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# *Dinophysis* spp. cells concentrated from nature for experimental purposes, using size fractionation and reverse migration

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ABSTRACT: A method based on size fractionation plus reverse phototactic migration was used to collect large *Dinophysis* spp.-dominated populations. Cells were active and survived handling and transportation. Experiments involving various treatments for establishing permanent cultures are described; none have yet been successful.

KEY WORDS: *Dinophysis* · Dense-cell suspension · Attempted culture

## INTRODUCTION

The recent increase, both in space and time, of damage related to the presence of dinoflagellates *Dinophysis* (Anderson 1989, Smayda 1990, Hallegraeff 1993) has considerably stimulated research on their life cycles, reproductive strategies, nutrition, toxin production and taxonomy, using newly developed methods. Failure to culture any of the *Dinophysis* species, however, has so far prevented the usual set of ecophysiological experiments, seriously limiting current knowledge. Notwithstanding these difficulties, various working hypotheses have been tested using material isolated, with considerable effort, from seawater.

In offshore areas, the thermocline has been reported as the layer where *Dinophysis* spp. accumulate (Delmas et al. 1992, and references therein); a research vessel is needed there to allow CTD and 'Niskin'-type sampling bottles to be operated. A few locations further inshore, however, represent more convenient sampling sites. Granéli et al. (1993), for instance, sampled natural populations of *Dinophysis* spp. a few tens of meters from the pier of Kristineberg Marine Station, Sweden, and Subba Rao & Pan (1993) sampled these organisms near the Bedford Institute of Oceanography, Canada.

In France, the port of Antifer, near Le Havre, provides exceptionally good conditions for collecting *Dinophysis* spp. cells, since high densities, up to 160000 cells $l^{-1}$, have occurred in summer nearly every year since 1987 (Lassus et al. 1993). The dominant species is similar to *D. acuminata*, though it is likely to be a different, undescribed species (hereafter referred as *D.* cf. *acuminata*; Lassus & Bardouil 1991). *D. acuta* and *D. sacculus* are usually also present.

Here we report (1) useful information gained during attempts to cultivate these dinoflagellates, and (2) a method we have developed to provide a substitute material for experiments, i.e. a dense cell suspension, obtained from the natural population.

## MATERIAL AND METHODS

**Sampling.** From 1989 to 1992, from July to September, as soon as the cell density reached 5000 $l^{-1}$ (Fig. 1), samples of up to 2000 l of surface water were collected during flood tide, using a bucket, from the pier of Antifer harbor (Fig. 2). Concentration (see below) was

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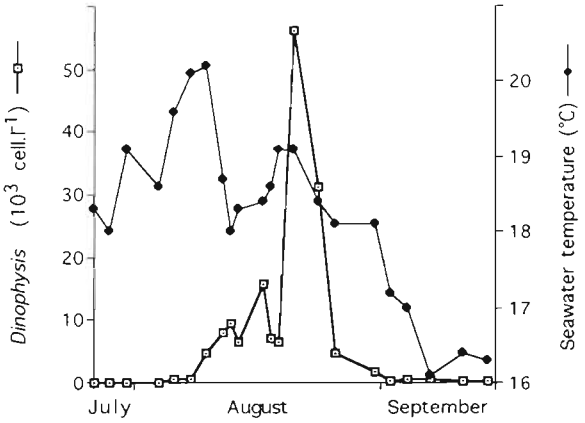


Fig. 1. Temperature and cell density of *Dinophysis* cf. *acuminata* in Antifer harbor, France, summer 1992

done immediately after sampling in uncontrolled-temperature conditions. Cultivation attempts were made in laboratory facilities, at least 1 d after sampling.

**Cultivation.** First attempts were made with 3 typical algal culture media free from silicate: medium f/2 of Guillard & Ryther (1962), Antia & Cheng's (1970) medium, Chan's (1978) medium; standard glassware and handling methods were used. Later, the following improvements were made: polycarbonate or Teflon bottles, cleaned according to the ultraclean protocol (Guillard & Keller 1984), were used in order to prevent leaching from the vessel walls of toxic substances such as heavy metals; offshore deep water, purified by passage on Florisil resin to remove potential organic inhibiting substances (Gentien & Arzul 1990), was used to make up media; ultrapure chemical compounds were employed; and sterilisation was by filtration or by autoclavation in Teflon bottles.

As supplements, various materials were tried according to results reported in the literature, including soil extract, humic acids (Carlsson & Granéli 1993) and various cocktails of either inorganic compounds such as those of Fe (Okaichi et al. 1989, Wells et al. 1991), Mn, Mo or Se (Lindström & Rodhe 1978, Keller et al. 1987, Harrison et al. 1988), or organic substances: dextrans at various concentrations (15 to 500 $\times$ $10^3$ M or 5 to 500 mg $l^{-1}$) (Tranvik et al. 1993), and organic nitrogenous substances such as urea, glutamic acid and hypoxanthin (Antia et al. 1975). Growth substances such as giberellic acid, indol acetic acid and kinetin (Bentley-Mowat & Reid 1969, Paster & Abott 1970) were tried with or without a mixture of 10 vitamins. Polyamines (cadaverin, dimethyl amine, putrescine, spermine, spermidine) at $10^{-6}$ to $10^{-9}$ M, lectins of *Phaseolus* at 1 to 100 mg $l^{-1}$, and porcine blood platelets at 10 to 500 µg $l^{-1}$ were also tested.

Several global concentration sets were used, from low (i.e. 5 µM nitrogen) to high concentration (i.e. 500 µM nitrogen).

During these trials, temperature was kept constant (18 to 20°C), and light intensity dim (200 µmol photons $m^{-2}$ $s^{-1}$) in view of reports that such conditions give better activity (Keller & Guillard 1985), while stirring was nil or reduced, since turbulence usually inhibits growth in dinoflagellates (White 1976, Thomas et al. 1991, Berdalet 1992).

The inoculum used was either a single or several *Dinophysis* cells isolated under a microscope with a micropipette, or a sample of a natural population.

On the basis of results obtained and those of Hansen's (1991), we ultimately tried to grow *Dinophysis* cf. *acuminata*, *D. acuta* and *D. sacculus* by providing varied food: bacteria, picoplankton, nanoplankton (*Cryptomonas salina*; according to Larsen 1988) and yeast. We also performed experiments with a double chamber well, with *Dinophysis* spp. cells in one chamber and 'companion' species in the other, so as to investigate any possible stimulating effect of external metabolites. *In situ* incubations in dialysis bags were also carried out in the field at the point where the cells were sampled.

## RESULTS

### Cultivation

All attempts to provide a culture failed. The best result we obtained was to keep several cells alive for 5 mo, and to obtain 16 cells from a single one in 4 mo (both in 400 µl wells, 20 µm filtered seawater, dim light, 12/12 h light-dark, 18°C). Generally cells became progressively depigmented and then died.

### Nutrition

We never observed any external phagotrophy with a peduncle or a velum, like we did several times with a *Protoperidinium* spp. which was frequently a companion species in samples. On the other hand, we observed several *Dinophysis* cf. *acuminata* cells which stopped swimming, sank to the bottom, attached the epitheca and the flagellar pore to the wall of the culture flask, and later resumed swimming.

### Cell behavior

All 3 species of *Dinophysis* appeared not to be fundamentally fragile: (1) they were collected several

times in very turbulent water and swam quite well soon afterwards in a laboratory vessel, whereas other motile species did not swim but sank; (2) they did not die when mailed in a 100 ml polyethylene bottle in a regular parcel taking 48 h, nor when transported by car for 8 h without temperature control in a 25 l polycarbonate bottle covered by a dark cloth. In contrast, they suffered badly from concentrated decayed material.

## DISCUSSION

Our results on cultivation are consistent with previous reports. Barker (1935), who first tried to cultivate *Dinophysis* species on the medium 'Erd-Schreiber' of Foyn (1934) prepared with aged seawater, maintained several cells alive for 1 mo. Later, Maranda & Shimizu (1987) tried, in addition to the medium f/50 of Guillard & Ryther (1962), several specific media prepared with


Fig. 2. Sampling of surface water from the pier of Antifer harbor


Fig. 3. Accumulation strips (white arrows) of *Dinophysis* spp. in the upper part of the polycarbonate bottle

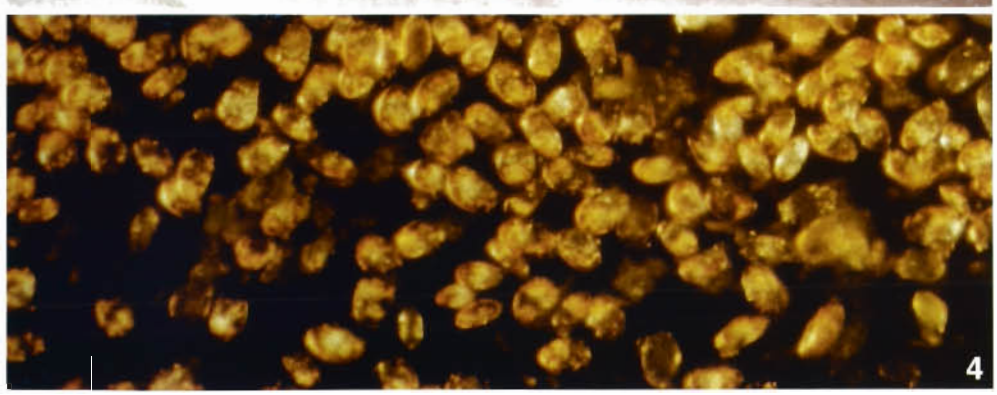


Fig. 4. Extremely concentrated population of *Dinophysis* cf. *acuminata* (not usual)

artificial or natural seawater to grow some dinoflagellates; namely, the media MLH/4 and MLH/40 that Tuttle & Loeblich (1975) devised to grow *Crypthecodinium cohnii*, a heterotrophic species, and the medium OX 7/20 of Droop (1959) supplemented with terpenoid quinones and steroids (Droop & Pennock 1971), originally used to grow the phagotrophic species *Oxhyrrhis marina*. In experiments by Durand Clément et al. (1988), *Dinophysis* spp. cells survived for 4 to 5 wk and some divided, in seawater enriched according to Provasoli et al. (1957) or simply with inorganic N and P compounds and soil extract. Sampayo (1993) reported better results by using the natural-seawater medium of Miquel (1890–93) and the artificial-seawater medium ASP-7 of Provasoli (1963); she could maintain alive several cells of *D. acuminata* and *D. acuta* for up to 5 mo, with some cellular divisions occurring.

Ishimaru et al. (1988) reported having obtained a culture of *Dinophysis fortii* and *D. acuminata* fed with the cryptomonad *Plagioselmis* sp. They obtained 22 cells from a single one in 3 wk, which is not sufficient to be regarded as a culture in the full sense. Their results are nevertheless important because they support the possibility of phagotrophy which was already suspected by many authors, including us, on the basis that *Dinophysis* spp. cells lose their pigments and divide best in the presence of small living particles. Furthermore, Granéli et al. (1995) reported they had observed *Dinophysis* cells which appeared to have captured a small *Thalassiosira* cell.

That *Dinophysis* cf. *acuminata* could be mixotrophic would not be surprising, since other dinoflagellates of different genera frequently are (Schnepf & Elbrächter 1992, Bockstahler & Coats 1993) and some *Dinophysis* species are heterotrophic (Hallegraeff & Lucas 1988) and even predatory (Hansen 1991). On this basis, further research should focus on both the photosynthetic and the heterotrophic capabilities of *Dinophysis* spp., and protistological approaches should be used as well,

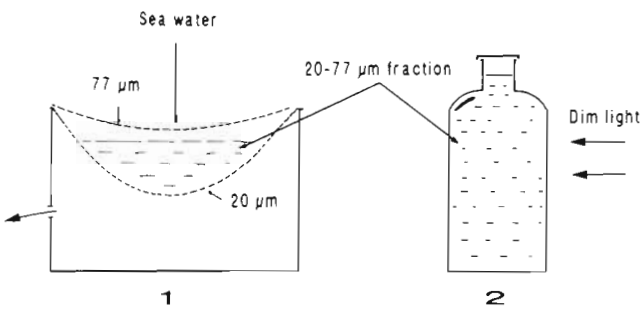


Fig. 5. Diagram of protocol used to concentrate *Dinophysis* spp. cells

Table 1. Typical cell concentrations ($10^3$ cells $l^{-1}$) and relative cell density (%, number within parentheses) of algal components in the natural population, the 20–77 µm fractionated fraction and the surface layer of the nonturbulent bottle. Experiment of 31 August 1992; sample taken at Antifer

| Alga | Natural seawater | 20–77 µm fraction | Surface layer |
|---|---|---|---|
| *Dinophysis* spp. | 2.5 (11) | 12.9 (14) | 317.4 (69) |
| Other dinoflagellates | 8.8 (39) | 17.5 (19) | 137.0 (29) |
| Diatoms | 11.3 (50) | 61.8 (67) | 7.7 (2) |

since results of Jacobson & Andersen (1994) suggest the prey could be in the >77 µm size fraction.

By taking advantage of their resistance and their motility, it is possible to concentrate *Dinophysis* spp. populations, with markedly reduced diatom components. The following protocol (Figs. 3, 4 & 5), based on that used by Vernet et al. (1989) for *Prorocentrum micans*, has proved efficient: (1) Seawater, preferably harvested with a bucket, or a peristaltic pump when necessary, is successively filtered through 77 µm and 20 µm meshes (Fig. 5), so as to concentrate the cells by a factor of 5 to 10. (2) The concentrated 20–77 µm fraction is gently siphoned into 10 l polycarbonate bottles and left to stand for at least 6 h; most of the dead cells and diatoms sink and collect at the bottom, while *Dinophysis* spp. and some other motile cells swim near the surface. (3) The surface layer is gently siphoned off; Table 1 shows a typical result. (4) In a few favorable cases, *Dinophysis* spp. concentrated in 5 to 6 mm strips on the side away from the light when the bottle was laterally illuminated at low intensity with 1 or 2 fluorescent tubes (Fig. 3); siphoning with a curved glass tube provided very dense *Dinophysis*, yet never totally free from co-occurring companion species (Fig. 4).

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