Decimeter scale heterogeneity in the plankton during a pycnocline bloom of *Gyrodinium aureolum*

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ABSTRACT: Vertical heterogeneity of pelagic organisms in a subsurface bloom of *Gyrodinium aureolum* (Hulburt) was studied on the decimeter scale by a high resolution sampler in the Kattegat, Denmark, during August 1990. Maximum concentrations of 3.25×10^6 *G. aureolum* l^{-1} and 72 µg chlorophyll a l^{-1} were recorded in the pycnocline at a depth of 14 m with 5 % of surface irradiance. Dinoflagellates were confined to a 1 m thick layer which contained more chlorophyll than the rest of the water column. In situ primary production rates suggested a phytoplankton turnover time of 1 d. Bacterioplankton production and turnover rate and microzooplankton abundance were significantly reduced in the dinoflagellate layer

INTRODUCTION

The Kattegat and the Skagerrak, situated between Denmark, Sweden and Norway, form the mouth of the Baltic Sea, which may be regarded as the world's largest estuary. During summer these waters are stratified into a surface layer of outflowing Baltic water with a salinity of 10 to 20 ppt and a bottom layer of counterflowing North Sea water with a salinity of 30 to 33 ppt. The stratification is further stabilized by solar heating of the surface layer.

The establishment of stratification and the increasing insolation in spring trigger a diatom spring bloom which rapidly depletes the surface water of nutrients, and the diatoms eventually precipitate to the bottom. Nutrient concentrations in the surface water remain low throughout summer and primary production becomes limited by nutrients supplied by remineralisation in the surface layer and via transport through the pycnocline. Nutrient inputs from riverine and diffusive runoff may support new production in the surface water. However, on a regional scale most of these inputs are assimilated and precipitated in the Baltic Proper (Larsson et al. 1985, Rydberg et al. 1990). Phytoplankton biomasses of less than a few µg of chlorophyll a l^{-1} are usually recorded during the summer in surface waters of the Kattegat and the Skagerrak (Lindahl & Hernroth 1983, Richardson & Christoffersen unpubl.).

Recently, attention has been drawn towards the occurrence of high densities of phytoplankton confined to a narrow subsurface stratum of the water column around the pycnocline. During May–June 1988, the toxic flagellate *Chrysochromulina polylepis* was found in a 1 to 2 m wide layer in the pycnocline throughout the Kattegat (Barth & Nielsen 1989, Nielsen et al. 1990). Cell densities reached 70×10^6 cells l^{-1}. A recurrent phenomenon in the Kattegat during early autumn is the occurrence of dinoflagellate subsurface blooms, including *Ceratium* spp. and the toxic *Gyrodinium aureolum* (Lindahl & Hernroth 1983, Nielsen 1991). These active phytoplankton layers seem to appear where the oppositely directed gradients of light penetrating from above and nutrients permeating the pycnocline from below meet to sustain primary production. The phytoplankton assemblage in the pycnocline thus acts as a 'filter' to the upwards flux of nutrients, and thereby impedes the build up of phytoplankton biomass in the surface layer that would reduce light penetration to the pycnocline.

Narrow subsurface layers of phytoplankton are easily recognized by in situ fluorometry, but they can be quite inaccessible to accurate sampling by conventional water samplers. Furthermore, narrow layers of phytoplankton are likely to be maintained by steep physico-chemical gradients, that may give rise to a biological stratification of the phytoplankton, and –

possibly in response to the phytoplankton – of the heterotrophic organisms as well. In order to improve quantitative sampling of subsurface phytoplankton layers and to examine a possible stratification within such layers, we constructed a high resolution gradient multi-sampler. We here report on the use of this sampler during a subsurface dinoflagellate bloom.

MATERIALS AND METHODS

The gradient multi-sampler takes 20 duplicate water samples simultaneously at 15 cm intervals (Fig. 1). Twenty plastic syringes of 150 ml (Monoject, Sherwood Medical, N. Ireland) are mounted on each side of a 300×75 cm aluminium frame. The pistons in each pair of syringes are hooked onto the frame by a modified plug, which is spring-loaded by rubber tubing. The loaded sampler is fixed on a wire and lowered to the desired depth. The assymmetrical position of the wire ensures that the syringe openings will face any existing current. Dropping a messenger weight releases all hooks simultaneously via a slide rod inside the frame, and the pistons are pulled by the rubber tubings until they reach a stop bar. Video recordings have shown that the syringes fill within 0.8 s.

The sampler was deployed on 15 August 1990 at a permanent station in the Southern Kattegat (56°15′42″N, 12°00′12″E). Continuous vertical profiles of fluorescence were recorded on 5 occasions at the same station by a Q Instruments in situ fluorometer connected to an X-Y plotter. Temperature and salinity were recorded at 2 m depth intervals using a Salinity-Temperature Bridge (Type MC5, Electronic Swithgear Ltd, London). Subsamples were taken from the gradient sampler for microscopy and measurements of chlorophyll a, primary production and thymidine incorporation.

Chlorophyll a was determined from 100 ml samples (1 per depth) filtered onto GF/C filters, extracted in ethanol and measured fluorometrically (Jespersen & Christoffersen 1987). The 5 densest samples were also measured spectrophotometrically according to Lorenzen (1967). Primary production was measured in 8 ml subsamples (2 per depth) contained in 10 ml plastic syringes. Two μCi of $NaH^{14}CO_3$ were injected into each sample. The syringes were fixed onto a string and incubated in situ at the original depth for 2 h around noon. Incubations were stopped by 200 μl of 1 N HCl. Samples were bubbled for 30 min and radioassayed. Parallel dark incubations were subtracted as blanks. Dissolved inorganic carbon (DIC) was estimated from an empirical relationship to salinity (authors' unpubl. results): DIC (mM) = 1.3 + 0.02 Salinity (ppt). Thymidine incorporation rate was measured in duplicate
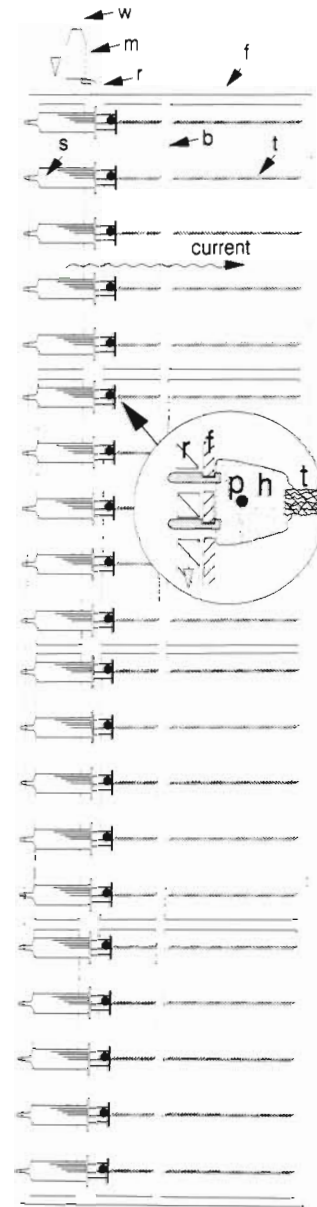

Fig. 1. Side view of the gradient sampler. f: aluminium frame; s: 150 ml syringe; t: rubber tubing; b: stop bar; r: release slide rod; m: messenger weight; w: wire. Inserted detail of release mechanism: h: hook plug; p: piston pull

10 ml samples at each depth according to Fuhrman & Azam (1980), using 5 nM [^{3}H]-Thymidine, formalin fixation, cold TCA extraction and pre-killed blanks. Autotrophic flagellates and cyanobacteria were enumerated by epifluorescence microscopy of 5 ml samples stained by proflavine hemisulphate, fixed by 0.5 % glutar aldehyde and filtered onto 0.2 μm filters (Haas 1982). Bacterioplankton were counted according to Hobbie et al. (1977), and microzooplankton were enumerated by inverted microscopy of settled 50 ml samples.

RESULTS

A pronounced fluorescence peak close to the pycnocline was observed at the permanent station during the entire month of August 1990 (Fig. 2). On 15 August the pycnocline was situated at 14 to 15 m depth and a strong peak in chlorophyll a was found at 14 to 14.5 m depth (Fig. 3), where irradiance was about 5% of surface irradiance (data not shown). One pair of sampling syringes contained 72 µg chlorophyll a l^{-1} as determined spectrophotometrically and the concentration decreased by a factor of 3 to 5 within 30 cm. Chlorophyll a concentration in the mixed surface and bottom layers was 3.5 and 0.7 µg l^{-1}, respectively. Hence, more than half of the chlorophyll a in the water column was confined within the pycnocline.

Microscopy revealed that phytoplankton in the pycnocline was completely dominated by the dinoflagellate *Gyrodinium aureolum* (Hulburt). Maximum
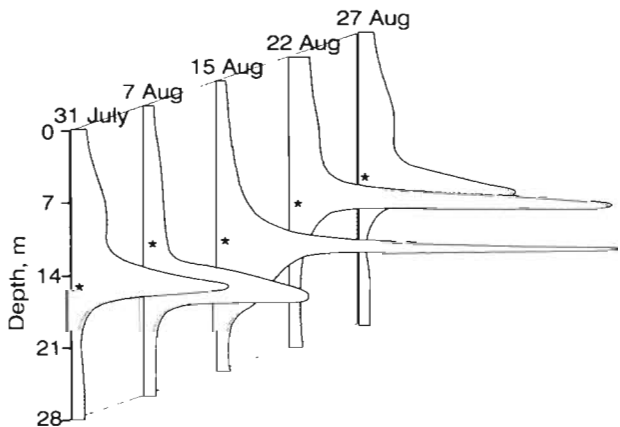

Fig. 2. Depth profiles of in situ fluorescence (arbitrary units) on 5 dates in 1990 in Southern Kattegat. Asterisks indicate position of the pycnocline
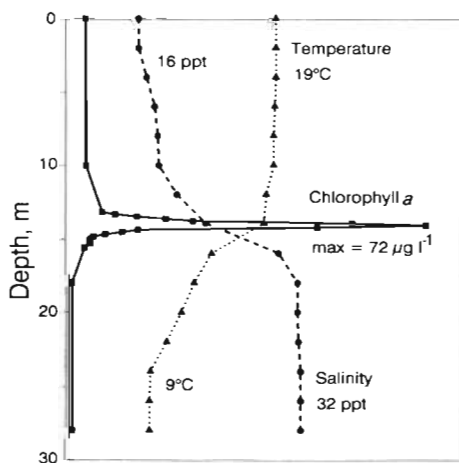

Fig. 3. Depth profiles of salinity, temperature and chlorophyll a on 15 August 1990 in Southern Kattegat

cell density was 3.25×10^6 cells l^{-1} compared to 0.03×10^6 cells l^{-1} in the surface water (Fig. 4a). Cyanobacteria were also abundant in the pycnocline with a maximum density of 175×10^6 cells l^{-1} compared to 90×10^6 cells l^{-1} in the surface water. However, their biomass contributed only by a few percent of the total phytoplankton in the pycnocline.

Primary production showed a peak of 192 µg C l^{-1} h^{-1} with a slight upwards displacement from the chlorophyll a peak (Fig. 4b). Specific primary productivity ('assimilation number' calculated as the ratio between in situ primary production and chlorophyll a concentration) also showed a distinct maximum of about 4 µg C h^{-1} per µg chlorophyll a at the upper shoulder of the production peak (Fig. 4b). Assuming a phytoplankton carbon to chlorophyll a ratio of 30 (Parsons et al. 1984), the turnover rate of the phytoplankton assemblage in the pycnocline was 0.09 h^{-1} at the given light conditions. Bacterioplankton cell number almost doubled in the *Gyrodinium aureolum* layer, whereas bacterial production measured as thymidine incorporation rate showed a decrease to about half of the surrounding values (Fig. 4c). Microzooplankton showed a distinct negative response to the *G. aureolum* layer (Fig. 4d). Naked oligotrichous ciliates and tintinnids were absent in the center of the layer, and density of the heterotrophic dinoflagellate *Protoperidinium* sp. was significantly reduced. Correlation analysis between phytoplankton biomass or production versus bacterial number and activity and microzooplankton abundance (Table 1) confirmed the trends in Fig. 4.

DISCUSSION

The vertical structure of the plankton observed on 15 August 1990 (Figs. 3 & 4) seemed to be a pattern of considerable temporal and geographical extension. At the permanent station, the fluorescence peak in the pycnocline persisted throughout August (Fig. 2), although significant quantitative variations (up to a factor of 2 in maximum fluorescence) were observed between successive casts (data not shown). A monitoring cruise carried out by the Danish Ministry of Environment in late July recorded a distinct subsurface bloom of *Gyrodinium aureolum* at all 4 stations visited in the Kattegat (Kurt Buck et al. unpubl., Gunni Ærtebjerg pers. comm.). We suppose that *G. aureolum* formed a more or less coherent 'magic carpet' in the pycnocline of the Kattegat. This phenomenon is fundamentally different from the small-scale 3-dimensional patchiness described by e.g. Derenbach et al. (1979) and Mitchell & Fuhrman (1989).

Several mechanisms may contribute to form and maintain a layer of high phytoplankton biomass in the
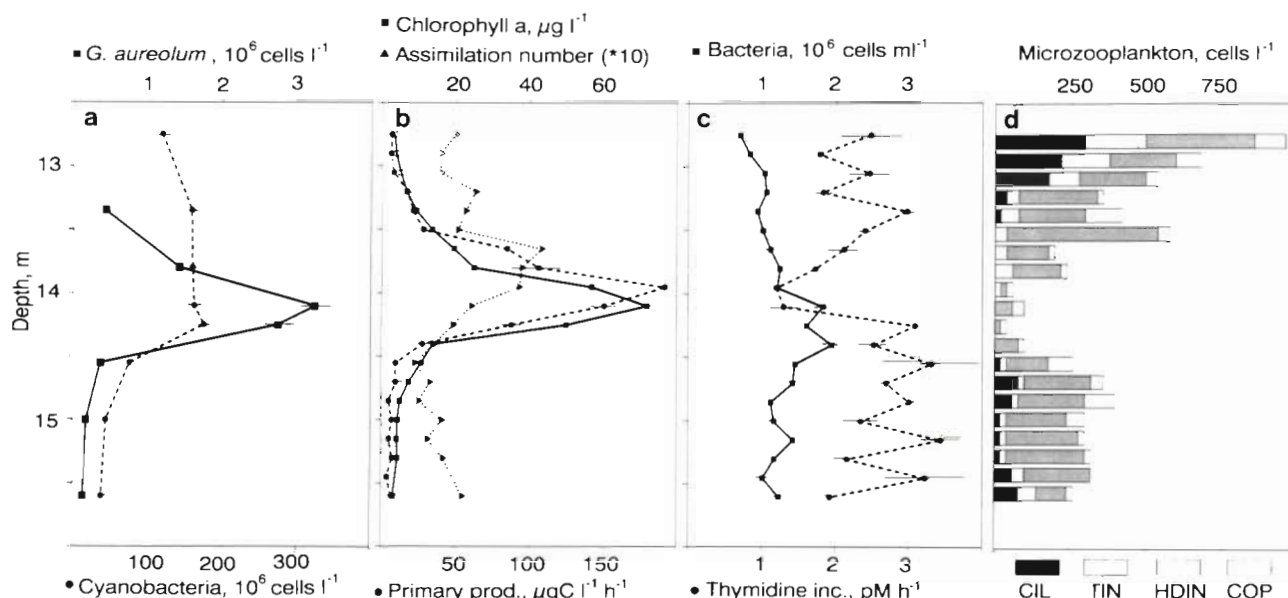
Fig. 4. Biological stratification within the pycnocline. (a) Densities of *Gyrodinium aureolum* and cyanobacteria. (b) Chlorophyll a, primary production and specific primary productivity ('assimilation number' defined as the ratio between primary production and chlorophyll a, μg C h^{-1} per μg chl a). (c) Bacterioplankton cell density and thymidine incorporation rate. (d) Microzooplankton density shown as stacked bars of: 'CIL = oligotrichous ciliates (size ca 40 μm), 'TIN' = tintinnids (ca 40 μm), 'HDIN' = *Protoperidinium* sp. (ca 100 μm) and 'COP' = juvenile copepods. Horizontal bars represent standard errors (a) or ranges of duplicates (b, c). Open symbols indicate interpolated values

Table 1. Correlation coefficients (r) between chlorophyll a (CHL) or primary production (PP) versus bacterial number (BACT), thymidine incorporation rate (TTI), bacterial turnover rate (BTO), and abundances of oligotrichous ciliates (CIL), tintinnids (TIN), *Protoperidinium* sp. (HDIN) and juvenile copepods (COP). All values were transformed to logarithms (microzooplankton abundance as log $(x+1)$) to obtain normal distributed data. Asterisks indicate significance (* p < 5%, ** p < 1%, *** p < 0.1%)

	CHL	PP
BACT	+0.545*	+0.407
TTI	−0.456*	−0.586**
BTO	−0.689***	−0.699***
CIL	−0.856***	−0.859***
TIN	−0.616**	−0.458*
HDIN	−0.729***	−0.661**
COP	−0.030	−0.033

pycnocline: (1) high irradiance and reduced wind-induced mixing; (2) passive accumulation of cells by sedimentation combined with a deceleration of sinking rate in the pycnocline (Bienfang et al. 1983); (3) phototaxis combined with active responses to gradients of salinity and nutrients (Rasmussen & Richardson 1989); (4) enhanced growth due to the combined effects of light and nutrients, cf. above; and (5) reduced mortality from zooplankton grazing due to avoidance of the phytoplankton layer (Fiedler 1982).

It seems unlikely that sedimentation would be a major factor in the establishment of the observed pronounced and narrow dinoflagellate peak. Phototaxis has been demonstrated for *Gyrodinium arueolum* and other dinoflagellates (e.g. Ekelund & Häder 1988), and laboratory studies have shown that the phototactic response of *Gonyaulax tamarensis* is suppressed by a salinity gradient of > 7 ‰ and by the absence of nutrients (Rasmussen & Richardson 1989). The steepest salinity gradient in the present study was ca 5 ppt over a few decimeters (Fig. 3). The 2-fold increase in assimilation number (Fig. 4b) suggests that phytoplankton growth was actually enhanced in the pycnocline. The phytoplankton layer would absorb about half of the irradiation (assuming a specific attenuation coefficient of 0.018 m^{-1} per mg chlorophyll m^{-3}; Taylor et al. 1986), so the lower shoulder of the assimilation number peak can be explained by light limitation, while the upper shoulder may perhaps be due to nutrient limitation. The maximum of about 4 μg C h^{-1} per μg chlorophyll a suggests that the dinoflagellates were well adapted to low light levels. The increased abundance of cyanobacteria in the pycnocline (Fig. 4a), though much less pronounced than for dinoflagellates, also indicated enhanced growth and/or reduced mortality

The apparent microzooplankton avoidance of the phytoplankton layer (Fig 4d) and the reduced thymidine incorporation despite higher bacterial cell numbers in the layer (Fig. 4c) indicate that high densities of

Gyrodinium aureolum may have toxic or inhibitory effects on these heterotrophic organisms. We have previously reported on similar effects on the planktonic community by a monospecific subsurface bloom of *Chrysochromulina polylepis* (Nielsen et al. 1990). Blooms of *G. aureolum* have often been associated with fish and invertebrate mortality (Tangen 1977), and laboratory studies have demonstrated toxic effects on a variety of marine organisms, including fish (Roberts et al. 1983), shellfish (Erard-le Denn et al. 1990), copepods (Gill & Harris 1987) and phytoplankton (Gentien & Arzul 1990). Inhibition of potential predators could be an important factor in the maintenance of a high phytoplankton biomass in the pycnocline. Phytoplankton may be transported from this 'refuge' to frontal areas and appear as a surface bloom (Richardson & Kullenberg 1987). Narrow subsurface phytoplankton layers may be missed or inadequately sampled by routine monitoring programmes, and the biological stratification within the layers is disrupted by conventional bottle sampling.

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