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Recent aspects of nutrition in the dinoflagellate *Dinophysis* cf. *acuminata*

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ABSTRACT: No species of *Dinophysis* has yet been cultured in the full sense. To determine the photosynthetic capability in *D.* cf. *acuminata*, the dominant morphotype of the genus along the French Atlantic coast, we took material from natural populations, in some trials by pipetting out single cells, and in others using plankton assemblages enriched in *D.* cf. *acuminata* by size fractionation and reverse sedimentation. Inorganic carbon uptake was measured at temperatures from 11 to 23°C and at a light intensity of 400 µmol photons m^{-2} s^{-1}. Total uptake was proportional to the number of cells and increased linearly with incubation time, the uptake rate showing a distinct maximum at 18°C, while at 23°C being only half this maximum. Photosynthetic capability was conclusively demonstrated for *D.* cf. *acuminata*, but mixotrophy remains a distinct possibility. Light microscopy of concentrated material showed numerous large, reddish-brown chloroplasts, which fluoresced yellow-orange. Their fine structure is rather unusual for dinoflagellates. No food vacuoles or residual bodies were seen, which suggests inability to ingest and digest prey directly, unless the required apparatus can be generated rapidly. Likewise, no feeding tubes were seen, but a tongue-like structure presenting microtubular bands was observed in the flagellar-pore region, which may be thus a simplified and reduced peduncle; this organelle, however, has not been seen protruded and active.

KEY WORDS: *Dinophysis* cf. *acuminata* · Nutrition · Ultrastructure

INTRODUCTION

The genus *Dinophysis* contains ca 200 species (Sournia 1986), which are all marine and planktonic. None has been reported to generate blooms responsible for seawater discoloration, although some are sometimes companion species of red-tide assemblages dominated by *Prorocentrum minimum* as well as *P. micans* and/or *P. triestinum* (Kat 1989).

Several *Dinophysis* species have been reported to show a photosynthetic cell structure, i.e. they are chloroplastic. Lessard & Swift (1986), however, described some species as heterotrophic, although the toxic *Dinophysis* species still appear autotrotophic as a whole (Lassus & Marcaillou-Le Baut 1991). Supporting this conclusion are Hallegraeff & Lucas' (1988) observations that some senescent cells of *D. acuminata* and *D. fortii* become very pale, apparently exhibiting reduced pigmentation. Accordingly, the group of oceanic species formerly included within the non-photosynthetic genus *Phalacroma* has been revisited and sorted as *Dinophysis* (Hallegraeff & Lucas 1988). Most of them usually show large digestive vacuoles containing incompletely digested particles, including on occasion eucaryotic algae; they are clearly phagotrophic.

Failure to culture any of the *Dinophysis* species (Maestrini et al. 1995 — this issue) has so far prevented the usual set of nutritional experiments, seriously limiting current knowledge. Nevertheless, in order to test various working hypotheses, material isolated from seawater has been used. Thus, Subba Rao & Pan (1993) measured the photosynthetic parameters of *D. norvegica* using natural samples largely dominated by this species, as well as using single cells isolated according to Rivkin & Seliger (1981). Similarly, Berland et al.

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(1994) derived the photosynthetic-light response (P-I) curve's parameters with an assemblage dominated by *Dinophysis* cf. *acuminata*. Granéli et al. (1993a) measured light and dark carbon uptake rates in single cells of *D. acuminata*, *D. acuta* and *D. norvegica*, and concluded that nutrition was mixotrophic. They further observed (Granéli et al. 1993b) a centric-diatom-like body inside 1 *D. acuminata* cell, which they concluded to have been prey captured by phagotrophy.

In order to estimate to what extent some *Dinophysis* species are able to show both autotrophy and phagotrophy, we here describe electron microscope (EM) observations and carbon uptake measurements made on cells taken from the same samples used for P-I measurements by Berland et al. (1994). Natural populations were enriched in *Dinophysis*, relative to co-occurring plankton, either by size fractionation and reverse sedimentation (Maestrini et al. 1995), or by pipetting out single cells of *D.* cf. *acuminata*, a dominant morphotype of the genus along the French Atlantic coast, and which Lassus & Bardouil (1991) considered to be a new species.

MATERIAL AND METHODS

Sampling. In August 1992, samples consisting of about 2000 l of surface water were collected with a bucket, in the harbor of Antifer, near Le Havre, France, and were immediately size-fractionated between 2 meshes, 20 and 77 µm. The 20–77 µm fraction was left overnight in graduated funnels to allow migration towards the surface; the surface population was then gently siphoned off (Maestrini et al. 1995). The resulting suspension contained few (0.2%) or no diatoms and was dominated by *D.* cf. *acuminata* (57% of the total number of cells) and *Dinophysis sacculus* (8%), but not totally free from co-occurring 'companion' dinoflagellates (Fig. 1).

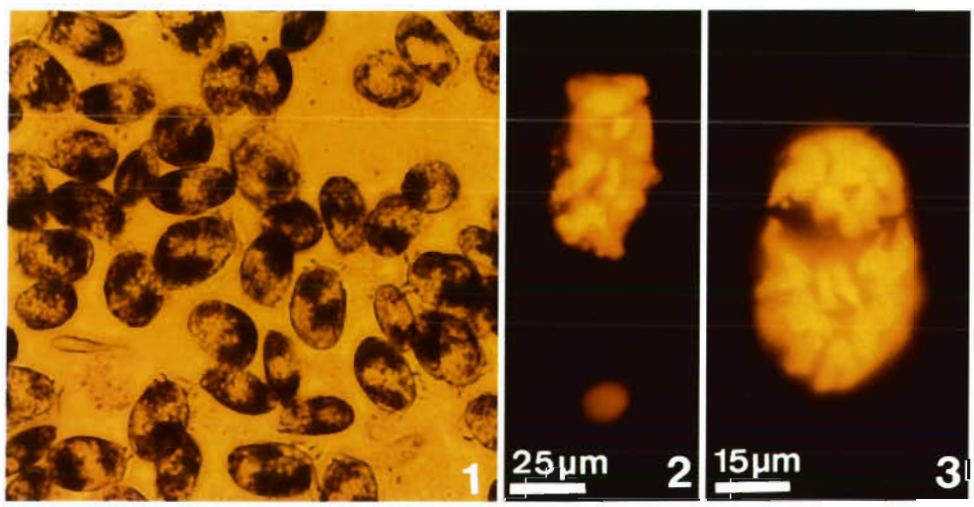
Microscopy. ***Light microscopy (LM):*** living cells were observed with a Leitz light epifluorescence microscope.

Scanning electron microscopy (SEM): fixed specimens were gently filtered and rinsed with distilled water and dehydrated through successive baths of ethanol (30, 50, 70 and 100%); then the filter was glued to an SEM stub, freeze-dried and coated with a gold palladium alloy.

Transmission electron microscopy (TEM): specimens were fixed at 4°C, for 1 h, in the following mixture: 1 vol. 25% glutaraldehyde, 4 vols. 0.4 M cacodylate buffer, 5 vols. sample; then cells were rinsed with 0.2 M cacodylate, 0.4 M NaCl for 2 h, and filtered seawater for 1 h. Postfixation was carried out in 2% osmic acid solution for 1 h, followed by rinsing in diluted seawater and then in distilled water; fixed material was dehydrated through successive baths of ethanol (30, 50, 70 and 100%), and embedded in 'Durcupan' (Fluka®) resin and sectioned; thin sections were stained with uranyl acetate before observation.

Measurement of inorganic carbon uptake rates. Triplicate aliquots (90 ml each) of *Dinophysis*-enriched natural populations were adapted to temperatures of 7, 11, 14, 18 and 23°C for 24 h, then incubated at the same respective temperatures in the presence of $NaH^{14}CO_3$ (0.5 µCi ml^{-1}) for 10 h in the light (400 µmol photons m^{-2} s^{-1}; fluorescent tubes, special for plant growth). After incubation, samples were filtered through glass-fiber membrane filters (Whatman GF/C) and excess $NaH^{14}CO_3$ was removed with 150 µl 6 N HCl.

Single cells of *Dinophysis* cf. *acuminata* were individually isolated with a micropipette (Rivkin & Seliger 1981) from an enriched 20–77 µm fraction, washed 2 or



Figs. 1 to 3. *Dinophysis* cf. *acuminata*. Fig. 1. Concentrated population obtained from size-fraction filtration and reverse sedimentation (exceptionally pure selection). Figs. 2 & 3. Yellow-orange fluorescent chloroplasts; the red fluorescent chloroplast shown for comparison belonged to the prasinophyte *Tetraselmis striata*

3 times, then pooled in batches of 50 cells and inoculated in 1 ml 0.45 µm filtered seawater in 10 ml scintillation counting glass phials. After addition of $NaH^{14}CO_3$ (1 µCi ml^{-1}), incubation was carried out at 18°C under continuous illumination (400 µmol photons m^{-2} s^{-1}), for 25 to 36 h. Incubation was stopped by adding 150 µl 6 N HCl, for a minimum of 6 h. As a check on the validity of using time t_0 as control, runs were made in triplicate with 50 cells of *Protoperidinium* sp., a non-photosynthetic genus (Jacobson & Anderson 1993); counts made after different incubation periods using these samples were not significantly different from those at time t_0.

RESULTS

Cytology

Cells of *Dinophysis* cf. *acuminata* contained numerous reddish-brown chloroplasts. In both the *D.* cf. *acuminata* and *D. sacculus* cells (not shown here), we observed a yellow-orange fluorescence (Figs. 2 & 3). The chloroplasts showed a clumped structure with pyrenoids packed in the center (Fig. 3).

In electron micrographs, cytoplasm appeared to contain little starch, located only around plastids and terminal pyrenoids. In contrast, the cells contained numerous rhabdosomes (Fig. 9) ordered in bundles. The bundles were disposed roughly radially; some reached the cortical cytoplasm under a flat peripheral sack producing a light, fibrous mucus; none reached the thecal pore area (Fig. 7), indicating they are unlikely to be ejectable.

Dinophysis cf. *acuminata* had large chloroplasts each showing 2 thylakoid stacks and 2 membranes in the chloroplast envelope (Fig. 10). The chloroplasts possessed a terminally positioned pyrenoid, and paired thylakoids (each with an electron-dense lumen) in the chloroplasts.

The theca was very clear, showing a stratified structure (Fig. 6). The thecal pores were of diameter 0.2 to 0.3 µm (Fig. 5 & 6). The pore bases, seen in tangential section, were star-shaped, and were constricted by a cupule-like structure which appeared to be an extension of the amphiesma, a perforated disk to which a mucus sac sometimes appears to lead (Figs. 6 & 8). In some pore cavities dense granula were present, probably resulting from ejection of the mucocyst's content. On the external face of the cytoplasmic membrane, dense granula were seen gathered together in streaks in several places; they resembled excreted mucus or micro-organisms captured as prey.

No food vacuoles or residual bodies were observed. In contrast, a tongue-like structure with microtubular bands and microbodies in the flagellar-pore region was seen in *Dinophysis* cf. *acuminata* (Fig. 11). The flagellar pore, located in the apical region (Fig. 4), had an opening size of roughly 1.5 µm. Bacterium-like organisms were present in the flagellar canal, and one was seen as if adhering to the tongue-like organelle (Fig. 8, transversal section).

Photosynthetic carbon uptake

At all temperatures, the total amount of inorganic carbon taken up by *Dinophysis*-dominated populations increased linearly with time and fitted a regression model (Fig. 12). The rate of uptake increased with temperature up to 18°C which appeared to be the optimal temperature (over the range of values we used); at 23°C, the carbon uptake rate was only half that at 18°C.

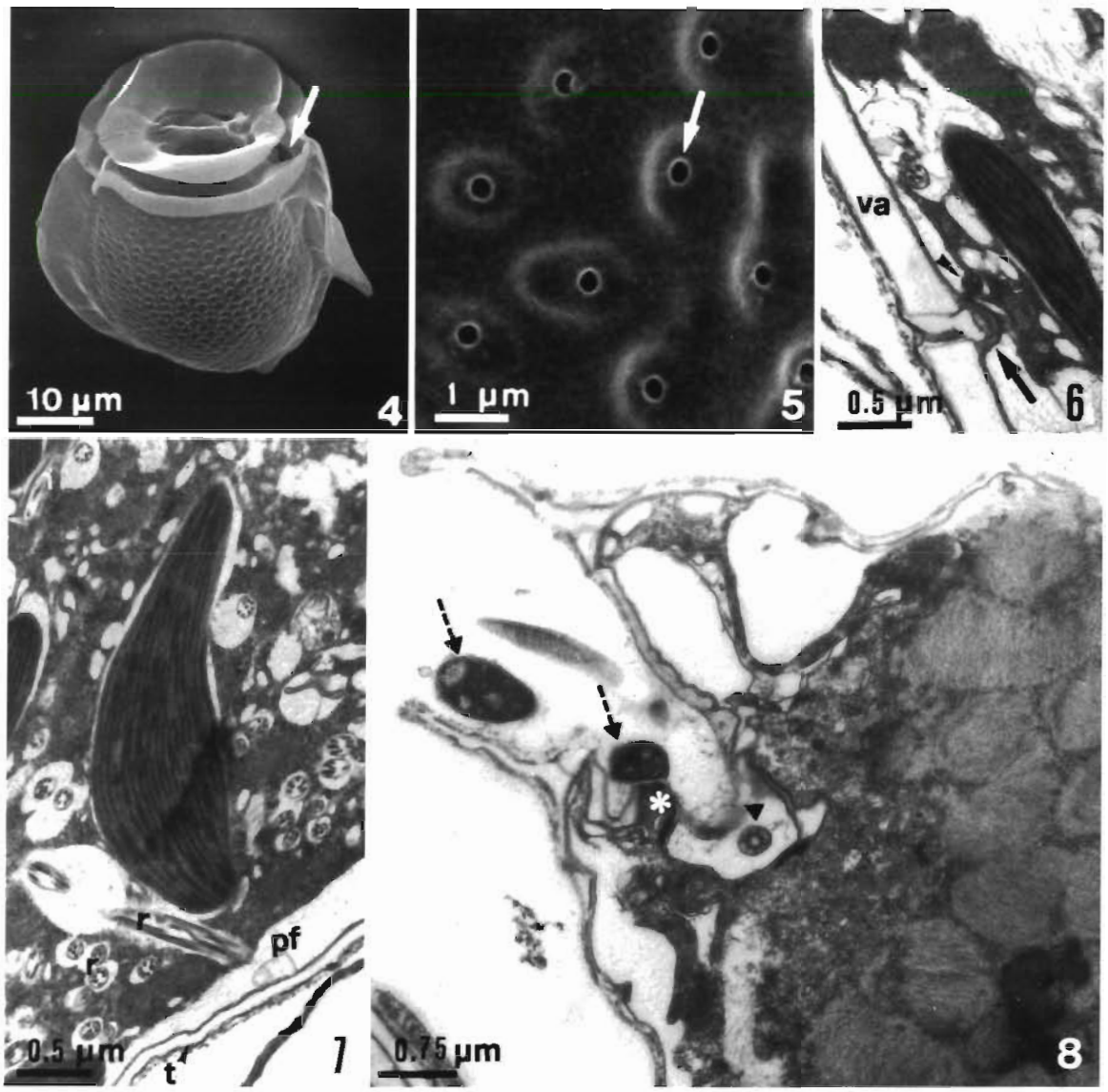
The net total amount of carbon taken up by single cells increased progressively with time (Fig. 13; r^2 = 0.80).

DISCUSSION

That cells of *Dinophysis* cf. *acuminata* contained numerous reddish-brown chloroplasts producing a yellow-orange fluorescence (Figs. 2 & 3) suggested they were actively growing. Lessard & Swift (1986) were the first to report bright red or yellow-orange fluorescence in *D. acuta* and *D. fortii*, and suggested it originated from the presence of cyanobacterial-like symbionts. Later, Schnepf & Elbrächter (1988) gave a list of 14 species with orange primary fluorescence, out of 30 *Dinophysis* species investigated, and Geider & Gunter (1989) brought the evidence for the presence of phycoerythrin in *D. norvegica*.

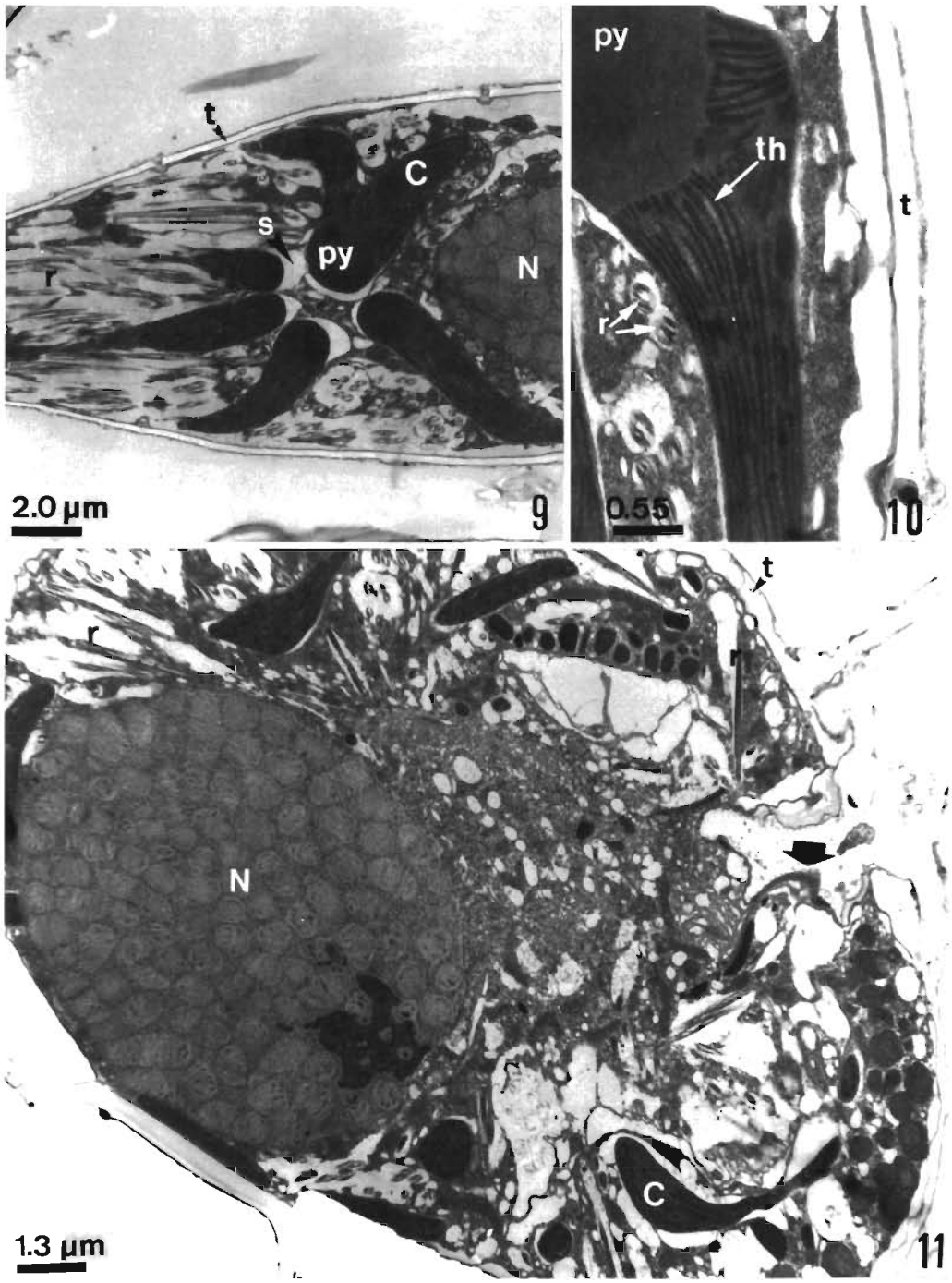
Furthermore, the clumped chloroplast ultrastructure we observed (Fig. 9) was also reported by Hallegraeff & Lucas (1988), who showed that the chloroplasts of *Dinophysis fortii* are often arranged in tight clusters (chromatospheres) located in the central part of the cells. Large chloroplasts showing 2 thylakoid stacks and 2 membranes in the chloroplast envelope, both unusual for dinoflagellates, were found in the *D. acuminata*, *D. acuta* and *D. fortii* investigated by Hallegraeff & Lucas (1988), Schnepf & Elbrächter (1988) and Lucas & Vesk (1990), as well as in the *Dinophysis* cf. *acuminata* that we studied (Fig. 10). Pyrenoids were terminal, and paired thylakoids with an electron-dense lumen were present in the chloroplasts, suggesting the presence of phycobilin pigments (Gantt et al. 1971).

Schnepf & Elbrächter (1988) suggested that *Dinophysis* chloroplasts might be cryptophycean-like cell



Figs. 4 to 8. *Dinophysis* cf. *acuminata*. Fig. 4. Scanning electron microscope (SEM) apical view showing the flagellar pore (arrow). Fig. 5. SEM view showing thecal pores (arrow). Fig. 6. Transmission electron microscope (TEM) section of 1 thecal pore, showing the perforated cupule-shaped disc (indicated by the arrow) allowing the end of a mucocyst (arrowhead) to protude; va: striated amphiesal vesicule (i.e. theca). Fig. 7. TEM micrographs of rhabdosomes (r), seen in transversal and longitudinal sections; one is seen touching the subthecal peripherical sacs (pf) which contained a light mucus under the theca (t). Fig. 8. TEM section at the sulcate level, showing trapped microorganisms (arrows); one of them is seen touching a tongue-like organelle (star); the star-shaped basis of 1 thecal pore is also seen (arrowhead)

structures captured from free cryptophyte cells by myzocytosis (i.e. sucking out the cell content without the prey plasmalemma), and might represent a relatively old symbiotic association, in the evolutionary sense. This hypothesis is consistent with the observation that dinoflagellates in several other genera host endosymbiotic cryptomonads (Wilcox & Wedemayer 1984). On the other hand, Larsen (1988) observed the dinoflagellate *Amphidinium poecilochroum* feeding on small cryptophytes by myzocytosis, and suggested that the ingested chloroplasts might remain photosynthetically active for a while, after the cytoplasm and other organelles had been digested. The ciliate *Mesodinium rubrum* also hosts a cryptomonad endosymbiont, whose origin is indicated by its typical cryptomonad nucleomorph and mitochondrial structure (Hibberd 1977). In contrast, cryptomonad organelles such as mitochondria, ejectosomes or nucleomorphs have never been observed in *Dinophysis* spp. (Schnepf & Elbrächter 1988, Lucas & Vesk 1990; this work). In



Figs. 9 to 11. *Dinophysis* cf. *acuminata*. Fig. 9. TEM longitudinal section showing chloroplasts (C) grouped in chromatospheres with terminal pyrenoids (py) and surrounding starch (s), the typical dinoflagellate nucleus (N), the theca (t), and several rhabdosomes (r). Fig. 10. Part of Fig. 9 enlarged to show thylakoids (th) with a dense lumen and a terminal pyrenoid. Fig. 11. TEM transverse section at the flagellar pore level, showing the tongue-like organelle (indicated by large arrow)

addition, the pigment signature of *D.* cf. *acuminata* indicates the simultaneous presence of peridinin, alloxanthin and phycobilins (Hallegraeff & Lucas 1988, Maestrini et al. unpubl.). Hence, the origin of chloroplasts in *Dinophysis* spp. is still an open question.

The steady uptake of inorganic carbon measured in both *Dinophysis* cf. *acuminata*-dominated populations (Fig. 12) and isolated cells of *D.* cf. *acuminata* (Fig. 13) clearly indicates that the species is photosynthetic. The optimal temperature, 18°C, is that of natural water

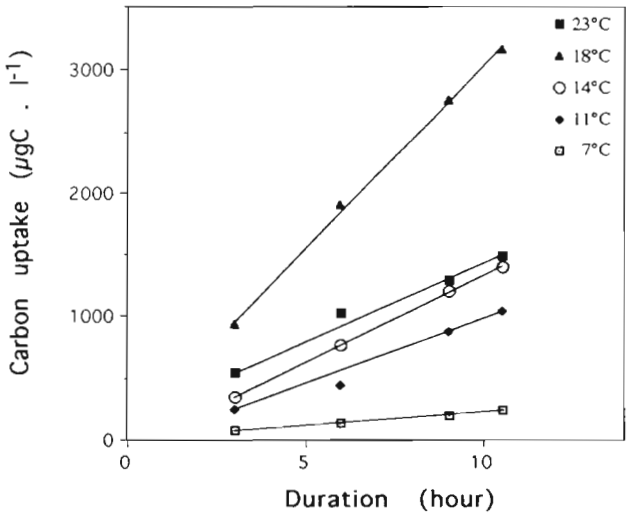


Fig. 12. Time course of inorganic carbon uptake by a *Dinophysis* cf. *acuminata* dominated population in the light (400 µmol photons m^{-2} s^{-1}), at different temperatures

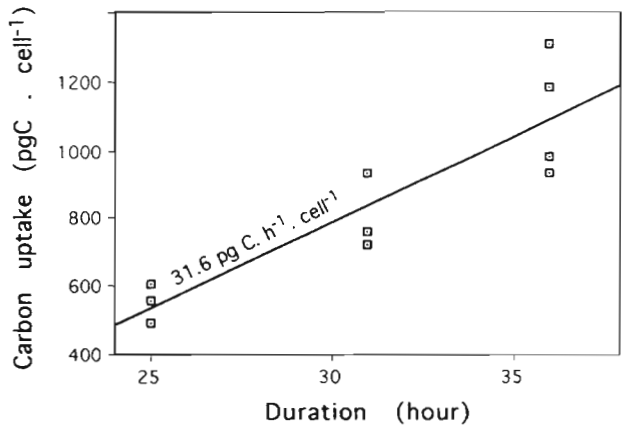


Fig. 13. Time course of inorganic carbon uptake of single *Dinophysis* cf. *acuminata* cells in the light (400 µmol photons m^{-2} s^{-1}) versus duration of incubation (r^2 = 0.80; N = 10)

at which *D.* cf. *acuminata* is usually most abundant (Lassus et al. 1993). Moreover, from data reported elsewhere (Berland et al. 1994), we calculated that the initial slope of the P-I curve given by a similar assemblage dominated by *D.* cf. *acuminata* was 0.053 µg C µg^{-1} chl *a* h^{-1} (µmol photons m^{-1} s^{-1})$^{-1}$, the maximum photosynthetic rate P_m^B was 16 µg C µg chl a^{-1} h^{-1}, and the photoadaptative index I_k was 300 µmol photons m^{-2} s^{-1}. These values are significantly higher than those reported by Subba Rao & Pan (1993), for a different *Dinophysis* species and with less contaminant algae present. Photosynthetic activity in *D.* cf. *acuminata* is therefore conclusively demonstrated, although it is lower than that typical of other algae of similar size (Rivkin et al. 1984, Rivkin & Voytek 1985, Subba Rao 1988, Han et al. 1992).

On the other hand, Granéli et al. (1993a, b) measured the light and dark carbon uptake rates in singly-isolated cells of several autotrophic *Ceratium* species and of *Dinophysis acuminata*, *D. acuta* and *D. norvegica*, all incubated *in situ* and in natural assemblage. These workers found that biomass-specific rates of carbon uptake in darkness were significantly higher for the *Dinophysis* species than for the *Ceratium* species. Light fixation rates in the *Dinophysis* species were not systematically less than in the *Ceratium* species. That carbon was nevertheless fixed in the dark led these authors to suggest a mixotrophic mode of nutrition for the 3 *Dinophysis* species. Furthermore, they observed 1 *D. acuminata* cell to contain a centric diatom cell, and suggested that it might have been captured by phagotrophy. *D. acuminata* and *D. norvegica* may be mixotrophic: other dinoflagellates frequently are (Schnepf & Elbrächter 1992, Bockstahler & Coats 1993), and some *Dinophysis* species are heterotrophic (Hallegraeff & Lucas 1988), or even predatory (Hansen 1991).

Although several results have suggested mixotrophic nutrition, including phagotrophy, most attempts to find structural equipment for digesting particles, including prey, have been unsuccessful for *Dinophysis* cf. *acuminata*. We also have not found any food vacuoles or residual bodies in our electron micrographs of this species (Fig. 8), while Hallegraeff & Lucas (1988) and Lucas & Vesk (1990) did similarly not find any in either *D. acuminata* or *D. fortii*. However, *D. hastata* and *D. schuettii*, both unpigmented phagotrophic species, show large food vacuoles (Hallegraeff & Lucas 1988, Lucas & Vesk 1990). Our results therefore also indicated that *D.* cf. *acuminata* in bloom stage does not have the capability to ingest and digest the whole prey inside the cell, unless the required equipment can form rapidly, perhaps depending on the life cycle and/or temporary environmental conditions. In contrast, Jacobson & Anderson (1994) have first reported the presence of food vacuoles in *D. acuminata* and *D. norvegica*. These organella, however, were observed in only a fraction of the populations: 36% and 6%, respectively, and were not seen at all in August–September in *D. norvegica*. On this basis, it can be hypothesized that the phagotrophic nutrition is probably highly variable, according to environmental conditions whose respective importances are still an open question.

Heterotrophic mechanisms other than phagocytosis, described in other species, could account for the apparently mixotrophic regime of this species. In *Dinophysis rotundata*, for instance, Elbrächter (1991) reported a myzocytosis nutrition. Moreover, Hansen (1991) observed *D. rotundata* drilling the membrane of

ciliates with a feeding tube and sucking out their cytoplasm; in contrast, he never observed pigmented *Dinophysis* species to actively ingest particles. We also never observed drilling activity in *D.* cf. *acuminata*, nor velum-like structures, although we saw the latter several times in some *Protoperidinium* species present in the same natural assemblages.

In micrographs of ultrastructure, both Lucas & Vesk (1990) and ourselves have nevertheless observed a tongue-like structure with microtubular bands, in the flagellar-pore region of *Dinophysis acuminata*, *D.* cf. *acuminata* (Fig. 10) and *D. fortii*. This may represent a simplified and reduced peduncle, an organelle not rare in dinoflagellates. Lucas & Vesk (1990) suggested that this reduced peduncle may indicate the loss of phagotrophic capability. This suggestion is challenged, however, by the findings of Jacobson & Anderson (1992) who reported the same simplified peduncular structure in 2 actively phagotrophic species, including *D. rotundata*, and suggested that it could be protruded and functional. No active protruded peduncle, however, has been observed so far in photosynthetic species of *Dinophysis*, although the phenomenon could be missed if it took place in only a few minutes and in the dark, as has been shown in *Amphidinium poecilochroum* (Larsen 1988).

In a few micrographs, microorganisms can be seen engaged in the flagellar pore area, including one bacterium-shaped body adhering to the tongue-like organelle (Fig. 8), which might produce digestive enzymes. The opening size of the flagellar pore, on the other hand, roughly 1.5 µm, would appear to allow only small particles to enter, yet sulcal and other plates have been seen to move slightly to one side (Lucas & Vesk 1990); which renders puzzling Granéli et al.'s (1993b) finding that *Dinophysis acuminata* had ingested a *Thalassiosira subtilis*-type cell, unless an unknown mechanism was acting

In contrast, Ishimaru et al. (1988) observed by LM the cytoplasm of *Dinophysis fortii* covering some cells of *Plagioselmis* sp. attached to the hypothecal plates, thus acting like a pallium. The dinoflagellate absorbed the cytoplasm of the cryptophytes within only a few hours. Generally these pallium-like structures have been seen in the flagellar-pore area of phagotrophic species (e.g. *Protoperidinium*), and are not associated with thecal pores. Moreover, the thecal pores we observed were narrow, obturated by a disc, and were connected only to mucus sacs, whose role, perhaps the production of enzymes, is still unknown. The structure of these thecal pores, also reported by Lucas & Vesk (1990), would seen to preclude completely any prey capture.

Potential phagotrophic mechanisms envisaged here, however, would not exclude other possible mechanisms such as endocytosis (i.e. uptake of macromolecules; Lucas & Vesk 1990) by the pusule system (Klut et al. 1987), and/or direct resorption uptake of dissolved organic substances through the plasmalemma. Altogether, the heterotrophic capability of *Dinophysis* cf. *acuminata* and other photosynthetic species of *Dinophysis* is highly probable, but its mechanism still remains to be demonstrated.

CONCLUSIONS

Dinophysis cf. *acuminata* and some other *Dinophysis* species involved in DSP (Diarrhoeic Shellfish Poisoning) episodes are chloroplastic and show an unusual chloroplast structure for dinoflagellates. Their photosynthetic pigments produce cryptophyte-like fluorescence which reveals the presence of phycobiliproteins. Their specific carbon uptake rate is also rather low compared to that in other algae of similar size. To what extent the uptake of inorganic carbon supplies synthetic processes in the cell remains unclear. Their phagotrophic capability is also still open to question.

A mixotrophic mode of nutrition is strongly suggested by indirect evidence from different sources including cell structure, *in situ* distribution and attempts at culture. The nutrient(s) and/or food they preferentially take up from the natural environment have still to be determined, as have any possible changes which might be externally modulated, or related to the life cycle. Attempts to cultivate or grow these organisms should thus not be limited to algal methods, but those developed for the culture of protozoa should also be investigated.

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