# Growth and grazing responses of two chloroplast-retaining dinoflagellates: effect of irradiance and prey species

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ABSTRACT: The effect of irradiance on growth and grazing responses of 2 phagotrophic dinoflagellates, *Gymnodinium gracilentum* Campbell 1973 and *Amphidinium poecilochroum* Larsen 1985, was studied. While *G. gracilentum* belongs to the plankton, *A. poecilochroum* is a benthic species that primarily feeds on prey associated with surfaces. Both organisms are able to retain functional chloroplasts from their prey. They are both able to grow heterotrophically in the dark, but growth rates increase in the light. The maximum growth and ingestion rates of *G. gracilentum* are much higher than those of *A. poecilochroum*. However, the growth rate of *A. poecilochroum* is saturated at a lower irradiance (~6 µmol photons $m^{-2} s^{-1}$) than to *G. gracilentum* (~60 to 80 µmol photons $m^{-2} s^{-1}$). Also, the irradiance required for saturation of growth for both dinoflagellates matched that found for the prey algae. The effect of light on ingestion and growth was also studied during the light and dark periods of the day. Ingestion rates of *G. gracilentum* were higher during the light period, while division rates were higher during the dark period. Offered a variety of prey items belonging to different algal classes, *G. gracilentum* selectively feeds on species belonging to the class Cryptophyceae.

KEY WORDS: *Gymnodinium gracilentum* · *Amphidinium poecilochroum* · Dinoflagellates · Mixotrophy · Feeding rates · Growth rates · Chloroplast

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## INTRODUCTION

Many planktonic heliozoans, foraminifers, ciliates and dinoflagellates are pure heterotrophs. However, some species within these groups have the ability to retain functional chloroplasts from ingested algal prey and thereby become mixotrophs. The most restricted form of this mixotrophy has been described in some heliozoans (Patterson & Dürrenschmidt 1987). In these heliozoa, the mixotrophy is transitory and the retention of plastids non-obligatory. More developed relationships can be found among the ciliates. Here the plastids are placed at the periphery of the cell, probably for effectiveness in light-harvesting, and are often quite constant in number (Laval-Peuto 1992). The plastids have only a limited lifespan within the ciliate cell, before they are either diluted out, digested or egested (Stoecker et al. 1988, Stoecker & Silver 1990). In many cases, it is not known whether the relationship is facultative or obligatory. Obligate mixotrophy has been demonstrated in only 1 chloroplast-retaining ciliate, *Laboea strobila* (Stoecker et al. 1988).

The importance and role of retained chloroplasts for the metabolism of mixotrophic dinoflagellates is largely unknown. A notable exception is a recent study by Skovgaard (1998) that suggested sequestered chloroplasts help *Gymnodinium gracilentum* resist starvation at low prey densities by providing an alternative source of carbon.

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The aim of the present study was to examine (1) the effects of irradiance on the growth and ingestion rates of 2 chloroplast-retaining dinoflagellates, *Gymnodinium gracilentum* and *Amphidinium poecilochroum*; (2) cell division and feeding rates of *G. gracilentum* in light and dark periods of the day, and (3) the range of prey organisms qualitatively and quantitatively accepted by *G. gracilentum*.

## MATERIALS AND METHODS

**Isolation and maintenance of cultures.** *Gymnodinium gracilentum* Campbell 1973 was isolated in July from surface water samples from the northern part of Øresund, Denmark. *Rhodomonas salina* was added as prey, and the cultures were maintained on a plankton wheel (1 rpm) at an irradiance of 50 μmol photons m$^{-2}$ s$^{-1}$ in a light:dark cycle of 16:8 h at 15°C.

Larsen (1985) originally described the benthic species *Amphidinium poecilochroum* as phototrophic. Later he found that *A. poecilochroum* was able to retain functional chloroplasts from ingested cryptophytes (Larsen 1988). The present culture of *A. poecilochroum* is the same strain as that originally isolated by Larsen (1985, 1988). Stock cultures were maintained by adding a few cells of *A. poecilochroum* to a culture of a small green cryptophyte *Chroomonas* sp., at intervals of 1 to 2 mo. The cultures were maintained in tissue bottles (Nunclon') placed on a transparent glass plate lit from below. The prey organism, *Chroomonas* sp., showed the same ability of adhering to surfaces as *A. poecilochroum*, suggesting that this species also lives in association with the sediment.

**Experimental conditions.** All experiments were conducted in autoclaved seawater based on B1 media (Hansen 1989), with a salinity of 30 psu and a temperature of 15 ± 0.5°C. All cells used in experiments were from cultures grown under a light:dark cycle of 16:8 h supplied from cool-light fluorescent tubes. Irradiance was measured using a radiometer (LI-COR LI-1000, Li-Cor®, USA), equipped with a flat sensor (L-192SA) inside a polystyrene tissue bottle (Nucleon®) for *Gymnodinium gracilentum* and through a multidish (Nucleon®) for *Amphidinium poecilochroum* in order to compensate for the absorption of light by the experimental containers. Irradiance was regulated by varying the distance between the plankton wheel and the light source in the experiments with *G. gracilentum*. In the case of *A. poecilochroum*, irradiance was varied by attenuation with Letratone® screening film or by varying the distance from the light source.

**Growth and ingestion rates as a function of irradiance.** Growth and ingestion rates for the 2 dinoflagellates were measured at different light intensities, ranging from 0 to 270 μmol photons m$^{-2}$ s$^{-1}$ for *Gymnodinium gracilentum* fed *Rhodomonas salina* and from 0 to 80 μmol photons m$^{-2}$ s$^{-1}$ for *Amphidinium poecilochroum* fed *Chroomonas* sp. Control experiments on growth rates for *R. salina* and *Chroomonas* sp. in monoculture were carried out parallel to experiments with the dinoflagellates. Growth rates of prey algae in monoculture were iteratively fitted to a 2nd-order equation using Sigmaplot® (Jandel Scientific, California, USA):

$$\mu = \frac{P_{\max}(I - I_{\min})}{I_{\max/2} + (I - I_{\min})} \qquad (1)$$

where $P_{\max}$ = maximum theoretically obtainable growth rate, $I$ = actual irradiance, $I_{\max/2}$ = irradiance that sustains 0.5 $P_{\max}$, and $I_{\min}$ = point of light compensation where growth is zero ($\mu = 0$)

Growth and ingestion rates were measured for *Gymnodinium gracilentum* and for *Amphidinium poecilochroum* at saturating prey concentrations of >170 μg$^{-1}$ C l$^{-1}$, estimated from cell concentration and cell volume using the carbon to volume relationship given in Strathmann (1967). Prior to the experiments, all organisms were pre-incubated at experimental conditions for at least 48 h.

Experiments with *Gymnodinium gracilentum* were carried out in triplicate batch cultures using 50 ml tissue bottles and paralleled by triplicate controls for prey growth. Sampling was done between 09:00 and 12:00 h at intervals of 24 h in order to eliminate interference from diurnal rhythms. Cells were fixed in Lugol's solution (1% final concentration) and counted in a Sedgwick-Rafter chambers using an inverted microscope.

Because of the adhesive properties of *Amphidinium poecilochroum* and *Chroomonas* sp., it was not possible to obtain homogeneous samples from tissue-culture bottles. Instead, experiments with *A. poecilochroum* and *Chroomonas* sp. together and *Chroomonas* sp. alone were carried out in 0.4 ml microwells (Nunclon®). The wells were illuminated from below using unidirectional light; all cells settled to the bottom of the micro-wells without adhering to the walls, and could thus be counted with an inverted microscope. At each irradiance, 8 wells containing a suspension of *A. poecilochroum* and *Chroomonas* sp. and 8 wells with only *Chroomonas* sp. cells as controls were set up. After pre-incubation, 4 wells with *A. poecilochroum* and 4 control wells were fixed in Lugols' solution and counted. Depending on the number of *A. poecilochroum* at the beginning of the experiment, the remaining 4 experimental and 4 controls wells were fixed and counted after 3 to 5 d.

Growth rates of *Amphidinium poecilochroum* and *Gymnodinium gracilentum* were assumed to be exponential and were estimated by linear regression of log-transformed data. Experiments with a correlation coefficient (r$^2$) below 0.90 were not used.

Ingestion rates ($U$) of both *Gymnodinium gracilentum* and *Amphidinium poecilochroum* were estimated from changes in prey cell numbers in treatments compared to prey densities in controls. These calculations were based on a model developed by T. Fenchel and used in previous studies (e.g. Jakobsen & Hansen 1997 and Skovgaard 1998):

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \mu_x x - Uy \qquad (2)$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \mu_y y \qquad (3)$$

This iterative model assumes that the concentration of predator ($y$) and prey ($x$) increase exponentially at constant rates $\mu_y$ and $\mu_x$ respectively. The prey mortality induced by predators is $Uy$ (where $U$ is per capita prey uptake per unit time), and was calculated iteratively on a computer with steps of 0.01 h. However, because of the use of wells in experiments with *Amphidinium poecilochroum*, the ingestion rates of this species were calculated based on average values for all 4 wells.

**Cell volume and number of retained chloroplasts.** Cell volumes ($V$) of all used organisms were estimated from linear dimensions of width ($w$) and length ($l$) assuming a longitudinal prolate with a spherical cross-section:

$$V = \frac{\pi}{6} \, lw^2 \qquad (4)$$

The cell volume was determined for *Gymnodinium gracilentum* grown at 4 different light intensities (6, 25, 50 and 80 µmol photons m$^{-2}$ s$^{-1}$) and pre-incubated at excess food concentration for at least 72 h. The cell volume of *Amphidinium poecilochroum* was measured at one single irradiance (25 µmol photons m$^{-2}$ s$^{-1}$). Unpublished TEM photographs of *G. gracilentum* grown in excess of food and satiated levels of irradiance reveal that food vacuoles are spherical and larger than retained chloroplasts, and that their numbers are fairly constant (<2). Retained chloroplasts are more or less fused, smaller, elongated bodies with orderly stacked cryptophyte thylakoids. In the epi-fluorescent microscope, the retained chloroplasts can be recognised and distinguished from food vacuoles through their shape and smaller size. All large spherical bodies within the cell were assumed to be food vacuoles according to their size, shape and lower fluorescence level, and they were not included in the counts. In the present study, we quantified the average total number of chloroplasts retained in *G. gracilentum* grown at light intensities from 7 to 75 µmol photons m$^{-2}$ s$^{-1}$. Prior to counting retained chloroplasts, cells were incubated at ambient light intensities for at least 4 generations with food concentrations of >170 µg$^{-1}$ C l$^{-1}$. The counts were done at a magnification of 1000×.

**Growth and grazing during light and dark periods of day.** These experiments were carried out at an irradiance of 60 µmol photons m$^{-2}$ s$^{-1}$ in a light:dark cycle of 16:8 h. Experiments were run as triplicates, according to the protocol described above. However, sampling was done at the beginning of the light and at the beginning of the dark period.

**Prey species selection in *Gymnodinium gracilentum*.** A number of algae representing different classes were offered to *Gymnodinium gracilentum* as prey (Table 1). The size of these algae ranged from 3 to 11 mm ESD (equivalent spherical diameter). Prey cultures were maintained in 250 ml tissue-bottles at an irradiance of 20 to 50 µmol photons m$^{-2}$ s$^{-1}$. Otherwise the experimental conditions were as above.

Table 1. *Gymnodinium gracilentum* growth and ingestion rates of dinoflagellate fed algae belonging to different classes at an irradiance of 60 µmol photons m$^{-2}$ s$^{-1}$. No growth indicates that *G. gracilentum* was unable to grow on prey. Values are means (SE); ESD: equivalent spherical diameter; nd: no data

| Prey species (taxa) | Prey | | Dinoflagellate | |
| --- | --- | --- | --- | --- |
| | Growth rate ($\mu$ d$^{-1}$) | Size (ESD, µm) | Growth rate ($\mu$ d$^{-1}$) | Ingestion rate (prey cells d$^{-1}$) |
| *Phaeodactylum tricornutum* (Bacillariophyceae) | nd | nd | No growth | nd |
| *Prymnesium patelliferum* (Prymnesiophyceae) | nd | nd | No growth | nd |
| *Tetraselmis suecida* (Prasinophyceae) | nd | nd | No growth | nd |
| *Isochrysis galbana* (Prymnesiophyceae) | nd | nd | No growth | nd |
| *Chroomonas vectensis* (Cryptophyceae) | nd | nd | No growth | nd |
| *Chroomonas* sp. (Cryptophyceae) | 0.19 (0.14) | 6 | 0.07 (0.05) | 1.70 (0.19) |
| *Rhodomonas marina* (Cryptophyceae) | 1.1 (0.07) | 10.3 | 0.29 (0.07) | 0.65 (0.05) |
| *Plagioselmis prolonga* (Cryptophyceae) | 0.24 (0.02) | 6.6 | 1.25 (0.1) | 1.8 (0.05) |
| *Teleaulax amphioxeia* (Cryptophyceae) | 0.31 (0.07) | 8.5 | 1.51 (0.07) | 1.58 (0.02) |
| *Rhodomonas salina* (Cryptophyceae) | 0.84 (0.072) | 7.8 | 0.55 (0.07) | 1.75 (0.05) |

## RESULTS

### Effects of irradiance on bioenergetics of *Gymnodinium gracilentum* and *Amphidinium poecilochroum*

*Gymnodinium gracilentum* was able to grow at a rate of 0.53 $d^{-1}$ in the dark when supplied with food in excess. The growth rate remained at this level up to an irradiance of ~25 µmol photons $m^{-2}$ $s^{-1}$. Above an irradiance of 25 µmol photons $m^{-2}$ $s^{-1}$, the growth rate increased to a maximum level of 1.2 $d^{-1}$ at an irradiance of 60 to 80 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 1a). Ingestion rates of *G. gracilentum* fed *Rhodomonas salina* were approximately 1.2 cells $d^{-1}$ at light intensities <25 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 2a). Ingestion rates increased with increasing irradiance to a maximum level of ~2.4 to 3.4 cells $d^{-1}$ at light intensities >80 µmol photons $m^{-2}$ $s^{-1}$.

*Amphidinium poecilochroum* grew at a rate of 0.06 $d^{-1}$ in the dark when supplied with food in excess. The growth rate of *A. poecilochroum* increased to a maximum of 0.17 $d^{-1}$ when exposed to light intensities >6 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 1b). Growth rate decreased at irradiancies >40 µmol photons $m^{-2}$ $s^{-1}$. Ingestion rates of *A. poecilochroum* when fed on *Chroomonas* sp. increased from 0.38 cells $d^{-1}$ in the dark to approximately 0.53 cells $d^{-1}$ at light intensities above 6 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 2b). At light intensities >40 µmol photons $m^{-2}$ $s^{-1}$, ingestion rate decreased to 0.34 *Chroomonas* sp. cells $d^{-1}$ at 80 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 2b).

### Cell volume and number of vacuoles

The cell volume of *Gymnodinium gracilentum* remained constant at ~500 $µm^3$ at all light intensities
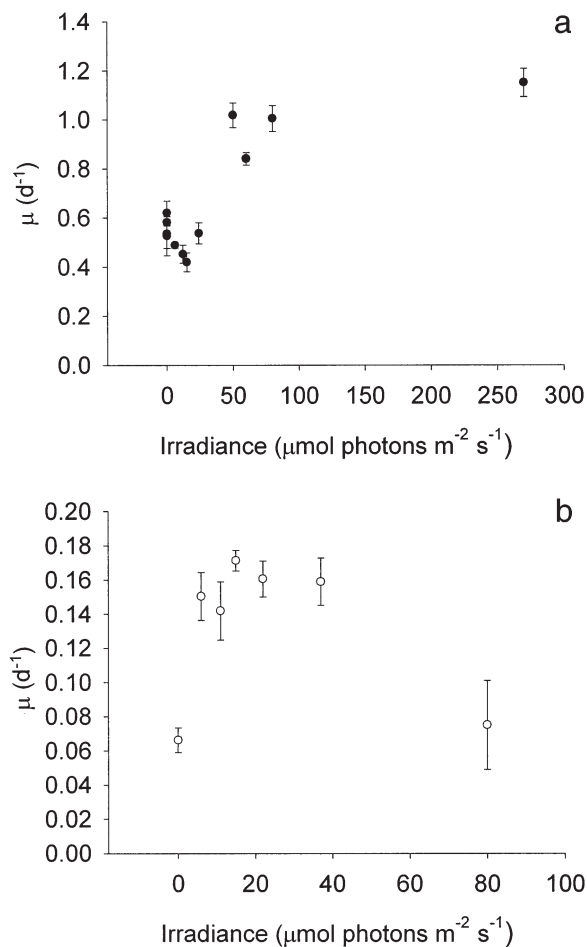


Fig. 1. (a) *Gymnodinium gracilentum*, and (b) *Amphidinium poecilochroum* growth rates as a function of irradiance. Data points are treatment means ± SE
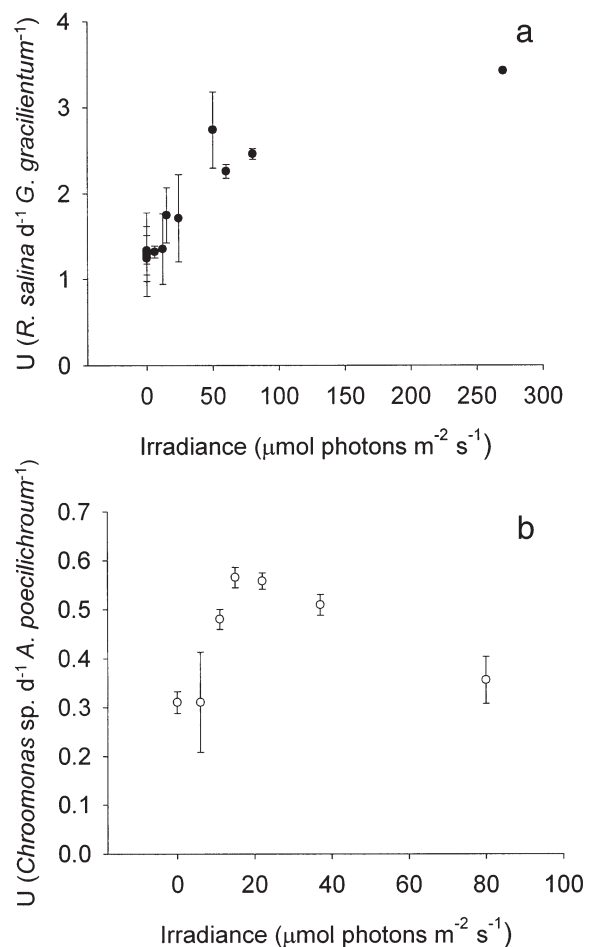


Fig. 2. (a) *Gymnodinium gracilentum*, and (b) *Amphidinium poecilochroum* ingestion rates, *U*, as a function of irradiance. Data points are treatment means ± SE
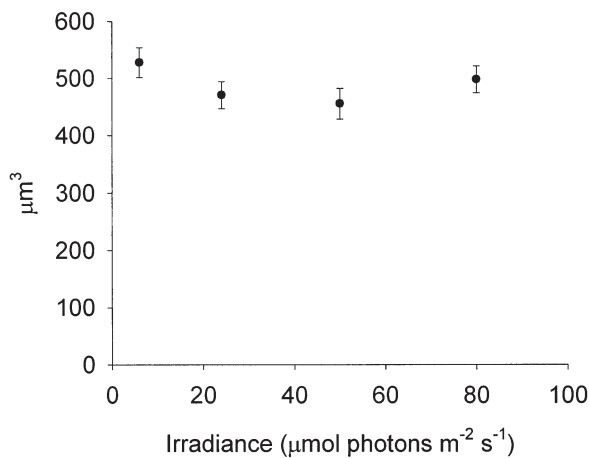
Fig. 3. *Gymnodinium gracilentum* cell volume as a function of irradiance. Data points are treatment means ± SE
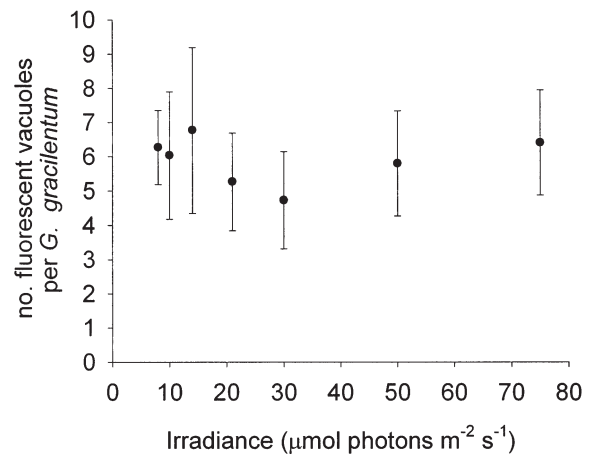


Fig. 4. *Gymnodinium gracilentum.* Number of food vacuoles as a function of irradiance. Data points are treatment means ± SE

(Fig. 3). The cell volume of *A. poecilochroum* was 1100 µm$^3$ at an irradiance of 25 µmol photons m$^{-2}$ s$^{-1}$. The number of retained chloroplasts (vacuoles) in *G. gracilentum* cells appeared to be unaffected by irradiance, and constant at about 6 chloroplasts per individual (Fig. 4).

### Growth rate of prey algae in monoculture

Growth rates of prey algae were studied as a function of irradiance. Data were fit to Eq. (1) using a sigma plot (Jandel Scientific, California, USA) and the results are shown in Table 2. The maximum growth rate of *Rhodomonas salina* was 6 times higher than that of *Chroomonas* sp. However, the light compensation point of *Chroomonas* sp. was 0.13 µmol photons m$^{-2}$ s$^{-1}$, while it was 7.8 µmol photons m$^{-2}$ s$^{-1}$ for *R. salina*. The light intensity sustaining 0.5 $\mu_{max}$ was 2.9 µmol photons m$^{-2}$ s$^{-1}$ for *Chroomonas* sp., that for *R. salina* was 35 µmol photons m$^{-2}$ s$^{-1}$ (Table 2). The growth of *Chroomonas* sp.; declined at a light intensity >37 µmol photons m$^{-2}$ s$^{-1}$. Growth for *R. salina* was not affected at an irradiance as high as 270 µmol photons m$^{-2}$ s$^{-1}$ (Fig. 5).

### Growth and grazing during dark and light periods of day

The growth rate of *Gymnodinium gracilentum* (Student's *t*-test: p = 0.0002; α = 0.05) and *Rhodomonas salina* (*t*-test: p = 0.0001; α = 0.05) was

significantly higher in the dark than in the light (Fig. 6). In contrast to the growth of *G. gracilentum*, its ingestion of *R. salina* was significantly higher in the light than in the dark (*t*-test: p = 0.0001; α = 0.05, Fig. 6).

### Prey species selection in *Gymnodinium gracilentum*

In experiments where *Gymnodinium gracilentum* was offered algae within the size range 3 to 10.3 µm from different algal classes, food uptake and growth was only observed with cryptophyte prey (Table 1). Ingestion rates, in terms of biovolume, showed little variation between the different cryptophyte species (*U* = 356 ± 126 µm$^3$ d$^{-1}$, mean ± SD). Despite this, the growth rate of *G. gracilentum* varied considerably (*μ* [d$^{-1}$] = 0.46 ± 0.43, mean ± SD). No relationship was found between growth of the prey algae and growth of the dinoflagellate.

Table 2. Fitted parameter of growth, $P$ ($\mu$ d$^{-1}$), as a function of irradiance (µmol photons m$^{-2}$ s$^{-1}$) of prey organisms used. $P_{max}$: maximum obtainable growth rate; $I_{min}$: minimum irradiance that sustains minimum growth; $I_{max/2}$: irradiance that sustains 50% of $P_{max}$. Values in table were used to draw regression lines in Fig. 5. Values in parentheses = SE of mean

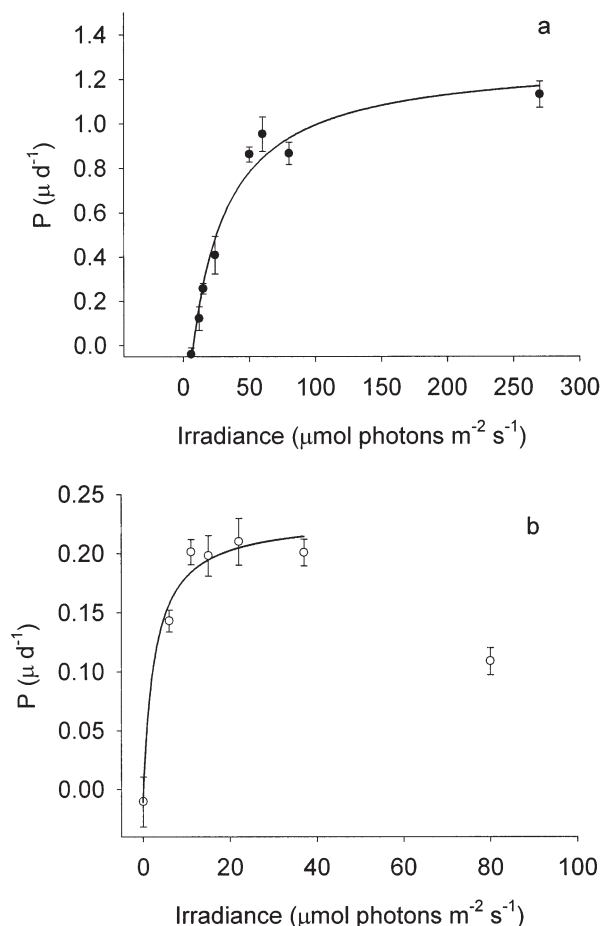| | $P_{max}$ (d−1) | $I_{min}$ (µmol photons m$^{-2}$ s$^{-1}$) | $I_{max/2}$ (µmol photons m$^{-2}$ s$^{-1}$) |
|---|---|---|---|
| *Rhodomonas salina* | 1.29 (0.1) | 7.75 (1.22) | 35 (8.2) |
| *Chroomonas* sp. | 0.23 (0.02) | 0.13 (0.17) | 2.89 (1.8) |

Fig. 5. (a) *Rhodomonas salina*, and (b) *Chroomonas* sp. growth rates, $P$ ($\mu$ d$^{-1}$), as a function of irradiance. Data points are treatment means ± SE
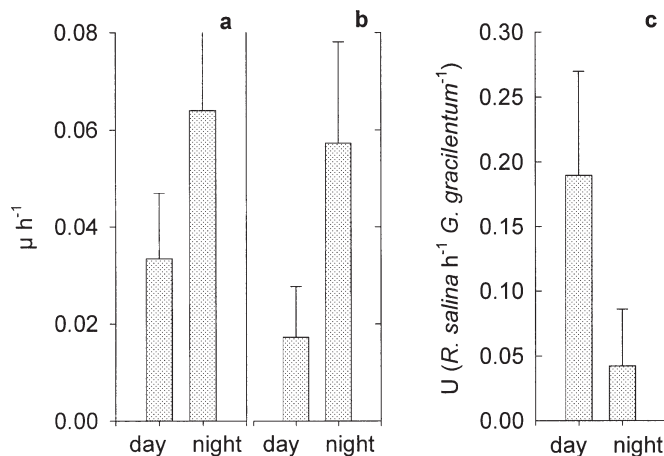


Fig. 6. Growth ($\mu$ h$^{-1}$) of (a) *Gymnodinium gracilentum* and (b) *Rhodomonas salina*, and (c) ingestion rates, $U$ (prey cells h$^{-1}$), of *G. gracilentum* during day and night. Error bars = SD

## DISCUSSION

### Growth and feeding rates as a function of irradiance

The 2 dinoflagellates studied here were able to grow in the dark when supplied with food. However, growth rates of both species were positively affected by light. Thus, both may be considered as facultative mixotrophs. Maximum growth of *Gymnodinium gracilentum* in the light was close to that expected from rate calculated from body size/max. growth rate relationships reported in the literature (Banse 1982, Hansen 1992, Sherr & Sherr 1994, Hansen et al. 1997). The maximum growth rate of the benthic dinoflagellate *Amphidinium poecilochroum* was however approximately 4 to 5 times slower than that of *G. gracilentum*. Its light-dependent growth saturated at lower irradiance, and became light-inhibited at irradiances exceeding 40 $\mu$mol photons m$^{-2}$ s$^{-1}$. *A. poecilochroum*

and its prey *Chroomonas* sp. live interstitially in the sediments, where light is limited, while *G. gracilentum* lives in the plankton. It therefore seems that both *A. poecilochroum* and *Chroomonas* sp. are adapted to a low-light environment.

A positive relationship was found between ingestion rate and irradiance for both of the studied dinoflagellates. Thus, the overall growth yield (= ingestion rate/ growth rate), in terms of volume, was largely unaffected by irradiance. At least 2 possibilities exist which might explain this observation. One possibility is that light acts directly by increasing the degradation rate of the food as suggested by Skovgaard (1998). Our data cannot support this hypothesis. While the maximum growth and ingestion rates of *Gymnodinium gracilentum* were achieved at an irradiance >80 $\mu$mol photons m$^{-2}$ s$^{-1}$, the maximum growth and ingestion rates of *Amphidinium poecilochroum* were reached at an irradiance of 10 $\mu$mol photons m$^{-2}$ s$^{-1}$.

Another possibility is that photosynthesis by the retained chloroplasts supplies the dinoflagellate with an additional carbon source which is rapidly incorporated into biomass, leading to an enhanced growth rate. Because the overall growth yield of both *Gymnodinium gracilentum* and *Amphidinium poecilochroum* does not differ as a function of irradiance, a larger fraction of the prey carbon must therefore be egested. This hypothesis is supported by the light:dark experiment. In this experiment, ingestion rates were substantially higher during the light period compared to ingestion rates in the dark period of the day. Thus, these dinoflagellates seem to act as mixotrophs during the light period and as a heterotrophs during the dark period.

We have shown that the number of fluorescent vacuoles retained is independent of irradiance (Fig. 4) and

that the growth response of the 2 dinoflagellates matches the growth response of the prey algae (cf. Figs. 1 & 5). This fact suggests that prey cells and retained chloroplasts must be turned over faster at higher irradiances. The retained chloroplast may therefore be an analogue to a chargeable unit, e.g. a battery, which is able to fix a finite amount of carbon. As light increases, the amount of carbon fixed per unit sequestered chloroplast increases, but the time the chloroplast is retained by the dinoflagellate decreases.

Little is known about the length of time chloroplasts can be retained and still be photosynthetically active in dinoflagellates. In the case of *Gymnodinium gracilentum*, it has previously been shown that the chloroplasts are photosynthetically active for about 48 h (Skovgaard 1998). In *Pfiesteria piscicida*, Lewitus et al. (1999) found retained chloroplasts in part of the population for at least 13 d. However, whether the retained chloroplasts were photosynthetically active is not clear from their study.

## Prey specificity of chloroplast-retaining dinoflagellates

The chloroplast retaining *Gymnodinium gracilentum* selectively fed on cryptophytes. Chloroplast retention is found in several species of dinoflagellates (Table 3). In all known cases, chloroplasts retained by dinoflagellates are derived from cryptophytes. Some species such as *G. gracilentum*, and possibly also *Amphidinium poecilochroum* and *A. latum*, are selective feeders on cryptophytes (Larsen 1988, Horiguchi & Pienaar 1992), indicating that they are able to recognize and distinguish between their prey. *Pfiesteria piscicida* differs slightly and can feed on a variety of prey organisms (Glasgow et al. 1998). Nevertheless, *P. piscicida* only retains and hosts chloroplasts from cryptophytes (Lewitus et al. 1999).

Other groups of protists are known to host chloroplasts obtained from their prey. Foraminifers, ciliates and heliozoans have all been shown to retain chloroplasts from a variety of algal classes, including diatoms, dinoflagellates or cryptophytes. Some difference does exist though. In ciliates and heliozoans, some species may retain chloroplasts from different algal classes, while in foraminifers species are known to retain 1 type of chloroplast. (Leutenegger 1984, Laval-Peuto & Febvre 1986, Patterson & Dürrenschmidt 1987, Stoecker & Silver 1990).

It was interesting to observe that G*ymnodinium gracilentum* in our experiments ingested all tested cryptophytes at an almost similar rate (in terms of biovolume), but that the growth response differed considerably. The main reason for this may be a combination of the ability of the dinoflagellate to utilise the chloroplasts of the prey, and the rate and degree by which the prey can be digested.

## Ecological implications

Our knowledge of the quantitative importance of dinoflagellates that retain chloroplasts in natural habitats is generally sparse, and biased by insufficient knowledge on the autecology of dinoflagellates. The main reason is that it is impossible to routinely distinguish these dinoflagellates from ordinary phototrophic dinoflagellates with the current techniques for quantification. In fact, *Amphidinium poecilochroum* was first described as a phototrophic dinoflagellate (Larsen 1985).

Nevertheless, some basic principles can be extracted from data on the few species studied in the laboratory so far. The studies of Skovgaard (1998) and Lewitus et al. (1999) and the present observations suggest that chloroplast-sequestering dinoflagellates grow well in the light, but only when food is available.

If sufficient food is available dinoflagellates such as the species studied will increase in number faster than obligate phototrophs when light is limited. In an environment with fluctuating food availability, a chloroplast-retaining dinoflagellate such as *Gymnodinium gracilentum* will survive better than pure heterotrophic dinoflagellates because of its ability to prolong growth and survival (Skovgaard 1998). Thus, this

Table 3. Prey items of chloroplast-retaining dinoflagellates. Data from the literature

| Predator | Prey species (class) | Source |
| --- | --- | --- |
| *Amphidinium latum* | (Cryptophyceae) | Horiguchi & Pienaar (1992) |
| *Amphidinium poecilochroum* | *Chroomonas* sp. (Cryptophyceae) | Larsen (1988) |
| *Amphidinium acidotum* | (Cryptophyceae) | Wilcox & Wedmayer (1984), Fields & Rhodes (1991) |
| *Gymnodinium aeruginosum*[a] | (Cryptophyceae) | Schnepf et al. (1989) |
| *Gymnodinium gracilentum* | (Cryptophyceae) see Table 1 | This study |
| *Pfiesteria piscicida* | *Rhodomonas* sp. (Cryptophyceae) | Lewitus et al. (1999) |
| [a]Suspected to retain chloroplasts from prey | | |

organism will have the potential to find a niche in a fluctuating environment where light or prey resources are sometimes limited. *Amphidinium poecilochroum* behaves in fairly much the same way as *G. gracilentum.* It does not grow very fast, but the turnover rate of the retained chloroplasts is accordingly lower, suggesting that this dinoflagellate is even more fit to manage fluctuating environments. However, with such a low growth rate, it will probably only thrive in habitats in which grazing is low.

## LITERATURE CITED

Banse K (1982) Cell volumes, maximal growth rate of unicellular algae and ciliates and the role of ciliates in the marine pelagial. Limnol Oceanogr 27:1059–1071

Fields SD, Rhodes RG (1991) Ingestion and retention of *Chroomonas* spp. (Cryptophyceae) by G*ymnodinium acidotum* (Dinophyceae). J Phycol 27:525–529

Glasgow HG, Lewitus AJ, Burkholder JM (1998) Feeding behavior of the ichthyotoxic estuarine flagellate, *Pfiesteria piscicida*, on amino acids, algal prey and fish vs mammalian erythrocytes. In: Blanco RB, Fernbndez ML, Wyatt T (eds) Harmful algae. UNESCO, Paris, p 394–397

Hansen PJ (1989) The red tide dinoflagellate *Alexndrium tamarense*: effect on behaviour and growth of a tintinnid ciliate. Mar Ecol Prog Ser 53:105–116

Hansen PJ (1992) Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Mar Biol 114: 327–334

Hansen PJ, Bjørnsen PK, Hansen B (1997) Zooplankton grazing and growth: scaling within the 2–2000 µm body range. Limnol Oceanogr 42:687–704

Horiguchi T, Pienaar RN (1992) *Amphedinium latum* Lebour (Dinophyceae), a sand-dwelling dinoflagellate feeding on Cryptomonads. Jpn J Phycol 40:353–363

Jakobsen HH, Hansen PJ (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

Larsen J (1985) Algal studies of the Danish Wadden Sea. II. A taxomonic study of psammobious dinoflagellates. Opera Bot 79:14–37

Larsen J (1988) An ultrastructural study of *Amphedinium poecilochroum* (Dinophyceae), a phagotrophic dinoflagellate feeding on small species of cryptophytes. Phycologia 27:366–377

Laval-Peuto M (1992) Plastidic protozoa. In: Reisser W (ed) Algae and symbioses: plants, animals, fungi, viruses, interactions explored. Biopress, Bristol, p 471–499

Laval-Peuto M, Febvre M (1986) On plastid symbiosis in *Tontonia apendiculariformis* (Ciliophora, Oligotrichina). Biosystems 19:137–158

Leutenegger S (1984) Symbiosis in benthic foraminifera: specifity and host adaptation. J Foraminifer Res 14:16–35

Lewitus AJ, Glasgow HG, Burkholder JM (1999) Kleptoplastidy in the toxic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). J Phycol 35:303–312

Patterson D, Dürrenschmidt M (1987) Selective retention of chloroplasts by algivorous heliozoa: fortuitous chloroplast symbiosis. Eur J Protistol 23:51–55

Schnepf E, Winter S, Mollenhauer D (1989) *Gymnodinium aeroginosum* (Dinophyta): a blue-green dinoflagellate with a vestigal, anucleate, cryptophycean endosymbiont. Plant Syst Evol 164:75–91

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223–235

Skovgaard A (1998) Role of chloroplast retention in a marine dinoflagellate. Aquat Microb Ecol 15:293–301

Stoecker DK, Silver MW (1990) Replacement and aging of chloroplasts in *Strombidium capitatum* (Ciliophora: Oligotrichida). Mar Biol 107:491–502

Stoecker DK, Silver MW, Michaels AE, Davis LH (1988) Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. Mar Biol 99:415–423

Strathmann RR (1967) Estimating the organic content of phytoplankton from cell volume or plama volume. Limnol Oceanogr 12:411–418

Wilcox LW, Wedemayer GJ (1984) *Gymnodinium acidotum* (Porrophyta), a dinogflagellate with an endosymbiontic cryptomonad. J Phycol 20:236–242