Phagotrophy by a plastidic haptophyte, *Prymnesium patelliferum*

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ABSTRACT: Observations and photographs of the previously unsuspected ingestion capacity of the phytoflagellate *Prymnesium patelliferum* are presented. *P. patelliferum* took up prey cells of different sizes by an elongated, U-shaped pseudopodium formed at the posterior part of the cells. While feeding, aggregates of several *P. patelliferum* cells were formed in which their pseudopodia come in close contact. Motile prey cells were immobilised or probably killed before ingestion, but not all protistan species of ingestible size were immobilised or attacked. It is suggested that *P. patelliferum* may secrete a toxin that serves as a killing agent for prey organisms.

KEY WORDS: *Prymnesium patelliferum* · Mixotrophy · Phagotrophy · Haptophyta

INTRODUCTION

Phagotrophy by photosynthetic flagellates is a well-known phenomenon described in the phycological literature as early as the late 19th century. This process has recently been 'rediscovered' and its potential ecological significance addressed as part of the rapid evolution in microbial food web ecology. Many higher algal taxa have species with both photosynthetic and phagotrophic nutrition (mixotrophy). This is most common in the dinoflagellates (Schnepf & Elbrächter 1992), chrysophytes (Sanders & Porter 1988) and prymnesiophytes (Green 1991, Jones et al. 1994). In the latter group, descriptions of mixotrophic species are nearly exclusively restricted to the genus *Chrysochromulina*. In only 1 case has phagotrophy been recorded in the coccolithophorids (Parke & Adams 1960).

Prymnesium Conrad, the type genus of Prymnesiophyceae, has a number of toxic bloom-forming species separated morphologically only by the fine-structural details of body scales (Green et al. 1982) and is well known to cause extensive fish kills in brackish ponds in Israel (Reich & Aschner 1947), Denmark (Otterstrøm & Steemann Nielsen 1940), Germany (Lenz 1933, Hickel

1976, Dietrich & Hesse 1990), Bulgaria (Valkanov 1964) and Norwegian coastal waters (Kaardtvedt et al. 1991). Because of its economic importance as an ichthyotoxin-producing genus, *Prymnesium* is an often-cultivated and well-studied organism. Nevertheless, for a long time, the only documentation of phagotrophy within this genus was an anecdotal description by Conrad (1941), who described particle ingestion by pseudopodia in amoeboid cells of *P. saltans* Massart. Little detail was given in that report and confirmatory observations were lacking (Green 1991). However, Nygaard & Tobiesen (1993) recently showed that *P. parvum*, among several other species of flagellates, was able to ingest bacteria both in the field and in the laboratory. In the present study, I show that particle ingestion by *Prymnesium* is not restricted to bacteria but that at least *P. patelliferum* is able to incorporate different sized particles, sometimes even larger than itself.

MATERIALS AND METHODS

In 1992, *Prymnesium patelliferum* was isolated from a brackish pond near Büsum, northern Germany. From this isolate, unialgal cultures were obtained by the dilution technique and were grown in natural seawater

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(S = 28‰) enriched with nutrients (Guillard & Ryther 1962). Species determination, based on TEM examination of the scales, was carried out by J. Green (Plymouth Marine Laboratories, UK). Cultures were maintained at 20°C under natural light in the laboratory. To study phagotrophy, senescent cultures were mixed with different species of other protists (Table 1). Live observations were made using an inverted microscope (Zeiss Axiovert 35) equipped with a Nikon photomicrography system and documented with a Panasonic video system. From video tapes, single frames were printed by a Sony video-printer.

RESULTS

In exponentially growing cultures, *Prymnesium patelliferum* is elongate and slender with 2 chloroplasts covering the whole cell in the longitudinal axis. In dense and senescent cultures, the cell shape changes to a more rounded form. The chloroplasts are smaller and the posterior part of the cell is often filled with a large body of reserve metabolite, probably chrysolaminarin. The cells are often filled with small oil droplets. In addition, cells with a large brownish pellet in the posterior occurred and sometimes excretion of this body by the cells was observed. These cellular features, with hindsight, possibly resulted from cannibalism, i.e. the brown bodies strongly resembled 'faecal pellets', suggesting the occurrence of phagotrophy.

After mixing senescent *Prymnesium patelliferum* cultures with other protist cultures, some *P. patelliferum* cells showed a swollen and transparent posterior, which afterwards formed an elongated U-shaped

Table 1 List of species tested for their suitability as prey for *Prymnesium patelliferum*

Species	Diameter (µm)	Ingestion observed
Unknown Chlorococcales	5–10	+
Dunaliella sp.	7	+
Cryptomonas sp.	10	–
Skeletonema costatum (Greville) Cleve	10	–
Thalassiosira rotula Meunier	30	–
Oxyrrhis marina Dujardin	30	+
Gyrodinium sp.	45	+
Unknown amoeba	12	+

pseudopodium (Fig. 1). Presumably, the formation of the pseudopodium is chemically stimulated and not triggered by direct contact with the prey, because it was often observed in cells not surrounded by algal food. If *P. patelliferum* with a pseudopodium encountered a food particle, the particle was completely enclosed by the pseudopodium and taken up into a food vacuole located at the posterior of the cell within about 1 min (Figs. 2 & 3).

In cultures of motile protists mixed with *Prymnesium patelliferum*, a varying number of potential prey cells lost their motility. This effect was not due to observation handling (e.g. microscopic illumination) as determined by prey-alone control cultures under the microscope. For example, Fig. 4a shows the immobilised and rounded form of an amoeba. This species can then easily be ingested by *P. patelliferum*, as shown in Fig. 4b, c.

Single prey cells were often attacked by several *Prymnesium patelliferum* cells simultaneously. Sometimes, when multiple *P. patelliferum* encountered a single prey, the membranous pseudopodia of *P. patelliferum* cells appeared to merge to enclose the prey between them. This process is illustrated with a series of sequential images taken from video (Fig. 5). Resulting feeding aggregates of the 2-cell type are most common (Figs. 4c & 6a–e), but 3 or more *P. patelliferum* cells may be involved (Fig. 6f). *P. patelliferum* also aggregated around relatively large prey. Both the heterotrophic dinoflagellate *Oxyrrhis marina* (Fig. 7) and the phototrophic *Gyrodinium* sp. (Fig. 8) were attacked. The predator cells formed a more or less complete coat over the prey. In such cases, the shape of a single *P. patelliferum* cell could be extremely deformed.

Differences in suitability of protistan prey for *Prymnesium patelliferum* were observed. While the Chlorococcales and an amoeba were ingested (Figs. 2 to 4), the similarly sized *Cryptomonas* sp. and *Skeletonema costatum*
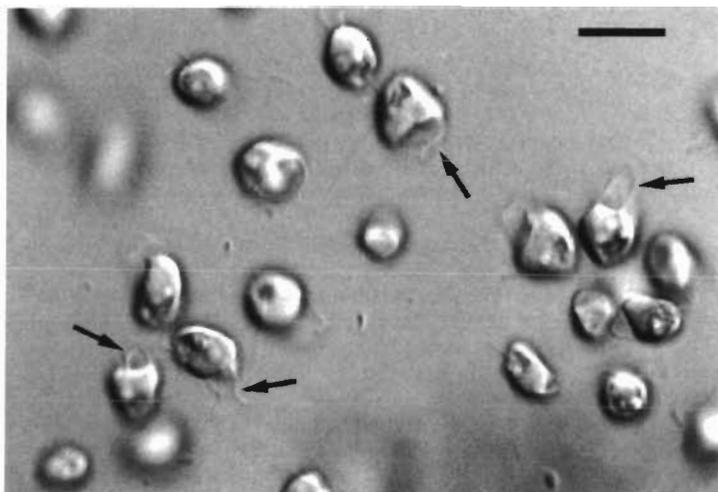

Fig. 1. *Prymnesium patelliferum* forming elongated pseudopodia (arrows) at the posterior part of the cell prior to feeding activity. Scale bar = 10 µm
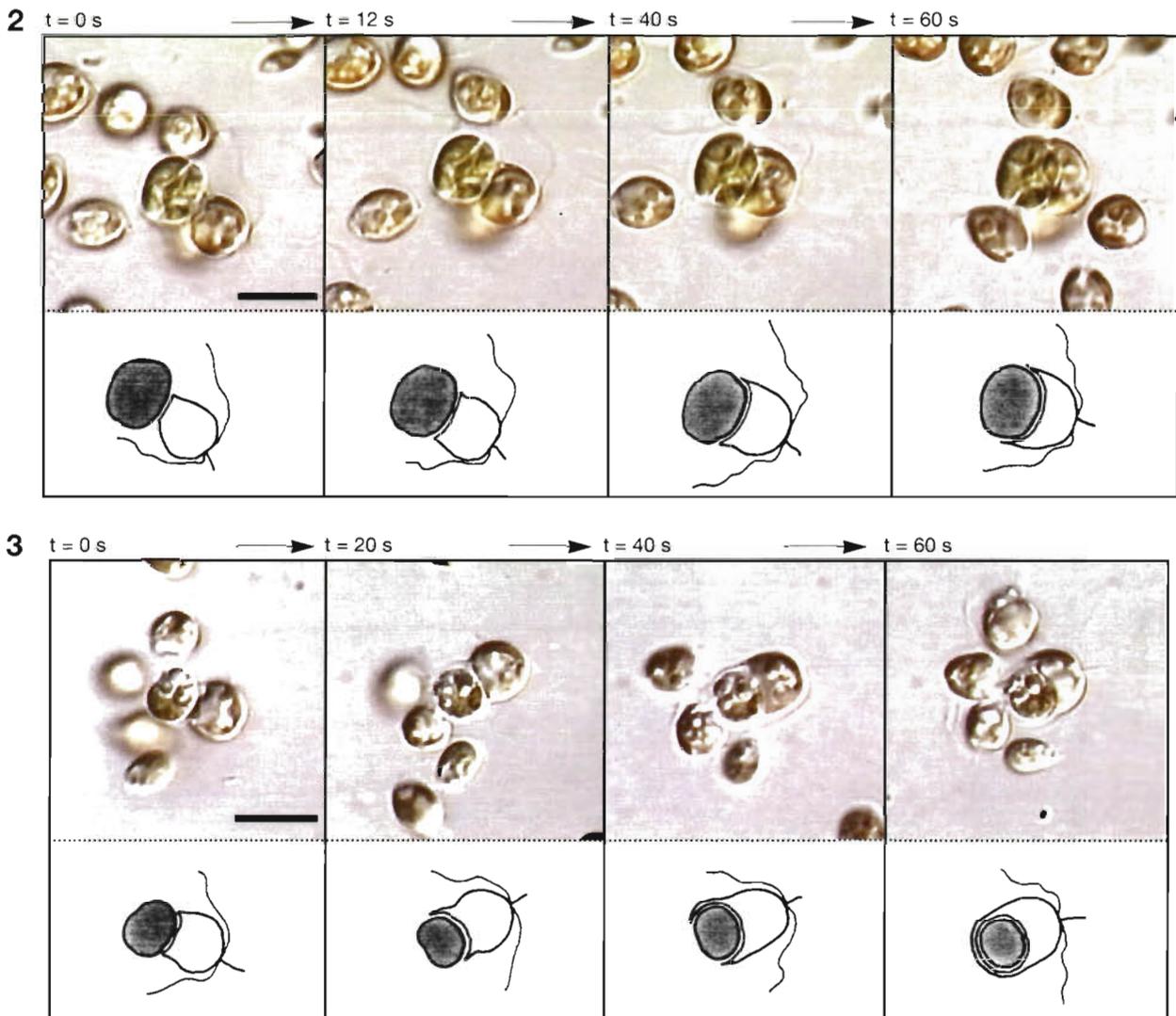
Figs. 2 & 3. Time course of prey ingestion by *Prymnesium patelliferum*. The drawings in the lower part give a schematic simplified view of pseudopod action. Scale bars = 10 µm. Fig. 2. *P. patelliferum* ingesting an unidentified chlorococcalean alga. Fig. 3. *P. patelliferum* ingesting *Dunaliella* sp.
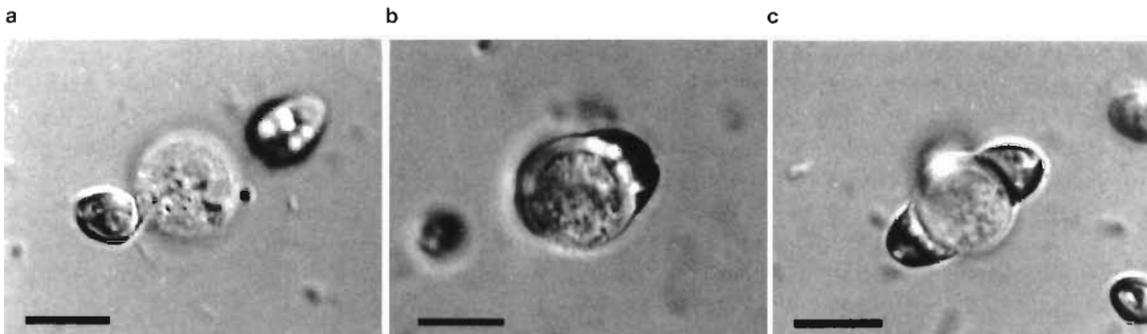

Fig. 4. *Prymnesium patelliferum* ingesting an amoeba. (a) After addition to a *P. patelliferum* culture, the amoebae lost their motility and became rounded. (b) After ingestion, the amoeba can be seen in a large food vacuole. (c) Two cells in close connection with the food between them. Scale bars = 10 µm
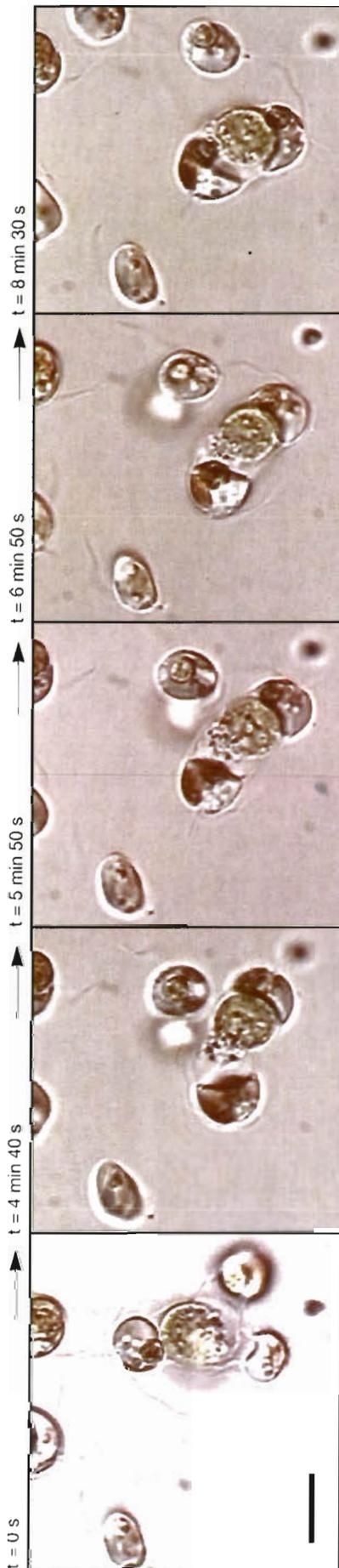
Fig. 5. *Prymnesium patelliferum*. Video sequence of the formation of a close connection between cells with the enclosed prey between them. In the first frame the prey item (*Dunaliella*) is surrounded by 3 *P. patelliferum* cells. Later on, 2 cells remain, forming a close connection around the food. Scale bar = 10 μm
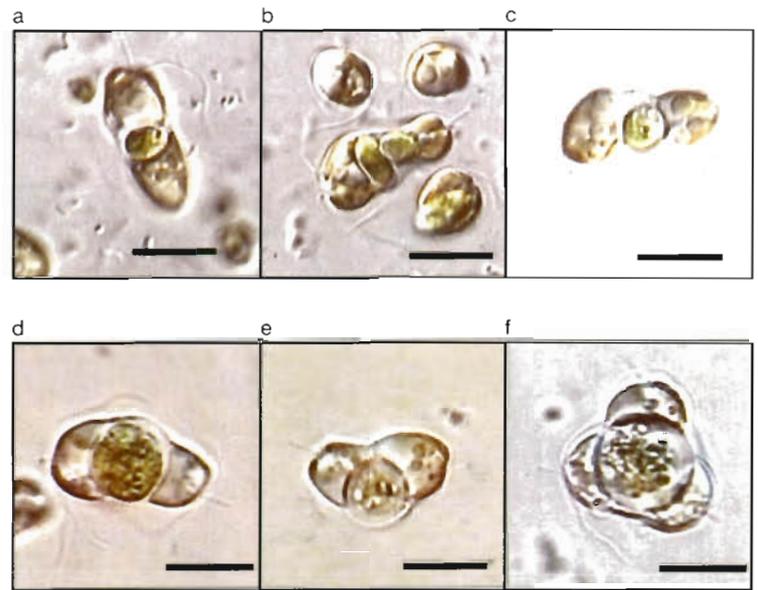

Fig. 6. *Prymnesium patelliferum*. Examples of communal phagotrophy by (a–e) pairs or (f) a triplet of *P. patelliferum* cells feeding on (a, b) unknown chlorococcalean algae or (c–f) *Dunaliella* sp. Scale bars = 10 μm

were not captured (Table 1). Although suitable prey items were apparently immobilised, *Cryptomonas* sp. motility was unaffected by the presence of *P. patelliferum*.

DISCUSSION

The mode of particle ingestion of *Prymnesium patelliferum* by means of pseudopodial development at the non-flagellar pole resembled that of *Chrysochromulina kappa* Parke & Manton and *C. ericina* Parke & Manton. Parke et al. (1955, 1956) described how a colourless, slightly granular substance seemed to flow out from the non-flagellar pole of *C. kappa* and enveloped the prey particles, which were then drawn quickly inside the cell. I did not observe involvement of the haptonema in the feeding process of *P. patelliferum*. This is in contrast to *C. hirta* Manton, in which the haptonema plays an important role in catching and taking up particles (Kawachi et al. 1991)

The observations from this study suggest that prey are immobilised or even killed by *Prymnesium patelliferum* before being ingested. Cells being ingested were, in every case, immobilised and rounded. Although the toxicity of the *P. patelliferum* strain used here has not been tested with standard toxicity tests, the rapid immobilisation of prey suggests toxin production, especially since the toxicity of 2 strains of *P. patelliferum* was demonstrated in a recent study by Larsen et al. (1993). Thus, it may be speculated that
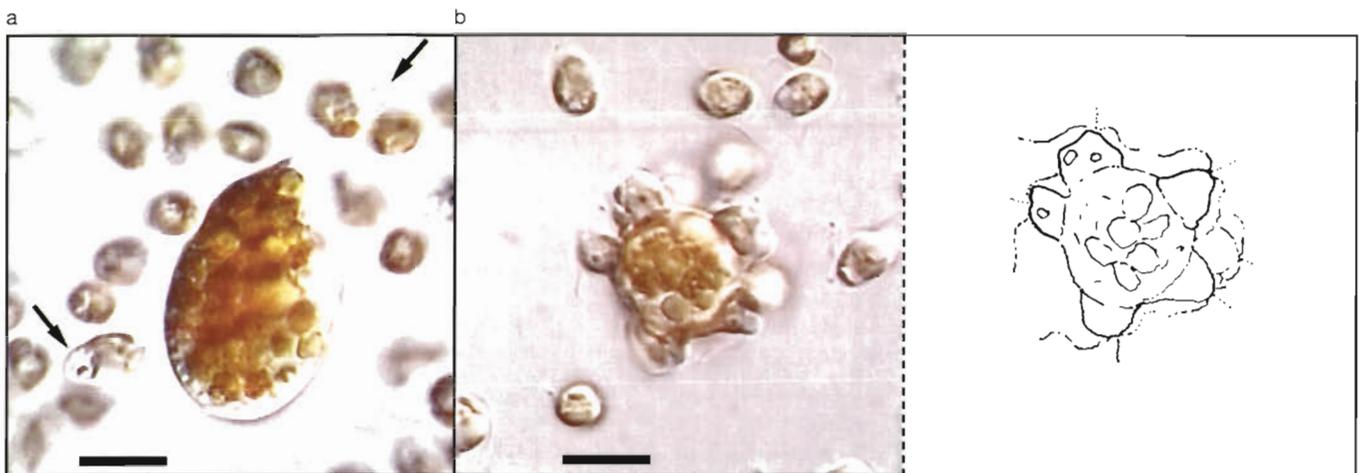
Fig. 7. *Prymnesium patelliferum* attacking a cell of the heterotrophic dinoflagellate *Oxyrrhis marina*. Scale bars = 10 μm. (a) The dinoflagellate cell, coloured due to ingested *Dunaliella* cells, is completely rounded off. Nearby, *P. patelliferum* cells 'ready to feed' with the typical swollen hyaline posterior part (arrows) can be seen. (b) Several *P. patelliferum* cells have settled on the surface of a smaller individual *O. marina*

Prymnesium toxin may be used to kill potential prey organisms prior to ingestion. Toxins of haptophytes are well known to affect organisms of their own size class, like amoeba, flagellates and ciliates (Valkanov 1964) and bacteria, phytoplankton and zooplankton (Moestrup 1994). Estep & McIntyre (1989) hypothesised that toxin released by *Chrysochromulina* spp. simply punches holes in the cell membrane of other organisms. According to this hypothesis of auxotrophy following induced osmosis (dasmotrophy), the prey is not killed, but an outflow of nutrients is induced, which *Chrysochromulina* spp. can take up and absorb. The advantage to species producing toxins is still under discussion.

Not all potential prey were ingested by *Prymnesium patelliferum*. *Cryptomonas* sp. retained their motility in the presence of *P. patelliferum* and thus are probably insensitive to *P. patelliferum* toxin. The failure of *P. patelliferum* to ingest the 2 tested diatom species may be due to chain formation (*Skeletonema costatum*), spines (*Thalassiosira rotula*) or in general to surface properties of silica shells. Handling problems seem unlikely, however, since *P. patelliferum* could ingest other species in the same size range of the diatoms (Table 1). Field studies have demonstrated that diatoms are not affected by either *P. parvum* (Otterstrøm & Steemann Nielsen 1940) or *Chrysochromulina leadbeateri* Estep et al. (Johnsen & Lømsland 1991).
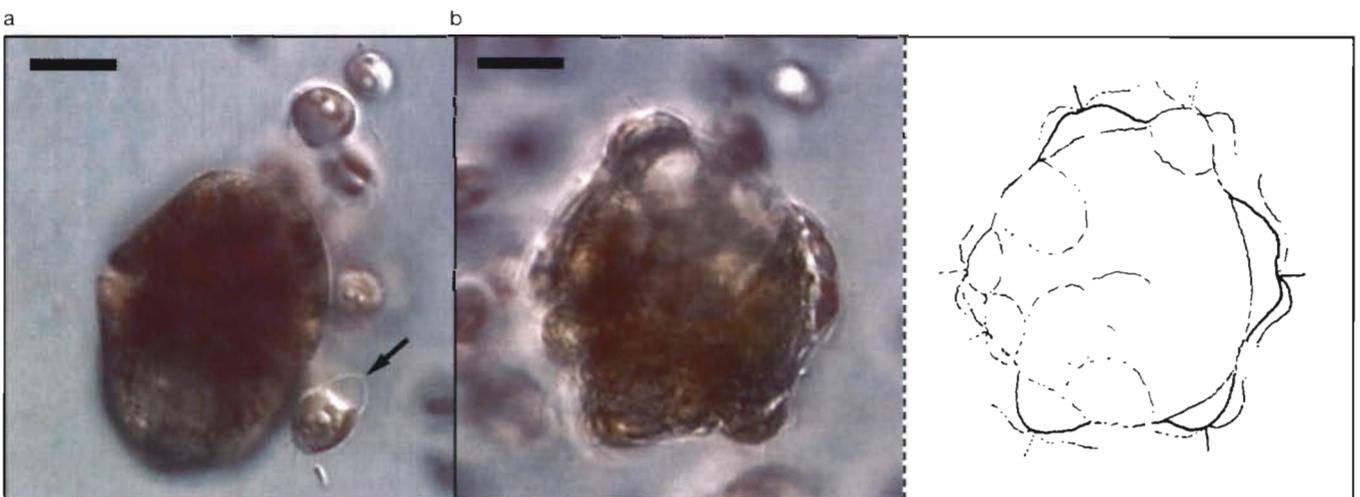

Fig. 8. *Pymnesium patelliferum* attacking the autotrophic dinoflagellate *Gyrodinium* sp. Scale bars = 10 μm. (a) Immediately after addition to the *P. patelliferum* culture, the dinoflagellate loses its motility, but the cell shape is still recognisable. Nearby, *P. patelliferum* cells 'ready to feed' with the typical swollen hyaline posterior part (arrow) can be seen. (b) In a later stage, several *P. patelliferum* cells form a dense layer around *Gyrodinium* sp. *P. patelliferum* cells are extremely deformed

When more than 1 *Prymnesium patelliferum* cell attacked a prey item, the membrane material of the predatory cells often appeared to fuse and form a common food vacuole (e.g. Fig. 6). Such feeding communities with concomitant cell fusion and a subsequent formation of common food vacuoles are known from the freshwater heliozoan *Actinophrys sol* (Patterson & Hausmann 1981). For *P. patelliferum* a cell fusion process remains speculative, since it was not possible to identify a continuous membrane unambiguously in the light microscope. Refined cytological techniques are needed to clarify this problem. However, formation of a fused common food vacuole will not be required in the case of pinocytosis of leaked dissolved nutrients or for phagocytosis of small cell particles which may eventually be released after disruption of the prey's cell wall.

It is unknown whether the formation of cell aggregates and feeding by *Prymnesium patelliferum* cells occur under natural conditions. The current observations of phagotrophy by senescent-stage cells suggest that the phenomenon may be most common towards the end of *Prymnesium* blooms. Further detailed microscopical inspection of natural *Prymnesium* blooms for uncommon cell aggregates will give more insight into these interesting questions.

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