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DIURNAL VARIATIONS IN RESPIRATION OF MAYFLY
NYMPHS (EPHEMEROPTERA)

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INTRODUCTION

Numerous behavioral studies, performed in the field and laboratory, have shown that activity (Elliott 1968) and drift rates (Waters 1962, 1965; Holt and Waters 1967; Chaston 1968; Bishop 1969) of many mayfly nymphs exhibit a diurnal rhythmicity with highest rates occurring during the night. However, no attempt has been made to correlate the behavioral cycles of mayflies, observed in their environment, with physiological endogenous rhythms, for example, diel variations in metabolic rates. As Enright (1970) has noted, while there is a tremendous volume of literature describing internal timing mechanisms, too little attention has been given to an evaluation of the ecological significance of endogenous

rhythmicity. Previous investigations of mayfly nymph respiration have been limited to the effects of habitat, oxygen concentration of the water, and substrate on oxygen consumption (Fox, Simmonds, and Washbourn 1935; Fox, Wingfield, and Simmonds 1937; Erikson 1963*a*, 1963*b*, 1964, 1966). This study was initiated to determine (1) whether rates of oxygen consumption varied on a diel basis and corresponded with reported behavioral patterns, and (2) if a daily rhythm of metabolic rate was present, whether it was controlled by exogenous or endogenous factors.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

The experimental animals used in the study were the mayfly nymphs, *Isonychia* sp. (Baetidae) and *Stenonema fuscum* (Heptageniidae). These were chosen primarily on the basis of their

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availability but also because they vary considerably with regard to morphology and behavioral characteristics. *Isonychia* sp. is a streamlined, torpedo-shaped, lotic species which was often observed darting rapidly among rocks and detritus. *Stenonema fuscum*, also a lotic species, is dorso-ventrally flattened and more cryptic in nature and was always found attached to the underside of rocks or in crevices. *Stenonema fuscum* was never observed swimming.

Nymphs of both species, 8–12 mm in length, were collected from Buffalo Creek, a trout stream approximately 5 miles west of Lewisburg, Union County, Pennsylvania. Collections were made from November 10, 1969 to March 5, 1970. Water temperature during the collection period ranged from 0 to 4 C.

The nymphs were held in the laboratory in an artificial stream which simulated field conditions of current temperature and photoperiod. The stream proper consisted of a fiberglass-lined plywood trough approximately 186 cm long, 30 cm wide, and 23 cm deep. Water was cooled and aerated in a reservoir (capacity approximately 200 liters) by a 1/3 hp cooling unit (Frigid Units, Toledo, Ohio) and circulated through the system by a pump (Little Giant, Oklahoma City, Okla. which delivered approximately 46 liters/minute. With the water in the trough 7.6 cm deep, the normal capacity of the stream was 43.4 liters. Water was obtained from Buffalo Creek and was changed about twice a month to prevent accumulation of dissolved organic matter. A glass wool filter box was inserted periodically to remove particulate matter.

Nymphs were kept in nylon screen baskets containing rocks and leaf detritus and were acclimated to $6.5\text{ C} \pm 0.5$ for at least 72 hr before metabolic studies were performed.

OXYGEN CONSUMPTION MEASUREMENTS

Oxygen consumption was measured using a closed-bottle technique with before-and-after Winkler titrations (azide modification [American Public Health Association 1965]). Atlas pint jars served as respiration chambers and were modified by leveling the bottom with epoxy resin and constructing a chamber which enclosed a magnetic stir bar. An artificial substrate of nylon screen and pieces of rubber were then sealed under water and placed on a magnetic stirrer in a low-temperature incubator adjusted to the temperature of the lab stream. Nine to 22 nymphs were placed in the respiration chamber. The stirring ensured thorough and constant mixing, although no effort was made to simulate natural stream currents.

The initial dissolved oxygen concentration of the water was estimated from the mean of duplicate Winkler titrations of water samples from 125-ml glass-stopped bottles that were filled at the same time as the respiration chamber. After 2 hr, water was siphoned from the chamber, and the dissolved oxygen concentration was again estimated from the mean of duplicate determinations. This value was subtracted from the initial determination and the difference was assumed to be the uncorrected oxygen consumption for a 2-hr period. Blank chamber values were periodically determined, and the mean used to correct for "bottle effects." In most cases, nymphs were used only once in metabolic rate experiments; however, severe winter conditions made collecting impossible at times, and a few nymphs were used a second time. Subjecting nymphs to the experimental procedure the second time had no apparent effect on metabolic rates.

PHOTOPERIOD AND LIGHT INTENSITY

The laboratory photoperiod was controlled by a poultry timer and corresponded approximately to local sunrise and sunset, with alternating 10-hr light and 14-hr dark periods. The light intensity at water level was approximately 40 ft-c and was provided by fluorescent lights. A 40-w incandescent bulb with an intensity of approximately 20 ft-c provided light in the incubator for experiments run during photophase. Elliott (1968) reports that nocturnal activity increases at light intensities lower than some value between 0.2 and 5 ft-c. The sides of the respiration chamber were covered with aluminum foil, thereby permitting light to enter only through the top of the jar. Dull red lights were employed in the laboratory while carrying out experiments during scotophase; no light was provided in the incubator during this period. Red light has no apparent effect upon the activity of mayfly nymphs (Elliott

1968). However, it has been reported by Bishop (1969) that intensity rather than wavelength governs the effect upon activity. In the present study, nymphs showing negative phototaxis under normal lights showed none under red.

STATISTICAL METHODS

All data were analyzed at the .01 level of significance, using the standard "Student" *t*-test.

RESULTS AND DISCUSSION

RESPIRATION RATES DURING A NORMAL PHOTOPERIOD

The relationship between respiration rate and time of day for *Isonychia* sp. and *Stenonema fuscum* can be seen in figure 1. The average nighttime respiration rate of 5.2 μ liters O_2 /nymph per hour for *Isonychia* sp. is significantly different from the average daytime rate of 3.6 μ liters O_2 /nymph per hour. The nighttime mean of 3.5 μ liters O_2 /nymph per hour for *S. fuscum* is not signifi-

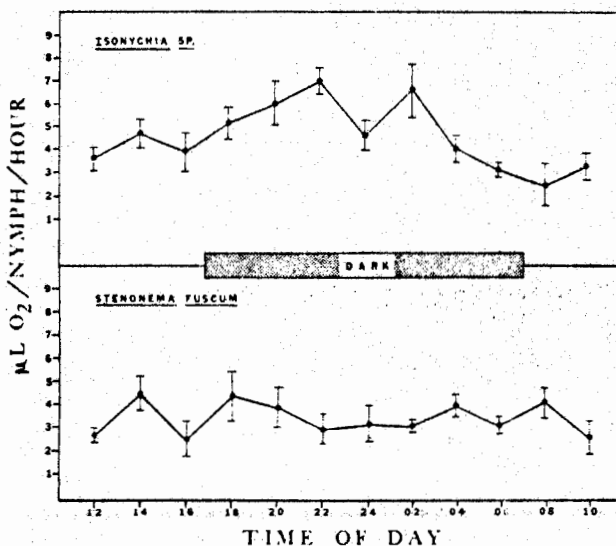


FIG. 1.—Oxygen utilization by *Isonychia* sp. and *Stenonema fuscum* during normal photoperiod. Vertical lines indicate 2 se's of mean. *Isonychia*: mean photophase—3.6 μ liters O_2 /nymph per hour.; mean scotophase—5.4 μ liters O_2 /nymph per hour; mean daily—4.5 μ liters O_2 /nymph per hour. *Stenonema fuscum*: mean photophase—3.3 μ liters O_2 /nymph per hour; mean scotophase—3.5 μ liters O_2 /nymph per hour; mean daily—3.4 μ liters O_2 /nymph per hour.

cantly different from the daytime mean of 3.3 μ liters O_2 /nymph per hour.

Few studies have been made of respiration rates of hemimetabolus aquatic insects, and no investigations over a diel period for any mayfly species have been reported. Fox et al. (1935) have recorded respiration rates of ephemeropterid nymphs from still and running water and reported one lotic form, *Ecdyonurus venosus*, with a mean dry weight of 6 mg, to utilize 1,321 μ liter O_2 /gram per hour at 10 C. *Isonychia* sp. and *S. fuscum* of comparable size were found to utilize 810 μ liters O_2 /gram per hour and 670 μ liters O_2 /gram per hour, respectively, at 6.5 C.

A large variety of factors are known to affect rates of oxygen consumption: activity, temperature, nutrition, body size, stage in life cycle, time of day, previous oxygen experience, and genetic background (Prosser and Brown 1961). In addition, oxygen consumption of mayfly nymphs is affected by substrate and oxygen consumption of the water. Eriksen (1963b, 1964, 1966) reported that when substrate was provided, the burrowing mayfly nymphs, *Ephemera simulans* Walker and *Hexagenia limbata* (Serville), were respiratory regulators, while in the absence of substrate they were respiratory conformers. An artificial substrate was provided in the present study. Fox et al. (1937) found a decrease in oxygen consumption with decreasing dissolved oxygen concentration of the water for several species of mayfly nymphs, and Knight and Gaufin (1964) reported similar results for stonefly nymphs. Dissolved oxygen concentration never fell below 95% of the initial in the present study; hence, no depression of metabolic rate is believed to have occurred as a result of oxygen depletion.

The oxygen consumption of *Isonychia*

sp. (810 μ liters O_2 /gram per hour) and *S. fuscum* (670 μ liters O_2 /gram per hour) for nymphs of approximately the same size is significantly different. This difference might be expected because of dissimilarities in behavioral activities. Heptageniidae, in general, are dorso-ventrally flattened and particularly well suited for a sedentary existence, while Baetidae are streamlined and presumably adapted to a more active existence. Elliot (1968) found higher rates of activity in Baetidae than in Heptageniidae. Such a variation in activity might also account for the significant difference between day and night respiration in *Isonychia* sp. and the absence of such a difference in *S. fuscum*.

Most ephemeropterid nymphs exhibit a strong negative phototaxis and positive thigmotaxis. Wodsedalek (1911) first reported this for the mayfly nymph *Heptagenia interpunctata*. More recently, negative phototaxis has been shown for many Ephemeroptera by a large number of authors (Hughes 1966; Elliott 1967). In addition, behavioral studies have shown that peaks of drift and activity occur at night in a large number of mayfly species.

Harker (1952) reported diurnal rhythms of activity for three species of mayfly nymphs. She found that the nymphs did not show the expected negative phototactic responses, and that the rhythms were unaltered by conditions of continuous light or dark or by reversed illumination. Elliott (1968), working with the same three species, also found activity patterns that continued under constant conditions. However, he reported that the nymphs showed a strong negative phototactic response with periods of peak activity occurring during the night. Furthermore, these activity patterns could be reversed by reversing periods of illumination. It should be

noted that while the species used in these experiments were swift-water forms, Harker's studies were conducted in still water.

Drift rate studies have revealed a significant increase in numbers of nymphs drifting downstream at night. Waters (1965) subdivided the phenomena of downstream drift into three components: (1) behavioral drift—a result of some behavioral characteristic of the animal, such as response to change in light intensity; (2) catastrophic drift—that which occurs as a result of floods or other physical disturbance; (2) constant drift—composed of occasional individuals that for various reasons lose their hold on the bottom and drift in low numbers without any regard to diurnal periodicity.

Investigations by Moon (1940) and Waters (1962) initially demonstrated the diurnal periodicity of downstream drift of stream invertebrates (behavioral drift), and subsequent studies have shown that light intensity is the controlling factor in drift patterns (Anderson 1966; Holt and Waters 1967; Chaston 1968). However, population density, temperature, and water-level fluctuations may also play minor but important roles (Pearson and Franklin 1968).

Holt and Waters (1967) used artificially induced light patterns in the field and were able to control drift patterns by rapidly alternating light and dark phases. They concluded that although an endogenous rhythm might exist, it was not evident, and if one existed at all, it was extremely weak and strongly influenced by environmental light conditions. Bishop (1969) found similar results in the laboratory. However Chaston (1968) suggests that under normal conditions variation in drift is due to both exogenous and endogenous

changes in activity, and that the endogenous variations are masked by exogenous increases in activity caused by changing light conditions.

In view of these diurnal periodicities of activity and drift in mayfly nymphs, a metabolic pattern which mirrors them might be expected, with higher oxygen consumption rates occurring during periods of peak activity. However, it should not be assumed that high activity and metabolic rate are necessarily correlated. Beck (1964) found a respiratory rhythm in the European corn borer larvae which continued during diapause. Ralph (1956) reported that variations of oxygen consumption in earthworms were not reflections of motor activity.

Oxygen consumption has been shown to exhibit a diurnal rhythmicity in many organisms. Metabolic rhythms have been found in earthworms (Ralph 1956), potatoes, carrots, and seaweed (Brown Freeland, and Ralph 1955), fiddler crabs (Brown et al. 1956), and corn borer larvae (Beck 1964). The subject of diurnal rhythms has been reviewed by Harker (1961, 1964) and Sollberger (1965). "Organisms in their natural physical environment possess adaptively adjusted rhythms of their various activities which are reflected in metabolic rate changes; since these periodic patterns tend to persist even in constant conditions in the laboratory, metabolic rate becomes a function of time in these recurring patterns" (Prosser and Brown 1961). It is generally thought that these rhythms depend upon a common external factor, normally light, which sets an "internal clock." Current hypotheses for the working of this internal mechanism can be found in Bunning (1964) and Pittendrigh and Minis (1964). However, attempts have been made to correlate rhythmic fluctuations in metabolism with such exogen-

ous controls as barometric pressure (Brown, Webb, et al. 1955; Brown et al. 1956) and cosmic radiation (Brown, Webb, and Bennett 1958; Brown 1959).

Since *Isonychia* sp. exhibits a diurnal periodicity of oxygen consumption with a well-defined nocturnal increase (fig. 1), experiments were conducted to determine if this periodicity was controlled by exogenous or endogenous factors.

OXYGEN CONSUMPTION OF "ISONYCHIA" SP.
DURING REVERSED ILLUMINATION

Reversed illumination is an exact reversal of the normal photoperiod regime; in this case, a 10-hr light period from 1900 to 0500 and a 14-hr dark period from 0500 to 1900 was used. The organisms were acclimated to this regime for a 72-hr period prior to data collection.

The nymphs responded to reversed illumination with higher oxygen consumption during the new dark period (fig. 2). The scotophase rate of 5.4 μ liters O_2 /nymph per hour is significantly different from the photophase rate of 3.1 μ liters O_2 /nymph per hour. A mean daily oxygen consumption rate of 4.5 μ liters O_2 /nymph per hour under reversed illumination was not found to be significantly different from the mean daily rate of 4.4 μ liters O_2 /nymph per hour under normal conditions. On the

basis of these results it can be concluded that light is an exogenous controlling factor which influences rates of oxygen consumption in *Isonychia* sp.

OXYGEN CONSUMPTION OF "ISONYCHIA" SP.
DURING CONSTANT CONDITIONS

In order to detect the possible existence of an endogenous factor, metabolic rate determinations were conducted under conditions of continuous light and continuous dark.

Under continuous darkness (fig. 3), a value of 4.3 μ liters O_2 /nymph per hour was found during the subjective night compared with 3.7 μ liters O_2 /nymph per hour during the subjective day. Differences between the means are not nearly as great as those found under conditions of normal or reversed photoperiod, but the means are significantly different.

Under continuous light (fig. 3), higher mean rates of oxygen consumption also occur during the subjective night period. The subjective nighttime mean of 4.3 μ liters O_2 /nymph per hour is significantly different from the subjective daytime mean of 3.5 μ liters O_2 /nymph per hour.

Harker (1961) defines a diurnal rhythm as "one which will persist for at least 2 to 3 days in a constant environment, little affected by temperature

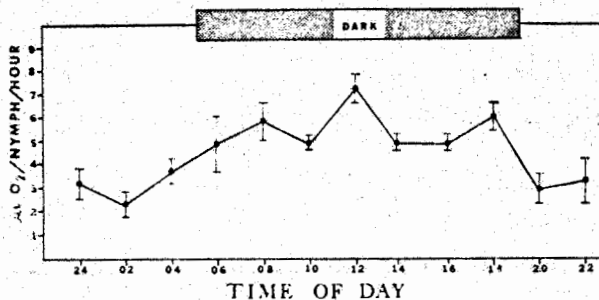


FIG. 2.—Oxygen utilization by *Isonychia* sp. during reversed illumination. Vertical lines indicate 2 SE's of mean. Mean photophase—3.1 μ liters O_2 /nymph per hour; mean scotophase—5.4 μ liters O_2 /nymph per hour; mean daily—4.5 μ liters O_2 /nymph per hour.

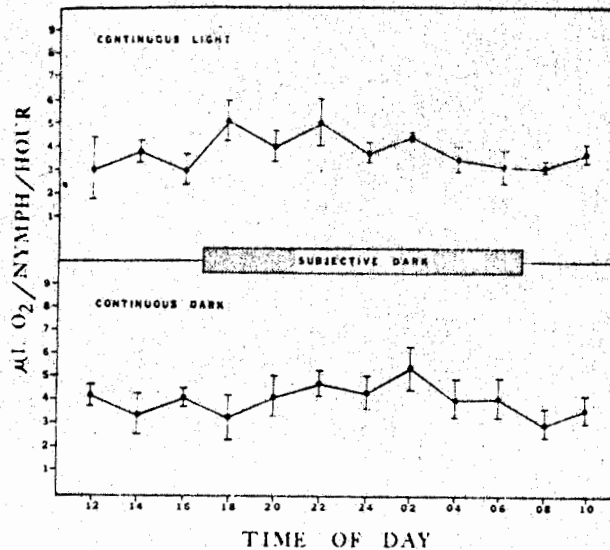


FIG. 3.—Oxygen utilization by *Isonychia* sp. during continuous dark and continuous light. Vertical lines indicate 2 SE's of mean. Continuous dark: mean subjective light—3.7 μ liters O₂/nymph per hour; mean subjective dark—4.3 μ liters O₂/nymph per hour; mean daily—4.0 μ liters O₂/nymph per hour. Continuous light; mean subjective light—3.5 μ liters O₂/nymph per hour; mean subjective dark—4.3 μ liters O₂/nymph per hour; mean daily—4.0 μ liters O₂/nymph per hour.

within biological limits, and the timing of peaks or phases of the rhythm in relation to solar time is originally determined by an environmental change, such as variation in light intensity or temperature." Since a diel pattern of consumption in *Isonychia* sp. did persist under constant conditions, at least for a 2-week period, it is a diurnal rhythm with timing originally determined by variations in light intensity.

Under constant conditions, mean daily rates of oxygen consumption are not significantly different from mean daily rates under normal and reversed illumination. However, the subjective nighttime respiration is significantly lower than dark-period respiration during normal and reversed conditions of illumination. This indicates that although an endogenous rhythm does exist, there is an exogenous override which results in a more pronounced expression of the rhythm.

Since no significant variation can be

seen in day/night oxygen consumption for *S. fuscum*, it is concluded that no discernable diurnal periodicity of metabolic rate exists in this species.

SUMMARY

1. *Isonychia* sp. nymphs with an average dry weight of 6.2 mg utilized 810 μ liters O₂/gram per hour under simulated winter conditions. *Stenonema fuscum* nymphs with an average dry weight of 5.2 mg utilized 670 μ liters O₂/gram per hour under identical conditions. These figures represent mean daily rates of oxygen consumption. Rates of oxygen consumption in the two species are significantly different. This difference can probably be attributed, at least in part, to differences in behavior.

2. Respiration rates for *Isonychia* sp. exhibited rhythmic fluctuations, with significantly higher nighttime oxygen utilization. No such variation was found for *S. fuscum*.

3. *Isonychia* sp. was studied under

conditions of reversed illumination, continuous light, and continuous dark, to determine whether the periodicity of oxygen consumption was controlled by exogenous or endogenous factors. Reversed illumination resulted in adjustment of metabolic rate with significantly higher oxygen consumption occurring during the dark period. Under condi-

tions of continuous light and continuous dark, significantly higher respiration occurred during the subjective night. This suggests that metabolic rates in *Isonychia* sp. are controlled by both an exogenous and endogenous factor, with the endogenous component being weak and strongly influenced by the external stimulus of light.

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