

Role of chloroplast retention in a marine dinoflagellate

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ABSTRACT: The dinoflagellate *Gymnodinium 'gracilentum'* feeds on the cryptophyte *Rhodomonas salina* and retains the chloroplasts of the prey as functional kleptochloroplasts. Using kleptochloroplasts, *G. 'gracilentum'* becomes a mixotroph, acquiring a proportion of its organic carbon demand through photosynthesis, but the kleptochloroplasts and their photosynthetic activity are lost within a few days. Photosynthesis seems, primarily, to be an important means of nutrition for *G. 'gracilentum'* during food depletion, thereby enhancing the survival of the species during food limitation and starvation. However, light has a positive effect on growth kinetics of *G. 'gracilentum'* in food replete cultures: growth and ingestion rates are higher at a high light intensity than at a low light intensity. This effect may be due to other factors than photosynthetic activity of kleptochloroplasts, since a control experiment with a supposed strictly heterotrophic dinoflagellate, *Gymnodinium* sp., also showed a dependence of growth kinetics on light intensity.

KEY WORDS: *Gymnodinium* · Dinoflagellate · Chloroplast retention · Cryptophyte · Mixotrophy · Heterotrophy

INTRODUCTION

A number of dinoflagellate species which lack chloroplasts of their own are known to host chloroplasts retained from cryptophyte prey. Such kleptochloroplasts (Schnepf & Elbrächter 1992) have been found in *Amphidinium poecilochroum* (Larsen 1988), *Gymnodinium acidotum* (Fields & Rhodes 1991), *A. latum* (Horiguchi & Pienaar 1992), and most recently in *Pfiesteria piscicida* (Lewitus et al. pers. comm.). Ultrastructural studies have likewise revealed that *A. wiggrense* (Wilcox & Wedemayer 1985), *G. aeruginosum* (Schnepf et al. 1989) and *Dinophysis* spp. (Schnepf & Elbrächter 1988) contain cryptophyte-like chloroplasts. However, whether these originate from ingested prey or constitute permanent endosymbionts has not yet been established. Schnepf et al. (1989) implied that the chloroplasts of *G. aeruginosum* were most likely obtained through chloroplast retention. This is indeed plausible, considering that Popovský & Pfiester (1990) believed *G. aeruginosum* and *G. acidotum* to be conspecific. As dis-

cussed by Schnepf & Elbrächter (1992), it is doubtful that *A. wiggrense* has genuine chloroplasts, since the species was originally described as being colourless. Also *Paulsenella* spp. might utilize kleptochloroplasts: these species are predators on diatoms and, due to the appearance of the chloroplasts following ingestion, Schnepf et al. (1988) suggested that these remained photosynthetically active for some time.

Chloroplast-retaining dinoflagellates at times play major roles in aquatic ecosystems (Burkholder & Glasgow 1995, Burkholder et al. 1995). However, surprisingly little is known about their ecology and, especially, about their nutrition and the importance of photosynthesis of the kleptochloroplasts for their survival and growth. In most cases it is unknown for how long the chloroplasts are retained and whether they are actually photosynthetically active. Fields & Rhodes (1991) showed that pigmented *Gymnodinium acidotum* cells underwent cell divisions for up to 10 d in monocultures and these remained viable for more than 15 d, but after 13 to 14 d the cells had lost the chloroplasts. Recently, Lewitus et al. (pers. comm.) using microautoradiography showed that the kleptochloro-

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plasts in *Pfiesteria piscicida* are photosynthetically active, and the authors suggested that photosynthesis is an important mechanism of survival during periods of low prey availability.

The present work describes the nutrition of a gymnodinoid, plastid-retaining dinoflagellate and represents the first attempt to quantify the relative importance of phagotrophy and kleptochloroplast photosynthesis for such an organism, thus gaining information on the autecology and trophic position of a dinoflagellate representative of this specialized mode of nutrition. The plastid-retaining dinoflagellate used in this study is believed to be identical to *Gymnodinium gracilentum* (Campbell 1973). However, since the description of this species has not yet been validly published, it will be referred to as *G. 'gracilentum'*. The findings are compared with those for another *Gymnodinium* species which is supposed to be strictly heterotrophic.

MATERIALS AND METHODS

Gymnodinium 'gracilentum' was isolated by H. H. Jakobsen from an unfiltered surface water sample from Øresund off the coast of Helsingør, Denmark, in July 1996. *Gymnodinium* sp. was originally isolated by Jakobsen & Hansen (1997). The dinoflagellates were cultivated in B-medium (Hansen 1989) based on autoclaved, 0.2 µm filtered seawater (salinity = 30‰) with the cryptophyte *Rhodomonas salina* as prey. *R. salina* was obtained from the culture collection of the Marine Biological Laboratory, Helsingør. Stock cultures were kept in polystyrene bottles mounted on a plankton wheel (1 rpm) at ~15°C in dim light (5 to 15 µE m⁻² s⁻¹, cool white fluorescent tubes). Experiments were carried out in 62 ml polystyrene culture tissue bottles mounted on a vertically rotating wheel (1 rpm) which was kept in a water-cooled incubator and exposed to a day:night cycle of 16:8 h light:dark. Photosynthetically active radiation (400 to 700 nm) was measured outside the culture bottles with a LI-COR LI-1000 data logger connected to a spherical LI-193SA underwater radiation sensor (LI-COR Inc., NE, USA). The temperature was monitored in 10 min intervals with an EBI data logger system (EBRO Electronics GmbH, Germany) and kept within the range of 15.5°C (dark period) to 16.5°C (light period). Experiments were carried out for both dinoflagellate species in order to quantify survival (or growth) in the light and in the dark during starvation, ability to fix inorganic carbon by photosynthesis, and growth and ingestion rates at low and high light intensities in food replete cultures.

Survival during starvation. Cultures of both dinoflagellate species were kept in 270 ml bottles at 90 µE m⁻²

s⁻¹ for 3 to 4 d until all prey organisms had disappeared from the cultures. Each culture was then split into 6 aliquots which were transferred to 62 ml bottles and these were filled with media. 3 replicates were kept in the light and 3 in darkness (wrapped in aluminum foil). Samples were taken periodically for 4 to 6 d by fixing 1 ml of each culture directly into a 1 ml Sedgewick-Rafter chamber containing 8 µl of 25% glutaraldehyde. The final concentration of 2‰ glutaraldehyde resulted in a fixation with a minimum of distortion. At least 400 cells were counted per sample using a Nikon Diaphot microscope. Cell volumes were estimated from linear dimensions (length and width) assuming the shape of a prolate ellipsoid and volumes were converted to carbon content according to the regression given by Strathmann (1967). All cell measurements were made within 30 min after fixation by using an ocular micrometer at ×400 (≥20 cells measured per replicate). Since starving heterotrophic flagellates are able to undergo divisions without any increase in biomass (Fenchel 1982b), population sizes were expressed as total biovolumes, i.e. cell numbers multiplied by mean cell volumes.

Photosynthesis. To determine the ¹⁴C incorporation rates, 4 replicate 23 ml glass scintillation vials were filled with >2 d acclimated *Gymnodinium* spp. culture. In addition 4 dark controls were set up. A NaH¹⁴CO₃ stock solution (specific activity = 100 µCi ml⁻¹, Carbon 14 Centralen, Denmark) was added, resulting in a specific activity of ~0.5 µCi ml⁻¹. The vials were incubated for 3 h at 90 µE m⁻² s⁻¹ (incorporation of ¹⁴C was linear with time for at least 4 h, data not shown) whereupon 8 ml from each vial was transferred to new scintillation vials and excess H¹⁴CO₃⁻ was removed by diffusion for 24 h after the addition of 100 µl of 1 M HCl. Total activities were determined in 100 µl of sample to which 200 µl phenylethylamine was added. To count radioactivity, 10 ml of Packard Insta-Gel scintillation cocktail was added and activity determined with a Packard 1500 Tri-Carb liquid scintillation analyzer with quench correction by external standards. Photosynthetic rates, *P*, were calculated following the equation given by Parsons et al. (1984). The photosynthesis experiment was carried out for *Gymnodinium 'gracilentum'* cultures which were food limited but not starved, i.e. *Rhodomonas salina* was still present, but in low number. The predator:prey concentration ratio was approx. 10:1. ¹⁴C incorporation of *G. 'gracilentum'* was determined as total incorporation minus the estimated prey contribution, as determined in control incubations containing *R. salina* only. This experiment was also performed at 6 µE m⁻² s⁻¹. In addition, experiments were made with *G. 'gracilentum'* cultures which had been starved for approx. 12 and 48 h, i.e. no prey was left. Finally, an experiment was performed with *Gymno-*

dinium sp. cultures which had been starved for approx. 48 h.

Growth and ingestion rates. Dinoflagellate and prey cultures were acclimated to either low light ($6 \mu\text{E m}^{-2} \text{s}^{-1}$) or high light ($90 \mu\text{E m}^{-2} \text{s}^{-1}$) intensity for >2 d. Following acclimation, 3 replicate cultures of predator with prey were set up (initial concentrations ~ 200 to 600 ml^{-1} and ~ 4000 to 7000 ml^{-1} , respectively) plus 3 replicate control cultures of prey only (initial concentration ~ 1000 to 3000 ml^{-1}). These cultures were thereafter incubated for 4 d. Samples were taken every 12 to 24 h and treated as described above. Data for calculation of growth and ingestion rates were obtained only from the part of the incubations where the prey cell concentrations were $>4000 \text{ cells ml}^{-1}$, which is well above the saturation limit for *Gymnodinium* sp. ($1300 \text{ Rhodomonas salina}$ cells ml^{-1} ; Jakobsen & Hansen 1997). Growth rates, μ (dimension: d^{-1}), were determined assuming exponential growth, taking changes in cell volume into account, i.e.:

$$\mu = \frac{1}{t} \left(\ln \frac{N_t \text{Vol}_t}{\text{Vol}_0} - \ln N_0 \right) \quad (1)$$

where N_0 and N_t denote the cell concentrations and Vol_0 and Vol_t the mean cell volume at the beginning and at the end of the incubation period (t), respectively.

Per capita ingestion rates, U (prey d^{-1}), were calculated on the basis of decrease in prey concentrations as compared with the controls using the equations given previously (Skovgaard 1996; see also Marin et al. 1986, Hansen & Nielsen 1997). Gross growth efficiency, GGE , was determined as increment of predator carbon per ingested amount of prey carbon. Thus (derived from Fenchel 1982a):

$$GGE = \frac{\mu C_{\text{pred}}}{UC_{\text{prey}}} \quad (2)$$

where C_{pred} and C_{prey} denote the estimated carbon content of the predator and the prey, respectively (pg C cell^{-1}). The mean prey volume was $265 \mu\text{m}^3$ (based on >300 cells measured at both light intensities); hence a prey carbon content of $43.5 \text{ pg C cell}^{-1}$ (Strathmann 1967) was used for all GGE calculations. It is important to note that Eq. (2) relates to strictly heterotrophic organisms only. However, one may hypothesize that by calculating GGE for a mixotrophic organism, it can be revealed whether phagotrophic carbon uptake is substantially supplemented by photosynthesis, since this is likely to result in an erroneously high GGE estimate.

Light microscopy. Pictures were taken of live cells using Zeiss Axiophot and Olympus BH-2 microscopes, both equipped with differential interference contrast light and a $\times 100$ oil emulsion objective.

RESULTS

Cell morphology

Cells of *Gymnodinium 'gracilentum'* measure approx. $14 \times 8 \mu\text{m}$ when grown in the presence of *Rhodomonas salina*. Under these conditions each dinoflagellate cell contains several reddish chloroplasts (Fig. 1a, b) and individuals are frequently seen swimming with a *R. salina* cell attached to their sulcal area while feeding. In the absence of prey, chloroplasts decrease in numbers (Fig. 1c–f) and the cells will therefore eventually become colourless and their size will reduce to approx. $11 \times 5 \mu\text{m}$ (Fig. 1f). After 4 to 5 d of starvation, most cells have lost their colouration. If food is then added, feeding will quickly commence and the dinoflagellates soon regain their original size and colouration. This will happen even after more than a week of starvation. In plastidic *G. 'gracilentum'* cells the chloroplasts appear 'healthy', i.e. they possess visible chloroplast lamellae (Fig. 1a, b) and show a colour resembling that of *R. salina*. Superficially, each dinoflagellate cell seems to contain up to 4–8 kleptochloroplasts (Fig. 1a, b). However, considering that the prey chloroplasts are two-lobed (Fig. 1h), it is more likely that the cells each contain 2 to 4 two-lobed chloroplasts. In fact, chloroplasts are often located 'in pairs' in *G. 'gracilentum'* (Fig. 1a–d). In addition to kleptochloroplasts, *G. 'gracilentum'* often contains amorphous food vacuoles in the posterior part of the cell (Fig. 1c, d), possibly representing digestive vacuoles. Starvation of the dinoflagellate is accompanied by a change in cell shape: the episoma becomes pointed in starved cells (Fig. 1f) versus rounded in well-fed cells (Fig. 1a, b). Feeding in *G. 'gracilentum'* involves a feeding tube emerging from the sulcus, and the predator is thus able to ingest prey of a size comparable to its own.

The heterotrophic dinoflagellate *Gymnodinium* sp. (Fig. 1g; Jakobsen & Hansen 1997) is slightly smaller than *G. 'gracilentum'*, measuring 6 to 11×4 to $8 \mu\text{m}$, depending on the nutritional state of the cells. The species seems to feed by direct engulfment and its food vacuoles appear rounded and amorphous without any lamellar structure (Fig. 1g; Jakobsen & Hansen 1997).

Survival during starvation

Light had a positive influence on the biomass of *Gymnodinium 'gracilentum'* during starvation. During the first 2 to 3 d following the onset of starvation, cell division in *G. 'gracilentum'* cultures kept in the light continued for about 1 division per cell (Fig. 2a). In the dark, fewer cell divisions occurred and only within the

first day. The cell divisions in the dark, however, do not represent any growth in biomass since mean cell volume was reduced drastically during this period (Fig. 2b). The total biovolume of *G. 'gracilentum'* thus decreased exponentially in the dark (Fig. 3), and after 6 d of incubation without food, total biovolume had

decreased by approx. 60%. In the light, in contrast, the reduction in cell volume was less pronounced and *G. 'gracilentum'* exhibited growth in total biovolume for at least the first 1.5 d of starvation. After 3 d, total biovolume in the light equaled the Day 0 values and after 6 d it had only decreased slightly.

The influence of light on the biomass of *Gymnodinium* sp. during starvation was less pronounced: cell concentration and cell volume were almost similar in light and in the dark for this species (Fig. 4). However, changes in total biovolume show that the species did exhibit some growth during the first 1.5 d of starvation in the light whereas no growth was observed in the dark (Fig. 5). After 4 d, total biovolume in both the light and the dark treatments had decreased by approx. 70%.

Photosynthesis

In cultures which were food limited but not yet fully deprived of food, *Gymnodinium 'gracilentum'* had photosynthetic carbon fixation rates of 123 and 3 $\mu\text{g C cell}^{-1} \text{d}^{-1}$ at the light intensities of 90 and 6 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively (Table 1), which correspond to daily photosynthetic rates of 187 and 5% of its own cell carbon content. *G. 'gracilentum'* cells which had been starved for approx. 12 or 48 h (at 90 $\mu\text{E m}^{-2} \text{s}^{-1}$) were capable of fixing only 81 and 3% of their cell carbon content d^{-1} , respectively. Incorporation of inorganic carbon by *Gymnodinium* sp. was similar in the light and in dark controls, i.e. no measurable photosynthesis occurred. *Rhodomonas salina* had carbon fixation rates of 439 and 17% its own cell carbon content d^{-1} at 90 and 6 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively.

Growth and ingestion rates

The development in cell concentrations is shown in Fig. 6. The predators exhibited exponential growth

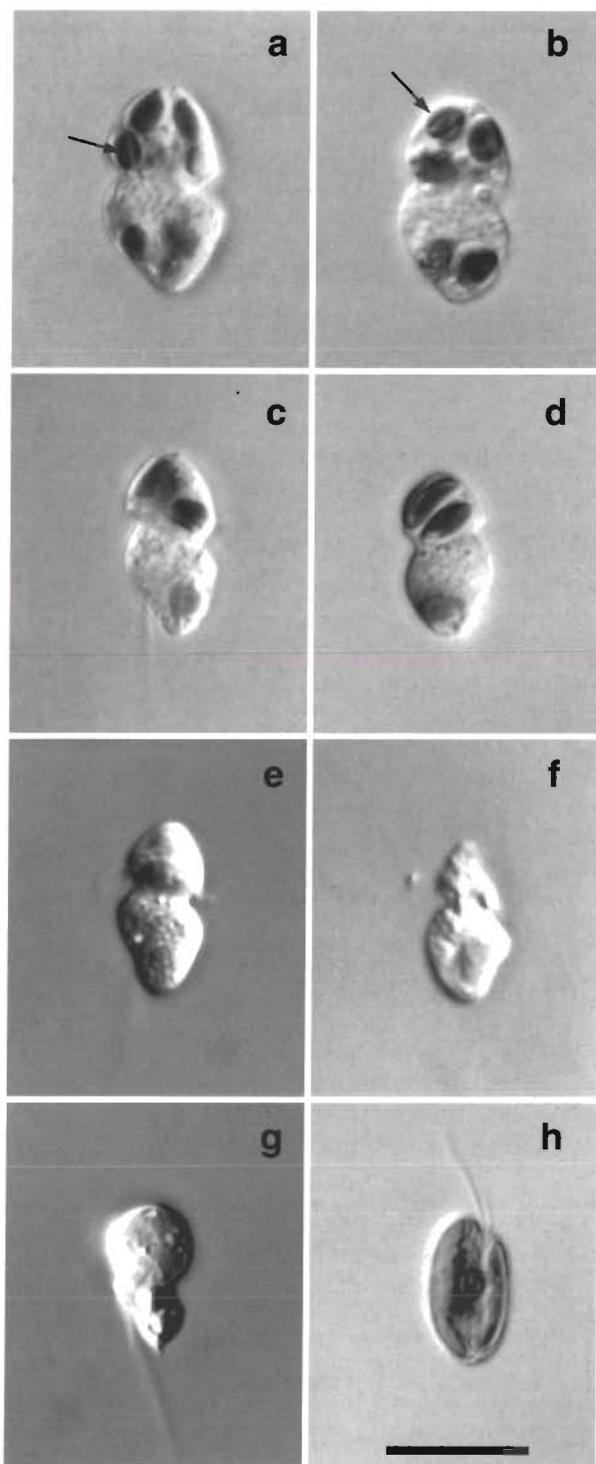


Fig. 1. Light micrographs of live cells. (a–f) *Gymnodinium 'gracilentum'*. (a, b) Well-fed cells with each several chloroplasts. The chloroplast ultrastructure seems intact (arrows). (c, d) Starved cells which have reduced their size and lost all but 2 chloroplasts. The vacuoles in the posterior end of the cells appear to be digestive vacuoles. (e) Following 2 to 3 d of starvation: cell size has been further reduced and the cell has only 1 chloroplast left. (f) Long-term (approx 1 wk) starved cell with all chloroplasts eliminated. The cell size is greatly reduced and the episoma has become pointed. (g) *Gymnodinium* sp.; remnants of food vacuoles are visible. (h) *Rhodomonas salina* with two-lobed chloroplast and a central pyrenoid.

Scale bar = 10 μm , all figures of same magnification

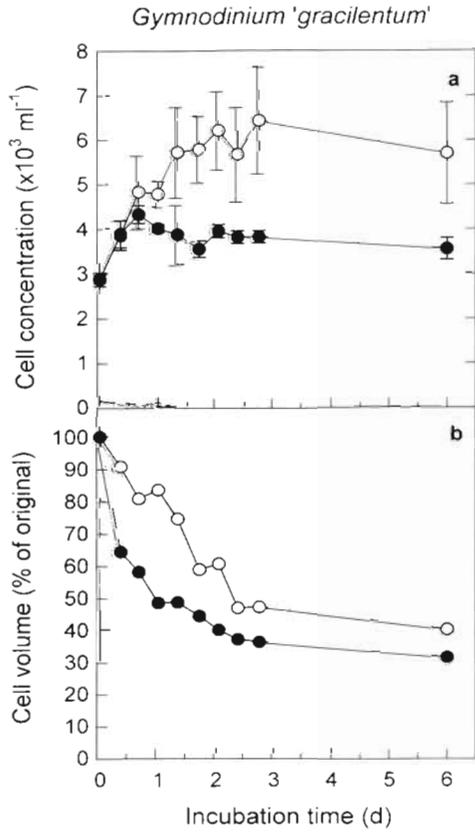


Fig. 2. Development in (a) cell concentrations and (b) mean cell volume of *Gymnodinium 'gracilentum'* in starved cultures in light ($90 \mu\text{E m}^{-2} \text{s}^{-1}$) (O) and in darkness (●). Dashed and dotted lines show residual prey concentrations. Symbols represent means of triplicate incubations \pm SE

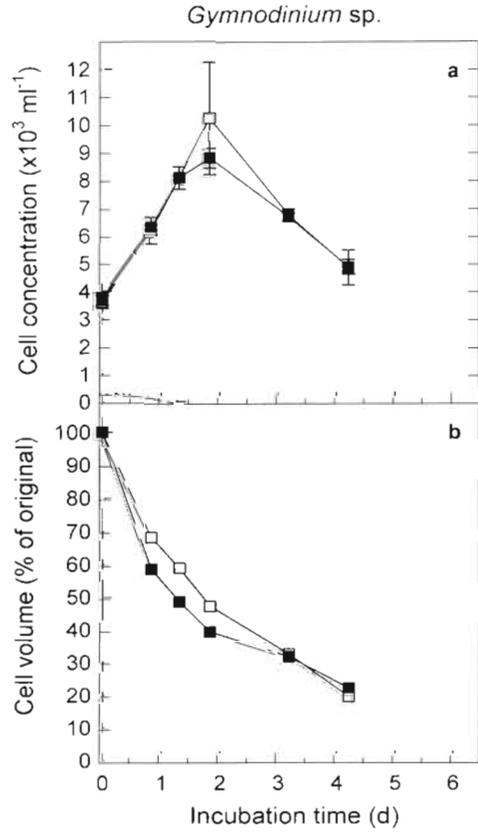


Fig. 4. Development in (a) cell concentrations and (b) mean cell volume of *Gymnodinium sp.* in starved cultures in light ($90 \mu\text{E m}^{-2} \text{s}^{-1}$) (□) and in darkness (■). Dashed and dotted lines show residual prey concentrations. Symbols represent means of triplicate incubations \pm SE

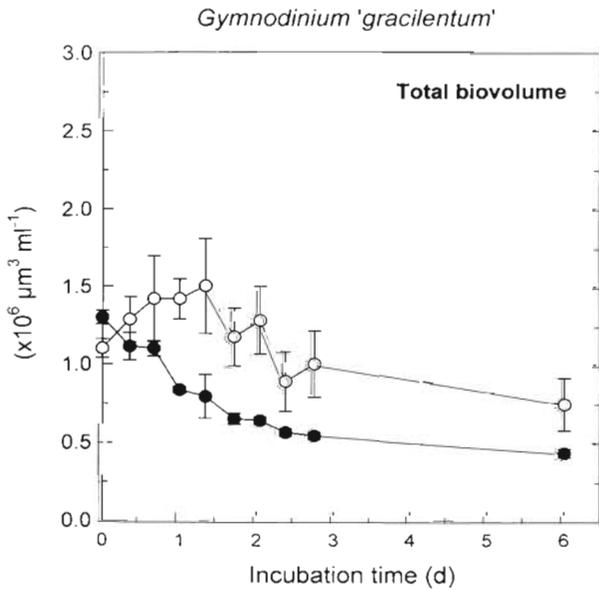


Fig. 3. Development in total biovolume of starved *Gymnodinium 'gracilentum'* populations in triplicate cultures in light and in darkness. Data from Fig. 2, symbols as in Fig. 2

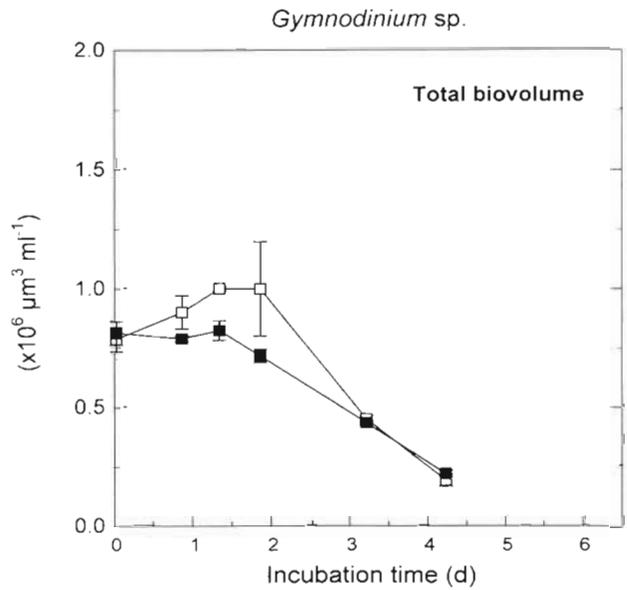


Fig. 5. Development in total biovolume of starved *Gymnodinium sp.* populations in triplicate cultures in light and in darkness. Data from Fig. 4, symbols as in Fig. 4

Table 1. Photosynthetic rates (P) for *Gymnodinium 'gracilentum'*, *Gymnodinium* sp. and their prey *Rhodomonas salina*. SE in parentheses ($n = 4$)

	P (pg C cell ⁻¹ d ⁻¹)	Cell volume (μm^{-3})	C content ^a (pg C cell ⁻¹)	% body C fixed d ⁻¹
6 $\mu\text{E m}^{-2} \text{s}^{-1}$				
<i>G. 'gracilentum'</i>				
Food limited	3 (0.5)	371	58.2	5
<i>R. salina</i>	5 (0.3)	163	28.6	17
90 $\mu\text{E m}^{-2} \text{s}^{-1}$				
<i>G. 'gracilentum'</i>				
Food limited	123 (5)	426	65.5	187
Starved for ~12 h	40 (4)	308	49.0	81
Starved for ~48 h	0.8 (0.03)	162	28.4	3
<i>Gymnodinium</i> sp.				
Starved for ~48 h	0 ^b	80	15.4	0
<i>R. salina</i>	156 (14)	210	35.6	439

^a Volume:carbon regression from Strathmann (1967), 30 cells measured per treatment
^b ¹⁴C uptake in the light was not different from dark controls

during the entire experiment, and so did *Rhodomonas salina* in the control bottles. Mean growth rate of *Gymnodinium 'gracilentum'* was 3.8 times higher at high light than at low light intensity and mean ingestion rate was 3.5 times higher (Table 2). These bioenergetic rates correspond to GGE s of 59 and 64% at low and high light intensity, respectively. It should be stressed again, however, that these figures do not represent true GGE s if a portion of the growth is

accounted for by photosynthesis rather than by phagotrophy.

On the basis of theoretical carbon:volume ratios for the predator and prey, it is possible to set up a carbon budget for *Gymnodinium 'gracilentum'*. However, the following assumptions must be made: (1) all photosynthate produced by the kleptochloroplasts is available for the dinoflagellate host; (2) there is no difference between photosynthetic rates of *G. 'gracilentum'* in food replete cultures and in the food limited cultures in which ¹⁴C incorporation was measured (Table 1); and (3) all the ingested carbon (Table 2) is digested and assimilated. Under these conditions, photosynthesis would contribute ~51% of the total carbon uptake of the dinoflagellate at the high light intensity and ~8% at low light.

Growth and ingestion rates of the control organism *Gymnodinium* sp. were less dependent on light intensity. Still, both μ and U were ~1.6 times higher at high light as compared with low light (Table 2). GGE was different in the 2 treatments: 32 and 41% at low and high light intensity, respectively, a difference which mainly reflects the difference in predator cell size between the 2 treatments. Growth rates of *Rhodomonas salina* were 0.07 (SE = 0.02, $n = 6$) and 0.77 d⁻¹ (SE = 0.04, $n = 6$) at the light intensities of 6 and 90 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively.

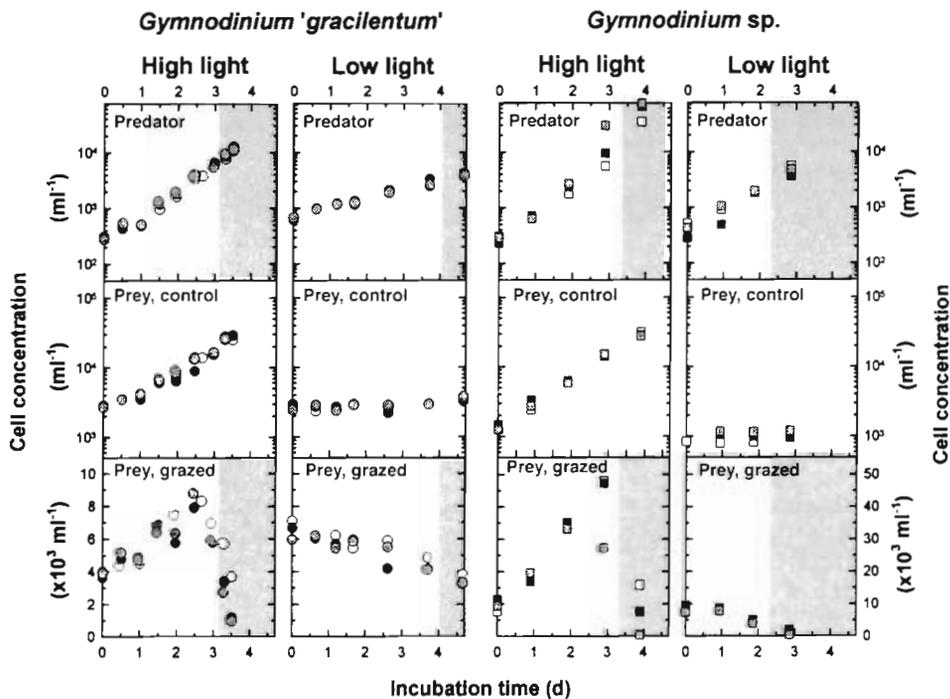


Fig. 6. Development in cell concentrations of predators (*Gymnodinium 'gracilentum'* and *Gymnodinium* sp.) and prey (*Rhodomonas salina*) in experimental bottles (upper and lower panels, respectively) and of prey in control bottles (middle panels) under 2 different light conditions (low light, 6 $\mu\text{E m}^{-2} \text{s}^{-1}$; high light, 90 $\mu\text{E m}^{-2} \text{s}^{-1}$). Data points in the shaded areas were not used in calculations of growth and ingestion rates of the predators, because the prey concentrations had become too low. Triplicate incubations are represented by different symbols; when not all triplicates are visible, it is due to overlap of symbols. Note different ordinate scales

Table 2. Growth rates (μ), per capita ingestion rates (U), cell volume, estimated carbon content and gross growth efficiency (GGE) for *Gymnodinium 'gracilentum'* and *Gymnodinium* sp. at 2 different light intensities. SE in parentheses ($n = 3$)

Species	Light intensity ($\mu\text{E m}^{-2} \text{s}^{-1}$)	μ (d^{-1})	U (<i>R. salina</i> d^{-1})	Cell volume (μm^3)	C content ^a (pg C cell ⁻¹)	GGE ^b (%)
<i>G. 'gracilentum'</i>	6	0.31 (0.03)	0.8 (0.1)	400	62.1	59
	90	1.19 (0.01)	2.8 (0.1)	415	64.2	64
<i>Gymnodinium</i> sp.	6	0.81 (0.09)	2.5 (0.6)	261	42.9	32
	90	1.25 (0.13)	4.1 (0.8)	373	58.5	41

^aVolume:carbon regression from Strathmann (1967)
^bDoes not represent true GGE if growth is partially photosynthetic

DISCUSSION

This study demonstrates that *Gymnodinium 'gracilentum'* exhibits a mixotrophic growth strategy using chloroplasts sequestered from algal prey for performing its 'own' photosynthesis. The phagocytotic origin of the chloroplasts of *G. 'gracilentum'* is proven by the cell's need for algal food in order not to lose the chloroplasts. That the kleptochloroplasts are photosynthetically active can be concluded from the measured ^{14}C uptake (Table 1) and from the fact that starved *G. 'gracilentum'* cells survive longer in the light than in the dark (Figs. 2 & 3). However, the kleptochloroplasts are photosynthetically beneficial for the dinoflagellate host for only ~2 d, whereupon they are digested and/or eliminated through cell division. The cell-specific photosynthetic rate of *G. 'gracilentum'* is lower than that of *Rhodomonas salina*, even though each *G. 'gracilentum'* cell contains 4 to 8 (or rather: 2 to 4 two-lobed) chloroplasts whereas *R. salina* cells contain only 1 large, two-lobed chloroplast. The photosynthetic efficiency of the chloroplasts thus decreases when they are separated from their original host cell and kept as kleptochloroplasts (for discussion, see Stoecker et al. 1988).

Gymnodinium 'gracilentum' exhibits higher GGE than the strictly heterotrophic control organism *Gymnodinium* sp. This might reflect differences in the efficiency of the 2 species at converting prey biomass into their own biomass, but, according to the above-mentioned hypothesis, it is plausible that GGE of *G. 'gracilentum'* is overestimated due to photosynthetic carbon fixation. Indeed, a GGE of 59 to 64% (Table 2) exceeds the upper end of the range of GGE s typically found for heterotrophic aerobic flagellates (20 to 50%; Caron & Goldman 1990). When interpreting the calculated GGE s, one should remember, however, that cellular C content was estimated from cell volume and due to the variation of, particularly, the prey volume, these figures may be subject to error.

The increased growth rate of *Gymnodinium 'gracilentum'* at high light intensity is accompanied by a cor-

responding increased ingestion rate, i.e. there is no evident effect of light on GGE . It seems, therefore, that photosynthesis does not contribute to growth when food is plentiful. Perhaps, *G. 'gracilentum'* is not C limited in the food replete cultures, but rather limited by other factors, e.g. nutrients. Since there is no evidence that chloroplast-retaining dinoflagellates are capable of taking up dissolved inorganic nitrogen or phosphorus, it is likely that kleptochloroplast photosynthesis is an advantage only by supplying organic carbon for covering respiratory needs during periods of unfavourable food conditions. More studies must be undertaken in order to understand fully the importance of photosynthesis under food replete conditions.

Gymnodinium sp. was included in this study as a representative of a strictly heterotrophic dinoflagellate and it was expected that its metabolism would not be influenced by light conditions. In fact, *Gymnodinium* sp. was affected less by light conditions than *G. 'gracilentum'*, but nevertheless, μ , U and GGE were actually enhanced by higher light intensity (Table 2) even though food concentrations were above the saturation concentration throughout the experiment. This could be due to a dependence of the nutritional value of the prey on light intensity, but it must also be considered that even though the ^{14}C -based photosynthesis experiment yielded negative results for *Gymnodinium* sp., one cannot exclude the possibility that food vacuoles containing phototrophic prey may photosynthesize for a short while until they become digested. Such a potential extreme short-term photosynthetic activity would not have been detected in this study, since *Gymnodinium* sp. had to be starved for 48 h in order to eliminate all prey cells. More sensitive methods for detecting photosynthesis (e.g. microautoradiography; Carman 1993) are needed to elucidate this. A third possibility is that light has a direct positive influence on digestion and growth. This is not improbable, considering that it is well established that dissolved organic matter is photochemically degradable (reviewed by Moran & Zepp 1997) and that light, thus, has an

indirect positive effect on bacterial production through the production of labile substrate (Lindell et al. 1996, Jørgensen et al. 1998). It needs to be studied whether such a photodegradation can occur even of particulate organic matter inside food vacuoles of heterotrophic protists and thereby enhance digestion. Although yet speculative, this would explain the beneficial effect of increased light intensity on growth of the heterotrophic *Gymnodinium* sp.

The ability to use ingested and retained chloroplasts for photosynthesis has been observed in many metazoa (e.g. Trench 1975) as well as in protists. Among protists, the phenomenon exists in marine ciliates, e.g. *Prorodon* sp. (Blackbourn et al. 1973), *Laboea strobila* (Jonsson 1987, Stoecker et al. 1988) and *Strombidium* spp. (Blackbourn et al. 1973, Jonsson 1987, Stoecker & Michaels 1991), and has also been found in foraminifera (Lopez 1979) and heliozoa (Patterson & Dürrschmidt 1987). The phenomenon is so far known for only a few dinoflagellate species, but many dinoflagellates belonging to the *Gymnodinium*/*Gyrodinium* species complex possess variable numbers of chloroplasts which are of uncertain origin, but which resemble the kleptochloroplasts of *G. 'gracilentum'* (e.g. Hansen & Larsen 1992, Larsen 1994, 1996). It is therefore probable that chloroplast retention among dinoflagellates is much more widespread than so far perceived.

The plastid retention time for *Gymnodinium 'gracilentum'*, i.e. the period of time for which the kleptochloroplasts remain functional, is relatively short (1 to 2 d). In contrast, the dinoflagellate *G. acidotum* appears to have a plastid retention time of up to 14 d (Fields & Rhodes 1991). Among chloroplast-retaining ciliates, different plastid retention times and different degrees of dependence on kleptochloroplasts have been found (Jonsson 1987, Stoecker & Silver 1987), and it now seems that this variability applies to dinoflagellates as well. As a consequence, it is not possible to determine a general importance of kleptochloroplasts for the growth of plastid-retaining dinoflagellates. In order to understand the ecology of these organisms and their possible role in their natural habitats, it is therefore necessary to gain considerably more knowledge about the physiology of individual species.

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