DIEL RHYTHMS IN THE RESPIRATION AND FEEDING RATES OF ZOOPLANKTON

by

Wayne Stuart Duval

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Name: W. e Stuart Duval

Degree: Doctor of Philosophy

Title of Thesis: Diel Rhythms in the Respiration and Feeding Rates of Zooplankton.

Examining Committee:

Chairman: Dr. J. M. Webster

G. H. Geen
Senior Supervisor

P. Belton

P. V. Fankboner

R. M. F. S. Sadleir

T. G. Northcote
External Examiner
Professor
Institute of Animal Resource Ecology
University of British Columbia

Date Approved: 21 December 1970

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Author:

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WAYNE DUVAL

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(date)

TO STONER HAVEN

Out of the emptiness of innocence

Out of the clear pool

Within the depths of lakes, a madrigal symmetry reigns

The rhythm falters - obsidian in spawning pools

Out of the fluid flows the form

Of dazzling patterns of light

red

blue

and green

Migrating critters relentlessly searching

Creating Life by their existence

The biological rhythm of the universe is the movement of life

All things emerge in the mystery of changes

- Katalin Burns

ABSTRACT

A number of aspects of diel rhythms in the feeding and respiration rates of zooplankton were examined. Emphasis was placed on the role of the environment in the maintenance and synchronization of rhythmic processes under natural conditions and the effects of various environmental parameters on the amplitude of rhythmic phenomena.

The existence of endogenous diel feeding and respiration rhythms under constant conditions was demonstrated in three zooplankton populations and at temperatures from 10 C to 22 C. The rhythms were typically bimodal; maximum rates were found at dawn and dusk, lowest values near midday. Respiration rates at dawn and dusk were, on the average, 2.3 times higher than during midday; the corresponding diel difference was 6.6 times in the case of feeding.

Light intensity, spectral composition, and temperature were found to have significant effects on the amplitude of diel feeding and respiration rhythms. It was concluded that under natural conditions, periodic changes in the light and temperature environment would reinforce endogenous rhythms, increasing their amplitude over and above that demonstrated under constant laboratory conditions. The effects of twilight duration and spectral composition of light on the maintenance and phase of diel respiration rhythms were not clearly defined.

Significant diel rhythms in feeding, respiration, phosphate excretion, and assimilation rate were demonstrated *in situ* and an energy budget constructed from the Eunice Lake zooplankton community. Maximum rates were found near dawn and dusk, although the precise timing of maxima and amplitude of rhythms varied with each parameter. Net assimilation efficiency varied from less than 5% at dawn and dusk to over 90% during midday; net growth efficiency was highest at dawn and dusk and lowest near midday.

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GENERAL INTRODUCTION

The 24-hr period of the earth's rotation has profound effects on the physiology and behavior of organisms. Many important daily life functions reoccur approximately every 24 hrs as diel rhythms that are either exogenous or endogenous. Under natural conditions an organism may exhibit a day-night periodicity which is purely exogenous, reflecting some response to the environment that disappears under constant laboratory conditions; such rhythms are diel but not circadian. Circadian rhythms, on the other hand, are endogenous, persisting under constant conditions but drifting out of phase with the external time scale. Some aspect of the environment, typically light or temperature, acts as a cue (*Zeitgeber*) to maintain synchrony under natural circumstances.

Innumerable examples of circadian activity cycles, usually characterized by one or more activity peaks at specific times of the day, have been described (Harker, 1957). The diel vertical migration of zooplankton is one behavioral rhythm that has received much attention in the last 100 years. The endogenous nature of plankton migration has been demonstrated by a number of authors (Harris, 1963; Ringelberg and Servaas, 1971).

There have been few studies of diel rhythms in the feeding and respiration rates of zooplankton and many of these results are contradictory. Fuller (1937) first proposed a diel feeding rhythm in planktonic copepods demonstrating that feeding rates were greatest during the evening when zooplankton inhabit surface waters. Similar observations have been made by a number of other authors (Ponomareva, 1971; Singh, 1972; and Wimpenny, 1938). However, these observations do not necessarily represent circadian phenomena since grazing rates will probably increase at the surface due to

the influences of increased temperature and food concentration. It is not strictly possible to establish the existence of circadian rhythms without controlling all the exogenous influences of the environment.

The purpose of this investigation is to ascertain whether circadian rhythms in zooplankton feeding and respiration exist and if so, to examine the role of the environment in the maintenance and synchronization of rhythmic processes under natural conditions and to describe the effects of various environmental parameters on the magnitude of rhythmic phenomena. Diel rhythms were studied *in situ* to determine their possible significance in the measurement of energy flow within natural zooplankton communities.

Chapter 1

ENDOGENOUS DIEL RHYTHMS IN THE FEEDING AND RESPIRATION OF ZOOPLANKTON

INTRODUCTION

Over the past 50 years a number of workers have demonstrated the existence of a 24-hr cycle of variation in the feeding and respiration of zooplankton. However, it has not been satisfactorily ascertained whether these rhythms are endogenous, in that rate changes occur in the absence of periodic environmental stimuli, or are exogenous rhythms which are a consequence of environmental stimuli.

The first suggestion of such a cycle in feeding rate was the observation of Marshall (1924) who noted that the copepod *Calanus* (Crustacea) captured in early morning had full guts, whereas the stomachs of those taken later in the day were empty. Similar observations have been made by a number of authors (Petipa, 1964; Ponomareva, 1971; and Singh, 1972) although their studies neither confirm nor deny the endogenous nature of feeding rhythms since their experimental design did not eliminate possible environmental stimuli.

Other workers have been unable to demonstrate rhythms in the feeding and respiration rate of zooplankton. Gauld (1953) found that if food was available, the majority of *Calanus* examined had full guts throughout the day; alternation of feeding and non-feeding periods only occurred when there was vertical migration into warmer, phytoplankton-rich surface waters. Similarly, Pearcy *et al.* (1969) and Richman and Rogers (1972) were unable to demonstrate diel feeding and respiration rhythms in freshwater zooplankton.

The purpose of the first phase of my thesis was to establish whether or not endogenous feeding and respiration rhythms exist in zooplankton obtained from three lakes.

MATERIALS AND METHODS

Study Areas

Samples of zooplankton used in this work were obtained from three lakes in British Columbia: Eunice Lake, Deer Lake, and Babine Lake.

Eunice Lake is a small oligotrophic lake in the University of British Columbia Research Forest near Haney. It has a surface area of 18.2 ha, a mean depth of 15.8 m, and a maximum depth of 42 m. *Diaptomus kenai* (Wilson) and *Diaptomus tyrelli* (Poppe) were the most common zooplankton collected from this lake.

Deer Lake, a shallow eutrophic lake in Burnaby, has a surface area of 28.0 ha and depths varying from 3 to 5 m throughout much of the lake. The principal zooplankters present were *Daphnia pulex* (de Greer) and *Cyclops scutifer* (Sars).

The third lake from which zooplankton were obtained for this investigation was Babine Lake, situated in north-central British Columbia. It is about 160 km long and has a surface area of 490 km^2. *Diaptomus ashlandii* (Marsh) and *Heterocope septentrionalis* (Juday and Muttowski), the most common zooplankton, were collected from the North Arm where the maximum depth is 46 m and the mean depth 20 m.

Collection of Samples

Zooplankton were collected with #6 or #10 mesh (239 μ and 158 μ pore diam.) nets. Samples from Eunice Lake were taken with a #10 net (30 cm diam.) towed approximately 25 m/min at a depth of 5 m. Babine Lake zooplankton were collected with a #10 net towed at 10 m. A #6 net towed at 1 m was used in Deer Lake. The plankton samples were transferred to 5-gal darkened insulated carboys containing lake water to minimize crowding. Carboys were maintained at lake temperature and returned to the laboratory.

Experimental Design

Three feeding and five respiration studies were conducted over a period of four years in an attempt to establish diel rhythms. Each investigation consisted of six consecutive 4-hr laboratory experiments with zooplankton from one of the lakes. Experiments were conducted at constant temperature under continuous darkness using a sample of mixed zooplankton from the lake rather than a single species.

Determination of Respiration Rate

Shortly after zooplankton samples were returned to the laboratory, 25-ml subsamples were transferred to darkened 50-ml beakers and acclimated for 18 to 24 hr at constant temperature and continuous darkness. A far red filter (Percival perspex) was used when zooplankton were handled and observed in the laboratory darkroom. Viaud (1951) has shown that zooplankton are insensitive to light of this wavelength (> 700 nm). A wide range of zooplankton densities was used in the experiment to allow least squares linear regression correction of all rates to values expected at a standard biomass of 50 µg dry wt/ml. Only active individuals were used in each experiment. The temperature chosen for each study was within 5 C of the surface temperature to minimize any concern for acclimation.

Thirty minutes before each 4-hr experiment, 200 ml of air-saturated dechlorinated water were measured into each of eight darkened 250-ml BOD bottles. The zooplankton and the 25-ml samples of lake water were added to six of the BOD bottles. The zooplankton from the remaining two beakers in each set were removed with 53 µ mesh and 25 ml of water added to two BOD bottles as controls. Streptomycin sulphate (100 mg/l) was added to each bottle to inhibit bacterial growth; this concentration did not affect the respiration rate of zooplankton ($P > 0.05$). All bottles were stoppered and

placed in an environmental chamber for 4 hrs. The bottles were inverted periodically to prevent settling of the zooplankton.

At the end of each 4-hr experiment the dissolved oxygen in each sample was fixed by addition of 2 ml of $MnCl_2$ (3M) and 2 ml of alkaline iodide (NaOH, 8N; NaI, 4M) with positive displacement type pipettes (Carritt and Carpenter, 1966). Preservation of zooplankton was accomplished by the addition of 2 ml of a 50% ethanol:50% formalin mixture. This preservation did not affect the stability of the iodide precipitate.

At the end of each 24-hr study the dissolved oxygen concentration was determined by titrating 5-ml aliquots of the liberated iodine solution with 0.0084 N thiosulphate from a 5-ml microburette.

Zooplankton were removed from each bottle by filtration with 53 μ mesh, stained with Lugol's solution, and counted by species on filter paper. The species composition of the zooplankters in each replicate did not vary over the 24-hr period ($P > 0.05$). The mean weight of 20 individuals of each species after drying at 60 C for 48 hr was determined on a Cahn Electrobalance.

A decrease in the dissolved oxygen concentration in experimental bottles relative to the controls was attributed to oxygen consumption by the zooplankton. The respiration rate of the individuals in each bottle was calculated with the following expression.

$$\text{RESPIRATION RATE } (\text{1 } O_2/\text{mg dry wt/hr}) = \frac{(\overline{CT} - \overline{ET})(F)(X/X\text{-}6)(CF)}{([N_1\overline{W}_1 + N_2\overline{W}_2 + N_x\overline{W}_x]/1000)(T)}$$

where $\overline{CT}$ = ml thiosulphate for controls (mean)

$\overline{ET}$ = ml thiosulphate for experimental bottle (mean)

F = calibration factor for thiosulphate solution

X = size of BOD bottle (ml)

CF = conversion factor (ml thiosulphate to μl O_2)

N_1 to x = no. zooplankton of each species (x)

$\overline{W}_1$ to x = mean dry wt (µg)/zooplankter of each species (x)

T = experiment duration (hr)

In one of the respiration experiments, oxygen consumption of a population of *Diaptomus kenai* was determined with a Gilson Differential Respirometer. Readings on the same zooplankton were taken every 1 - 2 hrs over the 24-hr study.

Since the number of zooplankters in each vessel was not the same and population density is known to influence weight-specific respiration rates (Zeiss, 1963), it was necessary to convert rates to those expected under standard biomass conditions. For each 4-hr experiment, an individual correction equation (log r/s = slope(log biomass) + intercept) was derived from a least squares linear regression of log respiration rate versus log biomass density. This expression (eq. Fig. 2-8) was used to correct all oxygen consumption values to those expected with a zooplankton biomass of 50 µg dry wt/ml.

One-way analysis of variance was used to test the null hypothesis that there were no differences in the respiration rate of zooplankton within a day. F-ratios were determined in each study by comparing the mean square of the deviation in rates due to time with variability (error) within each 4-hr experiment. The null hypothesis was rejected when the calculated F-ratio exceeded the critical F-value for the appropriate degrees of freedom (groups - 1; no. replicates - no. groups) at the 0.1% probability level.

Determination of Feeding Rate

Feeding experiments were set up and conducted in the same way as the respiration experiments except that all samples contained 10^5 cells/ml of *Chlamydomonas reinhardtii* as a food source. *Chlamydomonas* was ingested by all zooplankton examined during this investigation. At the end of each 4-hr

experiment, zooplankton were removed from the BOD bottles by filtering the water through 53 μ mesh and preserved with Lugol's solution for subsequent counting and dry weight determination. A 100-ml aliquot of the water was removed from each bottle and the algae preserved with ethanol. A concentration of 6 - 7% (v/v) ethanol killed the algal cells without causing plasmolysis.

Phytoplankton numbers in each sample were counted with a Model B Coulter Counter equipped with Model M Volume Converter. Feeding rate was determined by comparison of the total biomass (μ^3) in control and experimental vessels, following the procedure of Sheldon and Parsons (1966b). A 50 μ aperture tube calibrated with paper mulberry pollen and 5% saturated KNO_3 electrolyte was used in all cases. Decrease in algal biomass in experimental vessels was attributed to feeding by the zooplankton. The feeding rate of the zooplankters in individual bottles was computed from the following expressions.

$$\underset{(\mu^3 \text{ algal biomass/ml/mg dry wt/hr})}{\text{FEEDING RATE}} = FR$$

$$FR = \frac{\sum_{sc=1}^{n} \overline{CC}_{sc=1,n}(F) - \overline{EC}_{sc=1,n}(F)}{([N_1\overline{W}_1 + N_2\overline{W}_2 + N_x\overline{W}_x]/1000)(T)}$$

where $$F = \frac{(\text{abs. no. cells/ml @ SCM})(\text{MCV @ SCM})}{(\overline{CC} \text{ @ SCM})}$$

and $$MCV = (K)(S)30$$

sc = size class of algal cells (max = 16) where MCV of each successive size class (sc) increases logarithmically

n = total number of size classes used for distribution

$\overline{CC}_{sc=1,n}$ = mean control bottle phytoplankton count for each size class

$\overline{EC}_{sc=1,n}$ = mean experimental bottle phytoplankton count for each size class

F = biomass conversion factor

SCM = size class with highest cell count

MCV = mean cell volume (μ^3)

N_1 to x = no. zooplankters of each species (x)

$\overline{W}_1$ to x = mean dry wt (µg)/zooplankter of each species (x)

T = experiment duration (hrs)

K = calibration factor for given Coulter Counter aperture tube

S = sensitivity of Coulter Counter

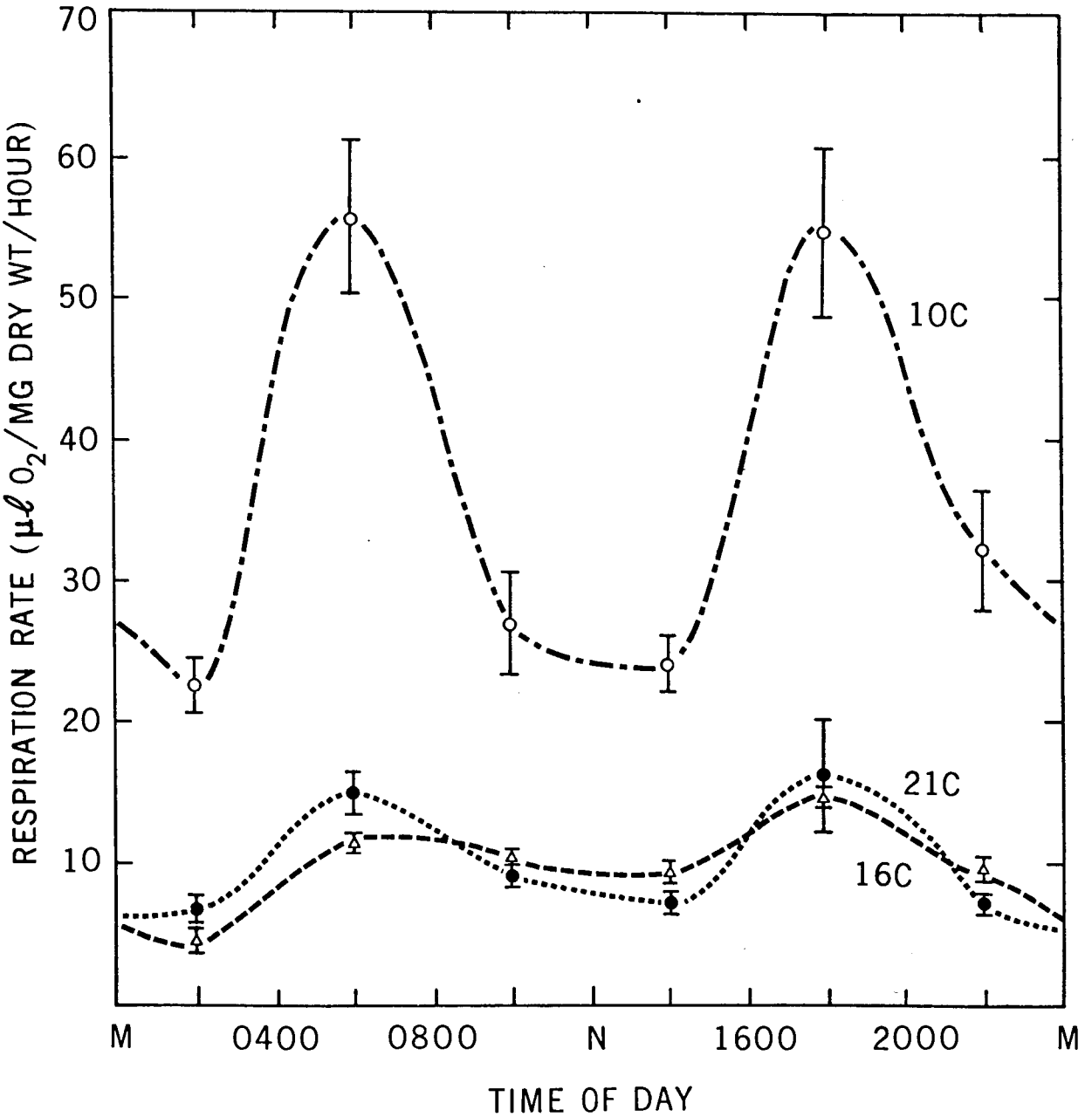
All feeding rates were corrected to values expected at a zooplankton biomass of 50 µg dry wt/ml.

RESULTS

Diel Changes in Zooplankton Respiration

Figure 1-1 presents data which show diel changes in the respiration rate of Eunice Lake zooplankton. The experiments were conducted over a two month period at temperatures of 10 C, 16 C, and 21 C. There was a significant diel change in oxygen consumption at all three temperatures (10 C: 12.31 > 5.98 @ $P < 0.001$, df = 5,24, 16 C: 26.28 > 6.08 @ $P < 0.001$, df = 5,23, 21 C: 4.18 > 4.10 @ $P < 0.01$, df = 5,20). The most pronounced diel changes were noted at 10 C. In all experiments there were maxima at 0600 and 1800 hr, although the dawn maximum was not well defined in the 16 C study. This study showed a large amplitude at 10 C and significantly ($P < 0.001$) higher rates at all times of the day at 10 C than at 16 or 21 C. There were no significant differences ($P > 0.05$) in the magnitude of the dawn and dusk

Figure 1-1 Diel changes in the respiration of zooplankton from Eunice Lake at three temperatures. All studies conducted in continuous darkness with rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. The most abundant species present were the copepods *Diaptomus kenai* (41.9 µg dry wt), *Diaptomus tyrelli* (13.5 µg dry wt), and the cladocerans *Holopedium gibberum* and *Daphnia rosea* (6.9 µg dry wt). Experiment dates: 10 C = 22 May 1969, sunrise at 0416 hr, sunset at 2202 hr; 16 C = 18 June 1969, SR at 0502 hr, SS at 2124 hr; 21 C = 8 August 1969, SR = 0412 hr, SS at 2021 hr. Mean ± standard error indicated.


70
60
50
40
30
20
10
RESPIRATION RATE (μℓ O2/MG DRY WT/HOUR)
10C
21C
16C
M
0400
0800
N
1600
2000
M
TIME OF DAY

maxima at any individual temperature or in respiration rates ($P < 0.05$) measured at 16 C and 21 C. In all experiments the rates determined near midday (1000 - 1400 hr) were lowest.

In another study of diel changes in the respiration of Eunice Lake zooplankton in 1970, a similar trend was observed using respirometry to measure the oxygen consumption of *Diaptomus kenai* at 22 C (Fig. 1-2). Respiration maxima were found at 0730 and 1730 hr and were more precisely defined than those shown in Fig. 1-1 since oxygen determinations were made every hour. The diel differences observed were significant at the 0.1% probability level ($10.85 > 4.52$ @ $P < 0.001$, df = 19,109). There was no significant ($P > 0.05$) change in respiration rate from 0830 to 1430 hr.

Babine Lake zooplankton (95% *D. ashlandii*) were used in studies of diel changes in oxygen uptake at 13 C. A significant ($9.78 > 5.66$ @ $P < 0.001$, df = 5,28) difference in oxygen consumption with time of day was demonstrated; respiration rates were highest at 0600 and 2200 hr (Fig. 1-3).

Diel Changes in Zooplankton Feeding

Diel differences in zooplankton feeding were also demonstrated. Eunice Lake zooplankton maintained at 21 C showed a diel difference in feeding rate ($16.38 > 5.53$ @ $P < 0.001$, df = 5,30) characterized by maxima at 0200 and 1800 hr (Fig. 1-4). The dawn maximum was significantly larger than the dusk maximum ($P < 0.001$). There were no significant differences in feeding rate from 0600 to 1400 hr.

Diel differences in the feeding rate of summer and winter populations of *Daphnia pulex* and *Cyclops scutifer* from Deer Lake were also examined (Fig. 1-5). Highly significant diel differences were found in both cases (winter: $5.01 > 3.22$ @ $P < 0.005$, df = 9,40; summer: $12.21 > 5.53$ @ $P < 0.001$,

Figure 1-2 Diel changes in the respiration rate of *Diaptomus kenai* from Eunice Lake, B.C. Rates were determined under continuous darkness at 22 C (16 August 1970; SR = 0501 hr, SS = 1931 hr). All rates were corrected to values expected at a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

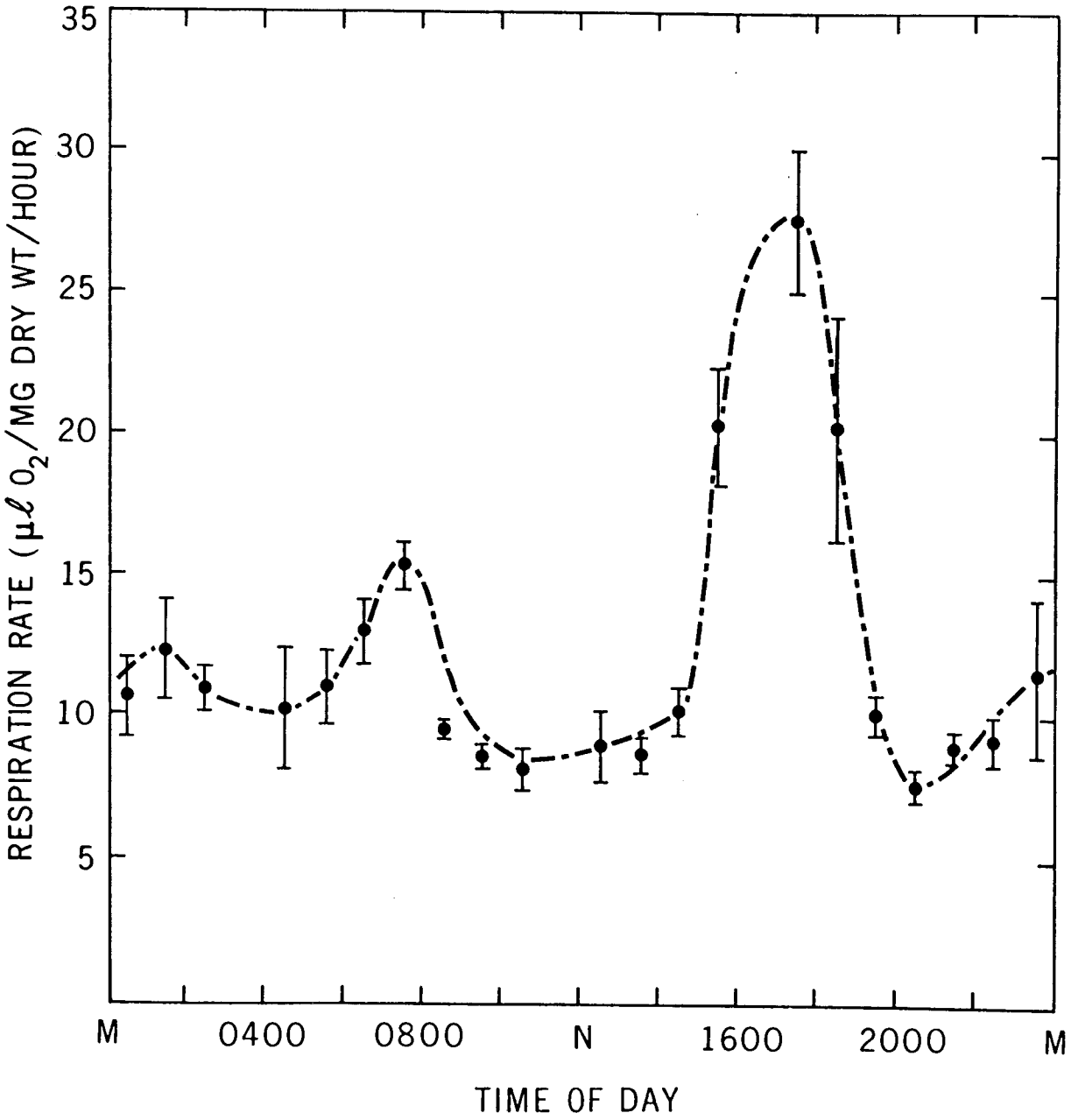

35
30
25
20
15
10
5
RESPIRATION RATE (μℓ O2/MG DRY WT/HOUR)
M
0400
0800
N
1600
2000
M
TIME OF DAY

Figure 1-3 Diel changes in the respiration rate of Babine Lake zooplankton (95% *Diaptomus ashlandii*, mean dry wt = 5.6 µg). Experiments were conducted at 13 C and under continuous darkness (12 Sept. 1969; SR = 0541 hr, SS = 1838 hr). All rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

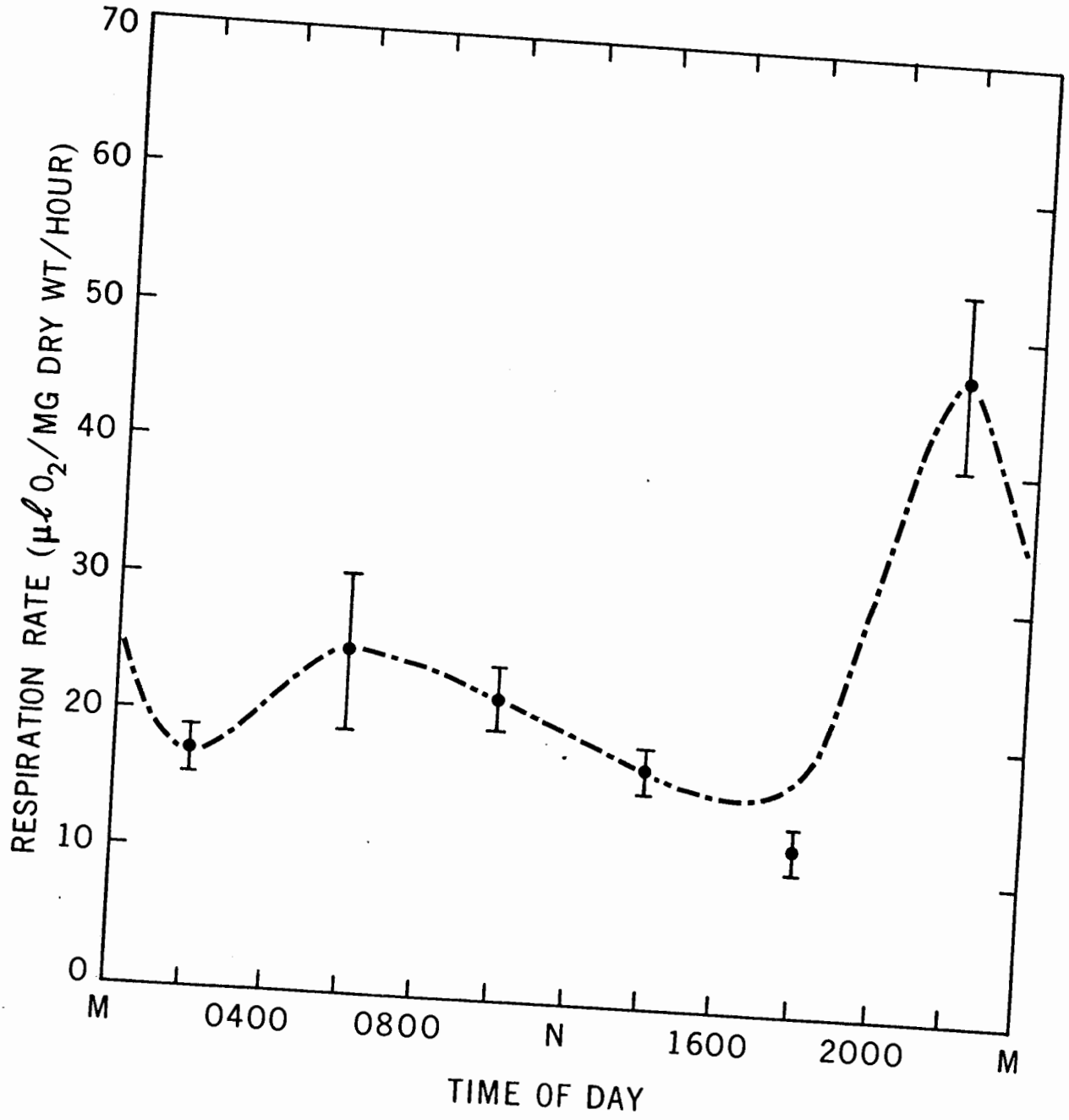

70
60
50
40
30
20
10
0
RESPIRATION RATE (μℓ O2/MG DRY WT/HOUR)
M
0400
0800
N
1600
2000
M
TIME OF DAY

Figure 1-4 Diel change in the feeding rate of Eunice Lake zooplankton. Investigation conducted at 21 C and under continuous darkness (8 July 1969; SR = 0412 hr, SS = 2021 hr). The principal species present were the copepods *Diaptomus kenai* (mean dry wt = 41.9 µg), *Diaptomus tyrelli* (mean dry wt = 13.5 µg) and the cladocerans *Holopedium gibberum* and *Daphnia rosea* (mean dry wt = 6.9 µg). All rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

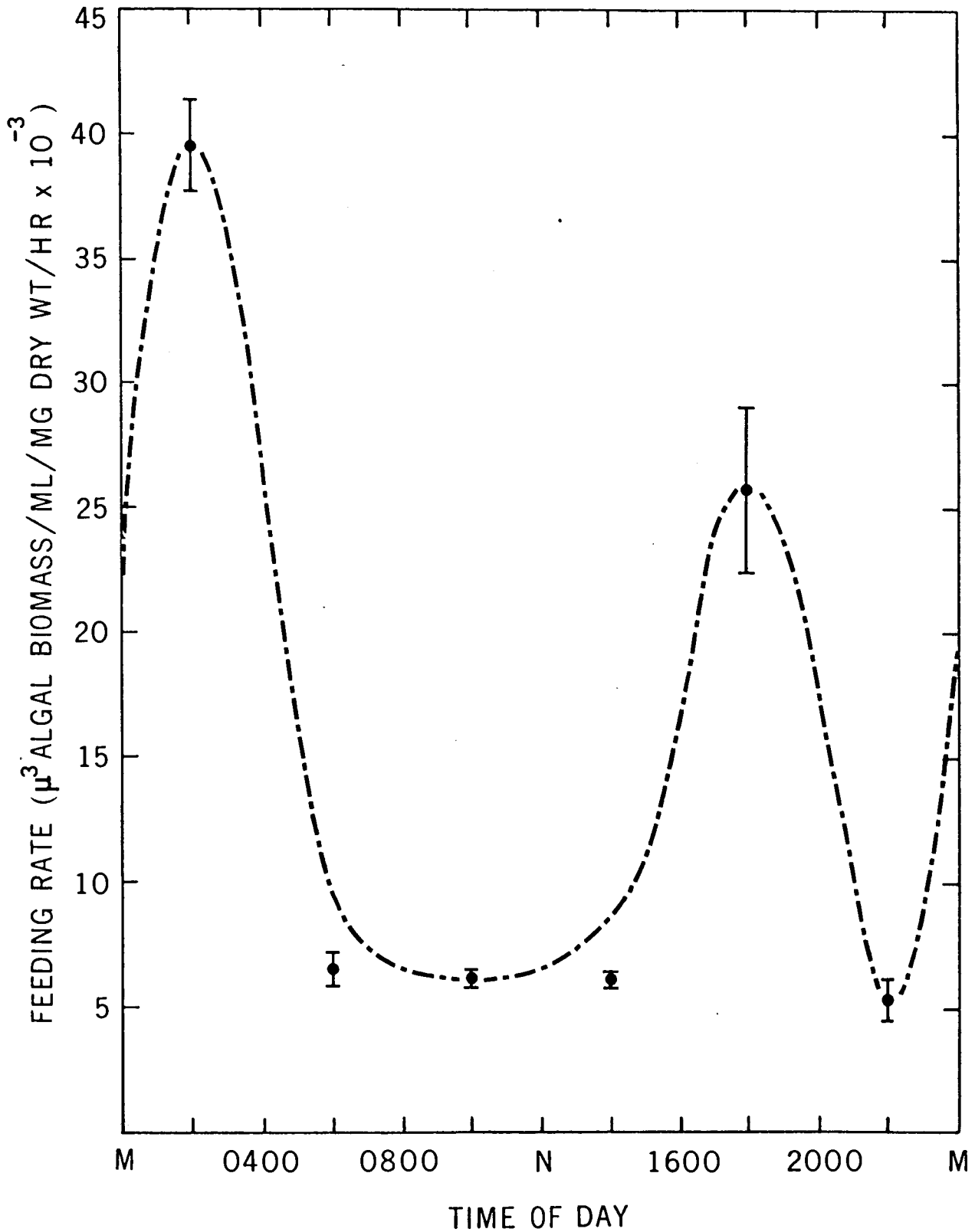
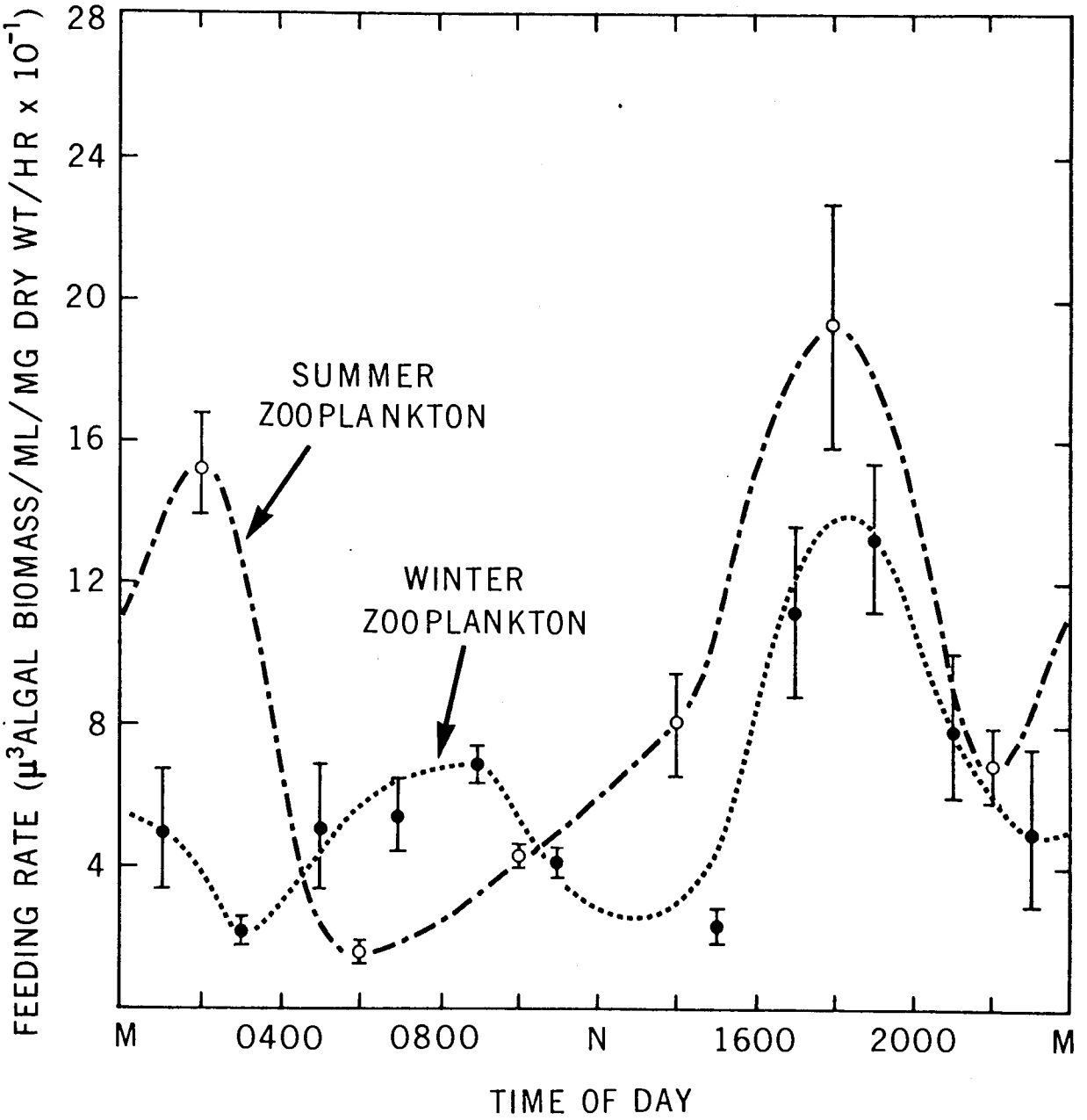

45
40
35
30
25
20
15
10
5
FEEDING RATE (μ^3 ALGAL BIOMASS/ML/MG DRY WT/HR x 10^{-3})
M
0400
0800
N
1600
2000
M
TIME OF DAY

Figure 1-5 Diel change in the feeding rate of Deer Lake zooplankton samples consisting primarily of *Daphnia pulex* (mean dry wt = 18.5 µg) and *Cyclops scutifer* (mean dry wt = 8.5 µg) collected in winter (5 Feb. 1972; SR = 0742 hr, SS = 1711 hr) and summer (23 June 1972; SR = 0500 hr, SS = 2121 hr) and feeding experiments conducted at 10 C and 20 C respectively. Experiments conducted under continuous darkness and all rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.


FEEDING RATE (μ³ALGAL BIOMASS/ML/MG DRY WT/HR x 10⁻¹)
28
24
20
16
12
8
4
SUMMER ZOOPLANKTON
WINTER ZOOPLANKTON
M
0400
0800
N
1600
2000
M
TIME OF DAY

df = 5,30). In both summer (20 C) and winter (10 C) experiments, the dusk maximum occurred at 1800 hr while the dawn maximum occurred much earlier in the summer experiment. At 10 C the dusk maximum exceeded the dawn maximum ($p < 0.001$) but was not significantly different from the dawn maximum at 20 C.

DISCUSSION

The results of the present investigation demonstrate significant diel changes in the respiration and feeding rates of zooplankton. The consistent repetitive pattern of these changes and occurrence in the absence of periodic environmental stimuli clearly establish their rhythmic and endogenous nature.

The limitations inherent in the present findings must be considered before discussion of the data themselves. This study does not establish that these rhythms are circadian - only that they are endogenous. Even this statement must be qualified to some extent since rhythms can only be called endogenous if all periodic environmental variables are controlled. In the present study light and temperature were constant; however, some authors suggest that periodic oscillations in other factors such as barometric pressure may still persist (Brown *et al.*, 1955). Before they can be called 'circadian' the rhythms must be followed under constant conditions for a minimum of five to seven days and the frequency of the 'free-running period' determined (Pittendrigh, 1960). If a rhythm is circadian the free-running period must deviate by a more or less constant amount from the period of the earth's rotation. The free-running period is not exactly 24 hrs since this would usually indicate the existence of an unknown periodic factor in the environment acting as a *Zeitgeber*. The period of the free-running rhythm is not constant but continuously influenced by both the

environment and the physiological state of the organisms. Since the free-running period was not determined, the feeding and respiration rhythms could not be defined as circadian.

The fact that significant diel rhythms were demonstrated for several different species under a variety of environmental circumstances suggests that these rhythms are a general phenomenon. Diel rhythms in feeding or respiration were shown at temperatures from 10 - 22 C, in zooplankton from both oligotrophic and eutrophic lakes, in both cladocerans and copepods, at different times of the year, and at different latitudes.

Several trends emerge from the experiments reported here. The diel rhythm in the feeding and respiration of zooplankton is typically bimodal. Respiration and feeding rates were always highest during the early morning and late afternoon or evening, and were lowest during the midday. The amplitude of the diel rate changes varied considerably but was usually two to four times greater than midday levels for respiration and even greater for feeding (Table 1-1). Respiration rates, on the average, decreased by a factor of 1.9 times from dawn to midday and increased by 2.7 times from noon to dusk. The corresponding average dawn to noon decrease for feeding rhythms was 6.1 times with a noon to dusk increase by a factor of 7.1.

The limitation of a 4-hr experiment period was apparent in attempts to establish the precise timing of these maxima. It is not possible to ascertain the exact time at which the increased activity occurs. The observed maxima may not represent the true maxima, which may occur slightly earlier or later; consequently, the observed amplitude changes at dawn and dusk may be underestimates of the true values.

The timing of the maxima appear to be correlated with dawn and dusk. The results of the two Deer Lake studies are consistent with this hypothesis

TABLE 1-1

Amplitude changes in zooplankton feeding and respiration rates under constant conditions. All rates are expressed relative to a rate of 1.0 at the midday minima. The zooplankton community was predominantly copepods in both Eunice and Babine Lakes, and mostly cladocerans in Deer Lake.

Sampling Area	Temp	Parameter Measured	Decrease from Dawn Maximum to Midday Minimum	Increase from Midday Minimum to Dusk Maximum
Eunice	10 C	Resp	2.3	2.3
Eunice	16 C	Resp	1.1	1.5
Eunice	21 C	Resp	2.1	2.3
Eunice	22 C	Resp	1.9	3.4
Babine	13 C	Resp	2.0	3.9
Eunice	21 C	Feed	6.4	4.2
Deer	10 C	Feed	2.8	5.6
Deer	20 C	Feed	9.1	11.4

(Fig. 1-5). Daylength during the winter study was approximately 10 hrs and 16 hrs during the summer. Since the two maxima observed in the winter study were separated by 10 hrs of daylight and 16 hrs in the summer, a close correlation to daylength is indicated, although the timing of the evening peaks at both seasons was similar. However, the differences in the time of occurrence of the maxima may not be entirely due to daylength since the period of circadian phenomena is temperature dependent to some extent (Sweeney and Hastings, 1960).

The studies of diel rhythms in the respiration of Eunice Lake zooplankters (Fig. 1-1) indicate that although the rhythms exist at the three temperatures examined, temperature has a profound influence on both the amplitude of the changes as well as the lowest midday rate. This is shown in Table 1-2 which indicates the integrated daily oxygen consumption and midday rates for each temperature. At 10 C the midday rates are 2.5 to 3.0 times higher than at 16, 21, or 22 C. The 13 C respiration rates of Babine Lake zooplankton are intermediate between the 10 and 16 C results obtained with zooplankton from Eunice Lake (Table 1-2) even though the species composition differed. The decrease in oxygen consumption with increased temperature is not predictable given the usual Q_{10} relationships. Similar observations were made by Halcrow (1963) who noted higher respiration rates in *Calanus* at 10 C than at 20 C after a 24-hr acclimation period. However, after 10 days acclimation, Halcrow (1963) found a more typical Q_{10} relationship in that rates at 20 C exceeded those measured at 10 C. Consequently, the results of the present investigation are probably an artifact of incomplete temperature acclimation.

The oxygen consumption of zooplankton at dusk was on the average 42% higher than at dawn; the evening maximum always equalled or exceeded morning

TABLE 1-2

Integrated daily oxygen consumption and midday rates determined for various zooplankton populations under constant conditions. Integrated daily respiration rates are determined on an hourly basis and expressed in µl O_2/mg dry wt/day. Midday level is expressed in µl O_2/mg dry wt/hr.

Sampling Area	Temperature	Integrated Daily Oxygen Consumption	Midday Oxygen Consumption
Eunice	10 C	875.3	24.0
Eunice	16 C	247.8	7.9
Eunice	21 C	255.7	10.2
Eunice	22 C	304.2	8.4
Babine	13 C	561.4	20.0

rates. However, the relationship between the respective amplitudes of dawn and dusk feeding maxima was not as clearly defined. Dusk rates exceeded dawn values by approximately 18% in two experiments, while in the third the dawn feeding maximum was 52% higher than the evening maximum.

The magnitude of diel periodicity in the feeding rate of zooplankton exceeds that for respiration (Table 1-1). This may have important implications to the diel energy flow within zooplankton communities. If the energy required for maintenance of zooplankton communities has smaller diel change than feeding rate, then there may be an enhancement of food assimilation relative to energy requirements at dawn and dusk. Consequently, the energy derived from increased feeding at dawn and dusk, with only slightly greater respiratory expenditure, may be made available for increased growth and production. This argument assumes that assimilation efficiency does not vary throughout the day as it may if zooplankton exhibit superfluous feeding (Beklemishev, 1961) at dawn and dusk. Investigations of diel rhythms in feeding, respiration, and assimilation *in situ* are required to substantiate this hypothesis.

Rhythms of the type demonstrated in this investigation may be of significance in three ways: to the organism, to the population, and to experimental design. Of fundamental significance to the individual is the selective value of biological clocks themselves. Here a rather important distinction must be made. A biological clock is a basic physical oscillator within the cells of virtually every organism. It is expressed as a number of behavioral and physiological rhythms which may or may not have selective advantage of their own. Bünning (1963) clearly makes this distinction by relating the 'clock' itself to the 'hands of the clock' which are the observed phenomena or the overt expressions of the fundamental oscillation.

Oscillations with 24-hr periods can only be called 'clocks' if they are actually used to measure time. Respiration and feeding rhythms do not measure time but are an indirect expression of basic cellular processes. The selective advantage of biological clocks is that they provide the mechanism whereby organisms continuously regulate their physiological activities, even in the absence of external time cues which rely on the earth's rotation.

The intrinsically higher respiration and feeding near dawn and dusk has an important implication to energy flow within many zooplankton populations. At dawn and dusk migrating plankton are often exposed to increased temperature and higher food concentrations which are associated with increased respiration and feeding rates. Inherent rhythms in feeding and respiration will reinforce this exogenous tendency for maximized rates near the surface. A distinct energetic advantage is achieved when zooplankton feed near the surface at dawn and dusk then migrate into deeper, colder waters during the day; the energy in excess of requirements for basal metabolism can be efficiently utilized for increased fecundity, development rates, and production, by virtue of both lower temperatures and inherent minimum energy expenditures during midday (McLaren, 1963).

A major significance of diel rhythms is to experimental design - large errors may result from ignoring intrinsic periodicities. Frequently oxygen consumption and feeding rates of zooplankton are expressed on a daily basis without consideration of possible diel differences. Rates determined at one time of day are often extrapolated to calculate daily values. Examples of the resultant errors in estimating oxygen consumption and feeding rates are given in Table 1-3 in which integrated daily rates are compared with extrapolated rates determined from 4-hr experiments conducted at midday.

TABLE 1-3

Total daily respiration and feeding rates of zooplankton under constant conditions. Actual rates, integrated over 24 hrs are compared with extrapolated daily rates based on 4-hr experiments conducted near midday (1000 - 1400 hr). Respiration rates are expressed as µl O_2/mg dry wt/day. Feeding rates are expressed as μ^3 algal biomass/ml/mg dry wt/day.

Sampling Area	Temp	Parameter Measured	Extrapolated Daily Rate	Integrated Daily Rate	% Error
Eunice	10 C	Resp	583.2	875.3	33.4
Eunice	21 C	Resp	190.2	255.7	25.6
Eunice	22 C	Resp	209.4	304.2	31.2
Babine	13 C	Resp	474.0	561.4	15.6
Eunice	21 C	Feed	150.0	537.4	72.1
Deer	10 C	Feed	739.2	1344.4	45.0
Deer	20 C	Feed	1454.4	2304.0	36.9

Extrapolated respiration rates on the average underestimate total daily oxygen conusmption by more than 25%; the underestimate of feeding by extrapolation ranged from 36.9 to 72.1%. Errors of this magnitude become very important when extrapolated rates, such as those determined by Richman (1958, 1964), provide the basis for further calculations of energy transformation in zooplankton populations.

Chapter 2

THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE AMPLITUDE OF ZOOPLANKTON RESPIRATION AND FEEDING IN THE LABORATORY

INTRODUCTION

Diel changes in environmental parameters, such as light or temperature, may affect feeding and respiration rhythms of zooplankton in two distinct ways. They may act as cues that maintain synchrony between endogenous rhythms and the exogenous time scale. In other words, the environment may affect the phase of rhythms. These diel changes may also influence the amplitude of respiration and feeding rhythms with the result that rates are increased at certain times of the day and lowered at other times relative to the endogenous rhythm under constant conditions. In this chapter I describe the effects of various environmental factors on the amplitude of respiration and feeding. Some consideration was also given to the persistence of endogenous rhythms under a variety of light environments and the possible roles of spectral composition and twilight duration on the maintenance of rhythmic phenomena.

Light and temperature are the dominant factors which vary in a consistent diel fashion and their effects on feeding and respiration rates will be considered in detail. A number of other variables require consideration since they affect rates of respiration and feeding measured in the laboratory and the subsequent correction of rates to standard conditions.

Temperature is an important variable in the environment of zooplankton, particularly in the case of migratory forms where temperature changes of 5 to 10 C are possible during diel vertical migrations. Non-migratory zooplankton are not exposed to significant periodic temperature fluctuation; however, the '*in situ*' temperature affects their metabolic rate and possibly their response to light. The effects of temperature on respiration rates of zooplankton are well documented (Comita, 1968), oxygen

consumption normally increasing with temperature. Several workers have also shown that the feeding rate of zooplankton is positively correlated with temperature (Burns, 1969; Schindler and Comita, 1966).

The effects of light intensity and spectral composition on zooplankton feeding and respiration are poorly documented and often contradictory. The majority of workers have been unable to demonstrate significant differences in feeding and respiration due to light intensity (see for example, McMahon, 1965; Schindler, 1968). Several authors have suggested that *Calanus* has higher respiration and feeding rates in darkness (Gauld, 1951; Marshall and Orr, 1955). Moshiri *et al.* (1969), on the other hand, have shown an increase in respiration in light. Buikema (1973) reported highest filtration rates in *Daphnia* at light intensities of 10 $\mu w/cm^2$ and significantly lower rates in darkness and at intensities greater than 160 $\mu w/cm^2$.

There have been few investigations which examine the effects of spectral composition on respiration and feeding rates. The locomotor activity of *Daphnia* varies with spectral composition of incident light (Smith and Baylor, 1953). They noted that *Daphnia* populations remained relatively inactive under red light (> 600 nm) and moved predominantly in a vertical vector, but when exposed to blue light (< 500 nm) the population moved actively in a horizontal vector. Harris and Wolfe (1956) noted that the phototactic responses of *Daphnia* were lost under red light leaving a response which was predominantly photokinetic. Buikema (1973) has shown that with the exception of a small increase in filtration rates of *Daphnia* under blue light, spectral composition did not influence feeding behavior.

Population density, food size, and concentration are factors whose effects must be determined prior to an evaluation of the impact of light and temperature on the amplitude of rhythms. The effects of these factors

on feeding and respiration are well documented, but were examined to provide a basis for correction of all laboratory results to standard conditions.

There are few environmental factors which affect the timing of the phases of a rhythm under natural conditions; one or more features of the day:night cycle are the most common Zeitgebers (Harker, 1964). Since the relative rate of intensity change (dI/Idt) has been shown to affect the vertical migration of *Daphnia* (Ringelberg, 1961), rate of intensity change, manifested as twilight duration, was the factor considered in the present investigation. Under natural conditions, changes in spectral composition at dawn and dusk, photofraction (hours daylight/hours darkness), threshold intensities, or any combination of these factors may act as a Zeitgeber or affect the phases of rhythmic phenomena. For example, a relative intensity change from dim to bright light has been shown to set the phase of chromatophore rhythms in the fiddler crab, *Uca*, providing the photofraction exceeds one hour of darkness (Brown *et al.*, 1950, 1954). In another case, a light flash of 5×10^{-4} sec duration was shown to be sufficient to set the phase of eclosion rhythms in *Drosophila* (Harker, 1964). With the exception of the effects of rate of intensity change on the vertical migration of *Daphnia* (Ringelberg, 1961, 1964), the role of environmental periodicities on the phase and maintenance of diel rhythms in zooplankton is unknown.

MATERIALS AND METHODS

Study Area and Collection of Samples

Zooplankton were collected from Eunice and Deer Lakes in the manner outlined in the previous chapter. Unless otherwise stated, all studies were conducted with a mixed-species sample of the zooplankton. After

collection, zooplankton were returned to the laboratory in darkened 5-gal plastic carboys and kept under constant temperature and darkness for 18 - 24 hrs.

Measurement of Feeding Rate of Zooplankton

Feeding rates were measured in a manner similar to that described in Chapter 1. To determine the effect of zooplankton abundance on weight-specific feeding rate, 10^5 cells/ml of *Chlamydomonas reinhardtii* were added to 12 darkened 250-ml BOD bottles and *Diaptomus kenai* with a biomass ranging from 2 - 115 mg dry wt/l added to 10 of the bottles. Two controls without zooplankton were used and the experiment was conducted at 20 C for 4 hr. Streptomycin sulphate (100 mg/l) and Penicillin G (50 mg/l) were used to inhibit bacterial growth.

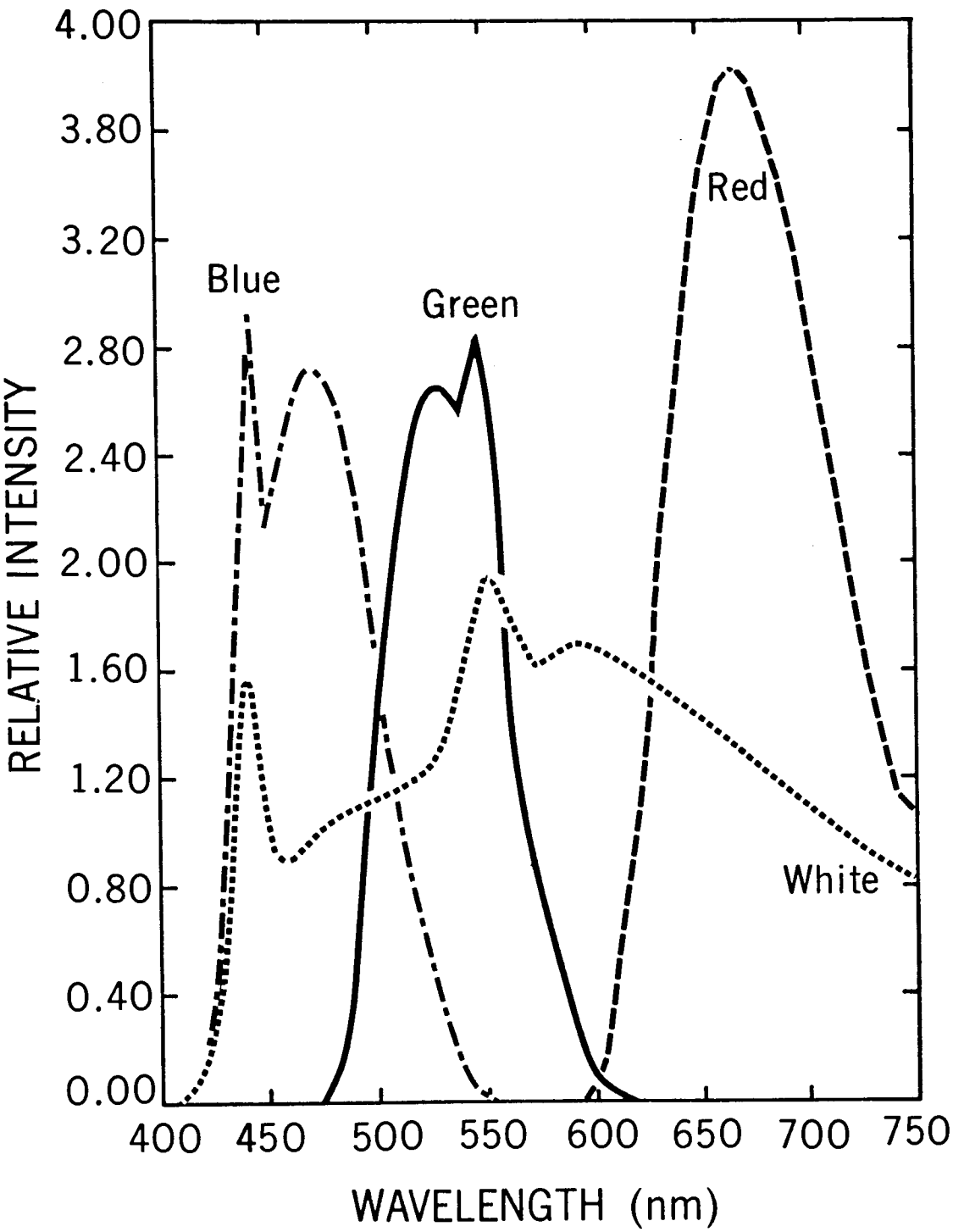
To determine the effect of food particle size on feeding, *Daphnia pulex* were provided with food in which the diameter of individual cells ranged from 1.3 to 90 μ. Suspensions of equal total biomass (μ^3 ml^{-1}) between food types were prepared using the following food sources: *Escherichia coli* (1.3 μ length), *Chlorella ellipsoidea* (3.3 μ diam.), *Chlamydomonas reinhardtii* (10.4 μ diam.), pecan (45 μ diam.), and corn (90 μ diam.) pollen. Cells were suspended in dechlorinated water previously filtered through a Millipore HA filter (47 mm, pore diam. 0.45 μ). Approximately 150 *Daphnia* were transferred into each of six 250-ml BOD bottles for each of the five foods; two controls, without zooplankton, were used in each case. In another experiment, equal biomass of the five food sources were combined. Experiments of 4-hr duration were conducted in the dark at 20 C. Cell numbers were determined at the conclusion of the experiments with a Coulter Counter equipped with either a 100 μ or 200 μ aperture tube.

The feeding rates of Eunice Lake zooplankton were examined at eight concentrations of *Chlamydomonas* ranging from 1.4×10^4 to 1.2×10^6 cells/ml. The desired cell concentrations were prepared by suspending the algae in Millipore filtered, dechlorinated water. Two replicates containing approximately 150 zooplankton were used at each concentration and weight-specific feeding rates determined in each case. A similar experiment was conducted using *D. pulex* from Deer Lake; four replicates were used at each of 10 concentrations from 2.8×10^2 to 1.4×10^6 *Chlamydomonas* cells/ml.

The effect of temperature on feeding rates in *D. pulex* was determined at 5 C intervals from 5 C to 25 C after 24-hr acclimation. Six replicates were used at each temperature and all experiments were conducted in the dark for 4 hr using *Chlamydomonas* (10^5 cells/ml) as a food source.

Two studies were conducted to determine the effects of light intensity and spectral composition on the feeding rates of zooplankton. In the first study, zooplankton collected from Eunice Lake (predominantly *Holopedium gibberum* and *Daphnia rosea*) were kept under constant darkness at 22 C for 18 hr, and then transferred to clear 250-ml BOD bottles containing *Chlamydomonas*. The various light quality regimes were achieved by placing Percival coloured plexiglass filters between 4-20 W Durotest Vitalite fluorescent lamps. The spectral composition of each source (Fig. 2-1) was measured with the system described by Duval *et al.* (1973a). Intensities were reduced to 20 - 40 $\mu w/cm^2$ with neutral-density filters. Feeding rate was measured for 4 hr under red, green, blue, and white light; six zooplankton samples and two controls were used for each quality. Rates determined in darkness served as a control. A similar experiment was conducted at 12 C with *Cyclops scutifer* collected from Deer Lake. In this case, the effect of spectral composition was examined at eight intensities from 0.1 to 1000 $\mu w/cm^2$.

Figure 2-1 Transmittance spectra of light energy from 4-20 W Durotest Vitalite fluorescent lamps passing through the plexiglass and/or neutral density filters used in the light quality studies.


4.00
3.80
3.20
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0.00
RELATIVE INTENSITY
Blue
Green
Red
White
400
450
500
550
600
650
700
750
WAVELENGTH (nm)

Layers of plastic film (Letraset Letratone LT-7) were used as neutral-density filters.

In each study, feeding rates were standardized to values expected with a zooplankton biomass of 50 µg dry wt/ml by least squares linear regression; individual corrections were applied for each experiment.

Measurement of the Respiration Rate of Zooplankton

The effects of the same parameters on respiration rates were examined in zooplankton collected from Deer and Eunice Lakes. Unless otherwise indicated, oxygen concentration was determined by the Micro-Winkler technique described in the previous chapter.

To test the effect of zooplankton abundance on weight-specific oxygen consumption, respiration rates of *Diaptomus kenai* from Eunice Lake were measured in 20 samples ranging in biomass from 15-115 mg dry wt/1. Streptomycin sulphate (100 mg/1) was added to samples to inhibit bacterial respiration.

The effect of phytoplankton concentration on oxygen consumption was determined using Eunice Lake zooplankton. The experiments were conducted at 20 C using *Chlamydomonas* in suspensions of 1.4×10^4 to 1.2×10^6 cells/ml. Two replicates with approximately 150 zooplankton in each bottle were used at each of 10 algal concentrations.

The effect of temperature on the oxygen consumption of *Daphnia pulex* was examined after 24-hr prior exposure of six zooplankton samples to each experimental temperatures ranging from 5 C to 25 C.

Experiments were conducted to determine the combined effects of light intensity and spectral composition on the weight-specific oxygen consumption of two species of zooplankton at different temperatures. In the first study, the respiration rate of *D. pulex* collected from Deer Lake was examined under

red, blue, and green light, at 5 and 12 C. Oxygen consumption was determined with a Gilson Differential Respirometer after the animals had been kept in darkness for 24 hr at the experimental temperature.

In another study, the respiration rate of *Diaptomus kenai* from Eunice Lake was determined under red, blue, green, and white light, at intensities from 1 - 100 $\mu w/cm^2$ and at three temperatures: 5, 10, and 15 C. All experiments were conducted for 4 hr in 250-ml BOD bottles after a 24-hr dark period at the experimental temperature. Dissolved oxygen concentrations were determined using the spectrophotometric technique of Duval *et al.* (1973b). Oxygen consumption of *Daphnia pulex* was examined under the same colours and intensities of 1 - 1000 $\mu w/cm^2$.

Respiration rates were corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml by the method previously described.

Measurement of Short-Term Changes in the Respiration Rate of Zooplankton (Phase Studies)

Diel rhythms in the oxygen consumption of Deer Lake zooplankton (predominantly *Daphnia pulex* and *Cyclops scutifer*) were determined for one week periods with a continuous flow respirometer/data acquisition system. Zooplankton samples were enclosed in a 7-l perspex vessel and aerated dechlorinated water flushed through the system at a flow rate of 300 ml/min. The apparatus and zooplankton were kept at constant temperature (15 C) in an environmental chamber equipped with a twilight simulation system. Light intensities were maintained at 1500 $\mu w/cm^2$ by a negative-feedback control system and temperatures continuously monitored with five thermistors placed throughout the system. Twilight duration was adjustable from 1 to 120 min with an electronic dimmer; the rate of intensity change followed the

exponential pattern characteristic of natural twilight transitions. Respiration rates were determined at 16-min intervals by comparing dissolved oxygen concentrations at each side of the respirator with four YSI Model 5331 oxygen probes placed in perspex holders. Five 1-sec readings were taken from each probe by a programmed data acquisition timer utilizing two YSI Model S3 Oxygen Monitors and punched on paper tape in ASCII code by a Datex DSR-1 data acquisition system. Further details of the system are described by Duval *et al.* (in prep.)

Each investigation was of one-week duration and examined the effects of a specific aspect of the light:dark cycle on the phase of respiration rhythms. In the first two studies, the persistence of diel rhythms was determined under continuous darkness and continuous light.

Three experiments were designed to examine the effects of twilight duration on the maintenance of respiration rhythms; twilight durations of 1, 60, and 120 min were tested. In each study, the zooplankters were kept in continuous darkness for two days before the light:dark cycle (L:D = 12:12) was initiated. The timing of the light:dark cycle was delayed 6 hr (90° phase shift) from the natural photoperiod to test the ability of zooplankton to resynchronize their endogenous rhythms to the altered light regime.

The role of red (> 650 nm) and blue (< 500 nm) light in the maintenance of respiration rhythms was also examined. In these experiments a 12L:12D photoperiod with 120-min twilight was given 6 hr out of phase with the natural light:dark cycle after two days continuous darkness.

RESULTS

The Effects of Temperature on the Feeding and Respiration Rates of Zooplankton

Temperature had a significant effect ($P < 0.001$) on the biomass of *Chlamydomonas* consumed by *Daphnia pulex* from Deer Lake. Feeding rates were highest at 15 C and decreased at temperatures above and below this point (Fig. 2-2). The range of cell sizes consumed also varied with temperature; the greatest range of cells was consumed at 15 C, the smallest at 5 and 25 C (Table 2-1).

The oxygen consumption of *D. pulex* increased in a linear manner with temperature; rates at 25 C were eight times higher than at 5 C (Fig. 2-3). Q_{10} calculated for temperatures between 5 - 15 C, 10 - 20 C, and 15 - 25 C were 4.6, 2.2, and 1.7 respectively.

The Effects of Spectral Composition and Light Intensity on the Feeding and Respiration Rates of Zooplankton

Spectral composition had a significant effect ($P < 0.001$) on the biomass of phytoplankton consumed by Eunice Lake zooplankton under approximately equal intensities (20 - 40 $\mu w/cm^2$) of red, blue, green, and white light. The biomass of algae consumed by the cladoceran-dominated samples were as follows:

LIGHT QUALITY	FEEDING RATE μ^3 biomass/ml/mg dry wt/hr x 10^3
Red	7.5 ± 0.8
Green	13.4 ± 0.8
Blue	23.7 ± 3.3
White	8.0 ± 0.5
Dark	11.8 ± 1.8

TABLE 2-1

The effect of temperature on the range of particle sizes consumed by *Daphnia pulex*. All investigations were conducted with a food concentration of 10^5 cells/ml. Feeding rates corrected to values expected with a zooplankton biomass of 50 µg dry wt /ml.

Temperature	Size Range Consumed µ diameter	Mean Cell Volume of Cells Consumed (μ^3)
5	1.3 to 2.6	2.4
10	1.3 to 3.3	4.5
15	1.3 to 10.4	8.0
20	1.3 to 2.6	2.0
25	1.6 to 2.6	2.0

Figure 2-2 The effect of temperature on the feeding rate of *Daphnia pulex* collected from Deer Lake. All rates are corrected to values predicted with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

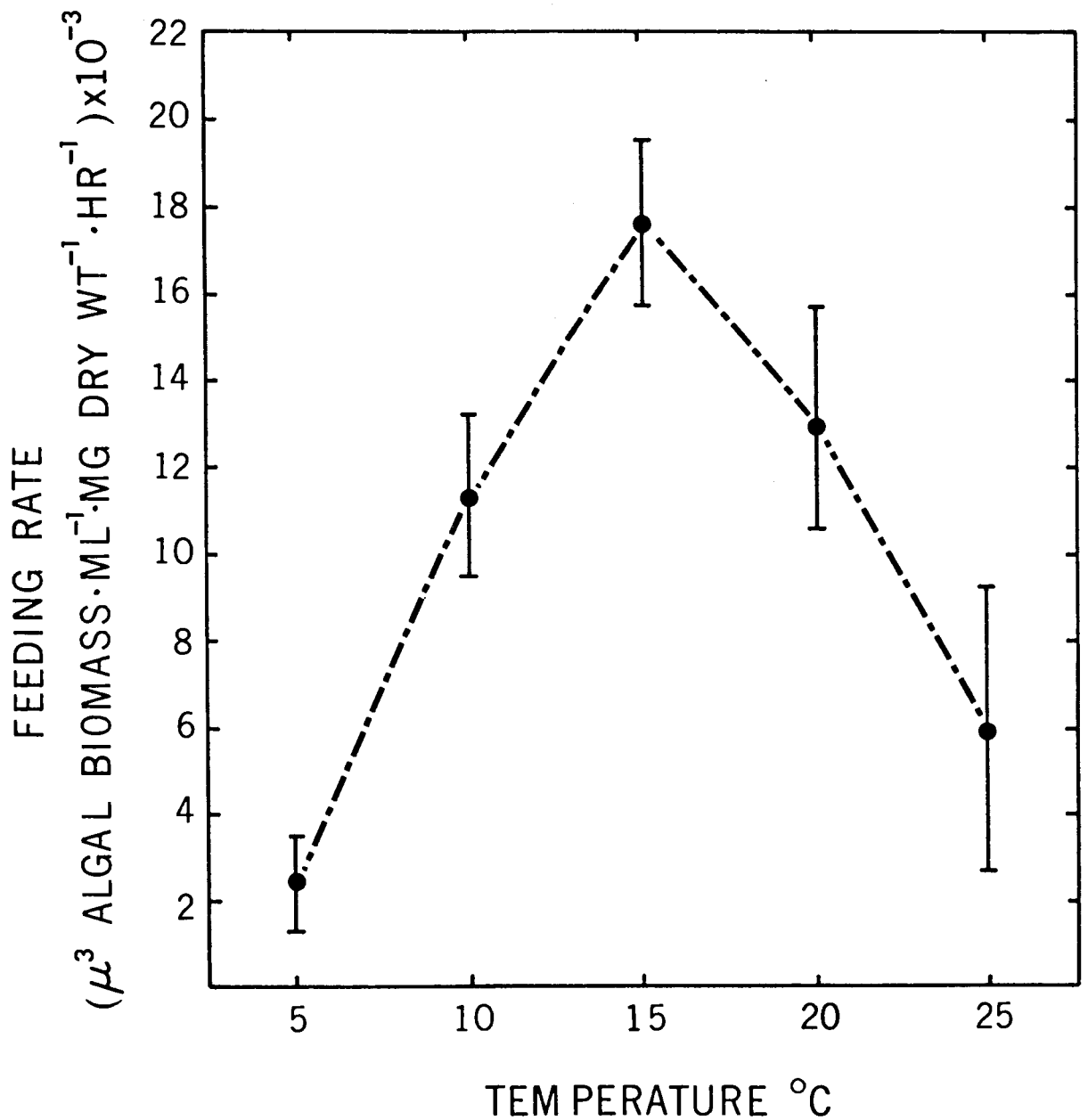
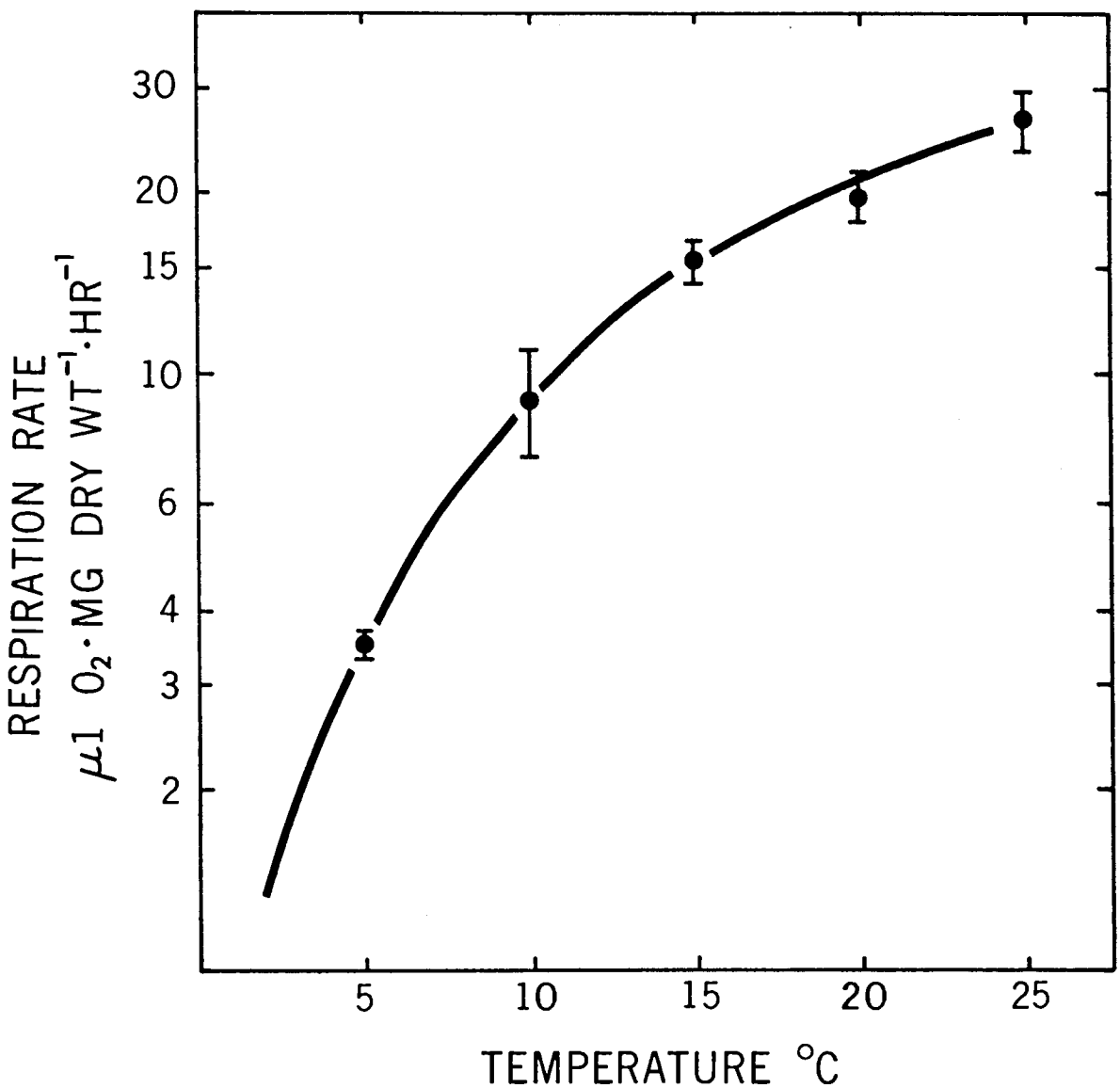

FEEDING RATE
(μ^3 ALGAL BIOMASS·ML^{-1}·MG DRY WT^{-1}·HR^{-1})x10^{-3}
22
20
18
16
14
12
10
8
6
4
2
5
10
15
20
25
TEMPERATURE °C

Figure 2-3 The effect of temperature on the respiration rate of *Daphnia pulex* collected from Deer Lake. All rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.


RESPIRATION RATE
μl O2·MG DRY WT-1·HR-1
30
20
15
10
6
4
3
2
5
10
15
20
25
TEMPERATURE °C

The feeding rate of *Cyclops scutifer* from Deer Lake was dependent on both spectral composition and intensity of light. Feeding rate generally decreased with increasing light intensity under all qualities (Fig. 2-4). Rates at intensities less than 1 $\mu w/cm^2$ were 50 - 90% higher than at intensities over 1000 $\mu w/cm^2$; the greatest percentage change was found in blue light, the least in green. The feeding rate of *Cyclops* was significantly higher in white light ($P < 0.001$). There were no significant differences ($P > 0.05$) in the rates determined in red, blue, or green light except at intensities less than 10 $\mu w/cm^2$. At low intensities (< 10 $\mu w/cm^2$) feeding rates were higher in red and white light than in either blue or green light.

Both spectral composition and intensity influenced the respiration rate of *D. pulex* from Deer Lake. There was a significant interaction between light intensity and temperature ($P < 0.001$); at intensities greater than 1 $\mu w/cm^2$ respiration rates in all spectral compositions were lower at 12 C than at 5 C; 12 C rates exceeded 5 C values at intensities less than 1 $\mu w/cm^2$ (Fig. 2-5). At 12 C, the highest rate of oxygen consumption was found in low intensity (< 1 $\mu w/cm^2$) blue light. At 5 C, rates determined under blue light were significantly higher than red and green rates at all intensities ($P < 0.01$) whereas oxygen consumption did not vary significantly between red and green light; maximum respiration rates were found at the intermediate intensity (2.1 - 3.4 $\mu w/cm^2$) in all light regimes.

The oxygen consumption of *Daphnia pulex* at 22 C was also dependent on both intensity and spectral composition. Respiration rates in blue light at intensities from 1 - 30 $\mu w/cm^2$ were highest; maximum rates were found at 8 $\mu w/cm^2$, more than 70% higher than in white light of equal intensity (Fig. 2-6). Spectral composition did not influence the respiration rate of this cladoceran at intensities greater than 500 $\mu w/cm^2$. At intensities less than 30 $\mu w/cm^2$

Figure 2-4 The effect of light intensity and spectral composition on the feeding rate of *Cyclops scutifer* collected from Deer Lake. Experiments conducted at 12 C and all rates are corrected to values predicted with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

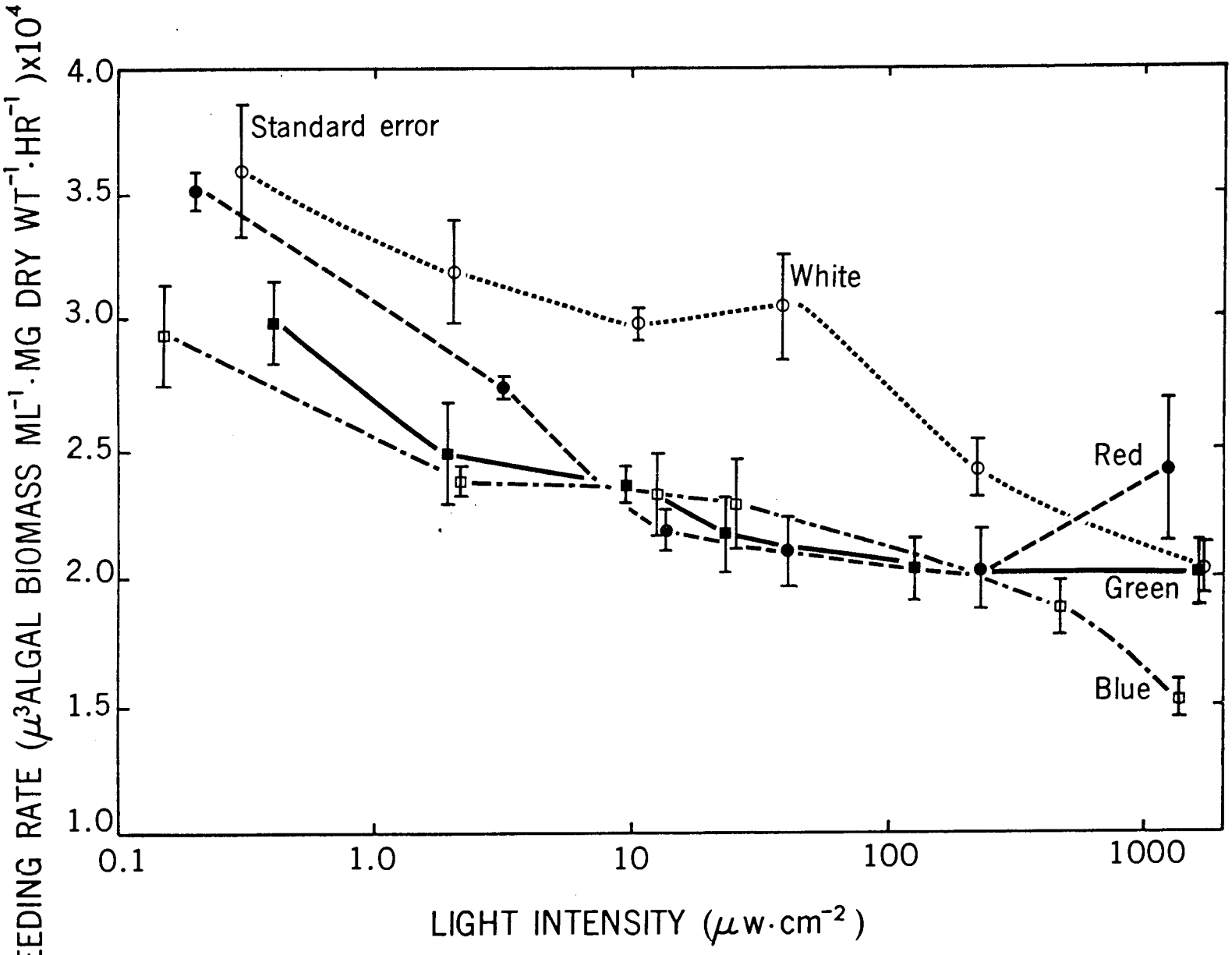

FEEDING RATE (μ^3ALGAL BIOMASS $ML^{-1}\cdot MG\ DRY\ WT^{-1}\cdot HR^{-1}$)x$10^4$
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Standard error
White
Red
Green
Blue
0.1
1.0
10
100
1000
LIGHT INTENSITY ($\mu w\cdot cm^{-2}$)

Figure 2-5 The effect of light intensity, spectral composition, and temperature on the respiration rate of *Daphnia pulex* collected from Deer Lake. All rates corrected to values predicted with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated. Upper graph (A) = 12 C, lower graph (B) = 5 C. Intensity ranges were as follows:

I = 0.9 - 1.3 $\mu w/cm^2$

II = 2.1 - 3.4 $\mu w/cm^2$

III = 16.3 - 22.9 $\mu w/cm^2$

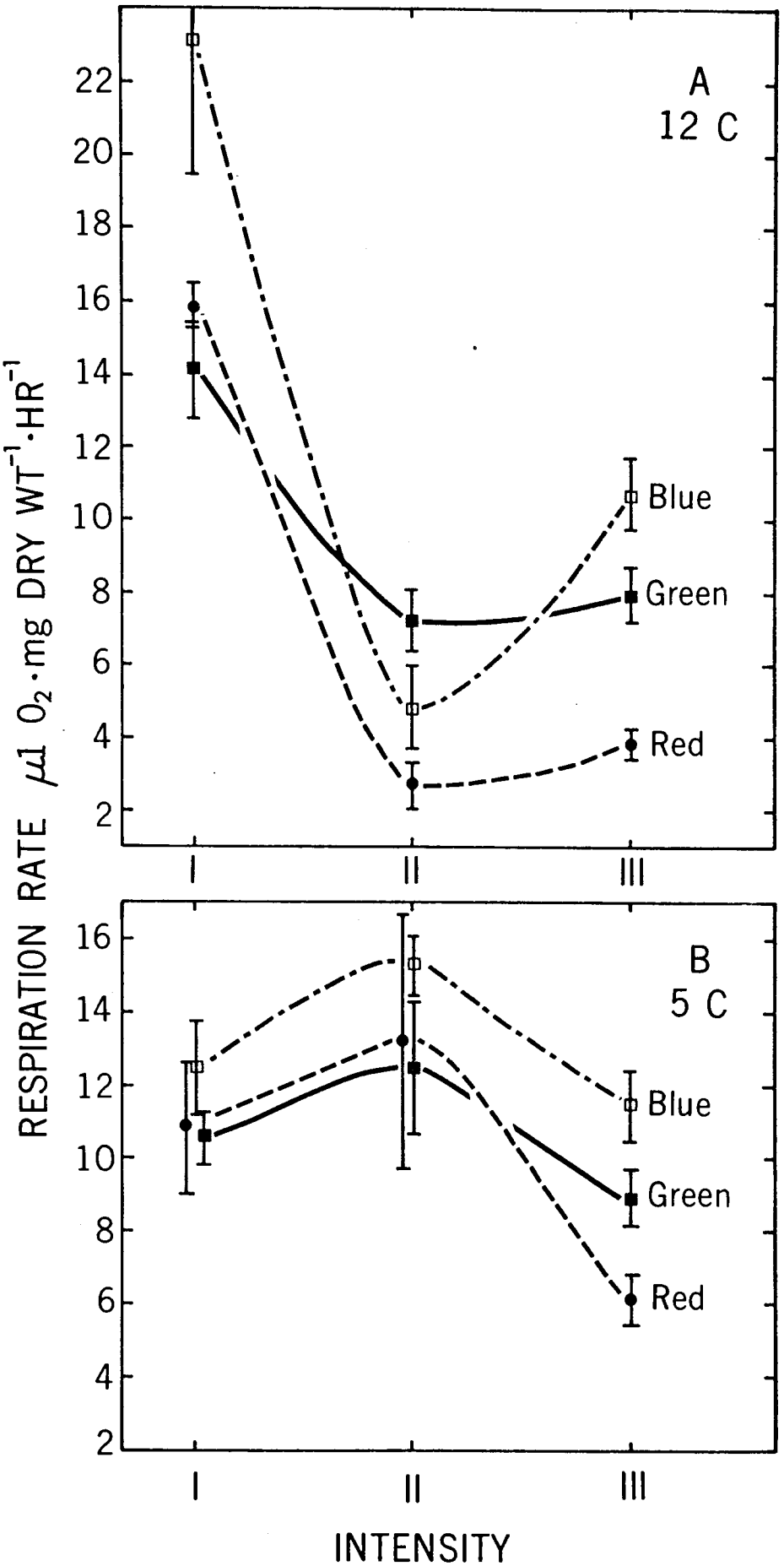
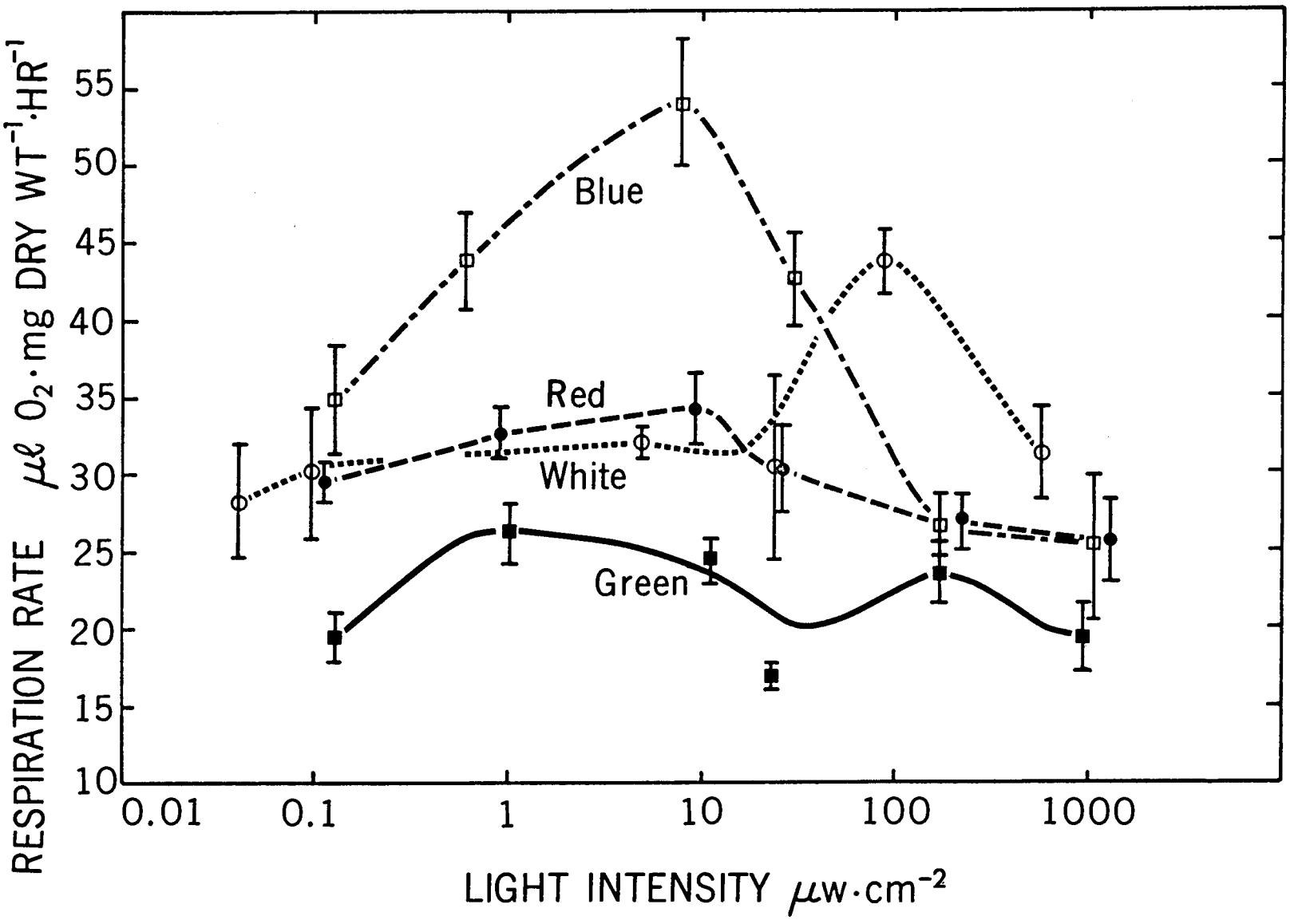

A
12 C
B
5 C
Blue
Green
Red
RESPIRATION RATE μl O2 · mg DRY WT-1 · HR-1
INTENSITY
I
II
III
2
4
6
8
10
12
14
16
18
20
22

Figure 2-6 The effects of light intensity and spectral composition on the respiration rate of *Daphnia pulex* at 22 C. All rates are corrected to values predicted with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.


RESPIRATION RATE $\mu\ell$ O_2·mg DRY WT^{-1}·HR^{-1}
55
50
45
40
35
30
25
20
15
10
Blue
Red
White
Green
0.01
0.1
1
10
100
1000
LIGHT INTENSITY $\mu w \cdot cm^{-2}$

there was no significant intensity effect in blue light. In general, respiration rates were lowest in green light, approximately equal in red and white light, and highest in blue light, although oxygen consumption at intensities near 100 $\mu w/cm^2$ was significantly higher ($P < 0.01$) in white light (Fig. 2-6).

The respiration rate of the copepod *Diaptomus kenai* was examined under eight light intensities between 1 and 100 $\mu w/cm^2$, in four colours, and at 5, 10, and 15 C. The results are presented in Table 2-2 and summarized below.

A. Temperature = 5 C

1. Light intensity had no significant effect on respiration rates in red and green light ($P > 0.05$).
2. In blue light, oxygen consumption was inversely related to light intensity; rates at 2.5 $\mu w/cm^2$ were 2.2 times greater than those at 60 $\mu w/cm^2$.
3. Respiration rates in white light were highest at 10 $\mu w/cm^2$, five times greater than at 2.5 $\mu w/cm^2$.
4. At intensities less than 10 $\mu w/cm^2$, rates were highest in red light, not significantly different between blue and green light, and lowest in white light ($P > 0.05$).

B. Temperature = 10 C

1. Light intensity had no significant effect ($P > 0.05$) on respiration rates in red and green light except at intensities less than 2.5 $\mu w/cm^2$.
2. In both blue and white light, minimum respiration rates were found at intensities of 5 $\mu w/cm^2$, increasing at intensities above and below this level.

TABLE 2-2

The effects of light intensity, spectral composition, and temperature on the respiration rate of *Diaptomus kenai* collected from Eunice Lake. All rates corrected to values predicted with a zooplankton biomass of 50 µg dry wt/ml. Units = µl O_2/mg dry wt/hr. Mean and standard error indicated.

TEMP	SPECTRAL COMP	LIGHT INTENSITY (µw/cm^2) 0.1	1	2.5	5	10	25	50	100
5 C	Red	17.0 1.4	18.2 0.1	17.3 1.0	14.6 0.3	15.0 0.6	16.7 1.6	16.1 0.6	15.9 1.2
	Blue	12.8 0.6	16.9 0.5	--	14.3 0.6	11.2 1.3	--	7.9 0.4	7.8 0.6
	Green	14.9 1.7	14.4 1.5	14.9 1.8	15.3 0.7	18.2 0.8	--	12.4 0.2	11.1 0.8
	White	7.5 0.9	2.6 0.6	4.3 0.7	11.4 1.3	14.5 0.8	13.7 2.2	11.2 1.3	10.7 1.6
10 C	Red	22.6 1.6	16.2 2.6	11.9 1.8	13.4 1.3	15.3 0.3	14.0 1.9	15.4 0.7	13.4 0.7
	Blue	--	14.4 0.6	6.4 0.8	11.1 1.3	13.9 1.9	8.1 0.6	7.9 0.4	9.6 1.0
	Green	16.0 0.8	14.4 2.2	14.7 1.1	14.8 1.4	12.5 1.0	11.7 1.8	15.0 1.6	13.6 2.6
	White	6.4 0.7	--	5.5 0.8	8.9 0.7	14.9 2.2	--	13.3 1.6	13.0 1.9
15 C	Red	28.3 0.6	31.4 0.9	29.2 1.3	29.8 0.9	25.6 0.6	27.2 0.4	22.8 0.8	26.5 1.2
	Blue	23.2 1.5	25.0 0.5	21.0 2.3	24.2 0.7	23.8 1.1	24.3 1.6	--	20.7 2.7
	Green	24.1 4.2	--	23.8 0.6	25.2 0.5	24.0 2.7	21.0 1.7	22.1 1.6	21.2 1.8
	White	25.2 1.5	25.4 1.1	25.7 0.1	29.0 0.7	24.8 1.5	23.8 0.8	30.2 0.2	25.1 1.1

3. At intensities greater than 10 $\mu w/cm^2$ there were no significant differences ($P > 0.05$) in rates observed in red, white, or green light; all rates were significantly greater ($P < 0.01$) than oxygen consumption in blue light.
4. At light intensities less than 10 $\mu w/cm^2$, respiration rates were highest in red and green light.

C. Temperature = 15 C

1. In general, oxygen consumption of *D. kenai* at 15 C was 80% higher than values determined at either 5 or 10 C.
2. Light intensity had no effect on rates in green, blue, or white light; a significant intensity effect was noted under red light where oxygen consumption was inversely related to intensity above 2.5 $\mu w/cm^2$.
3. There were no significant differences in respiration rate in blue, green, or white light at intensities below 30 $\mu w/cm^2$; oxygen consumption in red light always exceeded values found in other colours.

The Effects of Crowding, Food Concentration, and Food Size on the Feeding and Respiration Rates of Zooplankton

The effects of various laboratory variables were examined to provide the data necessary to standardize feeding and respiration rates to values expected under comparable conditions in the field. This enabled determination of the results of light and temperature on the amplitude of rhythms independent of lab variables such as population density, food concentration, and food size. The results presented in this section are representative of the types of corrections applied to the data; however, species-specific corrections were determined for each light and temperature experiment.

Feeding rate was inversely related to the biomass of zooplankton/liter (Fig. 2-7). Rates decreased 170 times when the biomass of zooplankton was increased from 2 to 115 mg dry wt/l. Crowding also had a significant effect on the weight-specific oxygen consumption of *Diaptomus kenai* (Fig. 2-8).

Particle size had a significant effect on both the biomass and number of cells consumed by *Daphnia pulex*. The number of cells consumed was inversely related to cell diameter (Table 2-3). The lowest feeding rate was observed with *Chalmydomonas reinhardtii*, less than 1.5% of the rate determined with the bacterium *Escherichia coli*.

Food concentration had a significant effect on the feeding rate of *Diaptomus kenai* and *Daphnia pulex* (Fig. 2-9). The biomass of algal cells consumed increased in a linear manner with food concentration up to 6 - 7 x 10^5 cells/ml depending on the species. Beyond this concentration feeding rate was reduced in both populations. Phytoplankton concentration did not affect ($P > 0.05$) the oxygen consumption of zooplankton.

The Effects of Light on the Persistence and Phase of Respiration Rhythms

Few consistent trends emerged from the studies reported here. In general, significant diel respiration rhythms were observed only on the first and second days of each study, decreasing in amplitude and becoming arrhythmic on the third and successive days. This loss of rhythmicity was most apparent in those investigations having a twilight duration of 120 min, irrespective of spectral composition (Table 2-4). The most pronounced rhythms were observed under continuous light and continuous dark, but even in these studies the timing of the maxima were not consistently correlated to the photoperiod prior to the experiments; in continuous darkness maximum rates were found between 2100 and 2400 hr and 1400 - 1600 hr. Under

TABLE 2-3

The effect of cell size on the feeding rate of *Daphnia pulex* as determined in 4-hr experiments in which each of five food sources were provided in equal total biomass (μ^3)/ml. All rates are corrected to values predicted with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

Food Source	Cell Diam. µ	Biomass Consumed μ^3/ml/mg dry wt/hr x 10^3	Cells Consumed no/ml/mg dry wt/hr
Escherichia coli	1.5	123.0 ± 36.0	4.3 ± 1.0 (x 10^4)
Chlorella ellipsoidea	3.3	5.0 ± 0.6	1.8 ± 0.1 (x 10^3)
Chlamydomonas reinhardtii	10.4	1.8 ± 0.2	1.5 ± 0.1 (x 10^3)
Pecan Pollen	45.0	5.0 ± 0.5	0.2 ± 0.05
Corn Pollen	90.0	15.0 ± 2.4	0.2 ± 0.03

TABLE 2-4

The persistence of diel respiration rhythms under various light regimes. All studies were one week in duration with rates determined at 16-min intervals. Times of each maxima (PST) indicated.

	Continuous Dark	Continuous Light	12L:12D with 1 min Twilight	12L:12D with 60 min Twilight	12L:12D with 120 min Twilight	12L:12D Red Light 120 min Twilight	12L:12D Blue Light 120 min Twilight
Day 1	2240 1600	1900 1300	1735 0135 0700	2400 1100	1300 0300	0200 0700	NR
Day 2	2200 1415	1500 1000	1445 2300 0800	0530 *	NR	0230 0900	NR
Day 3	* 1255	1800 *	1335 2300 0600	2250 *	NR	1730 0530	NR
Day 4	2215 1415	* 0800	1300 2200 0900	2130 *	NR	2100 1000	NR
Day 5	2400 1415	1800 0600	1600 0135 0830	2130 1400	NR	* 0600	NR
Day 6	2115 *	2130 *	1630 * *	* 1130	NR	* 0400	NR
Day 7	2215 0900	NR	NR	NR	NR	NR	NR

* = no discernable maximum

NR = no rhythm

Figure 2-7 The effect of crowding on the feeding rate of *Diaptomus kenai* collected from Eunice Lake. Feeding rate was related to population density by the following linear expression; log FR = - 0.945(log zooplankton biomass/liter) + 8.29, correlation coefficient (r) = - 0.997. Mean feeding rates indicated.

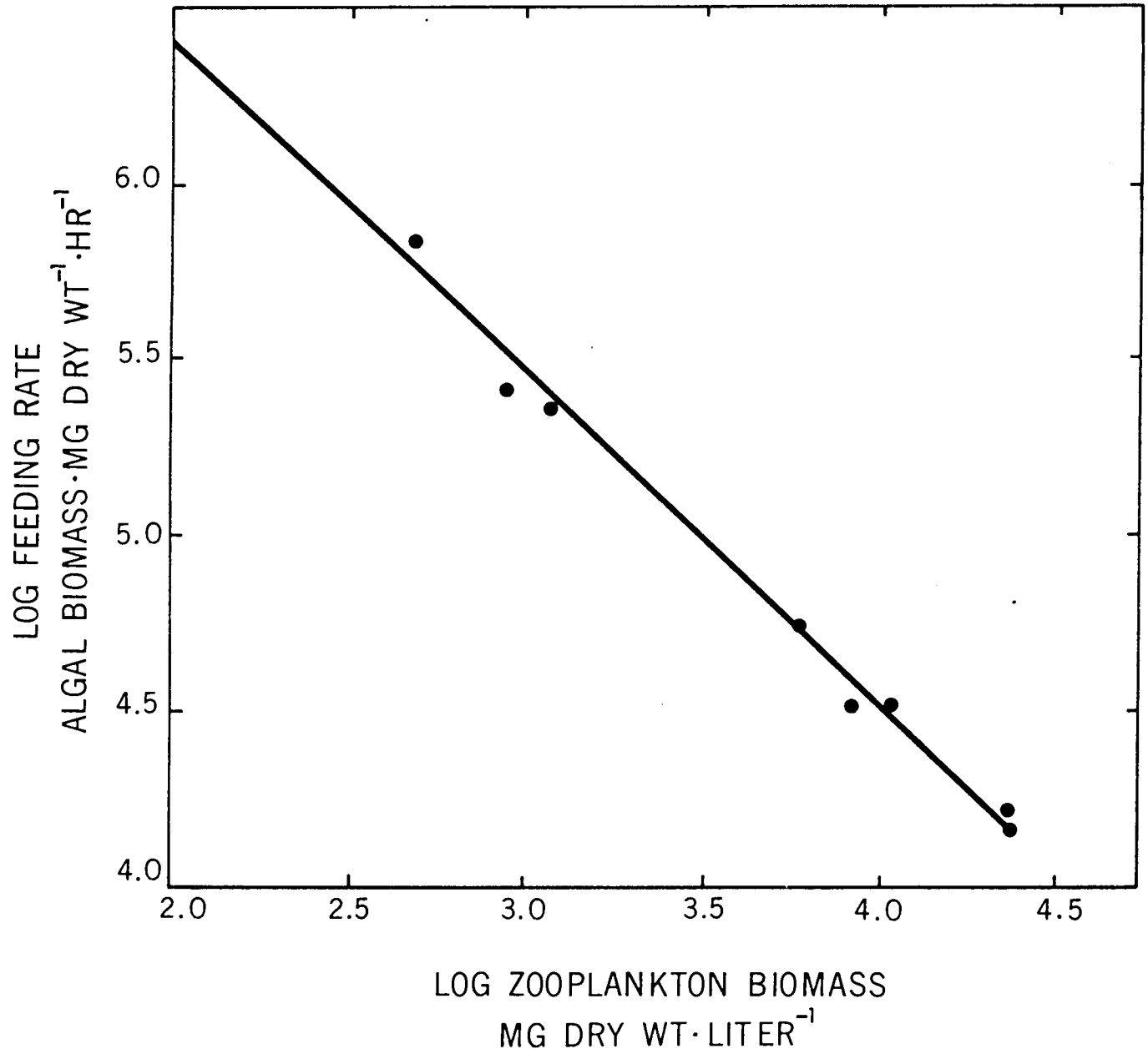

LOG FEEDING RATE
ALGAL BIOMASS·MG DRY WT^{-1}·HR^{-1}
6.0
5.5
5.0
4.5
4.0
2.0
2.5
3.0
3.5
4.0
4.5
LOG ZOOPLANKTON BIOMASS
MG DRY WT·LITER^{-1}

Figure 2-8 The effect of crowding on the weight-specific oxygen consumption of *Diaptomus kenai* collected from Eunice Lake. Respiration rate was related to population density by the following linear expression: log R/S = - 0.837(log zooplankton biomass/liter) + 2.545, correlation coefficient (r) = - 0.920. Mean respiration rates indicated.

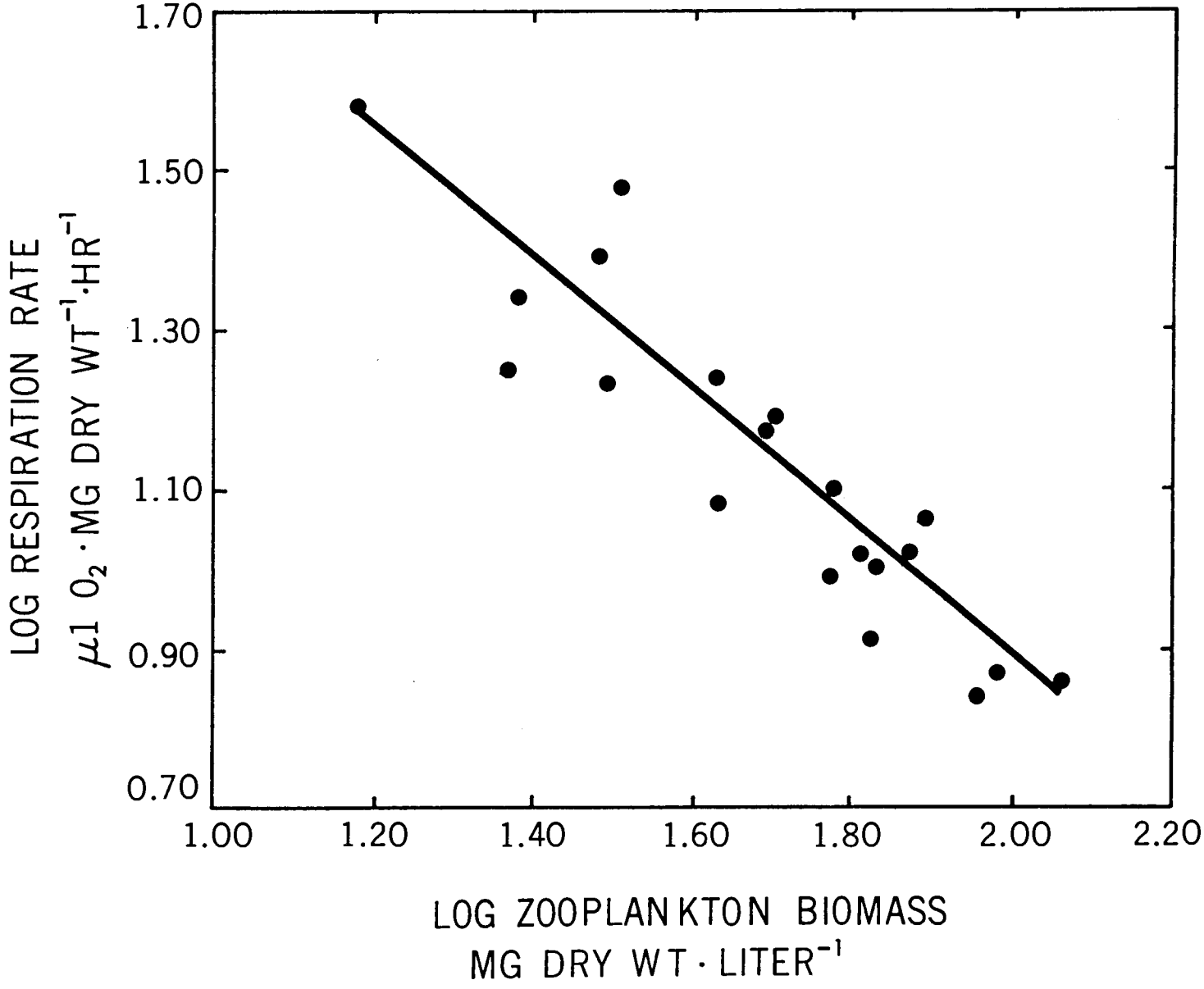
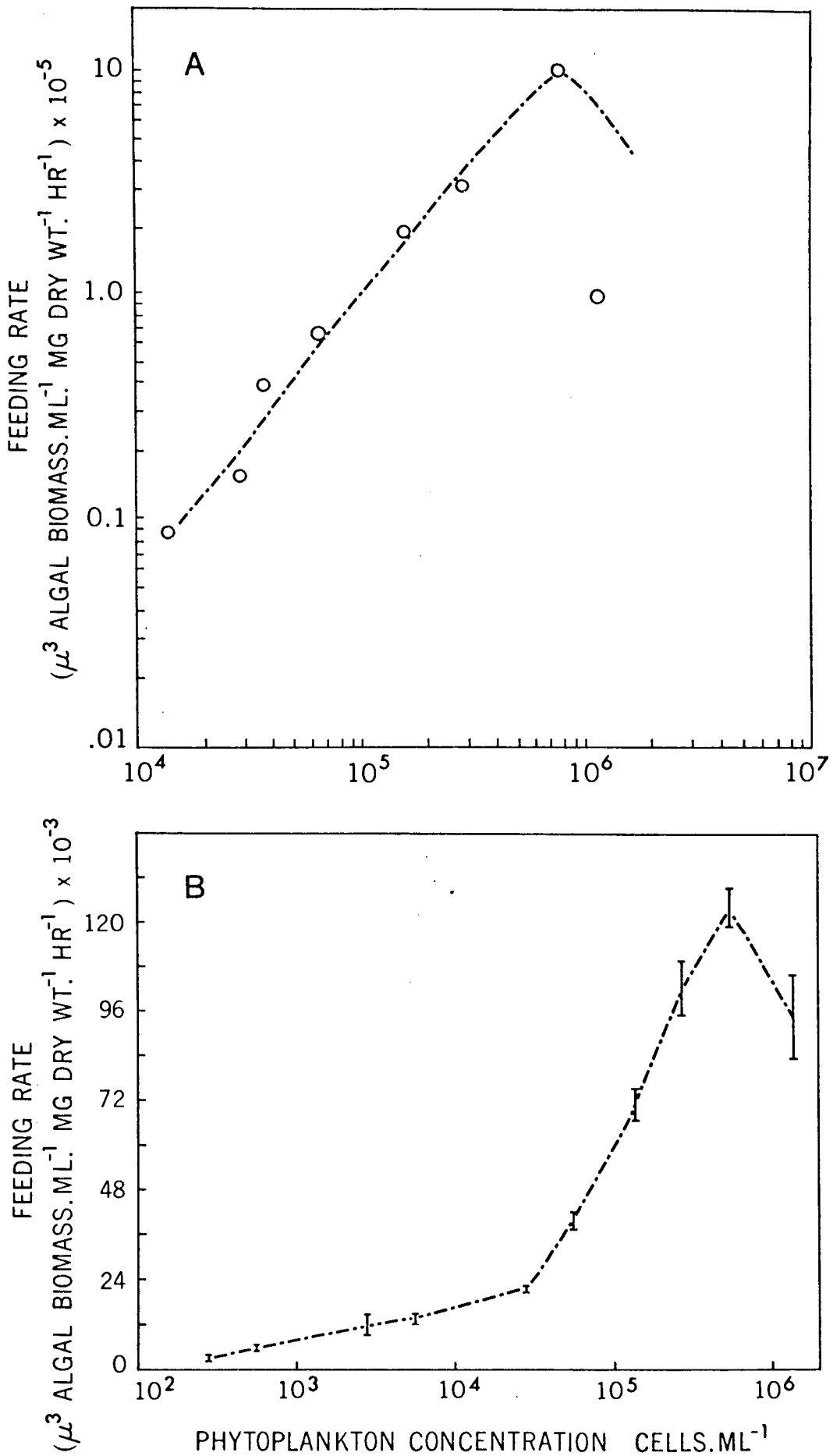

1.70
1.50
1.30
1.10
0.90
0.70
1.00
1.20
1.40
1.60
1.80
2.00
2.20
LOG RESPIRATION RATE
μl O2 · MG DRY WT-1 · HR-1
LOG ZOOPLANKTON BIOMASS
MG DRY WT · LITER-1

Figure 2-9 The effect of phytoplankton concentration on the feeding rate of *Diaptomus kenai* (A) collected from Eunice Lake and *Daphnia pulex* (B) collected from Deer Lake. The food source utilized for the investigation was *Chlamydomonas reinhardtii*. All rates are corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean rates indicated for *D. kenai* (A); mean ± standard error indicated for *D. pulex* (B).


A
B
FEEDING RATE
(μ^3 ALGAL BIOMASS.ML.$^{-1}$ MG DRY WT.$^{-1}$ HR^{-1}) x 10^{-5}
10
1.0
0.1
.01
10^4
10^5
10^6
10^7
FEEDING RATE
(μ^3 ALGAL BIOMASS.ML.$^{-1}$ MG DRY WT.$^{-1}$ HR^{-1}) x 10^{-3}
120
96
72
48
24
0
10^2
10^3
10^4
10^5
10^6
PHYTOPLANKTON CONCENTRATION CELLS.ML^{-1}

continuous light, maxima were observed between 1500 and 1900 hr and from 0600 - 1300 hr, showing no consistent pattern of shift in phase. There were no rhythms observed under either blue or white light when zooplankters were exposed to a photoperiod of 12L:12D with 120 min twilight duration. There was some suggestion of a rhythm in red light, but again the phase of rhythm shifted rapidly and bore little relation to the photoperiod examined.

DISCUSSION

The results presented in this chapter clearly indicate that light intensity, spectral composition, and temperature have a marked influence on the feeding and respiration rates of zooplankton. Consequently, daily changes in these parameters will affect the amplitude of any inherent feeding and respiration rhythms.

Vertically migrating zooplankton are often exposed to diel temperature fluctuations of 5 - 10 C for many months of the year, non-migrating species considerably less. Diel temperature changes can be expected to affect the amplitude of any diel rhythms in migrating species. The relationship between temperature and oxygen consumption demonstrated for *Daphnia pulex* in the present investigation is consistent with responses demonstrated for other zooplankters (Comita, 1968). Respiration rates of *Daphnia* increased with temperature in a curvilinear manner; since the zooplankters were kept at constant temperature for 24 hr prior to each experiment, a lack of acclimation is indicated. This is consistent with the observations of Halcrow (1963) who noted that *Calanus* could only reach an acclimated state after several days exposure to a particular temperature. Consequently, in nature, it is unlikely that the diel temperature changes zooplankton encounter would be accompanied by any significant degree of acclimation.

Thus, in those species in which vertical migrations are associated with a pronounced temperature change, significant effects on respiration rates can be expected. The effect of temperature on the amplitude of inherent diel respiration rhythms can be illustrated by re-examining data from the previous chapter (Fig. 1-1). In a hypothetical situation where zooplankton migrate from 5 C water at midday to 20 C water at dusk, the amplitude of the diel respiration rhythm would be increased four times over that due to the endogenous component alone (Table 2-5). In nature, the increase in amplitude may be even greater due to the simultaneous action of other environmental factors such as light and population density differences throughout the day.

Temperature also affected both the feeding rate and size of cell consumed by *D. pulex*. Maximum feeding rates were observed at 15 C (Fig. 2-2). If filtration rates of *Daphnia* increase linearly with temperature as shown by Burns (1969) and McMahon (1965), the high feeding rates at 15 C are attributable to the much wider range of cell sizes (1.3 - 10.4 μ diam.) consumed at this temperature. Similarly, the observed decrease in feeding rate above 15 C may be a consequence of the smaller range of phytoplankton ingested (1.3 - 2.6 μ diam.). In the case of vertically migrating zooplankters, temperature will increase the amplitude of feeding rhythms in a manner similar to that previously described for respiration.

In non-migratory zooplankton species, temperature will have little impact on the diel variability in either feeding or respiration rhythms since any diel temperature changes will be minimal.

Light intensity and spectral composition also have a marked effect on the behavior and physiology of zooplankton. Exposure of zooplankton to diel changes in either of these parameters will be reflected in relative

TABLE 2-5

The effect of temperature on the amplitude of diel respiration rhythms in a vertically migrating zooplankter. For the purpose of demonstration the effect of temperature shown in the present chapter (Fig. 2-1) is superimposed on the amplitude of the endogenous rhythm observed at a constant temperature (16 C) from the previous chapter (Table 1-1). Respiration rates at midday are given as 1.0 for the endogenous component.

Time	Depth (m)	Temp	Endogenous Rhythm	Diel Rhythm + Temp Effect
Midday	10	5	1.0	0.2
Dawn	1	20	1.1	1.5
Dusk	1	20	1.5	2.1

changes in the amplitude of diel rhythms.

In the present investigation I examined the combined effects of light intensity, spectral composition, and temperature on the respiration and feeding rates of both cladocerans and copepods. Several generalizations emerged from the trends observed in this study.

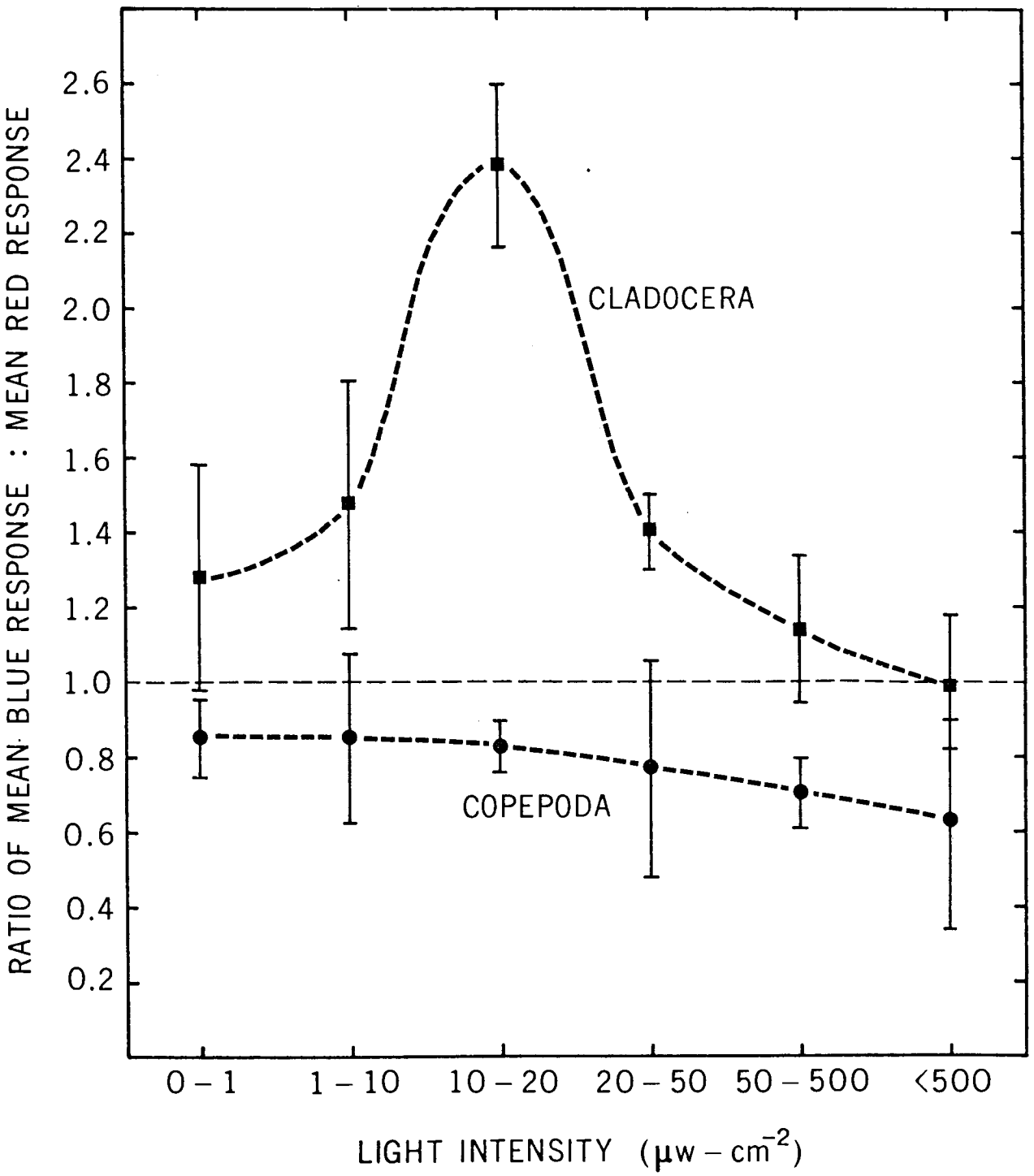
The first point is that spectral composition and intensity affect zooplankton feeding and respiration only at low light intensities; there were no significant colour or intensity effects above light intensities of 500 $\mu w/cm^2$ in either *D. pulex* or *Cyclops scutifer*. Zooplankton are only exposed to these low intensities before sunrise and after sunset; even in the case of the vertically migrating species observed in this study, light intensities exceed 1000 $\mu w/cm^2$ throughout most of the day (Fig. 3-3). Any effects of light intensity or spectral composition would be expressed only at dawn and dusk.

A second generalization is that the effects of light intensity are temperature and colour dependent. In the copepod *Diaptomus kenai*, changes in light intensity had little effect on respiration rates beyond 15 C (Table 2-4). The effects of temperature are not fully understood since a marked intensity effect persists in the cladoceran *Daphnia pulex* at 22 C (Fig. 2-6) and respiration rates at intensities greater than 1 $\mu w/cm^2$ are lower at 12 C than at 5 C (Fig. 2-5). A similar temperature-light interaction was shown by Smith and Baylor (1953) who noted that the characteristic behavioral response of *Daphnia* to blue light was lost at certain temperatures. It is possible that in some situations the effects of temperature may override any light responses, particularly in the case of surface-dwelling species which are exposed to the combination of low intensities and high temperatures at dawn and dusk.

The final generalization concerns the response of zooplankton to spectral composition. At all temperatures and at intensities less than 30 $\mu w/cm^2$ the cladocerans examined showed highest feeding and respiration rates in blue light. In the copepods *Diaptomus kenai* and *Cyclops scutifer*, feeding and respiration rates were highest in red or occasionally white light and lowest in blue. The distinction between the response of the cladocera and copepoda to spectral composition is indicated in Fig. 2-10 which illustrates the ratios of mean feeding and respiration rates in blue light to the corresponding red rates at six intensities; the marked effect of low intensity blue light (10 - 20 $\mu w/cm^2$) on the cladocerans examined is clearly apparent. The high ratios in cladocerans were due to high respiration and feeding rates in blue light rather than unusually low rates in red light. These results are consistent with the observations of Smith and Baylor (1953) who noted that *Daphnia* exposed to blue light became active, whereas populations kept under red light remained relatively inactive.

In studies where comparisons between light and dark were made, respiration and feeding rates between 1 and 30 $\mu w/cm^2$ exceeded dark rates; beyond 100 $\mu w/cm^2$ rates in the light were less than those measured in the dark. This observation may explain many of the conflicting results shown by other workers. For example, Gauld (1951) and Marshall and Orr (1955) found higher respiration and feeding rates in darkness. However, the light intensities used by these authors were well above those normally encountered by *Calanus*. The results of the present investigation are consistent with the observations of Buikema (1973) who has shown that filtration rates of *Daphnia* are highest at a light intensity of 10 $\mu w/cm^2$ and significantly lower in darkness and at intensities greater than 160 $\mu w/cm^2$. Many workers have observed that zooplankton respond to high light intensities by moving to regions of lower

Figure 2-10 Ratios of blue response:red response in the cladocerans and copepods examined. Equal respiration or feeding rates in blue and red light equals unity by definition. Mean values (± standard error) for both feeding and respiration are shown for each of six intensity ranges.


RATIO OF MEAN BLUE RESPONSE : MEAN RED RESPONSE
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
CLADOCERA
COPEPODA
0 – 1
1 – 10
10 – 20
20 – 50
50 – 500
<500
LIGHT INTENSITY (μw – cm⁻²)

intensity (Cushing, 1951); changes in respiration and feeding rate may be another manifestation of this photonegative response.

The most pronounced intensity effect was observed on the feeding rate of the copepod *Cyclops scutifer* (Fig. 2-4) where rates at less than 1 $\mu w/cm^2$ were 50 - 90% higher than at intensities over 1000 $\mu w/cm^2$. Feeding rate was inversely related to light intensity over the range examined (0.1 - 1000 $\mu w/cm^2$), the greatest intensity effect being shown in blue light. A similar relationship between intensity and oxygen consumption was observed for *D. kenai* in blue light at 5 C and in red light at both 10 and 15 C. However, the effect of intensity on respiration and feeding rates was both colour and temperature dependent. In all studies, there was no significant effect of intensity above 15 C in either red or green light; in the case of *D. kenai* this lack of intensity effect in red and green light persisted at 5 and 10 C. In white light, oxygen consumption of *Diaptomus* was exceedingly low when intensities were less than 10 $\mu w/cm^2$ at either 5 or 10 C. The significance of this effect in white light is not known and will require further study for clarification.

Spectral composition had a marked effect on the feeding rates of the cladocerans *Daphnia rosea* and *Holopedium gibberum*. The biomass of phytoplankton consumed was three times higher in blue light than in either red or white light of equal intensity (20 - 40 $\mu w/cm^2$). The fact that rates determined in red and white light were not significantly different and both lower than dark rates suggests that the presence of red light in the visible spectrum may have an inhibitory effect on the activity of cladocerans, overriding the presence of all other wavelengths. The results of the respiration study with *D. pulex* are consistent with this hypothesis (Fig. 2-6); oxygen consumption in red and white light was not significantly

different and less than 50% of the rates determined in blue light (1 - 30 $\mu w/cm^2$).

The increased activity of certain cladocerans in low intensity blue light may have an important consequence to the amplitude of inherent diel rhythms in feeding and respiration rate. During dawn and dusk most zooplankters are found near the surface and are exposed to a visible spectrum which is predominantly blue light of low intensity (Chapter 3). In cladocerans, feeding and respiration rates will be increased as much as 50%, reinforcing the rates which are already inherently high at these times of the day (Chapter 1). In copepods, the increase in the proportion of red light at sunrise and sunset may produce the same effect, except slightly later than the blue light enhancement predicted for cladocerans. The significance of spectral composition and intensity effects in terms of diel variability in zooplankton energy requirements and expenditure will thus be even greater than described in Chapter 1. In a thermally stratified water column, respiration and feeding rates of migrating species may be as much as ten times higher at dawn and dusk than at midday. Endogenous rhythms are reinforced by the effects of light and temperature to produce an amplitude of this size.

The effects of the light environment on the maintenance and phase of respiration rhythms observed in the present investigation are not clear. In general, rhythms shifted in phase rapidly and were unrelated to either the photoperiod under natural conditions or the photoperiod examined. To satisfactorily demonstrate the role of some environmental parameter as a *Zeitgeber*, it is necessary to achieve full synchronization of rhythms to a light: dark cycle at least 6 hr out of phase with the natural environmental cycle after a period of continuous darkness. This was not demonstrated in

the present investigation; consequently, the role of the light environment in the maintenance of respiration rhythms cannot be assessed from my results.

There are several possible reasons which may account for the failure to shift the phase of the rhythms in a consistent fashion during this study. Light intensities in the environmental chamber (1500 $\mu w/cm^2$) exceeded those to which diel migrating zooplankters are normally exposed (Table 3-1); results of the amplitude studies reported in this chapter clearly suggest that intensities of this magnitude produce lowered respiration rates. There was no significant zooplankton mortality during this investigation, although light avoidance reactions were observed in many cases.

It is possible that the high zooplankton densities (> 1000/l) required to achieve a measurable change in oxygen concentration in 16 min may have contributed to the problem; physical interference between zooplankters is unavoidable at these densities.

Further studies will be required to clarify the role of specific aspects of the light environment as a *Zeitgeber* and in the maintenance of respiration rhythms. There is every reason to expect that both the rate of intensity change at dawn and dusk and perhaps the marked changes in spectral composition observed at these times (Fig. 3-2) may play an important role in the synchronization of endogenous diel rhythms under natural conditions. The effects of light intensity and spectral composition on the amplitude of rhythms have been previously described; it remains to be demonstrated whether the phase of rhythms is also affected by these parameters.

Chapter 3

DIEL RHYTHMS IN THE FEEDING, RESPIRATION, EXCRETION, AND ASSIMILATION OF EUNICE LAKE ZOOPLANKTON *IN SITU*

INTRODUCTION

In the first chapter I demonstrated the existence of endogenous diel feeding and respiration rhythms. The results in the second chapter suggested that diel changes in light and temperature would reinforce these rhythmic phenomena under natural conditions. In the present chapter I have examined the possible existence of diel rhythms *in situ*. Vertically migrating zooplankton, in the normal course of events, are exposed to periodic differences in light intensity, spectral composition, temperature, and food concentration which may modify the final expression of rhythms. In an attempt to clarify the consequences of rhythmic phenomena to the secondary production and energetics of zooplankton communities, diel variability in assimilation, excretion, respiration, and feeding rates were examined and a diel energy budget constructed.

Although there have been numerous attempts to measure respiration, feeding, excretion, and assimilation in the field, they have never been monitored simultaneously throughout the day and under a periodic light and temperature regime. In most cases, energy budgets of zooplankton communities are based on short-term experiments extrapolated to 24 hrs (Kibby, 1971; Pavlova, 1961; Richman, 1958, 1964; and others). As indicated in Chapter 1, the existence of rhythms could lead to serious underestimates of the production of natural communities. Rates determined in the present investigation were compared with the results of other authors and the consequences of rhythmic phenomena to the energy expenditure and utilization of natural zooplankton communities predicted.

MATERIALS AND METHODS

Study Area and Collection of Samples

The investigation was conducted over a 24-hr period (May 28 - 29, 1973) at Eunice Lake in the University of British Columbia Research Forest. All experiments and sampling were conducted from a large float (16 ft x 20 ft), situated over the deepest portion of the lake (> 30 m). Zooplankton were collected every 2 hr with a #10 (156 μ mesh, 30 cm diam.) plankton net hauled vertically from a predetermined depth. Phytoplankton samples were collected from various depths with a 7-l Van Dorn bottle.

Measurement of Physical Parameters, Phytoplankton, and Zooplankton Distributions

Light spectra were measured from dawn to dusk at 1-m intervals from the surface to 20 m using the spectroradiometer/data acquisition system described by Duval *et al.* (1973a). Due to an equipment malfunction during this study, 1971 are presented; however, cloud cover and surface conditions were similar on both days. Spectra were normalized to 550 nm (intensity @ 550 nm = 1.0) to facilitate identification of changes in spectral composition with depth and time.

Water samples collected at 1-m intervals every 2 hr were used for measurement of temperature, dissolved oxygen concentration, and phytoplankton standing crop. Temperatures were taken immediately after samples were brought to the surface.

Dissolved oxygen concentrations were determined as follows. Water from the Van Dorn sampler was siphoned into 250-ml BOD bottles and 2 ml of $MnCl_2$ (3M) and alkaline iodide (NaOH 8N; NaI 4 M) added to each bottle with plastic syringes. Samples were returned to the laboratory for spectrophotometric determination of dissolved oxygen concentration (Duval *et al.*, 1973b).

A 100-ml aliquot from each sample was transferred to a 4-oz glass bottle and preserved with 5 ml of a 50% ethanol:50% formalin mixture. The standing crop, biomass, and dry weight estimation of phytoplankton were determined with a Model B Coulter Counter and Model M Volume Converter (Sheldon and Parsons, 1966a,b).

The vertical distribution of zooplankton was determined in two ways. Vertical hauls were taken every 2 hr at 2-m intervals from 0 - 20 m with a #10 plankton net. A Furono 200-kc/sec echo sounder was used to monitor the distribution of zooplankton every 15 min throughout the day.

Determination of Feeding Rate

Every 2 hr zooplankton (predominantly *Diaptomus kenai* (30%), *D. tyrelli* (40%), *Holopedium gibberum* (30%), and *Chaoborus* sp.) were collected from the depth of highest population density as indicated by the echo sounder. Any *Chaoborus* sp. were removed from the samples and discarded. Samples of water containing phytoplankton were taken from the same depth. Approximately 200 zooplankters of the above species composition were transferred into each of six 250-ml BOD bottles; the bottles were then filled with lake water containing phytoplankton and returned to the same position in the water column. Two bottles without zooplankton were used as controls. After 2 hr the bottles were brought to the surface and the zooplankton removed and preserved with 5 ml of Lugol's solution. The phytoplankton in 100 ml of the sample were preserved and returned to the laboratory where the cells were counted and feeding rate calculated.

Determination of Respiration Rate

The oxygen consumption of six zooplankton samples taken from the depth of maximum population density was measured every 2 hr. Zooplankton were not removed from the bottles at the end of each experiment; 5 ml of ethanol:

formalin were added to each bottle in addition to the Winkler reagents (Carritt and Carpenter, 1966). The addition of preservative had no significant effect ($P > 0.05$) on the dissolved oxygen content of the samples. Dissolved oxygen concentration was determined as described by Duval *et al.* (1973b) and respiration rates calculated as outlined in Chapter 1.

Determination of Rates of Inorganic Phosphate Excretion

At 2-hr intervals, approximately 200 zooplankton were transferred into six 250-ml BOD bottles; two bottles without zooplankton were used as controls. The samples were returned to the sampling depth for 2 hr. At the end of this period the bottles were brought to the surface, the zooplankton removed, and 100 ml of the remaining water preserved with 1 ml of Chloroform and quick-frozen in an acetone/dry ice mixture. On thawing in the laboratory, all particulate matter was removed from the water with a Millipore HA filter (47 mm, pore diam. 0.45μ). The concentration of dissolved inorganic phosphate in each sample was determined with a Beckman DU-2 spectrophotometer following the method of Strickland and Parsons (1972).

Determination of Assimilation Rate

To measure the assimilation rate of zooplankton, 50 μCi of $H^{14}CO_3^-$ were added to 1.0 L of a log phase culture of *Chlamydomonas reinhardtii* (5×10^6 cells/ml). After 24 hr the culture was diluted to 5 l with dechlorinated water. Every 2 hr 50 ml of radioactive *Chlamydomonas* were pipetted into each of eight 250-ml BOD bottles and approximately 200 zooplankton added to six of the bottles. All bottles were filled with unfiltered lake water and returned to the sampling depth. After a 2-hr experiment, the zooplankters were transferred into water containing non-radioactive food to allow them to clear their guts of ^{14}C-labelled phytoplankton. The zooplankters were then counted, dissolved in 2 ml of Protosol Solubilizer (New England Nuclear)

and transferred to scintillation vials for radiocarbon assay in the laboratory. The algae in all bottles were preserved with 5 ml of ethanol: formalin mixture and returned to the laboratory for a Coulter Counter determination of cell number and radiocarbon assay with a Packard Model 3003 Tri-Carb Scintillation Spectrometer. Phytoplankton numbers were converted to dry weight and assimilation rates determined following Sorokin (1968).

Determination of Caloric Equivalents

Respiration rates were converted to caloric equivalents with the factor of 0.005 cal/µl O_2 proposed by Swift and French (1954) assuming an RQ (Respiratory Quotient = + ΔCO_2/- ΔO_2) of 1.0 for zooplankters feeding off phytoplankton rather than living off fat reserves (Parsons and Takahashi, 1973). A sample calculation is given below.

If: Oxygen Consumption = 5.0 µl O_2/mg dry wt/hr

Assuming: RQ = 1.0 (Parsons and Takahashi)

and Caloric Conversion = 0.005 cal/µl O_2 (Swift and French, 1954)

Respiratory Expenditure = (0.005)(5.0)

= 2.5×10^{-2} cal/mg dry wt/hr

Feeding rates were converted to caloric equivalents in the manner outlined in the following example. Since Eunice Lake phytoplankton were predominantly Chlorophyceae, the mean dry weight/cell was taken as 1.67×10^{-4} µg (Parsons *et al.*, 1961) with a caloric equivalent of 5.3 cal/mg dry wt (Cummins and Wuycheck, 1971).

Feeding Rate = 50.0×10^3 μ^3/ml/mg dry wt/hr

Volume of Sample = 250 ml

Total Biomass Consumed = (50.0×10^3 μ^3/ml/mg dry wt/hr)(250 ml)

$= 1.25 \times 10^7\ \mu^3$/mg dry wt/hr

since the mean cell volume of each cell consumed (Fig. 3-7) was 50 μ^3

Total Cell Number Consumed = $(1.25 \times 10^7\ \mu^3)/50\ \mu^3$

$= 2.5 \times 10^5$ cells/mg dry wt/hr

Dry Wt Consumed = $(2.5 \times 10^5)(1.67 \times 10^{-4}$ µg/cell)

= 41.75 µg/mg dry wt/hr

Calories Consumed = (41.75 µg)(0.0053 cal/µg)

= 0.221 cal/mg dry wt/hr

Assimilation rates were converted to calories in a similar manner using a carbon:dry weight ratio of 0.52 (Parsons *et al.*, 1961).

Carbon Assimilated = 1.0 µg C/mg dry wt/hr

Total Assimilated = (1.0 µg C)/0.52

= 1.92 µg/mg dry wt/hr

Calories Assimilated = 1.92 µg(0.0053 cal/µg)

= 0.0102 cal

RESULTS

Vertical Distributions of Temperature, Light, Dissolved Oxygen, Phytoplankton, and Zooplankton

a) Light

Light intensity decreased in an exponential fashion with depth; 60 - 80% of the surface light was removed in the first meter with the greater surface extinction occurring near dawn and dusk. At no time during the day (May 27, 1971) did more than 1% of the incident light reach 7.5 m. The extinction coefficients at noon were as follows:

QUALITY	WAVELENGTH (nm)	EXTINCTION COEFFICIENT
Violet	400 - 430	0.58
Blue	430 - 500	0.34
Green	500 - 575	0.22
Yellow	575 - 600	0.24
Orange	600 - 650	0.25
Red	650 - 700	0.29
Far Red	700 - 750	0.49

The shifts in spectral composition of light with depth are more apparent when normalized spectral distributions are compared (Fig. 3-1). Virtually no red or blue light penetrated to depths greater than 10 m.

The spectral composition of incident light varied throughout the day. The proportions of all wavelengths varied to some extent with time, but marked changes in the relative amount of blue were evident at dawn (Fig. 3-2) and dusk. The proportion of blue light at the surface near midday was 29.5% of the total radiation and increased to over 48% at dawn and dusk. At 1 m the relative amount of blue light (400 - 500 nm) at noon was less than 16% but over 34% at dawn and dusk.

Changes in the total down-welling radiation at various depths throughout the day are shown in Fig. 3-3. The absolute rate of intensity change ($\Delta I/\Delta t$) varied with both depth and time of day (Fig. 3-4). The rate of intensity change was inversely proportional to depth and highest at sunrise and sunset. Relative rates of intensity change ($\Delta I/I\Delta t$) were highest at dawn and dusk but did not vary significantly with depth.

Figure 3-1 Spectral composition of down-welling light with depth in Eunice Lake, May 27, 1971, at 1240 hr (PST). All data are normalized to a value of 1.0 at 552 nm.

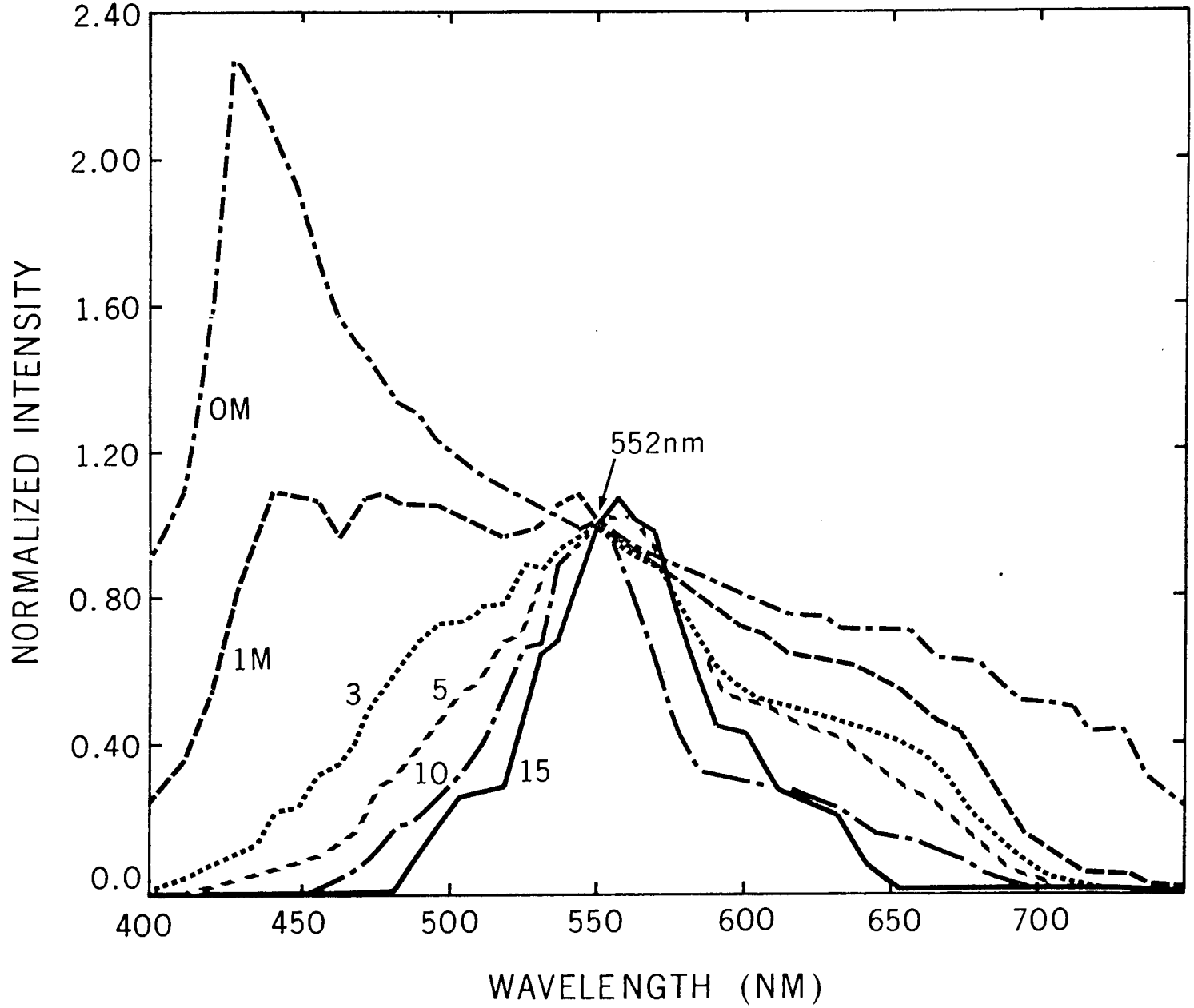

2.40
2.00
1.60
1.20
0.80
0.40
0.0
NORMALIZED INTENSITY
OM
1M
3
5
10
15
552nm
400
450
500
550
600
650
700
WAVELENGTH (NM)

Figure 3-2 Changes in the spectral composition of surface light from dawn to noon in Eunice Lake, May 27, 1971. Relative spectral distributions are normalized to a value of 1.0 at 600 nm.

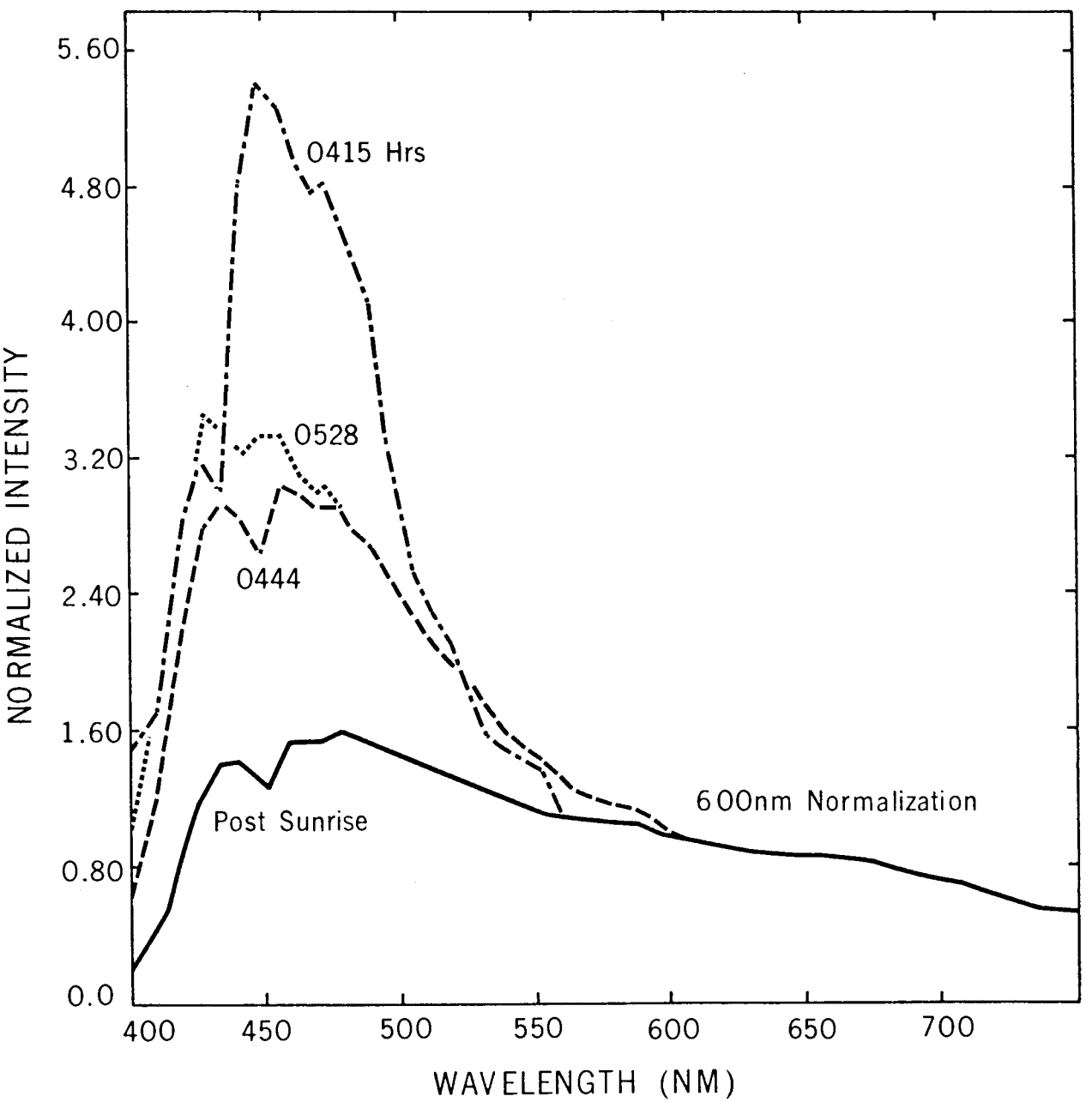

5.60
4.80
4.00
3.20
2.40
1.60
0.80
0.0
NORMALIZED INTENSITY
0415 Hrs
0528
0444
Post Sunrise
600nm Normalization
400
450
500
550
600
650
700
WAVELENGTH (NM)

Figure 3-3 Changes in the total down-welling radiation at various depths throughout the day in Eunice Lake, May 27, 1971. Sunrise at 0530 hr (PST), sunset at 1800 hr (PST). Energy units are $\mu w/cm^2$.

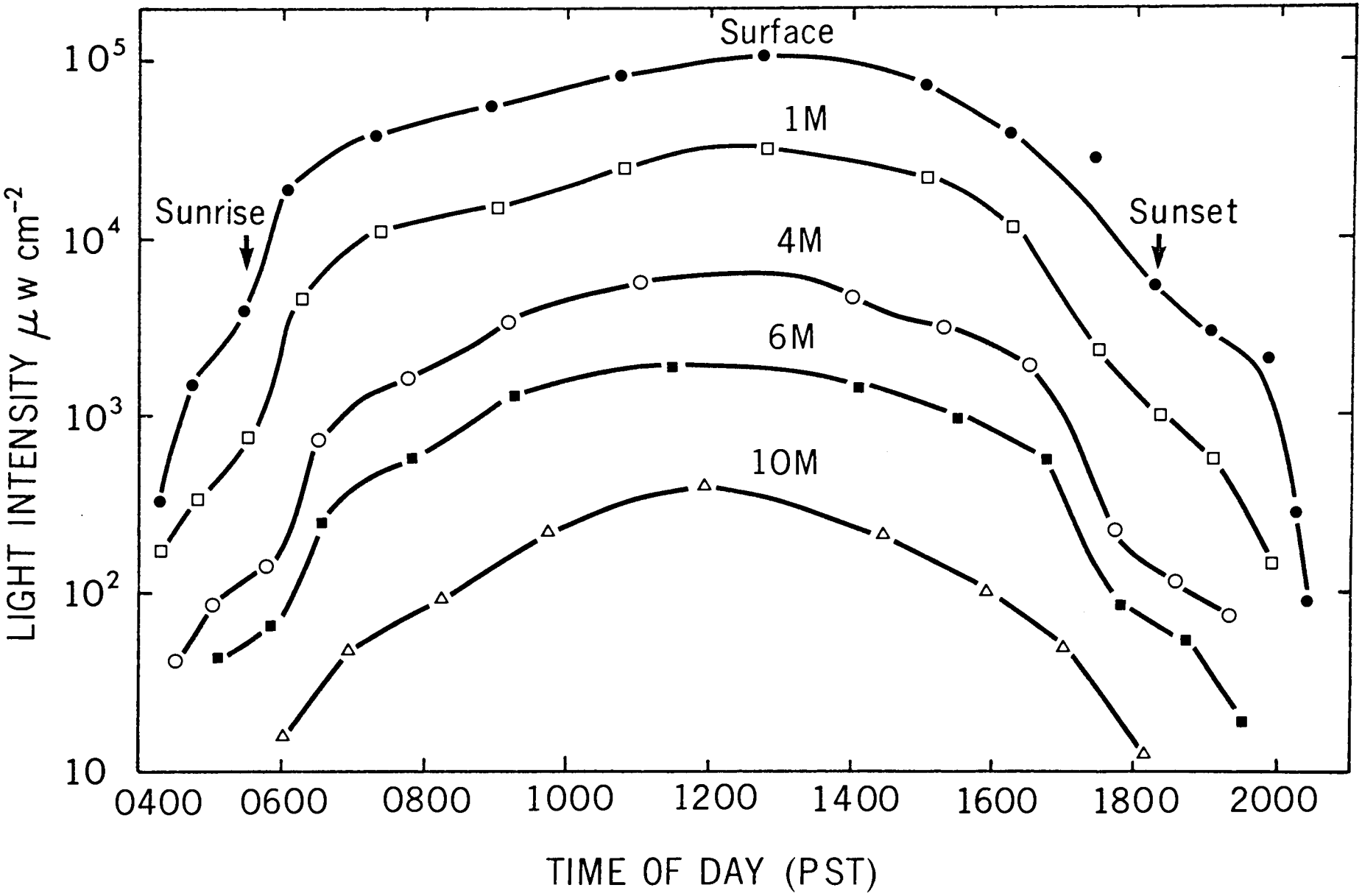
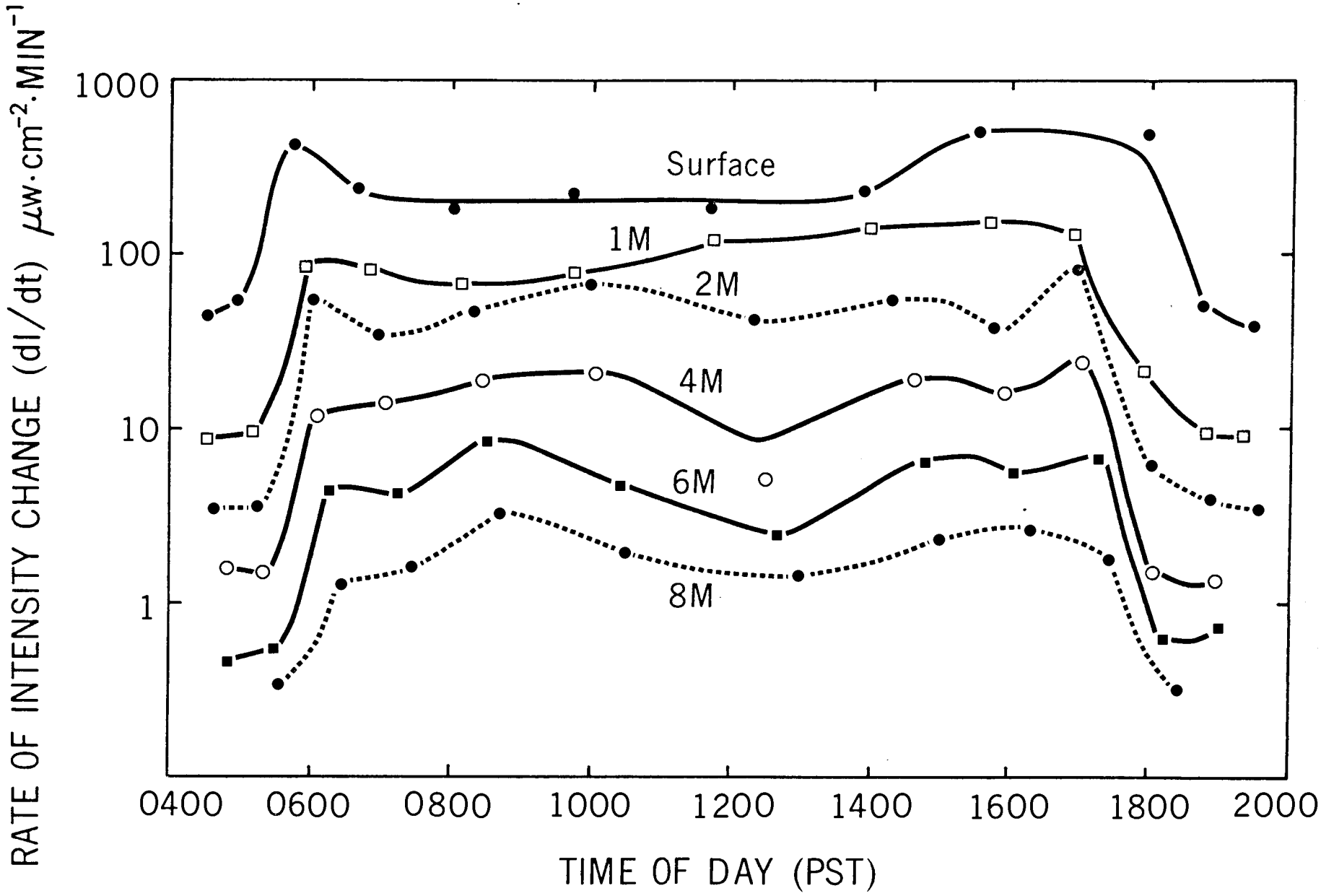

Surface
1M
4M
6M
10M
Sunrise
Sunset
10^5
10^4
10^3
10^2
10
LIGHT INTENSITY μw cm^{-2}
0400
0600
0800
1000
1200
1400
1600
1800
2000
TIME OF DAY (PST)

Figure 3-4 The rate of intensity change (dI/dt) at various depths and times of the day in Eunice Lake, May 27, 1971. Units are $\mu w/cm^2/min$ and time of day is PST.


RATE OF INTENSITY CHANGE (dI/dt) μw·cm⁻²·MIN⁻¹
1000
100
10
1
Surface
1M
2M
4M
6M
8M
0400
0600
0800
1000
1200
1400
1600
1800
2000
TIME OF DAY (PST)

b) Temperature and Dissolved Oxygen Profiles

With the exception of the first meter, there were no significant differences in the temperature profiles in Eunice Lake throughout the day. A gradual decrease in temperature was found between 4.5 m and 9.5 m (Fig. 3-5). Surface temperatures varied from 14.2 - 17.0 C throughout the day.

Dissolved oxygen concentration did not vary significantly with time of day. There was a slight increase in oxygen concentration from the surface to 6 - 8 m, but concentrations always exceeded 10 ppm above 20 m (Fig. 3-5).

c) Phytoplankton Standing Crop

The biomass of phytoplankton varied significantly with depth but not with time. The standing crop of phytoplankton and detritus at 2.5 m (94 µg dry wt/l) was approximately 50% of the surface value (180 µg dry wt/l) as indicated in Fig. 3-6. The highest standing crop was found at 5.5 m. The phytoplankton community was composed of at least three major species having mean cell volumes of 0.6, 18.7, and 149 μ^3 respectively (Fig. 3-7); the species composition of the entire phytoplankton community was not determined.

d) Diel Changes in the Vertical Distribution of Zooplankton

There were marked diel vertical movements of Eunice Lake zooplankton throughout the day (Fig. 3-8). The migration pattern indicated by the echo soundings was similar to that shown by the vertical hauls. During midday, the majority of zooplankters were found between 3 and 12 m, although the vertical position varied with species. The vertical haul data indicated that the most extensive migration (8 - 10 m) was undertaken by *Diaptomus kenai, D. tyrelli,* and *Chaoborus* sp. and the least by *Holopedium gibberum* and *Daphnia rosea.* In general, they began their ascent between 1345 and 1415 hr and reached the surface waters by 2300 hr. Shortly before midnight they sank

Figure 3-5 Temperature and dissolved oxygen profiles of Eunice Lake, May 28 - 29, 1973. Units for dissolved oxygen are ppm; mean values over the 24-hr period indicated.

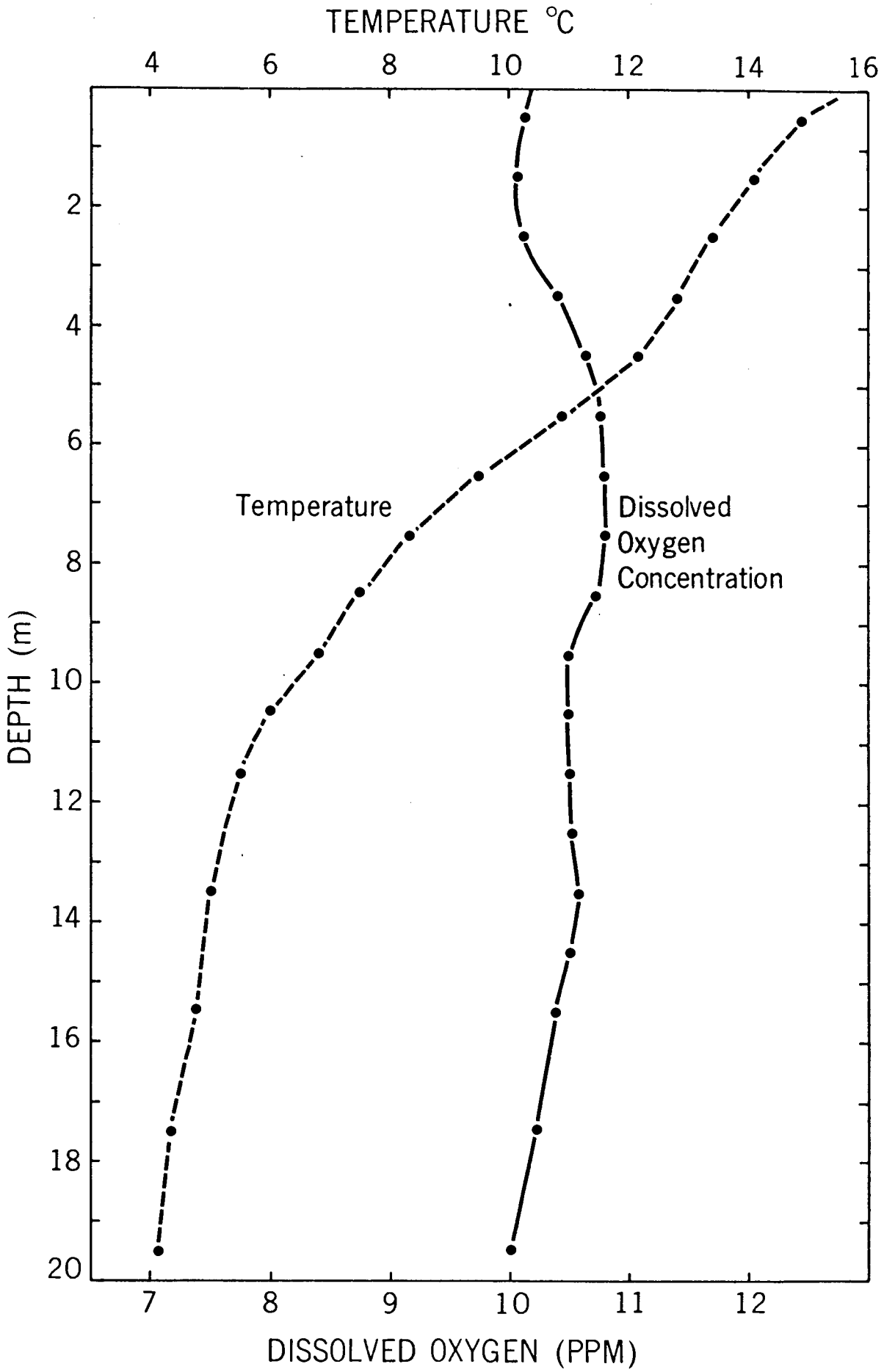

TEMPERATURE °C
4
6
8
10
12
14
16
2
4
6
8
10
12
14
16
18
20
DEPTH (m)
Temperature
Dissolved
Oxygen
Concentration
7
8
9
10
11
12
DISSOLVED OXYGEN (PPM)

Figure 3-6 The standing crop of phytoplankton and detritus ($\mu^3 \times 10^6/l$) in Eunice Lake at various depths on May 28 - 29, 1973. Mean values over the 24-hr period are indicated.

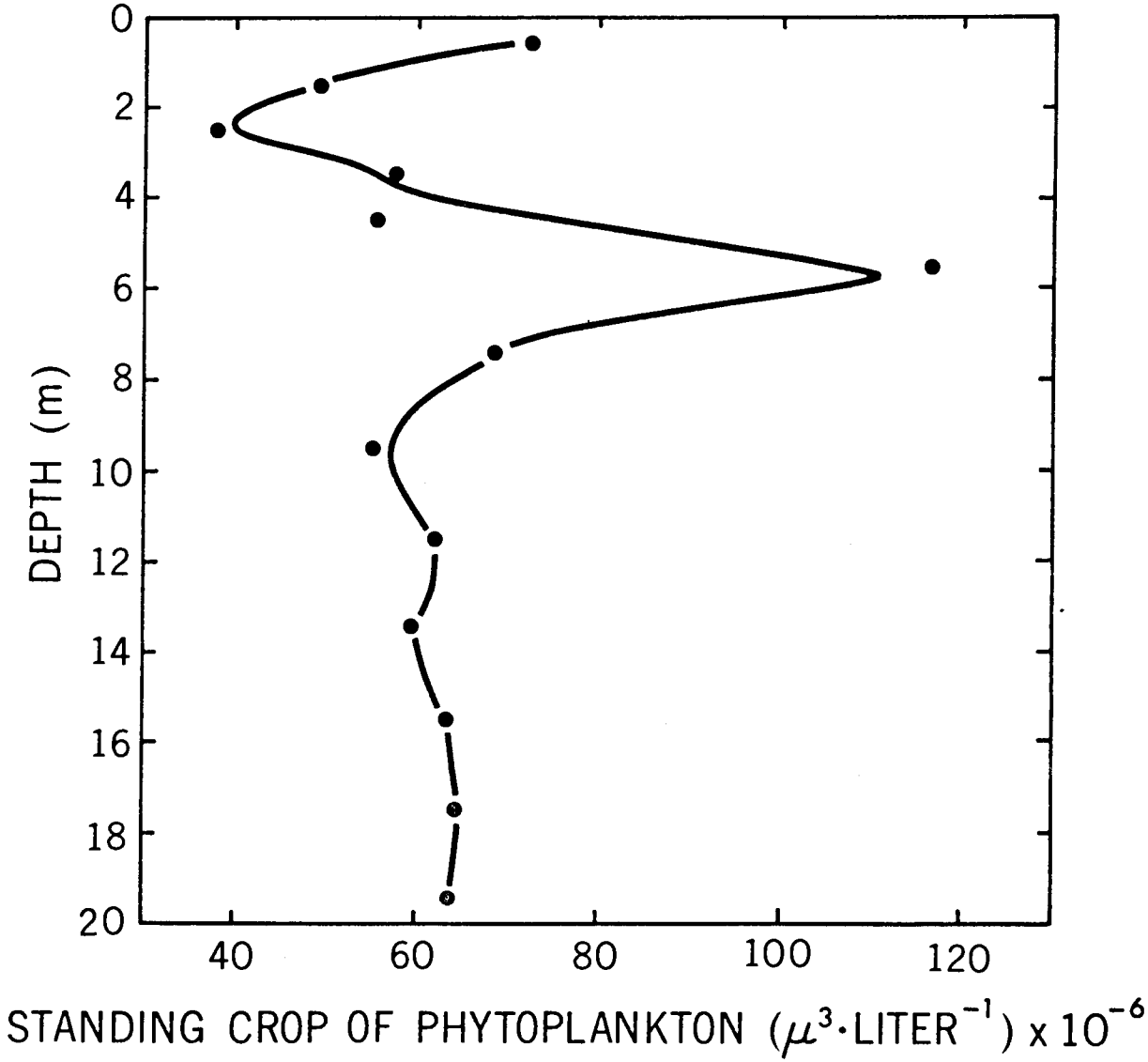

0
2
4
6
8
10
12
14
16
18
20
DEPTH (m)
40
60
80
100
120
STANDING CROP OF PHYTOPLANKTON ($\mu^3 \cdot$LITER^{-1}) x 10^{-6}

Figure 3-7 Size distribution of phytoplankton collected from Eunice Lake during a 24-hr period on May 28 - 29, 1973. Mean values indicated.

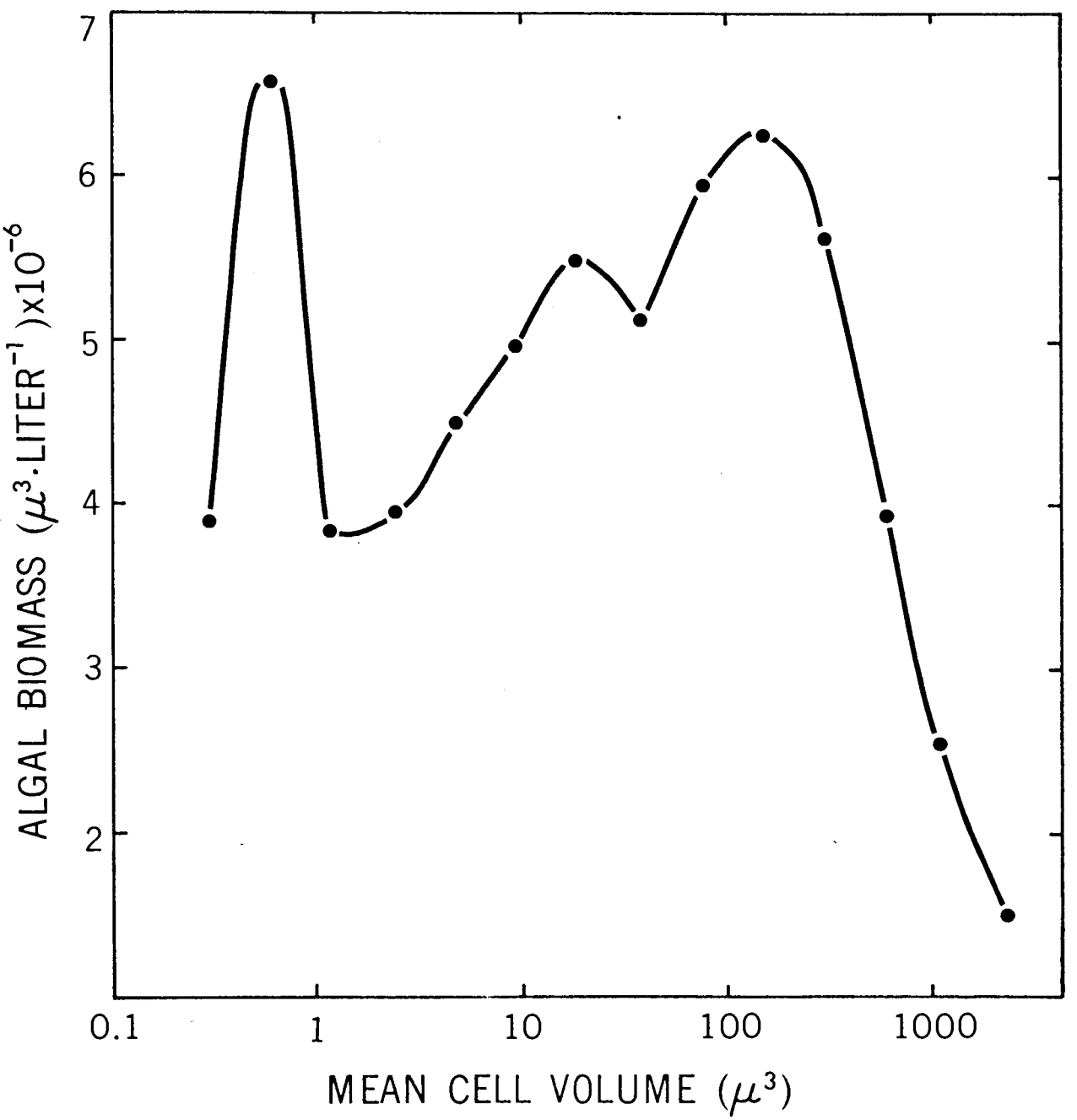
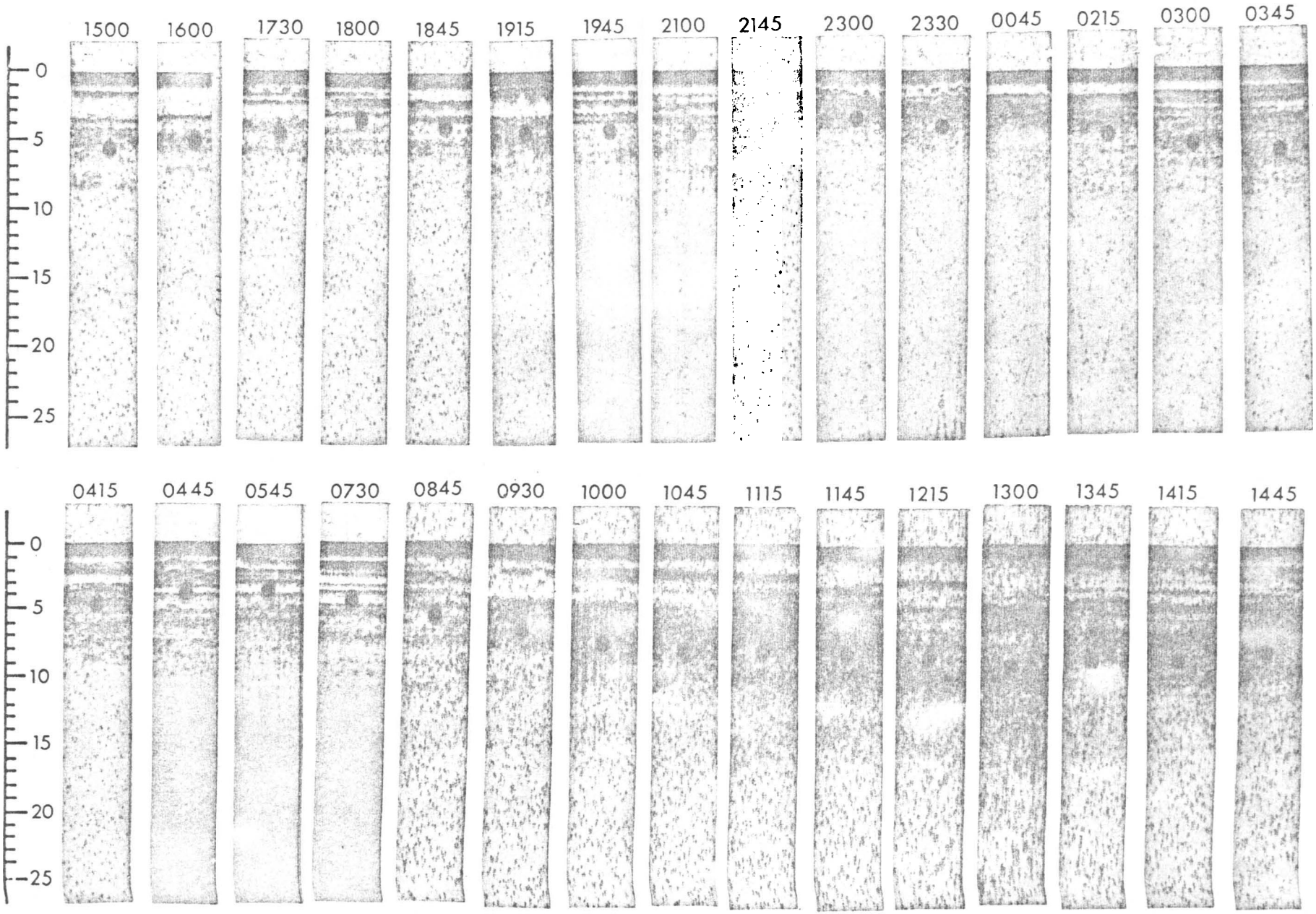

7
6
5
4
3
2
ALGAL BIOMASS ($\mu^3 \cdot LITER^{-1}$)x10^{-6}
0.1
1
10
100
1000
MEAN CELL VOLUME (μ^3)

Figure 3-8 Diel changes in the vertical distribution of Eunice Lake zooplankton (May 28 - 29, 1973). Data taken with a Furuno 200 kc/sec Echo Sounder.


1500
1600
1730
1800
1845
1915
1945
2100
2145
2300
2330
0045
0215
0300
0345
0
5
10
15
20
25
0415
0445
0545
0730
0845
0930
1000
1045
1115
1145
1215
1300
1345
1415
1445
0
5
10
15
20
25

slightly in the water column and between 0730 and 0845 hr after a slight dawn rise (0.5 m), descended to their day depth.

Diel Changes in the Feeding Rate of Zooplankton

Highly significant differences ($P < 0.001$) in the feeding rate of Eunice Lake zooplankton were found throughout the day. Feeding rates were highest at 0400 and 2115 hr and lowest near midday (Fig. 3-9). Noon rates were only 1% of values found near dawn.

Diel Changes in the Respiration Rate of Zooplankton

The respiration rate of Eunice Lake zooplankton varied throughout the day (Fig. 3-10). Oxygen consumption was highest at 0930 and 2130 hr and not significantly different ($P > 0.05$) between 1100 and 1900 hr. Rates at dusk were twice as high as midday levels; the morning maximum was 23% higher than the midday minimum.

Diel Changes in the Inorganic Phosphate Excretion of Zooplankton

Highly significant ($P < 0.001$) differences in the rate of inorganic phosphate excretion were found throughout the day. Rates were lowest at 1500 hr, increasing four times to a maximum at 1700 hr (Fig. 3-11). Two lesser maxima were found at 0130 and 0800 hr.

Diel Changes in the Assimilation Rate of Zooplankton

The assimilation rates of Eunice Lake zooplankters, as measured from the incorporation of labelled *Chlamydomonas*, were highest near dawn and dusk and lowest during midday (Fig. 3-12). Rates at noon were less than 50% of dawn and dusk values. There was no significant difference in the amplitude of dawn and dusk maxima.

Figure 3-9 Diel changes in the feeding rate of Eunice Lake zooplankton (May 28 - 29, 1973). All experiments conducted at the depth of maximum zooplankton density with *in situ* light, temperature, and food concentration. Rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

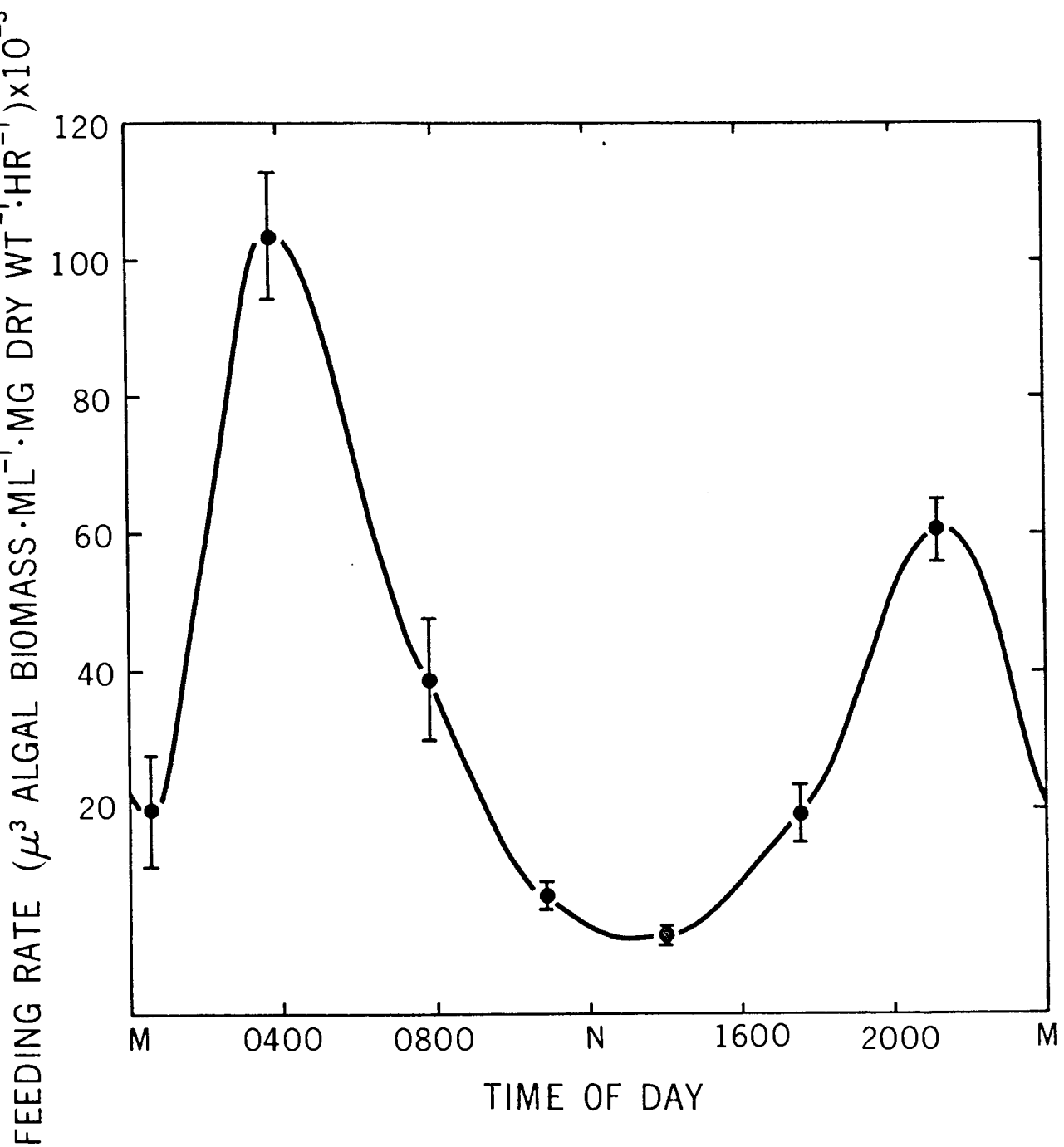

FEEDING RATE (μ^3 ALGAL BIOMASS·ML^{-1}·MG DRY WT^{-1}·HR^{-1})x10^{-3}
120
100
80
60
40
20
M
0400
0800
N
1600
2000
M
TIME OF DAY

Figure 3-10 Diel changes in the respiration rate of Eunice Lake zooplankton (May 28 - 29, 1973). All experiments were conducted *in situ* at the depth of maximum zooplankton density and rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

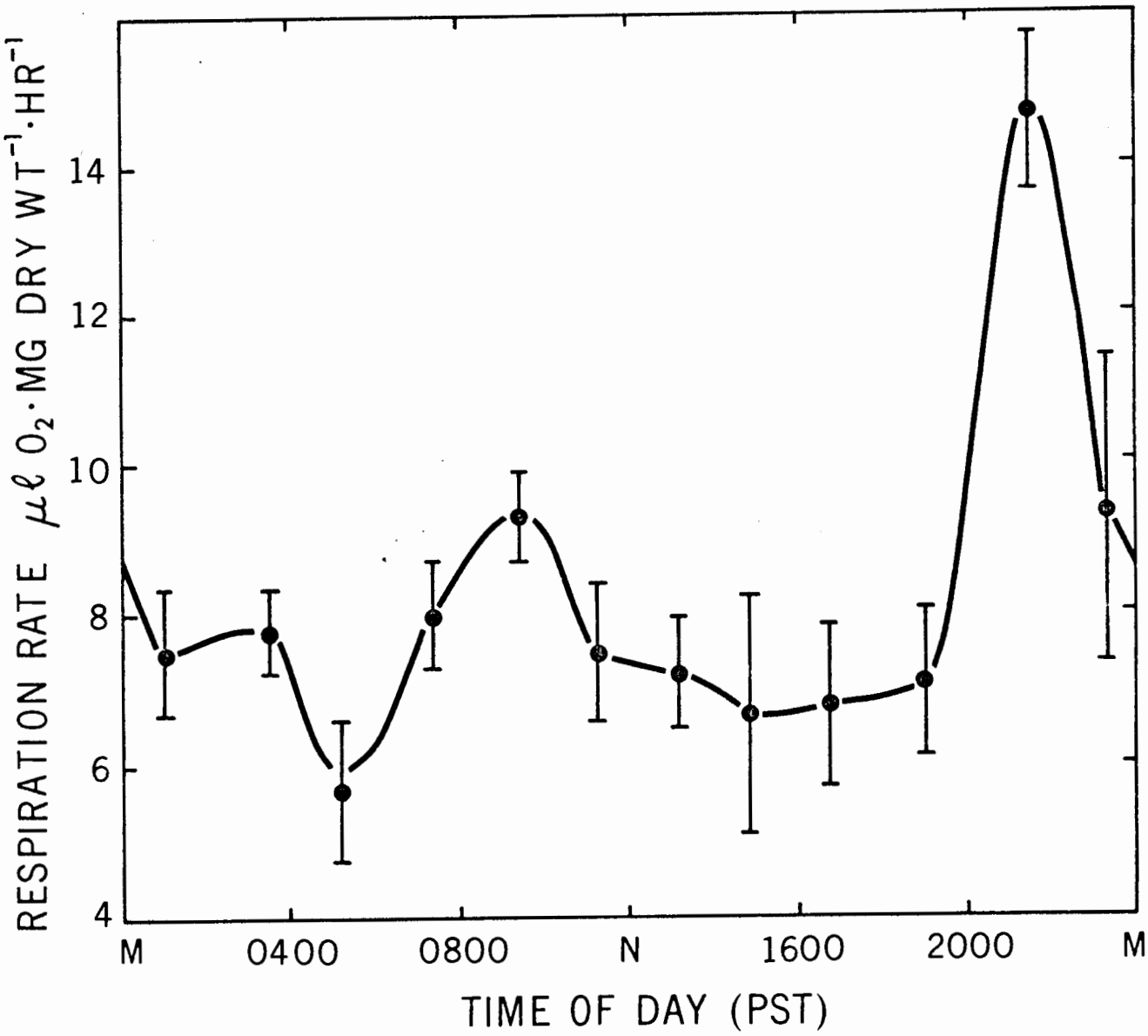

RESPIRATION RATE μℓ O2·MG DRY WT-1·HR-1
14
12
10
8
6
4
M
0400
0800
N
1600
2000
M
TIME OF DAY (PST)

Figure 3-11 Diel changes in the rate of inorganic phosphate excretion of Eunice Lake zooplankton (May 28 - 29, 1973). All experiments were conducted with *in situ* conditions at the depth of maximum zooplankton density. Rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.

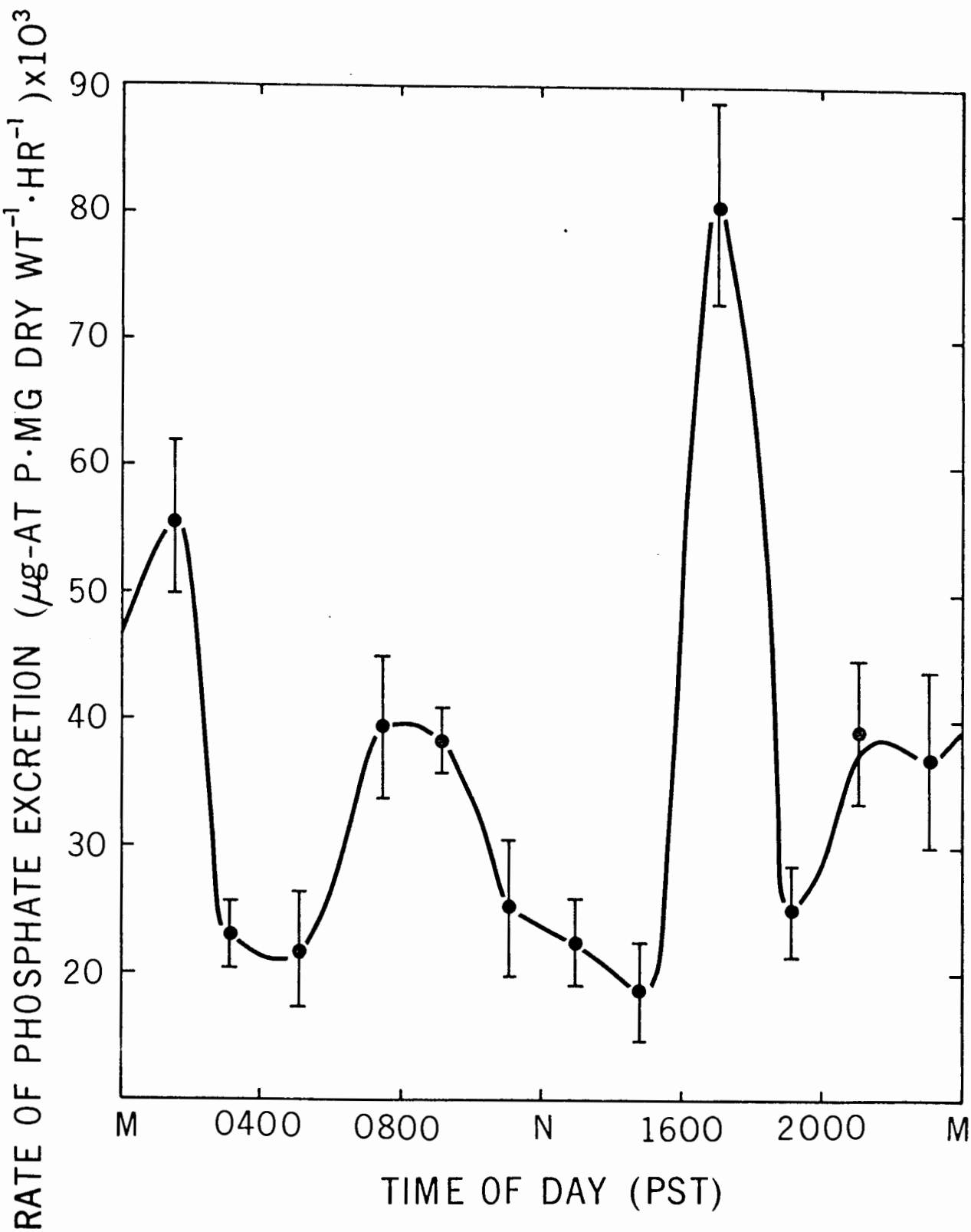
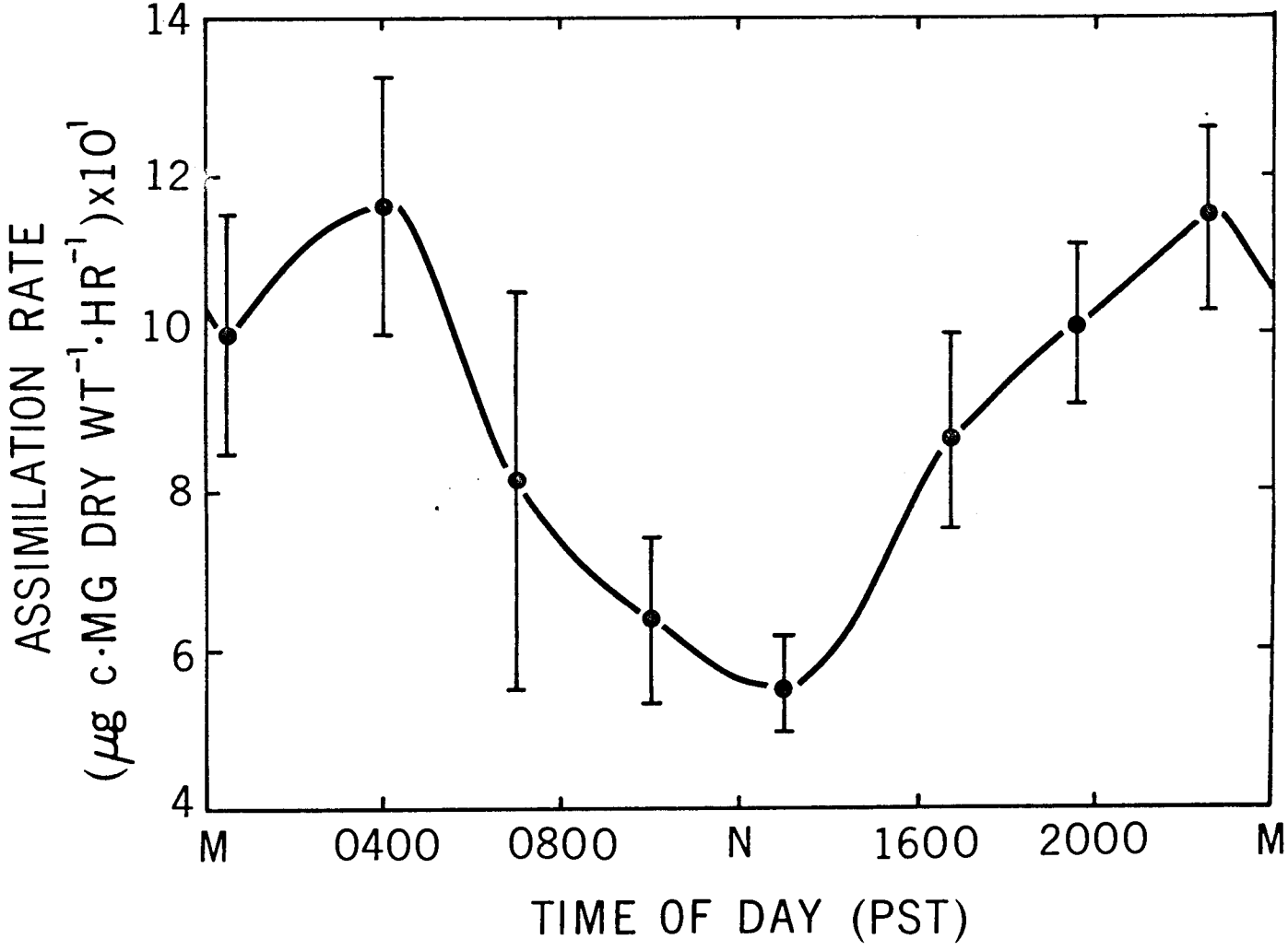

RATE OF PHOSPHATE EXCRETION (μg-AT P·MG DRY WT⁻¹·HR⁻¹)x10³
90
80
70
60
50
40
30
20
M
0400
0800
N
1600
2000
M
TIME OF DAY (PST)

Figure 3-12 Diel changes in the assimilation rate of Eunice Lake zooplankton (May 28 - 29, 1973). All experiments were conducted at the depth of maximum zooplankton density under *in situ* light and temperature conditions. Incorporation of ^{14}C-labelled *Chlamydomonas reinhardtii* was determined and rates corrected to values expected with a zooplankton biomass of 50 µg dry wt/ml. Mean ± standard error indicated.


14
12
10
8
6
4
ASSIMILATION RATE
(μg c·MG DRY WT⁻¹·HR⁻¹)x10¹
M
0400
0800
N
1600
2000
M
TIME OF DAY (PST)

DISCUSSION

The results of the previous chapter suggested that under natural conditions periodic changes in light intensity, spectral composition, temperature, and food concentration would reinforce endogenous diel rhythms by increasing rates even further at dawn and dusk, reducing rates during midday. The results of the present chapter clearly demonstrate significant diel rhythms in feeding, respiration, excretion, and assimilation *in situ*. Differences in the amplitude of each of these rhythms resulted in large variations in assimilation efficiency (A/I x 100) and net growth efficiency (A/(A + R) x 100) throughout the day.

Since a number of environmental factors affect feeding and respiration rates (Chapter 2) and would change the amplitude of *in situ* rhythms, several biotic and abiotic features in the environment of Eunice Lake zooplankton were examined at the depth of maximum zooplankton density (MZD) at 1 hr intervals throughout the day. These data, based on visual approximations of the mean depth of maximum zooplankton density (MZD) from echo soundings, are summarized in Table 3-1.

During this investigation, Eunice Lake zooplankton at the depth of maximum abundance experienced significant diel changes in light, including shifts in the spectral composition of incident light, changes in the total intensity of down-welling light, as well as marked changes in the rate of intensity change (dI/dt) throughout the day. Zooplankton were exposed to maximum light intensities at sunrise and sunset (ca. 1100 $\mu w/cm^2$) and experienced lower intensities during midday (Table 3-1). The intensities shown in the previous chapter to increase respiration and feeding rates (< 100 $\mu w/cm^2$) were only found at dawn and dusk; consequently, reinforcement

TABLE 3-1

Diel changes in various biotic and abiotic factors in the environment of Eunice Lake zooplankton at the depth of maximum zooplankton abundance (May 28 - 29, 1973). The mean depth of maximum zooplankton abundance (MZD) was determined from echo soundings by visual approximation. The standing crop of phytoplankton (cell no/ml) was calculated from total algal biomass (μ^3) assuming a mean cell volume of 50 μ^3. Direction of rate of intensity change (dI/dt) indicated by: (+) = increasing intensity and (-) = decreasing intensity. Light measurements taken May 27, 1971.

Time	MZD (m)	Temp	Dissolved Oxygen (ppm)	Phytoplankton Standing Crop (μ^3/1 x 10^6)	Phytoplankton Standing Crop (cell/ml x 10^3)	Light Intensity ($\mu w/cm^2$)	dI/dt ($\mu w/cm^2/m$)	λ 0.5 (nm)
2400	3.8	12.7	10.50	59.0	1.18	-	-	-
0100	4.0	12.5	10.55	61.0	1.22	-	-	-
0200	4.0	12.5	10.55	61.0	1.22	-	-	-
0300	5.0	11.5	10.70	86.0	1.72	-	-	-
0400	5.0	11.5	10.70	86.0	1.72	20	+ 0.3	500
0500	4.0	12.5	10.55	61.0	1.22	90	+ 1.2	560
0600	3.5	12.8	10.40	55.5	1.11	400	+ 5.2	580
0700	4.2	12.4	10.60	70.0	1.40	1000	+ 10.0	595
0800	5.2	11.3	10.75	100.0	2.00	1100	+ 1.7	596
0900	6.5	9.5	10.80	92.5	1.85	650	- 7.5	595
1000	7.5	8.3	10.80	68.5	1.37	900	+ 4.2	608
1100	8.0	7.9	10.75	65.0	1.30	750	- 2.5	614
1200	8.5	7.5	10.70	61.0	1.22	700	- 0.8	560
1300	8.5	7.5	10.70	61.0	1.22	700	0.0	560
1400	7.5	8.3	10.80	68.5	1.37	850	+ 0.8	567
1500	6.1	10.0	10.75	102.0	2.04	1050	+ 3.3	567
1600	5.5	10.9	10.75	116.5	2.33	1100	+ 0.8	580
1700	4.5	12.2	10.65	73.5	1.47	700	- 6.7	578
1800	4.3	12.3	10.60	68.0	1.36	150	- 9.2	565
1900	4.1	12.4	10.55	65.0	1.30	85	- 1.1	580
2000	4.0	12.5	10.55	61.0	1.22	35	- 0.8	525
2100	4.5	12.2	10.65	73.5	1.47	-	-	-
2200	3.5	12.8	10.40	55.5	1.11	-	-	-
2300	2.8	13.2	10.20	44.0	0.88	-	-	-

of endogenous rhythms would only occur at these times. Higher intensities found between sunrise and sunset, given the relationship established in the previous chapter, may be partially responsible for the low rates observed near midday, particularly in the case of feeding. There was no evidence of Eunice Lake zooplankton at the depth of maximum abundance (MZD) following similar or 'optimal' light intensities throughout the day as suggested by Cushing (1955).

Rates of intensity change were also highest at sunrise and sunset; however, the effects of this parameter on the feeding and respiration of zooplankton are not known. The vertical movement of zooplankton appeared to be related to rates of intensity change ($\Delta I/\Delta t$); rates of intensity change began to increase shortly after dawn (Table 3-1) and were accompanied by a general downward movement of zooplankton (Fig. 3-8). This phenomenon was not examined in detail in the present investigation; however, various other authors have demonstrated a strong correlation between rates of intensity change and vertical movement of zooplankton (McNaught and Hasler, 1964; Ringelberg, 1961).

At dawn and dusk when zooplankton were distributed near the surface (Fig. 3-8), populations were exposed to high proportions of low intensity blue (400 - 500 nm) light (Fig. 3-2). This was evident by dawn and dusk (0400, 0500, and 2000 hr) values of $\lambda_{0.5}$ (Table 3-1), the wavelength which divides the spectral distribution into two equal portions in terms of total energy. Jerlov (1954) demonstrated that the spectral distribution of daylight before sunrise and after sunset was predominantly short wavelength radiation (< 560 nm) due to a relatively high contribution from blue sky light in the absence of direct sunlight. As indicated in the previous chapter, blue light increased feeding and respiration rates in cladocerans

and may contribute to the higher rates observed at dawn and dusk.

In the previous chapter, food concentration was shown to have marked effect on the feeding rate of zooplankton. During the present investigation, Eunice Lake zooplankton encountered diel differences in the standing crop of particulate matter (Fig. 3-6, Table 3-1); concentrations increased approximately two times from 2.5 to 5.5 m. Consequently, during their morning descent and again during the evening ascent, zooplankton passed through water containing significantly higher food concentrations. This could result in an increase in feeding rate as indicated in Chapter 2 which, however, might be partially offset by the high intensities (> 1000 $\mu w/cm^2$) also experienced at these times.

Since temperature also affects feeding and respiration rates, the 5 C temperature change experienced by Eunice Lake zooplankton during their diel vertical migration may account for amplitudes which are greater than those demonstrated in endogenous rhythms under constant conditions (Chapter 1).

In summarizing the effects of diel changes in the environment of Eunice Lake zooplankton at the depth of maximum abundance, it is clear that the environment will reinforce the endogenous diel rhythms demonstrated in Chapter 1, increasing rates further at dawn and dusk, reducing them during midday. Feeding and respiration rates at dawn and dusk when zooplankters were distributed near the surface are increased by the combined influence of higher temperatures, low light intensities, and a high proportion of blue light. Rates at midday, already inherently low, are further reduced by lower temperatures, higher intensities, and incident light which was predominantly yellow-green.

Highly significant diel changes in feeding (Fig. 3-9), respiration (Fig. 3-10), inorganic phosphate excretion (Fig. 3-11), and assimilation

(Fig. 3-12) were found during the present investigation. However, both the amplitude and timing of the maxima of each of the four parameters differed. Feeding rates were highest at 0400 and 2115 hr and the amplitude of the diel rhythm large due to negligible feeding near midday. The high rates measured at dawn and dusk are not entirely due to the endogenous rhythm; the intrinsic component probably accounts for an increase of six to seven times (Chapter 1) with the environmental conditions previously described contributing to the remainder of these maxima.

In the case of respiration, the dawn maximum was later (0930 hr). However, since the migrating zooplankters did not reach the depth of maximum phytoplankton density until sunrise (Table 3-1), the delay in the respiration maximum may be related to the increasing food concentrations; respiration rates occasionally increase when zooplankton are actively feeding (Conover, 1966). This effect was not indicated in the previous chapter where food concentrations from 10^4 to 10^6 cells/ml had no significant effect on the oxygen consumption of zooplankton. Previous investigations (Chapter 1) indicated that the amplitude of respiration rhythms was always less than the amplitude of feeding rhythms. The results of the *in situ* observations are consistent with this trend; respiration increased only 2.0 times from the midday level. This amplitude is no greater than observed under controlled conditions in the laboratory and a consequence of the inherent rhythm itself. For the species examined in the present investigation, the environment did not reinforce endogenous respiration rhythms to the same extent as feeding rhythms.

The diel pattern of inorganic phosphate excretion by Eunice Lake zooplankton was similar to the pattern described for oxygen consumption. However, the amplitude of the excretion rhythm was greater than that

demonstrated for respiration; excretion of dissolved inorganic phosphate increased four times from midday to dusk. The amplitude and timing of the present rhythm was similar to that described for *Acartia* by Hargrave and Geen (1968). The relative importance of endogenous and exogenous components to the amplitude of the rhythm cannot be assessed since endogenous excretion rhythms were not considered in previous chapters.

The diel rhythm in assimilation rate was similar to that of feeding in timing but not in amplitude. Assimilation rates were highest at dawn and dusk and lowest at midday. The magnitude of dawn and dusk maxima were not significantly different and approximately 50% higher than midday levels.

A budget for the diel energy consumption and expenditure of the Eunice Lake zooplankton community is presented in Table 3-2; only zooplankters larger than 156 μ are considered, and no attempt has been made to estimate the energy losses incurred by the excretion of dissolved organic and inorganic compounds. It was not possible to estimate excretion of carbon based on losses due to inorganic phosphate excretion; there are no published C:P ratios for the excretion products of zooplankton. This represents a shortcoming of the present data since it has been estimated that in some cases as much as 30% of the energy incorporated is lost within 24 hr through excretion (Butler *et al.*, 1970).

Diel differences in the feeding, respiration, and assimilation rates of Eunice Lake zooplankton were converted to caloric equivalents and are presented in Fig. 3-13.

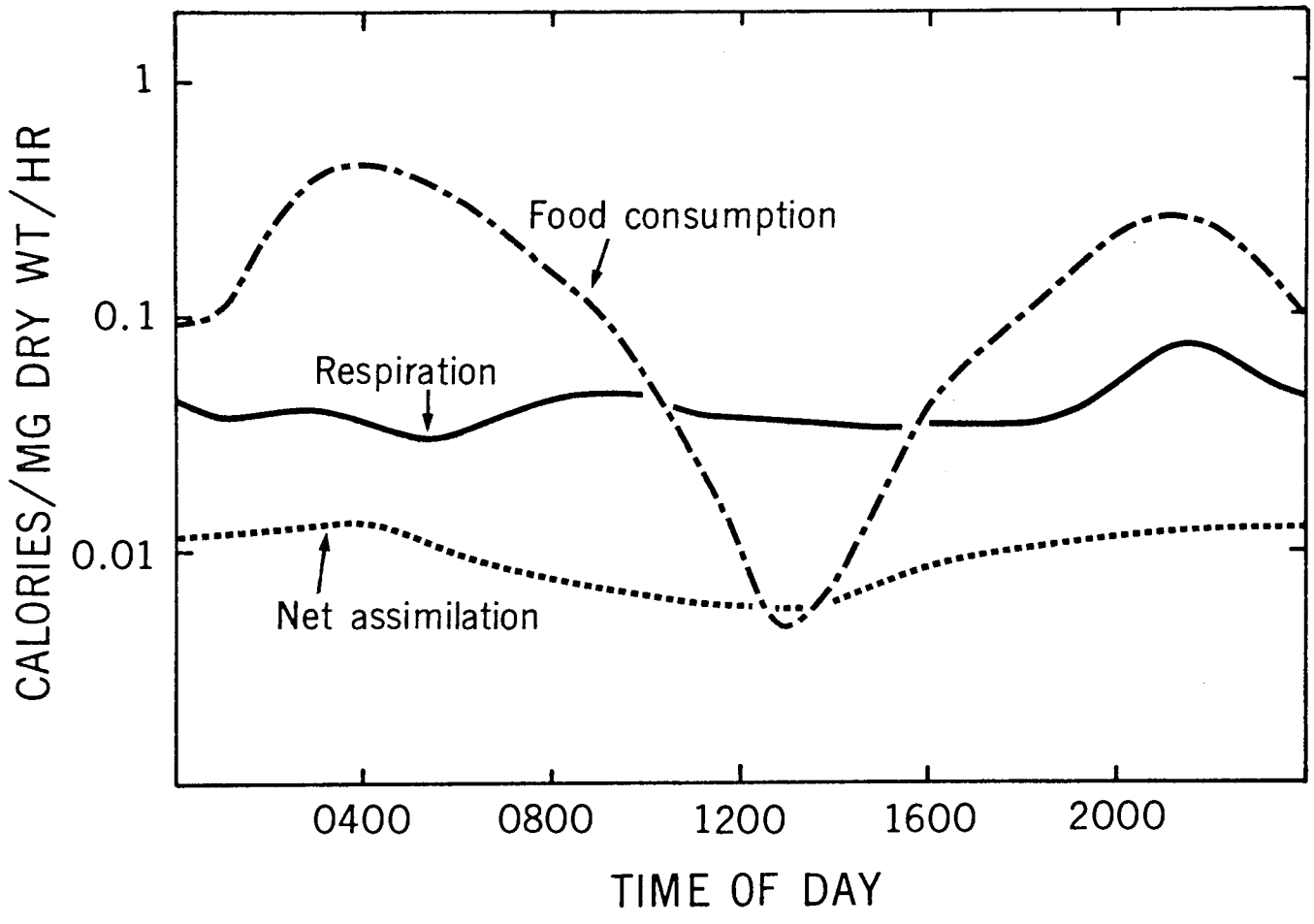
The results of the present investigation indicate that Eunice Lake zooplankton exhibit 'superfluous feeding' (Beklemishev, 1954) at certain times of the day; ingestion rates at dawn and dusk were well in excess of energy requirements (Fig. 3-13). It has often been suggested that when

TABLE 3-2

Budget for the diel energy consumption and expenditure of Eunice Lake zooplankton determined from a 24-hr study of the feeding, respiration, and assimilation rates *in situ*. Feeding rates were measured with natural phytoplankton and assimilation with a ^{14}C-labelled culture of *Chlamydomonas reinhardtii*. All rates were converted to caloric equivalents.

Time	Respiration µl O_2/mg/hr	Respiration cal/mg/hr	Feeding Rate μ^3/ml/mg/hr x 10^3	Feeding Rate cal/mg/hr	Assimilation µg C/mg/hr	Net Assimilation cal/mg/hr
2400	8.8	0.044	21.0	0.093	1.02	0.0104
0100	7.5	0.038	24.0	0.106	1.04	0.0106
0200	7.7	0.039	57.0	0.252	1.10	0.0112
0300	7.8	0.039	92.0	0.407	1.14	0.0116
0400	7.2	0.036	101.5	0.449	1.16	0.0118
0500	6.0	0.030	90.0	0.398	1.09	0.0111
0600	6.3	0.032	70.0	0.310	0.94	0.0096
0700	7.6	0.038	50.0	0.221	0.82	0.0084
0800	8.6	0.043	36.0	0.159	0.73	0.0074
0900	9.2	0.046	23.0	0.102	0.68	0.0069
1000	9.0	0.045	12.0	0.053	0.64	0.0065
1100	7.6	0.038	6.0	0.027	0.59	0.0060
1200	7.3	0.037	2.5	0.011	0.56	0.0057
1300	7.2	0.036	1.0	0.004	0.55	0.0056
1400	7.0	0.035	1.5	0.007	0.59	0.0060
1500	6.6	0.033	4.0	0.018	0.68	0.0069
1600	6.7	0.034	9.0	0.040	0.80	0.0081
1700	6.8	0.034	15.0	0.066	0.88	0.0090
1800	6.9	0.035	22.0	0.097	0.94	0.0096
1900	7.0	0.035	34.5	0.153	0.99	0.0101
2000	10.0	0.050	51.5	0.228	1.03	0.0105
2100	14.0	0.070	59.5	0.263	1.07	0.0109
2200	14.0	0.070	58.0	0.257	1.12	0.0114
2300	10.8	0.054	40.5	0.179	1.13	0.0115
TOTAL	197.6	0.991	881.5	3.900	21.29	0.2170

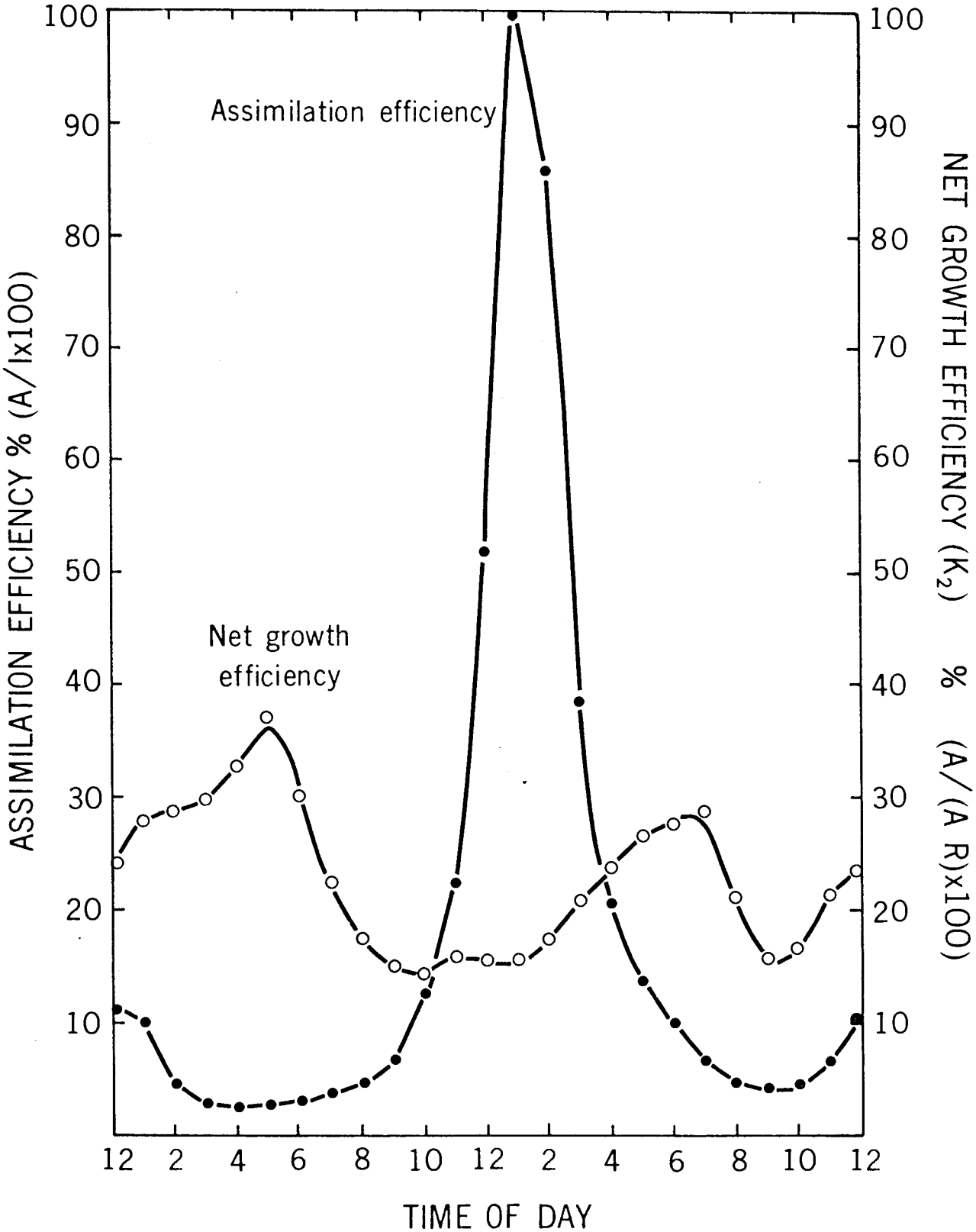
Figure 3-13 Diel changes in the caloric values for feeding, respiration, net and gross assimilation of Eunice Lake zooplankton *in situ* (May 28 - 29, 1973).


1
0.1
0.01
CALORIES/MG DRY WT/HR
Food consumption
Respiration
Net assimilation
0400
0800
1200
1600
2000
TIME OF DAY

concentrations of phytoplankton are high, zooplankton may ingest a great deal more food than is actually assimilated, the remainder passing through the gut undigested (Cushing and Vucetic, 1963). Conover (1966) stated that if superfluous feeding occurred, assimilation efficiencies would decrease but was unable to demonstrate any significant change in assimilation efficiencies over a range of food concentrations from 10^3 to 2 x 10^5 cells/l. Superfluous feeding has been the subject of much controversy over the past 20 years and has led to many discrepancies regarding assimilation efficiencies; estimates of assimilation vary from 6 to 99% (Conover, 1964). Inconsistency in the definition of 'assimilation' has also contributed to this problem; in the present study assimilation is defined as that amount of energy actually incorporated and remaining at the conclusion of the experiment. Consequently, the lack of excretion data previously mentioned will have no impact on the calculation of assimilation efficiencies (A/I x 100), only that energy actually incorporated is considered in calculations. As indicated in Fig. 3-14, assimilation efficiencies varied from less than 5% at dawn and dusk to 100% near midday. In fact, ingestion rates from 1000 - 1600 hr were insufficient to meet the respiratory expenditure of the zooplankton; energy incorporated at dawn and dusk must be used to support the population at this time.

In some cases, the high assimilation efficiencies demonstrated by other workers (Conover, 1964; and others) may be a consequence of experiments conducted near midday when feeding rates are inherently low and efficiencies possibly high. In fact, during this study, the average assimilation efficiency between 1100 and 1600 hr was 53%. This is consistent with values determined by other authors even though the total efficiency over the 24-hr period was less than 6%.

Figure 3-14 Diel changes in the net assimilation efficiency (A/I x 100) and net growth efficiency (A/(A + R) x 100) for Eunice Lake zooplankton (May 28 - 29, 1973).


Assimilation efficiency
Net growth efficiency
ASSIMILATION EFFICIENCY % (A/Ix100)
NET GROWTH EFFICIENCY (K2) % (A/(A R)x100)
TIME OF DAY
100
90
80
70
60
50
40
30
20
10
12 2 4 6 8 10 12 2 4 6 8 10 12

The net growth efficiency (A/(AR) x 100) remained highest at dawn and dusk (Fig. 3-14). In the present investigation, Eunice Lake zooplankton would be unable to sustain growth at the ingestion rates found near midday; some of the energy incorporated as a result of feeding at dawn and dusk must be used to maintain the population during midday. Energy assimilated at dawn and dusk, albeit at low efficiencies, may be utilized for increased production throughout the day as well as for maintenance of metabolic requirements at times when feeding rates are negligible.

There are a number of possible errors which may have influenced the present budget and consequently its interpretation. As previously mentioned, excretion of dissolved inorganic and organic compounds have not been included in this budget. High excretion losses would tend to underestimate the gross assimilation rate, resulting in net growth efficiencies (Fig. 3-14) which would be abnormally high. The efficiencies found in the present study (15 - 35%) are similar to the values presented by Conover (1968) for actively growing copepods (20 - 60%) based on the expression: $\Delta W = K_2 (A'R\Delta t)$ where ΔW is the energy equivalent of growth over any period of time (Δt) and A', R, and K_2 are the fraction of ingested food assimilated, energy acquired in feeding, and net growth efficiency, respectively.

Several other factors may have affected the assimilation rates measured in this study. If some unincorporated ^{14}C remained in the gut of zooplankters after the 2-hr clearing period, assimilation rates would be overestimated. A major consideration in the interpretation of the present data is that feeding rates were measured with natural phytoplankton populations, whereas assimilation was determined with a labelled culture of *Chlamydomonas reinhardtii* and lake water, albeit at similar concentrations. Since the species composition of phytoplankton ingested has been shown to affect

assimilation efficiencies (Fedorov and Sorokin, 1967; Schindler, 1971), this may be an important consideration here. Further studies are required to establish whether superfluous feeding is a general phenomenon under natural conditions.

Nevertheless, the existence of marked diel rhythms in feeding, respiration, and assimilation tend to cast doubt on the observations of several previous authors. Estimations of total daily consumption and assimilation based on experiments conducted during the day may account for a proportion of the discrepancy between these data and the values presented in other energetics studies. For example, Richman (1964) estimated the daily caloric intake of *Diaptomus* at approximately 1.7 cal/mg dry wt/day with a respiratory expenditure of 1.1 cal/mg dry wt/day. Similar values have been proposed for other zooplankters by a number of workers (Lasker, 1966; Pavlova, 1964; Richman, 1958; Pechen and Kuznetsova, 1966). The total daily caloric intake, assimilation, and respiratory expenditure measured in this study were 3.9, 0.22, and 1.0 cal/mg dry wt/day, respectively. Although respiratory expenditures suggested by other workers are not significantly different from the present one, presumably due to the small amplitude of respiration rhythms, the caloric intakes of other authors are less than 40% of the value determined for Eunice Lake zooplankton; assimilation estimates by other workers exceeded the present value by at least two times.

GENERAL DISCUSSION

In conclusion, it is clearly evident that diel rhythms in the feeding and respiration of zoo-plankton presist under both constant laboratory conditions and natural field conditions. These rhythms, although endogenous, are reinforced by periodic changes in light and temperature, increasing the magnitude of rhythms over and above that due to the intrinsic component alone. Further investigations will be required to determine whether or not rhythms of the type demonstrated in this study are circadian; these phenomena must be monitored continuously over a period of several days and the frequency of the free-running period determined.

Several other areas require further investigation. These include a more precise definition of the effects of temperature on the amplitude and persistence of respiration and feeding rhythms as well as clarification of the relationship between temperature and the responses of zooplankton to light intensity and spectral composition.

It is also not known to what extent endogenous respiration and feeding rhythms persist in other species of zooplankton or to what extent these phenomena are seasonally dependent; ubiquity of endogenous rhythms cannot be deduced from the results of the present investigation alone.

In this study, the magnitude of feeding rhythms always exceeded that of respiration rhythms. Since this has important ramifications to the diel energy budget of Eunice Lake Zooplankton, it is necessary to establish whether or not this too is a common phenomenon. In other words, does "superfluous feeding" commonly occur at dawn and dusk and is the food consumption during midday typically insufficient to meet the metabollic demands of the zooplankton community? It will also be

important to clarify the role of dissolved excretion products, omitted from the present investigation, to the diel energy budget of zooplankton.

The differential response of cladocerans and copepods to low intensity blue or red light is also worthy of further consideration. An electrophysiological examination of the visual system of zooplankton at different temperatures and at various times of the day may indicate that specific photosystems are more active at dawn and dusk when zooplankters are exposed to low intensity blue light.

One of the most important problems remains unresolved; what factors in the environment of zooplankton act as *Zeitgebers* to maintain synchronization of endogenous rhythms to the natural light : dark cycle? Rates of intensity change ($\Delta I/\Delta t$), absolute intensities, shifts in spectral composition of light with time and depth, photofraction, temperature or any combination of these factors may act as cues under natural conditions. Each of these variables must be examined in the laboratory to determine their ability to shift the phase of the free-running rhythm.

However, despite the seemingly large number of questions posed by the present investigation, several significant advances have been made. Endogenous diel feeding and respiration rhythms have been demonstrated under a variety of circumstances, the effects of several environmental parameters demonstrated from laboratory studies and the existence of diel rhythms in situ confirmed for at least one lake. The magnitude of the rhythms demonstrated in this study clearly suggests that rhythmic phenomena may be of great importance to the experimental design of future energetics studies and the diel energy budgets of zooplankton communities.

SUMMARY

1. Endogenous diel rhythms in feeding and respiration were shown at temperatures from 10 - 22 C, in zooplankton from both oligotrophic and eutrophic lakes, in both cladocerans and copepods, at different times of the year, and at different latitudes.

2. The diel rhythms demonstrated in the present investigation were typically bimodal; respiration and feeding rates were always highest during the early morning and late afternoon or evening, and were lowest during the midday.

3. The amplitude of the diel rate changes varied considerably but was usually two to four times greater than midday levels for respiration and six to seven times higher at dawn and dusk in the case of feeding.

4. The timing of respiration and feeding maxima were closely correlated with the onset of dawn and dusk.

5. The oxygen consumption of zooplankton at dusk was on the average 42% higher than at dawn; the evening maximum always equalled or exceeded morning rates.

6. Light intensity, spectral composition, and temperature had a marked influence on the feeding and respiration rates of zooplankton.

7. Respiration rates of zooplankton increased with temperature in a curvilinear manner; oxygen consumption of *Daphnia pulex* increased from 3.5 µl O_2/mg dry wt/hr at 5 C to 26.5 µl O_2/mg dry wt/hr at 25 C.

8. Temperature also affected both the feeding rate and size of cell consumed by *D. pulex;* maximum rates were observed at 15 C, lowest values at 5 and 25 C.

9. Temperature was expected to have pronounced effects on the amplitude of diel feeding and respiration rhythms; in migrating species temperature may reinforce endogenous rhythms, increasing rates further at dawn and dusk when zooplankters are found near the surface and lowering them during midday when zooplankton descend to colder water.

10. Spectral composition and intensity were shown to affect zooplankton feeding and respiration only at low light intensities; there were no significant quality or intensity effects above light intensities of 500 $\mu w/cm^2$.

11. The effects of light intensity were temperature and colour dependent; this interaction, however, varied with the species examined.

12. At all temperatures and at intensities less than 30 $\mu w/cm^2$ the cladocerans examined showed highest feeding and respiration rates in blue light. However, in copepods, rates were highest in red or occasionally white light and lowest in blue.

13. Feeding and respiration rates between 1 and 30 $\mu w/cm^2$ exceeded dark rates; beyond 100 $\mu w/cm^2$ light rates were less than those measured in the dark.

14. In the copepod, *Cyclops scutifer,* feeding rate was inversely related to light intensity over the range examined (0.1 - 1000 $\mu w/cm^2$).

15. In the cladocerans, *Daphnia rosea* and *Holopedium gibberum*, the biomass of phytoplankton consumed was three times higher in blue light than in either red or white light of equal intensity (20 - 40 $\mu w/cm^2$).

16. The effects of twilight duration and spectral composition of light on the maintenance and phase of diel respiration rhythms were not clearly defined in the present investigation.

17. Significant diel rhythms in the feeding, respiration, phosphate excretion, and assimilation of zooplankton were demonstrated *in situ*. Differences in the amplitude of each of these rhythms resulted in large variations in assimilation efficiency and net growth efficiency throughout the day.

18. Diel changes in the environment of Eunice Lake zooplankton were shown to reinforce the endogenous component of diel rhythms; at dawn and dusk, when migrating zooplankters were distributed near the surface, rates would be increased by the combined influence of higher temperatures, low light intensities, and a high proportion of blue light.

19. The results of the *in situ* investigation indicate that Eunice Lake zooplankton exhibit superfluous feeding at certain times of the day; ingestion rates at dawn and dusk were well in excess of energy requirements.

20. Net assimilation efficiencies varied from less than 5% at dawn and dusk to 100% near midday.

21. Ingestion rates from 1000 - 1600 hr were insufficient to meet the respiratory expenditure of the zooplankton; it is suggested that energy incorporated at dawn and dusk is used to support the population at this time.

22. The net growth efficiency of Eunice Lake zooplankton was also highest at dawn and dusk.

23. The total daily caloric intake, assimilation, and respiratory expenditure measured in this study were 3.9, 0.22, and 1.0 cal/mg dry wt/day, respectively.

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CURRICULUM VITAE

Name: WAYNE STUART DUVAL

Place and Year of Birth: Vancouver, British Columbia, 1945

Education: Simon Fraser University
B.Sc. (Honors First Class), 1969

Experience: Research Assistant, Department of Biological Sciences
Simon Fraser University, 1967 - 1973

Teaching Assistant, Department of Biological Sciences
Simon Fraser University, 1967 - 1973

Awards: B.C. Government Scholarship,
1967 - 1968

National Research Council Scholarship,
1968 - 1972

Simon Fraser University Graduate Scholarship,
1972 - 1973

Publications:

Duval, W. S., T. J. Brown and G. H. Geen. 1973. A submersible spectroradiometer/data acquisition system. J. Fish. Res. Board Can. 30: 313-316.

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