

Photosynthetic Capacity of the Toxic Dinoflagellates *Dinophysis* cf. *acuminata* and *Dinophysis acuta**¹

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Abstract: Natural phytoplankton assemblages from French Atlantic coastal waters were enriched in *Dinophysis* cf. *acuminata* and *D. sacculus* by size fractionation and reverse sedimentation, so that *D. cf. acuminata* became overwhelmingly dominating. *Dinophysis acuta* populations were also enriched by isolating individual cells. The rate of uptake of inorganic carbon was measured (¹⁴C) in enriched populations both of *D. cf. acuminata* and, separately, of *D. acuta*. In the light, carbon uptake increased linearly with incubation time and directly in proportion to the number of cells; the mean uptake rate per cell in *D. acuta* (21 pg C h⁻¹) was rather low. In *D. cf. acuminata*-dominated assemblages, the uptake rate was positively related to temperature from 7°C to 18°C, but negatively related between 18°C and 23°C. In the dark period, 36% to 48% of the carbon taken up was lost at 7°C-11°C, whereas the loss was only 12% at 18°C. When growth conditions were optimal, excretion of organic carbon represented only 0.5-2.3% of inorganic carbon taken up. At the end of the dark period, dissolved free amino acids (DFAA) represented 37% of total excreted carbon, which markedly increased the concentration of amino acids dissolved in the medium. The P-I parameters found, $\alpha = 0.053 \mu\text{gC } \mu\text{g Chl-}a^{-1} \text{ h}^{-1} [\mu\text{mole m}^{-2} \text{ s}^{-1}]^{-1}$, $\text{Pm}^{\text{B}} = 16 \mu\text{gC } \mu\text{g Chl-}a^{-1} \text{ h}^{-1}$ and $\text{I}_k = 300 \mu\text{moles m}^{-2} \text{ s}^{-1}$, are similar to those in other algal species; they reflect a high degree of tolerance to bright light. Carbon uptake rate data indicate the specific division rate of the *D. cf. acuminata*-dominated population to have been 0.35 division. day⁻¹.

It is concluded that the photosynthetic capacity of *D. cf. acuminata* and *D. sacculus* has been demonstrated. An overall mixotrophic mode of nutrition should nevertheless be considered a probability.

1. Introduction

The dinoflagellate genus *Dinophysis* has already been described by EHRENBERG in 1840. Its species remain poorly understood, however. Their biochemical and ecophysiological characteristics, such as their pigments, and their particulate organic carbon (POC) and particulate organic nitrogen (PON) contents, their division rates and their photosynthesis-light (P-I) relationships have not been fully determined, nor have their mode of nutrition. This lack of knowledge is mostly caused by their low relative abundance in natural assemblages and by their

complete resistance to laboratory culture.

Until the 1980s, research on the *Dinophysis* species was almost entirely limited to taxonomic studies. During the past two decades, however, the damage to human health caused by the diarrhetic toxin they produce, okadaic acid (YASUMOTO *et al.*, 1980; KAT, 1983), has progressively increased in absolute magnitude and in geographic extent (ANDERSON, 1989; SMAYDA, 1990; HALLEGRAEFF, 1993). Furthermore, cell densities of $> 10^5$ cells l⁻¹, two orders of magnitude higher than those mentioned in the old literature, have recently been reported from different regions of the world (FREUDENTHAL and JACOBS, 1991; BELIN, 1993; LASSUS *et al.*, 1993; SUBBA RAO *et al.*, 1993). Consequently, increasing research is now being focussed on their life cycles, reproductive strategies, nutrition, toxin production and taxonomy using newly developed methods.

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Table 1. Respective cell densities (number of cells per litre) of algal species in the *Dinophysis*-enriched assemblage used for experiment.

Species	Cell density
<i>Dinophysis</i> cf. <i>acuminata</i>	1,491,750
<i>D. rotunda</i>	1,350
<i>D. sacculus</i>	97,500
<i>Diplopsalis</i> sp.	1,650
<i>Dissodium</i> sp.	300
<i>Gymnodinium</i> sp. (green)	996,660
<i>Prorocentrum micans</i>	19,800
<i>Protoperidinium</i> sp.	5,400
<i>Leptocylindrus</i> sp.	1,800
<i>Nitzschia</i> sp. (small cells)	3,300

In order to compensate for failure to culture any of the *Dinophysis* species and nevertheless to test various working hypotheses, material isolated from seawater has been used with considerable effort. Thus, SUBBA RAO and PAN (1993) measured photosynthetic parameters in *D. norvegica* using natural samples largely dominated by this species, as well as using single cells isolated according to RIVKIN and SELIGER (1981). GRANELI *et al.* (in press) measured light and dark carbon uptake rates in single cells of *D. acuminata*, *D. acuta* and *D. norvegica*, and concluded that nutrition was mixotrophic. On the other hand, from electron microscope pictures, JACOBSON and ANDERSEN (1994) concluded that chloroplastic *D. acuminata* and *D. norvegica* are also phagotrophic.

In order to estimate to what extent the dominant species of the genus along the French Atlantic coast are autotrophic, we report here carbon uptake measurements made at (i) different temperatures and (ii) in a light-intensity gradient, with cells of *D. cf. acuminata* and *D. sacculus* taken from natural populations, enriched relative to co-occurring plankton either by size fractionation and reverse sedimentation, or by pipetting out single cells of *D. acuta*. Taking advantage of these experiments, (iii) excreted dissolved organic carbon (DOC) and dissolved free amino acids (DFAA) have been analysed, and (iv) the specific growth rate has been calculated.

2. Material and methods

The port of Antifer, near Le Havre, France, provides exceptionally good conditions for collecting cells of *Dinophysis* spp., since high densities (up to 160,000 cells l⁻¹ in some years) have occurred in summer nearly every year since 1987 (LASSUS *et al.*, 1993). The dominant species is similar to *D. acuminata*, although it appears to be a different underscribed species (LASSUS and BARDOUIL, 1991). In August 1992, we harvested a large number of cells by using a protocol of concentration by size fractionation and reverse sedimentation (MAESTRINI *et al.*, submitted *a*). The resulting population (2619 cells ml⁻¹) contained 60% *D. cf. acuminata* (Table 1); other important species present were *Prorocentrum micans* and a green dinoflagellate, *Gymnodinium* sp. (SOURNIA *et al.*, 1992). This population was used for (i) analysis of POC, PON (Carlo Erba CHN analyser) and chlorophyll *a* content (YENTSCH and MENZEL, 1963), and measured for carbon uptake rate (¹⁴C) over different (ii) temperatures and (iii) light intensities, and (iv) measurement of carbon uptake rate and carbon excretion rate over time at fixed temperatures and light intensities.

Temperature experiment: Triplicate aliquots (90 ml each) were incubated in the presence of HNa¹⁴CO₃ (18520 Bq ml⁻¹) for 10.5 hours in the light (400 μmoles m⁻² s⁻¹) followed by 10.5 hours in darkness, at 7°, 11°, 14°, 18° and 23°C. After incubation, samples were filtered through glass-fiber membrane filters ("Whatman" GF/C) and excess HNa¹⁴CO₃ was removed by adding 150 μl

6N HCl. The rate of carbon loss was calculated by subtracting the activity and the end of the dark phase from the activity at the end of the photophase.

Light experiment: 1-ml aliquots were incubated at 18°C, in a photosynthetron (LEWIS and SMITH, 1983), for 15 minutes, in the presence of $\text{HNa}^{14}\text{CO}_3$ (37040 Bq ml⁻¹), at light intensities from 1 to 1595 $\mu\text{moles m}^{-2}\text{s}^{-1}$. Excess $\text{HNa}^{14}\text{CO}_3$ was removed by adding 150 μl 6N HCl. β activity was counted directly in the incubation vial after addition of 10 ml scintillation cocktail ("Instagel", from Packard).

Carbon uptake and excretion rates over time: The *Dinophysis*-enriched mixture was diluted with filtered seawater: then nutrient-enriched with ANTIA and CHENG (1970) medium in order to obtain 1/40 the original nutrient content (*i.e.* $\text{N}=12.5\mu\text{M}$, and other elements at balanced concentrations), incubated in the presence of $\text{HNa}^{14}\text{CO}_3$ (18520 Bq ml⁻¹), at 18°C, for 9 hours in the light (400 $\mu\text{moles m}^{-2}\text{s}^{-1}$) followed by 9 hours in darkness. Subsamples were taken every three hours; estimation of carbon uptake rate was made as in the temperature experiment; carbon excretion rate was measured by counting the β activity in the filtrate in which excess of $\text{HNa}^{14}\text{CO}_3$ had been removed with 150 μl 6N HCl; the DFAA content was analysed in the filtrate by the method of PETTY *et al.* (1982), improved by the protocol of DELMAS *et al.* (1990).

In May 1993, from water samples collected near Douarnenez, south Brittany, single cells of *Dinophysis acuta* were individually isolated with a micropipette (RIVKIN and SELIGER, 1981) from an enriched 20–77 μm fraction, washed two or three 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 $\text{HNa}^{14}\text{CO}_3$ (37040 Bq ml⁻¹), incubation was carried out at 18°C under continuous illumination (400 $\mu\text{moles m}^{-2}\text{s}^{-1}$), for 15 hours. Incubation was stopped by adding 150 μl 6 N HCl, for a minimum of 6 hours. As a check on the validity of using time to as control, runs were made in triplicate with 50 cells of *Protoperdinium sp.*, a non-photosynthetic genus (JACOBSON and ANDERSON, 1993); counts made after different incubation periods using these samples were not

significantly different from those at time *t*.

3. Results

For all experiments, *Dinophysis*-dominated population or single *Dinophysis* cells took up inorganic carbon in the light; the measured carbon uptake was related both to elapsed time and to the number of pooled cells.

The specific carbon uptake rate strongly increased with temperature from 7° to 18°C (Fig. 1); at 7°C its value is only 5% that of 18°C which appeared to be the optimum in the range we used, the uptake rate at 23°C being only half this value. In the dark, an important fraction (36–48% at 7–11°C and 12–15% at 18–23°C) of carbon taken up was lost.

The specific inorganic carbon uptake rate first increased linearly with light intensity up to 200 $\mu\text{mole m}^{-2}\text{s}^{-1}$ (Fig. 2). In the 200–700 $\mu\text{mole m}^{-2}\text{s}^{-1}$ range, slope of the curve decreased, resulting in an absolute maximum in specific assimilation at about 700 $\mu\text{mole m}^{-2}\text{s}^{-1}$. Beyond this value, specific assimilation decreased. This graph shows that the P-I parameters were: $\alpha = 0.053 \mu\text{gC} \mu\text{g Chl-}a^{-1} \text{h}^{-1} [\mu\text{mole.m}^{-2}\text{s}^{-1}]^{-1}$, $\text{Pm}^{\text{B}} = 16 \mu\text{gC} \mu\text{g Chl-}a^{-1} \text{h}^{-1}$ and $\text{I}_k = 300 \mu\text{moles m}^{-2}\text{s}^{-1}$.

During the light-dark experiment, the uptake of inorganic carbon continued for 1.5 hour after the light was turned off (Fig. 3). Then, the total number of carbon atoms taken up declined by 30% over the following four hours. Excretion of organic carbon material occurred both during the light and the dark periods; the ratio of relative release to the uptake of inorganic carbon was low: 0.5–2.3%. During the light period, the concentration of DFAA decreased (Fig. 4), whereas during the night it increased and in four hours reached a value higher than that at the beginning of the experiment. During the dark period, both total organic carbon and DFAA-carbon concentrations increased by 3.94 $\mu\text{g C l}^{-1}$ and 1.44 $\mu\text{g C l}^{-1}$, respectively. Hence, DFAA represented 36% of the total amount of carbon excreted during the night.

The total carbon uptake rate of *D. acuta* cells, individually isolated before incubation, was directly proportional to the number of cells ($r^2=0.92$; Fig. 5). The mean uptake rate per cell was 21 pg C h^{-1} ($s=7.4$; $n=14$).

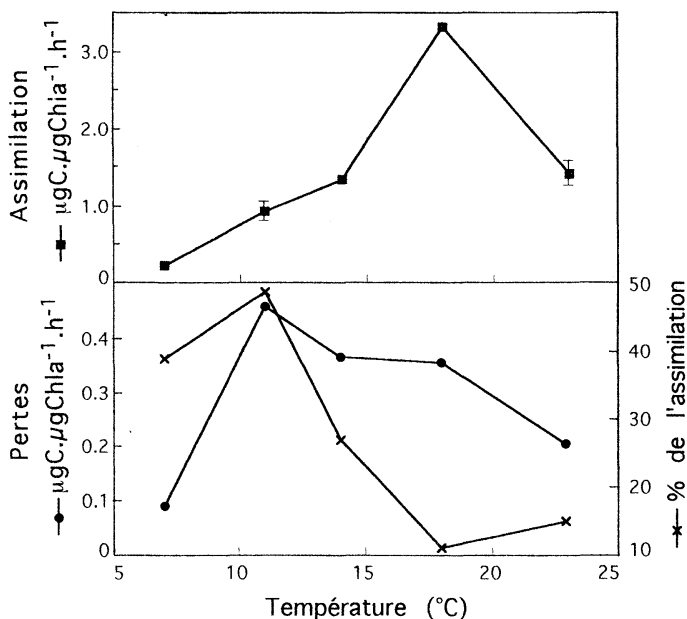


Fig. 1. Mean specific photosynthetic carbon uptake rate ($\mu\text{g C Chl-}a^{-1} \text{h}^{-1}$; bar indicates the standard deviation, whose three values are included in symbol; $n=4$), specific excretion rate ($\mu\text{g C Chl-}a^{-1} \text{h}^{-1}$) and relative excretion rate (%), in relation to temperature, in a *Dinophysis cf. acuminata*-dominated population.

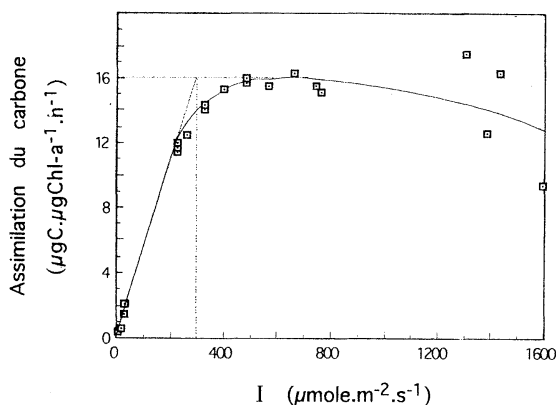


Fig. 2. specific photosynthetic carbon uptake rate ($\mu\text{g C Chl-}a^{-1} \text{h}^{-1}$), in relation to light intensity, in a *Dinophysis cf. acuminata*-dominated population.

4. Discussion

The uptake of inorganic carbon, measured using both *Dinophysis cf. acuminata*-dominated populations (Fig. 1 and 2) and isolated cells of *D. acuta* (Fig. 5), clearly indicated that both species are photosynthetic. This is consistent

with the presence of chloroplastic structures (HALLEGRAEFF and LUCAS, 1988; SCHNEPF and ELBRÄCHTER, 1988; LUCAS and VESK, 1990) and photosynthetic pigments (SUBBA RAO and PAN, 1993; MAESTRINI *et al.*, submitted *b*) reported previously. The mean carbon uptake rate per cell obtained with *D. acuta* (21 pg C h^{-1}) lies approximately in the range of values reported by SUBBA RAO and PAN (1993) in *D. norvegica* ($16\text{--}25 \text{ pg C h}^{-1}$), and by BERLAND *et al.* (submitted) in *Dinophysis cf. acuminata* (32 pg C h^{-1}). In contrast, they are significantly lower than those reported by GRANELI *et al.* (in press) in *D. acuminata* (41 pg C h^{-1}), *D. acuta* (68 pg C h^{-1}) and in *D. norvegica* (41 pg C h^{-1}). Values reported for other, but similar-sized dinoflagellate genera, are usually higher: 136 pg C h^{-1} in *Alexandrium tamarisense* (RIVKIN and SELIGER, 1981), 44 pg C h^{-1} in *Gonyaulax digitale* (SUBBA RAO and PAN, 1993), $55\text{--}390 \text{ pg C h}^{-1}$ in several *Ceratium* species (RIVKIN and VOYTEK, 1985).

In the *Dinophysis cf. acuminata*-dominated assemblage, the specific maximum carbon uptake rate, P_m^B , was $16 \mu\text{g C } \mu\text{g Chl-}a^{-1} \text{h}^{-1}$. This

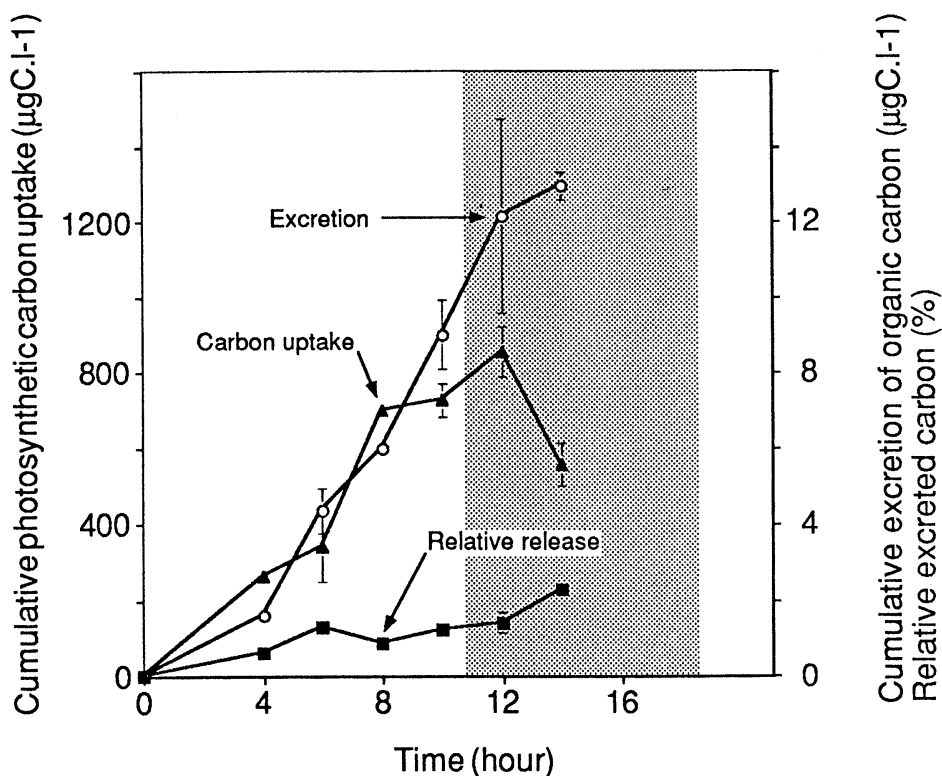


Fig. 3. Cumulative photosynthetic carbon uptake ($\mu\text{g C l}^{-1}$) and cumulative excreted carbon ($\mu\text{g C l}^{-1}$) and relative excreted release (%), over time, in light and dark conditions by a *Dinophysis cf. acuminata*-dominated assemblage.

value is significantly higher than the only other published for the genus *Dinophysis*: $0.45\text{--}1.09 \mu\text{g C } \mu\text{g Chl-}a^{-1} \text{ h}^{-1}$ in *D. norvegica*-dominated population (SUBBA RAO and PAN, 1993). It is likely that this discrepancy results from the different growth conditions in the two experiments: the temperature was 18°C in our experiment, whereas SUBBA RAO and PAN (1993) incubated their cultures at $10^\circ\text{--}12^\circ\text{C}$. In addition, dominant and companion species were different and the physiological status of the cells might also have been different. As a matter of fact, the photoadaptation indice I_k ($300 \mu\text{moles m}^{-2} \text{s}^{-1}$) suggests that the cells we used were light-adapted, while those of SUBBA RAO and PAN (1993) were shade-adapted ($I_k = 14\text{--}69 \mu\text{moles m}^{-2} \text{s}^{-1}$). The different initial slope values α : 0.053 and $0.013\text{--}0.047 \mu\text{g C } \mu\text{g Chl-}a^{-1} \text{ h}^{-1}$ [$\mu\text{mole m}^{-2} \text{s}^{-1}$], respectively, also suggests better photonic efficiency in our *Dinophysis cf. acuminata*-dominated population (PLATT *et al.*, 1980). Altogether the P-I parameter values

which have been recorded so far in *Dinophysis* species indicate a high degree of tolerance to high light levels when compared to other dinoflagellates (RICHARDSON *et al.*, 1983); although GARCIA and PURDIE (1992) reported a similar feature for *Gyrodinium cf. aureolum*.

Temperature appeared to be an important factor triggering the photosynthetic activity of *Dinophysis cf. acuminata*. At 18°C , their specific carbon uptake rate was roughly 15 fold higher than that at 7°C . The optimal temperature, 18°C , is that of natural water at which *Dinophysis cf. acuminata* is usually most abundant along the French Atlantic coast (DURAND CLEMENT *et al.*, 1988; DELMAS *et al.*, 1992; LASSUS *et al.*, 1993). Nevertheless, significant photosynthetic activity did occur at 7°C . This finding is consistent with those of other authors who reported *Dinophysis* species growing at rather lower temperature: OZAKA (1985) observed cells of *D. fortii* in the Mutsu Bay, as soon as the temperature of surface water

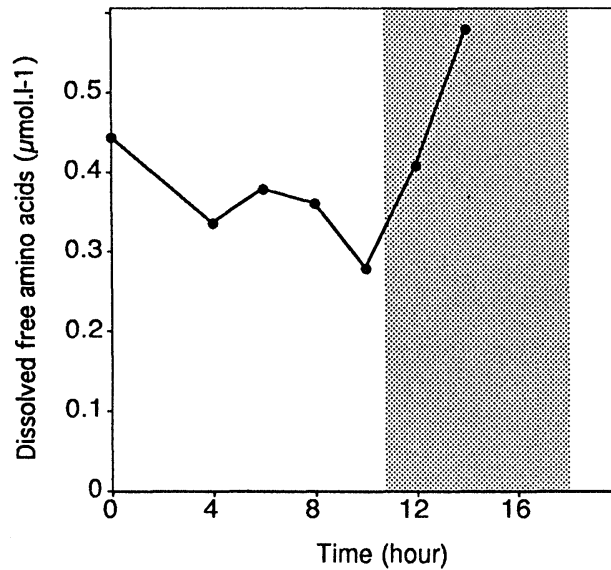


Fig. 4. Variation over time, of DFAA concentration (nM) in water containing a *Dinophysis cf. acuminata*-dominated assemblage.

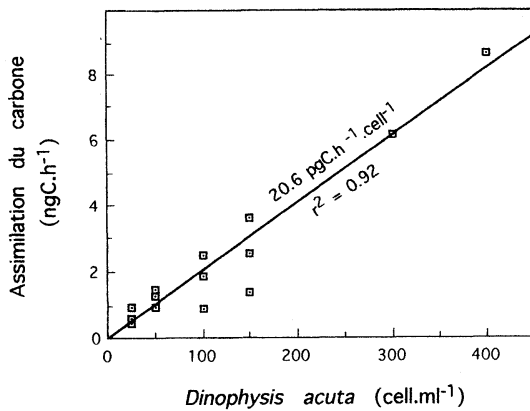


Fig. 5. Total photosynthetic carbon uptake rate in individually isolated cells of *Dinophysis acuta* in relation to the number of pooled cells.

exceeded 8°C, whereas they were undetectable in winter; REGUERA *et al.* (1993) observed proliferations of *Dinophysis cf. acuminata* over a wide range of temperature 12.5°–22°C; and GIACOBBE *et al.* (in press) found several species of *Dinophysis* in a range of temperature (10–28°C), although the maximum growth of *D. sacculus* was found at 19°C.

Losses of inorganic carbon taken up by *Dinophysis cf. acuminata*-dominated assem-

blages occurred at all temperatures (Fig. 1). However, the relative loss rate versus the carbon uptake rate was minimum (circa 10%) at the optimal photosynthesis temperature (18°C); at lower temperature, losses were significantly higher (almost 50%), while a higher temperature caused only a slight increase (circa 15%). Such photosynthetic-carbon losses are not uncommon in marine algae. According to LANGDON (1993), losses of carbon due to respiration may account for up to 40% of the light-saturated photosynthetic rate, and respiratory losses are even larger when the entire euphotic layer is considered. On the other hand, excretion of organic carbon (*i.e.* direct transfer of dissolved organic carbon from the algal cell to the water) in extreme cases may constitute 60–90% of the fixed carbon (LANCELOT, 1979; SAKSHAUG, 1993). At optimal conditions of temperature, light and nutrients, excretion accounted only for 2.5% of a total loss of 10%, thus leaving a 7.5% loss ascribed to respiration.

Such low losses might result from optimal conditions during growth (LANCELOT, 1983) and/or from heterotrophic assimilation and respiration by bacteria of the organic compounds released (LI and DICKIE, 1991). In addition, the uptake of organic compounds by *Dinophysis cf.*

acuminata itself should be considered a possibility. GRANIELI *et al.* (in press) reported that *D. acuminata* and *D. norvegica* are indeed able to assimilate radioactively labelled carbon in the dark, and suggested they may have utilize dissolved organic carbon exudated by other algae or feed on microorganisms through phagocytosis. Moreover, these species have been proved to be mixotrophic and preying upon ciliates (JACOBSON and ANDERSEN, 1994). In conclusion, the uptake of inorganic carbon which occurred during 1.5 hour in the dark phase (Fig. 3) might have resulted from dark fixation; according to LI *et al.* (1993), light-independent ^{14}C fixation in microalgae is accomplished by β -carboxylating enzymes and requires subst-rates originating from an intracellular carbohydrate pool.

The increase of extracellular DFAA during the dark phase (Fig. 4) is rather surprising. MOPPER and LINDROTH (1982), for instance, have shown that DFAA concentration in the Baltic Sea was maximum in the evening and minimum at dawn, and FLYNN and BUTLER (1986) who reviewed laboratory and field studies suggested that maximum rates of DFAA uptake would occur in dark conditions in waters depleted of dissolved inorganic nitrogen. We can at present suggest no clear explanation; we only can remark that DFAA excretion in our experimental cultures occurred under nutrient-replete conditions ($12.5\ \mu\text{mole l}^{-1}$ nitrogen and other nutrients at balanced concentrations were added prior to incubation).

From data on the total amount of inorganic carbon taken up versus POC content at the same time in the *Dinophysis cf. acuminata*-dominated population, we calculated a notional specific division rate of 0.37 division day^{-1} . This value is roughly in agreement with DELMAS *et al.* (1993) who calculated, from cell density data, a lower maximum apparent *in situ* growth rate (0.25 division day^{-1} , in a natural population where *D. sacculus*, *D. acuminata* and *D. rotundata* were the most abundant *Dinophysis* species. In contrast, the few other growth rates previously reported for the *Dinophysis* genus were rather higher: GRANIELI *et al.* (in press), from calculation also made with carbon uptake data growth rates, obtained 0.52–0.73 doublings day^{-1} in *D.*

acuminata, 0.25–0.38 doublings day^{-1} in *D. norvegica*, and 0.36–0.45 doubling day^{-1} in *D. acuta*; SAMPAYO (1993), trying to cultivate *Dinophysis* spp., obtained a growth rate of 0.6 division day^{-1} in both *D. acuminata* and *D. acuta*. From estimations by the cell cycle method, CHANG and CARPENTER (1991) reported an *in situ* growth rate (μ) of 0.67 day^{-1} (=0.97 division day^{-1}), in *D. acuminata*. The discrepancy between extreme values might have originated from the different growth conditions, but could also have been influenced by the different protocols of measurement; DELMAS *et al.*'s (1992) values of net growth rate may have been reduced by grazing, while CHANG and CARPENTER's (1991) method eliminated the effect of grazing.

5. Conclusion

The photosynthetic capacity of *D. cf. acuminata* and *D. acuta* is ascertained. Three of the species most frequently cited as being responsible for Diarrhetic Shellfish Poisoning (DSP) episodes (LEE *et al.*, 1989; YASUMOTO, 1990) are therefore photosynthetic organisms. Nevertheless, an overall mixotrophic mode of nutrition should be considered a probability.

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Résumé-Des populations phytoplanctoniques naturelles des côtes atlantiques françaises ont été enrichies en *D. cf. acuminata* and *D. sacculus* par filtration différentielle et sédimentation inverse. Le taux d'assimilation photosynthétique du carbone a été mesuré (^{14}C) sur une suspension cellulaire exempte de Diatomées et fortement dominée par *D. cf. acuminata* et sur des cellules de *D. acuta* isolées une à une et ensuite regroupées en nombre croissant. En présence de lumière, l'assimilation photoautotrophe a été directement proportionnelle au temps d'incubation ou au nombre de cellules. Le taux moyen d'assimilation par cellule de *D. acuta* est relativement faible (21 pg C h^{-1}). Le taux d'assimilation chez la population dominée par *D. cf. acuminata* s'accroît quand la température s'élève de 7°C à 18°C ; à 23°C , il est réduit de moitié par rapport à la valeur maximale. Une fraction importante du carbone assimilé est rejetée à l'obscurité (36-48% à $7-11^\circ\text{C}$ et 12% à 18°C). Dans des conditions optimales de croissance, l'excrétion de carbone organique est continue et faible (0.5-2.3% du carbone assimilé). A la fin de la phase obscure, la concentration des acides aminés libres dissous (DFAA) augmente significativement et représente 37% du carbone excrété total. Les paramètres P-I ont des valeurs semblables à celles mentionnées pour d'autres espèces phytoplanctoniques: $\alpha = 0.053 \mu\text{gC } \mu\text{g Chl-}a^{-1} \text{ h}^{-1} [\mu\text{mole m}^{-2} \text{ s}^{-1}]^{-1}$, $\text{Pm}^{\text{B}} = 16 \mu\text{gC } \mu\text{g Chl-}a^{-1} \text{ h}^{-1}$ et $\text{I}_k = 300 \mu\text{moles m}^{-2} \text{ s}^{-1}$. Le taux de croissance de la population dominée par *D. cf. acuminata* est estimé à 0.35 division. jour $^{-1}$.

Ces résultats établissent la capacité d'assimilation photosynthétique de *D. cf. acuminata* et *D. acuta*, sans exclure un comportement nutritionnel mixotrophe.

有毒渦鞭毛藻類 *Dinophysis cf. acuminata* および
Dinophysis acuta の光合成能

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要旨：フランスの大西洋沿岸域の天然植物プランクトン群から、サイズフラクションおよび逆沈降法によって *Dinophysis cf. acuminata* と *D. sacculus* を濃縮し、圧倒的に *D. cf. acuminata* が優占する群を得た。また細胞の個別分離により、*Dinophysis acuta* の個体群を濃縮した。*D. cf. acuminata* の優占する群およびこれとは別に *D. acuta* の優占する群について、無機炭素の取り込みを¹⁴C法によって測定した。明所においては、炭素の取り込みはインキュベーション時間に比例して増加し、また細胞数に直接比例して増加したが、個々の細胞当りの平均取り込み測定は *D. acuta* の群では低かった (21pg C・h⁻¹)。 *D. cf. acuminata* の優占する群では、取り込み速度は7℃から

18℃の範囲では温度に正の相関を示したが、18℃から23℃の範囲では逆に負の相関を示した。暗所では、7℃から11℃の範囲では取り込まれた炭素の36%から48%が失われたが、18℃ではわずかに12%が失われたにすぎなかった。最適生育条件下では、有機炭素の排出は取り込まれた無機炭素のわずか0.5-2.3%にすぎなかった。暗期の終わりには、溶存遊離アミノ酸 (DFAA) は全排出炭素の37%に達し、培地中に溶存するアミノ酸の濃度を著しく高めた。光合成-光曲線の諸特性、 $\alpha = 0.053 \mu\text{gC} \mu\text{gChl}^{-1} \text{h}^{-1}$ および $I_k = 300 \mu\text{moles m}^{-2} \text{s}^{-1}$ は他の種で得られている値とほぼ同様であり、強光に対する高い耐性を示した。炭素の取り込み速度から、*D. cf. acuminata* 優占個体群の分裂速度は1日あたり0.35と見積られた。

以上の結果から *D. cf. acuminata* および *D. sacculus* は光合成能を持つことが証明された。しかしながら、彼らが総合的な混合栄養を行う可能性も否定できない。