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Nutrition, growth rate and sensibility to grazing for the dinoflagellates *Dinophysis acuminata, D. acuta* and *D. norvegica*.[*1]

Edna GRANÉLI[*2], D. M. ANDERSON[*3], Per CARLSSON[*2], G. FINENKO[*4], S. Y. MAESTRINI[*5], Maria A. de M. SAMPAYO[*6] and T. J. SMAYDA[*7]

Abstract: Growth rates for three *Dinophysis* species were estimated by incubating whole plankton communities with high levels of ^{14}C bicarbonate, followed by transfer of single cells to scintillation vials. Based on ^{14}C uptake during light: dark incubations, growth rates (doublings/day) were 0.52-0.73 for *D. acuminata*, 0.25-0.38 for *D. norvegica* and 0.36-0.45 for *D. acuta*. *D. acuminata, D. norvegica*, and *D. acuta* may be mixotrophic, since they incorporated ^{14}C during dark periods. Dark uptake of carbon was not always observed, however, suggesting that this nutritional strategy may be sporadic and not continuous through time. *D. acuminata* showed better growth in the dark than in light when aspartic acid was added, providing yet another indication of a mixotrophic mode of nutrition. Grazing regulation of *D. acuminata* in concentrated whole phytoplankton communities to which copepods were added, was also investigated. *Centropages typicus* and *Isias clavipes* ingested *D. acuminata* cells in significant amounts (3 and 10% respectively, of total ingested carbon), but only after their preferred food (other phytoplankton species and nauplii) decreased in abundance in the experimental bottles. Maximum ingestion of *D. acuminata* cells occurred after 72 h (40-50% and 30% of total ingested carbon for *C. typicus and I. clavipes*., respectively). *Acartia clausii* was the only copepod which immediately started to ingest *D. acuminata* cells at levels above 30% of the total ingested carbon. However, *A. clausii* appeared unhealthy after 24 h in the experimental bottles; while *C. typicus* and *I. clavipes*., started dying only after 48 h of incubation. This suggests that copepods might be poisoned when *D. acuminata* constitutes a large percentage of their food in relation to other phytoplankton species.

1. Introduction

The dinoflagellate genus *Dinophysis* Ehrenberg includes several species which cause DSP (Diarrhetic Shellfish Poisoning). DSP is widespread in Europe, North and South America, Australia and Japan (YASUMOTO *et al.*, 1980; KROGH *et al.*, 1985; HALLEGRAEFF and LUCAS, 1988; SAMPAYO *et al.*, 1990; EDLER and HAGELTORN, 1990; SEDMAK and FANUKO, 1991; BONI *et al.*, 1993; LEMBEYE *et al.*, 1993, etc).

Within the genus *Dinophysis* there are both photosynthetic and non-photosynthetic species (LUCAS and VESK, 1990; HALLEGRAEFF and LUCAS, 1988). Because of the economic importance of *Dinophysis* much effort has been devoted to its autecology and bloom formation (ALVITO *et al.*, 1990; EDLER and HAGELTORN, 1990; LASSUS *et al.*, 1993; MARCAILLOU-LE BAUT and MASSELIN, 1990; MOITA and SAMPAYO, 1993; DELMAS *et al.*, 1992, etc.). However, our knowledge of *Dinophysis* is still scanty due to the difficulties of maintaining this genus in laboratory cultures (SAMPAYO, 1993).

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*2 Marine Ecology Dept., Univ. of Lund, Ecology Building, 223 62 Lund, Sweden
*3 Biol. Dept., Woods Hole Oceanogr. Inst., Woods Hole, MA 02543, U.S.A.
*4 Institute of Biology of South Seas, Sevastopol 333 000, Ukraine
*5 CREMA - L'Houmeau (CNRS - IFREMER), B. P. 5, 17137 L'Houmeau, France
*6 INIP. Av Brasilia 1600, Lisbon, Portugal
*7 Graduate School of Oceanography, Univ. Rhode Island, Kingston, R. I. 02881-1197, U.S.A.

2. Material and methods

This paper summarizes results of experiments using single-cell ^{14}C techniques to determine *D. acuminata, D. norvegica* and *D. acuta* growth rates as well as the potential of these species for mixotrophic growth. These data, as well as the dynamics of copepod grazing on these species, were obtained through experimental investigations of natural plankton communities from the Skagerrak and Atlantic waters outside Southern France. This paper is a summary only; details of these various studies will be published elsewhere and are referred to in the text.

3. Results and discussion

Recent research on Dinophysis spp. autoecology

Growth rates

Most attempts to estimate the growth rates of *Dinophysis* species have been done by cell counting (SAMPAYO, 1993; DELMAS *et al.*, 1992), and recently by the method of cell cycle analysis (CHANG and CARPENTER, 1991). Both methods gave daily growth rates of 0.61 and 0.58 divisions$\cdot d^{-1}$ for *D. acuminata* and *D. acuta*, respectively (SAMPAYO, 1993) and 0.78 and 0.97 divisions$\cdot d^{-1}$ for *D. acuminata* (CHANG and CARPENTER, 1991).

Based on RIVKIN and SELIGER's (1981) single-cell ^{14}C technique, with modifications of the post-incubation washing procedure, individual cells of *D. acuminata, D. norvegica* and *D. acuta* were isolated at various time periods (up to 42 h) after the addition of high levels of ^{14}C bicarbonate (1 $\mu Ci \cdot ml^{-1}$) to the whole plankton communities. During the 42 h incubation period phytoplankton were subjected to sequences of light and dark (Fig. 1). All three species incorporated ^{14}C during the light periods, with maximum uptake rates of 41 pg C$\cdot$cell$^{-1}\cdot h^{-1}$ for *D. acuminata* and *D. norvegica* and 68 pg C$\cdot$cell$^{-1}\cdot h^{-1}$ for *D. acuta*.

Based on ^{14}C uptake and using the same method as BERLAND *et al.* (1994) estimated growth rates (as doublings/day) were 0.52–0.73 for *D. acuminata*, 0.25–0.38 for *D. norvegica* and 0.36–0.45 for *D. acuta*. We are aware that algae release and / or exudate organic carbon (SAKSHAUG, 1993) might give a bias on our growth rates estimations. Hitherto, however, no measurement of carbon release has been obtained by *Dinophysis* spp. However, the growth rates for *D. acuminata* and *D. acuta* are in the same order of magnitude as obtained by some other authors based in the same method as us (BERLAND *et al.*, 1994) or by counting cells *in*

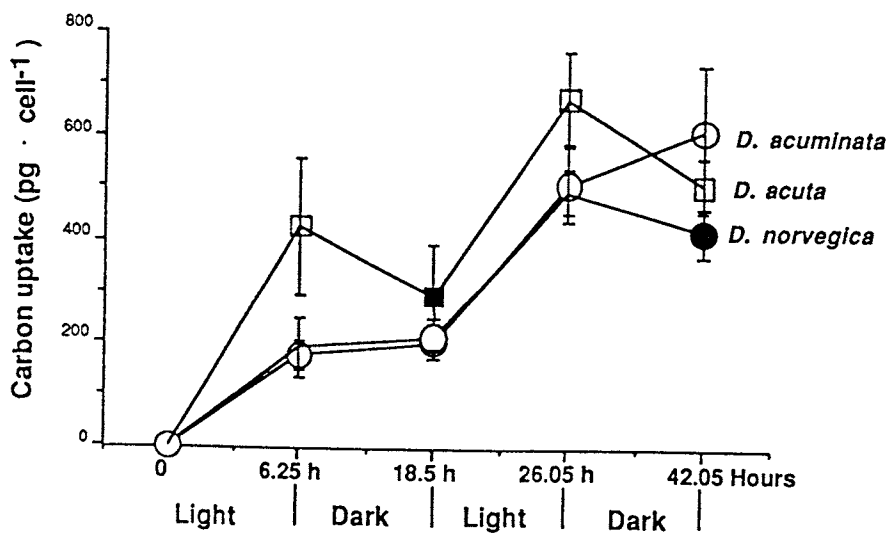

Fig. 1. Accumulated carbon incorporation for three *Dinophysis* species based on ^{14}C-uptake of individually isolated cells. To samples (4L) containing fractionated (20–70 μm size) and concentrated natural phytoplankton communities was added radioactively labelled ^{14}C bicarbonate (1 $\mu Ci \cdot ml^{-1}$). After incubation in light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) and darkness single cells of *Dinophysis* spp. were isolated and placed in scintillation vials (14–48 cells $\cdot$ vial^{-1}). Values are mean $\pm$ 1xsd, n = 3–10 (replicate vials).

situ SAMPAYO (1993) or DNA replication (CHANG and CARPENTER, 1991). There are no estimations of growth rate for *D. norvegica* in the literature.

Mixotrophy and phagotrophy

D. acuminata and *D. norvegica* assimilated radioactively labelled carbon during the dark period, unlike *D. acuta* (Fig. 1). *D. norvegica*, however, did not incorporate ^{14}C during the second dark period. This might be explained by the bad conditions of the cells at the end of the 42 h incubation as observed in the microscope during the single cells isolation. *Dinophysis* species have not been possible to keep in laboratory cultures, and even natural populations generally do not survive more than a few cell divisions *in vitro*. Dark uptake of ^{14}C found for *D. acuminata* and *D. norvegica* suggests that these species might be capable of mixotrophic nutrition. They may have utilised dissolved, radioactively labelled organic carbon released as exudates by other algae, or have fed on radioactively labelled algae/bacteria through phagocytosis.

An experiment was performed in which 10 μM aspartic acid was added to 1 L polythene bottles containing phytoplankton concentrated from Skagerrak coastal surface water on a 20 μm mesh size nylon net after passing through a 70 μm mesh size net (BERLAND *et al.*, 1994). Bottles were exposed either to 12:12 h L:D light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) or complete darkness. *D. acuminata* grew well without light, but positive growth was not observed when cells were exposed to light in the presence of aspartic acid (Fig. 2). The fact that *D. acuminata* did not grow in the presence of aspartic acid (A.A.) in the light might be explained by the fact that there were not enough inorganic nutrients available to the algae to perform photosynthesis and that heterotrophy is stimulated by dark conditions. Another possibility wouldd be that A.A. has an inhibithory effect upon the growth of this algae in light (we did not found in the literature any similar case, however). For cell division to occur there must be a sufficient supply of carbon, nitrogen, phosphorus, etc. During the dark phase, it was likely that heterotrophy provided carbon, nitrogen and

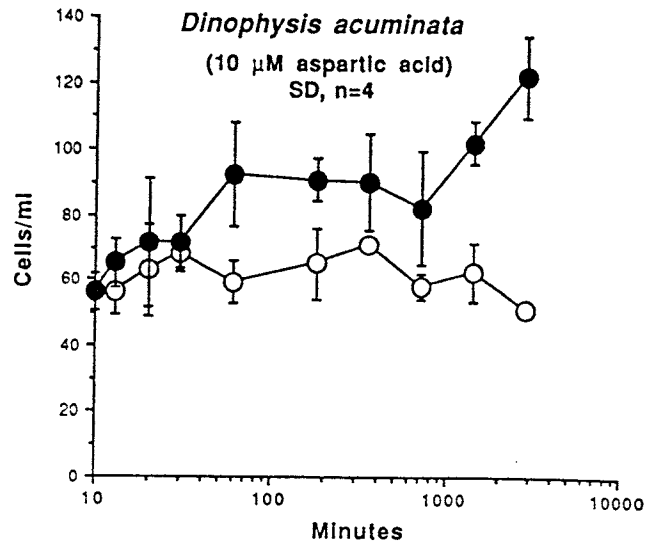

Fig. 2. Growth of *Dinophysis acuminata* in darkness or light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) in the presence of aspartic acid (10 μM).

phosphorus. When light was turned on, photosynthesis resumed and maybe inhibited the heterotrophy pathway. PINTNER and PROVASOLI (1968) have found heterotrophy in *Chrysochromulina strobilus* and *C. brevifilum*. However, these two species could use organic carbon only at low light levels (5 $\mu E \cdot m^{-2} \cdot s^{-1}$). This seems to have been the case for *D. acuminata* in our experiment. In the dark it grew effectively if aspartic acid was added to the medium, but when exposed to light it was able to maintain the population only at the initial cell concentrations (Fig. 2). As our experiments lacks proper controls, i.e. bottles where *Dinophysis* -cells were growing with inorganic nutrients additons in light and dark but no aspartic acid. Another interpretation might be that something else went wrong in the light experiment, and that growth on AA in the dark was an indirect effect, AA serving as a carbon source for bacteria, etc. that were eventually ingested by *Dinophysis*..

Phagotrophy has been demonstrated in the photosynthetic freshwater algae *Dinobryon cylindricum* and *Uroglena americana*. (BIRD and KALFF, 1986, 1987). MANTON and PARKE (1962) suggested, based on taxonomic features, that *Chrysochromulina polylepis* could ingest bacteria. This has been confirmed, not only for *Chrysochromulina* species but also for other flagellates (JONES *et al.*, 1993; NYGAARD and

HESSEN, 1990; NYGAARD and TOBIESEN, 1993). Phagotrophy in the genus *Dinophysis* has until now only been shown for the non-photosynthetic *D. rotundata* (HANSEN, 1991). According to HANSEN (1991) autotrophic *Dinophysis* species are grazed by the ciliate *Tiarina fusus*. However, *D. rotundata* could prey on *T. fusus* via a feeding tude originating from the flagellar pore. HALLEGRAEFF and LUCAS (1988), using transmission electron microscopy, examined three photosynthetic species of *Dinophysis*. Since they did not find food vacuoles, they concluded that these species are strictly autotrophic. However, recently JACOBSON and ANDERSEN (1994) using electron microscopy could identify food vacuoles in *D. acuminata* and *D. norvegica*. However, it was impossible for them to identify with certainty what kind of particles they were, although there was a strong resemblance of these particles to parts of ciliates similar to the ones found in the heterotrophic *Oxyphysis oxytoxoides*. In a sample from Parchal, Arade estuary (Portugal) preserved with Lugol *D. acuminata* was found with a circular particle resembling the centric diatom (*Thalassiosira subtilis* type) inside. The alga was photographed in three different positions to confirm that the particle was really inside the cell (Fig. 3). The mechanism by which this particle got inside *D. acuminata* is not yet known. FRITZ and NASS (1992) have found in the coastal waters of Nova Scotia, Canada the endoparasitic dinoflagellate *Amoebophrya ceratii* inside *D. norvegica*.. However this parasite bears no similarity to the particle found by us inside *D. acuminata* in the coastal waters of Portugal, but also found on several occasions during experiments performed by us during the autumn of 1994 in the coastal waters west of Sweden in the Skagerrak.

Grazing on dinophysis

Grazers have the potential capacity to control toxic phytoplankton and thus prevent the formation of a bloom. WATRAS *et al.*, (1985) studied the effect of grazing on the toxic dinoflagellate *Alexandrium tamarense* in two Cape Cod (U.S.A.) embayments. In years when *A. tamarense* did not bloom, the grazing rate exceeded the algal division rates. The main grazers of *A. tamarense* were polychaete larvae (*Polydora ligni*) and the ciliate *Favella* sp. HANSEN (1989), studying the effect of *A. tamarense* on *Favella ehrenbergii*, found that although the algae can be ingested by the ciliate, exudates from the algae act on the cell membranes of the ciliate, causing swelling and, eventually cell lysis.

We have investigated grazing by three copepods (*Centropages typicus*, *Isias clavipes*. and *Acartia clausii*) on the toxic *Dinophysis acuminata*., in comparison to other phytoplankton species and nauplii. The plankton that passed a 75 μm mesh size nylon net was concentrated on a 25 μm mesh size nylon net. *D. acuminata*, *Leptocylindrus danicus* together with nauplia made up to 70% of the plankton biomass initially. All three copepods ingested *D. acuminata* cells. *Acartia clausii* ingestion of *D. acuminata* was high, nearly 40% of the total ingested phytoplankton and nauplii cell carbon during the first 24 h of the grazing experiment (Fig. 4). However, after 24 h the *Acartia clausii* individuals were in bad condition and evidently dying. They were thus replaced by new individuals, and the experiment continued. The problem continued, however, such that this procedure had to be repeated every 24 hours. This suggests that when *D. acuminata* represent a high percentage of the ingested food *Acartia clausii* die. Individuals of *Acartia clausii* fed a diatom mixture were healthy, with

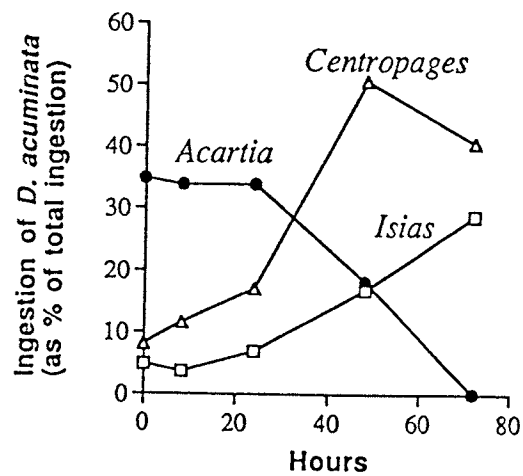

Fig. 4. *Dinophysis acuminata* ingested by the copepods *Acartia clausii*, *Isias clavipes*, and *Centropages typicus* (as carbon) as a percentage of total ingestion (phytoplankton and nauplii).

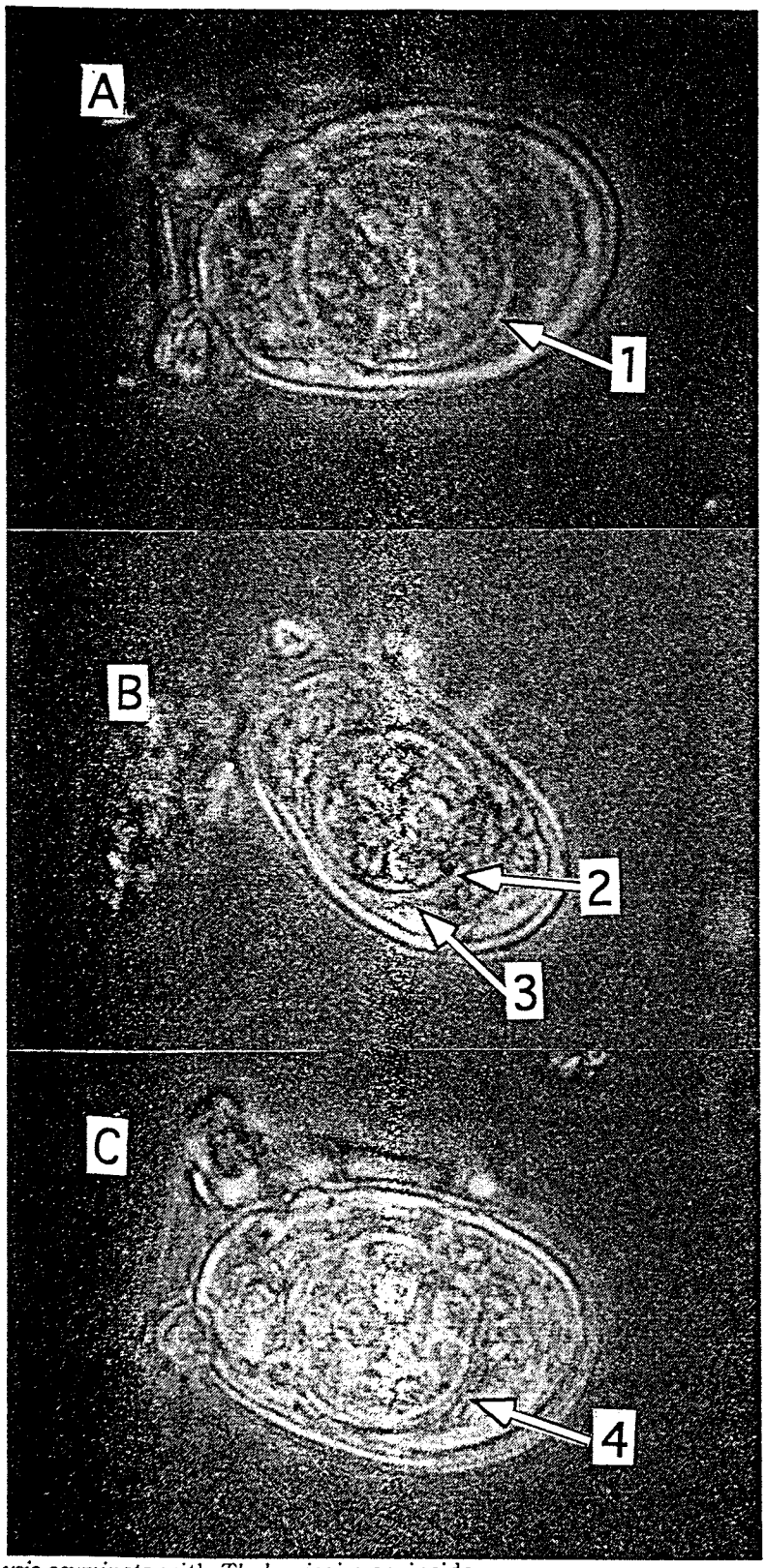

Fig. 3. *Dinophysis acuminata* with *Thalassiosira* sp. inside.
A: Right valve of *D. acuminata* and top valve view of *Thalassiosira* sp. (1). B: More or less antapical view of *D. acuminata* showing *Thalassiosira* sp. valve mantle (2) and the girdle (3). C: *D. acuminata* - right valve where the valvar mantle of *Thalassiosira* sp. is visible (4).

the females producing eggs. This indicates that the cause of death of *Acartia clausii*. was not the confinement in bottles, but rather a toxic effect from *D. acuminata*. The other two tested copepods, *C. typicus* and *Isias clavipes*, appeared unhealthy after 48 h of incubation with a concentrated phytoplankton community containing *D. acuminata*. Thus copepods might be negatively affected, probably through a toxin, when *D. acuminata* constitutes a significant proportion of their potential food resources. This suggests that growth of *Dinophysis acuminata*, and possibly other *Dinophysis* species as well, may occur with relatively low losses due to grazing.

4. Conclusions

D. acuminata, *D. norvegica* and *D. acuta* are photosynthetic dinoflagellates which seem to be able of both auxotrophy and phagotrophy. This mixotrophic mode of nutrition might enable these algae to obtain essential substances to growth not possible to obtain otherwise from most of the used media by the scientific community to growth marine phytoplankton in general.

When *D. acuminata* constitutes a significant proportion of the food ingested by copepods, the grazer is poisoned and even dead may occur. This suggests that growth of *Dinophysis acuminata*, and possibly other *Dinophysis* species as well, may occur with relatively low losses due to grazing.

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Résumé. Pour pallier l'absence de toute culture des espèces du genre *Dinophysis*, des populations naturelles ont été incubes en présence de bicarbonate marqué au ^{14}C, puis des cellules de *Dinophysis acuminata, D. acuta* et *D. norvegica* ont été isolées une à une avec une micropipette et la radioactivité de 5 à 20 cellules identiques mesurée au moyen d'un spectromètre à scintillation liquide. Le taux de croissance a été calculé en rapportant le taux d'assimilation du carbone pendant un cycle lumièreobscutité de 24 heures à la teneur moyenne en carbone de l'espéce, ellemême calculée à partir du volume

cellulaire. Les taux de croissance obtenus ont été de 0,52-0,73 division par jour (div•j-1) pour *D. acuminata*, 0,36-0,45 div•j-1 pour *D. acuta* et 0,25-0,38 div•j-1 pour *D. norvegica*. Pour *D. acuta* et *D. norvegica* l'incorporation de carbone marqué ayant été active pendant la phase obscure dans plusieurs échantillons et la croissance de *D. norvegica* ayant été plus active à l'obscurité qu'à la lumière quand de l'acide aspartique a été ajouté au milieu, il est conclu qu'un mode de nutrition mixotrophe est très probable pour ces espèces; l'hétérotrophie-phagotrophie n'étant pas permanente, cependant. La pression du broutage sur *D. acuminata* a été étudiée sur des communautés phytoplanktoniques enrichies en *Dinophysis*. *Centropages typicus* et *Isias clavipes* ont ingéré ce dinoflagellé en quantité notable: 3% et 10% du carbone total ingéré, respectivement, après toutefois que l'abondance de leur nourriture préférée (diatomées, nauplii) aît significativement diminué. Le nombre le plus important de cellules de *D. acuminata* capturées a été observée après 72 heures d'incubation, représentant alors 62% du carbone ingéré pour *C. typicus* et 24% pour *I. clavipes*. *Acartia clausii* a été le seul copépode ayant immédiatement ingéré *D. acuminata*, jusqu'à des quantités représentant plus de 30% de carbone total ingéré; 24 heures après, cependant, cette activité a cessé et les individus ont paru être malades. Pour *C. typicus* et *I. clavipes*, il y a eu simplement une baisse d'activité après 48 heures d'incubation. Ces résultats suggèrent un empoisonnement des copépodes par les toxines de *D. acuminata*, pouvant conduire à une forte baisse d'activité quand ce dinoflagellé représente une fraction significative de leur nourriture.

渦鞭毛藻 *Dinophysis acuminata*, *D. acuta*, および *D. norvegica* の栄養，増殖速度と捕食感受性

Enda GRANELI, D. M. ANDERSON, Per CARSSON, G. FINENKO, S. Y. MAESTRINI, Maria A. de M. SAMPAYO and T. J. SMAYDA

要旨：高濃度の $^{14}CO_2$ 条件下で植物プランクトンに光合成を行わせた後，渦鞭毛藻類 *Dinophysis* の3種について，細胞を単離測定することによりそれらの増殖速度を求めた。明期および暗期における細胞内への ^{14}C の取り込みから求めた，*D. acuminata*, *D. norvegica* および *D. acuta* の増殖速度（1日当たりの分裂速度）は，それぞれ 0.52–0.73，0.26–0.38 および 0.36–0.45 であった。暗期にも ^{14}C の取り込みが認められたことから，これらの藻類は混合栄養を行っていることが示唆された。しかし混合栄養は恒常的なものではなく，散発的に行っているものと考えられた。暗期に *D. acuminata* にアスパラギン酸を添加すると明期に比べ増殖速度が増加することからも，この藻類が混合栄養を行っていることが示唆された。濃縮した植物プランクトン群へかいあし類を移入した場合に，その捕食を *D. acuminata* がどのように制御するかについても検討した。*Centropages typicus* および *Isias clavipes* は，総摂取炭素量中のそれぞれ3％および10％を *D. acuminata* を捕食することで得たが，それは彼らがより好む他種の植物プランクトンや動物プランクトンの幼生が減少した後に摂取された。*D. acuminata* の摂取は実験開始から72時間後に最大となった（*C. typicus* および *I. clavipes* が *D. acuminata* の捕食により摂取した炭素量は，それぞれの総摂取炭素量の 40–50％および30％であった）。*Acartia crausii* のみは，実験開始後直ちに *D. acuminata* を捕食したが，捕食量は総摂取炭素量の30％以下であった。実験容器中の *A. clausii* は24時間後には健康状態が悪化したように見受けられ，一方 *C. typicus* および *I. clavipes* は，実験開始からわずか48時間後には死亡する個体が認められた。以上の結果は，*D. acuminata* が優占する植物プランクトン群は，かいあし類の餌としては有害である事を示唆している。