

# Light and dark carbon uptake by *Dinophysis* species in comparison to other photosynthetic and heterotrophic dinoflagellates

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**ABSTRACT:** The marine dinoflagellate genus *Dinophysis* includes species that are the causative agents of diarrhetic shellfish poisoning (DSP). Recent findings indicate that some *Dinophysis* species are mixotrophic, i.e. capable of both autotrophic and heterotrophic nutrition. We investigated inorganic (and organic) carbon uptake by several species of *Dinophysis* in the light and dark using the 'single-cell <sup>14</sup>C method', and compared uptake rates with those of photosynthetic *Ceratium* species and heterotrophic dinoflagellates in the genus *Protoperdinium*. Experiments were conducted with water from the Gullmar Fjord and from the Koster Strait (Swedish west coast). Nutrient-enriched phytoplankton from surface water samples were concentrated (20 to 70 µm) and incubated at *in situ* temperature under artificial light conditions with high concentrations of inorganic <sup>14</sup>C (1 µCi ml<sup>-1</sup>). Individual cells of each desired species were manually isolated under a microscope and transferred to scintillation vials. *C. tripos* showed net <sup>14</sup>C uptake only during light periods, whereas both *C. lineatum* and *C. furca* showed <sup>14</sup>C uptake in the light as well as uptake (and sometimes losses) in the dark. *Dinophysis* species had similar carbon fixation rates in light compared to *Ceratium* species. For *D. acuminata* and *D. norvegica*, net carbon uptake occurred in both light and dark periods. *D. acuta* showed a loss of carbon in the dark in one experiment, but in another, dark C uptake was significantly higher than uptake in light. When exposed to light, *C. furca*, *D. norvegica* and *D. acuta* had high specific carbon uptake rates. Growth rates for the different species were calculated from <sup>14</sup>C uptake by the cells during the first hours of incubation in light. *D. acuminata* and *D. norvegica* had similar maximum growth rates, 0.59 and 0.63 d<sup>-1</sup> (µ); the maximum growth rate of *D. acuta* was lower (0.41 d<sup>-1</sup>). The positive dark carbon uptake by *Dinophysis* may suggest a mixotrophic mode of nutrition. In one experiment, both *D. norvegica* and *D. acuta* showed a significantly higher carbon uptake in a dark bottle than in a light bottle, which would be consistent with uptake of <sup>14</sup>C-labeled organic matter by *D. norvegica* and *D. acuta*. Demonstration of direct uptake of dissolved and particulate organic matter would provide conclusive evidence of mixotrophy and this will require the development of new protocols for measuring organic matter uptake applicable to *Dinophysis* in the natural assemblages.

**KEY WORDS:** *Dinophysis* · Growth rate · Mixotrophy · Carbon uptake · *Ceratium* · *Protoperdinium*

## INTRODUCTION

Several species in the marine dinoflagellate genus *Dinophysis* are known to produce toxins responsible for diarrhetic shellfish poisoning (DSP) (Yasumoto et al. 1980, Krogh et al. 1985, Sedmak & Fanuko 1991, Lembeye et al. 1993, Subba Rao et al. 1993). The genus

occurs in many different tropical and temperate marine waters over a wide range of salinities (Hallegraeff & Lucas 1988, Hallegraeff 1993). Much of what is known of the ecology, biology, and toxicology of *Dinophysis* comes from studies of natural populations (Edler & Hageltorn 1990, Delmas et al. 1992, Carlsson et al. 1995) because it is not yet possible to culture any of the species for extended periods in the laboratory (Sampayo 1993, Maestrini et al. 1995, Subba Rao

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1995). The species linked directly to DSP (*D. acuta*, *D. acuminata*, *D. norvegica*, *D. fortii*, *D. mitra*) are all photosynthetic and contain functional chloroplasts. Nevertheless, *Dinophysis* species do not survive when cultured in light using culture media that support the growth of many other photosynthetic phytoplankton species. This may reflect the absence of some trace inorganic element missing from most culture media, such as cobalt or selenium, which have been shown to be essential elements for many phytoplankton species (Keller & Guillard 1985, Harrison et al. 1988, Edvardsen et al. 1990, Granéli & Haraldsson 1993, Granéli & Risinger 1994). Thus far, however, addition of selenium, cobalt or other trace elements has not made it possible to culture *Dinophysis* species (Maestrini et al. 1995). Alternatively, these species may be osmotrophic, relying on dissolved organic materials to supplement inorganic uptake. Many phytoplankton species are osmotrophic to a small extent, requiring vitamins such as biotin, B12, or thiamin that they cannot synthesize themselves (see Bonin & Maestrini 1981). Here again, addition of many different organic supplements to culture media has not resulted in long-lived cultures of several *Dinophysis* species (*D. cf. acuminata*, *D. acuta* and *D. sacculus*; Maestrini et al. 1995).

A third possibility is that the DSP-causing *Dinophysis* species are phagotrophic, meaning that they are able to ingest particles, perhaps as a survival strategy during times when photosynthesis and/or osmotrophy cannot support continued growth. One species of the genus *Dinophysis* (*D. rotundata*) lacks chloroplasts and is completely heterotrophic, feeding phagotrophically (Hansen 1991). Jacobson & Andersen (1994) have found food vacuoles in *D. norvegica* and *D. acuminata*, suggesting that a phagotrophic feeding mechanism exists.

The idea that photosynthetic phytoplankton might be able to supplement their nutrition through the direct uptake and utilization of organic material is not new. Evidence from older studies is based exclusively on microscopic observations (e.g. Manton & Parke 1962, Pintner & Provasoli 1968) or traditional bioassay experiments (Bonin & Maestrini 1981, Granéli et al. 1985). Study of the use of dissolved organic material or phagotrophic ingestion of algae or bacteria by photosynthetic phytoplankton species has recently gained new momentum with the advent of techniques such as fluorescent or radioactive labeling of dissolved/particulate substances (Rivkin & Putt 1987, Jones et al. 1993).

To demonstrate phagotrophy in phytoplankton, either radioactive  $^{14}\text{C}$ -labeled bacteria (Nygaard & Hessen 1990, Nygaard & Tobiesen 1993), labeled algae or bacteria (Nygaard & Hessen 1990), fluorescent beads (Porter 1988) or green flagellates (Jones et al.

1993) are given as a food source to the species in question. The above studies were typically performed with monocultures. Given our objective to gain information on the biology of the dinoflagellate genus *Dinophysis*, it was necessary to work with natural phytoplankton communities since these species cannot yet be cultured in the laboratory.

In an attempt to provide information on the possible heterotrophy of this genus, a series of experiments was performed using the natural phytoplankton communities containing several chloroplast-bearing *Dinophysis* species. Entire phytoplankton communities from waters along the East Skagerrak on the Swedish west coast were labeled with  $^{14}\text{C}$ , and incubated in the laboratory for several days. Thereafter *Dinophysis* and other dinoflagellate species were isolated as single cells, placed in scintillation vials and species-specific  $^{14}\text{C}$  uptake determined during light and dark periods. Uptake by *Dinophysis* species was compared to the uptake by the other dinoflagellates exposed to the same conditions, including photosynthetic and heterotrophic species.

## MATERIALS AND METHODS

Three experiments using East Skagerrak water containing natural phytoplankton communities were performed in 2 different years. Expts 1 and 2 (October 1992) were performed at the Tjärnö Marine Biological Station with water collected 4 km off the Koster Strait (Swedish west coast). Expt 3 (September 1993) used water from the Gullmar Fjord, outside the Kristineberg Marine Research Station.

One gallon (ca 4.5 l) polycarbonate bottles were filled with 4 l of seawater containing the phytoplankton communities which had been concentrated using aluminum frames (60 × 40 cm) to which nylon mesh was attached (see Maestrini et al. 1995 for details). By restricting the flow through the meshes using holes bored into the open box on which they were placed, cells were always suspended in a large volume of water and shear rates were low through the mesh. This has been shown to be a gentle means of concentrating populations and leaves most dinoflagellate cells swimming. Size fractionation was 20 to 70  $\mu\text{m}$  in Expt 1 and 40 to 70  $\mu\text{m}$  in Expts 2 and 3. The 40  $\mu\text{m}$  net was used in Expts 2 and 3 in order to let as many small cells as possible pass, since small cells present in large quantities made the rinsing of the isolated radioactive cells more laborious in Expt 1.

Before the experiments started, the concentrated phytoplankton were placed in 10 l polycarbonate bottles incubated in a cool room [temperature 16°C, light 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (photosynthetically active radiation,

PAR)] for at least 1 d. The algae swimming at the top of the bottles were then gently siphoned into the experimental bottles. In this way we were sure that only healthy cells that had survived the concentration process would be used in the experiments.

For Expts 1 and 2, a timer coupled to the lights was used to control light and dark hours. In Expt 3, double black plastic sheets were wrapped around the bottles to make them dark. In order to investigate dark uptake during a longer period without shaking and sampling, 1 bottle was left undisturbed in the dark in Expt 3. To accomplish this, 1 l was removed from the 1 gallon (4.5 l) bottle (Bottle A) at Hour 29 and poured into a polycarbonate bottle (Bottle B) covered with 2 layers of thick black plastic and kept in the dark under the same conditions as the original bottle, but without shaking. This prolonged darkness by 40 h until the last experimental sampling occasion (Hour 69).

The temperature, salinity and light intensities in the 3 experiments were as follows: Tjärnö 1992, Expt 1: 10°C, 28.5‰, 67  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Expt 2: 10°C, 31‰, 67  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Kristineberg 1993, Expt 3: 11.5°C, 30‰, 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The chosen temperatures were the approximate *in situ* temperatures when the water was sampled and the light levels were representative for the water at 3 to 6 m depth *in situ* at noon.

To each bottle  $^{14}\text{C}$ -labeled bicarbonate was added to give a final concentration of 1  $\mu\text{Ci ml}^{-1}$  ('25 mCi stock solution', Amersham). The method used to isolate cells following the incubations was that of Rivkin & Seliger (1981) with the following modifications: after incubating in light or dark, the phytoplankton cells were concentrated in a fume hood on a 6 cm diameter, 10  $\mu\text{m}$  mesh size nylon net. The cells retained on the net were then washed extensively with 4 l of Whatman GF/C filtered seawater in order to remove excess dissolved  $^{14}\text{C}$ . The collected plankton were then washed from the net and into a 10 ml test tube using filtered seawater from a squirt-bottle. The tube of cells was placed in a cooler containing crushed ice. Single cells of each target species were then isolated under the microscope from Sedgewick-Rafter or Palmer-Maloney chambers. By transferring the cells to a small volume of chilled, filtered seawater in clean Sedgewick-Rafter or Palmer-Maloney chambers, each cell was washed at least 3 times to remove contamination from other cells and from residual  $^{14}\text{C}$ . We learned from earlier trials that small phytoplankton cells in high abundance could contaminate the isolations to a high degree, making it necessary to wash each isolated cell several times in filtered seawater before it was placed into a scintillation vial. Between 15 and 50 cells of the same species were placed in each of 4 replicate scintillation vials containing 2 ml of Whatman GF/C filtered seawater. Blanks were taken by micro-pipetting water without cells from

the last slide and placing as many drops into the scintillation vials as the corresponding number of picked cells. The scintillation vials were then filled with 10 ml scintillation cocktail (Insta-Gel, Packard Inc.) and counted in a 1108 Beckman Liquid Scintillator.

Nutrients were added to the bottles in the following proportions: Expt 1:  $\text{NO}_3$ , 10  $\mu\text{M}$ ;  $\text{PO}_4$ , 0.5  $\mu\text{M}$ ; Expts 2 and 3:  $\text{NO}_3$ , 5  $\mu\text{M}$ ;  $\text{PO}_4$ , 0.5  $\mu\text{M}$ . Vitamins and trace metals were added according to F/10 (Guillard & Ryther 1962) in Expt 1 and as F/20 in Expts 2 and 3.

Phytoplankton cell counts in initial samples (before addition of  $^{14}\text{C}$ ) and at the end of the experiment (after acidifying some samples and bubbling them with air to remove remaining inorganic  $^{14}\text{C}$ ) were performed following the Utermöhl (1958) method. Cell volumes were estimated through measurements of the cell size and shape of 5 to 10 cells of each species and stoichiometric formulas as described in Edler (1979). To convert  $\mu\text{m}^3$  to pg C the conversion factor 0.13 was used (Smetacek 1975).

Prior to running the main experiments, an experiment was performed in 1991 to check if *Dinophysis* species were able to take up radioactively labeled inorganic carbon in the dark (without any prior light incubation). The natural algal communities were inoculated with 1  $\mu\text{Ci ml}^{-1}$   $^{14}\text{C}$ -labeled carbon and placed in the dark for 12 h. After this period single cells of different algal species including all 3 photosynthetic *Dinophysis* species were picked and placed in scintillation vials containing 2 ml of Whatman GF/C filtered seawater.

The amount of total inorganic carbon incorporated per cell ( $\Delta\text{C}_i$ ) was calculated as follows:

$$\Delta\text{C}_i = [(\text{DPM}_{\text{cell}}/\text{DPM}_{\text{added}}) \cdot \text{IC} \cdot k_1 \cdot k_2 \cdot k_3]/t$$

where  $\text{DPM}_{\text{cell}}$  = DPM (disintegrations per minute) obtained for each vial (corrected for background DPM) and divided by the number of cells per vial;  $\text{DPM}_{\text{added}}$  = added DPM  $\text{l}^{-1}$  (determined by dilution of the amount of added isotope and counting in the liquid scintillator);  $\text{IC}$  = concentration of total dissolved inorganic carbon ( $\text{mg l}^{-1}$ ), determined from tables (Ærtebjerg-Nielsen & Bresta 1984) after measuring pH, temperature, and salinity;  $k_1$  = correction factor for respiration (1.06) (Ærtebjerg-Nielsen & Bresta 1984);  $k_2$  = correction factor for slower uptake of  $^{14}\text{C}$  than for  $^{12}\text{C}$  (1.05) (Ærtebjerg-Nielsen & Bresta 1984);  $k_3$  = conversion factor to get carbon assimilated per cell in pg ( $10^9$ ); and  $t$  = time (h).

We have assumed that the incorporated radioactive carbon during the first light period (<8 h) of the experiment was only in inorganic form and calculated  $^{14}\text{C}$  uptake as carbon incorporated per cell for this time period (see Table 1). However, after this time period, the uptake could either have been in inorganic form

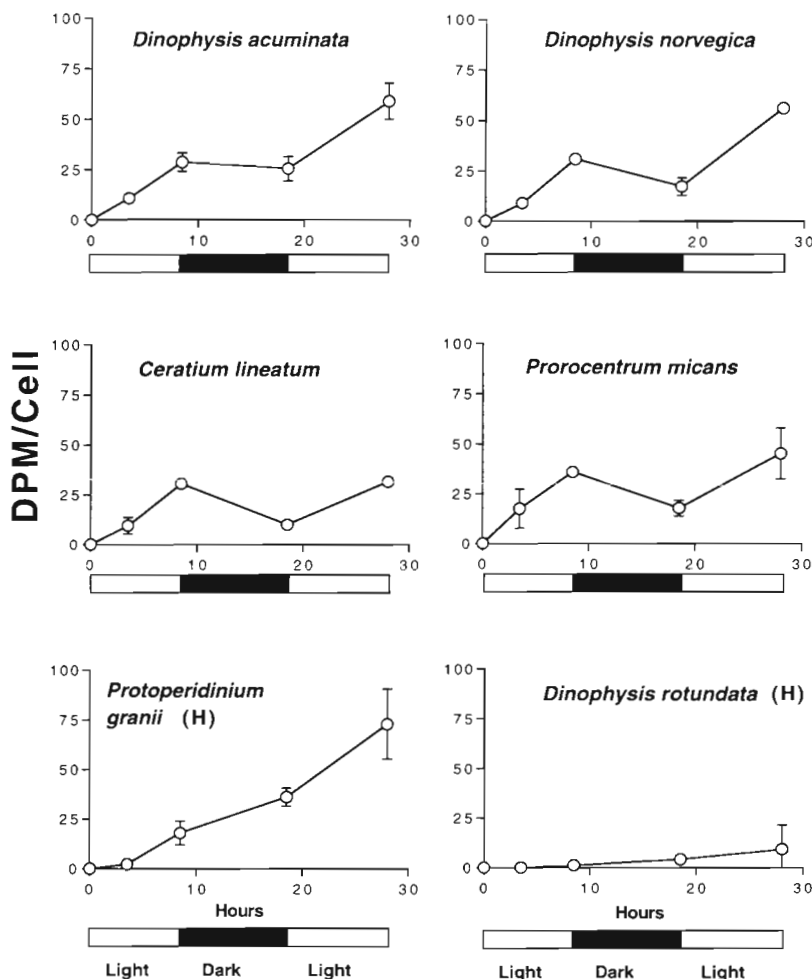


Fig. 1. Expt 1, water from the Tjärnö Strait, October 1992.  $^{14}\text{C}$  uptake by different dinoflagellate species after exposure of a natural plankton community to light and dark incubations. Cells were isolated under the microscope and placed in scintillation vials. Each point is the mean  $\pm$  SD of 4 scintillation vials, each vial containing 15 to 50 cells. H: species that are completely heterotrophic

(incorporated by photosynthesis) or in organic form (incorporation of secondarily labeled organic compounds by heterotrophy). We have therefore chosen to express the results obtained from the longer incubations (including dark periods) only as DPM cell $^{-1}$ .

Specific growth rates ( $\mu$ ) were calculated on the basis of the theoretical time necessary for each species to double its carbon content based on the  $^{14}\text{C}$  uptake during the first hours of incubation in light. Thus for *Dinophysis acuminata*, cell carbon was estimated to be 1194 pg C cell $^{-1}$  (by the measurements of its linear dimensions, calculation of volume and multiplying with the conversion factor mentioned before), so the calculated time for *D. acuminata* to accumulate carbon corresponding to 2388 pg C cell $^{-1}$ , would be the theoretical time for 1 division. In the calculation of  $\mu$  it was assumed that the cells were subjected to a theoretical

light:dark cycle of 10:14 h, with no increase of cell carbon in the dark. Thus

$$\mu = \frac{\ln(m + \Delta C_{i2}) - \ln(m)}{t_2 - t_1}$$

where  $m$  = cell carbon at  $t_1$  (calculated from measured cell volumes) and  $\Delta C_{i2}$  = amount of labeled carbon incorporated per cell after  $t_2 - t_1$  hours. It should be stressed that this calculated division rate is based on the uptake of inorganic carbon only for the first light period (less than 8 h of incubation).

## RESULTS

### Phytoplankton community composition

During the occasions when the 3 experiments were performed, the phytoplankton communities were dominated by dinoflagellates, representing Skagerrak waters typical for the autumn season. In the water collected for Expts 1 and 2, before concentration, *Dinophysis* species constituted a very small percentage of the total phytoplankton biomass (both as cell numbers and as carbon). The maximum cell numbers found *in situ* in Expts 1 and 2 were 400 cells l $^{-1}$  for *D. acuminata*, *D. norvegica* and *D. acuta* together. These cell numbers represented 0.28% of the total phytoplankton carbon and 0.32% of the total dinoflagellate carbon. During Expt 3 however, *D. norvegica* and *D. acuta* together reached densities up to 18 000 cells l $^{-1}$ , representing up to 5% of the total phytoplankton carbon. In 1992 *Gyrodinium aureolum* and *Ceratium* species (*C. furca*, *C. lineatum*, *C. tripos* and *C. fusus*) comprised the bulk of the dinoflagellate and phytoplankton cell carbon. During 1992 the diatom *Pseudonitzschia pseudodelicatissima* bloomed. Cell numbers were very high but this small species did not make up more than 10% of the total phytoplankton biomass. During 1993 the dinoflagellate *C. furca* constituted up to 94% of the total phytoplankton carbon.

### Light uptake of carbon

The  $^{14}\text{C}$  uptake, expressed as DPM cell $^{-1}$ , varied among species and between years (Figs. 1 to 3). The

highest carbon uptake values (calculated from uptake of inorganic carbon during the first hours of incubation in light) for the different phytoplankton species were: *Dinophysis acuta*  $62 \pm 19$  (mean  $\pm$  SD, Expt 2), *D. acuminata*  $40 \pm 4$  (Expt 1), *D. norvegica*  $108 \pm 11$  (Expt 1), *Ceratium lineatum*  $29 \pm 15$  (Expt 2), *C. furca*  $148 \pm 29$  (Expt 3), *C. tripos*  $34 \pm 11$  (Expt 2) pg C cell<sup>-1</sup> h<sup>-1</sup>, respectively (Table 1). For the heterotrophic dinoflagellates, the equivalent values were: *Protoperidinium granii*  $7.7 \pm 4.2$  (Expt 1), *P. divergens*  $5.2 \pm 0.3$  (Expt 3) and *Dinophysis rotundata*  $13.7 \pm 6.8$  (Expt 2) pg C cell<sup>-1</sup> h<sup>-1</sup> respectively (Table 1). The maximum carbon uptake values for *D. acuta* and *D. acuminata* were almost the same for the different experiments; however, *D. norvegica* had a much higher carbon uptake during Expts 1 and 2 than in Expt 3 (Table 1).

#### Dark uptake of carbon

In the September 1991 experiment to check for possible uptake of radioactively labeled inorganic carbon in the dark (without any prior light incubation) by the *Dinophysis* species, there was no uptake of inorganic radioactive carbon for any of the species. The radioactivity in all the scintillation vials containing the phytoplankton cells was at the same level (about 50 DPM) as the background or the blanks (water pipetted from the last rinse of the cells; volume corresponding to the pipetting of 50 cells) (Table 2).

In Expts 1 and 2 (1992) the dark <sup>14</sup>C uptake for the *Dinophysis* species varied from low to negligible or even negative, in relation to the uptake in light (Figs. 1 & 2). However, during Expt 3 (1993), the <sup>14</sup>C uptake by the *Dinophysis* cells in the bottle exposed to the dark (Bottle B) without mixing was significantly higher than in Bottle A, incubated in the light for the same period. *Dinophysis* species in the dark bottle reached maximum uptake values which were sometimes significantly higher than the uptake values for cells exposed to light in Bottle A (Mann-

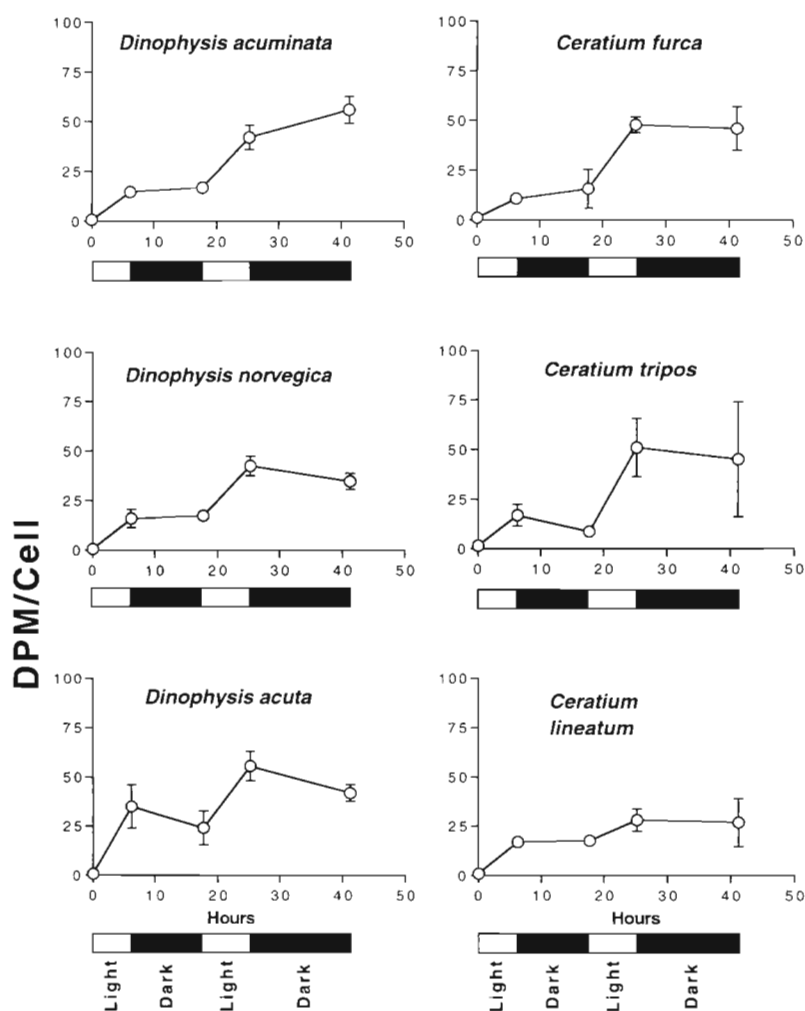


Fig. 2. Expt 2. Conditions as in Expt 1

Table 1. <sup>14</sup>C uptake (pg C cell<sup>-1</sup> h<sup>-1</sup>) by different dinoflagellates after the first hours of incubation (in light) of the natural plankton communities. Dinoflagellate cells were isolated under the microscope and placed in scintillation vials. Each value represents the mean  $\pm$  SD of 4 scintillation vials, each vial containing 15 to 50 cells. Expts 1 and 2: water from the Tjárnö Strait, October 1992. Expt 3: water from the Gullmar Fjord, September 1993. A: autotrophic species; H: heterotrophic species; M: possibly mixotrophic species

| Species                           | Expt 1            | Expt 2           | Expt 3            |
|-----------------------------------|-------------------|------------------|-------------------|
| <i>Dinophysis acuminata</i> (M)   | 40.12 $\pm$ 4.3   | 31.22 $\pm$ 4.0  | -                 |
| <i>D. acuta</i> (M)               | -                 | 61.81 $\pm$ 18.5 | 50.79 $\pm$ 5.6   |
| <i>D. norvegica</i> (M)           | 107.61 $\pm$ 11.3 | 32.23 $\pm$ 7.1  | 18.22 $\pm$ 2.7   |
| <i>Ceratium furca</i> (A)         | -                 | 21.69 $\pm$ 1.1  | 148.31 $\pm$ 28.7 |
| <i>C. lineatum</i> (A)            | 26.53 $\pm$ 5.4   | 28.66 $\pm$ 14.5 | -                 |
| <i>C. tripos</i> (A)              | -                 | 34.34 $\pm$ 10.6 | -                 |
| <i>D. rotundata</i> (H)           | 0.07 $\pm$ 0.3    | 13.70 $\pm$ 6.8  | -                 |
| <i>Protoperidinium granii</i> (H) | 7.68 $\pm$ 4.2    | -                | -                 |
| <i>P. divergens</i> (H)           | -                 | -                | 5.23 $\pm$ 0.3    |

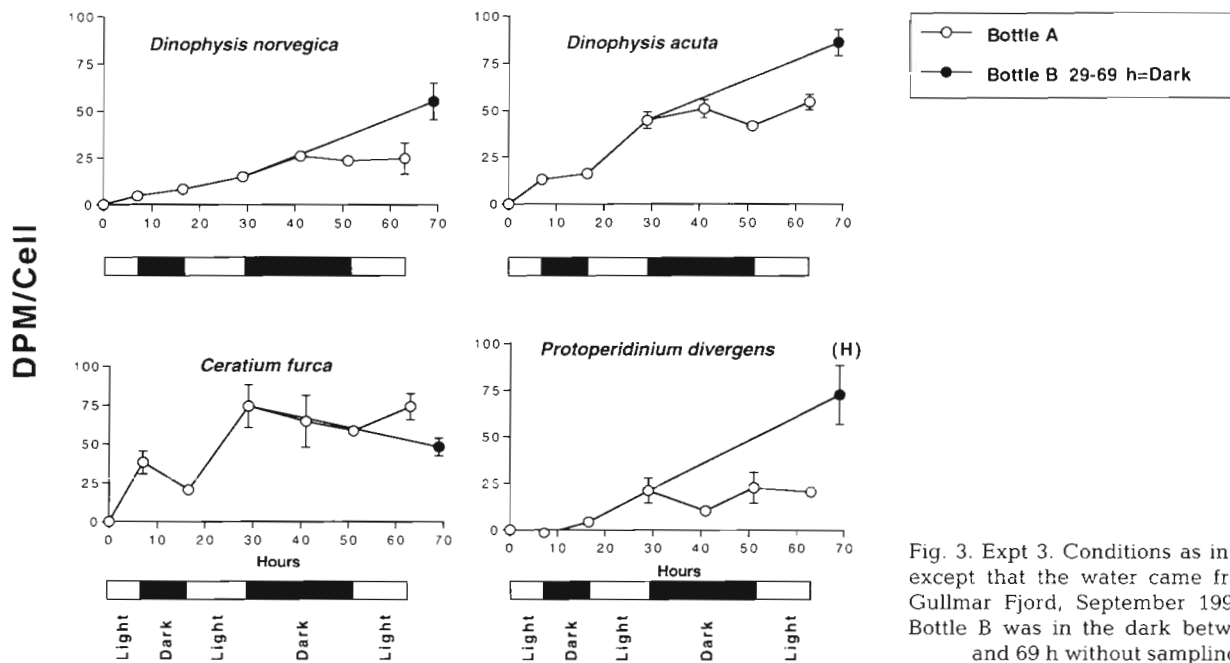


Fig. 3. Expt 3. Conditions as in Expt 1 except that the water came from the Gullmar Fjord, September 1993. The Bottle B was in the dark between 29 and 69 h without sampling

Whitney *U*-test,  $p < 0.05$ ). For example, *D. norvegica* assimilated  $1.0 \text{ DPM cell}^{-1} \text{ h}^{-1}$  during the dark incubation and  $0.1 \text{ DPM cell}^{-1} \text{ h}^{-1}$  in the light bottle. The difference was not so marked for *D. acuta*. During the first hours of incubation in the light, this species seems to have been in better condition than *D. norvegica*. Thus *D. acuta* assimilated  $1.8 \text{ DPM cell}^{-1} \text{ h}^{-1}$  in the light. The high dark uptake values found for *D. norvegica* in Bottle B might reflect the lack of mixing for that bottle. In the light bottles and in other dark bottle experiments,

cells were mixed prior to each sampling, and this is well known to be inhibitory to certain dinoflagellates (e.g. Thomas & Gibson 1990).

The uptake of  $^{14}\text{C}$  by *Dinophysis acuta* and *D. norvegica* in the dark (Bottle B) was the same as the maximum uptake for the heterotrophic dinoflagellate *Protoperdinium divergens* ( $1.3 \text{ DPM cell}^{-1} \text{ h}^{-1}$ ). *Ceratium* species had both negative and positive carbon uptake in the dark (Figs. 1 to 3).

### Growth rates

Growth rates ( $\mu$ ), estimated from carbon uptake during the first incubation hours in relation to cell carbon content, varied for the 3 experiments for the different *Dinophysis* species (Table 3). The maximum growth rates ( $\mu$ ,  $\text{d}^{-1}$ ) for *D. acuminata*, *D. norvegica* and *D. acuta* were  $0.59 \pm 0.13$ ,  $0.63 \pm 0.15$ , and  $0.41 \pm 0.04$ , respectively.

### DISCUSSION

Measurement of *in situ* growth rates for individual species among complex phytoplankton communities is not an easy task (Furnas 1990). Rivkin & Seliger (1981) first used the method of incubating the entire phytoplankton communities with radioactive  $^{14}\text{C}$  labeled carbon followed by single cell isolation of certain species in order to obtain the carbon fixation rate of these species growing in their normal environment. After this

Table 2. Radioactivity (DPM) in vials containing either dinoflagellate cells or the water surrounding the cells (blank, corresponding to the water volume when 50 cells were pipetted from the last rinse) or just the scintillation cocktail plus filtered non-radioactive water (background). The natural phytoplankton communities from the Gullmar Fjord (September 1991) were exposed to 12 h dark and radioactively labeled inorganic carbon ( $1 \mu\text{Ci ml}^{-1}$  of sample) before cell isolation

| Species                      | Cells/vial | DPM/vial<br>(n = 4 vials) |
|------------------------------|------------|---------------------------|
| Background                   |            | 52.1                      |
| Blank                        |            | 53.2                      |
| <i>Dinophysis acuminata</i>  | 63         | $52.9 \pm 7.1$            |
| <i>D. acuta</i>              | 50         | $52.4 \pm 9.5$            |
| <i>D. norvegica</i>          | 50         | $53.6 \pm 10.6$           |
| <i>Ceratium furca</i>        | 50         | $52.6 \pm 5.2$            |
| <i>C. lineatum</i>           | 50         | $52.6 \pm 14.2$           |
| <i>C. tripos</i>             | 50         | $53.4 \pm 8.9$            |
| <i>D. rotundata</i>          | 42         | $53.1 \pm 4.6$            |
| <i>Protoperdinium granii</i> | 50         | $52.5 \pm 4.8$            |
| <i>P. divergens</i>          | 50         | $53.4 \pm 5.2$            |

Table 3. Growth rates (specific  $\mu$ ) for the different dinoflagellate species, based on carbon uptake per cell for the first hours (<8 h) of incubation (light period only). Each value is a mean  $\pm$  SD for 4 replicate vials. H: species that are completely heterotrophic

| Species                           | Expt 1            | Expt 2          | Expt 3          |
|-----------------------------------|-------------------|-----------------|-----------------|
| <i>Dinophysis acuminata</i>       | 0.59 $\pm$ 0.13   | 0.49 $\pm$ 0.05 | –               |
| <i>D. acuta</i>                   | –                 | 0.41 $\pm$ 0.04 | 0.35 $\pm$ 0.06 |
| <i>D. norvegica</i>               | 0.63 $\pm$ 0.15   | 0.29 $\pm$ 0.06 | 0.18 $\pm$ 0.05 |
| <i>Ceratium furca</i>             | –                 | 0.28 $\pm$ 0.01 | 0.60 $\pm$ 0.08 |
| <i>C. lineatum</i>                | 0.48 $\pm$ 0.09   | 0.81 $\pm$ 0.22 | –               |
| <i>C. tripos</i>                  | –                 | 0.17 $\pm$ 0.02 | –               |
| <i>D. rotundata</i> (H)           | 0.001 $\pm$ 0.001 | 0.01 $\pm$ 0.01 | –               |
| <i>Protoperidinium granii</i> (H) | 0.008 $\pm$ 0.003 | –               | –               |
| <i>P. divergens</i> (H)           | –                 | –               | 0.03 $\pm$ 0.00 |

initial work, a series of experiments was performed in order to estimate not only the carbon uptake, but heterotrophic behavior for different phytoplankton species as well (Rivkin 1985, Rivkin & Voytek 1985, 1986, Rivkin & Putt 1987). Here we used the single cell isolation method to investigate carbon uptake by natural phytoplankton communities, including several enigmatic *Dinophysis* species for which nutrition is poorly understood. By measuring uptake repeatedly during both the light and the dark, it was hoped that signs of mixotrophic nutrition could be detected through positive and increasing uptake during the dark when photosynthetic uptake would not be possible. For the 3 *Dinophysis* species examined, carbon uptake in the light was roughly equivalent to that of photosynthetic dinoflagellates such as *Ceratium*. No clear trend was observed for the dark periods, however. Sometimes the *Dinophysis* cells behaved like other photosynthetic species, decreasing their carbon quota through respiration. On other occasions, however, especially in one experiment when the dark bottle was left undisturbed for 40 h, dark uptake of carbon was significant. These experiments have not resolved the enigma of *Dinophysis* nutrition, but the results do suggest that mixotrophy may be occurring for several species.

#### <sup>14</sup>C uptake

Carbon uptake for the different dinoflagellates in our experiments varied from year to year but, as expected, the maximum carbon uptake in the light was mostly dependent on cell size. For the large *Ceratium furca*, a maximum rate of 148 pg C cell<sup>-1</sup> h<sup>-1</sup> was found during Expt 3. This species did not seem to be in good condition during Expt 2 when a lower carbon uptake was recorded (22 pg C cell<sup>-1</sup> h<sup>-1</sup>). *C. lineatum* had low carbon uptake in Expts 1 and 2 (26 and 29 pg C cell<sup>-1</sup> h<sup>-1</sup>) compared to values found by Rivkin & Voytek (1985),

who reported 55 to 78 pg C cell<sup>-1</sup> h<sup>-1</sup> for this species. The smallest of the species we studied, *Dinophysis acuminata*, had an uptake of 31 to 40 pg C cell<sup>-1</sup> h<sup>-1</sup>. This is higher than rates reported by Berland et al. (1994), but similar to those of Berland et al. (1995b) (26 pg C cell<sup>-1</sup> h<sup>-1</sup>). Carbon uptake by *D. acuta* in our Expts 2 and 3 was 62 and 51 pg C cell<sup>-1</sup> h<sup>-1</sup>, respectively. Subba Rao & Pan (1993), using the same method as we did, found uptake rates for *D. norvegica* varying between 16 and 25 pg C cell<sup>-1</sup> h<sup>-1</sup>, which is in the same range as the values found by us in Expts 2 and 3 (32 and 18 pg C cell<sup>-1</sup> h<sup>-1</sup>, respectively). However, our value for the *D. norvegica* in Expt 1 was much higher (108 pg C cell<sup>-1</sup> h<sup>-1</sup>). In summary, the different dinoflagellate species exhibited different carbon uptake from experiment to experiment, presumably as a result of their different physiological status at the start of the incubations as well as the different conditions of the incubations themselves. Since these were natural communities under study, nutrient limitation or other stresses were likely, and cells would not be expected to respond equally fast to the nutrient enrichments and laboratory incubation conditions.

#### Heterotrophy

Our experiments were designed to reveal mixotrophic behavior through the uptake of labeled carbon during the dark. The pattern to be expected is exactly that exhibited by the exclusively heterotrophic *Protoperidinium* species and *Dinophysis rotundata* used in our experiments (Figs. 1 & 3). The insignificant initial carbon uptake in the light was followed by a significant and steady increase in the dark, a result of the consumption of labeled photosynthetic algae which only became a significant fraction of the labeled biomass after several hours of light exposure. The *Dinophysis* species in our experiments did not show this consistent behavior, although signs of dark uptake were observed. Care was taken throughout to have numerous replicates and several time points for each light or dark period in order to better resolve trends. Even with this care and the experience and improvement in precision gained from repeated experiments, dark uptake was significant on some, but not all, occasions. The most significant results are for *D. acuminata* in Expt 2 (Fig. 2) and *D. norvegica* and *D. acuta* in Expt 3 (Fig. 3). The most notable dark uptake was observed in a modification of our normal experimental protocol

when the dark bottle was left unsampled for an extended interval. Then, *D. norvegica* showed a high dark uptake, significantly higher than for the same species in the light (Fig. 3). This result suggests that the repeated mixing and subsampling from our experimental flasks may have inhibited the uptake process. The negative effects of turbulence on dinoflagellates is well known (e.g. Thomas & Gibson 1990).

Another explanation for the high dark uptake by *Dinophysis norvegica* observed in Expt 3 is that heterotrophy may be induced by prolonged exposure to low light, as has been reported by other authors (e.g. Jones et al. 1995) either through a stimulation of auxotrophy (Pintner & Provasoli 1968, Rivkin & Putt 1987) or phagotrophy (Jones et al. 1993). However, inhibition of the bacterivory of a freshwater Chrysophyte exposed to darkness or low light has also been found (Caron et al. 1993).

Until recently, within the genus *Dinophysis*, only the non-pigmented *D. rotundata* was known to be heterotrophic (Hansen 1991). However, Jacobson & Andersen (1994) used transmission electron microscopy to demonstrate the existence of food vacuoles in *D. norvegica* and *D. acuminata*. Although these authors could not identify the prey inside the vacuoles, the particles inside *D. acuminata* vacuoles resembled those in the totally heterotrophic *Oxyphysis oxytoxoides*, suggesting that *D. acuminata* was preying on ciliates. Berland et al. (1995a) found no food vacuoles in *D. acuminata*, but did observe a tongue-like structure in the flagellar region that had the appearance of an atrophied peduncle. These authors believe this apparatus might be involved in nutrition. Reguera et al. (1995) believe it may function in sexual reproduction. Carpenter et al. (1995) found *D. norvegica* in high concentrations during the summer of 1992 and 1993 at depths of 12 to 15 m in the Baltic Sea. Measurements of their photosynthesis showed that the cells would take 4 to 9 mo to double their cellular carbon (if based only on inorganic carbon uptake). The doubling time for this species has been found by us (in this article) and by other authors to reach 0.2 to 0.7 d<sup>-1</sup> (Berland et al. 1995b, Subba Rao & Pan 1993, Granéli et al. 1995). Thus as Carpenter et al. (1995) concluded, mixotrophy seems to be the explanation for growth of *D. norvegica* in situations when photosynthesis cannot provide sufficient carbon. In the Kattegat *D. norvegica* increased from 700–1000 cells l<sup>-1</sup> at the beginning of October to 3500–9000 cells l<sup>-1</sup> at the beginning of December 1995 (1 to 5 m depth-integrated water samples), when the noon light intensity had decreased to 5  $\mu\text{E m}^{-2} \text{s}^{-1}$  at 1 m depth (Per Olsson, TOXICON, Landskrona, Sweden, pers. comm.). Once again, this suggests that this species may survive (and even grow) through difficult periods using mixotrophy.

Among the other photosynthetic dinoflagellates investigated by us, *Ceratium furca* and *C. lineatum* were the only species that exhibited possible carbon uptake in the dark (Fig. 3). Chang & Carpenter (1994) found inclusion bodies that stained with a DNA-specific fluorochrome in several species of *Ceratium* from the Caribbean Sea. Among the individuals containing inclusion bodies were cells of *C. furca*. The authors, however, could not definitively conclude whether these inclusion bodies were from ingested phytoplankton, bacteria cells, or were due to parasites. However, in cells of *C. furca* collected from Chesapeake Bay, USA, Bockstahler & Coats (1993) were able to identify the remains of ingested oligotrich ciliates.

### Cell division rates

Growth rates were calculated for our targeted species using carbon uptake rates determined during the first few hours of light. We chose not to calculate these rates over longer intervals since we could not be sure the uptake was from the inorganic carbon pool, of which we knew the specific activity. It is possible that some of the cells divided during the first incubation period in light. In this instance, inadvertent isolation of recently divided cells would give an underestimation of the true growth rate, since those cells would be small and consequently would contain less radioactively labeled carbon than 'normal', larger cells. Carpenter et al. (1995) found that *Dinophysis norvegica* divided asynchronously, so cell division may have occurred at any time during our experiments.

In our experiments, *Ceratium lineatum*, *C. furca* and *C. tripos* had maximum growth rates of 0.81, 0.60 and 0.17 d<sup>-1</sup>, respectively. Rivkin & Voytek (1986) found maximum division rates for 5 *Ceratium* species, including *C. tripos*, isolated from the Chesapeake Bay to vary between 0.20 and 0.31 doublings d<sup>-1</sup> or 0.14 to 0.21 d<sup>-1</sup> ( $\mu$ ) which is in the same range as or lower than our values for different *Ceratium* species. These authors also found that *C. tripos* isolated from coastal waters grew twice as fast as the same species isolated from the mouth of Chesapeake Bay, i.e.  $\mu = 0.34$  versus 0.18 d<sup>-1</sup>. Thus our values for that species ( $\mu = 0.17$  d<sup>-1</sup>) are similar to those of Rivkin & Voytek (1986).

*Dinophysis acuminata* and *D. norvegica* had similar maximum growth rates of 0.59 and 0.63 d<sup>-1</sup>, respectively. For *D. acuta* the maximum growth rate was 0.41 d<sup>-1</sup>. Our estimates of *D. acuminata* growth rates are thus similar to those of Chang & Carpenter (1991) estimated for *D. acuminata* using the mitotic index approach on natural phytoplankton communities in Long Island Sound, USA. These authors found *D. acuminata* to grow at 0.53 and 0.67 d<sup>-1</sup>. Subba Rao &



Pan (1993) also measured  $^{14}\text{C}$  uptake by *D. norvegica*, but did not calculate the growth rate for this species.

### Summary

This study provides several insights into the nutrition of *Dinophysis* species, which remains largely unknown. First, our results make it clear that *D. norvegica*, *D. acuta*, and *D. acuminata* are all capable of photosynthetic carbon uptake rates and growth rates in the light that are comparable to rates for other autotrophic dinoflagellate species and that are consistent with published measurements for these species. Mixotrophy, which could only have been detected during the dark intervals using our methods, was indicated on several occasions with these species but, as is common with *Dinophysis*, the results were variable and inconsistent. Since natural communities were studied, the prior nutritional history of the cells and the extent to which those cells were capable of heterotrophic uptake at the time of the experiments could neither be known nor controlled. The strongest evidence for mixotrophic uptake comes from a population that was kept in the dark for 40 h, suggesting that mixotrophy may be induced only when photosynthesis is not able to meet the needs of the cells. This is consistent with the observations of Carpenter et al. (1995) on a subsurface population of *D. norvegica* that was persisting in the Baltic despite photosynthetic rates that were exceedingly low. All of these data suggest that the heterotrophy of *Dinophysis* species may be a means to survive unfavorable conditions, or to provide some special compound found only in organic material. This also may explain the lack of success in the many efforts to culture these organisms in the laboratory (Sampayo 1993, Maestrini et al. 1995). Uptake of particulate or dissolved organic carbon under conditions of darkness or low light may allow these species to maintain high cell numbers under adverse conditions, as exemplified by the high cell numbers found for *D. acuta* and *D. norvegica* during the 'low-light' winter in the Kattegat and Skagerrak (Pettersson 1994, Per Olsson pers. comm.).

**Acknowledgements.** Our thanks to Christer Nylander, Department of Marine Ecology, Lund, for the calculations of carbon uptake, growth rates and statistics. Thanks to Roger Finlay who corrected our English. This study was supported by funds (to E.G.) from the Swedish Board for Agricultural and Forestry Research (SJFR) and the Swedish Natural Science Research Council (NFR), and by the National Science Foundation through award OCE-9415536 (to D.M.A.). The 'Programme National Efflorescences Algales toxiques' (France) provided funds (to S.Y.M.). This is contribution no. 9459 from the Woods Hole Oceanographic Institution.

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