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# Light-aided digestion, grazing and growth in herbivorous protists

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ABSTRACT: The effect of light on digestion, grazing and growth rates of herbivorous protists was studied in a series of laboratory experiments. Relative to complete darkness, bright light (900 µmol photons $m^{-2}$ $s^{-1}$) resulted in a 40-fold increase in food vacuole loss rates (a proxy for digestion) in the heterotrophic dinoflagellate *Noctiluca scintillans* when fed phytoplankton prey. However, light had no effect on vacuole loss rate when *N. scintillans* was fed heterotrophic (non-pigmented) prey. Ingestion rates of 2 ciliate species feeding on phytoplankton were enhanced by factors of 2 to 7 in moderate light relative to darkness, and one of the ciliates (the tintinnid *Coxliella* sp.) exhibited large light-dependent increases in population growth rate. At very low prey concentrations, the presence of light allowed modest growth (0.12 $d^{-1}$) in populations that otherwise died rapidly (–0.46 $d^{-1}$); at high prey concentrations, growth rate was nearly 20 times higher (0.36 vs 0.02 $d^{-1}$) in light versus dark treatments. Light-aided growth at both low (subsaturating) and high (saturating) prey concentrations indicates that light affected both the extent of digestive breakdown and the rate of digestive throughput. A possible mechanism for the observed light enhancement is photooxidative breakdown of ingested organic matter in these nearly transparent grazers. Photooxidation may be sensitized by chlorophylls and phaeopigments in ingested cells, and should be favored by the oxygen- and lipid-rich environment of phytoplankton chloroplasts. Light-aided digestion, ingestion and growth of protist grazers has important ecological implications, including the possible systematic underestimation of rates of protist herbivory and growth in laboratory experiments, which are typically conducted in dim light or darkness. In aquatic ecosystems, the spatial and temporal coupling of phytoplankton production and grazing losses by a single abiotic resource — light — should lead to reduced temporal variation in oceanic phytoplankton biomass, and may also influence prey selectivity by protists, the ocean's dominant herbivorous grazers.

KEY WORDS: Grazing · Growth · Digestion · Ciliate · Dinoflagellate · Herbivory · Light · Active oxygen



## INTRODUCTION

The role of light in regulating phytoplankton photosynthesis is probably one of the most intensively studied aspects of biological oceanography. The degree of light penetration into ocean waters and its spectral composition at depth define the zone in which photosynthesis can occur (Kirk 1994) and establish a depth gradient of habitat types for different phytoplankton species (e.g. Campbell & Vaulot 1993, Moore et al. 1995). The variation of light with time of day, latitude, and season drives profound variation in primary production over these temporal scales (Banse 1992, Longhurst 1998). Light, along with nutrient supply, is thought to constitute the principal 'bottom-up' control on phytoplankton biomass and growth rate in the sea.

Over the last 15 yr or so, protist grazers have been shown to consume a large fraction of primary production in both coastal and oceanic regions (reviewed by Lynn & Montagnes 1991, Sherr & Sherr 1994). Protist grazing is generally believed to be the major sink for phytoplankton production in open ocean surface waters (Banse 1992). In conjunction with their impor-

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tance as grazers, the dissolved waste products of grazing protists are the dominant source of recycled nutrients in ocean surface waters (Johannes 1965, Caron & Goldman 1990), while protist biomass in turn provides food for organisms at higher trophic levels (Stoecker & Capuzzo 1990, Gifford & Dagg 1991, Fessenden & Cowles 1994). While considerably less well studied than 'bottom-up' processes, protist herbivory appears to be the primary 'top-down' control on phytoplankton biomass and growth rate in the sea.

Aside from behavioral phenomena such as diel vertical migration and phototaxis, light is thought to affect rates of zooplankton activity only indirectly, through the abundance and physiological condition of phytoplankton prey. Long-term study of planktonic protist grazers and their interaction with phytoplankton pigments suggested that light might interact with the digestion of pigmented prey in these consumers, many of which are transparent to visible light. Such an interaction was intimated in the work of Klein et al. (1986), who found that light enhanced the degradation of alloxanthin and chlorophyll *c* by the protist grazer *Oxyrrhis marina*. The goal of this study was to determine whether light exerts a direct effect on rates of digestion, feeding and growth in heterotrophic protists.

## METHODS AND MATERIALS

**Protist culture.** Heterotrophic protists were isolated from local waters (northern Puget Sound, Washington, USA) and grown in 'ciliate medium' (0.2 µm filtered, autoclaved seawater with dilute trace metal addition) according to Gifford (1985). Cultures were kept in dim light (<10 µmol photons $m^{-2}$ $s^{-1}$) on a 14:10 h light:dark cycle at either 12 or 15°C, and fed mixed diets of phytoplankton (Strom & Morello 1998). None of the protist isolates were mixotrophic in the sense of possessing their own chloroplasts or retaining chloroplasts from ingested prey. Phytoplankton were obtained from culture collections and grown in f/2 without added Si; they were maintained at 15°C on a 14:10 h light:dark cycle (irradiance range 70 to 100 µmol photons $m^{-2}$ $s^{-1}$). Light for experiments was provided by 'cool white' fluorescent bulbs, and all light intensities were measured with a Biospherical Instruments QSL 100 4π PAR sensor. In general, grazer species for experiments were chosen to represent common coastal heterotrophic protist species that are transparent and feed on phytoplankton. Phytoplankton prey were chosen to represent a range of taxa and thus pigment composition.

**Digestion experiments.** In separate experiments, the heterotrophic dinoflagellate *Noctiluca scintillans* (non-bioluminescent strain) was fed the autotrophic dinoflagellate *Prorocentrum micans* (pigmented, UTEX1993) or the tintinnid *Coxliella* sp. (non-pigmented). For the *P. micans* experiment, *N. scintillans* was fed *P. micans* exclusively for 5 d, then concentrated using reverse filtration (100 µm mesh), resuspended, and reconcentrated to remove nearly all prey from the medium. For the *Coxliella* sp. experiment, *N. scintillans* was starved for 5 d, then fed the tintinnid *Coxliella* sp. for 2 d. Prey was then removed from the *N. scintillans* culture as above. *Coxliella* sp. in turn was reared on a mixture of microalgae, then starved for 1 d (prey removed by sieving) before being added to the *N. scintillans* culture. Epifluorescence microscopy showed few or no algal cells inside individual *Coxliella* sp. after 1 d without food.

For each experiment, *Noctiluca scintillans* (1 to 2 cells $ml^{-1}$), preconditioned as above, were distributed into 300 ml polycarbonate bottles filled with ciliate medium. Triplicate bottles were prepared for each of 3 light treatments (9 per experiment). All bottles were held in the same water bath (temperature 14.5°C) and illuminated continuously from above and below for 2 d. Irradiance levels in the bottles were 0, 450, and 900 µmol photons $m^{-2}$ $s^{-1}$. Subsamples were removed at the beginning and end of the incubation period and preserved with acid Lugol's (final conc. 1.5%). Food vacuole dimensions (length and width) were measured using a video camera mounted on an inverted microscope, and Bioscan Optimas image analysis software. Food vacuole volumes were calculated assuming each vacuole was a prolate spheroid; there were typically 4 to 6 vacuoles per cell (range 2 to 32). All food vacuoles in the first 30 *N. scintillans* cells encountered were measured for each sample, and an average total vacuole volume per individual was then calculated for the sample.

Food vacuole loss rates for each bottle were estimated from the change in average vacuole volume per individual between the initial and final (2 d) sampling times. The rate of decrease in food vacuole volume was considered a measure of digestion rate. Vacuole loss rates do not account for qualitative changes in ingested prey, which were also observed to vary among treatments, but do provide a measure of throughput rate, analogous to a gut passage time for a metazoan grazer.

**Grazing experiments.** In separate experiments, the effect of light on the ingestion rate of 2 ciliate species (*Coxliella* sp. and *Strombidinopsis acuminatum*) was measured. Neither of the ciliates retains chloroplasts. Both experiments were conducted at 13°C, and bottles were incubated on a bottle roller (3 to 4 rpm) to ensure that non-motile fluorescently labeled algae (FLA) remained in suspension. For the tintinnid *Coxliella* sp., a stock culture was sieved to remove remnant maintenance prey cells and individuals were resuspended in

ciliate medium (100 ml in triplicate polycarbonate bottles) for an experimental concentration of 5 to 6 ciliates $ml^{-1}$. The autotrophic dinoflagellate *Gymnodinium simplex* (UBC119a) was then added to a concentration of 560 cells $ml^{-1}$. After 3 h acclimation to experimental irradiance levels (0 and 100 µmol photons $m^{-2}$ $s^{-1}$), fluorescently labeled (DTAF) *G. simplex* were added at a tracer level of 79 cells $ml^{-1}$ (14 % of unstained *G. simplex* abundance). FLA were prepared according to Rublee & Gallegos (1989) except that buffer NaCl concentration was 3.0 %, and FLA were removed from the phosphate buffer and resuspended in ciliate medium before being added to experimental bottles. After FLA addition, samples (20 ml) were taken immediately and, at 15, 30, and 45 min, preserved by addition to 1 ml cold 10 % glutaraldehyde and 0.5 ml DAPI solution (10 µg $ml^{-1}$), filtered (8.0 µm pore-size polycarbonate filter) and slide-mounted for epifluorescence microscopy. All *Coxliella* sp. (>100) in each sample were examined under blue light excitation for enumeration of FLA in food vacuoles. Ingestion rates in each bottle were calculated from the slope of average FLA/*Coxliella* sp. versus time, scaled to total *G. simplex* availability.

For the oligotrich *Strombidinopsis acuminatum*, ingestion rates were determined as described above, except that (1) 5 irradiance levels were used instead of 2; and (2) the prey species was the autotrophic dinoflagellate *Prorocentrum minimum* (UTEX1995), added at an average unstained concentration of 813 cells $ml^{-1}$ and an average FLA concentration of 250 cells $ml^{-1}$ (23 % of unstained algal abundance).

**Growth experiment.** The tintinnid *Coxliella* sp. was fed the coccolithophorid *Emiliania huxleyi* (non-calcifying strain CCMP374, previously shown to support growth of *Coxliella*) at high and low concentrations (20 000 cells $ml^{-1}$ [= 200 µg C $l^{-1}$], and 2000 cells $ml^{-1}$ [= 20 µC $l^{-1}$], respectively). Both food levels were offered in the dark and in the light (see below). Changes in *Coxliella* sp. abundance were monitored over 48 h to estimate population growth rates. To prevent differences in algal 'food quality' (i.e. biochemical composition) from developing in the different light treatments, a semi-continuous culture approach was adopted in which *E. huxleyi* in each bottle were largely replaced with fresh cells at regular intervals (Fig. 1).

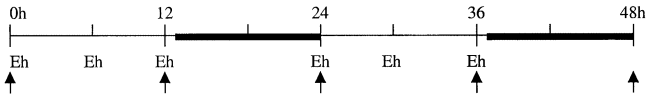


Fig. 1. Time line showing times of *Emiliania huxleyi* replacement (Eh), and times of *Coxliell*a sp. sampling (arrows) for *Coxliell*a sp. growth experiment. Thick line segments represent dark periods (see text for details of method)

To initiate the experiment, *Coxliella* sp. cells were removed from residual maintenance prey by sieving; ciliates were resuspended into fresh sterile filtered seawater (300 ml per bottle) in 12 polycarbonate bottles (3 per treatment). *Emiliania huxleyi* cells (concentration determined by haemocytometer count at the beginning of each light period) were added at high or low densities as described above. Flasks were incubated at 13°C either in continuous darkness, or on a 13:9 h light:dark cycle with an irradiance level of 100 µmol photons $m^{-2}$ $s^{-1}$ during the light period. *Coxliella* sp. were sampled and preserved (50 ml added to acid Lugol's, final conc. 10 %) at 12 h intervals during the 48 h experiment (arrows, Fig. 1); all *Coxliella* sp. cells in each sample (n = 50 to 150 except for final time point at low food concentration) were counted using the Utermöhl technique. Population growth rates were calculated from regression of ln(abundance) on time in each bottle (note that changes in *Coxliella* sp. cell size over the incubation were not accounted for in these growth rate estimates). *E. huxleyi* cells in both 'light' and 'dark' treatments were replaced every 6 h during light periods ('Eh' symbols, Fig. 1). Gentle reverse concentration through a 20 µm mesh was used to remove >90 % of filtered seawater and *E. huxleyi* cells from each bottle; *Coxliella* sp. was then resuspended in an equivalent volume of fresh filtered seawater and *E. huxleyi* from a single source culture was added to bring algal cell densities back up to target levels. Preliminary tests showed that this reverse concentration technique did not result in loss of *Coxliella* sp.

## RESULTS

### Digestion

Digestion of pigmented prey (the autotrophic dinoflagellate *Prorocentrum micans*) by *Noctiluca scintillans* was strongly enhanced by light (1-way ANOVA, p = 0.012). Food vacuole loss rates ranged from 70 $\mu m^3$ $h^{-1}$ in the dark to 2930 $\mu m^3$ $h^{-1}$ in the light (Fig. 2). Qualitative changes (e.g. loss of cell structure) in ingested material were observed over time in all treatments, indicating that darkness did not cause a complete cessation of digestion. However, food vacuole loss rates in *N. scintillans* fed *P. micans* approached zero in the dark.

In contrast, digestion of non-pigmented prey was unaffected by light treatment (1-way ANOVA, p = 0.814). Food vacuole loss rates averaged 2060 $\mu m^3$ $h^{-1}$ for all 9 bottles in which *N. scintillans* was fed the tintinnid *Coxliella* sp. (Fig. 2). Note that the food vacuole volume occupied by *Coxliella* sp. includes volume occupied by the tintinnid lorica: cell cytoplasm of the tintinnid constitutes less than half of the lorica volume.

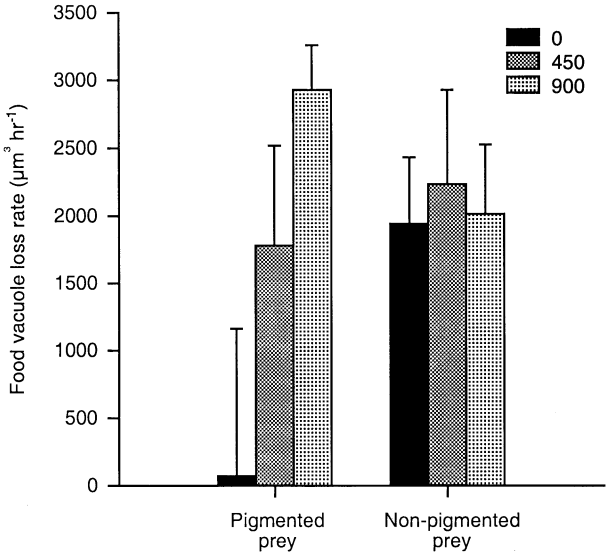


Fig. 2. Average food vacuole loss rates (a proxy for digestion; n = 3) for the heterotrophic dinoflagellate *Noctiluca scintillans* as a function of diet and irradiance. Error bars represent 1 standard deviation. Irradiance levels: 0, 450 and 900 µmol photons $m^{-2}$ $s^{-1}$. Pigmented prey: autotrophic dinoflagellate *Prorocentrum micans*; non-pigmented prey: tintinnid *Coxliella* sp.

## Grazing

The tintinnid *Coxliella* sp. ingested *Gymnodinium simplex* at significantly higher rates in the light than in the dark (1-way ANOVA, p = 0.0013). Rates of FLA ingestion were 2.2 times higher in the light (Fig. 3); scaled to unstained algal abundance, *Coxliella* sp. ingestion rates (±1 SD) averaged 1.52 (±0.29) cells ciliate$^{-1}$ $h^{-1}$ in the dark, and 3.35 (±0.13) cells ciliate$^{-1}$ $h^{-1}$ in the light.

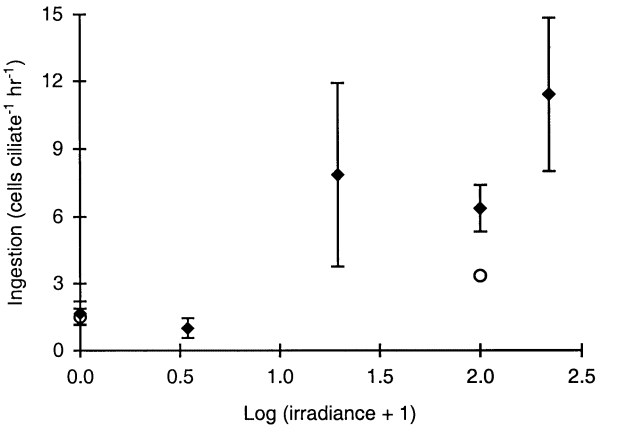


Fig. 3. Ingestion rates (average ±1 SD) as a function of irradiance (µmol photons $m^{-2}$ $s^{-1}$) for *Strombidinopsis acuminatum* fed *Prorocentrum minimum* (◆) and *Coxliella* sp. fed *Gymnodinium simplex* (○)

Light also significantly enhanced phytoplankton ingestion rates for the oligotrich *Strombidinopsis acuminatum* (1-way ANOVA, p = 0.0019). Rates of total *Prorocentrum minimum* ingestion averaged 1.67 (±0.53) cells ciliate$^{-1}$ $h^{-1}$ in the dark, and increased to 11.41 (±3.42) cells ciliate$^{-1}$ $h^{-1}$ at the highest experimental light intensity (Fig. 3). This represents a 7-fold increase. Over the same irradiance range utilized in the *Coxliella* sp. grazing experiment, *S. acuminatum* ingestion rates increased by a factor of 4.

## Growth

Light exerted a positive influence on survival and growth of the tintinnid *Coxliella* sp. (Fig. 4). At high food levels, population growth rates were near zero in the dark, and averaged 0.36 $d^{-1}$ in the light (Table 1). Statistically, these growth rate differences were suggestive ($0.05 < p < 0.10$). The low food levels used in this experiment were below the concentration required to allow sustained survival. However, the effect of light on *Coxliella* sp. at low food concentrations was striking. In the dark, *Coxliella* sp. experienced high rates of mortality in all 3 bottles throughout the incubation period (Fig. 4, Table 1). In contrast, *Coxliella* sp. incubated in the light survived and sustained a low growth rate (average 0.12 $d^{-1}$) for the first 24 h of the incubation. The difference in growth rates for this food treatment over the first 24 h was 0.64 $d^{-1}$, or nearly a doubling per day, and was statistically significant at the 95% confidence level. Over the final 12 h at low food concentration, mortality rates in both light and dark treatments were high and statistically indistinguishable. Note that, while prey cell densities in experimental bottles were not measured directly, maintenance of constant *Emiliania huxleyi* densities was aided by the fact that, under these culture conditions, *E. huxleyi* divides synchronously once per day (several hours after the end of the light period; Wolfe et al. unpubl.) Thus prey concentrations in experimental bottles, if they differed at all during the hours between

Table 1. Average population growth rates ($d^{-1}$) of tintinnid *Coxliella* sp. fed coccolithophorid *Emiliania huxleyi* (n = 3, SD in parentheses) and probabilites from 2-tailed *t*-tests. High food = 200 µg C $l^{-1}$; low food = 20 µg C $l^{-1}$. Irradiance for light treatments was 100 µmol photons $m^{-2}$ $s^{-1}$

| | High food (all data) | Low food (first 24 h) | Low food (last 12 h) |
|---|---|---|---|
| Light | 0.36 (0.10) | 0.18 (0.09) | −3.01 (0.28) |
| Dark | 0.02 (0.24) | −0.46 (0.28) | −2.41 (0.54) |
| p | 0.087 | 0.019 | 0.165 |

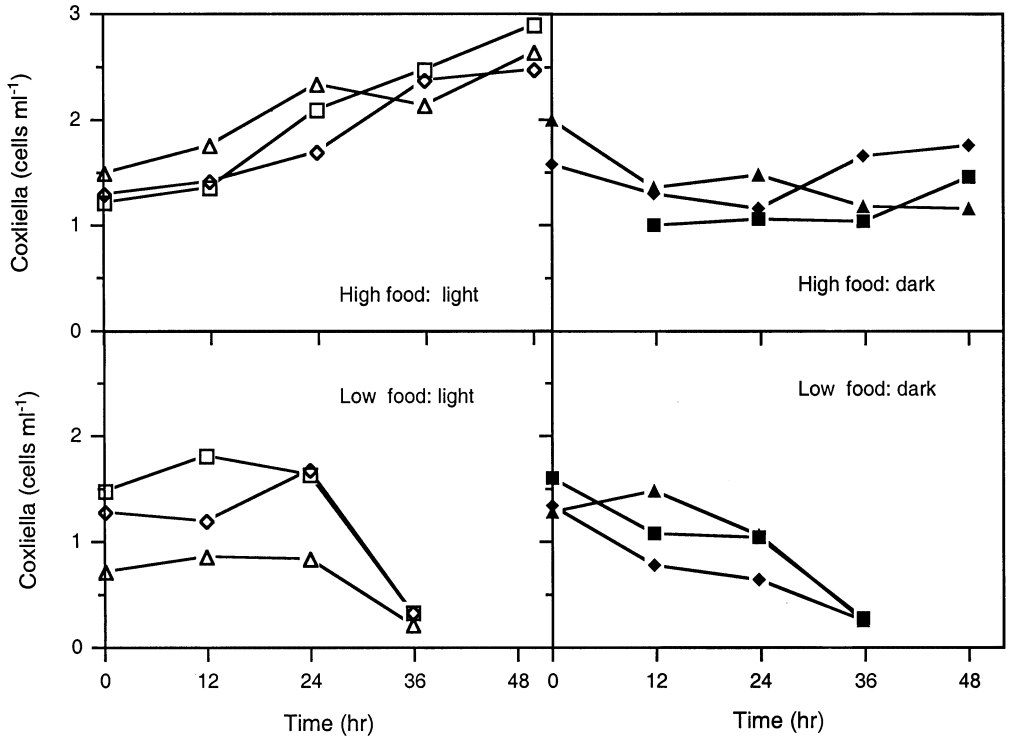


Fig. 4. *Coxliella* sp. concentrations over time during growth rate experiment. Each line represents an individual bottle. Open symbols: light; filled symbols: dark

prey replacement, should have been lower in light treatments due to the higher *Coxliella* sp. densities there. This indicates that differences in prey concentration are unlikely to have been the cause of observed differences in mortality.

## DISCUSSION

### Overview

Algal pigments are known to be highly labile in the presence of light and oxygen (Porra et al. 1997). The goal of this research was to determine whether light-enhanced degradation extends to the bulk organic matter of the phytoplankton cell. In other words, can light provide a significant 'boost' to digestion and metabolic utilization of phytoplankton cells as a whole?

These results show a substantial light enhancement of digestion, grazing and growth rates in heterotrophic protists fed phytoplankton prey. Light increased vacuole loss rates up to 40-fold in *Noctiluca scintillans* and enhanced grazing in 2 ciliate species by factors of 2 to 7. Perhaps most striking was the effect of light on growth of the tintinnid *Coxliella* sp. Here light allowed survival and moderate growth, whereas darkness led to near-zero growth or immediate high mortality, depending on prey concentration. Not only was the light effect substantial, it was rapid and apparently direct. Grazing experiments were short, with less than 4 h between initial exposure to light treatments and final rate measurements. Frequent prey replacement was employed in the growth experiment, to prevent light-related differences in phytoplankton growth rates and 'food quality' (i.e. through biochemical alterations) from affecting ciliate growth rates. Finally, the effect of light was broad and general, influencing a number of fundamental biological rates across a range of grazer and phytoplankton taxa.

### Previous findings

While the influence of light on mixotrophic protists has been reasonably well studied, almost nothing is known about light effects on strictly heterotrophic species. In the laboratory, the vast majority of reported grazing and growth experiments were conducted in dim light or in the dark, often with the goal of minimizing changes in prey cell abundance. While these rates are generally assumed to represent physiological maxima (e.g. Banse 1982, Hansen et al. 1997), my results show that such experiments can underestimate rates achievable in the light. In other words, if the results of this study apply across a range of planktonic protist taxa, our notions of maximum cellular growth rates for herbivorous protists may need to be revised upward. Anecdotally, investigators have known for years that herbivorous protists 'don't do well' when kept in complete darkness. In a study of the herbivorous dinofla-

gellate *Gymnodinium* sp. (an obligate heterotroph), Skovgaard (1998) found that light enhanced both growth rate and growth efficiency. His incubations lasted 3 d, however, and he was unable to rule out light-induced changes in 'food quality' as an explanation for the results. In complete contrast to the data reported here, the oligotrich *Lohmanniella* sp. was recently reported to experience a rapid and sizeable *decrease* in ingestion rate in the light (Chen & Chang 1999). Light intensities were similar to those employed here, and ingestion was measured by uptake of FLA. One possible explanation is that this species retains chloroplasts, and thus responds to light as a mixotroph, feeding preferentially in the dark (Jones et al. 1993, Skovgaard 1996). Alternatively, some protist grazer taxa may show different responses to light than those described here.

There have been relatively few field studies of light-related variation in protist herbivory. Community grazing methods such as the widely employed dilution technique (Landry & Hassett 1982) require lengthy incubations which prevent the examination of short-term responses, and confound direct light-aided digestion responses with the many other processes (photosynthesis, photoadaptation, mixotrophy) driven by light. Most studies examining diel cycles in microzooplankton herbivory are inconclusive for another reason as well: they relied on phaeopigment cycling to indicate grazing, and phaeopigments themselves are highly labile in the light (Litaker et al. 1988, Tsuda et al. 1989, Taguchi et al. 1993). Two recent field investigations using direct enumeration of prey cells do support the hypothesis that light enhances protist grazing rates. Kuipers & Witte (2000), working in the deep chlorophyll maximum of the (sub)tropical North Atlantic, found that grazing on *Prochlorococcus* occurred almost exclusively during daylight hours. Similarly, nanoflagellate grazing on *Synechococcus* in the northwest Mediterranean peaked during midday hours (Dolan & Šimek 1999).

### Physiological response of grazers

Light may affect both the rate and the extent to which pigmented material is digested. More rapid breakdown of ingested cells should lead to increased 'throughput', as suggested by the *Noctiluca scintillans* digestion experiment. This experiment literally measured the rate of food vacuole loss. The large difference in the absolute rate of loss for different prey types in the dark has 2 possible explanations. First, a large portion (over half) of the tintinnid prey cell volume, as defined by the size and shape of the lorica, is not occupied by living cell material. Thus much of the food vacuole volume loss does not require digestion of organic matter for this prey type. Second, the cell structure of the autotrophic dinoflagellate *Prorocentrum micans* prey is substantially different than that of the tintinnid prey, notably in having a thin cellulose theca covering the cell, and in containing membrane-rich chloroplasts. Both could affect the rate at which digestive processes operate.

Effects of throughput rate on grazing should only be manifest at high (saturating) food concentrations, where the rate of ingestion is thought to be controlled by the rate of food vacuole processing. At low (limiting) food levels, ingestion rates are dictated by the rate of encounter between grazers and prey cells. Light might also, however, promote more extensive breakdown of ingested material, for example to smaller, more readily assimilable compounds. This effect could be important at any food level, potentially increasing the gross growth efficiency, or the efficiency with which the grazer can utilize ingested matter for growth. The observation that light enhanced the growth of *Coxliella* sp. even at very low food levels (Fig. 4) indicates that light does, somehow, make ingested material more readily utilizable for growth, perhaps by increasing assimilation efficiencies and hence gross growth efficiencies. An alternative explanation is that behavioral changes in the light, such as increases in swimming speeds of either phytoplankton or ciliates, increased predator-prey encounter rates and led to elevated rates of ingestion and growth. Additionally, while none of the grazer isolates in this study were mixotrophic in the conventional sense, the possibility that photosynthesis by ingested phytoplankton cells continues to occur in grazer food vacuoles cannot be ruled out.

### A proposed mechanism

A probable mechanism for light-aided digestion of phytoplankton is the formation of reactive oxygen species in the protist food vacuole, photosensitized by chlorophyll and chlorophyll degradation products. Breakdown of organic matter by reactive oxygen, including superoxide, singlet oxygen, and hydroxyl radical, occurs in a number of biological systems. Of particular relevance are systems that generate reactive oxygen using photosensitization, a process by which light energy, absorbed by a photoactive compound, is passed to oxygen to generate active oxygen species. Chlorophylls and phaeopigments are particularly potent photosensitizers of active oxygen formation (Halliwell & Gutteridge 1989, Kohen et al. 1995). That phytoplankton pigments play a key role in light enhancement of organic matter degradation is indicated by the digestion experiment (Fig. 2), in which *Noc-*

*tiluca scintillans* evinced no irradiance effect when strictly heterotrophic prey were used.

Compound classes especially prone to breakdown by reactive oxygen include lipids (particularly polyunsaturated fatty acids), proteins, and nucleic acids. Lipids and proteins constitute substantial fractions of phytoplankton biomass, so their degradation is of particular interest for herbivory. Unsaturated fatty acids are readily peroxidized in the presence of reactive oxygen and, particularly in the presence of metals, can degrade further into a host of low molecular weight organic compounds (Halliwell & Gutteridge 1989). Peroxidized lipids are themselves free radical compounds and can initiate further oxidation reactions, so that the presence of unquenched reactive oxygen in a biological system creates a chain reaction of organic matter breakdown. Proteins also react with active oxygen, resulting in aggregation, denaturation, and fragmentation (Davies 1987). Once oxidized, proteins exhibit increased susceptibility to enzymatic breakdown (Davies & Delsignore 1987, Starke & Oliver 1989, Casano et al. 1994).

Chloroplasts, which contain high levels of oxygen, chlorophylls, polyunsaturated fatty acids and proteins, must deal with oxidative stress whenever exposed to light. Indeed, Halliwell & Gutteridge (1989) describe the chloroplast as a 'system with problems.' Normally, chloroplasts have a number of solutions to these problems, and are able to suppress the formation of reactive oxygen and to scavenge whatever reactive oxygen is produced. These solutions include the binding of chlorophyll to proteins, metal sequestration, free radical quenching by carotenoids, and the antioxidative activity of enzymes such as superoxide dismutase, catalases, and peroxidases (Krinsky 1978, 1979, Asada & Takahashi 1987, Polle 1997). Under various types of stress, however, these protective mechanisms may be overwhelmed, and damage by reactive oxygen is thought to be the process underlying plant cell damage during exposure to excess light or cold temperatures (Foyer et al. 1994, Lesser 1996, Polle 1997, Vardi et al. 1999). Digestion is, of course, a rather profound form of stress, and conditions in protist digestive vacuoles may disrupt the normal flow of electrons to photosynthetic pathways or carotenoid quenchers within the chloroplast, promoting the formation of reactive oxygen species in high-light environments. In other words, by disrupting chloroplast structure and function, protist grazers may get a 'digestive boost', as the oxidation reactions to which the system is already prone proceed to break lipids and proteins into smaller, more readily assimilable compounds.

Digestive processes are likely to disrupt chloroplast structure by, for example, disrupting pigment-protein complexes and compromising chloroplast membrane integrity (Nagata & Kirchman 1992, Strom 1993). Chemical conditions within ingested prey cells are likely to be altered as well, due to the acidic environment of the protist food vacuole (Nilsson 1979). A study of algal digestion by Zemke-White et al. (1999) found that the major structural effect of acid (pH 2) treatment was the breakdown of thylakoid membranes in the green macroalga *Ulva rigida*.

## Ecological consequences

A direct relationship between irradiance and rates of herbivore growth and grazing has important ecological consequences. Gradients in light intensity drive variation in marine primary production over time of day, season, depth and latitude. My data suggest that herbivory, as determined by individual grazing rate and grazer community biomass, has the potential to vary over similar time and space scales. The regulation of primary production and phytoplankton consumption at least in part by the same abiotic resource — light — should increase the extent of coupling between these processes. That is, irradiance conditions favoring high phytoplankton photosynthesis and growth rates should also favor high rates of herbivore grazing and growth, and vice versa. In as much as protist grazers are thought to be the major consumers of marine phytoplankton, this coupling could have a profound dampening effect on phytoplankton biomass variation in the sea, favoring a relative biomass constancy of the type actually observed in many ocean regions (e.g. Miller et al. 1991, Landry et al. 1997).

The growth rate experiment suggests that light could play a particularly important role for grazers at times of phytoplankton scarcity or patchiness. In the light treatment, *Coxliella* sp. survived and sustained a low rate of population growth for 24 h at very low food levels; presumably an encounter with a patch of prey cells during this time would have allowed the population to respond more strongly than the comparable dark-incubated population, whose density was reduced to just over half its initial level after 24 h (Fig. 4).

Finally, these data indicate that, all other factors aside, ingestion of phytoplankton prey might be energetically favored over ingestion of heterotrophic prey. This could influence prey selection by protist grazers. On the other hand, phytoplankton might be inherently more difficult to digest than heterotrophic eukaryotes, given their high content of chloroplasts rich in layered membranes. Such a possibility is suggested by the *Noctiluca scintillans* digestion rate data, as well as by work showing that bacterial membrane fragments (similar in composition to chloroplast membranes) are a major waste product arising from digestion by

nanoflagellates (Nagata & Kirchman 1992). In that case, light could be a means of utilizing a prey type otherwise relatively resistant to digestion. A potential cost of light-aided digestion is the presumed need for the grazer to defend itself against the potentially damaging products of photooxidation. Further work is needed to determine the extent to which these findings can be generalized, to verify the mechanism of action, and to understand the implications for utilization of the prey spectrum encountered by planktonic protist grazers.

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