

# SEASONAL DYNAMICS OF BACTERIA, AUTOTROPHIC PICOPLANKTON AND SMALL NANOPLANKTON IN THE INNER OSLOFJORD AND THE SKAGERRAK IN 1993

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## SARSIA



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Seasonal dynamics of bacteria, autotrophic picoplankton and small nanoplankton ( $< 5 \mu\text{m}$ ) were studied by direct counts using an epifluorescence microscope. Samples were collected from the euphotic zone in the inner Oslofjord from April to September and in the Skagerrak from May to August during 1993. The first exponential growth period of picocyanobacteria was observed during May and June in the Skagerrak and 2 to 3 weeks later in Oslofjorden. Eukaryotic picoplankton isolated by serial dilution cultures were dominated by the prasinophyte *Micromonas pusilla* (BUTCHER) MANTON & PARKE with cell numbers  $> 10^4$  cells  $\text{ml}^{-1}$  from April to May. Biomass of phytoplankton ( $< 5 \mu\text{m}$ ) and heterotrophic bacteria were 25-56  $\mu\text{g C litre}^{-1}$  in Oslofjorden, with increasing biomass of small nanoplankton towards the inner fjord. In the Skagerrak biomasses were 17-52  $\mu\text{g C litre}^{-1}$ , with increasing biomass of picocyanobacteria from May to August. The importance of competition for nutrients between autotrophic picoplankton and heterotrophic bacteria is discussed.

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## INTRODUCTION

Following the report by WATERBURY & al. (1979) demonstrating the presence of considerable amounts of picoplanktonic cyanobacteria, there has been an increased focus on the dynamics of picoplankton in open waters. The photosynthetic picoplankton, normally defined as the size category 0.2-2  $\mu\text{m}$  (sensu SIEBURTH & al. 1978) include high abundances of both prokaryotic and eukaryotic species (JOHNSON & SIEBURTH 1982; JOINT & PIPE 1984; SIEBURTH & al. 1988); cyanobacteria, prochlorophytes and prasinophytes being the most common groups reported from recent investigations (LI & al. 1992; VELDHUIS & al. 1993).

The prasinophyte *Micromonas pusilla* (BUTCHER) MANTON & PARKE appears to be the most commonly found eukaryotic species, whereas *Synechococcus* NÄGELI is the dominating genus among the prokaryotes. The former species grows easily in culture and quantitative information on *Micromonas pusilla* is dating back to KNIGHT-JONES (1951) who applied the serial dilution culture method for detection of nano- and picoplankton

algae. Later studies carried out with similar techniques have shown the species to be common in almost all marine waters (see e.g. THRONDSSEN 1976; THRONDSSEN & KRISTIANSEN 1991), and a number of investigations by direct EM methods has provided further evidence for its ubiquitous presence in the marine environment.

The morphological characteristics of the *Synechococcus* species are less conclusive, and the detection and identification usually depend on pigment characteristics combined with size (and shape) of the cells. In addition the picocyanobacteria from oligotrophic waters appears too sensitive for the standard growth media. Many species from inshore waters, however, grow well in serial dilution cultures. The complex taxonomy of this genus is illustrated by the clonal differences shown in cultured material (see e.g. WATERBURY & al. 1986).

Primary production in the picoplankton size-fraction has been shown to be 50-80 % for oligotrophic waters and 2-25 % in neritic waters (STOCKNER & ANTIA 1986). Regular seasonality of phycoerythrin-rich picocyanobacteria of *Synechococcus* -type has been described from the North-west Atlantic (WATERBURY & al. 1986), and they have also

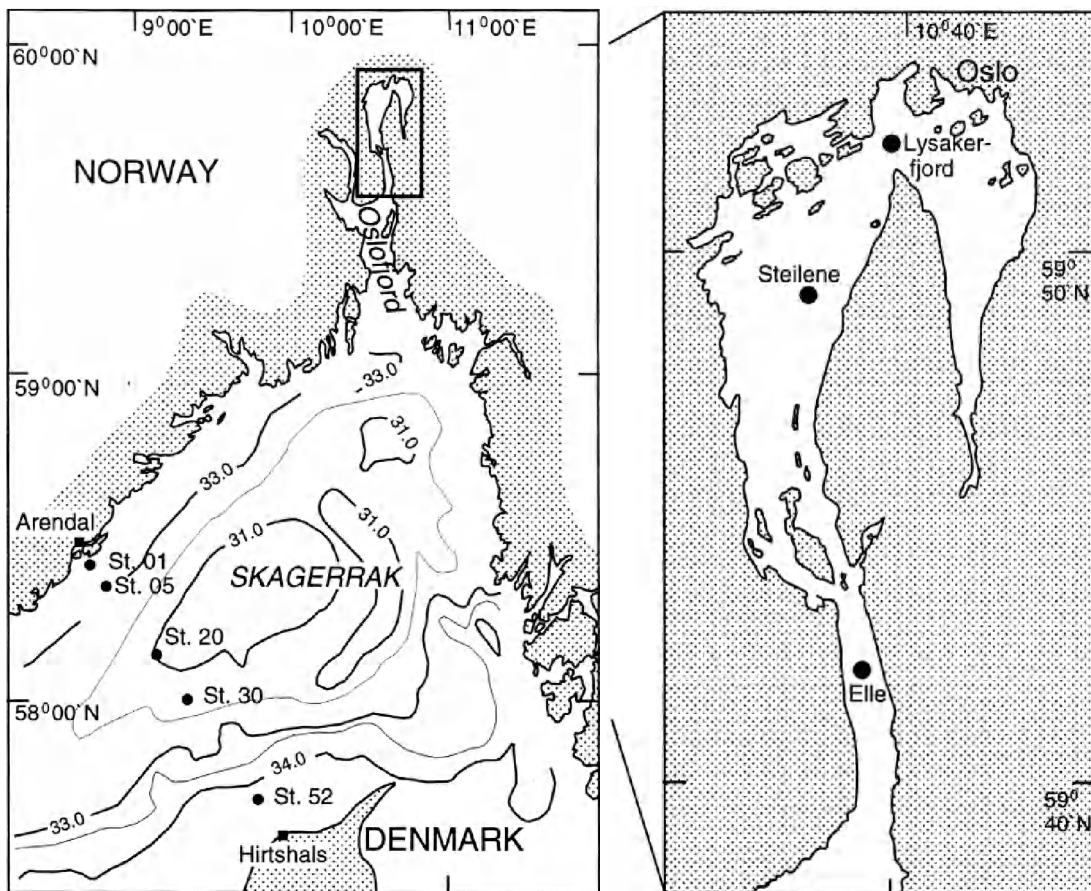


Fig. 1. Location of the sampling sites. The Skagerrak transect at left, the Oslofjord at right. Salinity isoplots are modelled data (NORWECOM) for 30 m depth at 19 May 1993.

been reported from the eastern Skagerrak (KARLSON & NILSSON 1991; KUYLENSIERTNA & KARLSON 1994). For Oslofjorden the information on cyanobacteria is meagre, in the inner Oslofjord this group has only once been included in the plankton studies (TOBIESEN 1991). It has been reported, however, that the ultraplankton ( $< 5 \mu\text{m}$ ) size fraction may be responsible for up to 70-90 % of the primary production in Oslofjorden (THRONDSSEN 1976; THRONDSSEN 1978a), and it is likely that a large part of this may be due to the picoplankton.

Initiated by the reports mentioned above the present investigations were aimed at studying more explicitly the seasonal dynamics of picocyanobacteria and eukaryotic picoplankton respectively in the spring and summer in Oslofjorden and the western part of the

Skagerrak. The phycoerythrin-rich picocyanobacteria are relatively easily distinguished from other picoplankton by their size, shape, and characteristically orange-yellow fluorescence in blue light excitation. In the following the picocyanobacteria are reported as an ecological and taxonomical entity. Abundance and biomass of phycoerythrin-rich picocyanobacteria and autotrophic picoeukaryotes together with small autotrophic nanoplankton ( $< 5 \mu\text{m}$ ) and heterotrophic bacteria have been calculated. The serial dilution culture method (SDC) was used to bring autotrophic picoplankton from the inner Oslofjord and Skagerrak into culture. We here report results from nine cruises in Oslofjorden and four cruises in the Skagerrak during spring and summer 1993.

## MATERIAL AND METHODS

### Sampling

Three stations were sampled in Oslofjorden, and five stations in the Skagerrak along a transect from Torungen in Norway to Hirtshals in Denmark (Fig. 1). The position of the selected stations were as follows:

#### Oslofjorden:

Lysakerfjorden 59°53'1 N, 10°38'9 E  
Steilene 59°49'0 N, 10°33'8 E  
Elle 59°38'6 N, 10°37'7 E

#### Skagerrak:

St. 01 58°23' N, 8°49' E  
St. 05 58°20' N, 8°54' E  
St. 20 58°08' N, 9°11' E  
St. 30 58°00' N, 9°22' E  
St. 52 57°42' N, 9°47' E

In Oslofjorden sampling was made from R/V *Trygve Braarud* from the surface, the euphotic zone (4 m), and the bottom of the euphotic zone (20 m) on 1 April, 13 April, 28 April, 24 May, 15 June, 16 August, and 24 September. Sampling on 6 July and 29 July was made by a manual water sampler from a smaller vessel. Sampling in the Skagerrak were made from R/V *G.M. Dannevig* from three depths; the surface, the euphotic zone (5 m), and bottom of the euphotic zone (30 m) on 19 May, 2 June, 13 July, and 5 August. All samples were collected with 1.8 l Niskin water bottles connected to a Neil Brown (Mark IIIB) CTD.

### Cell counts

Water samples (100 ml) for cell counts were drawn directly from the Niskin bottle and preserved with 4 ml cold 25 % EM grade glutaraldehyde to 1 % final concentration. A 10-ml subsample of this was vacuum filtered ( $\sim 130$  mBar) onto a black 0.2- $\mu\text{m}$  polycarbonate Nuclepore filter mounted on 5.0- $\mu\text{m}$  Nuclepore support filter to achieve an even distribution of cells. Cells were stained with  $10 \mu\text{g ml}^{-1}$  DAPI (4',6'-diamidino-2-phenylindole). Immediately mounted in paraffin on glass slides, and frozen for later enumeration by epifluorescence microscopy. Direct count of cells was done not later than two days after freezing. Twenty random positions were chosen on the filter, and the cells observed within a grid mounted in the ocular were counted. Each grid representing  $13456 \mu\text{m}^2$  corresponding to  $0.76 \mu\text{l}$  of the sample when 10 ml were filtered. The average count of cells from 20 fields were: picocyanobacteria 270, picoeukaryotes 60, nanoplankton ( $< 5 \mu\text{m}$ ) 46, and bacteria 100. A Leitz Laborlux S equipped with filterblock I 2/3 for blue light excitation and filterblock A for UV-excitation of DAPI-stained cells, was used. DAPI/DNA-complex excited with  $\text{UV}_{365 \text{ nm}}$  gives a bright blue fluorescence (PORTER & FEIG 1980).

### Carbon biomass estimates

Cell numbers of picocyanobacteria were converted to biomass of organic carbon by using  $0.25 \text{ pg C cell}^{-1}$  (KANA & GLIBERT 1987). Bio-volume of autotrophic eukaryotic picoplankton

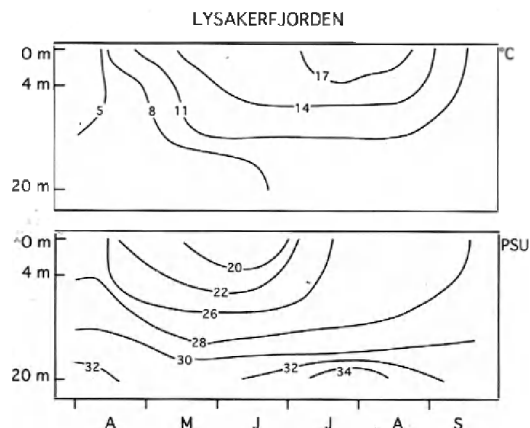


Fig. 2. Temperature and salinity isopleths from station Lysakerfjorden in Oslofjorden 1993.

and autotrophic nanoplankton in the size range  $2\text{--}5 \mu\text{m}$  was standardized to  $1.5 \mu\text{m}^{-3}$  (THRONDSEN 1976) and  $22 \mu\text{m}^{-3}$  (HANSEN 1992) respectively. Eukaryotes were converted to biomass by the factor  $0.22 \text{ pg C } \mu\text{m}^{-3}$  as given by BØRSHEIM & BRATBAK (1987). The heterotrophic bacteria were converted to biomass using  $0.02 \text{ pg C cell}^{-1}$  (LEE & FUHRMANN 1987).

### Serial dilution cultures

Samples for the serial dilution cultures were collected in Oslofjorden and the Skagerrak at the surface with a manual water sampler and pre-filtrated by a Nyltal monofilament HD-55 ( $55 \mu\text{m}$ ) and HD-05 ( $5 \mu\text{m}$ ) before it was inoculated to the modified Erd-Schreiber medium (THRONDSEN 1978b). Dilutions were usually in five steps, however in Oslofjorden 16 August and 29 September six dilutions (lowest inoculum 1 nl) were used. The cultures were incubated with white light (fluorescent tubes Philips TL-/33 and TLD-/84) at  $65 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  and  $15^\circ \text{C}$ . Incubation time varied from 5 to 7 weeks in 16 hour light: 8 hour dark cycle.

## RESULTS

### Inner Oslofjorden

**Hydrography.** Warm and sunny weather during April and May increased the process of stratification and a pronounced pycnocline was recorded towards the end of May at all stations in Oslofjorden. July and August were mostly clouded, with relatively cold weather, and the surface temperature did not increase noticeably during this period. Cold weather in September caused destratification of the surface layers, and during the cruise on 24 September a

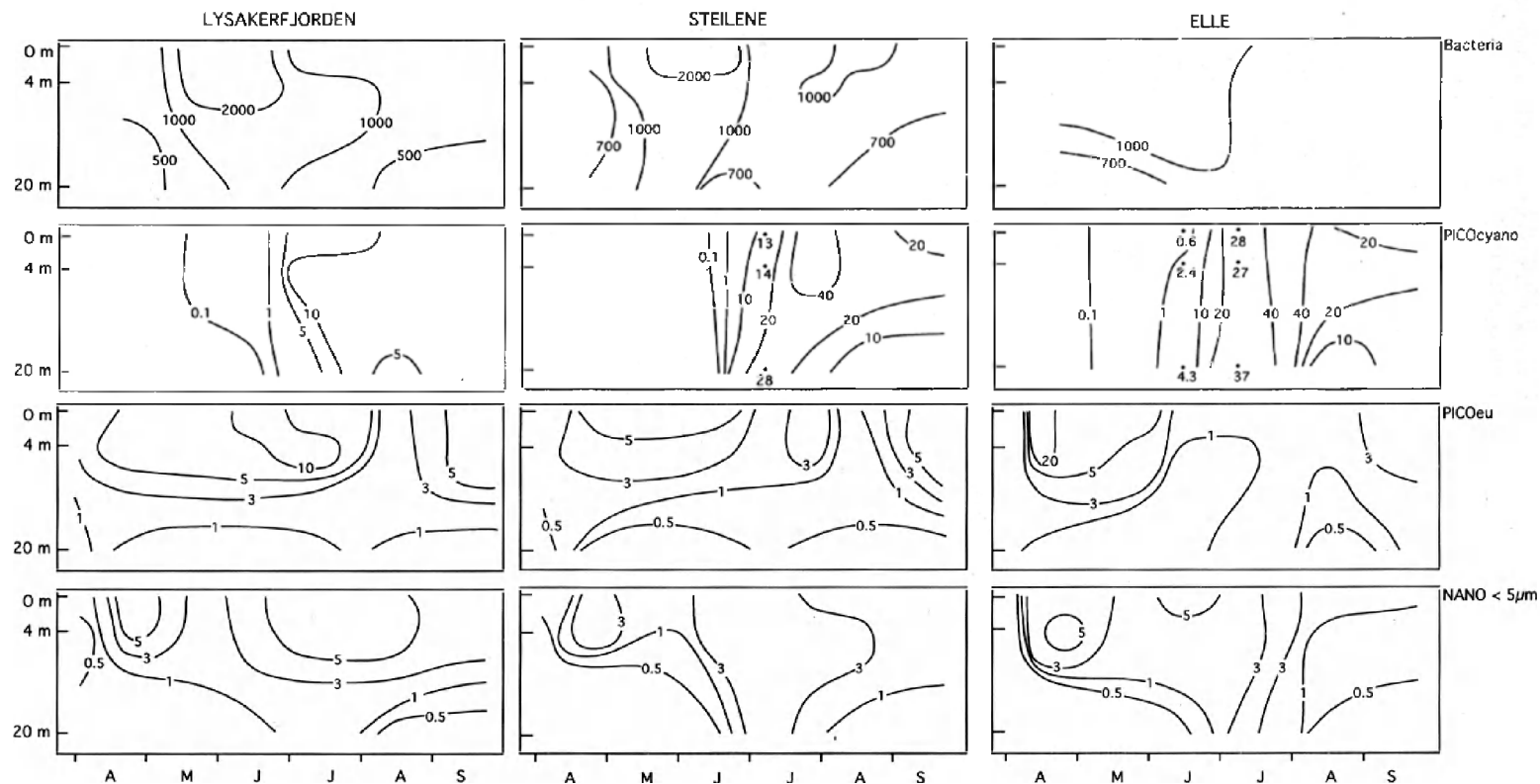


Fig. 3. Cell numbers ( $10^3 \text{ ml}^{-1}$ ) for heterotrophic bacteria, picocyanobacteria, picoeukaryotes and small nanoplankton ( $< 5 \mu\text{m}$ ) at station Lysakerfjorden, Steilene and Elle from April to September in Oslofjorden 1993.

pycnocline was not evident. The timing of stratification at the outermost station Elle was not very different from that at Steilene and Lysakerfjorden, but the surface water masses in Lysakerfjorden had the highest summer temperatures (Fig. 2).

**Seasonal abundance and biomass.** The exponential growth phase of picocyanobacteria in late June caused a shift in the ratio between picoeukaryotes and picocyanobacteria cell numbers (Fig. 3). Distribution and dispersal of picoeukaryotes seems to be different from that of picocyanobacteria. At Lysakerfjorden high numbers were found in the upper euphotic zone during June and July with a drop to low numbers in August (Fig. 3 left). The picoeukaryotic cell numbers at Elle were highest during spring with a drop to values around  $10^3$  cells  $\text{ml}^{-1}$  during the summer (Fig. 3 right).

A significant picocyanobacterial biomass was first observed in the samples from the deep euphotic zone at Elle in May. This was not observed at Lysakerfjorden and Steilene. The exponential growth phase of picocyanobacteria therefore seems to start earlier at station Elle than at the innermost stations. Disregarding few observations, the average net growth rate between 15 June and 6 July is calculated to 0.28 divisions per day for the picocyanobacteria, which gives an average generation time of 3.6 days. The highest biomasses of picocyanobacteria concurred with the maximum surface temperature in July. Cell numbers at Lysakerfjorden were lower than those found at Steilene and Elle. Small colonies of relatively large ( $1.5 \times 2.5 \mu\text{m}$ ) elliptical cells were observed at all stations. The cell numbers of picoeukaryotes and small nanoplankton ( $< 5 \mu\text{m}$ ) decreased while picocyanobacteria increased along a transect from the innermost station during summer (Fig. 4). The integrated phytoplankton biomass in the fraction  $< 5 \mu\text{m}$  and bacteria varied between 25 and  $56 \mu\text{g C litre}^{-1}$  (Fig. 5A-C). With highest bacterial biomass during May and June at Lysakerfjorden and Steilene.

Picocyanobacteria were not observed in the serial dilution cultures. The prasinophyte *Micromonas pusilla* on the other hand grew well and estimated cell numbers (MPN, most probable number) were often exceeding  $10^4$  cells  $\text{ml}^{-1}$  (Table 1). The prasinophyte *Bathycoccus*

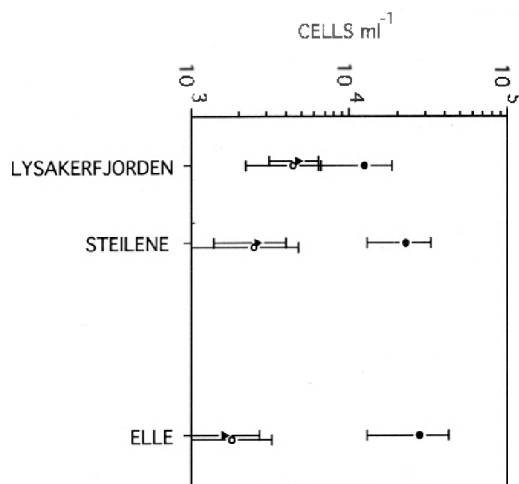


Fig. 4. Average cell numbers ( $\text{ml}^{-1}$ ) from 3 depths. Picocyanobacteria (●), picoeukaryotes (▲) and small nanoplankton (○) from July to September ( $n = 12$ ) at 3 stations in Oslofjorden 1993. Error bars indicate standard deviation.

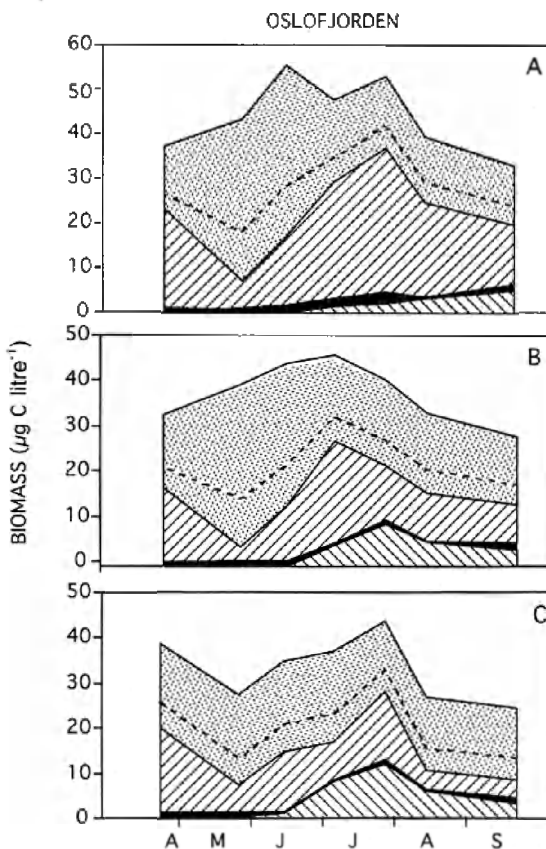
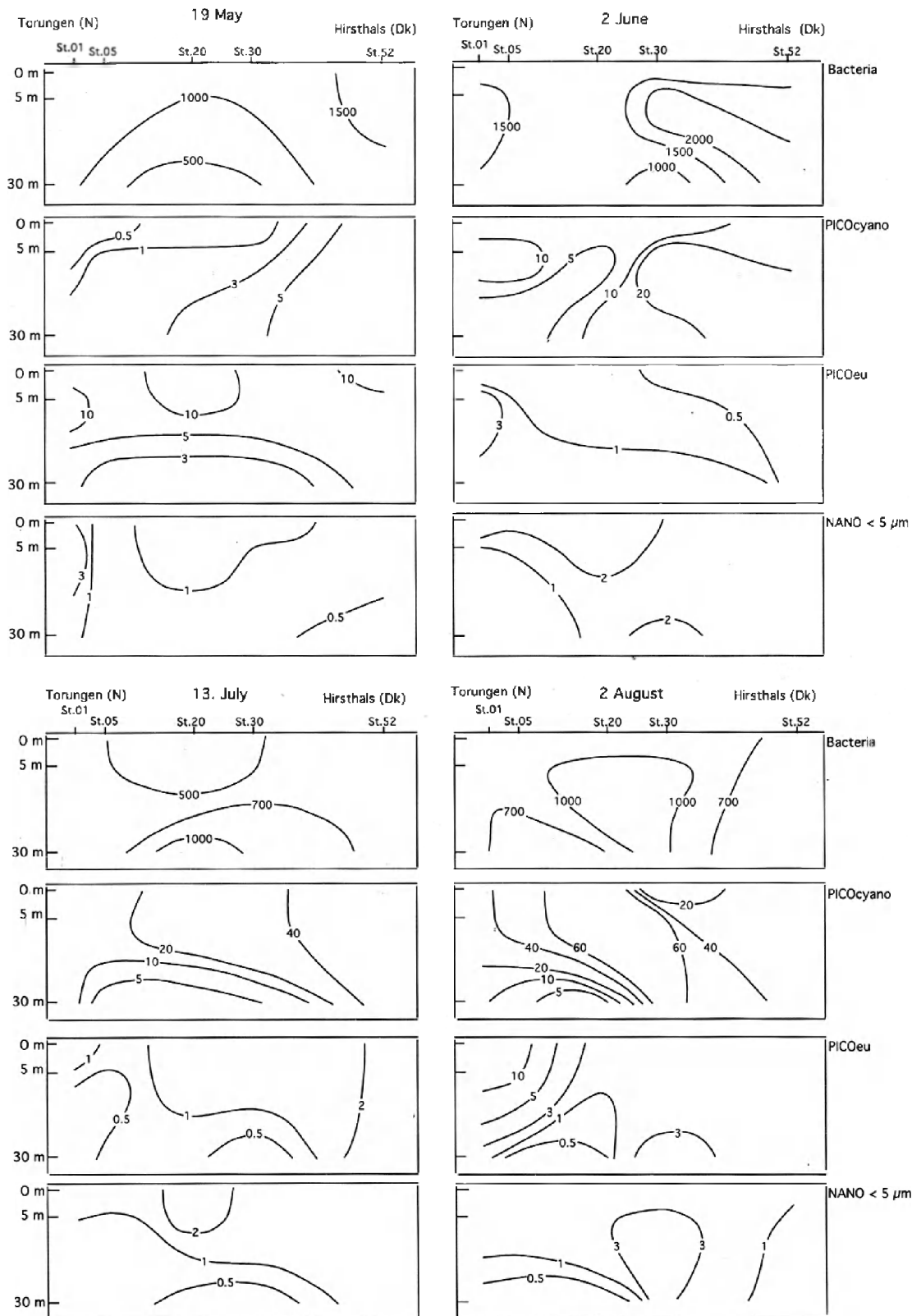


Fig. 5. Average biomass ( $\mu\text{g C litre}^{-1}$ ) of heterotrophic bacteria, picocyanobacteria, picoeukaryotes and small nanoplankton ( $< 5 \mu\text{m}$ ) at 3 stations from April to September in Oslofjorden 1993. A. Lysakerfjorden, B. Steilene, C. Elle. The broken line in the bacterial biomass indicates the 30% range reported by Zweifel & Hagström (1995).



*prasinus* EIKREM & THRONDSSEN was also recorded in serial dilution cultures. However, several different auto-fluorescence characteristics were observed in the epifluorescence microscope, and could denote that the picoeukaryote community were composed of more than these two species.

### Skagerrak

**Hydrography.** Freshwater from rivers and of Baltic origin influenced the surface layers in the Skagerrak during May and June and salinities of 20–22 psu were recorded. The salinity increased throughout the summer and were always lowest in the central part of the transect. Towards the Danish coast salinities of 31–34 psu were recorded (see Fig. 1). Doming of the nutricline (PINGREE & al. 1982) caused a 2-layer system with a deep chlorophyll maximum at 20–30 meters depth from May to July.

**Seasonal abundance and biomass.** There was a marked change in the picoplankton community between May and June. Samples in May (Fig. 6 upper left) had a majority of eukaryotic picoplankton while the June samples (Fig. 6 upper right) had more picocyanobacteria. The onset of the exponential growth phase of picocyanobacteria in Skagerrak therefore seems to start 2–3 weeks earlier than in Oslofjorden. Picocyanobacterial cell numbers increased throughout the summer at all stations with maximum numbers in the central and southerly parts of the transect (Fig. 6). Small colonies (10–50 cells) of picocyanobacteria were observed in samples from July and August. It was also observed one aggregate of about 1 500–2 000 small cells of 1–1.5  $\mu\text{m}$  in diameter. Average phytoplankton biomass in the fraction < 5  $\mu\text{m}$  and bacteria varied between 17 and 52  $\mu\text{g C litre}^{-1}$  with increasing biomass of pico-cyanobacteria from May to August (Fig. 7A–D).

### DISCUSSION

The annual cycle of picocyanobacteria in temperate waters is characterized by an early summer exponential growth phase correlated to increase in temperature and solar radiation (WATERBURY & al. 1986; JOCHEM 1988). In the Woods Hole harbour through several years of investigations, the onset of this growth phase averaged to 10 May with a duration of 35 days (WATERBURY & al. 1986). Our results show that the early exponential growth phase of picocyanobacteria occurred during early June in Skagerrak and

some weeks later in the inner Oslofjord in 1993. In the initial phase we observed that the highest cell numbers of picocyanobacteria were related to the deeper parts of the euphotic zone. Cell numbers in surface waters were lower despite considerably higher temperatures. This indicates that a well developed pycnocline and the low salinities in surface water probably prevented advection of waters rich in picocyanobacteria to the upper euphotic zone. In the Skagerrak picocyanobacteria was first observed with high cell numbers in the deeper part of the euphotic zone and towards the Danish coast in water with salinities of 31–34 psu. Which is water from the southern and central North Sea (AKSNES & al. 1989).

During summer the structure of the autotrophic picoplankton community changed; total cell numbers increased and the dominance shifted from eukaryotic to prokaryotic organisms. Depending on the content of essential elements in the organic substrates there may also be competition for nutrients between heterotrophic bacteria and phytoplankton (FENCHEL 1988; THINGSTAD & al. 1993). KARLSON (1989) showed that 70–90 % of the total

Table 1. Cell numbers ( $\text{ml}^{-1}$ ) for *Micromonas pusilla* (MPN) compared to total counts of autotrophic picoeukaryotes in Oslofjorden and the Skagerrak 1993.

Date	MPN	PICOeu	Station
OSLOFJORDEN			
13 April	450	1900	Lysakerfjorden
	160	1500	Steilene
	> 24000	27100	Elle
24 May	> 24000	4600	Lysakerfjord
	180	7000	Steilene
	1700	5700	Elle
15 June	3500	10500	Lysakerfjorden
	4600	6700	Steilene
	100	1600	Elle
6 July	2400	6200	Lysakerfjorden
	1800	1600	Steilene
	700	1400	Elle
SKAGERRAK			
19 May	5400	6200	St.01
	> 24000	10100	St.52
13 July	200	1300	St.01
	1600	2000	St.52

← Fig. 6. Cell numbers ( $10^3 \text{ ml}^{-1}$ ) for heterotrophic bacteria, picocyanobacteria, picoeukaryotes and small nanoplankton (< 5  $\mu\text{m}$ ) in the Skagerrak from samplings 19 May, 2 June, 13 July and 5 August in 1993.

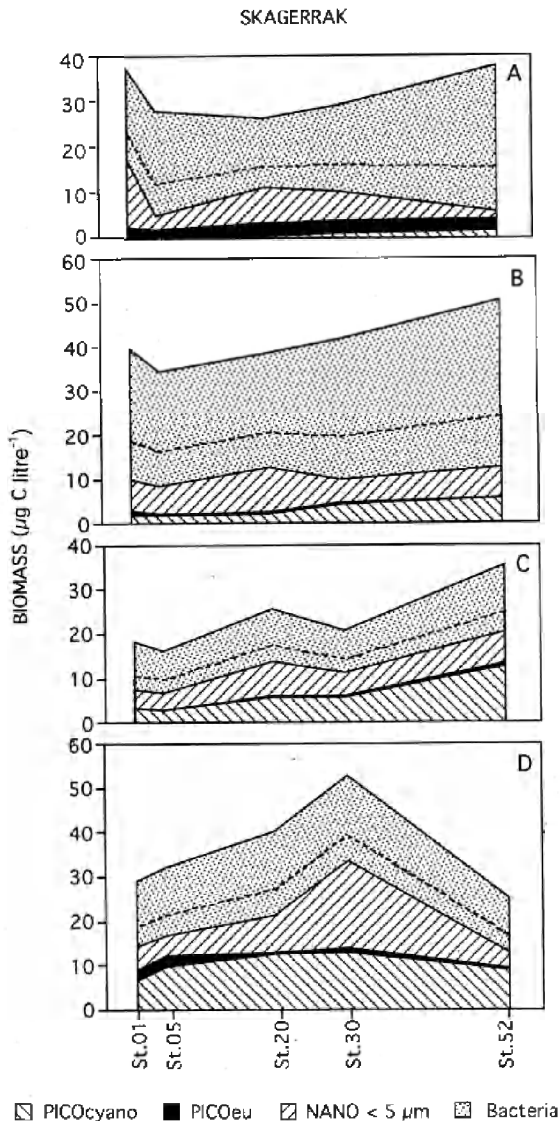


Fig. 7. Average biomass ( $\mu\text{g C litre}^{-1}$ ) of heterotrophic bacteria, picocyanobacteria, picoeukaryotes and small nanoplankton ( $< 5 \mu\text{m}$ ) from the transect Torungen-Hirtshals in the Skagerrak 1993. A. 19 May, B. 2 June, C. 13 July, D. 5 August. The broken line in the bacterial biomass indicate the 30 % range reported by ZWEIFEL & HAGSTRÖM (1995).

phosphate uptake from May to September in eastern Skagerrak is due to the  $0.2\text{--}3 \mu\text{m}$  fraction when picocyanobacteria constituted about 50 % of the total phytoplankton biomass (KARLSON & NILSSON 1991). ROSENBERG & al. (1990) emphasized the importance of

picoplankton ( $< 3 \mu\text{m}$ ) for the nitrogen flow in the pelagic food web.

Increased abundance of autotrophic picoplankton and a decreasing biomass of bacteria to picocyanobacteria towards the open Skagerrak could indicate competition between these two groups of important picoplankters throughout the summer. The picoprochlorophytes (CHISHOLM & al. 1988) have yet to be reported from the Skagerrak, but their importance in the Northeast Atlantic was documented by VELDHUIS & al. (1993). By application of adequate methods their presence will probably be revealed also in the Skagerrak. The biomass ratio between bacteria, pico-cyanobacteria, and picoprochlorophytes would probably give a more realistic indication of the nutrient cycling among the smallest phytoplankton in Oslofjorden and the Skagerrak during summer. Competition for nutrients among the prokaryotes could also be of more relevance than that between prokaryotes and eukaryotes.

Earlier studies give reason to believe that the abundance of phytoplankton decreases along an outward gradient in Oslofjorden (THRONDSSEN 1969, 1978a). Our results correspond with these studies and show a decreasing phytoplankton biomass towards the outer fjord, but with an increasing abundance of picocyanobacteria both relatively and in absolute numbers. The picoeukaryote *Micromonas pusilla* was very abundant during the spring period. Maximum of *M. pusilla* is also reported during this season by KUYLENSTIERN & KARLSON (1994). In the low chlorophyll period after the diatom spring bloom these picoeukaryotes could therefore be of major importance as primary producers in the water column. *M. pusilla* has a remarkable adaptability both with regard to temperature and salinity change (THRONDSSEN 1976) and these properties could explain why this species was so abundant during the spring period. LI (1994) also points out the importance of eukaryotes possessing high specific rates of  $^{14}\text{C}$  uptake, and that they are generally the dominant primary producers among the ultraphytoplankton community ( $< 5\text{--}10 \mu\text{m}$ ). Their high rates of extracellular release of dissolved organic compounds compared to larger cells also make them an important biological component of carbon flux (LEGRAND & MALINSKY-RUSHANSKY 1992). Studies by PAASCHE & ERGA (1988) of nutrient absorption capacity of summer phytoplankton show that phosphorus is probably more limiting than nitrogen, and that phosphorus limitation probably increases towards the outer Oslofjord. Increased importance of picocyanobacteria towards the outer fjord is therefore in concurrence with increasing probability of phosphorus limitation.

The concurrence of the exponential growth phase of picocyanobacteria with the regular abundances of mixotrophic and potentially toxic *Chrysochromulina* species in Skagerrak (E. Dahl pers. comm.) stresses the importance to follow the quantitative and qualitative change



in the phytoplankton and bacterial community during the early summer. Picocyanobacteria could also be understood as an important and easy recognizable indicator of the summer phytoplankton community which can be characterized by high turnover rates and low biomass (MALONE & al. 1991).

We were not able to recognize the phycocyanin-rich picocyanobacteria with the exitation filters used in this study and they are reported in coastal temperate waters by RAY & al. (1989). In the eastern Skagerrak they are reported to be not more than 10 % of the phycoerythrin-rich pico-cyanobacteria (KARLSON & NILSSON 1991). If our estimations of picocyanobacteria abundance are low they probably are of minor importance. Picocyanobacteria were dominated by spherical cells of 1.5–1.8 µm in size, but some larger ellipsoidal cells were also observed. It is known that the cell size of picocyanobacteria increase with decreasing irradiance (KANA & GLIBERT 1987), and the pico-cyanobacteria reported in this paper from the Skagerrak and Oslofjorden correspond well within the range of biovolume reported by these authors from their *Synechococcus* WH7803 experiments. It also support the view that picocyanobacteria in the Skagerrak and Oslofjorden are of oceanic origin and are smaller than cells reported from the Kiel Bight (JOICHEM 1988).

ZWEIFEL & HAGSTRØM (1995) have recently shown that only a minor fraction (2–32 %) of total counts of DAPI-stained particles can be scored as bacteria with nucleoids. The remaining particles are regarded as cell residues of virus-lysed bacteria or remains of protozoan grazing. We have therefore indicated in our biomass figures their upper range of 30 % when converting DAPI-stained particles into bacterial biomass. This means that a relative small numbers of active bacteria growing at high rates are responsible for the bacterial production reported (see e.g. MALONE & al. 1991). Heterotrophic nanoflagellates were often observed with picocyanobacteria in their food vacuoles. This group is reported to have high abundances in the North Sea (VAN DYUL & al. 1990) and should also be included in future seasonal studies in ultraplankton ecology.

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