Circadian cycles in growth and feeding rates of heterotrophic protist plankton

Hans H. Jakobsen[1] *and Suzanne L. Strom*
Western Washington University, Shannon Point Marine Center, 1900 Shannon Point Road, Anacortes, Washington 98221

Abstract

Growth and feeding rates of four species of planktonic marine heterotrophic protists showed pronounced diel cycles. In most cases, rates were higher during the day and lower at night. However, for the ciliate *Strobilidium* sp., growth was highest at night. In another ciliate species, *Balanion comatum,* no day–night difference in growth and feeding rates was found. Maintenance of day–night rate differences during 24-h exposures to continuous darkness demonstrated that most of these protists had circadian cycles. The heterotrophic dinoflagellate *Oxyrrhis marina* exhibited a clear irradiance threshold for maintenance of the circadian cycle: day–night differences in growth and feeding rates were observed at irradiances as low as 2.6×10^{-3} μmol photons m^{-2} s^{-1} but not at 3.1×10^{-4} μmol photons m^{-2} s^{-1}. We also studied growth and feeding in transition from complete darkness to culturing in a day : night light cycle in *O. marina* and found that resetting the circadian cycle in this dinoflagellate temporarily arrested growth and feeding. We suggest that protists use a time-integrated light threshold rather than an instantaneous irradiance to maintain the circadian cell cycle. This allows them to avoid temporary arrests in growth and feeding when they are mixed over depth across the 3.1×10^{-4} μmol photons m^{-2} s^{-1} irradiance threshold. Overall, higher rates of feeding and growth during the light period, when phytoplankton are photosynthetically active, may strongly influence predator–prey cycles in the euphotic zone.

Autotrophic dinoflagellates have long been known to exhibit a circadian cycle in mitosis cued by variations in environmental light. Persistence of the mitotic cycle for one to several days in continuous light or darkness demonstrated that, while the circadian cycle is entrained by environmental light cycles, it is ultimately controlled by biochemical processes endogenous to the dinoflagellate cells (e.g., Sweeney and Hastings 1958). Sweeney and Hastings' discovery led to a burgeoning of research on autotrophic dinoflagellates, such that circadian cycles in these organisms are better understood than most other group of single-celled eukaryotes. In the field, day–night variations in mitosis and in feeding have been reported for autotrophic, mixotrophic, and heterotrophic protists (Wikner et al. 1990; Butow et al. 1997). Whether these diel variations are triggered by environmental changes in light or whether they represent circadian cycles is poorly understood, and, in general, almost nothing is known about the circadian cycle in heterotrophic protists and its potential coupling to the flow of matter.

In the current work, cyclic processes are considered circadian if they follow the definitions given by, e.g., Aschoff (1981). These are as follows: a circadian rhythm must be a biological rhythm with a period of approximately 24 h, the rhythm must be endogenously generated (although modulated by environmental factors), and the rhythm must be entrainable by a naturally occurring environmental cycle with a period of 24 h. Contrasting with this are diel cycles, processes studied in light and darkness under a day : night scheme. Diel cycles may be endogenously regulated, but the day : night light regimes typifying most experiments do not allow us to distinguish between direct environmental regulation and circadian rhythms.

In addition to mitosis, physiological functions in autotrophic dinoflagellates that follow circadian cycles include bioluminescence, photosynthesis, superoxid-dismutase, nitrate metabolism, and phototaxis (reviewed in Roenneberg 1996). For most of the studied dinoflagellates cell division occurs in the dark phase, although exceptions exist (Van Dolah and Leighfield 1999 and references therein). The persistence of the circadian cycle in the face of changes in the light regime varies among autotrophic flagellate species. For example, the autotrophic dinoflagellate *Prorocentrum* sp. maintained a circadian cycle in cell division for 3 d after transition from a light : dark cycle to constant light. In contrast, *Amphidinium carterae* lost its diel cycle only 1 d after transition to constant light (Chisholm and Brand 1981).

The circadian cycle in autotrophic dinoflagellates appears to be controlled by a light-cued endogenous regulatory mechanism, but the underlying biochemistry is as yet poorly understood (Roenneberg 1996). The action spectrum for the mitotic circadian response in the autotrophic dinoflagellate *Gonyaulax polydra* shows two peaks. One peak is in the blue waveband (475 nm), and the other and smaller is in the red waveband (650 nm) (Hastings and Sweeney 1960). Apparently spectral composition dictates the length of the circadian period of mitosis in *G. polydra*: the circadian period is shortened by the addition of blue wavelengths and lengthened by the addition of red (Roenneberg and Hastings 1988). In addition to light quality, dissolved substances such as nitrate (Sweeney and Folli 1984) and other algal exudates (Roenneberg et al. 1991) are able to shorten or lengthen the circadian cycle. This plasticity has been interpreted as a means to respond to and optimize fitness in a dynamic environment (Roenneberg 1996).

Table 1. Heterotrophic protist predators, prey, and their size (ESD ± SD) used.

Predator	ESD (±SD) (μm)	Prey
Oxyrrhis marina	21 (±14)	*Rhodomonas* sp. *Heterocapsa rotundata*
Balanion comatum	13 (±9)	*Rhodomonas* sp.
Strombidium sp.	41 (±27)	*Rhodomonas* sp.
Strobilidium sp.	48 (±30)	*Isochrysis galbana*
Mesodinium pulex	18 (±1)	*Heterocapsa rotundata*

Because nearly all studies of diel and circadian cycles in single-celled eukaryotes have been conducted on autotrophic organisms, much is left to learn about such cycles in heterotrophic protists. At the same time, heterotrophic and mixotrophic protists are key components in marine planktonic food webs. They fix carbon and graze on primary producers, providing a food resource for metazoans such as copepods and thereby linking primary production to higher trophic levels (Strom et al. 2001). Because heterotrophic protists may display day : night differences in their activity similar to those of autotrophs, circadian variations in growth and feeding may profoundly affect the transfer of matter in the aquatic food web over the course of the diel period.

Study of the circadian cycle in heterotrophic protists offers some advantages compared to autotrophic protists. In strictly heterotrophic protists, light potentially matters much less in terms of food uptake than in autotrophs; i.e., heterotrophic protists may be able to feed and undergo endless numbers of cell divisions in complete absence of light. Hence experiments can be carried out under stressful light regimes that would minimize growth of or even kill autotrophs.

In this study we examined the feeding and growth rate responses of common coastal heterotrophic protists to the diel light cycle. Cultures acclimated to a light : dark cycle were studied in both a light : dark cycle, to look for possible diel cycles, and in continuous darkness, to determine whether day–night rate differences were the result of circadian cycles. Through stepwise reductions in experimental light level, we determined the minimum irradiance required for maintenance of diel cycles in feeding and growth rates. This irradiance threshold is compared to natural ocean irradiance levels to explore the potential ecological significance of the threshold effect in relation the physical environment.

Material and methods

Heterotrophic protist cultures—Cultures were isolated from local seawater following the protocol given by (Gifford 1985). The species employed are listed in Table 1. Cultures were maintained in 250-ml Erlenmeyer flasks on mixed algal diets in dim light (10–20 μmol quanta m^{-2} s^{-1}) in order to avoid significant prey growth. Light for maintenance cultures, provided by cool white fluorescent bulbs, was on a 14 : 10 light : dark (L : D) cycle, hereafter called "diel light." Cell sizes ($n > 20$) were estimated from live cells using a video imaging system. All studied heterotrophic protist species except one appeared to be strictly heterotrophic. The
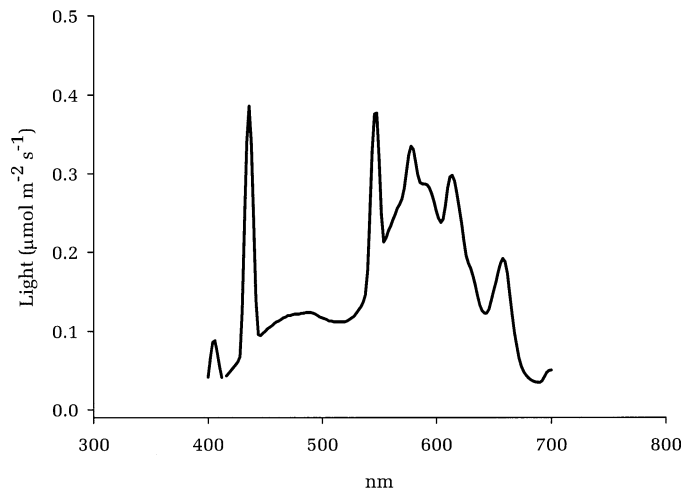

Fig. 1. Culture cabinet irradiance (μmol quanta m^{-2} s^{-1}) in the photosynthetically active range (PAR) as obtained with the LI-1800 spectroradiometer.

exception was *Strombidium* sp., in which fluorescent chloroplasts originating from the prey *Rhodomonas* sp. were found in large numbers throughout the ciliate cells.

Phytoplankton culture—Phytoplankton were obtained from various culture collections and routinely grown in autoclaved seawater with added f/2 nutrients, omitting Si. Cultures were maintained at 15°C at an irradiance of 70–100 μmol quanta m^{-2} s^{-1} from fluorescent cool white light bulbs.

Experimental conditions—Growth and grazing experiments were carried out in a climate-controlled cabinet at 15°C. Temperature during experiments was logged with a HOBO H8 temperature data logger (Onset Computer Corp.) and never changed more than ±1°C.

Grazing experiments were incubated in transparent 50- or 250-ml NUNC tissue flasks (Nalge Nunc International). All experiments were rotated slowly (0.5 rotations per minute) on a plankton wheel to keep cells in suspension but minimize turbulent shear. The plankton wheel was illuminated with a combination of Hagen Sun-glo and Hagen Aqua-glo fluorescent light bulbs (Rolf C. Hagen Inc.). The bulbs (spectral quality shown in Fig. 1) were mounted along the longitudinal axis of the plankton wheel, thus providing illumination from four sides. In this fashion omnidirectional light was found at almost any location on the plankton wheel. Illumination was kept constant at 80 μmol quanta m^{-2} s^{-1} in a diel light cycle. Samples were taken at the beginning and at the end of the light period, allowing calculation of growth and feeding rates during both dark and light periods. Samples were fixed in acid Lugol's solution (final concentration 4%). Heterotrophic protists were counted under an inverted microscope by settling subsamples in 3-ml microwell trays, while phytoplankton cells were counted in gridded 1-ml Sedgwick-Rafter chambers on a compound microscope.

Calculations—Growth rates (μ) were assumed to be constant and exponential during each incubation step and were calculated as

$$\mu = \frac{\ln N_{\text{end}} - \ln N_{\text{begin}}}{t} \qquad (1)$$

where N_{end} and N_{begin} are the number of cells at the end and beginning of each incubation experiment, respectively, measured over the time interval t. Ingestion rates were calculated using the iterative approach given in, e.g., Skovgaard (1996). Ingestion rates (I) were estimated from the decrease in prey cells in grazing flasks compared to parallel prey-only controls and assuming growth rates of predator (y) and prey (x) were constant and exponential, with rate constants μ_y and μ_x, respectively:

$$\frac{dx}{dt} = \mu_x X - Iy \qquad (2)$$

$$\frac{dy}{dt} = \mu_y y \qquad (3)$$

The prey mortality induced by predators, $I \times y,$ was calculated iteratively using a computer with time steps of 0.01 h. To ensure that predator growth and feeding rates represented conditions of balanced growth, we used only data from experiments in which the prey concentration remained within ±30% of initial prey concentration.

Diel and circadian growth and ingestion—The potential diel and circadian cycles in five heterotrophic protist species (Table 1) were investigated. Heterotrophic protists were preadapted to experimental prey at food-satiated concentrations and diel light conditions for a period corresponding to five cell divisions at maximal growth rates. Acclimatization was done under the exact physical regime described in the section experimental conditions (above). Prey concentrations during acclimatization and grazing experiments were always sufficient to sustain maximal growth rates during the entire incubation period. After adaptation, grazing experiments were carried out. Prey was offered initially in concentrations as close as possible to 150 μg C ml^{-1} and was never lower than 50 μg C ml^{-1} at termination of experiments. Prey carbon in these and subsequent experiments was determined from published relationships between cell size and carbon content (Montagnes et al. 1994; Menden-Deuer and Lessard 2000). Experiments were run either in triplicate or in replicates of six. One set of replicates was incubated in diel light, and a parallel set of replicates was incubated in complete darkness. Because 24 h in darkness may change prey quality, experiments were always started at the end of the light period and terminated after 24 h, hence reducing the time that the prey was exposed to unnatural light conditions to ca. 12 h.

Transition light experiment—The grazing and growth responses of the heterotrophic dinoflagellate *Oxyrrhis marina* were studied in transition from continuous darkness to a diel light cycle and back again to continuous darkness. A culture of *O. marina* was grown in complete darkness and fed *Rhodomonas* sp. ad libitum for 2 weeks. After the 2-week adaptation period, the culture was divided into two triplicate groups. One set of triplicates was kept in continuous darkness as a control, and the other set was transferred to diel

light conditions. Fresh prey was added to a final concentration of 150 μg C ml^{-1}. Samples for estimation of predator and prey cell abundance were retrieved at 12-h intervals as described above. *O. marina* was diluted and prey was replaced to a final concentration of 150 μg C ml^{-1} at 24-h intervals in order to keep the culture healthy and food concentrations constant. Growth and feeding of *O. marina* in the diel light cycle was followed until the diel pattern had established. After establishment of a diel pattern in growth and feeding, the culture was again transferred into complete darkness and growth and feeding were followed until the circadian cycle was no longer evident.

Circadian light threshold experiment—This experiment addressed the question: how sensitive are the diel light receptors in the heterotrophic dinoflagellate *O. marina*? Dinoflagellates were preadapted to the desired irradiance and fed *Rhodomonas* sp. ad libitum for a period corresponding to least five cell divisions at maximum growth rate prior to trials. Measurements of growth and feeding rates were carried out in triplicate with parallel prey controls, and samples were retrieved at 12-h intervals as described above. Irradiance was progressively reduced with neutral-density screens (Cinemills Corporation) until the diel cycle was absent. Irradiance in the waveband 400–700 nm (e.g., photosynthetically active radiation [PAR]) was measured inside the experimental bottles with a LI-1400 data logger (LI-COR, Inc.) equipped with a flat LI-190SA Quantum Sensor (LI-COR Inc.). Although we attempted to maintain uniform, omnidirectional light at all positions on the plankton wheel, light may vary. To account for possible light variations, the flat 190SA quantum sensor was mounted inside an experimental bottle on the plankton wheel and light was logged over the entire incubation period. Using a flat sensor may introduce erroneous photon flux estimates because light was omnidirectional. Therefore we made parallel instantaneous readings with a spherical LI-193SA quantum sensor (LI-COR Inc.). These parallel readings allowed calculation of a conversion factor between the two sensors. Because the sensitivity of *O. marina* turned out to be lower then the sensitivity of the sensor available, we determined a screening factor for each additional layer of neutral-density screen applied. This allowed us to reduce irradiance stepwise until the threshold for maintenance of the circadian cycle was found without being able to measure the photon flux directly.

Because irradiance quantity and quality affect the length of the circadian period in protists (Roenneberg and Hastings 1988), we did a spectral analysis (Fig. 1) with a LI-COR LI-1800 spectroradiometer (LI-COR Inc.) in the climate cabinet. The spectroradiometer measures incoming light as both energy per wavelength (Watts m^{-2} nm^{-1}) and irradiance (μmol quanta m^{-2} s^{-1}). For solar radiation between 400 and 700 nm, the average relationship between irradiance and energy per wavelength ($I:W$) is 2.77×10^{18} quanta W^{-1} s^{-1}; this value varies by only a few percent for individual wavelengths within the PAR spectrum (Kirk 1994). By estimating the $I:W$ ratio for each wavelength in the PAR spectral range, an overall average $I:W$ factor of 2.51×10^{18} quanta W^{-1} s^{-1} was found for our experimental light regime. In this fash-
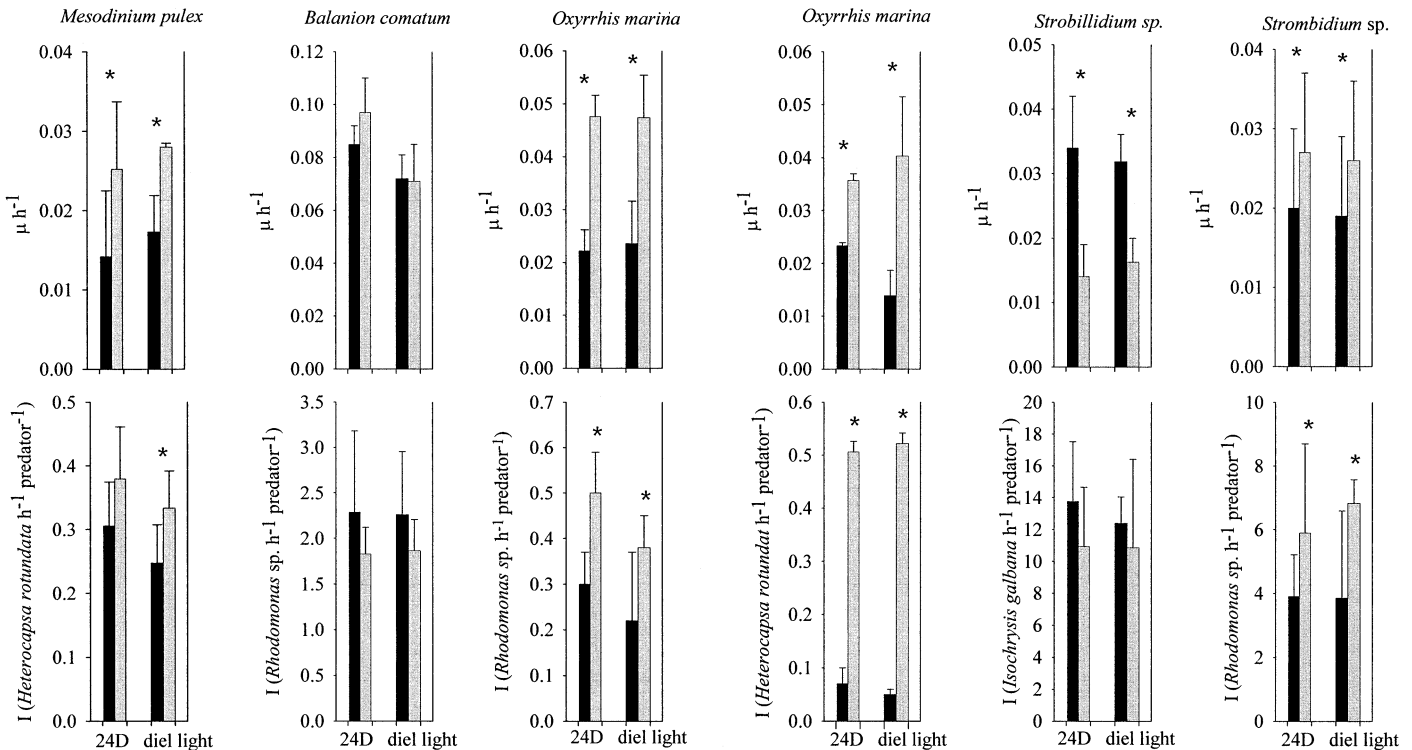
Fig. 2. Growth rates (upper panels) and ingestion rates (lower panels). Black bars denote rates measured at night and gray bars denote rates obtained during the day. 24D indicates 24 h of complete darkness, and diel light indicates a 14 : 10 L : D illumination cycle. Asterisks denote significant differences between day and night rates (*t*-test, $p \leq 0.05$).

ion we were able to convert irradiance estimated with the LI-1400 data logger to energy for the PAR waveband.

Results

Daytime rates were tested against the corresponding night rates in both diel light and 24-h darkness treatments (Student's *t*-test, $p \leq 0.05$). Differences between day and night rates were found for all studied heterotrophic protist species except the ciliate *Balanion comatum* (Fig. 2). In most cases growth and ingestion were both higher in the day than in the night. The only exception was growth in the ciliate *Strobilidium* sp., which was significantly higher at night than during the day. Although ingestion rates in *Strobilidium* sp. tended to be higher during the night too, no significant difference between day and night was found. All species that showed a difference between day and night rates also exhibited a significant circadian cycle when grown in 24-h darkness (Fig. 2). Growth and ingestion rates in 24-h darkness did not differ from the corresponding rates obtained in diel light (*t*-test, $p < 0.05$), at least for the first 24 h.

Although *Strombidium* sp. retained prey chloroplasts, ingestion and growth rates were the same whether the ciliate was incubated in diel light or in 24-h darkness. Additional starvation experiments, however, showed prolonged survival by this species when compared to other non–chloroplast-retaining oligotrichs, suggesting that chloroplasts in this case might function as source of stored energy (H. H. Jakobson unpubl. data).

Oxyrrhis marina in light cycle transition—Preadapting *O. marina* to complete darkness for 2 weeks erased the diel difference in growth and ingestion rates. When reintroduced to diel light, growth and feeding per day decreased to very low values after ca. 80 h (Fig. 3). The decreases in growth and feeding appeared parallel. The decline in growth and feeding was followed by an increase in both rates (Fig. 3) until a diel cycle was reset after ca. 170 h (Figs. 3 and 4). Computed separately, growth in day and night and ingestion in day and night decreased to very low rates in a similar fashion when diel light was introduced to dark-adapted *O. marina* (Fig. 4). The increases in growth and ingestion from the time when rates were minimal (ca. 80 h) to the time when the circadian cycle was reset and light was turned off (ca 170 h) were similar for both day and night (*t*-test on slopes; night growth vs. day growth, $t = 0.0066$, $p < 0.001$; night ingestion vs. day ingestion, $t = 0.0007$, $p < 0.001$, df $= 19$). After 196 h the light was turned off. The circadian oscillations slowly faded away and was absent after 230 h.

Circadian light threshold experiment in O. marina—The ratios between day and night rates remained approximately 2 : 1 until the irradiance was reduced below a PAR level of 2.6×10^{-3} µmol photons m^{-2} s^{-1} (Fig. 5), indicating that this PAR level approximates the irradiance threshold for maintenance of the circadian cycle in *O. marina*. Screening incubations to 3.1×10^{-4} µmol quanta m^{-2} s^{-1} led to day : night rate ratios of 1, indicating that no diel differences in growth or feeding were present (Fig. 5). Because the plank-
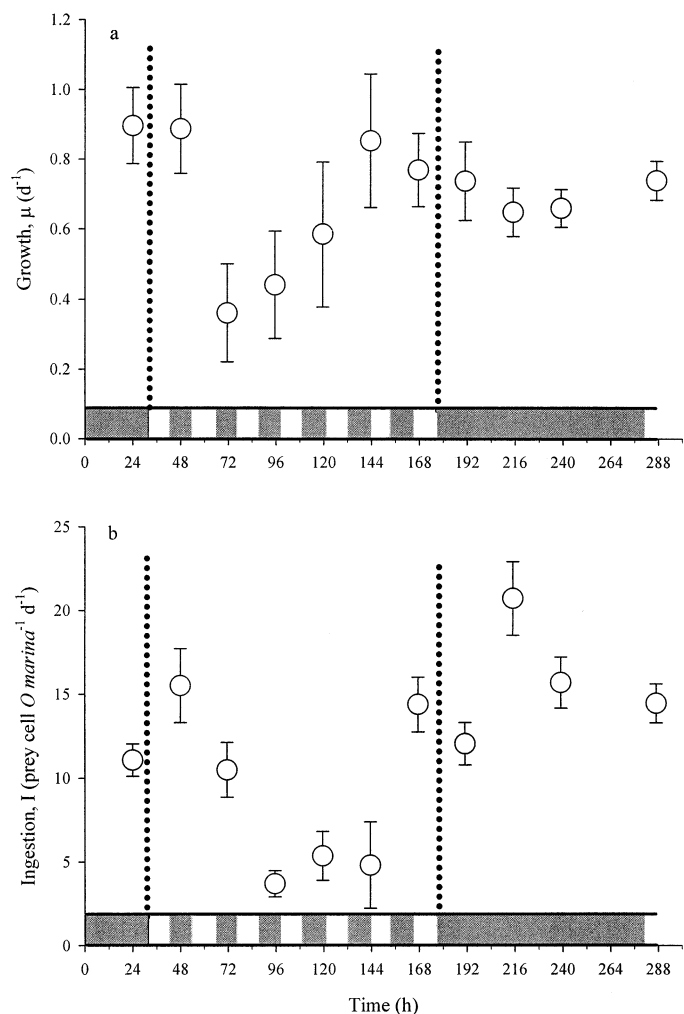
Fig. 3. (a) Net growth rates (d^{-1}) and (b) net ingestion rates versus time for the heterotrophic dinoflagellate *O. marina* in transient light. Rates are 24-h averages. Shaded areas along the *x*-axis indicate times when light was off, while nonshaded areas indicate when light was on.

Fig. 4. (a) Growth rates (h^{-1}) and (b) ingestion rates versus time for *O. marina* in transient light. Filled symbols, night rates; open symbols, day rates. *X*-axis shading as for Fig 3.

ton wheel was illuminated from all directions, the irradiance threshold of 3.1×10^{-4} μmol quanta m^{-2} s^{-1} can be converted to the photon dose required to maintain the circadian cycle. By multiplying the irradiance by the surface area of an *O. marina* cell (1.39×10^{-3} m^2) and Avogadro's number, a light dose of 2.6×10^{17} quanta *O. marina*$^{-1}$ s^{-1} was found. This in turn corresponds to a daily dose of 1.31×10^{22} quanta *O. marina*$^{-1}$ d^{-1}. Dividing by the *I* : *W* factor of 2.51×10^{18} photon W^{-1} s^{-1} estimated with the LI-1800 spectroradiometer, the instantaneous incoming light energy *W* (J s^{-1}) or total energy per circadian cycle received by one *O. marina* cell can be calculated as 1.04×10^{-1} W *O. marina*$^{-1}$ or 5.22×10^3 J cell^{-1} circadian cycle^{-1}.

Discussion

Our data demonstrate that a number of common heterotrophic protist species selected from a wide range of planktonic genera divide and feed with a diel cycle. In the dino-
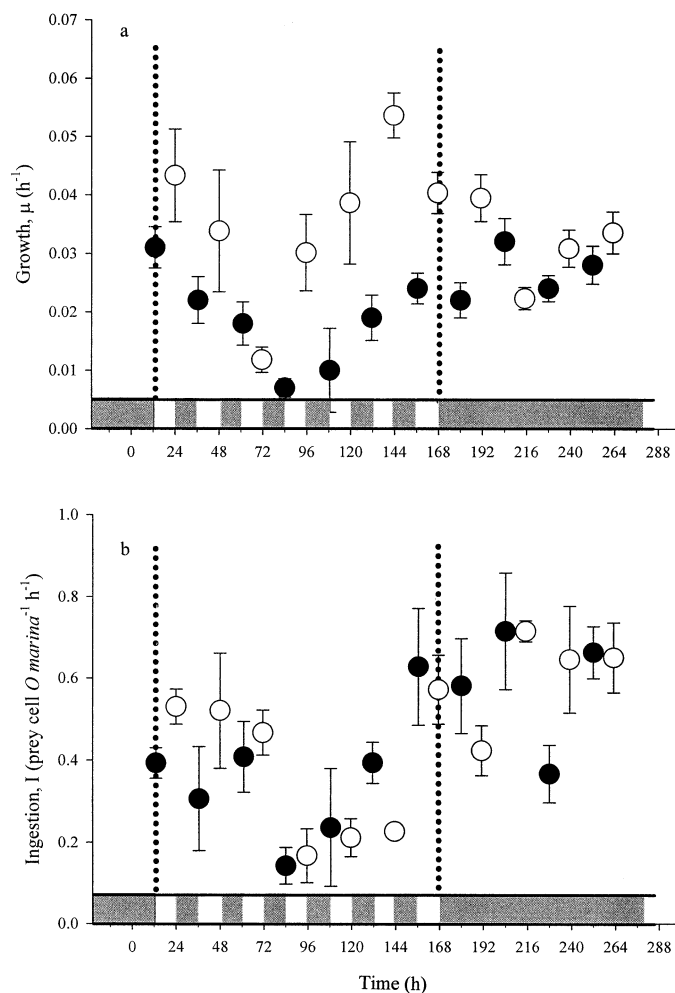
flagellate *O. marina* we detected diel differences in feeding and growth although we screened light to very low irradiances. Most of the studied heterotrophic protists grew at a rate less than one doubling per diel cycle. The only exception to this pattern was the prostomatid ciliate *B. comatum*. This ciliate grew and fed at equal rates during all portions of the diel light cycle, displaying two doublings per 24 h. In most of the studied heterotrophic protists, feeding and growth rates peaked during day. However, in one species, *Strobilidium* sp., the division rate was highest in the night period. Diel variations in feeding rates are found in the field in heterotrophic nanoflagellates feeding on bacteria plankton and, as in our study, feeding could peak during the day (e.g., Wikner et al. 1990) or during the night (e.g., Christaki et al. 2002).

For almost all the heterotrophic protists that exhibited a diel difference in growth and feeding, the diel difference persisted in incubations conducted in 24 h darkness. Because the diel difference persisted in continuous darkness without the modulating environmental cue (Fig. 2), our data strongly indicate that cell division and feeding in most heterotrophic
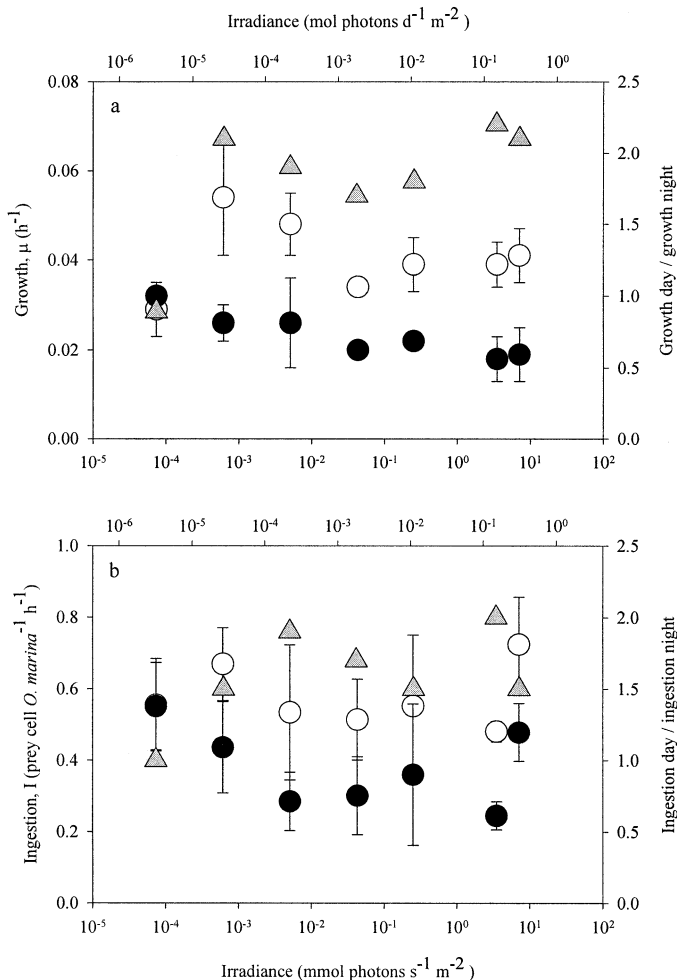
Fig. 5. (a) Growth rates (h^{-1}) and (b) ingestion rates (h^{-1}) in *O. marina* in the diel light threshold experiment (light was progressively screened until no difference between day and night rates was found). Filled symbols, night rates; open symbols, day rates; gray triangles, ratio between day and night rates.

protists are governed by a light-modulated endogenous circadian cycle. This is supported by our observations in the transition light experiment. Removing the external modulating light cue after 168 h in *O. marina* dampened the circadian oscillations in growth, which slowly faded away (Fig. 4a) such that the cycle was absent after about 230 h. This time scale (<3 d) in degradation of the circadian cycle is in accordance with the observations by Chrisholm and Brand (1981). They observed that the dinoflagellate *A. carterae* lost its diel cycle only 1 d after removing the modulating external light cue.

In autotrophic protists such as in dinoflagellates, cell division is typically cued by the "dusk" signal, which is the transition from light to darkness. Cell divisions begin typically 4 to 6 h after the cue but are usually finished before dawn (Van Dolah and Leighfield 1999). Thus the process of cell division may be viewed as a dark process. In our study we found that cell division and feeding straddled the light and night period. Such overlap of growth and feeding across the dawn or dusk transition may be interpreted in several

ways. One interpretation is that the population is unsynchronized so that the timing of feeding and division is variable relative to the dawn/dusk cue. This is not supported by data from the transition light experiment. Here, we saw that the entire population was affected by the introduction of a potential entraining light cue, causing the entire population to arrest its growth and feeding 96 h after introduction of the entraining light cue. Assuming that the entire population is under circadian control, our data suggest that cell division finishes later relative to the dusk signal than is the case for autotrophic dinoflagellates. It remains to be determined, however, whether it is the dusk or the dawn transition that is the cueing signal for cell division and feeding in *O. marina.*

Introducing or removing the diel light cue imposes stress on the circadian cell cycle (Fig. 4a,b). In a study with the facultative mixotrophic dinoflagellate *Fragilidium subglobosum,* the growth but not the feeding was arrested ca. 48 h after *removing* the light cue (Skovgaard 1996). A similar response in growth was observed in *O. marina,* ca. 50 h after removing the light cue (Fig. 4a). The decline in growth was not as dramatic and probably shorter in duration than that observed in *F. subglobosum,* but this may only be a reflection of the much higher growth rate in *O. marina.* Although Skovgaard (1996) suggested that change in the nutritional mode of *F. subglobosum* was responsible for the growth arrest, it is also possible that the arrest was a result of stress in the circadian regulated cell cycle. *F. subglobosum* was able to survive in complete darkness for an extended period and resume growth because of its mixotrophic physiology. Autotrophic dinoflagellates such as *Gymnodinium breve* do not have this option. Van Dolah and Leighfield (1999) observed that growth of *G. breve* was arrested in the absence of an entraining dusk cue. They suggested that the arrest was due to a direct coupling between photosynthesis and the cell cycle. In heterotrophic dinoflagellates such coupling is less obvious, and some dinoflagellates perform post-feeding cell divisions upon depletion of food supply (Jakobsen and Hansen 1997). Hence an alternative explanation of the observations by Van Dolah and Leighfield (1999) is that the diel cell cycle in *G. breve* is sensitive to removal of the circadian entraining light cue as suggested by the observations of Skovgaard (1996) and our experiment.

The light threshold for maintenance of the circadian cycle in *O. marina* is low! Much lower physiological sensitivities, however, have been described. A single photon was found to contain sufficient energy to initiate neurological firing of single light receptor cells in amphibian eyes (Baylor et al. 1979).

Implications of light quantity and quality—The light receptor system that entrains the circadian rhythms of autotrophic protists is complicated, consisting of specialized light receptors sensitive to red light (Lipps 1973) that work in conjunction with receptors activated by blue light (Hastings and Sweeney 1960). In addition to light quality, the intensity of the individual spectral bands may affect the frequency modulation of the circadian cycle in protists (Roenneberg and Hastings 1988).

Light is attenuated increasingly with depth, with the de-

Table 2. Circadian light threshold irradiance for entrainment of circadian growth cycles calculated from the literature for the flagellate *Euglena gracilis* in comparison with the current study.

Circadian light threshold irradiance (I_{ct}) (μmol quanta m^{-2} s^{-1})	W cell^{-1}	J d^{-1} cell^{-1}	Cell divisions d^{-1}	Species	Source
1.8×10^{-1}	8.8×10^{-4}	2.1	0.67	*E. gracilis**	Carre et al. (1989)
4.9×10^{-6}	2.3×10^{-4}	1.0	N/A	*E. gracilis*†	Edmunds (1966)
3.1×10^{-4} (PAR)	1.0×10^{-1}	5.2×10^{3}	1	*O. marina*	This study

* Heterotrophic clone.
† Autotrophic clone.

gree of attenuation dependent on the amount of suspended material in the water column. Hence, most of the light that penetrates below 15 m in the oceans consists of light in the 400–550-nm spectrum with a maximum in the 440–490-nm wavelength range (Kirk 1994). If heterotrophic protists living below this depth use light to maintain a circadian cycle, their circadian light receptors may be most sensitive to 440–490-nm wavelengths. The 400–550-nm wavebands that constitute most of the light below 15 m in the oceans only made up approx 27% of the incoming light in our experiments. Hence, if circadian light receptors are sensitive only to 400–550-nm wavelengths, then the circadian light threshold of *O. marina* may be correspondingly lower than the 3.1×10^{-4} μmol quanta m^{-2} s^{-1} (PAR) estimated here.

Our experiment was not designed to study whether the circadian cycle is maintained by the instantaneous irradiance or by an integrated light dose perceived per cell. Carre et al. (1989) entrained a heterotrophic clone of the flagellate *Euglena gracilis* in a circadian cycle by employing a 1:23 h L:D illumination scheme. Maintenance of a circadian rhythm in growth with 1 h of illumination implies that illumination time, on the scale of a natural day on Earth, is less important than the total light dose received by an organism. By accumulating a light dose over a period of time, undesirable responses to short-term variations in irradiance may be prevented.

The irradiance used to entrain a circadian cycle in *E. gracilis* growth (Carre et al. 1989) was almost 10× lower than that employed in our study with *O. marina* (Table 2). Even lower circadian thresholds are found in the literature (Table 2), suggesting large differences in sensitivity among species. Such sensitivity differences might also be due to methodical differences among studies, particularly in the nature of the light source and the method of light measurement.

Ecological consequences—Light penetration through the water column is described by the vertical attenuation coefficient (k_d),

$$k_d = \frac{\ln I_z - \ln I_0}{z} \qquad (4)$$

where I_0 and I_z are the irradiance at the surface and at z meters depth, respectively. The daily surface irradiance varies with meteorological condition, latitude, and time of the year. For example, the surface irradiance at 48°28′N, 122°42′W varies between 5 [max 11; min 2] mol photons m^{-2} d^{-1} in December and 46 [max 62; min 20] mol photons m^{-2} d^{-1} in June (data from Shannon Point Marine Center meteorological database). By rearranging Eq. 4, inserting the circadian light threshold as the daily irradiance at depth (z) and the seasonally varying surface irradiance, the critical threshold depth for maintaining the circadian cycle in *O. marina* is estimated (Table 3). Using the two annual irradiance extremes from northwestern Washington demonstrates that this critical threshold depth changes by about 10% over the year, assuming that the attenuation coefficient is held constant (Table 3). Organisms transported across the critical threshold depth may respond with arrested growth and feeding as suggested in Figs. 3 and 4. However, assuming protists respond to a time-integrated light dose rather then the instantaneous irradiance, light exposure during mixing can be accounted for using the model approach of Sverdrup (1953), in which a mixing-corrected critical threshold depth (Z_{dt}) is calculated (Eq. 5).

$$z_{dt} = \frac{I_0}{I_{ct} \times k_d} \qquad (5)$$

Instead of the compensation irradiance employed in the orig-

Table 3. The threshold depth calculated from locations with different attenuation coefficients. Daily surface irradiances for summer and winter are obtained from the Shannon Point Center meteorological observation database. Attenuation coefficients are reviewed in Kirk (1994). $z_{dt} = (I_0/(I_{ct} \times k_d))$ and is calculated accordingly to Sverdrup (1953).

Critical diel depth, z, summer (m)	Critical diel depth, z, winter (m)	Z_{dt} (m)	Attenuation coefficients K_d	Locations
497	423	9.83×10^{7}	0.03	Sargasso Sea
36	31	7.19×10^{6}	0.41	North Sea, offshore
7	6	1.44×10^{6}	2.05	Chesapeake Bay
2	1	3.43×10^{5}	8.60	Shannon estuary, Ireland

inal model by Sverdrup (1953), the circadian light threshold irradiance (I_{ct}) is used. The estimated mixing-corrected critical threshold depth is shown in Table 3. All mixing-corrected critical threshold depths estimated are outside the depth scales found in the oceans, which suggests that mixing and the relative low threshold light value (I_{dt}) would eliminate any effect such as periodic arrests in growth and feeding (Figs. 3 and 4). In fact, even a thousandfold decrease in the threshold light sensitivity will not reduce the critical depth to a level where protozoans would be temporarily affected given the depths encountered in the oceans.

The presence of a circadian cycle in heterotrophic protists may cascade both up and down through the planktonic food web, acting on food web dynamics over the 24-h day. The circadian cycle in small heterotrophic nanoflagellates, marine bacterioplankton, and copepods is well studied. Yet very little focus has been drawn to larger heterotrophic protists such as ciliates and dinoflagellates. This group of protist grazers is an important intermediate link between primary produces and metazoans (Strom et al. 2001). Further research is necessary, however, to understand how the circadian cycle in heterotrophic dinoflagellates and ciliates may match or mismatch these organisms with the activities of their prey and predators.

References

Aschoff, J. 1981. A survey on biological rhythms, pp. 3–10. *In* J. Aschoff [ed.], Biological rhythms. Plenum.

Baylor, D. A., T. D. Lamb, and K. W. Yau. 1979. Response of retinal rods to single photons. J. Physiol. **288:** 613–634.

Butow, B. J., D. Wynne, and E. Tel-Or. 1997. Antioxidative protection of *Peridinium gatunense* in Lake Kinneret: Seasonal and daily variation. J. Phycol. **33:** 780–786.

Carre, I. A., A. S. Oster, D. L. Laval-Martin, and L. J. Edmunds. 1989. Entrainment and phase shifting of the circadian rhythm of cell division by light in cultures of the achlorophyllous ZC mutant of *Euglena gracilis*. Curr. Microbiol. **19:** 223–229.

Chisholm, S. W., and L. E. Brand. 1981. Persistence of cell division phasing in marine phytoplankton in continuous light after entrainment to light : dark cycles. J. Exp. Mar. Biol. Ecol. **51:** 107–118.

Christaki, U., C. Courties, H. Karayanni, A. Giannakourou, C. Maravelias, K. A. Kormas, and P. Lebaron. 2002. Dynamic characteristics of *Prochlorococcus* and *Synechococcus* consumption by bacterivorous nanoflagellates 12. Microb. Ecol. **43:** 341–352.

Edmunds, L. J. 1966. Studies on the synchronously dividing culture of *Euglena gracilis* Klebs (strain Z). III. Circadian components of cell division. J. Cell Physiol. **66:** 147–158.

Gifford, D. J. 1985. Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). Mar. Ecol. Prog. Ser. **23:** 257–267.

Hastings, J. W., and B. M. Sweeney. 1960. The action spectrum for shifting the phase of the rhythm of luminescence in *Gonyaulax poluedra*. J. Gen. Physiol. **43:** 697–706.

Jakobsen, H. H., and P. J. Hansen. 1997. Prey size selection, grazing and growth response of the small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate *Balanion comatum*—a comparative study. Mar. Ecol. Prog. Ser. **158:** 75–86.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems, v. 2. Cambridge Univ. Press.

Lipps, M. J. 1973. The determination of the far-red effect in marine phytoplankton. J. Phycol. **9:** 237–242.

Menden-Deuer, S., and E. J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. **45:** 569–579.

Montagnes, D. J. S., J. A. Berges, P. J. Harrison, and F. J. R. Taylor. 1994. Estimating carbon, nitrogen, protein, and chlorophyll *a* from volume in marine phytoplankton. Limnol. Oceanogr. **39:** 1044–1060.

Roenneberg, T. 1996. The complex circadian system of *Gonyaulax polydra*. Physiol. Plant. **96:** 733–737.

———, and J. W. Hastings. 1988. Two photoreceptors control the circadian clock of a unicellular alga. Naturwissenschaften **75:** 206–207.

———, H. Nakamura, L. D. Cranmer, K. Ryan, and J. W. Hastings. 1991. Gonyauline: A novel endogenous substance shortening the period of the circadian clock of a unicellular algae. Experientia **47:** 103–106.

Skovgaard, A. 1996. Mixotrophy in *Fragilidium subglobosum* (Dinophyceae): Growth and grazing responses as function of light intensity. Mar. Ecol. Prog. Ser. **143:** 247–253.

Strom, S. L., M. A. Brainard, J. L. Holmes, and M. B. Olson. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in costal North Pacific waters. Mar. Biol. **138:** 355–368.

Sverdrup, H. U. 1953. On conditions for the vernal blooming of phytoplankton. J. Con. Perm. Int. Exp. Mer. **18:** 287–295.

Sweeney, B. M., and S. I. Folli. 1984. Nitrate deficiency shortens the circadian period in *Gonyaulax*. Plant. Physiol. **75:** 242–245.

———, and J. W. Hastings. 1958. Rhythmic cell division in populations of *Gonyaulax polyedra*. J. Protozool. **55:** 217–224.

Van Dolah, F. M., and T. A. Leighfield. 1999. Diel phasing of the cell-cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*. J. Phycol. **35:** 1404–1411.

Wikner, J., F. Rassoulzadegan, and Å. Hagström. 1990. Periodic bacterivore activity balances bacterial growth in the marine environment. Limnol. Oceanogr. **35:** 313–324.