was a heightened locomotor activity response to light decrease. There were no significant differences in BRITE activity or DARK activity between treatments (all $p >> 0.05$, ANOVA).

Because light could not be reliably measured much below the experimental minimum light intensity, it was not possible to test the effect of a light decrease over a comparable 4 log-unit range in the BRITE-reduced treatments. Because the adaptation light intensity was lower during the BRITE-reduced treatments, the duration of the light decrease was smaller than in the BRITE-noontime-ambient treatments. The magnitude of the activity change was therefore expected to be smaller in the BRITE-reduced treatments. When the treatments were pooled into two groups by adaptation light intensity, there was no significant difference in the amount of the activity change between groups ($p = 0.3$, $F_{1,14} = 1.4$, ANOVA), but there was a significantly smaller activity change in the BRITE-reduced/AM treatment when compared with the other three treatments (Tukey-Kramer (HSD) multiple-comparisons test, $\alpha = 0.05$). The DARK activity was somewhat higher in the PM experiments, regardless of the BRITE-adaptation light intensity (Fig. 10), suggesting an effect of the endogenous clock, but even when the PM and AM experiments were pooled and tested as two groups, the difference was not significant ($p = 0.09$, $F_{1,14} = 3.3$, ANOVA).

These results demonstrate that the onset of heightened locomotor activity was modified by adaptation light intensity although the absolute value of light intensity did not determine the timing of the activity change. In contrast, the overall change in the activity between the "daytime" and "nighttime" levels was not strongly affected by any of the treatments.

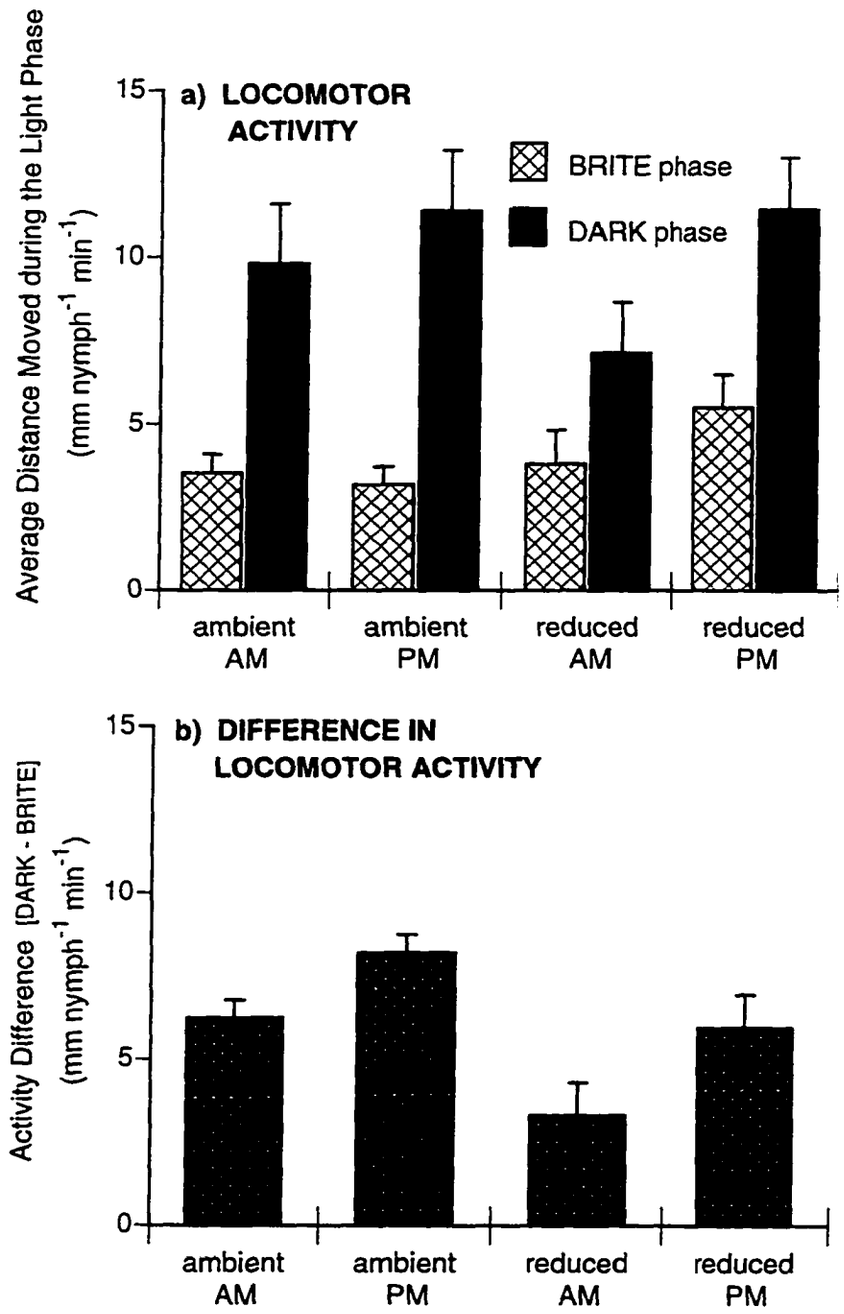

Figure 10. Locomotor activity before (BRITE) and after (DARK) the light-decrease phase compared between four treatments (listed in Fig. 9). (a) Average locomotor activity during the BRITE and DARK phases. Differences between the BRITE and DARK activity were significant ($p < 0.05$, ANOVA) within all treatments. (b) Average change in locomotor activity between the BRITE and DARK phases. There were no significant differences ($p > 0.05$, ANOVA) between pairwise comparisons of the activity change between treatments. Data are means ± SE ($n = 4$ for each treatment).

Movement Between Substrate Surfaces

Nymphs began to leave the tile undersides during the DECRS phase of the light/dark cycles and fewer were visible during the DARK phase as expected (Fig. 11). Nymphs that left the tile undersides did not always return to the tile undersides during the INCRS phase (Fig. 11). There was no relationship ($R^2 = 0.08$, $p = 0.16$, $n = 16$) between rate of light change and the total numbers of nymphs that left the tiles. There was also no relationship between rate of light change and the timing when nymphs began to leave the tile undersides ($R^2 = 0.03$, $p = 0.99$). However, most of the variability in timing took place during experiments in which the light stimulus was relatively weak ($S < -1.7 \times 10^{-3}$ s^{-1}). This was attributed to a combination of lengthy periods of light decrease and small numbers of nymphs used in the tests; nymphs tended to leave and return again to the tile undersides when the light reduction was slow, making it difficult to clearly define the timing of leaving the undersides (example, see Fig. 11 top panel, $S = \pm 1.2 \times 10^{-3}$ s^{-1}). Re-examination of the relationship using only rates of change $\geq -1.7 \times 10^{-3}$ s^{-1} indicated a significant correlation between the strength of the light stimulus and the length of the delay (Fig. 12), suggesting that light stimulus controls phototactic movements. The rheobase, or minimum rate of light change capable of eliciting phototactic movements between the substrate surfaces, was calculated as 6.0×10^{-4} s^{-1} from equation (1) in a similar fashion as for the locomotor activity response. (The best fit regression using Eq. (1), $R^2 = .71$, $p < 0.005$, $n = 9$, yielded a disintegration constant (c) of 9.0×10^{-5} s^{-1}.) This rheobase value is substantially greater than the rheobase value of 1.0×10^{-4} s^{-1} for the locomotor activity response, suggesting that although both the photokinetic locomotor activity and the phototactic vertical movements are controlled by relative light change, the locomotor activity response is much more sensitive to light stimulus than is the vertical location response. For example, during light decrease at a constant rate of -1.7×10^{-3} s^{-1}, the estimated time between the beginning of light decrease and the onset of heightened

locomotor activity is about 38 minutes, whereas for the onset of leaving the substrate undersides is about 46 minutes.

There were significant differences in the response curves across all light phases due to time of day (Table IV). This difference between the AM and PM experiments was attributed to significantly fewer ($p < 0.009$, $F_{1,14} = 9.3$, ANOVA) nymphs visible during the DARK phase in the PM experiments than in the AM experiments, regardless of the BRITE-adaptation light intensity (Fig. 13). The effect of Time was significant but there were no significant interactions between Time and either Time of Day or BRITE-adaptation light level (Table IV), an indication that light changes were the strongest influence on the movements between the tile surfaces.

The delay following the beginning of the DECRS period was somewhat longer ($p = 0.07$, $F_{1,14} = 3.6$, power = 0.43, ANOVA) in the AM treatments regardless of BRITE-adaptation intensity (Fig. 14), indicating another effect of the endogenous clock in addition to fewer nymphs remaining on the tile undersides during the DARK phase. The average light intensity when nymphs began to leave was significantly lower ($p < 0.03$, $F_{1,11} = 6.3$, ANOVA) in the BRITE-reduced treatments than in the BRITE-ambient treatments ($9.6 \pm 2.3 \times 10^{-7}$ vs. $8.4 \pm 2.7 \times 10^{-5}$ W cm^{-2}, respectively), demonstrating that absolute light intensity did not control location on the substrate.

TABLE IV. Analysis of variance with repeated measures[a] of the effects of time of day (Day) and adaptation light intensity (BRITE) on numbers of nymphs visible underneath the tiles.

	Source of variation	MS	F	df	P
Between treatments	Day	44.1	6.1	1	0.03
	BRITE-level	0.1	0.01	1	0.9
	Day x BRITE	6.3	0.9	1	0.4
	Error	7.2		12	
Within treatments	Time	56.8	51.1	3	0.0001
	Time x Day	1.2	1.1	3	0.4
	Time x BRITE	1.8	1.6	3	0.2
	Time x Day x BRITE	0.03	0.03	3	0.9
	Error (Time)	1.1		36	

[a]Repeated measures (time) = average number of nymphs visible during the BRITE, DECRS, DARK, and INCRS phases.

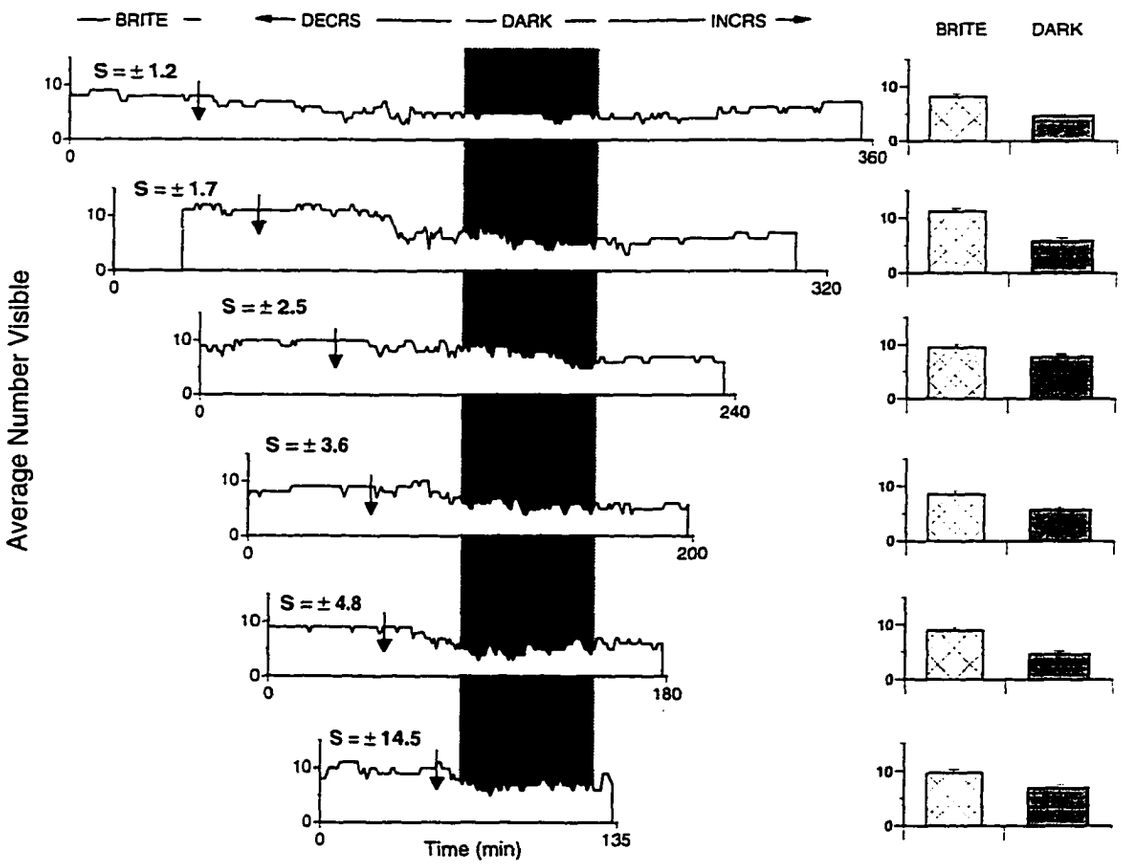

Figure 11. Sample time-series illustrating the differences in number of nymphs visible on the tile undersides for the same experiments shown in Fig. 7. Left panels). Time-series of the number of nymphs visible during each artificial light/dark cycle. Arrows mark the beginning of the DECRS phases, shaded area represents the DARK phases. Right panels). Mean number of nymphs visible during the BRITE and DARK phases. Values were significantly different between phases ($p < 0.0001$, ANOVA) for all experiments. Bars are mean ± SD.

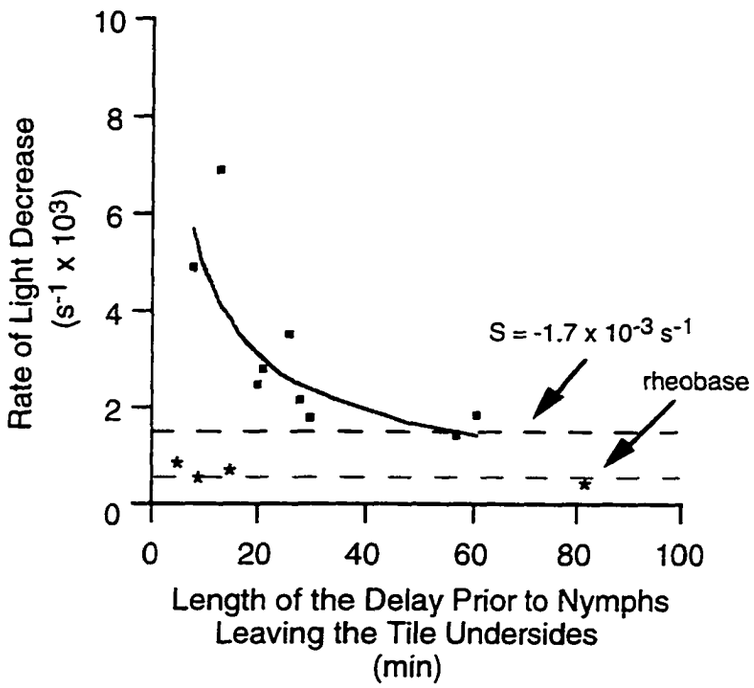

Figure 12. Relationship between the rate of light decrease (S) and the length of time before nymphs began to leave the tile undersides (the delay period). The equation of the line is: ln[delay period (min)] = -3.51 -1.15 x ln |S (s^{-1})|, (R^2=.71; p< 0.004; n=9), and represents data points for rates of light decrease ≥ -1.7 x 10^{-3} s^{-1} (large symbols). Data points at weaker rates of light change (*) were excluded to demonstrate the presence of a threshold rate of light change for the onset of phototactic movement. The line was fitted using log-log transformed data.

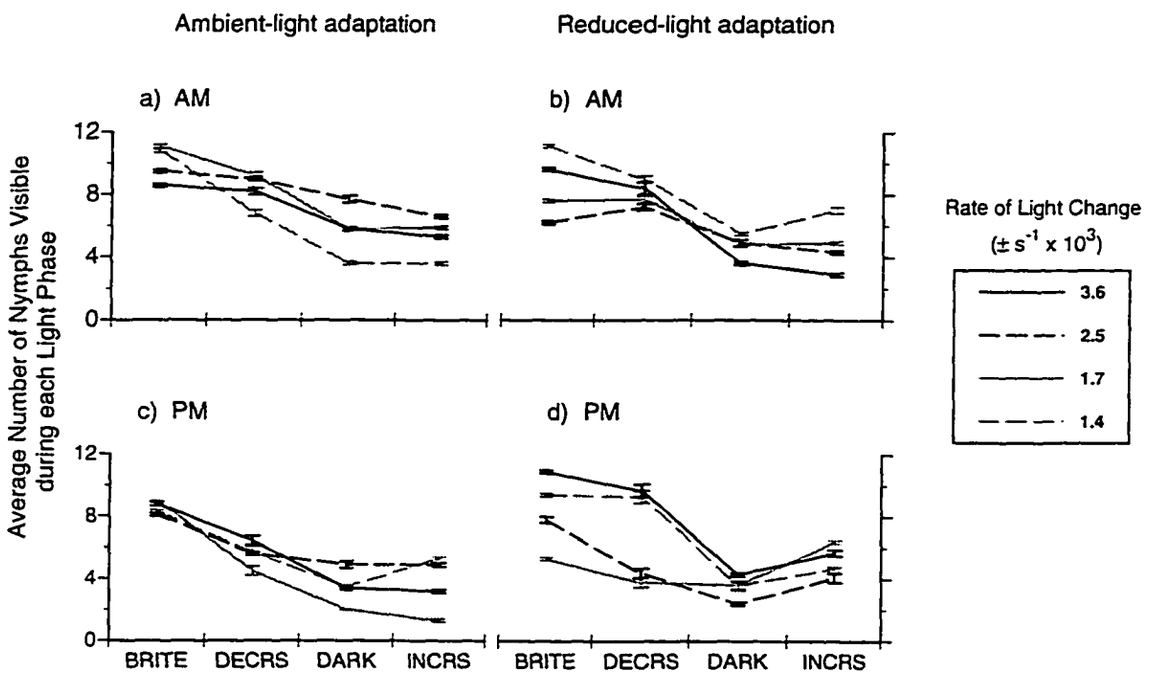

Figure 13. Response curves of average number of nymphs visible during each of the four light phases grouped by treatment (listed in Fig. 9). Nymphs did not return to the tile undersides during the INCRS phase as predicted, regardless of BRITE-adaptation light intensity or time of day. Error bars are ± 1 SD.

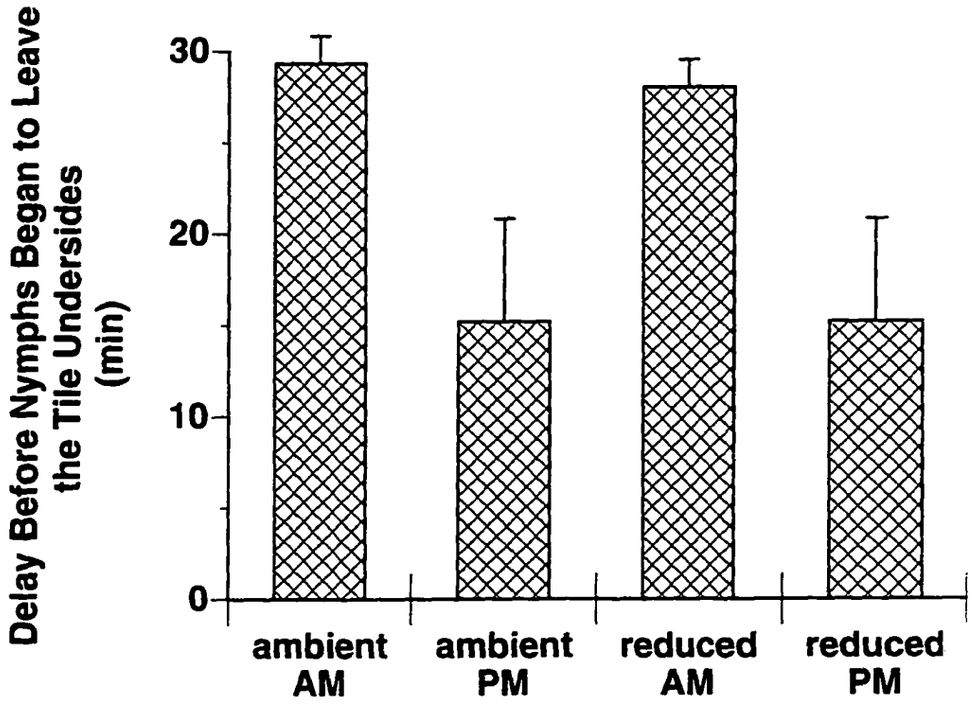

Figure 14. Comparisons of the average delay between the beginning of the DECRS phase and the moment when nymphs began to leave the tile undersides between treatments. (listed in Fig. 9). Delays were longer in both AM treatments, but differences were not significant. Bars are mean + SE.

Effect of Abbreviated and Discontinuous Periods of Light-Decrease

A key assumption of the STAR model is that the light stimulus, which is the rate of relative light change, must take place over a sustained period of time in order to elicit a locomotor activity response. In addition, the magnitude of the activity response should be proportional to both the strength of the stimulus (i.e., the rate at which light changes) and the length of time over which the stimulus is applied. Time-series of locomotor activity during artificial light/dark cycles when light was reduced over one, two, or four equal steps at $S = \pm 2.5 \times 10^{-3}\ s^{-1}$ are discussed in support the hypothesis (Fig. 15).

During the 1-step experiment, locomotor activity increased from the BRITE-adaptation level of 6.7 ± 0.5 mm nymph^{-1} min^{-1} $\pm$ SE to a DARK level of 17.0 ± 1.0 mm nymph^{-1} min^{-1} $\pm$ SE, an average increase of 10.3 mm nymph^{-1} min^{-1} (Fig. 15a); during the 2-step experiment, locomotor activity increased from the BRITE-adaptation level of 3.3 ± 0.3 mm nymph^{-1} min^{-1} $\pm$ SE to an intermediate level of 6.1 ± 0.5 mm nymph^{-1} min^{-1} $\pm$ SE during the LOW-1 phase following the initial light decrease phase, then to a high of 7.5 ± 1.9 mm nymph^{-1} min^{-1} $\pm$ SE during the DARK phase following the final light decrease, an overall increase of 4.3 mm nymph^{-1} min^{-1} (Fig. 15b). All activity changes between light phases were significant ($p < 0.05$, ANOVA) except the final activity increase during the 2-step experiment. Although locomotor activity increased sharply following the second light decrease step, there was a large drop in locomotor activity during the DARK phase (Fig. 15b) that accounts for both the lack of significance between the level of activity between the DARK and LOW-1 phases, and for the overall smaller change in locomotor activity compared to the 1-step experiment. In contrast, during the 4-step experiment, there were no significant changes in locomotor activity during any of the light phases (Fig. 15c).

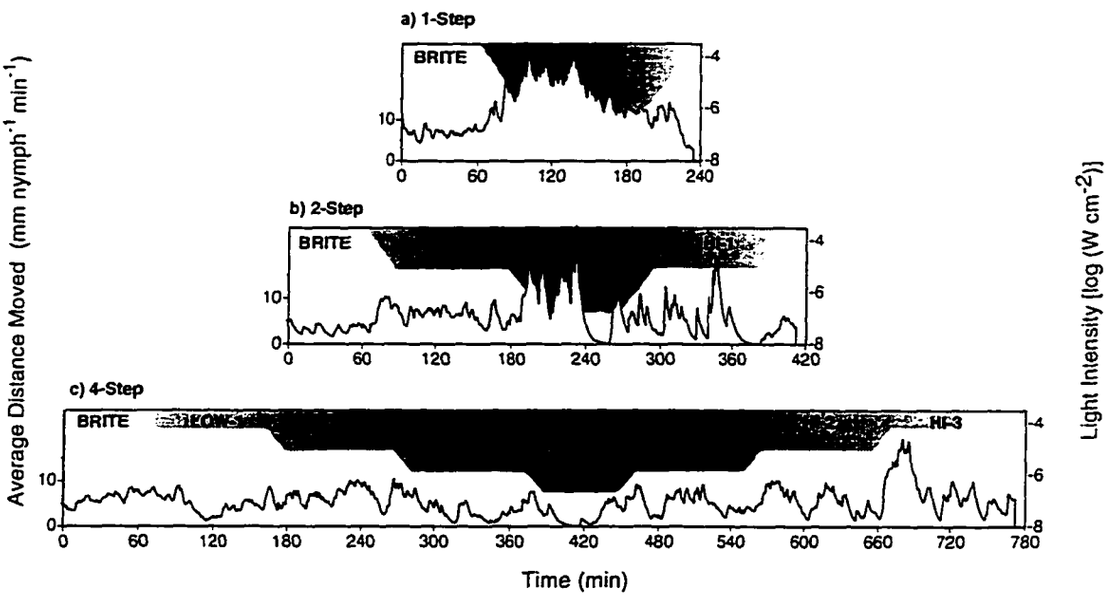

Figure 15. Time-series of average nymph locomotor activity ($^{-}$) during three artificial light/dark cycles when the applied rate of light change was $S = \pm 2.5 \times 10^{-3}$ s^{-1}. In each panel, light intensity is represented by the shaded areas, and periods of no light change are labeled. (a). 1-step experiment: Locomotor activity increased and decreased as predicted during the light decrease and light increase phases.

(b). 2-step experiment: Locomotor activity increased during both light decrease phases, reached a maximum during the DARK phase, then decreased during both light increase phases. (c). 4-step experiment: Changes in locomotor activity did not correspond with changes in light, as the lowest amount of activity took place during the DARK phase. The light-change steps were 56 min long in the 1-step experiment, 28 min each in the 2-step experiment, and 14 min each in the 4-step experiment. $Time_0$ = 1700 EST.

Response Curve

There were unexpected differences in the response curves between the three rates of light change tested during the 2-step and 4-step experiments (Fig. 16). In the 2-step experiments, locomotor activity increased above the BRITE-adaptation level during the first light decrease phase (DECRS-1) for all three rates of change tested (Fig. 16a). However, at the Ringelberg stimulus (-1.7×10^{-3} s^{-1}), locomotor activity did not continue to increase as a consequence of the second light decrease phase (DECRS-2) as was the case for the two larger rates, but decreased to the BRITE level, and stayed low throughout the remainder of the light/dark cycle (Fig. 16a). In the 4-step experiments, there was a complete breakdown of the stimulus-activity response at the two larger rates, in that changes in activity did not correspond with changes in light (Fig. 16b); whereas, at the Ringelberg stimulus (-1.7×10^{-3} s^{-1}), locomotor activity rose during the last light decrease (DECRS-4), then declined during and after the third light increase phase (INCRS-3). These results suggest that locomotor activity was more strongly regulated when both the rate of light change and the time interval of the light change were large (i.e., 2 larger rates of light decrease in the 2-step experiments), such as occurs during natural twilight, than when one or both were small (i.e., the weaker rate of light change ($S = \pm 1.7 \times 10^{-3}$ s^{-1}, and all of the 4-step experiments), as occurs during transient cloud events.

Locomotor activity during the BRITE and DARK phases, which represents the level of activity before and after the completion of all phases of the light decrease, was examined in order to test which factors were more important to the stimulus activity-response (Table V). Significant differences in the response could not be attributed solely to rate of light change, number of light-change steps, nor length of step (Table V), suggesting a complex relationship between stimulus strength (e.g., rate of light change) and length of time over which the light stimulus was applied, in producing a characteristic stimulus-activity response. Overall, larger changes in locomotor activity occurred with larger rates,

and smaller changes in locomotor activity occurred with more steps (Fig. 17). The shape of the relationship between the magnitude of the activity change and the length of the light-decrease steps suggests that there may be an optimal length of time (~40-60 min) that can trigger the largest, sustained changes in locomotor activity (Fig. 17). Because locomotor activity failed to increase in the DARK period at the shortest steps (10 and 14 min, S = -2.5 and -3.6 x 10^{-3} sec^{-1}, respectively; Fig. 17), the role of light intensity as the primary control of diel activity changes was again not supported.

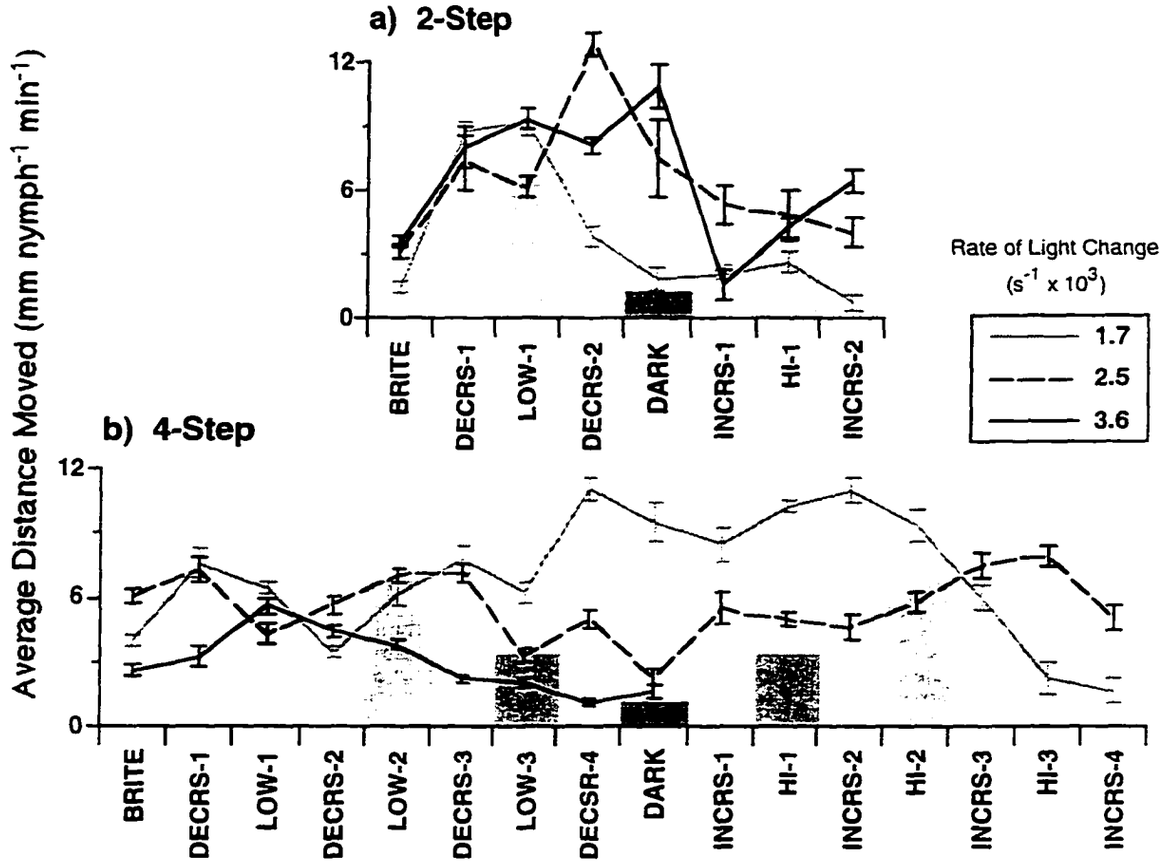

Figure 16. Response curves of locomotor activity during each light phase for the multiple-step experiments. (a). Locomotor activity during each of the experiments for which light was decreased over two separate periods (DECRS-1, DECRS-2), interspersed with a 90-min period of no light change (LOW-1). Following the 60-min DARK period, light was then increased over two separate periods (INCRS-1, INCRS-2) interspersed with a 90-min period of no light change (HI-1). (b). Locomotor activity during each of the experiments for which light was decreased over four separate periods (DECRS-1, DECRS-2, DECRS-3, DECRS-4), interspersed with 90-min periods of no light change (LOW-1, LOW-2, LOW-3). Light increase following the DARK period took place over four separate periods (INCRS-1, INCRS-2, INCRS-3, INCRS-4) interspersed with 90-min periods of no light change (HI-1, HI-2, HI-3). Due to a power failure, the light increase phases were not recorded for the 4-step experiment at the largest rate of change, S = ± 3.6 x 10^{-3} s^{-1}. Shaded areas represent the light environment during the periods of no light change. Error bars are ± 1 SD.

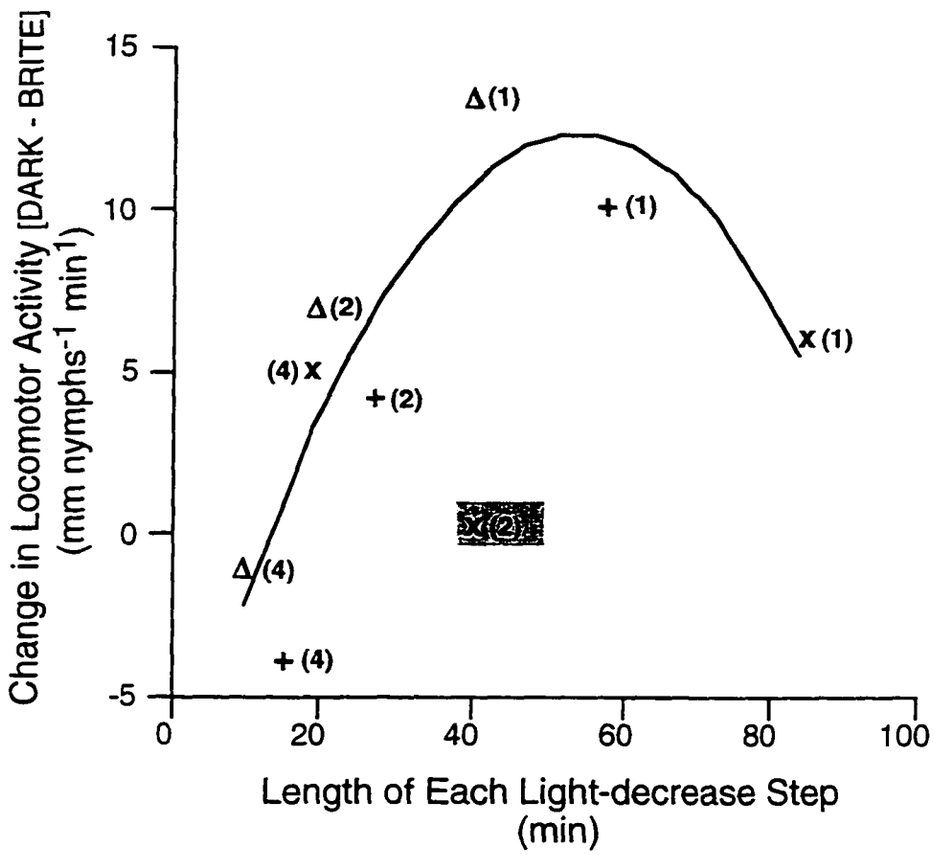

Figure 17. Relationship between the length of each light-decrease step and the change in locomotor activity as a consequence of the complete 4 log-unit decrease in light intensity. The data were fitted to a two-degree polynomial ($R^2 = .66$, $p < 0.03$, $n = 8$). The shaded point (representing the 2-step experiment when $S = -1.7 \times 10^{-3}$ s^{-1}; see Fig. 16a) was not included in the fit of the line because the value of standardized residual was > 3 SD from zero (Neter et al. 1990). Symbols represent the rates of light decrease: $S = -3.6$ (Δ), -2.5 (+) and -1.7 (x) $\times 10^{-3}$ s^{-1}. Values in parentheses indicate the number of light-decrease steps.

TABLE V. Analysis of variance with repeated measures[a] for the effects of number of steps, the length of each step (min) and the rate of light change (S) for the discontinuous light-decrease experiments.

Source of Variation	MS	F	df	P
Between subjects				
S	15.19	1.81	1	0.24
Steplength	34.22	4.08	1	0.10
S x Steplength	72.50	8.65	1	0.03
Error	41.89		5	
Within subjects				
Time	2.53	0.41	1	0.55
Time x S	5.14	0.84	1	0.40
Time x Steplength	15.35	2.51	1	0.17
Time x S x Steplength	37.08	6.05	1	0.06
Error(Time)	6.13		5	
BLOCKED BY NUMBER OF STEPS				
Between subjects				
Steps	38.98	11.17	2	0.04
Steplength	1.08	0.31	1	0.62
Steps x Steplength	22.75	6.52	2	0.08
Error	3.49		3	
Within subjects				
Time	21.00	6.56	1	0.08
Time x Steplength	1.08	0.34	1	0.60
Time x Steps	29.07	9.08	2	0.05
Time x Steplength x Steps	11.32	3.53	2	0.16
Error(Time)	3.20		3	
BLOCKED BY S CLASS[b]				
Between subjects				
S Class	4.58	0.30	2	0.76
Steplength	121.24	7.93	1	0.07
S Class x Steplength	29.85	1.95	2	0.29
Error	15.29		3	
Within subjects				
Time	7.29	1.28	1	0.34
Time x Steplength	87.04	15.29	1	0.03
Time x S Class	7.73	1.36	2	0.38
Time x Steplength x S Class	24.73	4.34	2	0.13
Error(Time)	5.69		3	

[a]Repeated measures (time) = average locomotor activity during the BRITE and DARK phases.
[b]The three applied rates of light change, S = ± 1.7, 2.5 and 3.6 x 10^3 s^{-1}, were used as class variables.

Timing (Latent Period)

The lengths of individual light decrease steps were a few minutes longer than the baseline estimates of the latent periods (see Fig. 5) in the 2-step experiments, but were shorter than the estimated latent periods in the 4-step experiments. Characteristic latent periods in which light stimulus was built up were supported, and during the 4-step experiments, the light stimulus was not applied over a long enough period of time to elicit the response.

During the 2-step experiments, the length of the latent period between the beginning of the initial light decrease and the onset of heightened locomotor activity was estimated as 22.2 ± 3.6 min $\pm$ SE at the Ringelberg stimulus ($S = -1.7 \times 10^{-3}\ s^{-1}$), and 22.0 ± 6.7 and 16.6 ± 2.7 min $\pm$ SE at the two larger rates of light decrease, $S = -2.5$ and $-3.6 \times 10^{-3}\ s^{-1}$, respectively. Predictions calculated from the baseline model (equation of Fig. 5) were 37.7, 28.0 and 21.0 min. The measured latent periods were all somewhat shorter than those predicted by the baseline model, more so at the Ringelberg stimulus (15.5 min) than for the two larger rates (6.0 and 4.4 at $S = -2.5$ and $-3.6 \times 10^{-3}\ s^{-1}$, respectively). The estimates at the two larger rates were within the 95% confidence interval of the baseline, whereas the estimate at the Ringelberg stimulus was not, suggesting that the response is more synchronous at larger rates of light decrease, and thus at stronger stimuli.

Magnitude of the Activity Change

The amount of change in locomotor activity was also expected to be a function of both rate of light decrease and the amount of time over which the light decrease took place. Once the stimulus-activity response was activated, (e.g., during the 2-step experiments), changes in locomotor activity following each light-decrease step did occur and were smaller than the changes in locomotor activity observed during the 1-step experiments (example, see Fig. 15 a, b). The direction of the activity change following each step was as expected for the two larger rates, but not for the Ringelberg stimulus, in which all of the activity

increase took place following the initial light-decrease step (Fig. 18). The magnitude of the actual activity changes were compared with those predicted by the baseline relationship (equation of Fig. 8) for each rate of light change tested (Table VI). The expected and actual activity changes were not significantly different for the first light-decrease step (Step1, Table VI), but the actual activity change was somewhat lower than expected ($p < 0.07$) for the second light-decrease phase (Step2, Table VI). In every case, more of the activity increase took place during the first step (BRITE to LOW-1) than during the second step (LOW-1 to DARK), suggesting that once the response is triggered, further reduction in light at that rate of light change has a lesser effect.

TABLE VI. Comparison of the magnitude of locomotor activity following each light-decrease step and that predicted by the baseline model for the two-step experiments.

Rate of light decrease S ($s^{-1} \times 10^3$)	Locomotor-activity Δ between phases (mm $nymph^{-1}$ min^{-1})	Expected Δ[a] (mm $nymph^{-1}$ min^{-1})	Difference (actual - expected)	t-Ratio ($p > t$; $p < t$)[b] [DF = 2]	
Step1	Δ = LOW1 - BRITE			0.52	(0.3; 0.7)
1.7	7.8	4.0	3.8		
2.5	3.0	4.7	-1.7		
3.6	5.7	5.3	0.4		
Step2	Δ = DARK - LOW1			-2.40	(0.9; 0.07)
1.7	-7.4	4.0	-11.4		
2.5	1.4	4.7	-3.3		
3.6	1.5	5.3	-3.8		

[a]Expected differences (Δ) are those estimated using the equation in Fig. 8 for each rate of light decrease (S).
[b]Paired t-tests were performed on the actual and the expected differences in locomotor activity for all rates of light change combined for each light-decrease step.

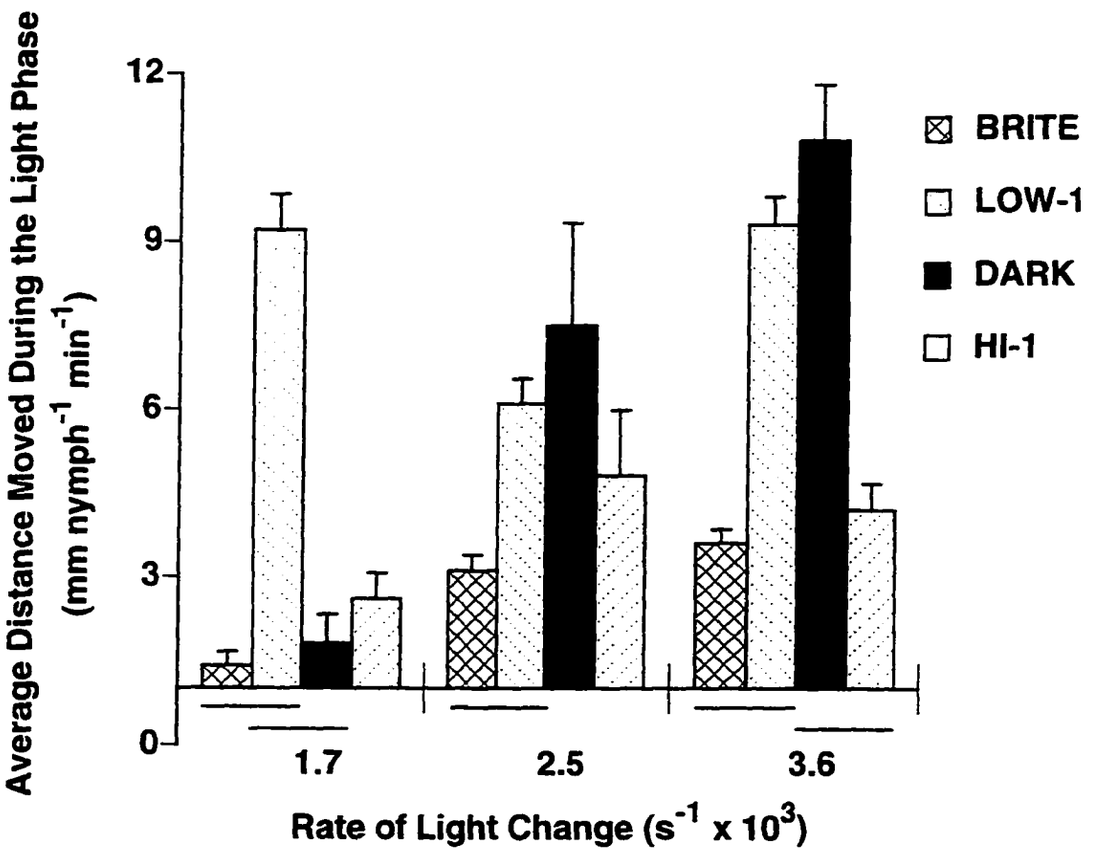

Figure 18. Comparison of locomotor activity following each phase of light change in the two-step experiments. Bars represent mean locomotor activity ± SE during the BRITE-adaptation phase, the 90 min interval (LOW-1) following the first light-decrease phase, the DARK phase following the second light-decrease phase, and the 90 min interval (HI-1) following the first light-increase phase. Locomotor activity during adjacent phases that are significantly different from each other ($p < 0.05$, ANOVA) are underlined.

Movement Between the Substrate Surfaces

Significantly fewer nymphs were visible during the DARK phase than during the BRITE phase in both the 2- and 4-step experiments ($p < 0.0001$, $F_{5,12} = 19.3$, Fig. 19). Repeated measures ANOVA of the BRITE and DARK periods detected an interaction between Time x Rate of Light Change within subjects ($p < 0.05$, $F_{2,6} = 5.1$) that was attributed to significantly higher numbers of nymphs leaving the tile undersides at the Ringelberg stimulus, regardless of number of light-decrease steps (7.5 ± 0.3 nymphs $\pm$ SE compared to 5.5 ± 1.0 and 4.5 ± 0.6 at S = -2.5 and -3.6×10^{-3} s^{-1}, respectively). There were no differences detected between or within subjects that could be attributed to the number of light-decrease steps ($p = 0.88$, $F_{2,6} = 0.93$). Numbers of nymphs visible on the tile undersides were examined during the intervals of no light change to determine during which light-phase the majority of leaving took place during the 2- and 4-step experiments (Fig. 19). Most nymphs left following the first light-decrease phase (DECRS-1), even in the 4-step experiments when the length of the light-decrease phase was shorter than the latent period for the stimulus-activity response. Also, in the 4-step experiments, nymphs continued to leave during the second light-decrease phase (DECRS-2), and fewer left thereafter (Fig. 19b).

The delay between the beginning of the light decrease and the moment nymphs began to leave the tile undersides was measured for all of the step experiments at each rate of light change as 18.7 ± 4.4 min $\pm$ SE at the Ringelberg stimulus, and 9.3 ± 1.8 and 11.7 ± 1.2 min $\pm$ SE at S = -2.5 and -3.6×10^{-3} s^{-1}, respectively. Although the delay was shorter at the two larger rates, the differences were not significant ($p = 0.13$, $F_{2,6} = 3.0$). There was no significant effect of number of steps ($p = 0.57$, $F_{2,6} = 0.6$) on the length of the delay (10.3 ± 0.3, 13.3 ± 5.5, and 16.0 ± 3.1 min $\pm$ SE at one, two and four steps, respectively), although there was a trend towards longer delays with shorter periods of light decrease. The lengths of the delay compared to those estimated from the baseline

model (see Fig. 12) were significantly shorter ($p << 0.05$, t-Test), by 22.4 ± 1.0 min ± SE at the Ringelberg stimulus, and 19.9 ± 3.7 and 7.1 ± 2.2 min ± SE at S = -2.5 and -3.6 x 10^{-3} s^{-1}, respectively. Smaller differences between the expected and the actual delays at larger rates of light change suggest that the likelihood an individual will exhibit phototactic movement increases at stronger stimuli. All step experiments were performed in the PM, and significantly earlier onsets of leaving the tile undersides than those estimated by the baseline model, which was produced from AM experiments, suggest an effect of the endogenous clock.

Results from the step experiments indicate that there are different mechanisms by which relative light change controls locomotor activity and vertical movements between the substrates. There are different requirements for which the duration of a light stimulus can elicit a locomotor activity response or a phototactic response. Although the baseline relationships indicated that the locomotor activity response was more sensitive to light stimulus than was the phototactic response, results of the step experiments suggest that the phototactic response may not be as dependent on the length of time over which a light stimulus is applied as is the locomotor activity response.

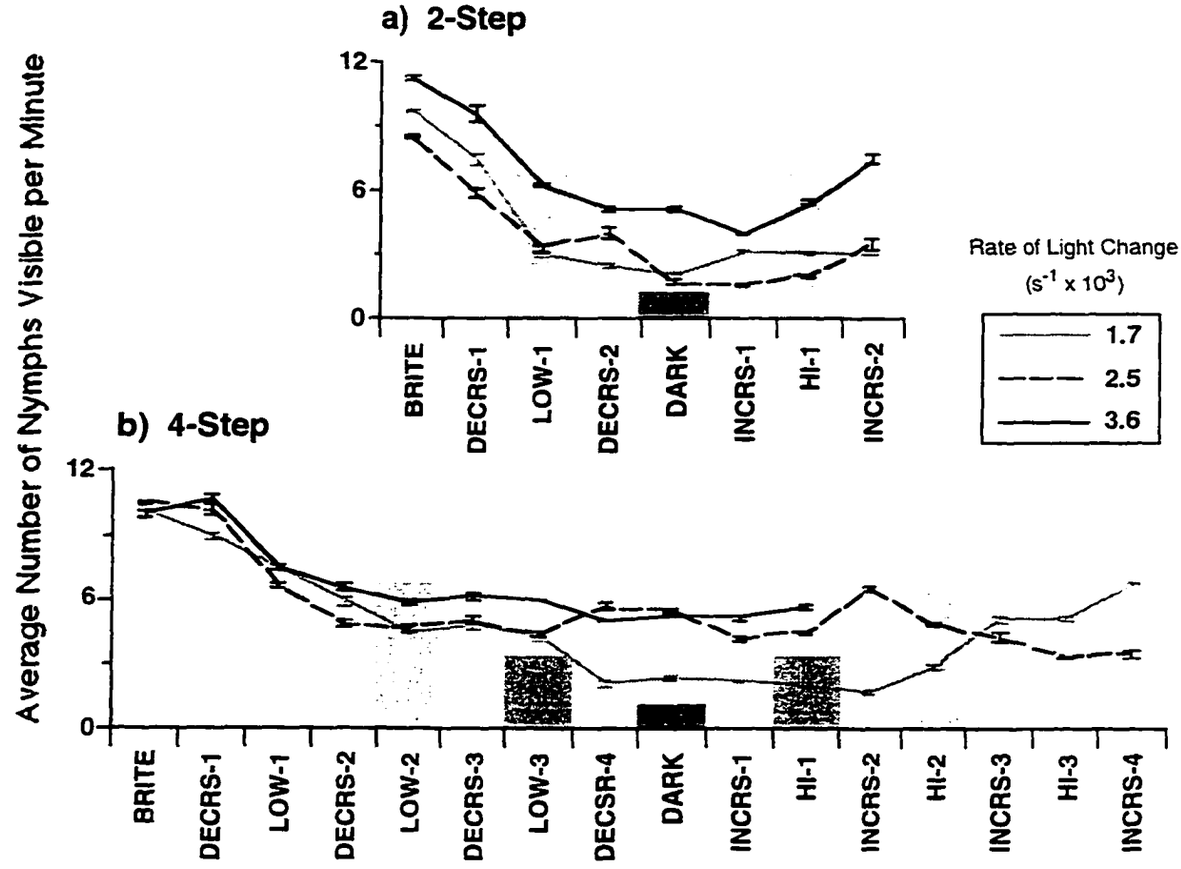

Figure 19. Response curves of nymph movements between the substrate surfaces during each light phase for the multiple step experiments. (a). Number of nymphs visible on the lower tile surfaces during each of the 2-step experiments. (b). Number of nymphs visible on the lower tile surfaces during each of the 4-step experiments. Light phases are as in Fig. 16. Shaded areas represent the light environment during the periods of no light change. Error bars are ± 1 SD.

Discussion

Relative change in light intensity was the most important environmental cue initiating changes in locomotor activity and vertical movements on the substrate in nymphs of the mayfly, *Stenonema modestum*, prevailing over the influence of either time of day or absolute light intensity. Light manipulations carried out over equivalent ranges of light intensities, in which the dominant variable was the rate of relative light change, elicited increases in locomotor activity for which the timing, magnitude, and time-course of the initial peak in activity corresponded to the rate at which the light changes took place. Responses proportional to the rate of light change, or the stimulus strength, strongly support relative light change as a regulator of locomotor activity (the photokinetic activity) as well as a cue for the diel activity cycle. In addition, relative light change provides the releasing stimulus allowing nymphs to leave the substrate undersides (the phototactic activity).

Relative light change has been described in regulating the swimming velocity and vertical swimming direction during evening twilight of two other aquatic genera, the water flea, *Daphnia* (Ringelberg 1964; Buchanan and Haney 1980), and the phantom midge, *Chaoborus* (Haney *et al.* 1990), indicating that relative light change is useful in both regulating locomotor activity and as a cue initiating phototactic movements in other aquatic organisms. Specific aspects of the response to such a light stimulus may have different purposes in each species, resulting in the observed differences in the timing of the response, the minimum rate of light change capable of eliciting the response, or in the particular behavior that is being regulated. For example, the length of the latent periods, the amount of time between the light stimulus and the initiation of a change in behavior, were much longer in *Stenonema* than recorded for *Daphnia magna* (Ringelberg 1964), for which delays were estimated in seconds rather than in minutes. Comparable latent periods

to those for *Stenonema* mayflies have been measured for the onset of daily flight of the nocturnal moth, *Plusia gamma* (data from Dreisig's Fig. 6, 1980), suggesting that a wide range in the length of latent periods in response to a common light stimulus may be prevalent among different species. The estimated 6.0 x 10^{-4} s^{-1} minimum rate of light change capable of eliciting the phototactic response in *Stenonema* was however, very similar to the 6.7 to 8.3 x 10^{-4} s^{-1} estimated for initiating the phototactic swimming response in *Daphnia* (Ringelberg 1964, 1991b), even though daphnids may have to swim upwards over considerable distances to reach their food source whereas the distance over a rock surface that mayflies must travel to reach their food source may be much shorter. It is remarkable that despite the many differences in the environments in which stream insects (*Stenonema*) and zooplankton (*Daphnia* and *Chaoborus*) live, vertical movements within those environments have similar adaptive consequences for each group, and adaptive goals, such as maximizing food availability while minimizing threat of predation, can be reached using the same light cues. Differences between species in the latent periods subsequent to the light stimulus probably reflect habitat differences and the different adaptive strategies necessary for success in those habitats.

A light response in organisms that exhibit periodic behaviors should function as an exogenous timekeeper capable of predicting the daily onset of darkness or light. The strength-duration relationships between rate of light change and the timing of heightened locomotor activity and vertical movements to the upper substrate surfaces suggest that the build-up of an excitatory state is the physiological basis of the periodicity in the behaviors and that the buildup over time may then be used as the exogenous timekeeper. Changes in light intensity during twilight are exponential with time (Ringelberg 1964, Dreisig 1980, Haney *et al.* 1983, Grace 1990), making relative light change a very reliable predictor of the light environment of the immediate future, and therefore a valuable external cue for timekeeping. Furthermore, relative light change is not altered by differences in the value of the beginning light intensity (Ringelberg 1964, Haney *et al.* 1983, Grace 1990, Baldwin

1993), making it an excellent cue in different environments such as lakes and streams, particularly streams, where the light intensity under any given rock depends on such local effects as shading, channel depth, and angle from the sun. Such an exogenous timekeeper would be successful in nature, as stronger stimuli are associated with a more imminent onset of darkness at evening twilight or of light at morning twilight, than are weaker stimuli. In stream invertebrates, longer latent periods due to a longer buildup period prior to initiating a response at weaker stimuli protect the animal from behavioral changes at inappropriate times.

The influence of the endogenous clock on the mechanism that measures light stimulus appears to be unequal for the photokinetic locomotor activity and the phototactic movements between the substrate surfaces. At any particular rate of light decrease, the magnitude of the change in locomotor activity following the light decrease was somewhat enhanced in the PM as expected, but contrary to the STAR prediction, there was no advance in the timing of heightened locomotor activity that would indicate greater sensitivity to a light change. However, vertical movements between the substrate surfaces were initiated earlier in the PM and significantly more nymphs left the tile undersides in the PM than in the AM, suggesting that these movements are more strongly influenced by an endogenous cycle. This conflicts directly with Elliott's (1968) conclusion that vertical movements in *Baëtis rhodani* kept for 8 days in continuous darkness were strictly phototactic, but was indicated for *Stenonema modestum* kept in light reduced by 3 log units during tests of the PK-PT model (Grace 1990). In both species, weak diel cycles in vertical location on the substrates were maintained. Vertical movements on the substrate are probably primarily driven by hunger (Wiley and Kohler 1981, Kohler 1984, Glozier and Culp 1989), although diel changes in oxygen levels have been related to positioning changes in lake (Rahel and Kolar 1990) and stream-dwelling mayflies (Wiley and Kohler 1980), and caddisflies (Kovalak 1976). During tests of the STAR model, there were no diel fluctuations the controlled physical environment in the laboratory stream; in particular,

oxygen saturation was maintained around 93%, indicating that oxygen stress was not a factor in positioning on the substrate in this study. Because the level of hunger was greater in the PM than in the AM (*Stenonema* feed in darkness but were collected early in the morning), and there were no diel fluctuations in the controlled physical environment in the laboratory stream, the enhancement of the response may have been related to hunger rather than directly driven by an endogenous cycle.

Light intensity, although not supported as the primary regulator of diel locomotor activity and vertical movements on the substrate in *Stenonema,* did influence the timing of locomotor activity changes. Nymphs adapted at reduced light were quicker to respond to equivalent relative light changes than those adapted at a noontime light intensity. Such advancements in timing of nocturnal activity, such as drift in stream invertebrates (Haney *et al.* 1983, Baldwin 1993), and flight times of nocturnal moths (Edwards 1962) have been reported, suggesting that reduced light intensity may signal that conditions are appropriate for the activity change.

The most striking effect of light intensity, however, was not in the response to the light decrease, but in the response to the subsequent period of light increase. Activity of the dark-adapted nymphs did not return to the initial level, as was the case for the bright-adapted nymphs. If locomotor activity is strictly regulated by relative light change, then the levels of beginning and ending light intensity should have no effect on the activity response-curve, which was not the case. Light intensity has recently been implicated in the regulation of the circadian clock controlling locomotor activity in the fruit fly, *Drosophila melanogaster* (Lee *et al.* 1996, Myers *et al.* 1996). The clock is regulated by two proteins, TIM and PER, that must form a complex to be effective. Both TIM and PER are produced cyclically, but TIM is rapidly degraded by light, regardless of where in the cycle the light is applied. The light-induced destruction of TIM causes a rapid breakdown of the TIM-PER complex and subsequent resetting of the circadian pacemaker (Barinaga 1996). In my reduced-light experiments, the adaptation light level was comparable to the illumination

level at local sunset (~ 4-6 x 10^{-6} W cm^{-2}). If a similar regulatory system operates in mayflies, the failure of nymphs in reduced light to lower their activity during the light-increase phase may have been a result of an ending light intensity that was too low to reset the circadian clock.

Regulatory pathways comparable to the TIM-PER complex in fruit flies may exist in other insects, as suggested by the pacemaker system that controls circadian flight activity in the mosquito, *Culex pipiens* (Jones 1982). The system consists of two pacemakers, labeled as labile and stable, that show differential responses to light intensity and may represent a variation of the fruit fly TIM-PER regulatory pathway. Continuing research in the physiological basis of circadian rhythms in insects will certainly yield new insights into the mechanisms by which relative light change controls diel behaviors.

Currently, the process by which relative light change and the duration of twilight combine to produce characteristic patterns in locomotor activity is not entirely clear. In the step experiments, the expected change in locomotor activity following periods of partial light decrease should have been smaller as the length of time over which the light decrease took place was shortened. In the 2-step experiments, locomotor activity increased as expected, following both light-decrease phases, and the magnitude of each activity change was smaller than the change in activity when the entire decrease in light was uninterrupted, as in the 1-step experiments. It was not expected that the change in activity would be larger following the initial phase than following the second light-decrease phase. Because the 90-min interval periods were not long enough for the initial reaction to fade away, subsequent responses were not independent. The subsequent responses varied by stimulus strength, and at the larger stimuli, the change in activity following the second light-decrease phase was sharper than the initial reaction, but faded away much more quickly. This suggests that both the magnitude and time-course of the stimulus-activity response are not simply multiplications of rate of light change and time interval, but are also dependent on light history. This is in concordance with alterations in the light response of *Daphnia magna*

(Daan and Ringelberg 1969), larvae of the estuarine crab *Rhithropanopeus harrisii* (Forward 1985), the copepod *Acartia tonsa* (Stearns and Forward 1984), and several species of terrestrial moths, (Edwards 1962, Dreisig 1980) under different light-history regimes.

Periods of light decrease as short as 10 minutes resulted in significant migrations away from the tile undersides, and when the completion of the light-decrease was broken up into discrete phases, the majority of nymphs that responded did so during the initial phase of light decrease. Similar behavior was shown in the onset of flight activity of the nocturnal moth, *Plusia gamma* (Dreisig 1980). When exposed to discontinuous periods of light decrease, the distribution of flight onsets rose steeply just after the initial light change and then more gradually (Dreisig 1980). *P. gamma* also responded to instantaneous changes in light, but there was a characteristic delay of approximately 7 minutes. This compares to the 6 to 14-minute delays estimated for *Stenonema* at the two larger stimuli in the 4-step experiments, and is probably indicative of a minimum reaction time in both species. It is not clear if the strength-duration curve between rate of light change and the timing of leaving the substrate undersides that was defined over an uninterrupted period of light decrease can be applied during periods of fluctuating light intensity. Contrary to the predictions of Elliott (1968) and Haney *et al.* (1983) that individuals become activated prior to leaving the substrate undersides, there appears to be no mandatory sequence between the cycle of locomotor activity and the movements between the substrates in *Stenonema*, as there were significant movements away from the tile undersides following periods of light change that were too short to trigger the locomotor activity response. Thus, nymphs left the substrate undersides without increasing their level of locomotor activity, suggesting that these two activities serve different adaptive purposes.

Sensitivity to light history, light intensity, and short-term fluctuations in light complicate attempts to predict in nature precisely when behavioral changes will take place and how pronounced the changes will be. Large scatter in the onset of nocturnal activity on

the same evening within a population is widespread. For example. large dispersion in the onset of DVM and the distribution of the population in the water column of *Chaoborus* (Haney *et al.* 1990) and *Daphnia* (Ringelberg *et al.* 1991a), in the onset of stream invertebrate drift (Haney *et al.* 1983), and in the onset of flight in nocturnal moths (Dreisig 1980) and birds (Daan and Aschoff 1975) have been recorded. Differences in the probability that an individual will express a particular behavior in response to an external cue depends on internal factors, such as individual variations in level of hunger or genetic differences that result in differences in the endogenous rhythm, and external factors, including food conditions, and immediate risk of predation (Dill 1987, Ringelberg *et al.* 1991a). Fluctuations in the light environment can compound these multiple individual differences and result in greater dispersion of the response over time. For example, cloudiness near twilight may cause striking differences in patterns of an activity that is regulated by a stimulus-based activity response (examples, Grace 1990, Baldwin 1993). The results of the step experiments verify that nymphs will not increase their locomotor activity during abbreviated periods of light decrease, such as cloud events, when they are shorter than the latent period of the response. But changes in activity in response to cloud events long enough to trigger the stimulus-activity response, when combined with subsequent twilight period, may result in variable timing, magnitude and duration of diel activities.

Specific predictions of the STAR model regarding locomotor activity changes, vertical movements between the substrate surfaces, and light stimulus were supported. There were significant correlations between rate of light change and the timing of and the amount of the change in heightened locomotor activity, and the timing when nymphs began to leave the undersides of the substrate. Preliminary results indicate that the time-course of the initial peak of nocturnal activity was also a function of the rate of light change. Adaptation light intensity altered the timing of nocturnal locomotor activity, resulting in nymphs initiating their activity increase earlier in reduced light than in bright light.

Shortened periods of light decrease resulted in smaller changes in locomotor activity, indicating that both stimulus strength and duration are important in regulating locomotor activity. There were minimum time limits below which relative light changes did not elicit changes in locomotor activity, but no such limits were detected for leaving the tile undersides. Thus, mayflies may be able to take advantage of food resources on cloudy days by frequent movements to the upper substrate surface without having to also increase their metabolic rate. Perhaps the diel increase in locomotor activity protects mayflies from predation by actively foraging stoneflies, tactile predators that also feed at night (Peckarsky and Cowan 1995, Peckarsky 1996), rather than as a prerequisite to the onset of feeding (Chapter Three presents tests of the light response under different predation regimes).

Particular predictions of the STAR model that were not supported were those regarding the influence of the endogenous clock on the stimulus-activity response. There was little evidence that the endogenous clock influenced either the timing or change in nocturnal locomotor activity. However, the timing and numbers of nymphs that left the tile undersides were altered at different times of the day, suggesting that these movements may be influenced by an endogenous rhythm. The most unexpected result was the failure of locomotor activity to return to a low level during light increases, when nymphs had been adapted at a reduced light intensity, suggesting that there may physiological responses to light that play a role in regulating diel behaviors.

Geographical and seasonal differences in the angle at which the sun crosses the horizon cause the strength of the twilight light stimulus to vary seasonally and with latitude. The regulation of locomotor activity by a seasonally changing light stimulus has fitness consequences throughout the entire life-cycle of these organisms. Future modifications to the STAR model will include the effects of non-constant rates of light change typical of natural twilight, and external factors, such as food availability and predators, on the light response. The effect of light stimulus on the duration of nocturnal locomotor activity must

be tested during dark periods long enough to represent normal nights in nature before any definitive conclusions regarding duration and light stimulus can be drawn.

Studies of relative light change as a control of diel cycles in aquatic species have been largely confined to diel vertical migration in plankton. This study demonstrates that relative changes in light intensity are an important regulator of the diel locomotor activity of a common mayfly species. *S. modestum* mayflies can be added to a small but growing list of aquatic organisms (*Daphnia, Chaoborus,* and calanoid copepods), for which relative light change has been shown to influence diel behaviors (Hart and Allanson 1976, Stearns and Forward 1984, Haney *et al.* 1990, Ringelberg 1991a, Ringelberg *et al.* 1991a). Interactions between species timed to the 24-hr light/dark cycle are widespread in lakes, streams, and marine systems; therefore it would be useful to examine the predictions of the STAR model with the full variety of aquatic prey, and their predators, both invertebrate and vertebrate.

CHAPTER III

LOCOMOTOR ACTIVITY AND LOCATION ON THE SUBSTRATE OF *STENONEMA MODESTUM* (EPHEMEROPTERA) IN RESPONSE TO RELATIVE LIGHT CHANGE IN THE PRESENCE OF FISH ODORS AND STONEFLIES

Introduction

Light and predation are important proximate and ultimate factors influencing diel changes in locomotor activity, vertical location on the substrate, and drift in stream invertebrates. Of these activities, drift has been the most thoroughly studied (reviews by Waters 1972, Müller 1974, Brittain and Eikeland 1988). Drift in most taxa is primarily nocturnal and has been considered to be a fixed response to ambient light levels (Holt and Waters 1967, Bishop 1969, Chaston 1969, Glozier and Culp 1989). It has been proposed that this response evolved as a predator-avoidance strategy, in species such as mayflies, that are prey to visual-foraging fish (Allan *et al.* 1986, Flecker 1992). Recent findings that the numbers of mayflies drifting correspond to the density of visual-foraging drift-feeding fishes (Flecker 1992, Douglas *et al.* 1994, Forrester 1994), indicate that the drift response can change in response to changing predation pressure. In mayfly populations from naturally fishless streams, rapid shifts in aperiodic or weakly periodic drift to nocturnal drift in the presence of fish (Douglas *et al.* 1994, McIntosh and Townsend 1994), support predation as an important causal reason to drift, rather than purely as an evolutionary force from the past.

Light strongly influences drift, as illustrated by the suppression of drift in continuous illumination and initiation of drift in artificial darkness (Holt and Waters 1967, Chaston 1968, Bishop 1969). However, the diel drift pattern has been shown to persist in continuous darkness, indicating an endogenous component. Endogenous activity cycles are common in insects (reviewed by Page 1985). An endogenous clock would serve to maintain the activity level on a 24 hr cycle, thus ensuring that an individual is in a state of readiness to perform the activity change in response to the appropriate external cue. Mayflies removed from fish streams continue diel cycles in drift under day/night illumination in the absence of predators (Flecker 1992, McIntosh and Townsend 1994, McIntosh and Peckarsky 1996), indicating that such internal and external controls may be in place. Once established, the diel cycle of activity appears to be maintained in individuals by a combination of external light control and an internal circadian clock. Local conditions, such as the risk of predation, are capable of altering an individual's propensity to drift, and possibly to become more active and move to the exposed upper substrate surface to feed.

Factors affecting behaviors that may precede the drift response are also important, such as locomotor activity level and movement to the exposed upper surfaces of the substrate to feed (Kohler 1983, Glozier and Culp 1989, Grace 1990, Wilzbach 1990, Culp and Scrimgeour 1993, Cowan and Peckarsky 1994, Peckarsky 1996). Diel periodicity in mayfly locomotor activity and vertical position on the substrate are well established (Elliott 1968, Kohler 1985, Malmqvist 1988, Glozier and Culp 1989, Cowan and Peckarsky 1994, McIntosh and Peckarsky 1996). The diel periodicity in feeding and vertical position on the substrate in mayflies from fish inhabited and fishless streams has been shown to change in ways analogous to the changes reported for drift (Cowan and Peckarsky 1994, McIntosh and Townsend 1994), indicating that these diel behaviors are under similar controls.

The locomotor activity and vertical movements of *S. modestum* nymphs on artificial substrates have been examined in laboratory experiments designed to investigate the role of

light as a proximate control of the diel activity of mayflies (Chapt. II). Relative light change, defined as the rate at which light intensity changes over time (Ringelberg 1964, Ringelberg 1991b, Ringelberg *et al.* 1991a), appears to control the timing of both heightened locomotor activity on the substrate and vertical migrations away from the lower substrate surfaces during simulated twilight periods. Relative light change also regulates the amount of change in locomotor activity. This leads to heightened locomotor activity during the nighttime (Chapt. II). These relationships between rate of light change and locomotor activity form the basis of the Stimulus-based Timing and Activity Rate (STAR) model, that predicts linear correlations between relative changes in light intensity and the timing of diel changes in activity and location on the substrate, and the difference between daytime and nighttime levels of locomotor activity (Chapt. II). During lengthy periods of rapid light changes as occur during natural twilight, changes in locomotor activity are triggered after a characteristic delay during which an excitatory state is built up. The length of this so-called latent period is proportional to the strength of the light stimulus, defined as the rate at which the light is changing. At stronger stimuli, or larger rates of light change, the latent periods are shorter, and at weaker stimuli, or smaller rates of light change, the latent periods are longer. Because of this dependence of the length of the latent period on the strength of the stimulus, the STAR model predicts that organisms will not respond to abbreviated periods of light change such as occur during transient periods of cloudiness.

Because of the evidence that fish odor enhances the extent of diel behaviors in mayflies, the light response was initially tested in water that contained fish (Chapt. II). However, stream invertebrates are under multiple predation pressures. Other important predators, such as stoneflies, may also alter the activity response in mayflies. Small shifts in the timing of the activity change or mechanical interference related to encounters with tactile predators such as stoneflies are possible effects. Currently there is no evidence that mayflies alter their diel pattern of behavior in the presence of stoneflies (Peckarsky and Cowan 1995, Peckarsky 1996), although drift as a response to encounters with actively

foraging stoneflies has been reported (Peckarsky 1980, Malmqvist and Sjöström 1987, Peckarsky 1996). Stonefly nymphs are also prey of various fish (Moore and Gregory 1988, Feltmate and Williams 1989), and their behavior may be altered in water with and without fish odors. Activity of predatory *Megarcys* stoneflies from a trout stream was reduced in the presence of trout odor in a recent study (Peckarsky and McIntosh 1997), an indication that fish odors influence stonefly behavior. If the behavior of stoneflies brings about changes in the behavior of the mayflies, then differences in stonefly behavior with and without fish odors should result in detectable differences in the behavior of the mayflies.

This study examines the light response of *Stenonema modestum* mayfly nymphs exposed to fish odors and stoneflies, separately and together, and predator-free water. These combinations were chosen because fish odor is capable of eliciting changes in diel behavior without confounding the effect from interference by mechanical interactions between predator and prey; in contrast, the most probable detectable effect of stoneflies would be due to physical encounters with the mayflies. The objectives of this study were to compare differences in the stimulus-activity responses, measured as the timing and magnitude of changes in mayfly locomotor activity, in response to relative changes in light to those predicted by the baseline relationships developed during initial tests of the STAR model (Chapt. II). Further, vertical movements on the substrate were examined to test for predator-mediated changes in timing and preference for a particular surface. Nymph movements were monitored over 1-minute time-intervals so that subtle differences in the timing of nocturnal locomotor activity and movement to the upper substrate surfaces could be detected.

Methods

Experimental Design and Handling of Experimental Animals

Three replicates were performed for each of four treatments: no predators, fish water, stoneflies, and fish water + stoneflies (Table A.3 in the Appendix). The relationships between locomotor activity and relative changes in light defined by the Stimulus-based Timing and Activity Rate (STAR) model were developed in fish water (Chapt. II), and this treatment was considered as the reference. For each experiment, light was manipulated in sequence through the same four phases of a simulated light/dark cycle as the initial tests of the STAR model: (1) an adaptation period at the brightest light intensity (BRITE) of at least 60 min, (2) a period of light decrease (DECRS) at a constant rate of light change, (3) a 60-min dim-light adaptation period (DARK), and (4) a period of light increase (INCRS) at the same, but opposite rate of light change used to decrease the light.

Experiments were carried out in two channels of a clear acrylic laboratory stream (dimensions 0.15 m wide x 2.4 m long). Well water (18 ± 2.0 °C) was continuously filtered (150-mm net) and recirculated from a tank located at the lower end of the stream at a flow rate of 5 cm-sec^{-1} (O_2 saturation measured at 5 min intervals over an ~24 hr period was 93 ± 4, % ± SD, n = 280). *Stenonema* nymphs (average length = 6.97 ± 0.73, mm ± SE, n = 144) were hand-picked daily from stones, avoiding last instars, from riffles located just below a dam in the 3rd order Oyster and Bellamy Rivers, in Durham and Madbury, NH. Nymphs were used once to avoid unknown effects of light history on their behavior. Six nymphs were transferred to an unglazed tile situated in each of the two stream channels (10 x 10 x 0.5 cm, raised 0.5 cm above the streambed) by 1400 Eastern Standard Time (EST) and acclimated at the highest (BRITE-adaptation) light intensity. The light decrease (DECRS) phase commenced at about 1800 EST.

For treatments to test the effects of fish odor, fish common to the area, two shiners (*Notropis cornutus*) and two longnose dace (*Rhinichthys cataractae*), were taken from the Oyster River and kept in the water-recirculation tank. Density of fishes (2.5 fish m^{-2}) was kept at the same level as for the initial tests of the STAR model (Chapter Two). Two-thirds of the volume of water in the tank was replaced with fresh water every week.

Large, predatory stoneflies, *Paragnetina media* (average headwidth = 3.90 $\pm$ 0.22 mm $\pm$ SE, n = 5) were also collected from the Oyster River. Stoneflies were not particularly abundant in either river during the summer of 1996; therefore stoneflies were kept in pans of aerated water and used for multiple experiments over a period of ten days. Stoneflies thus retained were kept with a variety of small invertebrate prey as a source of food. The effects of light history on the stoneflies was not as much of a concern, because only their presence was required as a perceived threat to the mayflies. However, stoneflies used in an experiment were not used again for two days to allow them to spend 24 hours in a natural light regime before being subjected to another simulated light/dark cycle.

Stoneflies were placed on the tiles first and allowed to move to the undersides, after which the mayflies were placed on the tiles. This sequence was preferred as more mayflies remained on the tiles than did so when the mayflies were placed first followed by the stoneflies. One stonefly was placed on each tile. Stoneflies were placed on the same tiles with the mayflies because there was doubt that olfactory cues alone would produce detectable changes in the mayfly activities, although *Stenonema fuscum* have been shown to move away from areas of high chemical stimulus when placed downstream from *Acroneuria lycorias* stoneflies (Peckarsky 1980). A preliminary experiment in which stoneflies were placed on a rock immediately upstream from the experimental tiles did indicate that the activity of the mayflies was not altered until the stonefly appeared on the tile surface with the mayflies.

Stoneflies were allowed free range over the tiles and were not prevented from feeding. Several recordings over 24 hr periods in natural light taken between 1987 - 1997,

revealed that *Paragnetina* nymphs taken from the Oyster River were most active during the nighttime and individual stoneflies consumed only one or two mayflies per evening, an indication that there would be few, if any mayflies consumed during the 60-min simulated "nighttime" periods. This was the case, as only one incident of successful predation was recorded during all six experiments with stoneflies.

As in all tests of the STAR model, the entire stream was enclosed in black plastic to block out all natural light. Four 500 W halogen lamps controlled by computer provided light (the system is described in detail in Chapt. I). Light intensity was sampled every second with a light sensor placed facing upwards adjacent to the tiles and mean values for every minute were calculated and saved on computer-disk. Illumination from two arrays of wide-angle GaAIAS infrared emitters (average power of 20 mW at peak width ± 50%, 940 ± 20 nm) allowed videotaping of the mayflies from underneath the tiles in the dark.

Ambient light intensity was manipulated between 7.9×10^{-4} W cm^{-2} and 1.2×10^{-7} W cm^{-2}. These values were chosen to simulate the summertime light environment between noontime and about 30 min after the period of largest light changes post-sunset. This range of illumination was adequate in eliciting responses in locomotor activity and migrations away from the tile undersides during the initial tests of the STAR model (Chapts. I, II).

Tests were carried out at one target rate of light change; $S = \pm 2.5 \times 10^{-3}$ s^{-1}. (Negative sign represents decreasing light and positive sign represents increasing light). This value is larger than the Ringelberg stimulus value of -1.7×10^{-3} s^{-1} defined as the minimum rate of light decrease, or smallest light stimulus, capable of initiating diel vertical migration (DVM) in the water flea, *Daphnia* (Ringelberg 1964, 1991b). At the particular light stimulus chosen, the estimated length of the simulated evening and morning twilight periods (DECRS and INCRS phases) was ~ 60 min, comparable to the length of local twilight during the summer (Old Farmer's Almanac, 1988-1996). Therefore, the chosen rate of light change was both large enough to elicit a response in *Stenonema* and the time-period of light decrease resembled actual twilight. These efforts along with starting the

light-decrease close to the beginning of local twilight, were undertaken to provide the best possible conditions that would elicit the anticipated responses.

Video-tapes were recorded in time-lapse from the tile undersides (recording speed = 1 frame s^{-1}, time compression = 1:72). Locomotor activity was measured as the distance moved by each nymph between consecutive video-frames captured at 30 s intervals. Individual nymphs were tracked by hand using the NIH-Image software package (NIH-Image v1.6, 1996, the macro-language code is written out in Appendix B). One-minute-interval time-series of the average locomotor activity of the nymphs visible underneath the tiles were produced for each experiment from pooled data of individuals (see detailed methods in Chapt. II).

Data Analysis

Response Variables

Locomotor activity was expected to start out at a low level during the BRITE-adaptation phase, increase during the DECRS phase, remain elevated during the DARK phase and decrease again during the INCRS phase. Numbers of nymphs visible beneath the tile surfaces were expected to be highest during the BRITE-adaptation phase, decline during the DECRS phase, remain low during the DARK phase, and increase during the INCRS phase. These expected responses were based on observed diel cycles in locomotor activity and vertical position on the substrate in *Stenonema* and other mayfly species (Elliott 1968, Glozier and Culp 1989, Grace 1990, Chapt. II).

For each experiment, mean locomotor activity was calculated for each light phase. Means for each light phase were compared between treatments with the Tukey-Kramer multiple comparisons test (HSD). The means for all four light phases were used together to define a response curve to light changes and as repeated-measures in a multi-variate analysis of variance (e.g., the mean locomotor activity during each light phase was a measurement of locomotor activity across time). Response variables tested among

treatments included the differences in the shape of the response curves, the timing of the increase in locomotor activity, and the magnitude of the change in locomotor activity from before and after the light-decrease phase. Similar aggregation and analyses of the number of nymphs visible underneath the tiles during the artificial light/dark cycles were also performed. Statistical analyses were made with SAS (SAS Version 5, or JMP Version 3.1.5, SAS Institute Inc., 1989-95).

Timing of the activity change was estimated from time-series data of individual nymphs as the latent period, or the length of time between the beginning of the light decrease and the moment when each individual's locomotor activity rose above their BRITE-adaptation mean. Only activity increases that lasted for a minimum of 10 minutes (unless the nymph left the tile underside within 10 minutes of increased activity), were considered a response to the light decrease. Ten minutes has been shown to be the shortest amount of time necessary to elicit a response in *S. modestum* under cloud conditions in natural light (Grace 1990), and was considered to be a conservative estimate of the timing of the actual change in activity. These latent periods were estimated only for nymphs that were considered to be inactive during the BRITE-adaptation phase. Differences in the BRITE-adaptation behaviors of the nymphs were observed in all experiments and categorized into three types: non day-active (average activity ≤ 6 mm min^{-1}); day-active (average activity > 6 mm min^{-1}); and other (either the nymph was not visible on the lower tile surface at all during the BRITE-adaptation period, or the nymph was visible during some portion of the BRITE-adaptation period, but left before the beginning of the light decrease). There is evidence that an individual's state of excitement affects the expression of a light response, as in *Daphnia* (Daan and Ringelberg 1969), and in some nocturnal moths (Dreisig 1980). Individuals such as the day-active nymphs were already in a heightened state of excitement so would be less likely to respond with increased activity to a light stimulus. For this reason, data from non day-active individuals were used to estimate the timing of the locomotor activity response because their responses to decreasing

light were very distinct compared with mayflies in the other classifications (illustrated in Chapter One). In the initial tests of the STAR model, correlations between rate of light decrease and the timing of the locomotor activity response were examined for time-series of individual non day-active nymphs and for the "population" (using the time-series of the averaged activity of data pooled for all nymphs). The shape of the relationships between rate of light decrease and the timing of the onset of heightened locomotor activity were the same, but significant differences ($p < 0.05$, ANCOVA) in the intercepts indicated that the timing of the increase in locomotor activity of the "population" was a few minutes earlier than the timing of the non day-active nymphs (Chapt. II). For this initial test of predator effects, individual data were considered a more sensitive comparison than the combined data. In future tests with larger numbers of mayflies, averaged data will be used in anticipation of testing the model predictions in the natural environment.

Estimates of the magnitude of the change in locomotor activity following the light decrease were made by subtracting the mean BRITE activity from the mean DARK activity for each experiment. The moment when nymphs began leaving the tile undersides was estimated using the technique outlined by Haney *et al.* (1983), as the mid-point between the first two of three points having decreasing numbers of nymphs below the BRITE-adaptation average.

Comparisons Between Treatments

Before the effects of treatments could be tested, it was necessary to determine if the responses of nymphs in the fish water were comparable to those in previous tests of the STAR model (cf. Chapt. II, the BRITE-noontime ambient/PM treatments). Both sets of experiments represent the same treatment (i.e., same illumination level during the BRITE-adaptation phase, same time of day that the light decrease began, and same predator regime), but the initial STAR tests were carried out in 1995 and the current study took place in 1996. Possible differences between years were considered. MANOVA performed for

all locomotor activity response variables (locomotor activity during the four light phases, and the difference in locomotor activity between the BRITE and DARK phases), was not significant (Pillai's trace = 0.72, p = 0.6, $F_{1,4} = 1.0$), nor for all positioning response variables (Pillai's trace = 0.98, p = 0.22, $F_{1,4} = 11.6$), indicating that the 1996 fish water treatments were within the limits of the 1995 baseline STAR results described in Chapt. II.

Next, the percentages of nymphs in each activity class were compared between all treatments in the current study (Table I). Some differences were detected in the percentage of day-active nymphs and in the numbers of nymphs visible during the BRITE-adaptation phase. There were significantly fewer nymphs visible and a significantly smaller percentage of day-active nymphs in both treatments containing stoneflies (Table I), suggesting that mayflies were less likely to move to the tile undersides when a stonefly was already there or to be active in the presence of a stonefly. There were no significant differences in the percentage of non day-active nymphs by treatment (Table I), ensuring that the estimates of the latent periods for each treatment would be made from equivalent numbers of nymphs.

One effect of stoneflies was to chase the mayflies off the tile undersides. This activity resulted in lengthy periods in which there were no mayflies visible on the tile undersides, and consequently, zero locomotor activity. For comparison of activity levels, those time periods were subtracted from the number of minutes used to calculate the mean locomotor activity for each light phase, so that mean locomotor activity was estimated only from time periods when nymphs were underneath the tiles.

TABLE I. Comparison of numbers of nymphs in each classification by treatment. Values with different letters were significantly different from each other ($\alpha = 0.05$, Tukey-Kramer (HSD) mean separation test[a]). Classifications are defined in the text. The total number of nymphs per treatment was 36.

Treatment	Non day-active (%)	Day-active (%)	Not visible (%)	Left prior to light decrease phase (%)
Fishwater (F)	41.7 ± 4.8 a	19.4 ± 2.8 a	11.1 ± 7.4 a	27.8 ± 7.4 a
No Predators (N)	52.8 ± 5.6 a	19.4 ± 2.8 a	11.1 ± 5.6 a	16.7 ± 4.8 a
Stoneflies (S)	33.3 ± 4.8 a	5.6 ± 5.5 b	33.3 ± 4.8 b	27.8 ± 5.6 a
Mixed (SF)	33.3 ± 9.6 a	8.4 ± 4.8 a,b	33.3 ± 4.8 b	25.0 ± 8.3 a

[a]Data were arc-sine transformed.

Parameters of the STAR-Model Used in the Comparison

The average rate of light change that was applied over the entire DECRS and INCRS phases was calculated for each experiment from the light intensities measured at 1-minute intervals as:

$$S = \frac{\sum_{j=1}^{n} \left[\frac{\ln\left(\frac{I_{j+1}}{I_j}\right)}{\Delta t} \right]}{n} \qquad \text{(from Ringelberg 1964)} \qquad (1)$$

where, S = the rate of relative light change per second, I = light intensity in W cm^{-2} at time period j or $j+1$, n = total minutes of decreasing (or increasing) light, t = length of the time-interval in s (in this study, $t = 60$ s).

The stimulus-activity responses predicted by the STAR model, measured as the latent period during the light decrease phase and as the magnitude of the change in locomotor activity from before and after the light decrease, were calculated for each experiment from the baseline equations developed in Chapter Two:

$$\ln[\text{latent period}] = -1.32 - 0.776 \times \ln|S| \quad (R^2 = .93;\ p < 0.001;\ n = 14) \qquad (2)$$

$$\text{magnitude of the change in locomotor activity} = 15.39 + 1.79 \times \ln|S| \quad (R^2 = .38,\ p < 0.02,\ n = 14) \qquad (3)$$

where *latent period* is the length of time in minutes between the beginning of the light decrease phase and the onset of heightened locomotor activity, *magnitude of the change* is the difference in the mean activity between the DARK and BRITE phases in mm per minute, and *S* is the average applied rate of relative light change per second, calculated from Eq. (1). The STAR model assumes that the magnitude of the change in locomotor activity depends on both the strength of the light stimulus (the rate at which light changes), and the duration of time over which the light stimulus is applied (Chapt. II). In the present study, both the range of light intensity and time-duration of the light decrease were the same as used to develop Eq. (3), therefore the baseline estimates of the magnitude of change in locomotor activity were used without modifications for shorter or longer periods of light decrease.

Tests of the STAR model using small numbers of nymphs were more appropriate to the locomotor activity response than to the timing of vertical movements on the substrate, because individual nymphs could be tracked, whereas fewer nymphs made it difficult to detect the time when nymphs began to leave the tile undersides. For this reason, comparison of location on the substrate between treatments was limited to the differences between the numbers of nymphs visible during each light phase and no attempt was made to compare the timing of leaving the substrate undersides with the STAR predictions (Chapt. II).

Results

Locomotor-Activity Response to Light Changes

Examination of the locomotor activity response curves of *S. modestum* nymphs during the artificial light/dark cycles revealed that nymphs responded to light decrease in all predator regimes, but there were treatment effects noticeable as differences in the shapes of the response curves (Fig. 1). Locomotor activity was higher during the BRITE-adaptation phase in fish water than in the other three treatments. Increased activity as a response to the light decrease (DECRS phase) was smaller in the no predator treatment than in fish water (Fig. 1). Once the stimulus-activity response was triggered during the DECRS phase and locomotor activity had increased, there were continued increases in the locomotor activity during the DARK-adaptation and light-increase (INCRS) phases in the stonefly treatment that were unrelated to changes in the light environment (Fig. 1). Locomotor activity also did not diminish in response to light increase (INCRS phase) in the fish water treatment as was the case in the no predator and mixed predator treatments (Fig. 1), indicating that locomotor activity was not controlled solely by relative light change.

Repeated measures ANOVA indicated no significant effect of either fish odor or stoneflies on the mayflies response to the light/dark cycle (Table II). There was, however, a significant interaction between the two predator regimes, Fish x Stonefly (Table II). This interaction resulted in depressed locomotor activity during the DARK and INCRS phases when both predators or no predators were present, compared to the heightened locomotor activity under single predator treatments (Fig. 1).

Light was a strong influence on locomotor activity within all treatments, as shown by the significant effect of Time (Table II). A significant interaction between Time x Fish x Stonefly suggests that the responses of the mayflies to light were altered in the different predator treatments, and that neither the presence or absence of fish odor alone or stoneflies

alone was powerful enough to solely determine the response of the mayflies to light (Table II, Fig. 1), suggesting some facilitation was occurring between the predators.

Within the light decrease (DECRS) phase, the STAR model predicts that there should be a characteristic delay, or latent period, between the beginning of the light decrease and the moment when locomotor activity begins to increase (see Eq. 2 in the Methods). Differences between the observed and STAR-predicted latent periods were compared for the fish water treatment. The observed latent periods were 6.77 ± 0.8, min ± SE, earlier than predicted, a difference that was weakly significant ($p < 0.07$ that 6.77 min > 0.0 min, Wilcoxon Signed-Rank Test), suggesting that there was some variability in the timing between the original tests of the STAR model and the fish water treatments that did not alter the overall activity levels during the light phases.

Differences between the observed and STAR-predicted latent periods were compared by treatment to determine if nymphs began to move around earlier or later than predicted in the presence or absence of different predators. No significant differences ($p = 0.28$, $F_{3,8} = 1.5$) between treatments were detected (Fig. 2). However, variability in the timing of the activity change within treatments was large, and the power of the test was low (power = 0.24), indicating that a significant difference would not be detected. There was a tendency for nymphs in the no-predator and mixed-predator treatments to leave later than nymphs in the fish water treatment, and this tendency was weakly significant between the fish water and the mixed predator treatments ($p < 0.06$, $F_{1,4} = 6.5$, power = 0.48), indicating that mayflies may indeed initiate locomotor activity later when in the presence of both fish odor and stoneflies than in the presence of fish odors alone.

The STAR model also predicts that the difference between daytime and nighttime locomotor activity in response to light decrease at a particular rate of light change will be a function of the rate of change (see Eq. 3 in the Methods). Differences in the observed and STAR-predicted changes in locomotor activity were not significantly different ($p = 0.31$, $F_{3,8} = 1.4$) for any of the treatments (Fig. 3). Here again the power of the test was low

(power = 0.25), indicating that a significant difference probably would not be detected. Of the relationships developed between rate of light change and stimulus-activity response in *S. modestum*, the magnitude of the change in locomotor activity was the weakest (the correlation coefficient for the baseline relationship was significant, but not highly predictive, see Eq. 2 in the Methods). The comparison between predator treatments was confounded by a lack of a significant difference in locomotor activity between the BRITE and DARK phases in the fish water and no predator treatments (both p values > 0.05, ANOVA, data square-root transformed, see Fig. 1). Although the level of activity was significantly higher during the DECRS phases than during the BRITE-adaptation phases (Fig. 1), the baseline relationship was developed using differences between the locomotor activity during the BRITE and DARK phases. Rapid decline in locomotor activity following the end of the light decrease may be indicative of response to predators that should be further investigated.

TABLE II. Analysis of variance with repeated measures[a] table for the locomotor activity response to the artificial light/dark cycle compared by treatment. Treatments were presence or absence of fish odors (Fish) and presence or absence of stoneflies (Stonefly). Data were square-root transformed.

	Source of variation	MS	F	df	P
Between treatments	Fish	23.9	1.8	1	0.2
	Stonefly	0.8	0.1	1	0.8
	Fish x Stonefly	290.8	21.8	1	0.002
	Error	13.4		8	
Within treatments	Time	59.8	1.3	3	0.001
	Time x Fish	7.5	0.7	3	0.6
	Time x Stonefly	5.2	1.0	3	0.5
	Time x Fish x Stonefly	32.0	7.4	3	0.019
	Error (Time)	9.7		24	

[a]Repeated measures (Time) = average locomotor activity during the BRITE, DECRS, DARK, INCRS light phases.

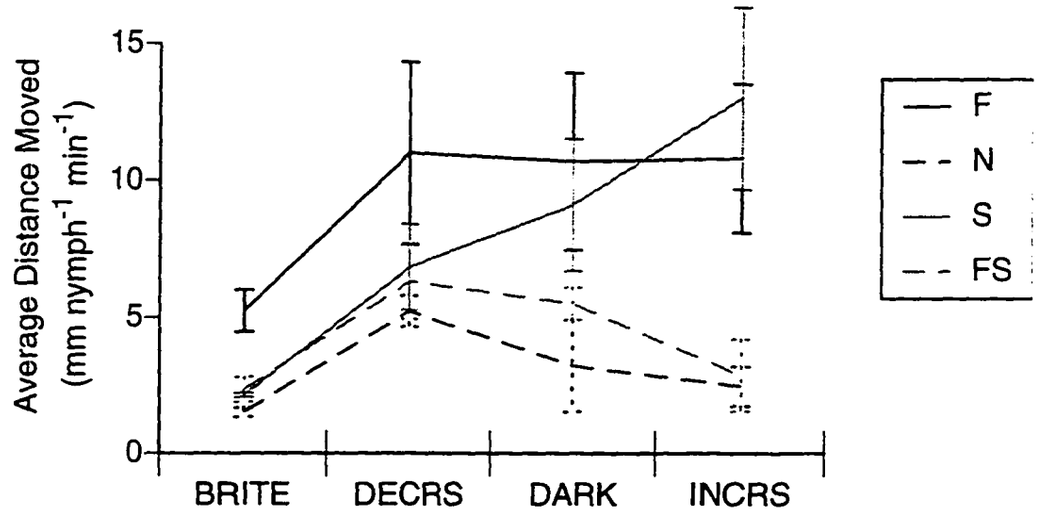

Figure 1. Response curves of the locomotor activity during the artificial light/dark cycles by treatment. Treatments are: fish water (F), no predators (N), stoneflies (S), and mixed fish water + stoneflies (FS). Bars are ± SE, (n=3). Mean separation tests (α = 0.05, Tukey-Kramer (HSD) multiple comparisons test) indicated that locomotor activity during the BRITE-adaptation phase was significantly higher in fish water than in the other three treatments; locomotor activity during the DARK phase was significantly higher in fish water than in the no predator treatment; and locomotor activity during the INCRS phase was significantly higher in the fish water and stonefly treatments vs. the mixed predator and no predator treatments.

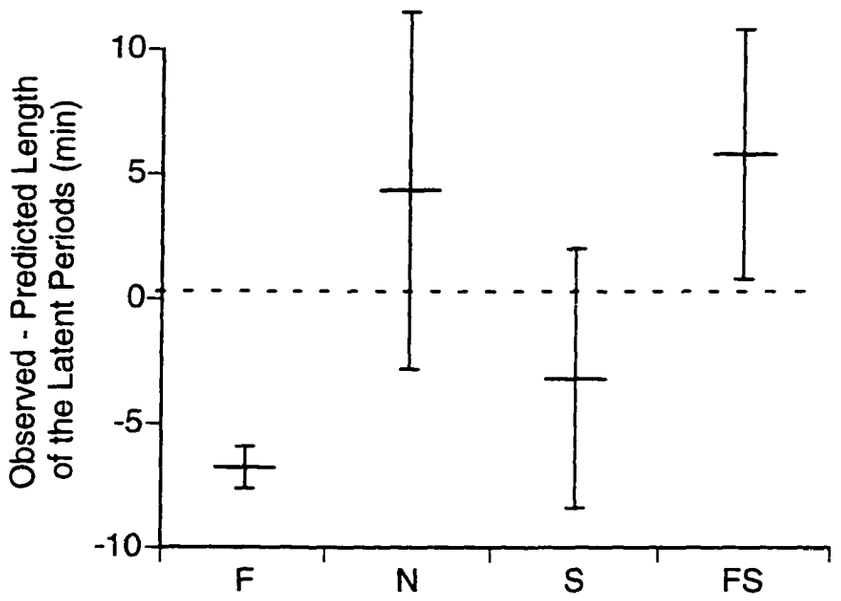

Figure 2. Comparison of differences between the observed and predicted length of the latent period, the amount of time between the light decrease and the beginning of heightened locomotor activity, by treatment. (Treatments are listed in Fig. 1). Negative values represent earlier onsets of heightened locomotor activity and positive values represent later onsets than predicted by the STAR model. At the target rate of light decrease, $S = -2.5 \times 10^{-3}\ s^{-1}$, the STAR-predicted latent period was 27.9 minutes. Values were not significantly different from each other ($p < 0.05$). Data are treatment means ± SE, (n=3).

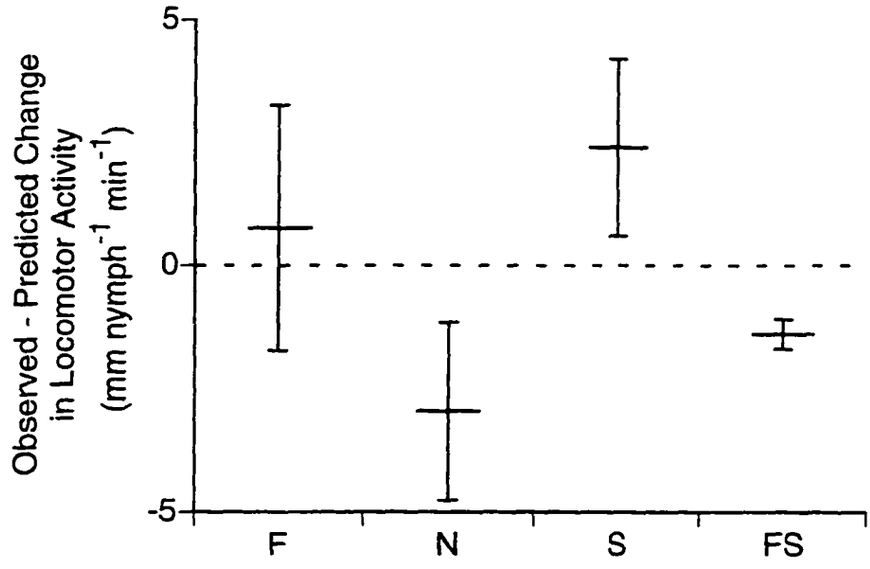

Figure 3. Comparison of differences between the observed and predicted magnitude of the change in locomotor activity from before and after the period of light decrease, calculated as mean DARK activity minus mean BRITE activity, by treatment. (Treatments are listed in Fig. 1). Negative values represent a smaller change and positive values represent a larger change than predicted by the STAR model. At the target rate of light decrease, $S = -2.5 \times 10^{-3}\ s^{-1}$, the STAR-predicted change in locomotor activity was 4.66 mm $nymph^{-1}$ min^{-1}. Values were not significantly different from each other ($p < 0.05$). Data are treatment means ± SE, (n=3).

Vertical Movements on the Substrate in Response to Light Changes

Nymphs began to leave the tile undersides during the DECRS phase of the artificial light/dark cycle in all treatments, as expected (Fig. 4). Numbers of nymphs visible during the DARK phases were significantly lower ($p < 0.05$, ANOVA) than numbers visible during the BRITE-adaptation phases in the two treatments without stoneflies, but differences were not significant in the two treatments containing stoneflies (Fig. 4). Nymphs did not return to the substrate undersides during the light-increase (INCRS) phase, contrary to that expected (Fig. 4). The same pattern of nymph movements during the artificial light/dark cycles were observed in the original tests of the STAR model (Chapter Two), and it was proposed that there may be an effect of the endogenous clock that inhibits mayflies from responding to increasing light by moving to the darker underside of the tiles after the much abbreviated artificial "nighttime" in these tests.

Within treatments, there were no significant interactions between predator regime and time (Table III), indicating that the light response was stronger than the effect of predators on mayflies preference for a particular substrate surface. Between treatments, the presence or absence of stoneflies was the only significant effect on the numbers of nymphs underneath the tiles (Table III). Mayflies avoided stoneflies and were less likely to utilize the lower tile surfaces during periods of high light intensity when the surface was occupied by a stonefly. The significant effect of stoneflies indicated that for movements between the substrate surfaces, the mayflies response to light change was the same regardless of the presence or absence of fish odors. Thereby, the two stonefly treatments (S and SF) and the two non-stonefly treatments (F and N) were combined for further analysis. Examination of the number of nymphs visible during the BRITE-adaptation phase in the combined treatments revealed significantly fewer nymphs visible ($p < 0.02$, $F_{1,10} = 6.8$, ANOVA) when stoneflies were present than when stoneflies were absent. There were, however, no significant differences in the numbers of nymphs that left the tile undersides during the

light-decrease period ($p = 0.2$, $F_{1,10} = 2.3$), indicating that those mayflies that remained under the tiles with a stonefly were just as likely to leave the tile undersides in response to decreasing light as those mayflies that remained under the tiles with no stonefly.

TABLE III. Analysis of variance with repeated measures[a] table for the numbers of nymphs visible underneath the tiles compared by treatment. Treatments were presence or absence of fish odors (Fish) and presence or absence of stoneflies (Stonefly). Data were square-root transformed.

	Source of variation	MS	F	df	P
Within treatments	Time	2.5	16.1	3	0.0001
	Time x Fish	0.1	0.7	3	0.6
	Time x Stonefly	0.1	0.9	3	0.4
	Time x Fish x Stonefly	0.1	0.4	3	0.7
	Error (Time)	0.2		24	
Between treatments	Fish	0.1	0.2	1	0.7
	Stonefly	2.4	102.0	1	0.01
	Fish x Stonefly	0.5	2.2	1	0.2
	Error	0.2		8	

[a]Repeated measures (Time) = average number of nymphs visible underneath the tiles during the BRITE, DECRS, DARK, INCRS light phases.

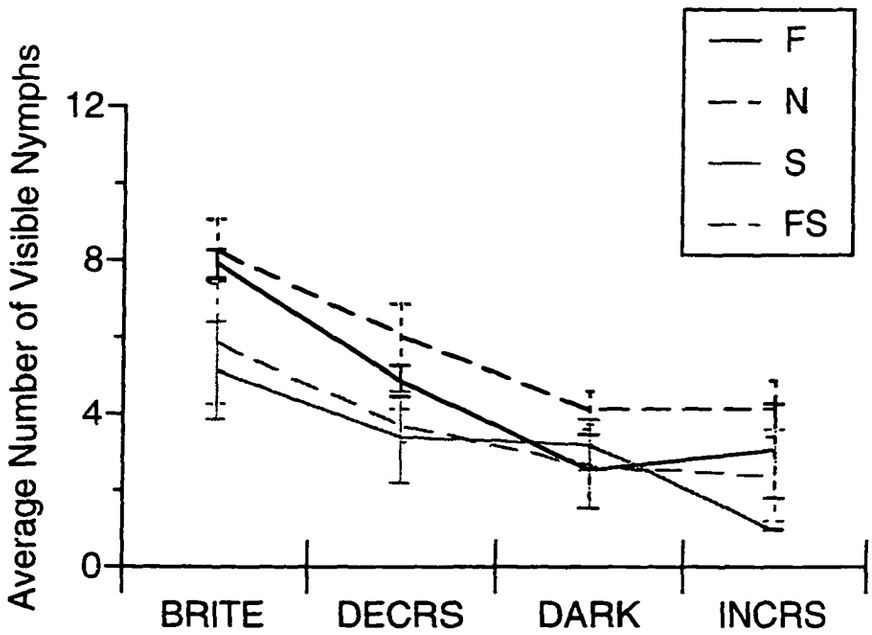

Figure 4. Response curves of the number of nymphs visible on the tile undersides during the artificial light/dark cycles by treatment. (Treatments are listed in Fig. 1). The maximum possible number of nymphs visible during any light phase was 12. Bars are ± SE, (n=3). Mean separation tests (α = 0.05, Tukey-Kramer (HSD) multiple comparisons test) indicated there were significantly fewer nymphs visible during the DECRS phase for the stonefly treatment than for the no predator treatment; and significantly fewer nymphs visible during the INCRS phase for the stonefly treatment than for the other three treatments. Data were square-root transformed.

Time-series of Locomotor Activity and Vertical Movements on the Substrate

Examination of one time-series from each treatment demonstrates the variability in locomotor activity and vertical movements on the substrate of *S. modestum* and the differences between treatments (Fig. 5). Locomotor activity during the BRITE-adaptation phase fluctuated around a low value in all treatments (Fig. 5). When stoneflies were present, there were abrupt changes in mayfly locomotor activity during the three subsequent light phases that were not related to changes in light (Fig. 5, Stoneflies and Mixed predators). There were also periods when several nymphs left the tile undersides within 1 or 2 minutes during the DECRS period (Fig. 5, Mixed predators) or during the INCRS period (Fig. 5, Stoneflies), suggesting that the activity of the stoneflies may have been the cause of these abrupt mayfly movements.

Time-series of stonefly activity was examined to determine if the observed abrupt changes in mayfly activity were directly related to activity of the stoneflies (Fig. 6). Stonefly behavior throughout the light/dark cycle was very similar to that of the mayflies. Stoneflies were quiescent during the BRITE-adaptation period, then became active during the DECRS period, and heightened locomotor activity continued through the DARK phase. In contrast to the pattern of activity in the mayflies, once activated, the stoneflies were extremely mobile, often traversing more than the entire width of a tile within a 1-minute interval (Fig. 6). Stoneflies did not appear to lower their activity during the INCRS phase, as the mayflies typically did. It was possible to track individual stoneflies through the entire light/dark cycle, revealing that the stoneflies spent long periods away from the tile undersides and presumably on top of the tiles, and cycled between the tile surfaces often, resulting in the abrupt changes in mayfly activity and in mayflies leaving the tile undersides over very short time intervals (Fig. 6).

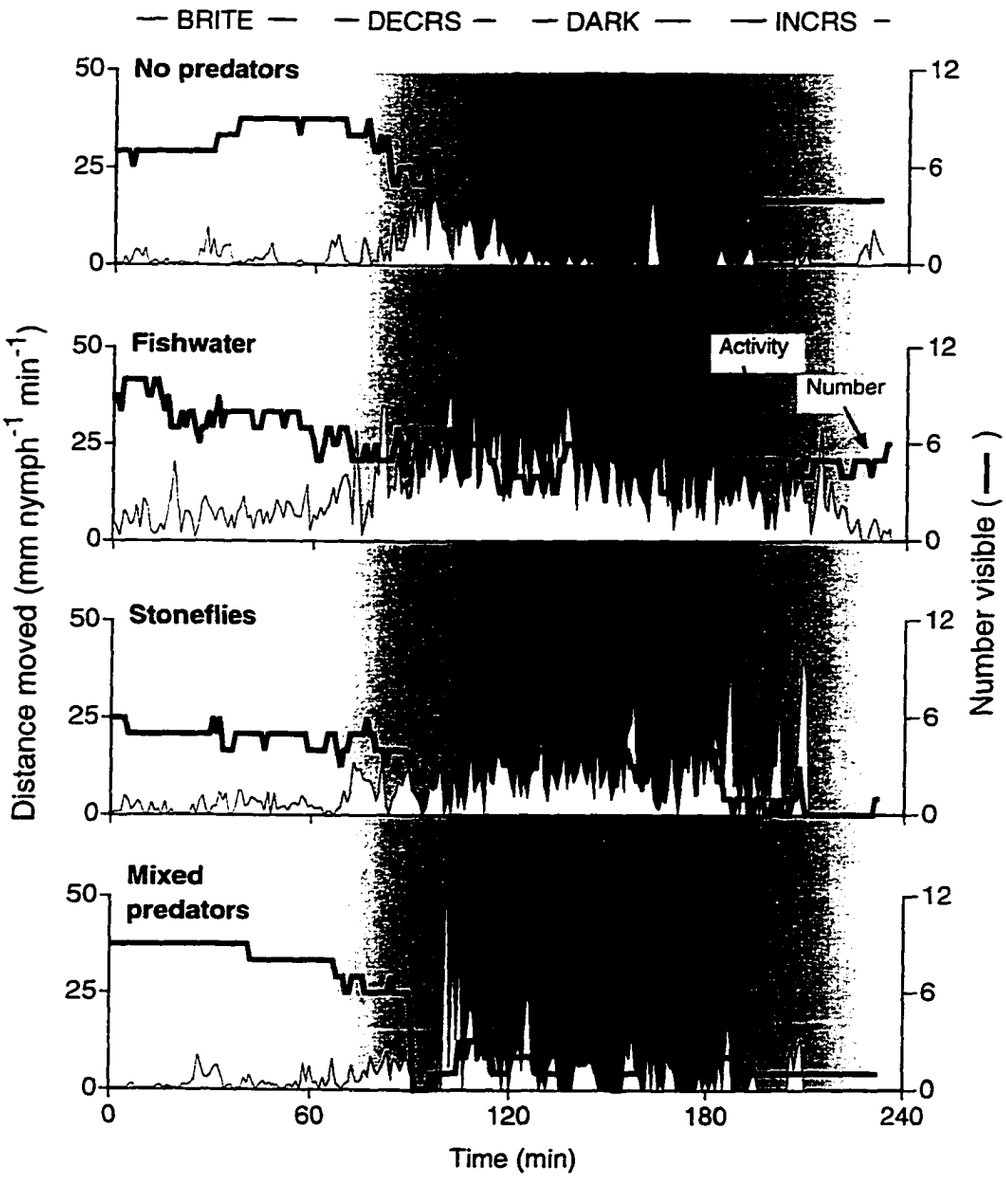

Figure 5. Sample time-series of locomotor activity and number of nymphs beneath the tiles for each treatment. From left to right, the time-series represent the 60 min BRITE-adaptation period, the light decrease (DECRS) phase, the 60 min DARK-adaptation period and the light increase (INCRS) phase. Shading represents the light environment. $Time_0$ = 1700 EST.

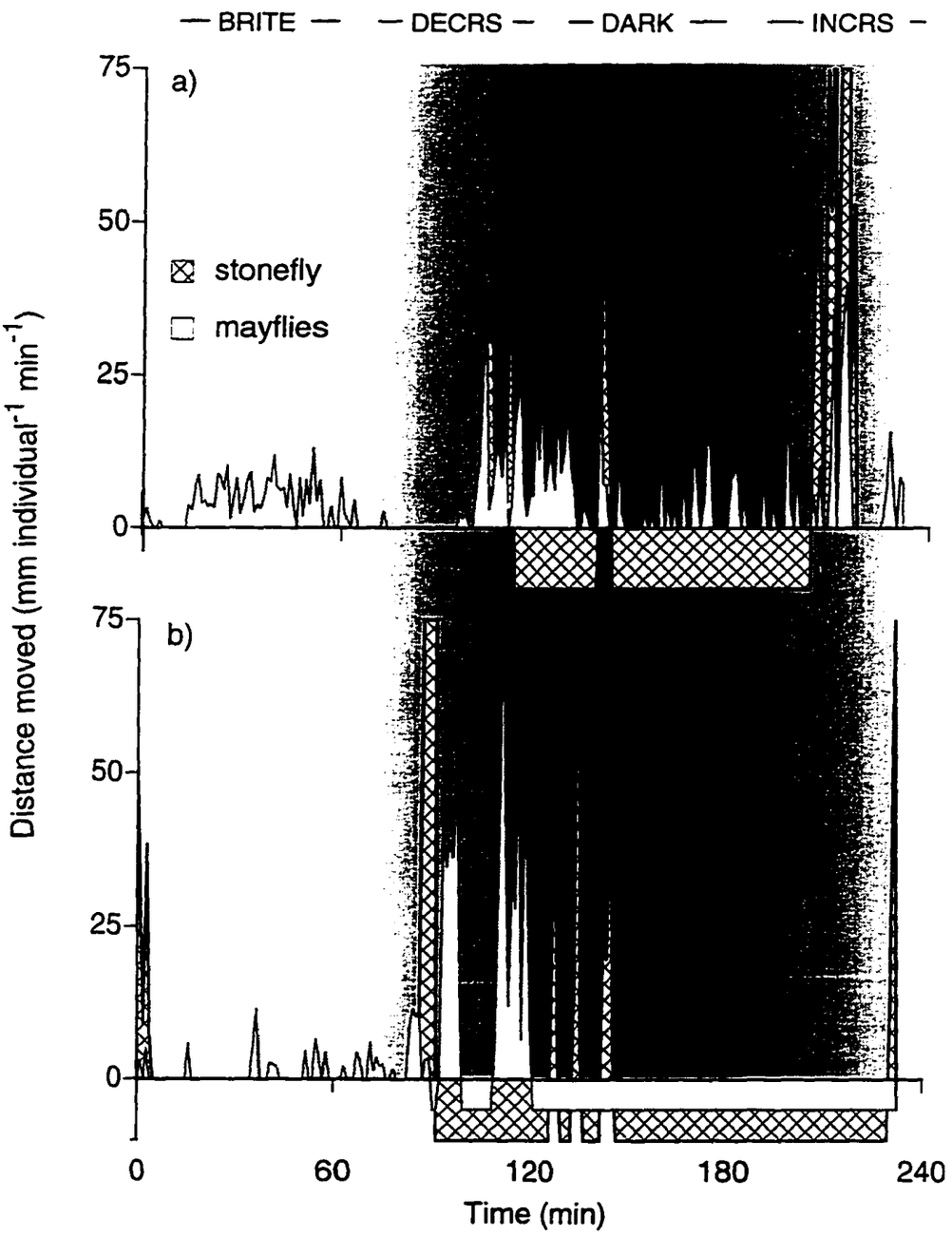

Figure 6. Time-series of individual stoneflies and the average locomotor activity of mayflies underneath each tile in a mixed predator experiment. Filled areas below the zero line represent periods when there were no individuals, either mayflies (white areas) and/or stonefly (hatched areas) visible beneath the tile. Where hatching is not visible, that particular stonefly was visible, but inactive. For plotting purposes, distances moved by individual stoneflies were clipped above 75 mm min^{-1}, but occasionally reached as high as 200 mm min^{-1}. Shading represents the light environment. $Time_0$ = 1700 EST.

Activity of the Stoneflies in Response to Light Change

The activity of the stoneflies with and without fish water was examined to determine if the stoneflies altered their behavior in fish water. There were no significant differences in the response curves between treatments ($p = 0.75$, $F_{3,1} = 0.12$, ANOVAR), indicating that there was no consistent difference in stonefly activity whether or not fish odor was present. There was a significant effect of Time ($p < 0.01$, $F_{3,3} = 28.0$), indicating that stoneflies responded to the light changes. A significant interaction between Time x Treatment was detected ($p < 0.02$, $F_{3,3} = 19.5$), suggesting that stonefly activity during particular light phases of the artificial light/dark cycle was different depending on whether or not fish odor was present. However, differences in activity were not detected between individual light phases ($p > 0.05$, ANOVA), indicating that the interaction between time x treatment shown by the repeated measures test was not due to significant differences in locomotor activity between any one particular light phase, but by smaller differences during some of the light phases, such as the DARK and INCRS phases, not detectable by ANOVA (Fig. 7).

Because stoneflies became activated during the DECRS phase (Fig. 7), the latent period between the beginning of the light decrease and the onset of heightened activity was compared to that predicted for the mayflies by the STAR model (see Eq. 2). Latent periods were within 3 to 10 minutes of predicted, suggesting that stoneflies may also use relative light change as a timing cue. Stoneflies in the fish water treatment became active later ($p = 0.06$, power = 0.5) than stoneflies in well water without fish odor (Fig. 8a), and left the tile undersides significantly later (Fig. 8b), suggesting there were fish odor effects on the timing of both of these stonefly activities.

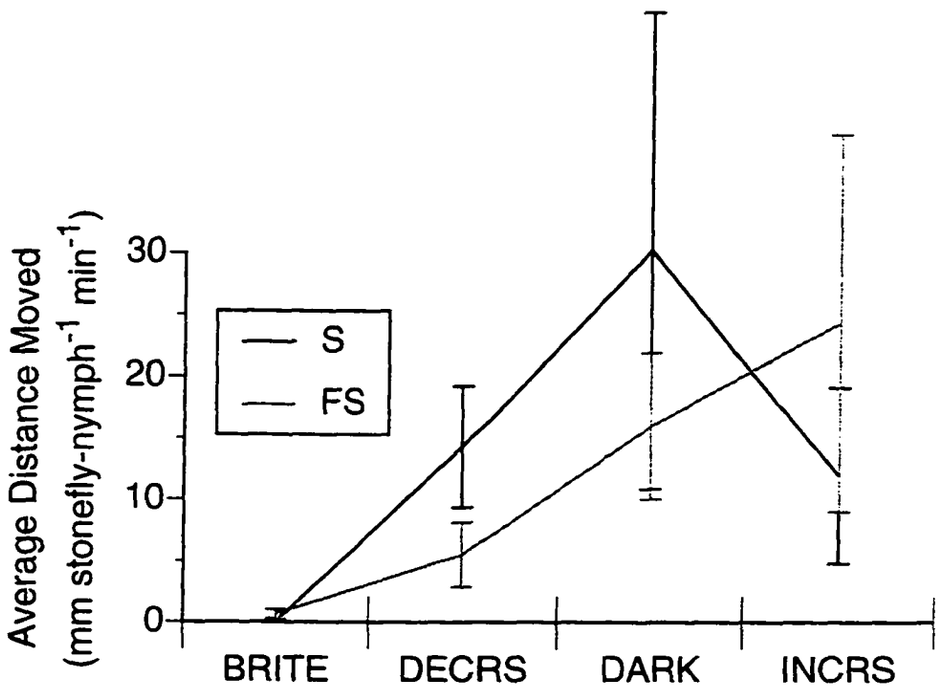

Figure 7. Response curves of stonefly locomotor activity during the artificial light/dark cycles with (FS) and without (S) fish odors. Bars are ± SE, (n=3). Activity during each light phase was not significantly different ($p < 0.05$, ANOVA) between treatments.

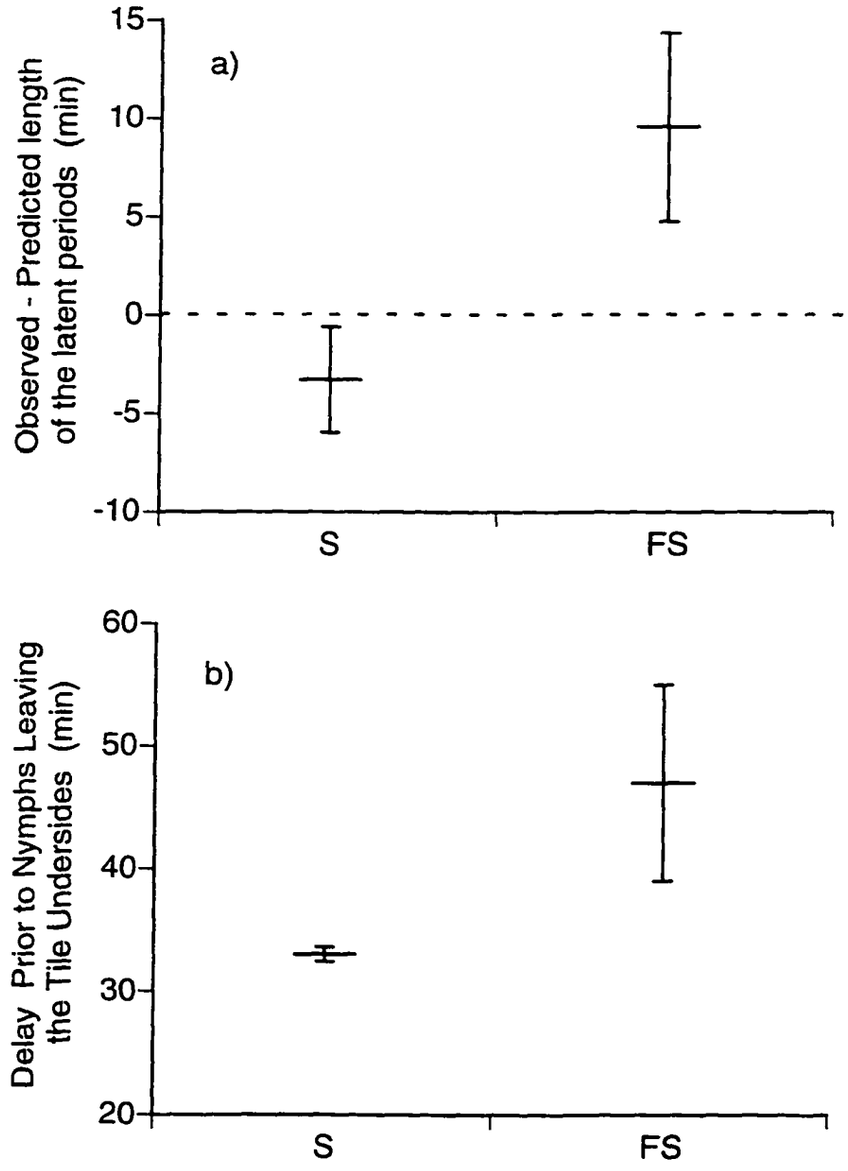

Figure 8. (a) Comparison of differences between the STAR-predicted latent period (the length of time between the beginning of the light decrease and the onset of heightened locomotor activity), and the observed latent period for stoneflies with (FS) and without (S) fish water. Negative value represents earlier onset of heightened locomotor activity and positive value represents later onset than predicted. Differences were weakly significant ($p < 0.06$, ANOVA). (b) Comparison of the delay between the beginning of the light decrease and the moment when stoneflies left the tile undersides. Differences were significant ($p < 0.046$, ANOVA, data square-root transformed) between fish water treatments. Values are mean ± SE, (n=3).

Discussion

Responses of *Stenonema* to light stimulus, the relative change in light, were altered in the presence and absence of fish and stonefly predators. Although changes in light during natural twilight are not constant as they were during these tests, the baseline relationships developed to test the STAR model provided a benchmark from which changes in behavior could easily be assessed. Mayflies modified their behavior in response to chemical cues from the fish and mechanical cues from the stoneflies. Mayflies were more active in the fish water than in the other three treatments. If activities such as locomotion, feeding, and drift are under similar internal and external controls, then the increased activity during the simulated night in the presence of fish odors was expected, as nocturnal drift and feeding have been shown to increase in mayflies exposed to fish or fish odors (Cowan and Peckarsky 1994, Douglas *et al.* 1994, McIntosh and Townsend 1994, Tikkanen *et al.* 1994).

A different result was observed when stoneflies were present. Mayflies initially avoided surfaces occupied by a stonefly, regardless of whether or not the stonefly was actively moving about. This is consistent with the reported avoidance by *Stenonema fuscum* of areas containing high chemical cues from caged *Acroneuria lycorias* stoneflies (Peckarsky 1980), and suggests that chemical cues from the stoneflies influence the preference for a particular substrate in mayflies. Once the mayflies were settled on the substrate, they were often situated on the tiles in positions downstream from the stoneflies, and locomotor activity was lower in the presence of stoneflies than in fish water alone. During the simulated twilight, the stoneflies began to move about and roam over both the lower and upper tile surfaces. The mayflies actively avoided the stoneflies, and there were abrupt changes in the locomotor activity of the mayflies that did not correspond with changes in light. The most prevalent stonefly avoidance strategy in *Stenonema modestum*

was crawling away from the stonefly rather than swimming or drifting as has been observed in some *Baëtis* species (Peckarsky 1980, Malmqvist and Sjöström 1987). Peckarsky (1980) and Ode and Wissinger (1993) recorded similar behavior in *Stenonema fuscum* and *Paraleptophlebia adoptiva*, respectively, in response to *Acroneuria spp.* stoneflies. Ode and Wissinger (1993) noted that *Paraleptophlebia adoptiva* remained motionless in the presence of chemical cues from *Acroneuria* stoneflies and responded only to physical contact. Malmqvist (1992) also reported reduced activity of *Baëtis*, and a caddisfly, *Agepetus*, in response to *Dinocras* stoneflies. These observations suggest that chemical cues or close proximity to a stonefly may not significantly disrupt the daytime activity of the mayflies, but mayflies lower their activity when situated on substrates occupied by stoneflies. In a natural stream, every rock does not harbor a stonefly, and the overall effect of the reduction of activity is not known, nor the fitness consequences of reduced daytime activity. However, avoidance of stoneflies has been related to reduced overall fitness of mayflies (Peckarsky *et al.* 1993, Peckarsky and McIntosh 1997), suggesting that reduced daytime activity does not compensate for energy consumed in avoiding stoneflies during the night. The short bursts of large increases in mayfly activity observed during treatments containing stoneflies did not translate into significantly higher locomotor activity during the simulated night periods, but such bursts of activity may consume more energy than when locomotor activity is sustained over a more consistent level, resulting in reduced fitness.

In the stonefly treatments, the latent period between the beginning of the light decrease and the onset of heightened locomotor activity in the mayflies was either a few minutes later or earlier than predicted, depending on whether the water also contained fish odors (later) or not (earlier). Although the differences in timing were not all significantly different, the same trend was observed in the timing of the onset of heightened locomotor activity in the stoneflies, suggesting that the activity of the stoneflies influenced the timing of the change in locomotor activity in the mayflies. If the lower daytime locomotor activity

was a strategy by the mayflies to avoid disturbing and thus provoking an attack by the stoneflies, perhaps mayflies also wait to become active to avoid encounters with stoneflies. In the tests by Ode and Wissinger (1993), mayflies were more reactive to stonefly encounters in water that contained both stoneflies and stonefly odors than in water that contained only stoneflies, suggesting that mayflies are aware of the presence of stoneflies and react accordingly.

Differences in the activity response of stoneflies in the two treatments suggest that the behavior of the stoneflies may have been altered by fish odors. Strong similarities between mayflies and stoneflies in the timing of heightened locomotor activity in response to decreasing light suggest that behaviors in both prey and predator species are under a similar suite of controls. Diel periodicity in stonefly behavior, vertical location on the substrate, and feeding has been documented in some species including *Dinocras* (Malmqvist and Sjöström 1980), *Megarcys* (Peckarsky and Cowan 1995) and *Kogotus* (Walde and Davies 1985, Peckarsky and Cowan 1995), although some stoneflies may feed during the daytime (Walde and Davies 1985, Feltmate and Williams 1989b, Peckarsky and Cowan 1995). Because the larger and more conspicuous stoneflies are also prey of visual-foraging fish (Allan 1981, Moore and Gregory 1988, Feltmate and Williams 1989a, 1989b, 1991), modification of their behavior by becoming active and leaving the refuge of the tile underside later in the presence of fish is consistent with a predator-avoidance strategy timed to the 24 hr light/dark cycle. Synergistic effects between stonefly and fish predators have been reported elsewhere (Soluk and Collins 1988a, 1988b, McIntosh and Peckarsky 1997), suggesting that particular predator-prey interactions may be modified depending on the assemblage of species that are present in a stream ecosystem, and interactions must therefore be assessed in the context of the larger environment.

Some understanding may come from observations from lakes and marine environments that suggest a similarity in the mechanisms by which predator and prey species in aquatic systems interact with each other. Diel vertical migration of zooplankton

is an analogous example of a predator avoidance strategy timed to the 24 hr light/dark cycle (reviewed by Haney 1988). Predation regimes under which DVM is present or absent parallel those reported for the presence or absence of invertebrate drift in streams. DVM occurs in lakes containing planktivorous fish (Gliwicz 1986, Haney 1988), but not necessarily in fishless lakes (Gliwicz 1986). There are numerous examples of rapid induction of vertical migrations of prey species in freshwater and marine environments following the introduction of predators or water that had previously contained predators (Neill 1990, Bollens and Frost 1991, Ringelberg 1991a, 1991b, Forward and Rittschof 1993, Loose 1993). The phototactic swimming response of the water flea, *Daphnia*, and the phantom midge, *Chaoborus*, is enhanced in water containing fish odors (Ringelberg 1991a, 1991b, Tjossem 1990), as is the drift response in mayflies. *Chaoborus*, like stoneflies, are both predator and prey (Fedorenko 1975, Luecke 1986), and like stoneflies, their impact on the diel activity of their prey species (cladocerans and copepods), may be masked in lakes containing fish (review by Haney 1988).

This study demonstrated how interactions between predator and prey, and between predators, can alter the response of prey organisms to a strong environmental cue, such as the 24 hr light/dark cycle. Stoneflies, the tactile predators, were capable of overriding the preference of mayflies for the darkened surface of the substrate during daytime light conditions and disrupted their activity response to light by forcing the mayflies into actively avoiding encounters. In streams containing both stonefly and visual fish predators, the trade-offs between avoiding stoneflies and avoiding fish may be considerable. Synergistic effects between predators may override the effect of any single predator type on the overall response of the prey species in natural systems, where the implications of alterations in timing and amount of nocturnal activity, or positioning on substrates of mayflies have not yet been assessed. Predation also affects individual behavior and differences are recognizable as variability in observed activities. More knowledge about how organisms use relative changes in light as a daily cue to change their behavior, and how local

environmental conditions can modify the timing or extent of the behavior change, is needed to fully understand how the interactions between internal and external cues in individuals combine to produce the observed behaviors of stream populations. Laboratory studies such as this one, can reveal detailed interactions between predators, prey, and light, important to understanding the function of the ecosystem.

CHAPTER IV

PRELIMINARY EVALUATION OF THE PREDICTIONS OF THE *STAR* MODEL WITH RESPECT TO DIEL ACTIVITY CYCLES OF NYMPHS OF THE MAYFLY, *STENONEMA MODESTUM*, IN NATURAL LIGHT

Introduction

The Stimulus-based Timing and Activity Rate Model (STAR) was developed to explain the mechanisms by which mayfly nymphs become active and move to the upper substrate surfaces to feed during evening twilight. The basis of the STAR model is that light stimulus regulates changes in locomotor activity on the lower substrate surfaces (a photokinetic response) and causes individuals to leave the darkened lower substrate surfaces and move to the brighter upper substrate surfaces (a phototactic response). Light stimulus is defined as the rate of relative light change, described by Ringelberg (1964). Relative changes in light are most pronounced and long-lasting during the twilight periods, and it is during twilight that largest changes in locomotor activity and vertical movements between substrate surfaces are observed (Kohler 1983, Allan *et al.* 1986, Casey 1987, Grace 1990, Wilzbach 1990).

According to the STAR model the timing and magnitude of nocturnal locomotor activity are determined during periods of light decrease by stimulus strength (measured as the rate at which light decrease takes place) combined with the length of time over which

the light decrease takes place. The differences between the levels of daytime and nighttime activity is proportional to the strength of the stimulus (e.g., the magnitude of the change will be larger when the rate of light decrease is larger and vice-versa). These predictions were tested in Chapter Two and mathematical relationships were developed between rate of light decrease and both the timing of the onset of heightened locomotor activity and the magnitude of the change in activity as a consequence of light decrease. Similarly, the timing of migrations away from the tile undersides during periods of light decrease were correlated with the rate at which the light decreased.

Tests of the STAR model were carried out in the laboratory in an artificial light environment. During simulated twilight periods of artificial light/dark cycles, light changes were produced over a constant rate of light change, which resulted in the same relative change in light over the entire light decrease phase of the artificial light/dark cycle. During natural twilight, relative changes in light are not constant, but become larger as the sun approaches the horizon and thereafter the largest relative changes in light take place following sunset (Haney *et al.* 1983, Ringelberg 1991b, Ringelberg *et al.* 1991a). The objective of this chapter is to compare the stimulus-activity response predictions of the STAR model with activity changes of nymphs of the mayfly, *Stenonema modestum*, in natural light as a preparation to testing the model in the natural environment.

Methods

Overview

All observations were carried out in the Anadromous Fish and Aquatic Invertebrate Research Laboratory at the University of New Hampshire, in Durham. Two channels of a clear acrylic laboratory stream were used (0.15 m wide x 0.25 m high x 2.4 m long). A tank located at the lower end of the stream was filled with water that was recirculated at a rate of 5 cm sec^{-1} (24 l min^{-1}) and aerated by flowing over upstream barriers. Water depth in the channels was 10 cm. Well water (temperature controlled at 18 ± 2.0 °C by immersion coolers), plus fish odor (fish density = 2.5 fish m^{-2}, two common shiners, *Notropis cornutus*, and two longnose dace *Rhinichthys cataractae*, kept in the tank throughout the experimental period) was used during the artificial light/dark cycles, which were carried out in the summer of 1995. Oyster River water was used during the natural light experiments, which were carried out during 1987-1988. Temperature varied between the natural-light experiments and averaged 22.4 ± 0.1 °C ± SE (n = 297) in October 1987, 12.6 ± 0.03 °C ± SE (n = 115) in February 1987, and 27.2 ± 0.1 °C ± SE (n = 54) in August 1988.

During the artificial light/dark cycles, the stream was completely enclosed in black plastic to ensure that light came only from the four halogen lamps located directly above the tiles (see Chapter One). Light intensity was manipulated between 7.9 x 10^{-4} W cm^{-2} and 1.3 x 10^{-7} W cm^{-2}. During the natural light experiments, floor-to-ceiling south-facing windows provided ambient light (see Grace 1990).

Tests of the STAR model used in this comparison were those that were carried out during the evening (light reduction began at 1800 EST), in which nymphs were placed on one large (10 x 10 x 0.5 cm) unglazed tile substrate and adapted for 1 hour to a noon-time

ambient light intensity typical of July (n = 4). Natural light experiments (October, February, and August) were each carried out over three consecutive days and nights, and nymphs were placed on four small (5 x 5 x 0.5 cm separated by 0.5 cm) unglazed tile substrates.

Activity of the nymphs was recorded from underneath the tiles on time-lapse video (time compression was 1:120 in the natural-light experiments, and 1:72 in the artificial light/dark cycles). Locomotor activity was measured as the average distance moved per nymph during 1-minute intervals for the STAR model tests (see Chapt. I), and as the percentage of nymphs that moved during 10-min intervals for the natural-light experiments (Grace 1990). Detailed descriptions of the methods for measuring activity are located elsewhere (Chapt. I, Grace 1990). Comparisons of distance moved and percent active nymphs indicate that these two measures of activity are comparable when used for estimations of the timing of activity changes over the short time intervals tested (Fig. 1).

Rate of light change was calculated as:

$$S = \frac{\ln\left(\frac{I_{j+1}}{I_j}\right)}{\Delta t} \qquad (1)$$

where S = rate of light change per second, I = light intensity in W m^{-2} during time interval j or $j+1$, and t = the time interval between light measurements in s (from Ringelberg 1964).

Baseline Relationships in the STAR Model

Two of the stimulus-activity responses predicted by the STAR model were tested: the latent period during the light decrease phase and the magnitude of the change in locomotor activity from before and after the light decrease. The latent period is the length of time between the beginning of the light decrease and the onset of heightened locomotor activity; the magnitude of the change in locomotor activity is a measurement of the difference in average locomotor activity before and after the period of light decrease.

Baseline relationships between the rate of light decrease and both of these response variables were developed in Chapt. II:

$$\ln[\text{latent period}] = -1.32 - 0.776 \times \ln|S| \qquad (R^2 = .93;\ p < 0.001;\ n = 14) \qquad (2)$$

$$\text{magnitude of the change in locomotor activity} = 15.386 + 1.79 \times \ln|S| \qquad (R^2 = .38,\ p < 0.02,\ n = 14) \qquad (3)$$

where *latent period* is the length of time in minutes between the beginning of the light decrease phase and the onset of heightened locomotor activity, *magnitude of the change* is the difference in the mean activity between the artificial night and artificial daytime phases in mm $nymph^{-1}$ min^{-1}, and *S* is the average applied rate of relative light change per second, calculated from Eq. (1). Both relationships can be applied to the natural light environment, but it will be important to carefully define the boundaries of each light phase (i.e., daytime, light decrease, and nighttime phases) before meaningful comparisons between dates can be made.

Vertical movements between the substrate surfaces were also correlated with the rate of light decrease (Chapt. II). There was a delay between the beginning of the light decrease and the moment when nymphs began to leave the tile undersides. A baseline relationship between the length of the delay and rate of light change was defined as:

$$\ln[\text{delay period}] = -3.51 - 1.15 \times \ln|S| \qquad (R^2 = .71;\ p < 0.004;\ n = 9) \qquad (4)$$

where *delay period* is the length of time in minutes between the beginning of the light decrease phase and the moment when nymphs began to leave the tile undersides, and *S* is the average applied rate of relative light change per second, calculated from Eq. (1).

Because the rate of light change during natural twilight is not constant, a direct comparison of the timing of the change in locomotor activity and the rate of light decrease was not possible. However, because the expression of the behavior depends on an accumulation of light stimulus over time as indicated by the shape of the strength-duration curve, in which longer latent periods correspond to smaller rates of change and shorter latent periods correspond to larger rates of change (defined by Eq. 2 and illustrated in Chapt. II, Fig. 5), then it is possible to compare the amount of accumulated light stimulus at the onset of heightened locomotor activity under both constant and variable light stimuli.

The accumulated light stimulus was calculated as:

$$S_{LP} = \sum_{j=1}^{LP} S_j \qquad (5)$$

where S_{LP} = the accumulated light stimulus between the beginning of the light decrease and the onset of nocturnal locomotor activity per second, j = time-interval in minutes, LP = the length of the latent period in minutes, and S_j = the light stimulus at time interval j, measured as the rate of light decrease per second during time interval j , from Eq. (1). A similar calculation was made to estimate the accumulated stimulus between the beginning of the light decrease and the moment when nymphs began to leave the tile undersides, by substituting the length of the delay period for LP, and summing the light stimuli over the delay period.

Results

Estimates of S_{LP}, the accumulated light stimulus prior to the onset of heightened nocturnal locomotor activity, were not significantly different ($\chi^2 = 10.9$, $p > \chi^2 = 0.01$, 3 df, Wilcoxon/Kruskal-Wallis test for different n sizes) between the STAR model tests and the natural-light experiments during August and October (Table I), suggesting that the timing of nocturnal activity may be a function of the amount of light stimulus accumulated during twilight. A significantly larger amount of stimulus was accumulated in February before nymphs initiated higher evening locomotor activity (Table I), suggesting that greater light stimuli are required to elicit a locomotor activity response in nymphs during the winter than during summer or fall.

Comparison of the magnitude of the activity change between the artificial light cycles and natural light was not as straightforward as for the timing of the nocturnal change in locomotor activity. This is because the magnitude of the change in locomotor activity is a function of both rate of light decrease and the amount of time over which the light decrease takes place (Chapt. II). The baseline curve (see Eq. 3 and Chapt. II, Fig. 8) was developed for absolute changes in light over a factor of 10^4, a subset of the actual range in natural light intensity (a range of about 10^{12}, RCA 1968).

For this initial comparison, the magnitude of the change in locomotor activity was estimated for the data of Aug. 4, 1988 (Fig. 1). The daytime level of locomotor activity was determined from a half-hour period before the rapid light changes (5:30 - 6:00 PM) and the nighttime value was determined from a half-hour period after the period of rapid changes (8:00 - 8:30 PM, Fig. 1). A change in activity of 10.1 mm was estimated between the daytime and nighttime values. This is considerably larger than that predicted by the STAR baseline relationship, where the maximum change in activity for large rates of light decrease, those greater than the maximum measured locally in natural twilight, was about

7.5 mm (Chapt. II, Fig. 8), indicating that locomotor activity responses were greater during the natural light changes than in the artificial light changes.

The delay between the beginning of light decrease and the moment when nymphs began to leave the tile undersides was also correlated with rate of light change during tests of the STAR model (Eq. 4, Chapt. II Fig. 12), a demonstration that this phototactic response also requires the buildup of light stimulus prior to the expression of the behavior. The amount of light stimulus accumulated before nymphs left the tile undersides was compared between tests of the STAR model and natural-light experiments in August and October. Experiments in February were not included because very few nymphs left the tile undersides making the estimation of the timing difficult (Grace 1990). There were no significant differences between tests of the STAR model and the natural light experiments ($\chi^2 = 3.8$, $p > \chi^2 = 0.2$, 2 df, Wilcoxon/Kruskal-Wallis test for different n sizes), suggesting that nymphs left the tile undersides after accumulating similar amounts of light stimulus during the light decrease phase in natural and artificial twilight.

TABLE I. Cumulative stimulus[a] for three seasons in natural light and tests of the STAR model in artificial light. Means with different letters were significantly different by the Tukey-Kramer (HSD) multiple comparisons test ($\alpha = 0.05$).

Test Period	Locomotor Activity Response (S_{lp} x 10^3 s^{-1})		Leaving the Tile Undersides (S_{lp} x 10^3 s^{-1})		n[b]
August	-42.0 ± 1.8	a	-60.2 ± 6.9	a	12
October	-34.3 ± 4.0	a	-56.5 ± 8.0	a	11
February	-63.4 ± 7.7	b	N/A		9
STAR model[c]	-36.0 ± 9.5	a	-35.9 ± 5.8	a	4

[a]See Eq. 1 for the method by which light stimulus (rate of light decrease, S_{lp}) was accumulated.
[b]Daily values are only reported for tiles that contained at least three nymphs (see Grace 1990).
[c]STAR model experiments used here are those labeled as "BRITE-ambient/PM" in Chapt. II.

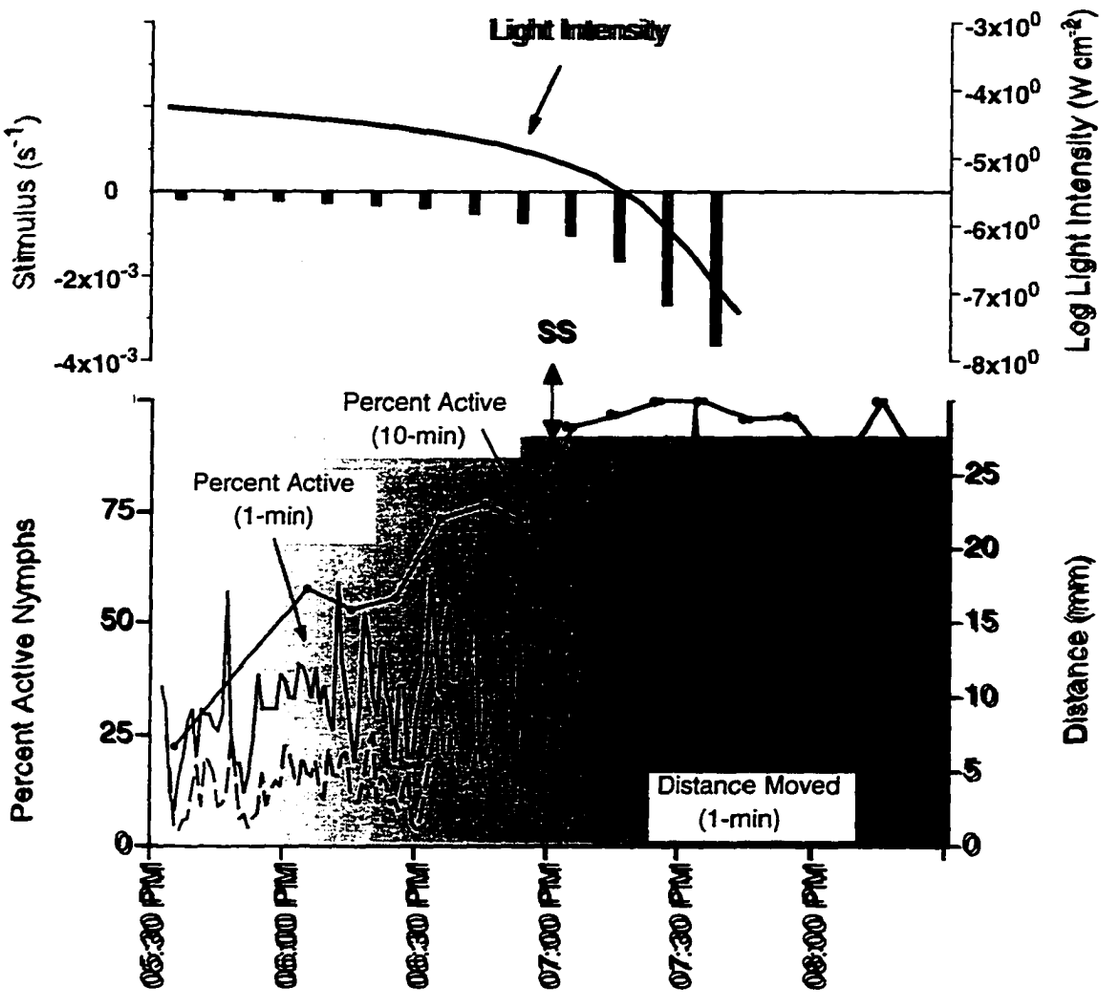

Figure 1. Comparison of measurements of locomotor activity as distance moved over 1-min intervals (- - -), percent of nymphs visible underneath the tiles that moved during 1-min intervals (__) and 10-min intervals (•). Data were originally analyzed at 10-min intervals for another purpose, then re-analyzed at 1-min intervals for comparison with tests of the STAR model. Correlation between distance moved and percent active nymphs during the 1-min intervals was highly significant ($R2 = .73$, $p < 0.0001$, $n = 179$). Data were collected on August 4, 1988.

Discussion

A goal of this research is to apply the STAR model to the natural situation. *Stenonema modestum* nymphs appear to initiate nocturnal increases in locomotor activity after the accumulation of a particular amount of light stimulus, an environmental variable that is easily measured in the laboratory and in the field. There appear to be seasonal differences in the amount of light stimulus required to elicit a locomotor response, a result that is consistent with an earlier study (Grace 1990, Fig. A.1 in the Appendix).

The relationship between the change in locomotor activity in response to light decrease and rate of light decrease is not simply a summation of light stimulus over time. During tests of the STAR model, increases in locomotor activity were shown to be proportional to the rate at which the light changed (Chapt. II), rather than solely the result of accumulated stimulus. If accumulated stimulus regulated the magnitude of the activity change, then the expected locomotor activity changes during the tests of the STAR model would have been equal, regardless of the rate of light change, because the light decrease during the model tests always occurred over the same range of light intensity, and thus, the accumulated light stimulus from the beginning to the end of the light decrease periods was always the same.

The much larger change in activity in natural light than that predicted by the baseline relationship suggests that there are other influences on nocturnal locomotor activity that are not present when light decrease occurs at a constant rate. Recent tests with *Daphnia* suggest that phototactic responses are enhanced when relative changes in light increase over time such as occurs during natural twilight (Ringelberg, pers. comm.). A similar response in *Stenonema* could explain the larger change in locomotor activity during natural twilight than during the tests of the STAR model.

The baseline relationship between rate of light change and the magnitude of the stimulus-activity response was developed for absolute changes in light intensity over a factor of 10^4. In natural light the range is much larger, up to a factor of 10^{12} between day and night, and this difference may explain the disagreement between the predicted and the observed stimulus-based changes in locomotor activity. Most of the entire range of light intensity is detectable by various aquatic species, such as the calanoid copepod, *Acartia tonsa*, that responds by positive phototaxis to illuminations as low as 5.6×10^{-12} W cm^{-2} (Stearns and Forward 1984, value reported as 2.8×10^{11} photons m^{-2} s^{-1}, conversion from Wetzel, 1983), and the phantom midge, *Chaoborus americanus*, for which a lower intensity threshold for action spectra was recorded at 1×10^{-9} W cm^{-2} (Bradshaw 1974). It is not known whether or not visual acuity in low light increases sensitivity to light stimulus beyond the period of most rapid light changes, and thus, a larger accumulation of stimulus during the twilight period.

Comparisons of the timing of *S. modestum* nymphs leaving the tile undersides in the laboratory stream with the timing of nymphs arriving on the uppersides of tiles placed in the Oyster River indicated that the timing of these activities are not different in the laboratory and in the field (Grace 1990), and lends support that the assumptions of the STAR model will be applicable to the natural situation. Attempts to correlate the stream drift with rate of light change (Haney *et al.* 1983, Baldwin 1993), have demonstrated a relationship between the timing of the onset of evening drift and light intensity at the time when the rate of light change surpassed the Ringelberg threshold value ($S = -1.7 \times 10^{-3}$ s^{-1}) for the onset of phototactic swimming in *Daphnia*. Because drift is such an important component of many stream ecosystems and these studies support the hypothesis that the initiation of drift is regulated by light stimulus, it would be useful to test the STAR model in the laboratory and in the natural environment with other mayfly genera, such as *Baëtis* or *Leptophlebia*, that drift regularly. As methods improve for observing stream invertebrates in natural streams, such as videotaping under infrared illumination (Grace 1990), using a

fiber optic scope (Wilzbach 1990), or other methods not yet in current use, the mechanisms by which organisms initiate diel behavior changes in response to light and how local conditions alter the light response can be better resolved.

GENERAL DISCUSSION

Relative change in light intensity during twilight is a reliable proximate cue that plays an important role in the diel locomotor activity and vertical location on the substrate in nymphs of the mayfly, *Stenonema modestum*. The light response can be modified in the presence of predators such as fish and stoneflies, so providing plasticity in timing the activity change when a mayfly is faced with immediate risks. Considering the day to day variability in predators and predation risk faced by mayflies, a strictly fixed light response would have little adaptive value.

Adaptive Considerations of a Stimulus-Activity Response

Timing of the Activity Change

Although the adaptiveness of such a light-response mechanism is apparent, it is not readily apparent that the actual outcome is adaptive, because timing of both the nocturnal increases in locomotor activity and moving to a presumably riskier location on the exposed surfaces of the substrate occur well before darkness. If the presence of visually foraging day-active predators was the only proximate cue driving the system, the nocturnal activity change in the prey should take place when illumination is lowest, rather than during the period of rapid changes in light, as observed. I suggested in Chapter Three that the activity of stonefly predators directly influenced the timing of nocturnal locomotor activity in *Stenonema*, indicating that non-visual predators may also be important in the stream environment. Other studies have demonstrated that actively-foraging benthic-feeding fish modify the locomotor activity and amount of time nymphs of various species of mayflies spend on the top of substrates (Kohler and McPeek 1989, Culp et al. 1991), indicating that these non-visual predators also influence the activity of their mayfly prey. Initiation of

nocturnal activity prior to darkness supports the hypothesis that other factors in addition to visual-foraging day-active predators influence the timing of diel activity changes.

Another factor affecting the early movement to the upper rock surfaces may be the diel abundance of food resources available to grazers such as mayfly nymphs (Sladecek and Sladeckova 1964, McIntyre and Phinney 1965). To maximize efficient location and use of unevenly distributed food resources, foragers should be most active at the time when food is most abundant (Pyke 1979, McNamara 1982, Zimmerman 1982). Invertebrate grazers in temperate and northern streams are often limited by food quality, quantity, and seasonal constraints on metabolism and food abundance (Warren *et al.* 1964, Richardson and Tartar 1976, Mayer *et al.* 1987, Webb and Merritt 1987, Rader and Ward 1990, Peterson *et al.* 1993, Palmer 1995). Food resources are often patchy spatially and grazers are capable of depleting algal patches over periods of days (Colletti *et al.* 1987) to weeks (Lamberti and Resh 1983, Hart *et al.* 1991, Scrimgeour *et al.*, 1991). Mayflies cope with patchiness by colonizing areas where food is abundant (Clifford *et al.* 1992, Hinterleitner-Anderson *et al.* 1992), actively searching for suitable patches (Wiley and Kohler 1984, Kohler 1984), and drifting downstream when food becomes scarce (Hildebrand 1974, Kohler 1985, Clifford *et al.* 1992). It may thus be adaptive to be the first to arrive on the stone tops, to assess the patch quality and either take advantage of the daily growth of algae or quickly move on to better patches.

Another possibility is that the timing of nocturnal locomotor activity and movements to the substrate uppersides takes advantage of diel changes in the effectiveness of predators. Fish that locate their prey visually can more quickly locate and attack prey at illumination levels above 10 lux than in darkness (reviewed by O'Brien 1987). Fish and invertebrates that are not exclusively nocturnal feeders may nevertheless be constrained to forage in darkness by their primary predators that hunt by sight. Examples include some dace that naturally forage in darkness but are much better able to detect prey at twilight light

intensities (Beers and Culp 1989), and *Dinocras* stoneflies that starve rather than expose themselves under lighted conditions (Sjöström 1980).

Even visual foragers are not prevented from feeding in the darkness (Allan 1980), but because of the dynamics of visual adaptation, the twilight period may be a time of especially low visual acuity. The dynamic range of a photoreceptor covers about 2 or 3 log units of light intensity (Laughlin 1981), necessitating a period of adaptation when the predominant light conditions shift to a higher or lower range (Menzi 1987). Sensitivity to light and the process of adaptation are under some circadian control (some examples: ants, Menzi 1987; beetles, Jahn and Wulff 1943; butterflies, Swihart 1963; horseshoe crabs, Barlow 1983; and moths, Edwards 1962, Nilsson *et al.* 1991). Light adaptation requires physiological changes in the photoreceptor, such as migration of pigments and other photomechanical changes in the photoreceptor cells within the eye (for insects reviewed by Järvilehto 1985). The time course of full adaptation to a new illumination level varies according to species, cell types and stimulus conditions (reviewed by Järvilehto 1985). Complete dark adaptation has been shown to take up to 60 minutes in nauplii of the shrimp, *Eualus gaimardii,* (Nordtug and Krekling 1989), and 2 hr. in *Camponotus* ants (Menzi 1987). Recorded circadian movements of the cones in the retina of the Midas cichlid (*Cichlasoma citrinellum*), a teleost fish, demonstrated that cones begin to elongate after light reduction at dusk and begin to contract shortly before dawn. Therefore, the period when the photoreceptors are undergoing a shift in photosensitivity coincides with the period of most rapid light changes at twilight.

Temporal partitioning of prey resources between competing species is not uncommon (Johnson 1981, 1982, Allan 1983, Angradi *et al.* 1991). It is possible that predation may be somewhat lessened within the twilight period of rapid light changes when the efficiency of visual foragers begins to fall off but the non-visual foragers have yet to reach their maximum activity. The lower 10-lux illumination limit for the highest efficiency of visual predators occurs at sunset (10 lux $\cong 4.2 \times 10^{-6}$ W cm^{-2}, conversion from

Westlake, 1965; illumination measured at the Oyster River during the summer of 1988). The period between sunset when the visual foragers become less efficient, and darkness when the non-visual predators become most active, is therefore not only the time when food resources are most abundant, but perhaps is also a time of maximum safety for mayfly prey.

Magnitude and Time-Course of the Initial Peak of Nocturnal Locomotor Activity

The adaptiveness of using light stimulus as a regulator of the magnitude and time-course of the initial peak in nocturnal locomotor activity is also not readily apparent. Seasonal changes in the strength and duration of twilight correspond with seasonal changes in food availability and temperature. Condensed, stronger twilight periods precede longer nights and lengthy, weaker twilight periods precede shorter nights. The positive relationship between light stimulus and the magnitude of the increase in nocturnal locomotor activity (Chapter Two, Fig. 8) indicates that higher nocturnal activity would occur following shorter twilights, such as in late fall, winter, and early spring. The negative relationship between the duration of heightened nocturnal locomotor activity and light stimulus (Chapter Two) indicates that bursts of activity during these same seasons would also then be short-lived. Conversely, the magnitude of the activity change during late spring, summer, and early fall would be relatively lower but the heightened activity would persist longer into the night. There is some evidence that these scenarios do occur for locomotor activity (Grace 1990) and drift (Chaston 1968, Koetsier and Bryan 1992), although data are not always consistent (Elliott 1968). In summer, a long-lasting period of heightened locomotor activity may be necessary for individuals to take advantage of the entire night for feeding. As food availability declines in the fall and winter, food intake may meet only the requirements for maintenance and a lowered metabolism, so a quick burst of activity may be all the energy that is possible or necessary to allocate to foraging.

Interactions between light stimulus, temperature, and food availability have not been explored, but most certainly are important components in regulating the diel activity of mayflies.

Physiological Considerations of a Stimulus-Activity Response

The mechanism by which mayflies monitor the light environment over long periods of time prior to responding to light changes has not been established. Pathways of communication between photoreceptors and the circadian oscillators are subjects of recent research. The TIM-PER complex controlling the circadian cycle of locomotor activity in the fruit fly, *Drosophila melanogaster*, is one example (Lee et al. 1996, Myers et al. 1996, discussed in Chapter Two). Hormonal substances also play an important role as neurotransmitters, neurohormones and neuromodulators of various activities in insects (reviewed by Pener 1985). For example, in the leech, *Hirudo medicinalis*, serotonin decreases the latency period before the onset of swimming towards a vibrating point, and also increases the biting frequency and food ingestion rate (Lent and Dickinson 1984). Octopamine increases the excitability of the cockroach, *Periplaneta americana*, and as one consequence, lowers the threshold for the initiation of flight in response to wind-stimuli (Weisel-Eichler and Libersat 1996). Increased levels of octopamine in the haemolymph have been measured during flight of locusts (Goosey and Candy 1980), intensifying the activity of the leg muscles (reviewed by Orchard 1982). Serotonin and octopamine both modulate the response of motion-sensitive neurons in the visual system of the honey bee, *Apis mellifera,* (Kloppenburg and Erber, 1995).

Only recently, have cyclical fluctuations in the concentration of hormones been reported, indicating some hormonal function in regulating circadian changes in behavior. Dopamine content in the retinas of fish and frogs shows circadian rhythmicity (Pierce and Besharse 1985, Koblinger et al. 1990, McCormack and Burnside 1992). Octopamine has been identified as a circadian neurotransmitter in the photoreceptor organs of the horseshoe

crab, *Limulus polyphemus* (Renninger and Farrell 1996). Octopamine increases the response to light, thus improving vision in the dark (Barlow 1983). The enhanced response to light is slow to develop and slow to disappear after removal of the hormone (Renninger and Farrell 1996). A similar slow time-course for the stimulus-based locomotor activity response in *Stenonema* (i.e., lengthy latent periods prior to the response and heightened locomotor activity for several hours following the stimulus), may indicate that the response has some hormonal basis.

For activity to be controlled by cyclical levels of hormones there must be a circadian clock. Tests of the STAR model during the early evening did not strongly support the importance of an endogenous rhythm on the locomotor activity response during the time periods tested (Chapter Two). A rigorous test of the stimulus-based locomotor activity response in *Stenonema* was carried out over a 24-hr period. Light was decreased and increased continuously at a constant rate of change with no intervening adaptation periods (Fig. C.1, Appendix C). The change in locomotor activity in response to light decrease gradually became larger from 10:00 AM until about 10:00 PM, after which the response began to lessen, indications of the influence of an endogenous rhythm on the 24-hr pattern of locomotor activity (Fig. C.1, Appendix C). The shape of the curve (Fig. C.1, Appendix C) suggests that nymph locomotor activity is enhanced during the nighttime, an indication that the endogenous component must be considered when interpreting diel locomotor behaviors.

SUMMARY AND CONCLUSIONS

Relative change in light plays a dual role as a timer for regulating locomotor activity and vertical movements on the substrate, as well as controlling the overall level of locomotor activity in nymphs of the mayfly, *Stenonema modestum*. Cyclic behavior is an important strategy that prepares the organism for regularly changing conditions (Rapp 1987), such as the time of day when predator activity or food abundance is greatest. Large relative changes in light reliably signal the approach of evening darkness and morning light, as the largest changes take place post-sunset and pre-sunrise (Ringelberg *et al.* 1991a). Light cues and local conditions, such as predator-prey assemblages and predator density, are therefore important in structuring interactions between predators and prey in stream ecosystems.

Through experimental tests of the STAR model, significant correlations were found between relative light change and the timing and amount of heightened nocturnal locomotor activity, and the timing when nymphs began to leave the undersides of the substrate. The difference between daytime and nighttime locomotor activity and the extent of the initial peak of nocturnal activity were also functions of relative light change. Shortened periods of light decrease resulted in smaller changes in locomotor activity, indicating the importance of both stimulus strength (i.e., the rate of light change) and duration of the light changes in regulating locomotor activity. Adaptation at a reduced light intensity advanced the timing of diel locomotor activity changes, but did not alter the timing of vertical movements between surfaces. The presence of predators (fish odor and stoneflies), also modified *Stenonema* locomotor activity. Mayflies were most active during the daytime in the presence of fish odors and least active in the presence of stoneflies. Nighttime activity was lower in the absence of predators and in presence of mixed predators than in the presence of either predator alone. Stoneflies began their activity later in the presence of fish odors, an

interaction between fish and stonefly nymphs that influenced the timing of the mayfly activity. The endogenous clock had no measurable influence on the timing or amount of nocturnal locomotor activity. However, time of day influenced the timing and numbers of nymphs that left the tile undersides, suggesting that position on the substrate may be influenced by an endogenous rhythm.

To allow for its application in the natural environment, the STAR model must now be expanded to include the changing rates of light decrease during natural twilight. The STAR model may provide a mechanistic model of the behavior leading up to the entry of mayflies into the drift. As is the case with locomotor activity and vertical movements on the substrate, the timing of drift must be in phase with local conditions, such as the time of sunset, to ensure that nymphs restrict their time in the water column to safe periods, such as those when predators are less active (Allan *et al.* 1986, Glozier and Culp 1989). A stimulus-based drift response would therefore be a useful and appropriate mechanism of control of the timing and likelihood that individuals will enter the drift under the varying conditions in stream environments.

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APPENDIX A

Lists of Experiments Used to Test the STAR Model Predictions

TABLE A.1. List of experiments used to test the predictions of the STAR model: development of the baseline model and treatment[a] effects.

Target rate of light change ($sec^{-1} \times 10^3$)	Minutes Required for DECRS phase	Time of Day	Date (m-d-yr)	Collection Site[b]
Baseline model				
10.0	15	AM	07-04-95	Oy
7.3	20	AM	08-12-96	Be
4.8	30	AM	07-09-95	Oy
3.6	40	AM	07-12-95	Oy
2.9	50	AM	07-13-95	Oy
2.7	55	AM	08-03-95	Oy
2.5	58	AM	07-11-95	Oy
2.3	63	AM	07-07-95	Oy
2.1	70	AM	07-25-95	Oy
1.9	76	AM	07-16-95	Oy
1.7	84	AM	07-24-95	Oy
1.4	100	AM	07-17-95	Oy
1.2	120	AM	08-16-95	Oy
1.0	150	AM	07-22-95	Oy
0.080	167	AM	08-15-96	Be
0.073	200	AM	08-04-95	Oy
Treatments				
Bright-adaptation/PM				
3.6	40	PM	07-12-95	Oy
2.5	58	PM	07-16-95	Oy
1.7	84	PM	07-03-95	Oy
1.4	100	PM	08-10-95	Oy
Reduced-light adaptation AM/PM				
3.6	20	AM	08-07-95	Oy
		PM	08-07-95	Oy
2.5	29	AM	07-26-95	Oy
		PM	07-31-95	Oy
1.7	42	AM	08-02-95	Oy
		PM	07-24-95	Oy
1.4	50	AM	08-11-95	Oy
		PM	08-01-95	Oy

[a] Treatments were time of day (AM and PM) and level of adaptation light intensity (bright and reduced).
[b] Oy=Oyster River, Be=Bellamy River

TABLE A.2. List of experiments used to test the predictions of the STAR model, con't: Tests of abbreviated and discontinuous periods (steps) of light decrease.

Target rate of light change ($sec^{-1} \times 10^3$)	Minutes Required for each step of the DECRS phase	Number of Steps Needed to Complete the Light Reduction	Time of Day	Date (m-d-yr)	Collection Site
3.6	40	1	PM	07-12-95	Oy
	20	2	PM	08-14-96	Oy
	10	4	PM	08-15-96	Be
2.5	58	1	PM	07-16-95	Oy
	28	2	PM	08-12-96	Be
	14	4	PM	08-13-96	Be
1.7	84	1	PM	07-03-95	Oy
	41	2	PM	08-17-96	Be
	20	4	PM	08-16-96	Be

TABLE A.3. Experiments used to test for predator-induced changes in *Stenonema's* response to relative light change. The target rate of light change was± 2.5 x 10^{-3} s^{-1} and light decreases began at 1800 EST.

Treatment	Date	Collection Site[a]
None (N)	07-23-96	Be
	07-25-96	Be
	07-26-96	Oy
Fish water (F)	08-06-96	Be
	08-08-96	Be
	07-16-95	Oy
Stonefly (S)	07-31-96	Be
	08-01-96	Be
	08-02-96	Be
Fish water + Stonefly (FS)	08-03-96	Oy
	08-07-96	Oy
	08-09-96	Oy

[a]Oy=Oyster River, Be=Bellamy River

APPENDIX B

NIH-Image Macro Code for MacIntosh Computers Used in Tracking Movements of Individual Mayflies

```
(© 1995 annette schloss; runs with NIH-Image ≥ v1.57)

{
A set of macros to locate mayflies in a stack of images, to measure mayflies,
and to output the results to a file for later use:
The program will mark up your images, so don't save them, or make a copy.

TRACK MAYFLIES:
  Lets you follow one mayfly. You select the # to track and then follow each individual.
Always (if possible) follow a mayfly through all slices. Results are saved in the results
window until the final mayfly is finished, then the results get saved to a file. You can quit the macro
without finishing:
 counter is only reset if you re-start with mayfly # 1, otherwise the counter increments.
 You tell the macro which mayfly to start with.
 the macro marks the mayfly with it's # as it is tracked.
 When a mayfly leaves the rock, hold down the <shift> key and click the mouse, and
        that mayfly will be counted as missing.
You can toggle between a slice and the slice before it by holding down the
        'option' key whilst macro is running.
If some mayflies are never present in the stack, use 'Mayflies Out Of Here' to fill in the output data file
with missing values (the post-processing routines expect complete data for all mayflies).

IMPORTANT: use 'Get-Corners' to get the edges of the tiles, so that the program will
 correctly calculate the distance when mayflies move off/onto the tiles!
 Code is setup to measure 1 or 2 tiles, if there are more, modify as necessary.
IMPORTANT: 'TrackMayflies' adds extra rows to the bottom of the results, which
  contain the top, bottom, left and right boundaries (in pixels) of the tiles.

IF YOU GET BEEPS when clicking the mouse, then you have reached max measurements; reset to a
larger value in the options dialogue box in analyze menu

 OUTPUT TO FILE:  minsize,maxsize,nslices
                        slice#,nmayflies,x1,y1,x2,y2....xn,yn      one line per slice
}

{INITIALIZATION OF GLOBAL VARIABLES}
var {Global variable, initially zero}
 RoiLeft,RoiTop,RoiRight,RoiBottom,xloc,yloc,rC:integer;
 x1,x2,y1,y2,top,left,width,height,dist,minarea,maxarea,myf,myfhalf,myfq1,myfq3:integer;
 myfile:string;
 ulx1,llx1,urx1,lrx1,ulx2,llx2,urx2,lrx2:integer;
 uly1,lly1,ury1,lry1,uly2,lly2,ury2,lry2,ntiles, kkk:integer;
```

```
up1,lo1,rt1,lf1,up2,lo2,rt2,lf2,value1,value2,value3:integer;
 up3,lo3,rt3,lf3,up4,lo4,rt4,lf4:integer;
 upa,loa,rta,lfa:integer;
{PROCEDURES (called by macros below)
 Note. procedures marked with 'nih' were not written by A. Schloss and are available on the NIH web site}

procedure CheckForStack;                                                    {nih}
begin
 if nPics=0 then begin
  PutMessage('This macro requires a stack.'); exit; end;
 if nSlices=0 then begin
  PutMessage('This window is not a stack.'); exit end;
end;


procedure GetDist;
var
  minx,miny,up,lo,rt,lf:integer
begin
   rC:=rCount-1;
   if myf <= myfhalf then begin
     up:=up1; lo:=lo1; rt:=rt1; lf:=lf1;
  end
  else begin
    up:=up2; lo:=lo2; rt:=rt2; lf:=lf2; end;

  {No mayfly at all}
 if ((rX[rC] = 0) and (rY[rC] = 0) and (rX[rCount] = 0) and (rY[rCount] = 0)) then
   rUser1[rCount]:=0;

 {Mayflies in from the edge}
 if ((rX[rC] = 0) and (rY[rC] = 0) and (rX[rCount] <> 0) and (rY[rCount] <> 0)) then begin
 if abs(rX[rCount] - rt) <= abs(rX[rCount]-lf) then minx:= abs(rX[rCount] - rt) else
     minx:= abs(rX[rCount]-lf);
  if abs(rY[rCount] - up) <= abs(rY[rCount]-lo) then miny:= abs(rY[rCount] - up) else
     miny:= abs(rY[rCount]-lo);
  if minx < miny then rUser1[rCount]:=minx else rUser1[rCount]:=miny;
 end;

 {Mayflies go off the edge}
 if ((rX[rC] <> 0) and (rY[rC] <> 0) and (rX[rCount] = 0) and (rY[rCount] = 0)) then begin
 if abs(rX[rC] - rt) <= abs(rX[rC]-lf) then minx:= abs(rX[rC] - rt) else
     minx:= abs(rX[rC]-lf);
  if abs(rY[rC] - up) <= abs(rY[rC]-lo) then miny:= abs(rY[rC] - up) else
     miny:= abs(rY[rC]-lo);
  if minx < miny then rUser1[rCount]:=minx else rUser1[rCount]:=miny;
end;

 {Mayflies not on the edge}
 if ((rX[rC] <> 0) and (rY[rC] <> 0) and (rX[rCount] <> 0) and (rY[rCount] <> 0))then
   rUser1[rCount]:=sqrt(sqr(rX[rCount]-rX[rC])+sqr(rY[rCount]-rY[rC]));
 end;
end;
```

```
procedure GetLoc;
begin
  xloc:=0;  yloc:=0;
repeat
  SetCursor('cross');  GetMouse(xloc,yloc);
  MakeOvalRoi(xloc,yloc,5,5);
  Drawboundary;
  Undo;
   while KeyDown('option') do begin
       if i >1 then begin
         Selectslice(i-1);  wait(0.5);
         Selectslice(i);    wait(0.5);
       end;
   end;
  until button;
 MakeOvalRoi(xloc,yloc,5,5); Drawboundary;  Measure;
 rX[k+1]:=xloc;  rY[k+1]:=yloc;
 KillRoi;
end;


procedure Getxy;
begin
  xloc:=0;  yloc:=0;
repeat
  SetCursor('cross');  GetMouse(xloc,yloc);
  until button;
  Measure;  MakeOvalRoi(xloc,yloc,5,5); Drawboundary;
  rX[kkk]:=xloc;  rY[kkk]:=yloc; Updateresults;
 KillRoi;
end;


procedure GetCorners;
var
 nc:integer;
begin
 Resetcounter;
 PutMessage('Enter the ul, ur, ll, lr corners of each tile[start with left tile]');
 ntiles:=GetNumber('Enter the number of tiles to measure corners:',2);
 if ntiles > 2 then begin
   PutMessage('Too many tiles, need to rewrite macro!',ntiles);  exit;  end;

 nc:=ntiles * 4;     {number of corners to get,  NOTE  kkk var is used by Getxy}
    kkk:=1; Getxy;  wait(0.15);  ulx1:=xloc; uly1:=yloc;
     kkk:=2; Getxy;  wait(0.15); urx1:=xloc; ury1:=yloc;
     kkk:=3; Getxy;   wait(0.15);  llx1:=xloc; lly1:=yloc;
    kkk:=4; Getxy;   wait(0.15);   lrx1:=xloc; lry1:=yloc;
  if ((ulx1 >= urx1) or (llx1 >= lrx1) or (uly1 >=lly1) or (ury1 >= lry1)) then begin
      Putmessage('Bad corners 1, try again'); exit; end;
    up1:=trunc((rY[1] + rY[2])/2);  lo1:=trunc((rY[3]+rY[4])/2);
    rt1:=round((rX[2]+rX[4])/2); lf1:=round((rX[1]+rX[3])/2);
 if ntiles > 1 then begin
   kkk:=5;    Getxy;   wait(0.15);   ulx2:=xloc; uly2:=yloc;
```

```
    kkk:=6;    Getxy;  wait(0.15);   urx2:=xloc; ury2:=yloc;
    kkk:=7;    Getxy;  wait(0.15);   llx2:=xloc; lly2:=yloc;
    kkk:=8;    Getxy;  wait(0.15);   lrx2:=xloc; lry2:=yloc;
      if ((ulx2 >= urx2) or (llx2 >= lrx2) or (uly2 >=lly2) or (ury2 >= lry2)) then begin
        Putmessage('Bad corners 2, try again'); exit; end;
    up2:=trunc((rY[5] + rY[6])/2);  lo2:=trunc((rY[7]+rY[8])/2);
    rt2:=round((rX[6]+rX[8])/2); lf2:=round((rX[5]+rX[7])/2);
  end;  {if ntiles > 1}
end;
{ MACROS}

macro 'Select Slice... [S]';
var
  n:integer;
begin
 CheckForStack; n:=GetNumber('Slice Number:',trunc(nSlices/2));  SelectSlice(n)
end;


macro 'GetLength ...[L]';
var
x1,x2,y1,y2,top,left,width,height:integer;
 xcenter,ycenter,radius,w,h:integer;
begin
 GetLine(x1,y1,x2,y2,width);
 if x1<=0 then begin
   PutMessage('Make a line selection of average-sized mayfly.');
   exit;
 end;
  xcenter:=x1+(x2-x1)/2;  ycenter:=y1+(y2-y1)/2;
  radius:=sqrt(sqr(x2-x1)+sqr(y2-y1))/2;
  dist := radius * 2;
 end;


macro 'Track Mayflies ...[K]';
var width,height,MyStackId,istart,isl,nmyflies,myfstart,k,w,h,i,n:integer;
      reswin:string;
begin
 CheckForStack;
 GetPicSize(w,h);
 MyStackId:=PidNumber;
 SetOptions('User2; User1; Headings; X-Y center; Min');  SetPrecision(1,9);
 SetUser1Label('distance'); SetUser2Label('10E4myfXtile');

nmyflies:=GetNumber('Enter the max # of mayflies',12);
if odd(nmyflies) then begin  PutMessage('nmayflies must be even ',nmyflies); exit; end;

 k:=rCount;
PutMessage('Hold "Shift" and click mouse when mayfly has left');
reswin:=concat(WindowTitle,'.trk');
if ((myf < 1) or  (myf > 12)) then myf := 1;
myfstart:=GetNumber('Enter the # of the mayfly to start',myf);
if myfstart > nmyflies then begin
```

```
   PutMessage('start myf less than n-mayflies',myfstart,nmyflies); exit; end;

{GetCorners: assumes same tot # myfs per tile, ie. 12 myfs = 6 on left, 6 on right}
if myfstart = 1 then begin GetCorners;
    Putmessage('top,bot,rt,left',up1:4,lo1:4,lf1:4,rt1:4,up2:4,lo2:4,lf2:4,rt2:4); end;
if myfstart = 1 then ReSetCounter;
myfhalf := nmyflies;   if ntiles > 1 then myfhalf:=nmyflies/2;
for myf:=myfstart to nmyflies do begin
 for i:=1 to nSlices do begin
   istart:=k + 1;
  SelectPic(MyStackId);  SelectSlice(i);   if i=1 then Beep;
  xloc:=0;  yloc:=0;
  repeat
     SetCursor('cross');  GetMouse(xloc,yloc);
        while KeyDown('option') do begin
         if i >1 then begin
           Selectslice(i-1);  wait(0.5); Selectslice(i);  wait(0.5);
         end;
       end;
    until button;
    Measure;  rUser2[rCount]:=10000*myf+i;  KillRoi;  wait(0.15);
 if KeyDown('shift') then begin
   rX[rCount]:=0;   rY[rCount]:=0;
 end
 else begin
  rX[rCount]:=xloc;   rY[rCount]:=yloc;
  rUser1[rCount]:=0;
end;  {else begin}
if i > 1 then GetDist;
 if (rX[rCount] <> 0) and (rY[rCount] <> 0) then begin
     MoveTo(xloc,yloc);  DrawNumber(myf); end;
 end;   {nslices loop}
   k:=rCount;
 end;     {myf loop}

{add tile boundaries to the end of the results}
Measure;
rX[rCount]:=up1; rY[rCount]:=lo1; rUser1[rCount]:=lf1; rUser2[rCount]:=rt1;
Measure;
rX[rCount]:=up2; rY[rCount]:=lo2; rUser1[rCount]:=lf2; rUser2[rCount]:=rt2;
UpdateResults;  SelectPic(MyStackId);
SetExport('Measurements'); SetOption;  Export(reswin);
 myf:=1;
end;


macro '(-' begin end;                                                    {nih}


macro 'Mayflies Out of Here ...';
var width,height,MyStackId,istart,isl,nmyflies,myfstart,myfstop,myfst,myff,k,w,h,i,n:integer;
     reswin:string;
begin
 CheckForStack;
```

```
 GetPicSize(w,h);
 MyStackId:=PidNumber;
 SetOptions('User2; User1; Headings; X-Y center; Min'); SetPrecision(1,9);
 SetUser1Label('distance'); SetUser2Label('10E4myfXtile');

nmyflies:=GetNumber('Enter the max # of mayflies',12);
if odd(nmyflies) then begin PutMessage('nmayflies must be even ',nmyflies); exit; end;

reswin:=concat(WindowTitle,'.trk');
myfstart:=GetNumber('Enter the # of the mayfly to start',myf);
myfst := nmyflies;  if (myf <= 6) then  myfst := myfhalf;
myfstop:=GetNumber('Enter the # of the mayfly to end',myfst);
if myfstop > nmyflies then begin
   PutMessage('stop myf > than n-mayflies',myfstop,nmyflies); exit;  end;

{GetCorners: assumes same tot # myfs per tile, ie. 12 myfs = 6 on left, 6 on right}
if  myfstart = 1 then begin GetCorners;
    Putmessage('top,bot,rt,left',up1:4,lo1:4,lf1:4,rt1:4,up2:4,lo2:4,lf2:4,rt2:4); end;
if  myfstart = 1 then ReSetCounter;
myfhalf:=nmyflies/2;
for myff:=myfstart to myfstop do begin
 for i:=1 to nSlices do begin
   Measure;
   rX[rCount]:=0;  rY[rCount]:=0;  rUser2[rCount]:=10000*myff+i;  rUser1[rCount]:=0;
 end;  {nSlices loop}
end;      {myf loop}
 myf:=myff+1;

if myfstop = nmyflies then begin;
  {add tile boundaries to the end of the results}
   Measure;
   rX[rCount]:=up1;  rY[rCount]:=lo1;  rUser1[rCount]:=lf1;  rUser2[rCount]:=rt1;
   Measure;
   rX[rCount]:=up2;  rY[rCount]:=lo2;  rUser1[rCount]:=lf2;  rUser2[rCount]:=rt2;
   UpdateResults;  SelectPic(MyStackId);
   SetExport('Measurements'); SetOption;  Export(reswin);
 myf:=1;
SelectPic(MyStackId);  SelectSlice(nSlices);
end; {if myff}
end;
```

APPENDIX C

Auxiliary Results

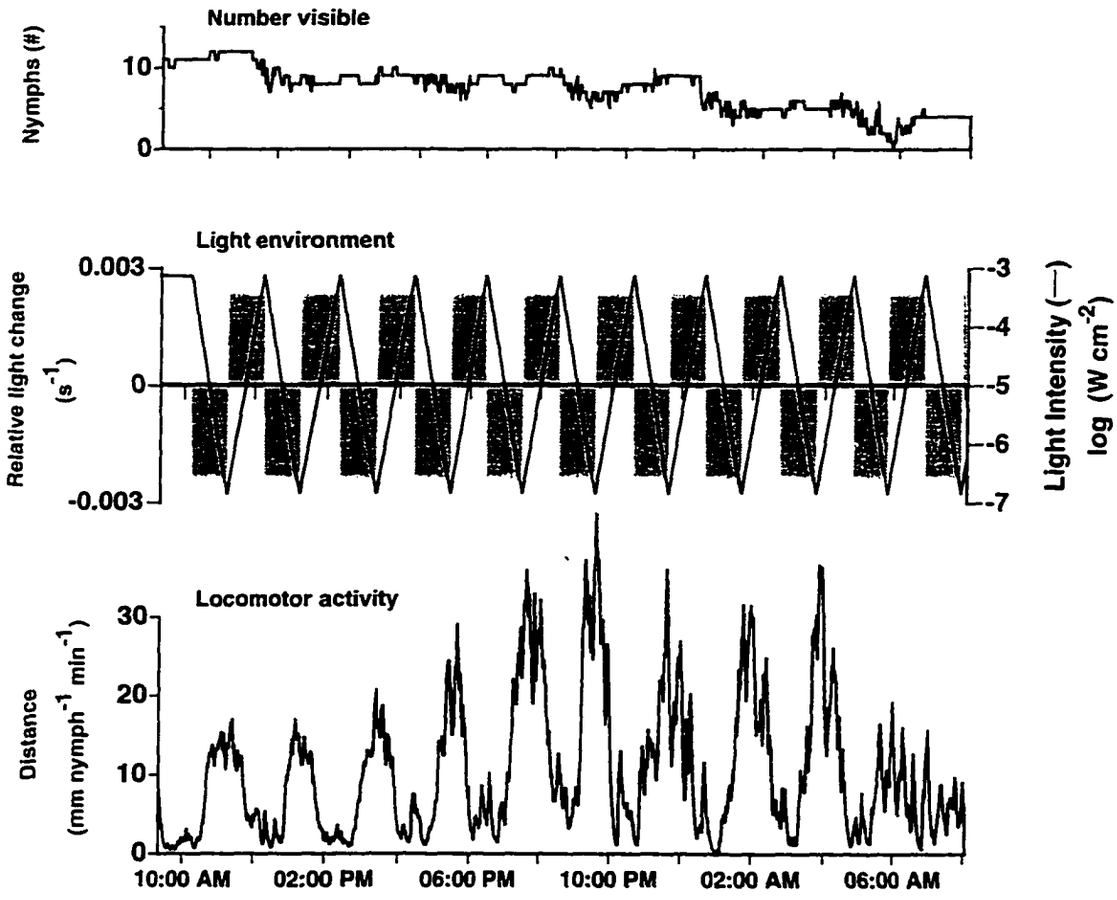

Figure C.1. Time-series of number of nymphs visible and locomotor activity during light decrease and increase cycles continued over 23 hours. The rate of relative light change (S) was ± 2.4 x 10^{-3} s^{-1} during the upward and downward portions of each cycle. Data are from June 17, 1994. (Top) nymphs visible beneath the tile surface. (Center) Light intensity (—) and relative light change (shaded area). (Bottom) Locomotor activity.