Berichte
aus dem Institut für Meereskunde an der
Christian-Albrechts-Universität zu Kiel

Nr. 201

DIE ROLLE DER NANOFLAGELLATEN IM NAHRUNGSNETZ EINES BRACKWASSERGEBIETES (WESTLICHE OSTSEE)

THE ROLE OF NANOFLAGELLATES IN THE FOOD WEB OF A BRACKISH WATER ENVIRONMENT (WESTERN BALTIC)

DOI 10.3289/IFM_BER_201

von

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ISSN 0341-8561

Acknowledgements

To my `Doktorvater', Prof. Rheinheimer, go my deepest thanks for his unwavering support throughout the research and writing of this work. I am also very grateful to Dr. J. Kuparinen from the University of Helsinki for his expertise and guidance in the development of techniques, which he contributed during a stay in Kiel as a visiting scientist. Dr. Helge Thomsen from the University of Copenhagen graciously helped in the identification of loricate choanoflagellates. Valuable technical assistance was provided by several people in different departments, in particular Regina Koppe and Hans Sell in Marine Microbiology, as well as Peter Fritsche in Planktology. Thanks also go to Prof. Hoppe, and Drs. R. Schmaljohann, K. Lochte and K. Gocke for helpful discussions and, to Rolf especially, for his invaluable assistance at the electron microscope. The comradeship of fellow `Doktoranden' in the department was truly appreciated, among others I owe P. Kähler and M.-N. Hermin many fun moments. Finally, for many hours spent in the lab or out on the pier, or simply for being there, I thank W. Weishar.

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Abbreviations

AO	acridine orange
BDW	bidistilled water
BE	Boknis Eck
BMP	Baltic monitoring program
DAPI	4',6'-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
DOM	dissolved organic matter
FB	Fehmarn Belt
HNF	heterotrophic nanoflagellates
IfM	Institut für Meereskunde
KB	Kieler Bucht
MCC	mean carbon content
MCV	mean cell volume
PNF	pigmented nanoflagellates
TBN	total bacteria number
TCA	trichloroacetic acid
TEM	transmission electron microscopy
TTI	tritiated thymidine incorporation
SEM	scanning electron microscopy

SUMMARY

In a two-year study (1987-89) at a station (Institut für Meereskunde pier) located in an eutrophied fjord (Kiel Fjord) of the Western Baltic Sea, total bacteria number (TBN) and mean cell volume (MCV) revealed a negative correlation on both long-term (seasonal) and short-term (weekly) scales at both sampling depths, sub-surface and near-bottom (5 m). The seasonal trend of small MCV in summer (0.03 - 0.06 μm^3) and large in winter (0.06 - 0.10 μm^3) could be explained by growth physiology and to a lesser extent by grazing. Both TBN and heterotrophic nanoflagellates (HNF) showed well-defined seasonality with pronounced oscillations in the spring and summer, which often revealed a trophic relationship. Trophic coupling was particularly clear in May 1988, when HNF minima and TBN maxima occurred simultaneously during a bloom of the silicoflagellate Dictyocha speculum. The presence of large numbers of naked D. speculum cells (5.8×10^3 cells ml^{-1}) appeared to have an inhibitory effect on HNF, stemming from the release of large numbers of ultra-structures resembling discharged discobolocysts. The most distinct feature in HNF seasonal distribution was a predominance of loricate choanoflagellates in early spring, which could compete effectively with the spring diatom bloom for silicates, particularly near the bottom. Very small HNF cells (2 μm^3) represented the size class with highest mean relative abundance for the entire study (32%).

At three stations in the Western Baltic sampled at 3 depths in a total of 14 cruises, bacteria and HNF populations also exhibited seasonality but with smaller biomass than inside the fjord, reflecting decreased eutrophication. In contrast, cyanobacteria formed large blooms of colony-forming chroococcoid cells with larger MCV and concentrations than in the Kiel Fjord. Cyanobacteria constituted 24% to 47% of total phytoplankton biomass in the summer at these stations, but only 1% in the fjord. These blooms probably sustained an important fraction of heterotrophic activity in summer Baltic waters and represented the single best determinant for TBN outside the Kiel Fjord. HNF abundances were also correlated with cyanobacteria and numerous flagellates were observed attached to chroococcoid colonies.

In a total of seven in situ grazing experiments using diffusion chambers performed at different times of the year, growth rates of 0.3 - 1.1 d^{-1} (no growth in winter) were estimated for HNF (unfiltered treatments) and 0.3 - 0.7 d^{-1} (0.02 d^{-1} in winter) for bacteria (1 µm filtered controls). Ingestion rates ranged from 12 to 90 bac. flag.$^{-1}$ h^{-1}. Highest grazing rates were measured in May, after senescence of D. speculum inside grazer chambers, as well as maximum bacteria production in control chambers, reflecting unusual environmental conditions caused by the bloom. Lowest grazing rates were estimated at the same time with maximum growth efficiency (based on bacterial biomass grazed) in the summer experiments (112% in July and 40% in August), when blooms of pigmented nanoflagellates (PNF) occurred in grazer chambers providing an alternative food source for HNF. Since the most frequently encountered bacterial size classes were also the most frequently removed (middle sizes, 0.05 μm^3 avg. volume), HNF grazing seemed to follow a random encounter strategy rather than true size-selectivity.

Tritiated thymidine incorporation (TTI) was measured during four grazing experiments. TTI tended to overestimate bacterial production relative to net increases in biomass, but underestimated ingestion rates relative to observed changes in cell counts, when an empirical conversion factor of 1.1 X 10^9 cells $nmol^{-1}$ was used. With an experimentally determined average factor of 0.5 X 10^9 cells $nmol^{-1}$ for unfiltered treatments and 1.7 X 10^9 cells $nmol^{-1}$ for filtered treatments, TTI-based grazing rates could be adjusted to a range in better agreement with cell-based estimates. Based on TTI, 40 - 82% of bacterial production was grazed by HNF, whereas changes in bacterial biomass indicated that grazing compensated or exceeded bacterial production depending on HNF growth phase. Bacterial carbon demand constituted < 35% of total primary production and became a major fraction only in the October 1988 experiment (54%). Flagellate carbon demand compensated or exceeded bacterial production, except in May 1988 during the D. speculum bloom. Assessment of carbon fluxes indicated that the microbial `loop' in the Kiel Fjord functioned as a varying energy sink of modest to moderately high importance, but could constitute a more continuous and significant sink outside the fjord due to the predominance of cyanobacteria.

ZUSAMMENFASSUNG

Über den Zeitraum von zwei Jahren (1987-89) erfolgten Untersuchungen zum Bakterio- und Nanoplankton aus zwei veschiedenen Tiefen (Wasseroberfläche und über dem Grund in 5m Wassertiefe) an einer Station (Pier des Instituts für Meereskunde) in der eutrophierten Kieler Förde (westliche Ostsee). Zwischen Gesamtbakterienzahl (GBZ) und mittlerem Zellvolumen (MZV) wurde dabei sowohl langfristig (saisonal) als auch kurzfristig (von Woche zu Woche) eine negative Korrelation festgestellt. Saisonale Unterschiede in der Größenverteilung mit kleinen Zellen (0.03 - 0.06 μm^3) im Sommer und großen (0.06 - 0.10 μm^3) im Winter waren hauptsächlich wachstumsphysiologisch und weniger durch größenselektives Grazing bestimmt. Sowohl die GBZ als auch die Zahl der heterotrophen Nanoflagellaten (HNF) wiesen eine ausgeprägte Saisonalität mit starken Oszillationen im Frühjahr und Sommer auf; häufig zeigte sich hier eine Räuber-Beute-Beziehung. Trophische Zusammenhänge wurden im Mai 1988 besonders deutlich, als, während einer Blüte des Silicoflagellaten Dictyocha speculum, HNF-Minima gleichzeitig mit GBZ-Maxima auftraten. Große Dichten nackter D. speculum-Zellen (5.8 X 10^3 ml^{-1} Maximum) hatten anscheinend einen negativen Effekt auf die HNF, möglicherweise wegen der Abgabe großer Mengen von Kleinstpartikeln (ähnlich wie Discobolocysten). Auffällig war bei den HNF das Vorherrschaften loricater Choanoflagellaten zu Beginn des Frühjahrs; diese waren während der Phytoplankton-Frühjahrsblüte in der Lage, mit Diatomeen um Silikat zu konkurrieren, insbesondere im bodennahen Wasser. Über den gesamten Untersuchungszeitraum wurde für sehr kleine HNF-Zellen (2 μm^3) die höchste relative mittlere Häufigkeit (32%) ermittelt.

Auf insgesamt 14 Ausfahrten wurden Proben von drei Stationen in der westlichen Ostsee aus drei Wassertiefen bearbeitet; auch dort zeigten Bakterien- und HNF-Populationen saisonale Unterschiede bei allerdings geringeren Biomassen als in der Förde; eine Folge des geringeren Trophiegrades. Dagegen bildeten hier chroococcale koloniebildende Cyanobakterien Blüten mit höheren Dichten und MCV als in der Förde. Sie machten über den Sommer an den drei Stationen 24-47% der Phytoplankton-Biomasse aus (in der Förde nur 1%). Diese Sommerblüten waren möglicherweise die Grundlage für einen bedeutenden Teil der heterotrophen Aktivität; sie waren die einzige erkennbare Steuergröße für die GBZ außerhalb der Kieler Förde. Auch die Zahlen der HNF korrelierten mit der Dichte der Cyanobakterien, und viele Flagellaten waren mit deren Kolonien assoziiert.

Sieben Grazing-Experimente wurden mit *in situ* exponierten Diffusionskammern zu verschiedenen Jahreszeiten durchgeführt. Die von Frühjahr bis Herbst ermittelten Wachstumsraten lagen zwischen 0.3 und 1.1 d^{-1} für HNF (unfiltrierte Ansätze) und zwischen 0.3 und 0.7 d^{-1} für Bakterien (filtriert, 1µm Porenweite). Über Winter wuchsen HNF nicht, Bakterien hingegen mit 0.02 d^{-1}. Die Ingestionsraten lagen zwischen 12 und 90 Bakterien pro Flagellat und Tag; die höchsten Raten wurden im Mai nach dem Absterben von *D. speculum* in den Kammern mit unfiltriertem Wasser bei gleichzeitig höchster Bakterienproduktion in den Kammern mit filtriertem Wasser gemessen. Die niedrigsten Grazing-Raten ergaben sich zu Zeiten der höchsten Wachstumseffizienz (bezüglich der eliminierten Bakterien-Biomasse) während des Sommers: 112% im Juli und 40% im August. Während dieses Zeitraumes traten Blüten von pigmentierten Nanoflagellaten (PNF) als alternative Nahrungsquelle für HNF in den Kammern mit unfiltriertem Wasser auf. Die am häufigsten auftretenden Bakterien-Größenklassen waren auch die am intensivsten eliminierten (mittlere Größen mit durchschnittlich 0.05 μm^{-3} Größe); Grazing durch HNF war also eher wahllos als selektiv nach der Größe.

Die Inkorporation von tritiiertem Thymidin (TTI) wurde während vier Grazing-Experimenten gemessen. Bei Verwendung des empirischen Umrechnungsfaktors von 1.1 x 10^9 Zellen $nmol^{-1}$ Thymidin überschätzte diese Methode gegenüber den Biomassemessungen die Bakterienproduktion, unterschätzte aber die Ingestionsraten im Vergleich mit den beobachteten Änderungen der Bakterienzahlen. Mit den experimentell ermittelten mittleren Umrechnungsfaktoren von 0.5 x 10^9 Zellen $nmol^{-1}$ (unfiltrierte Ansätze) und 1.7 x 10^9 Zellen $nmol^{-1}$ (filtrierte Ansätze) konnte eine bessere Übereinstimmung mit den aufgrund von Zellzählungen gewonnenen Ergebnissen erreicht werden. Nach der TTI-Methode wurden 40 - 82% der Bakterienproduktion durch HNF eliminiert. Die ermittelten Änderungen in der bakteriellen Biomasse ergaben, daß das Grazing die Bakterienproduktion kompensierte oder übertraf, je nach HNF-Wachstumsstadium. Der Kohlenstoffbedarf der Bakterien entsprach mehr als 35% der gesamten Primärproduktion; das Maximum (54%) wurde im Oktober 1988 gemessen. Der Kohlenstoffbedarf der Flagellaten entsprach der Bakterienproduktion oder übertraf sie, außer während der *D. speculum*-Blüte und des Winters. Die Kohlenstoffflüsse zeigen, daß die `microbial loop' in der Kieler Förde eine Energiesenke von wechselnder Bedeutung ist. In der offenen Kieler Bucht ist ihre Bedeutung wegen der Dominanz von Cyanobakterien möglicherweise größer und weniger variabel.

INTRODUCTION

Non-pigmented flagellated protozoa have long been recognized as an important component of marine microplankton and their role as bacterivores has been the subject of intensive investigation for the last two decades (eg. Sorokin, 1975; 1977; Haas and Webb, 1979; Fenchel, 1982c; Sieburth and Davis, 1982; Sherr et al., 1984; Coffin and Sharp, 1987; Taylor and Joint, 1990). Evidence of high heterotrophic production from bacteria was gathered with the development of sensitive techniques for estimating size and productivity of populations, such as epifluorescence microscopy (Zimmermann and Meyer-Reil, 1974; Hobbie et al., 1977) and tritiated thymidine incorporation (Fuhrman and Azam, 1982). Coupled to this high bacterial production, heterotrophic nanoplankton - the 2 to 20 μm size fraction constituted mostly by flagellates - occupy an intermediate trophic step between bacteria and larger zooplankton. Reliable estimates of the numerical importance and grazing impact of heterotrophic nanoflagellates were made possible by a series of improvements in epifluorescence microscopy (eg. Porter and Feig, 1980; Haas, 1982; Caron, 1983; Sherr and Sherr, 1983) and in experimental conditions (eg. Landry and Hassett, 1982; Sherr et al., 1987).

The recognition of the importance of nanoplankton led to a reformulation of the classic concept of a linear food chain based on algae grazing by zooplankton into a more dynamic and complex model of a microbial food web, which incorporated dissolved organic matter (DOM) cycling via bacteria and nanoplankton (Pomeroy, 1974; Azam et al., 1983). However, since the efficiency of an ecosystem decreases with increasing number of trophic levels, the `microbial loop' as postulated by Azam et al. (1983) constitutes a rather inefficient system of energy transfer. The extent to which this `loop' transfers lost primary production (DOM) back to higher trophic levels still remains to be resolved despite considerable debate (Ducklow et al., 1986; Sherr et al., 1987). Whether bacteria represent a `link' or a `sink' for organic matter is a central question for the analyses of carbon fluxes in the sea.

Apart from its basic role in the flux of carbon in aquatic ecosystems, heterotrophic nanoplankton may serve other important functions, such as remineralization of nutrients and homeostatic regulation for standing stocks of both heterotrophic and

autotrophic picoplankton, the 0.2 to 2 µm size fraction constituted mostly by bacteria. Therefore, its study is vital to the understanding of planktonic ecosystems. Yet, relatively scant information on heterotrophic nanoplankton exists for the Baltic Sea in general. This component has been so far neglected in both microbiological and planktonic studies, particularly in the context of long-term comprehensive monitoring programs. Considering the tight coupling between primary production, bacteria and flagellates, an assessment of eutrophication conditions in the Baltic cannot be adequately made until the functioning of the `microbial loop' is thoroughly investigated.

Thus, the present study attempted to contribute to a better understanding of the microbial food web in the Western Baltic. In collaboration with the Planktology Department, monitoring of heterotrophic nanoplankton was performed on an intensive basis in the Kiel Fjord and more extensively at three stations in the Western Baltic. Intensive sampling in the fjord was planned to describe temporal variations of the microbial community as thoroughly as possible and, thus, provide a background for the interpretation of results from in situ grazing experiments. Monitoring outside the fjord would enable a comparison with decreased eutrophied conditions and furnish preliminary information on spatial variations of heterotrophic nanoplankton. Experimental investigations were designed to yield estimates of growth and grazing rates of heterotrophic nanoplankton under conditions approximating the natural environment. Different techniques were also compared to estimate bacterial productivity and grazing in an effort to evaluate methodological bias. Finally, on a broader perspective, the unstable hydrography and high productivity of Baltic waters in the Kiel area provided the opportunity to characterize the microbial food web in a highly dynamic brackish water environment.

MATERIALS AND METHODS

1. Environmental Study

1.1 Institut für Meereskunde monitoring

A monitoring program of both pico- and nanoplankton was carried out in collaboration with the Planktology Department at a fixed station in an eutrophied fjord of the Western Baltic Sea (see map Fig. 1). A thorough description of the research area, particularly with respect to conditions affecting microorganisms can be found in Rheinheimer (1977). From the end of August 1987 to the end of August 1989, water samples were taken approximately once a week in the morning from the pier of the Institut für Meereskunde (IfM) at two depths, sub-surface (0.2 - 0.3 m) annotated 0 m, and at 5 m (ca. 1 m above bottom).

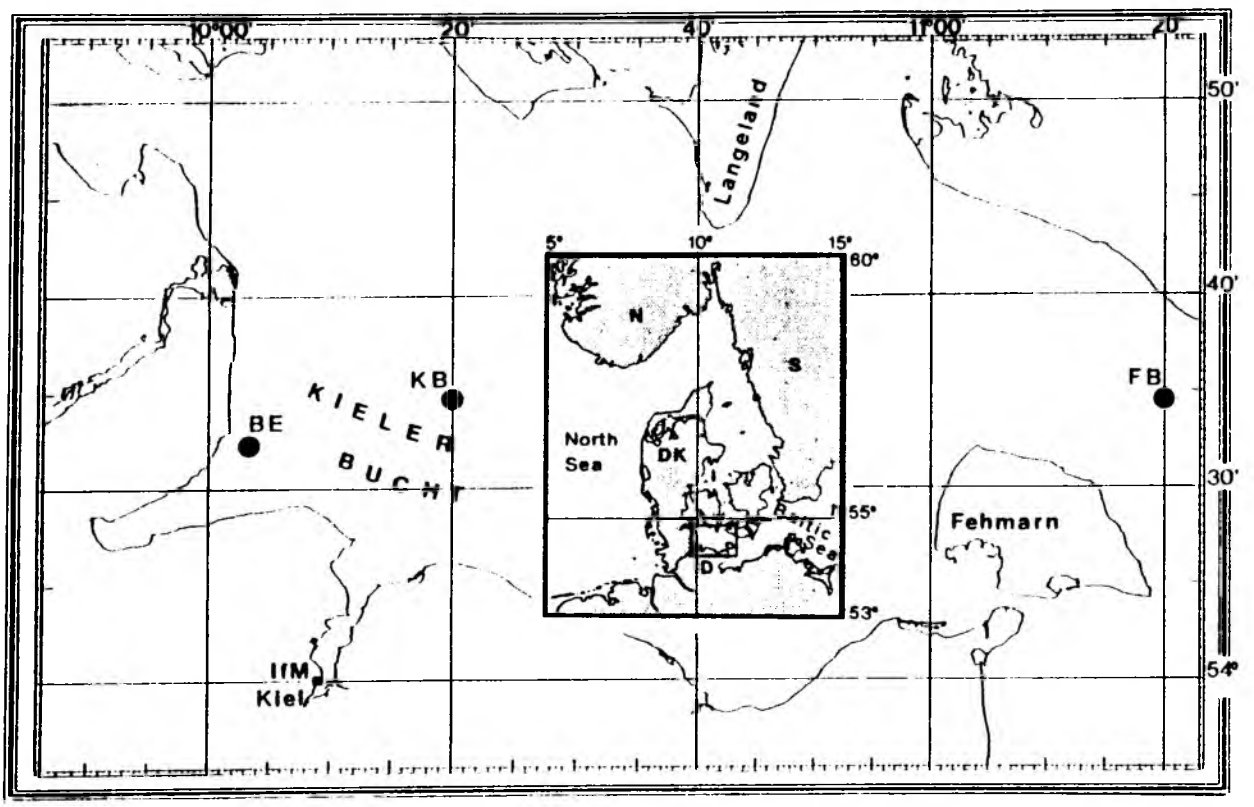


Figure 1. Map of Western Baltic Sea with location of monitoring stations.

a) Basic environmental measurements

Routine water analyses for dissolved inorganic nutrients

(NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, SiO_4^{4-}, standard colorimetric methods according to Grasshoff, 1976), chlorophyll a (acetone extracted, spectrophotometric method, Jeffrey and Humphrey, 1975) as well as primary productivity measurements (in situ bottle incubations with ^{14}C-bicarbonate following Baltic monitoring guidelines (cf. Gargas, 1975) were performed by the Planktology Department. Temperature and salinity were measured with a conductivity probe (WTW, F.R.G.).

b) Pico- and nanoplankton enumeration

For direct cell counts, samples were fixed with 50 µl per ml of 6% glutaraldehyde (25% synthesis grade solution, Merck; diluted in 32 S X 10^3 aged seawater; 0.2 µm filtered in sterile Nalgene apparatus) and refrigerated until further processing, usually within two days of sample collection. A varying volume of sample, (2 to 5 ml) depending on the season, was stained with proflavin (3,6-diaminoacridine hemisulfate, Sigma Chemical Co.) following the procedure from Haas (1982) and filtered onto 0.2 µm Nuclepore filters (pre-stained with Irgalan black or with Sudan Schwartz). Filters were mounted on glass slides with a drop of immersion oil (Cargille, type A) and frozen until counting. A Zeiss Standard epifluorescent microscope was used equipped with a high pressure mercury HBO 50W lamp, blue light excitation filter set, X63 Neofluar objectives and X16 oculars. This staining method gives reliable counts for both heterotrophic and autotrophic nanoplankton, since autofluorescence is not masked by the green fluorescence of proflavin. Most cell loss as well as autofluorescence fading occurs during the storage period prior to filtration. Epifluorescence counts remain stable up to one year of frozen storage of Nuclepore filters (see Galvão, 1984).

Cyanobacteria, unpigmented and pigmented nanoflagellates were counted in a range of 25 to 50 whole fields. Length and width of each flagellate cell was measured using a standard ocular micrometer. Other cell characteristics, such as number and position of flagella as well as general cellular appearance (i.e. red/orange/faded autofluorescence, pale or brightly stained cytoplasm, presence of ingested particles) were also recorded. The limit of detection of this method is approximately 100 cells ml^{-1}, therefore less abundant microorganisms such as

ciliates and dinoflagellates require filtration of much larger volumes to obtain reliable counts. Relative differences between duplicate sub-samples performed at various times of the year averaged 13 % for 1 ml and 9 % for 2 ml replicates. However, it was observed that certain environmental conditions such as attachment to diatom cells or inclusion in cyanobacteria colonies during phytoplankton blooms could reduce counting reproducibility. Certain types of choanoflagellates can also form colonies, probably in the process of active cell division (see sections 3.1 and 3.2). This clustered distribution would require different counting strategies to be properly assessed.

c) Heterotrophic nanoflagellate biomass

Heterotrophic nanoflagellates (HNF) were divided into 8 size classes with roughly doubling mean cell volumes. Volumes of rounded cells were calculated as spheres and elongated cells as rotational ellipsoids with circular cross-section given by

(1) $V = (\pi \times L \times D^2)/6$ L: length, D: diameter

Mean cell volume (MCV) per sample was estimated as the sum of the relative frequencies of the eight size class multiplied by average class volume. A description of size classes and corresponding cell sizes and volumes is given in Table 1. A carbon conversion factor of 0.220 pg C μm^{-3} determined with epifluorescent microscopy measurements (Bϕrsheim and Bratbak, 1987) was used. Thus, biomass in $\mu g\ C\ l^{-1}$ was calculated by

(2) $Biomass_{(HNF)} = HNF/ml \times MCV \times 0.220$

Table 1. Description of heterotrophic nanoflagellate size-classes.

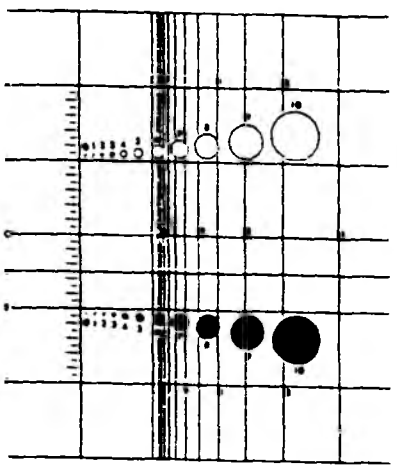
Class	Spheres Cell Diam. (µm)	Ellipsoids Diam. / Length (µm)	Range Cell Volume (µm³)	Average Class Volume (µm³)
1	1.2 - 1.8	1.2-1.6 / 1.2-2.5	0.9 - 3.2	2
2	1.9 - 2.5	1.6-2.4 / 1.9-4.8	3.5 - 8.5	6
3	2.7 - 3.2	1.9-3.2 / 2.7-6.7	9.0 - 20.5	14
4	3.5 - 4.5	2.4-4.0 / 3.5-11	21 - 44	32
5	4.7 - 5.7	3.2-5.2 / 4.7-16	45 - 95	68
6	5.9 - 7.2	3.7-6.4 / 5.9-19	96 - 200	140
7	7.5 - 9.0	4.8-8.0 / 7.5-20	205 - 430	288
8	9.5 - 12	7.2-11 / 9.5-22	450 - 950	617

d) Total bacteria number and biomass

For total bacteria number (TBN) and biomass determination, glutaraldehyde fixed samples (2 ml) were filtered onto Sudan Schwartz darkened 0.2 µm Nuclepore filters and stained with 1 ml acridine orange (5 mg in 50 ml bidistilled water (BDW), 0.2 µm filtered), as a slight modification of the method by Hobbie et al. (1977). For cell counting and measuring, a relatively simple and easy method was developed with the help of J. Kuparinen, University of Helsinki. A New Porton G12 graticule (Graticules, Ltd, U.K.) was installed in X12.5 oculars (Fig. 2). Bacteria were counted on the left side of the grid, which was divided into a vertical row of 6 equally sized squares. A total of 20 grid fields were counted covering as large an area of the filter as possible. Relative differences between duplicate sub-samples from various collection dates averaged less than 10%.

The Porton grid also contained two series of circles (clear and black), which increased in diameter following a root 2 progression (see Fig. 2). Circles were overlaid over randomly encountered bacteria, so that both cell length and width were given by sphere diameters (coded 0 to 10). With X100 Neofluar objectives, circle 0 had a diameter of ca. 0.2 µm, circle 1 ca. 0.3 µm, and successively with relative diameter size given by (1)

(1) $$D_n = \sqrt{2^n}$$ n: sphere number



New Porton G12

Figure 2. Diagram of ocular grid used for bacteria measurements.

For cocci, cell volume was calculated as a perfect sphere and for rod-shaped cells as a cylinder with rounded edges as in (2)

(2) $V = (\pi/4)D^2 (L-D/3)$ D: diameter, L: length

For vibrio type bacteria with curved shapes, cell length was measured by the fraction of curved circle perimeter covered by the cell. The smallest vibrios encountered measured 0.76 µm or 1/2 of circle 2 perimeter. Cell volume was calculated as for rod-shaped cells (formula 2). Mean cell volume (MCV) was estimated from a total of 100 bacteria measured in each sample. MCV measurements of replicate samples from various collection dates differed by 7% on the average. Bacteria were divided into different size classes with approximately doubling average volume. A description of size classes with ranges and average class volume is given in Table 2.

Table 2. Description of bacteria size-classes.

Class	Cocci Diam. (µm)	Rods Diam. / Length (µm)	Range Cell Volume (μm^3)	Average Class Volume (μm^3)
1	0.24	--	0.007	0.007
2	--	0.24 / 0.34	0.012	0.012
3	0.34	0.24 / 0.48	0.018-0.021	0.019
4	0.48	0.24-0.34/0.48-1.07	0.034-0.059	0.047
5	--	0.34-0.48/0.68-1.51	0.078-0.128	0.100
6	0.68	0.48 /0.96-1.51	0.147-0.247	0.195
7	0.96	0.48-0.68/0.96-2.73	0.268-0.469	0.384
8	--	0.48-0.68/1.93-3.03	0.523-0.910	0.684

The carbon content of each cell was calculated individually as a non-linear function of cell volume (equations 3 and 4) according to Simon and Azam (1989).

(3) $C_p = 88.6 \times V^{0.59}$ C_p: protein content, V: cell volume

(4) $C_c = C_p \times 1.04878$ C_c: carbon content

Bacteria biomass was then estimated as the mean carbon content (MCC) multiplied by total bacteria number (TBN).

(5) Biomass (µg C/l) = MCC (fg C/cell) X TBN (cells/ml) X 10^3

The range of MCC encountered for IfM pier samples varied from 10.1 fg C per cell for a sample with 0.0274 μm^3 MCV and 21.3 fg C per cell for a sample with 0.103 μm^3 MCV. Apart from substantially reducing microscopy time, this method had the advantage of taking into account cellular density increasing inversely to cell size, which has recently been reported in several studies using various techniques (Lee and Fuhrman, 1987; Norland et al., 1987; Simon and Azam, 1989). The method gave carbon conversion factors ranging from 0.70 pg C μm^{-3} for the smallest bacteria observed (0.007 μm^3) to 0.10 pg C μm^{-3} for large rods (0.9 μm^3). The most widely used carbon conversion factor for aquatic bacteria has been updated from 0.12 pg C μm^{-3} (Watson et al., 1977) to 0.35 pg C μm^{-3} (Bjørnsen, 1986; Lee and Fuhrman, 1987), both of which fit within the range of the method used here.

1.2 Western Baltic monitoring

During the Baltic monitoring program (BMP) conducted monthly by the Marine Microbiology Department, which included the routine investigation of three stations (Kieler Bucht, Boknis Eck and Fehmarn Belt) in the Western Baltic Sea (see map Fig. 1), water samples were taken for nanoplankton direct cell counts at three depths (2, 10 and 15/20 m). Samples were obtained from a total of 14 cruises from November 1987 to May 1989. Samples were fixed on board with 6 % glutaraldehyde and refrigerated until arrival at the laboratory, where they were further processed for epifluorescence microscopy following the procedure described in section 1.1-c).

Of the different physico-chemical and microbiological measurements performed by different departments during these cruises (see Berichte zum Biologischen Monitoring der Ostsee, 1987 and 1988), only temperature, salinity, chlorophyll a and bacteria MCV will be reported here. For BMP samples, TBN was determined from proflavin stained preparations, because TBN obtained with acridine orange (AO) were generally lower (average difference 29%, n= 102). This discrepancy could stem from inherent subjectivity of microscopy counts performed by different persons, and/or from prolonged storage of samples at room temperature prior to acridine orange processing. Proflavin and AO preparations of IfM pier samples made within one week of sample collection gave TBN esti-

mates which differed by an average of less than 2% (n= 192). In any case, it is recommendable to keep samples refrigerated during storage and to avoid prolonged storage periods (> 1 month), during which substantial cell breakdown can develop.

TBN from proflavin slides was estimated by counting cells encountered on one or two lines of a 20 X 20 grid installed in X16 oculars and randomly changing fields until a total of 300 bacteria was achieved. With this method, the relative difference between replicates averaged less than 5%. Bacteria MCV and biomass were calculated as described in section 1.1 - d).

2. Grazing Experiments

In order to determine the grazing impact of heterotrophic nanoplankton on bacteria under environmentally representative conditions, an incubation method with diffusion chambers was developed to approximate *in situ* conditions.

2.1 Diffusion chamber design

A chamber design, first developed by McFeters and Stuart (1972) and improved by Rhodes *et al.* (1983) to study *in situ* survival of coliform bacteria, was further modified to study growth and grazing potentials of natural populations of microprotozoans, as previously applied by Fritz (1986). The original design accomodated a 20 ml culture volume contained by plexiglas `donut'-shaped retainer and spacer plates, which held Millipore microweb membranes (McFeters and Stuart, 1972). Chamber volume was increased to 40 ml and polycarbonate, a more inert and robust construction material, was used as well as Nuclepore membrane, which resists autoclaving and improves diffusion (Rhodes *et al.*, 1983). The addition of `O-rings' between membrane and retainer plates eliminated leakage (see Fig. 3).

In the present study, chamber volume was increased to 170 ml by enlarging the width of the retainer plate to 3 cm and the lumen diameter to 8.5 cm (Fig. 3). This resulted in decreasing the area:volume ratio to 0.67, which constituted a considerable reduction from the original ratio of 2.84 (McFeters and Stuart, 1972). This would increase the time required for a solute to reach equilibrium dialysis when its concentration inside equals the concentration outside. This point, however, was probably

never reached in the in situ experiments for dissolved substances accumulating inside the chamber as a product of microbiological activity, if ambient water can be considered as a dilute solution which was continuously being replaced. Therefore, a positive gradient was probably continuously maintained between microbiologically enriched media inside chambers and surrounding water in terms of waste products (eg. ammonium).

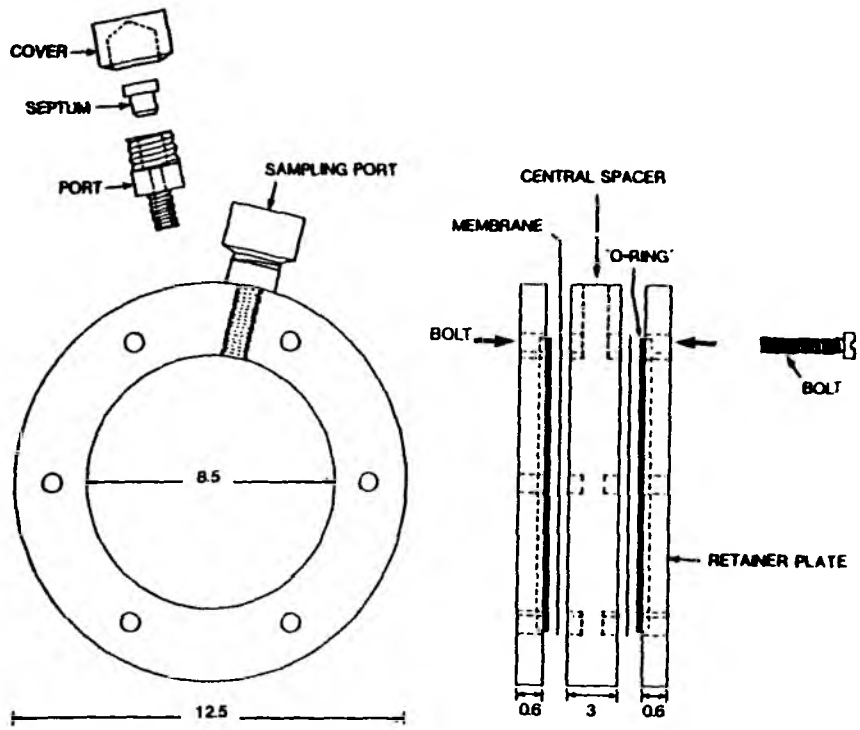


Figure 3. Diagram of a diffusion chamber. All measurements in cm.

2.2 Preliminary diffusion testing

a) Saffranin

To measure the effects of chamber design on diffusion efficiency, a plexiglas prototype with 6.5 cm width and 8.0 cm lumen diameter (0.31 area:volume ratio) equipped with 1.0 μm Nuclepore membrane was filled with a saffranin solution and submerged in an agitated tap water bath at 19 °C. At different time intervals, samples were taken from both chamber and aquarium water to measure the change in dye concentration. Samples were read with a spectrophotometer at 520 nm. The saffranin solution in the chamber had a starting optical density of 1.0. Dye concentration decreased by 16% after 2 h, 26% after 8 h and 85%

after 19 h. Dye diffusion approached steady-state conditions around this time, since 5 h later dye concentration inside the chamber had decreased only to 90%, after which virtually no change could be measured. A two-fold end-point gradient existed between dye concentrations inside and outside the chamber.

A similar dye diffusion test performed with 3 μm pore size membrane gave a decrease of 87 % in optical density after only 5 h. After this point, practically no further change could be detected. A two-fold gradient between internal and external concentrations had also been established by this time. Finally, two chamber prototypes with 3 and 4.5 cm widths (area:volume 0.67 and 0.45) equipped with 1 μm Nuclepore were tested filled with a saffranin solution of 0.5 optical density and submerged in a 19°C agitated freshwater bath. After 5 h, dye concentration in the 3 cm-width chamber had decreased by 63% and in the 4.5 cm-width chamber by 59%. After 8 h, near steady-state was reached in the 3 cm chamber with a dye density decrease of 87%.

From these diffusion tests, it could be calculated that a three-fold reduction in pore size (from 3 to 1 μm) resulted in a four-fold increase in the time taken to reach steady-state conditions (from 5 to 19 h) in a chamber with 0.31 area to volume ratio. For comparison, in 40 ml chambers (1.575 area:volume) equipped with 0.2 μm Nuclepore membrane and submerged in 13 °C seawater, a 86 % decrease in saffranin concentration was observed after 6 hr, at which point near steady-state conditions were reached (Anderson et al., 1983). Therefore, the effect of restricting diffusion by a five-fold decrease in pore size (1 to 0.2 μm) was cancelled with a five-fold increase in area to volume ratio (0.31 to 1.575).

These effects have to be taken into consideration when designing chambers. It could be expected that using 0.2 μm Nuclepore membrane with the chamber design in the present study (0.67 area:volume) would increase the time needed to reach steady-state to 36 h. However, this effect would be mitigated under environmental conditions, where a strong gradient was probably maintained across the membrane, thus keeping diffusion at maximum rates. Thus, the final chamber design constituted a compromise to achieve as large a volume of culture as possible, while keeping area:volume ratio close to 1 to promote equilibrium dialysis.

b) Glucose

In McFeters and Stuart (1972), comparison of glucose diffusion between chambers with 0.45 µm membrane and dialysis bags with comparable area to volume ratio showed that the initial diffusion rate into chambers was more than double that into dialysis bags. Furthermore, at steady-state conditions, the concentration inside chambers was higher than in dialysis bags. This was not surprising considering that standard viscose bags have an average pore size of 0.003 µm.

To compare the response of microbial populations outside and inside chambers to an external pool of glucose, two sterilized plexiglas chambers (3 and 4.5 cm widths) equipped with 0.2 µm Nuclepore membrane were filled with IfM pier water taken on 22 June 1987 at 1 m (12.2 °C, 13.9 S X 10^3) and placed in a sterile glass aquarium containing 3 l of the same water, to which was added 15 mg l^{-1} glucose. Incubation was done at room temperature in the dark with continuous agitation of aquarium water. Samples were taken at different times and prepared for epifluorescence miscroscopy as described in 1.1-b). Development of heterotrophic nanoflagellate and bacteria populations is plotted in Figure 4.

The same experiment was repeated on 10 July 1987 with water taken at 2 m (16.7 °C, 14.6 S X 10^3) and prefiltered through 3 µm sterile Nuclepore membrane. Results are plotted in Figure 4. In both experiments, population trends were parallel in ambient and chamber media, although some discrepancy existed in cell counts, particularly during the June experiment when intact water was used. It was also interesting that the 4.5 cm-width chamber showed lowest final concentrations of flagellates in both experiments. With the glucose concentration added, osmotrophy by flagellate cells was probably significant to sustain a large fraction of their oxidative metabolism. Therefore, lower population density in the chamber with smaller area:volume could reflect lower internal concentrations of glucose. In conclusion, these experiments demonstrated that the response of ambient and chamber populations to an ambient pool of nutrients was for the most part simultaneous and that population trends were virtually identical. This would argue in favour of chamber cultures being able to maintain sufficient chemical continuum with the environment for the development of natural microbial assemblages.

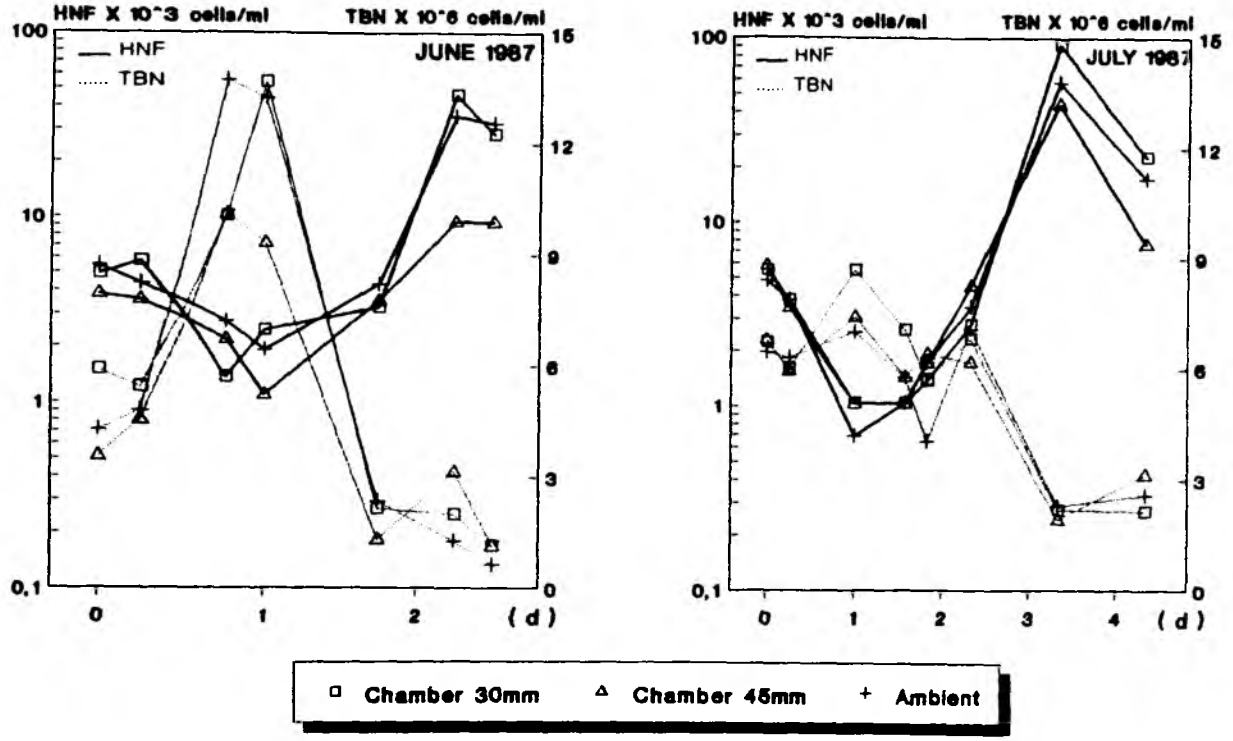


Figure 4. Development of heterotrophic nanoflagellates (HNF) and bacteria (TBN) in two chambers with different widths and in ambient water spiked with glucose.

2.3 In situ experiments

a) Chamber preparation

Prior to assembly, chamber parts were scrubbed and soaked overnight with Edisonite laboratory grade detergent. Then, after a dilute acid wash (5 % HCl), all pieces were repeatedly rinsed with deionized water and, after a final BDW rinse, allowed to air dry. Assembled chambers were then autoclaved (100 °C, 30 min.) in separate recipients. Five chambers were prepared for each experiment. Two were equipped with 0.2 µm Nuclepore membrane and filled with Whatman GF/C (ca. 1 µm particle size retention) filtered IfM pier water under sterile conditions. These chambers (coded c1 and c2) provided measurements of bacteria growth and production in the absence of grazing. The other three chambers were mounted either with 1.0 µm or with 0.2 µm Nuclepore (2 + 1) and filled with unfiltered IfM pier water, thus, providing estimates of nanoflagellate growth and grazing impact on bacteria. These will be referred to as c3 and c4 (1.0 µm

membrane) and c5 (0.2 µm membrane). Chamber c5 provided a control for bacteria loss across 1.0 µm membrane of c3 and c4.

Intact water was used for grazer cultures, since pre-filtration (5 to 20 µm) not only can remove loricate choano-flagellates and flagellates associated to detrital particles or diatom frustules (see sections 3.1 and 3.2), but also can cause damage to cells. Experimental water was collected off the IfM pier at 1 m with a sterile bottle sampler. Chambers were rinsed once and then filled inside sterile recipients containing the same water. Sterile silicon septa were inserted in the sampling port and sterile covers screwed on (see Fig. 3). Finally, chambers were hung from the pier at 1 m depth.

A chamber holder equipped with a protective teflon cylinder and bottom grate was at first used. This set-up floated freely by means of a large plastic buoy along a steel cable attached to a pier platform. However, this mechanism was exposed to surface wave action and rupture of chamber membranes occurred during strong wind events. Therefore, the holder was abandoned after the first two experiments and chambers were simply hung individually with a nylon rope tied to the end of two poles attached transversely to the end of the pier. Rope length was adjusted once or twice a day to maintain chambers at 1 m depth. No further membrane rupture occurred with this method. The chamber holder, nevertheless, would be useful in protected tidal environments, where it would be essential to allow for continously changing water levels.

b) Sampling strategy

Chambers and ambient water were sampled once or twice a day. Temperature and salinity was measured at 1 and 5m with a conductivity probe (WTW, F.R.G.) at every sampling. A five ml sample was taken from each chamber, after rotating it 6 -8 X, by piercing the silicon stopper using sterile needles (0.9 X 50 mm, 20 gauge) and syringes. The sample was then injected into vials containing 300 µl of 6% glutaraldehyde prepared as in 1.1-b). Vials were kept refrigerated, no longer than two days, until prepared for epifluorescent counting. A 3.2 ml fraction from each sample was filtered for nanoplankton counting (see section 1.1-b) and the remaining 2.1 ml filtered for TBN and bacteria biomass determination (see section 1.1-d).

For MCV measurement, a total of 50 bacteria was measured in each sample. The difference between MCV_{50} and MCV_{100} in a series of samples averaged 12% (n = 43). To analyse grazing impact on bacteria sizes, bacteria were divided into eight size classes with approximately doubling average volumes. Table 2 gives a description of classes and cell volumes.

A total of seven *in situ* grazing experiments were performed at different times of the year, namely October 1987, and January, March, May, July, August and October 1988. Experiments lasted about a week or until a sustained growth period of heterotrophic nanoflagellates had been observed. For the October 1987 experiment, a sixth chamber (6.5 cm width, plexiglas) was deployed to supply a third bacterial control. It is to be noted that, one control chamber was lost due to membrane rupture in the middle of October 87 and January 88 experiments.

c) Determination of growth and grazing rates

Growth of heterotrophic nanoflagellates (HNF) and bacteria was calculated assuming exponential growth by

(1) $$\mu = (\ln N_t - \ln N_o)/t$$

where N_o and N_t are cell counts per ml at the beginning and end of a sampling interval t. Bacteria growth rates were calculated in two ways: 1) from c1 and c2 with N_t as the maximum TBN observed, and 2) from c3, c4 and c5 during the initial lag period of HNF populations, which usually lasted one or two days. Although exchange of small bacteria is possible across 1.0 µm membranes and could result in net loss of small bacteria from chambers c3 and c4, this in fact did not occur to any detectable extent. Since no significant differences were observed in TBN or bacteria MCV in c3, c4 and c5, results from the three grazer chambers were treated as an average.

Grazing rates were calculated with equations derived from Landry *et al.* (1984) and Fritz (1986). The observed decrease in bacteria in chambers c3, c4 and c5, assumed due to grazing, was calculated by

(2) $$r = (\ln N_t - \ln N_o)/t \qquad r < 0$$

Since bacteria were growing while being grazed, then r is a result of the difference between bacteria growth rate (µ) and

grazing rate (g), thus

(3) $$r = \mu - g$$

The clearance rate (F) or volume cleared of bacteria per flagellate per unit time was defined as

(4) $$F = g/P$$

with P being the mean density of HNF during the sampling interval, which was calculated assuming exponential growth as

(5) $$P = (X_t - X_o)/(\ln X_t - \ln X_o) \qquad X = \text{HNF/ml}$$

Ingestion rate (I), expressed as average number of bacteria consumed per flagellate per unit time, was given by (6) with B in (7) being mean TBN assuming exponential growth

(6) $$I = F \times B$$

(7) $$B = (Y_t - Y_o)/(\ln Y_t - \ln Y_o) \qquad Y = \text{TBN/ml}$$

Bacterial production in c1 and c2 was estimated by the increase in biomass (see section 1.1-d) during a sampling interval. Total biomass grazed was calculated as the difference between biomass production in c1 and c2 and the observed decrease in biomasss in c3, c4 and c5 for a given sampling interval. Then, dividing by the mean HNF abundance present during this time, given by equation (5), an estimate of bacteria biomass grazed per flagellate per unit time was obtained. Finally, HNF growth efficiency could be assessed as the increase in HNF biomass relative to bacteria biomass grazed for a given sampling interval, namely

(8) $$E\ (\%) = \{(C_t - C_o)/t\}_{HNF}/\{(C_t - C_o)/t\}_{BG}$$

with $\{(C_t - C_o)/t\}_{HNF}$: HNF biomass production in µg C/l/d

$\{(C_t - C_o)/t\}_{BG}$: Bacteria biomass grazed ,, given by the difference between $(C_t - C_o)/t$ in control chambers (c1 & c2) and $(C_t - C_o)/t$ in grazer chambers (c3, c4 & c5). HNF biomass was estimated as in section 1.1 - c).

2.4 Tritiated thymidine incorporation

To obtain independent estimates of bacterial production, incorporation of ^{3}H-thymidine was measured during four grazing experiments (October 1987, and July, August and October 1988). A

modification of the Fuhrman and Azam (1982) method was employed. Samples taken from the chambers and ambient water were incubated as 7 ml triplicate aliquots (2 experimental + 1 blank) in 20 ml acid-washed polyethylene scintillation vials with 50 µl of a freshly prepared X10 dilution of the [methyl-^{3}H]-thymidine stock solution (40 Ci/mmol, Amersham), giving 18 nM end-point concentration.

After 1 h incubation at <u>in situ</u> (± 2 °C) temperature, incorporation was stopped with 0.5 ml of 5 M NaOH. Samples were then neutralized with 2.8 ml ice cold 100% (w/v) trichloroacetic acid (TCA, analysis grade, Merck). Preliminary testing revealed that this method reduced high blank counts, which occur when formalin was used as fixative. Neutralized samples were filtered using a stainless steel ramp and 0.2 µm cellulose nitrate filters (Sartorius) pre-soaked in 5% TCA. Filters were then rinsed 10X with 1 ml ice cold 5% TCA and placed in 5 ml Lumagel scintillation cocktail (J.T. Baker). Vials were counted after 4-5 days to allow complete solubilization of filter material and stabilization of scintillation counts, which increased substantially within this period.

At the beginning and end of grazing experiments, five replicate 10 ml aliquots were assayed (3 experimental + 2 blank) from each chamber with three concentrations of ^{3}H-thymidine (6, 12 and 18 nM) to check saturation kinetics. Tritiated thymidine incorporation (TTI) was performed during four grazing experiments (October 87, July, August and October 1988). In October 1987, particle-free formalin was used as fixative (1% final concentration) and incorporation results were obtained from J. Kuparinen (University of Helsinki). Chambers were sampled usually once a day in the morning. More frequent sampling was not possible, since it would introduce too large a dilution factor in the chamber cultures. TTI in pmol ml^{-1} h^{-1} was calculated as

$$TTI = dpm_C/(V \times t \times SA) \times F \quad (1)$$

with dpm_C: decays per min. or sample activity determined from scintillation counts (cpm) corrected for quenching and blank, V: sample volume, t: incubation time, SA: thymidine specific activity, F: 4.5 X 10^{-7} µCi dpm^{-1}.

Bacteria production adjusted to µg C l^{-1} h^{-1} was given by

(2) $$BP = TTI \times CF \times MCC$$

where CF: conversion factor of 1.1 X 10^9 cells $nmol^{-1}$ (Riemann et al., 1987), MCC: mean carbon content (fg C/cell) calculated as in section 1.1-d).

3. Descriptive Study

3.1 Epifluorescence microscopy

Several epifluorescent staining techniques were compared for the observation of heterotrophic nanoplankton, namely proflavin (Haas, 1982) described in section 1.1-b), DAPI or 4',6-diamidino-2-phenylindole (Porter and Feig, 1980) and acridine orange described in section 1.1-d). A double-epifluorescent staining with proflavin and DAPI was tested, whereby 400 µl of a 0.4 µg ml^{-1} freshly prepared dilution from frozen 1 mg ml^{-1} stock solution was added at the same time as proflavin and stained for 5 min. Sample was then filtered onto Sudan Schwartz stained 0.2 µm Nuclepore filters and observed with a Zeiss microscope equipped with switchable filter set 47 77 09 (blue excitation) for proflavin fluorescence and filter set 47 77 02 (UV excitation) for DAPI fluorescence. Photomicrographs were taken with Kodak Ektachrome 400 ASA film.

3.2 Scanning electron microscopy

Samples were fixed with glutaraldehyde (analysis grade, two-step fixation 0.3 % and 1% final concentration) and filtered onto 13 mm diam. 0.2 µm Nuclepore filters. Filters were then processed through an ethanol dehydration series (30, 40, 70, 90% and absolute) allowing 10 min for each step. Filters were then critical point dried in 100% ethanol with liquid CO_2 in a model 010 Balzer C.P.D. and mounted on aluminum stubs with conductive carbon cement (Leit C, Neubauer Chemikalien). Stubs were coated with Au-Pd under vacuum in a model 004 Balzer Sputter Coating Device and observed with a Zeiss Nanolab 7 scanning electron microscope.

RESULTS

1. Environmental Study

1.1 IfM monitoring

a) Temperature and salinity

Water temperatures followed a well-defined seasonal trend at both 0 m (sub-surface) and 5 m at the IfM station (Fig. 5) with largest fluctuations in the summer. It was also noted that spring warming exhibited some variability as opposed to more continous fall cooling. Significant differences in temperature between surface and bottom existed only in the summer. At 0 m, maximum water temperature observed in 1987 was 16.2 °C (26 August), in 1988 18.3 °C (10 August) and in 1989 19.6 °C (24 August). Surface winter minima reached 2.3 °C at the beginning of March 1988 and 4.4 °C from early February to early March 1989. At 5 m, summer maxima reached 16.3 °C on 26 August 1987, 16.6 °C on 16 June 1988 and 19.5 °C on 21 June 1989. Winter minima in bottom waters were 4.4 °C in February 1988 and 1989.

In general, salinity exhibited substantial variations at both depths, somewhat dampened at 5 m (Fig. 5). No pronounced seasonal trends were evident, although salinities tended to decrease in the spring. The water column oscillated frequently from stratified to destratified conditions, as given by maximum and minimum surface to bottom salinity differences. The pattern indicated aperiodical wind-driven circulation. Two sustained periods of destratification lasting over several weeks were observed in February 1988 and 1989.

b) Phytoplankton

Chlorophyll a and pigmented nanoflagellates (PNF) revealed considerable variation with higher values observed usually at the surface (Fig. 6). High variability tended to mask seasonal trends. Maximum chlorophyll concentrations in surface waters reached 47.1 $\mu g\ l^{-1}$ in 1987 (30 September), 33.3 $\mu g\ l^{-1}$ in 1988 (18 May) and 22.4 $\mu g\ l^{-1}$ in 1989 (30 May). PNF at the surface exhibited maximum abundances of 14.3 10^3 cells ml^{-1} in 1987 (30 September), 22.7 X 10^3 cells ml^{-1} in 1988 (29 June) and 12.1 X 10^3 cells ml^{-1} in 1989 (27 April). Correlation between chlorophyll and phytoflagellates was low at both depths with a somewhat higher regression coefficient at the surface (r^2 = 0.317, $P < 0.001$) than in bottom waters (r^2 = 0.214, $P < 0.001$).

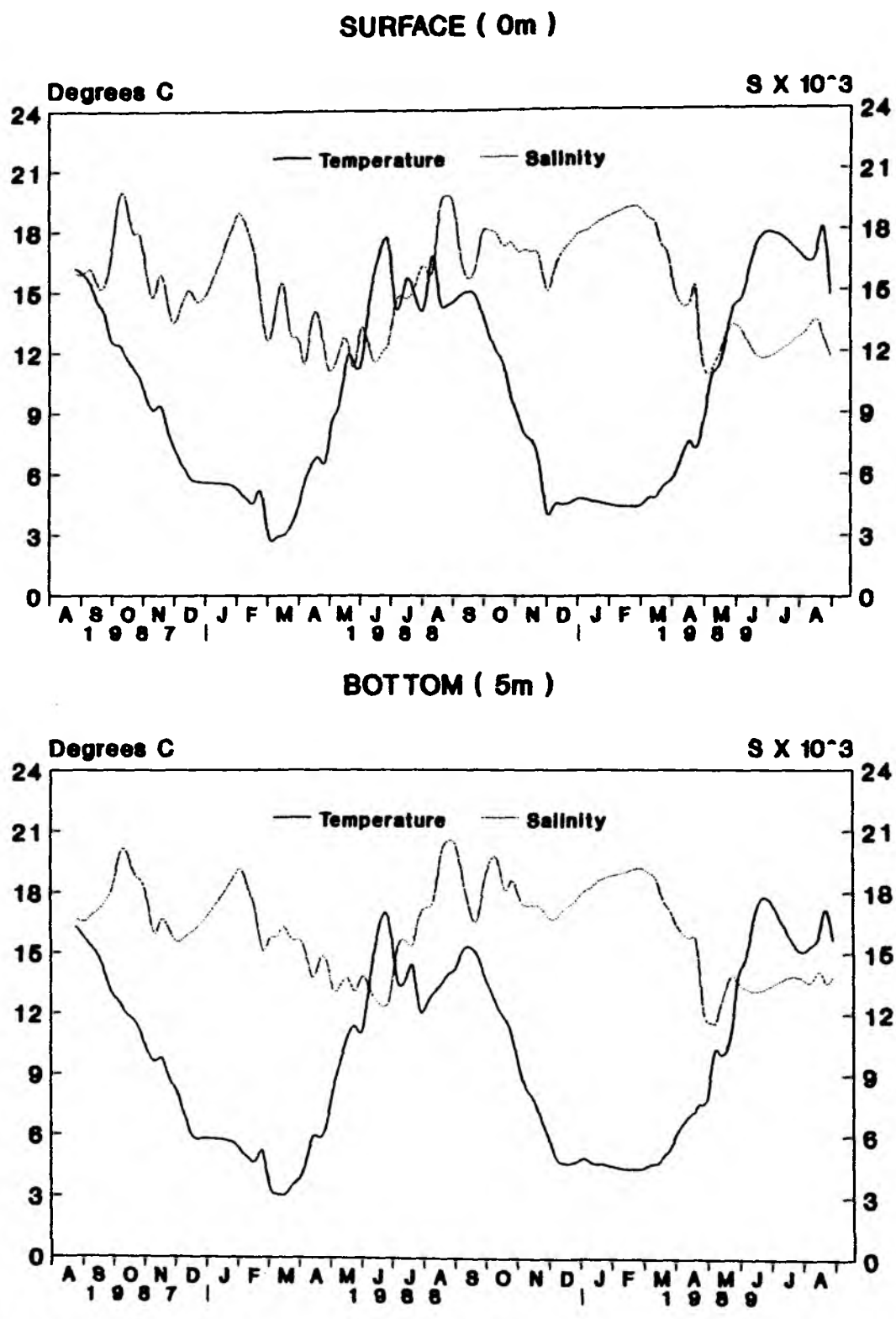


Figure 5. Water temperature and salinity at the IfM station (curved lines).

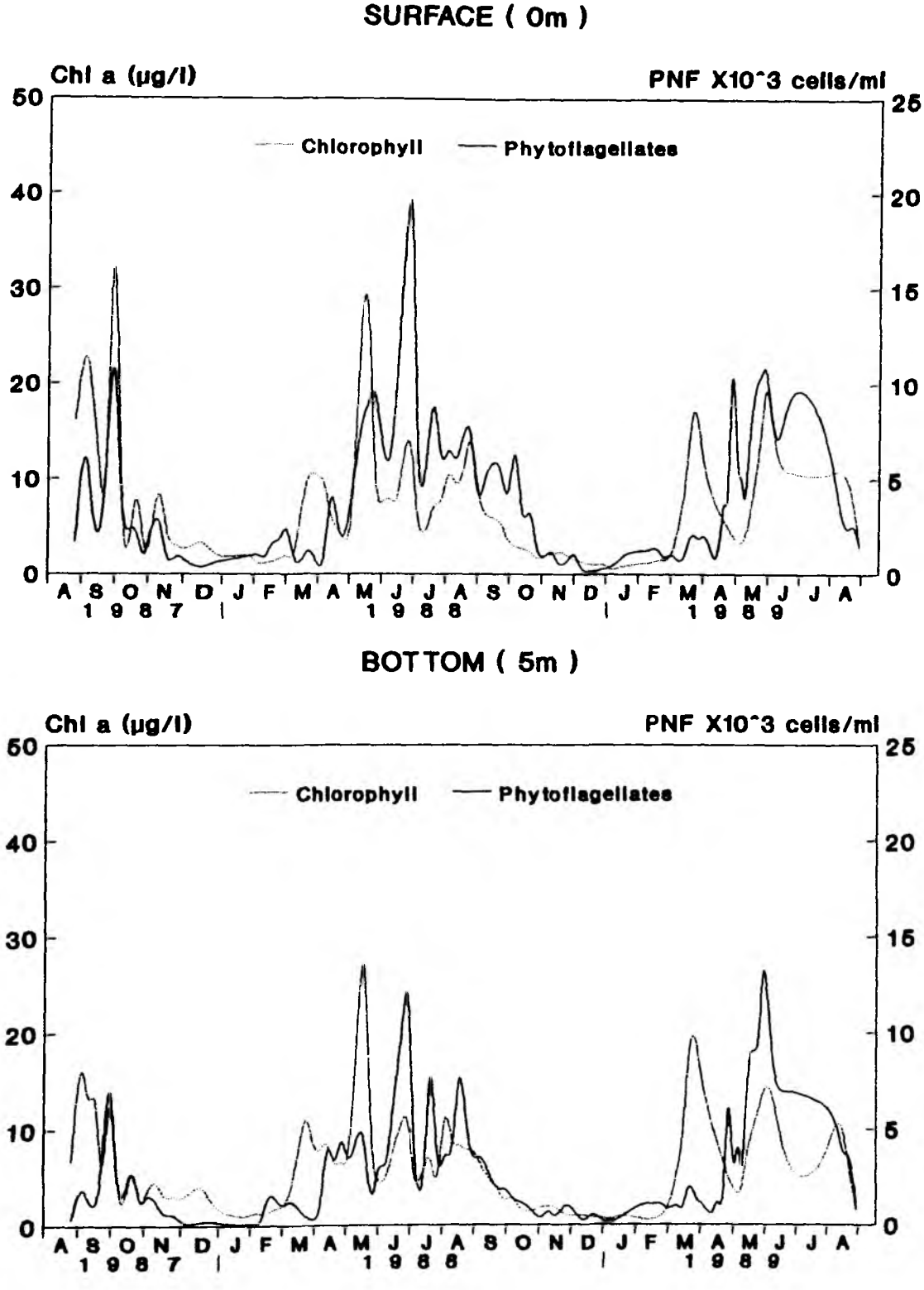


Figure 6. Chlorophyll a and pigmented nanoflagellates (PNF) at the IfM station (curved lines).

Chlorophyll predicted primary production better (r^2 = 0.546, P < 0.0001) than phytoflagellate counts (r^2 = 0.402, P < 0.0001) at the surface. There was on the average a 20-fold difference in primary production between 0 m and 5 m. PNF blooms were usually dominated by orange-fluorescing cryptomonad forms (2-6 µm width, 4-10 µm length) with the exception of May 1988, when naked cells of the silicoflagellate Dictyocha speculum (syn. Distephanus speculum, Ehrenberg) predominated (10-20 µm diam.). This bloom was present from 3 to 25 May at both depths reaching a maximum abundance of 7.3 $X10^3$ cells ml^{-1} on 18 May at the surface with 88% naked cells (see micrographs in sections 3.1 and 3.2). It was further observed that another unusual `bloom' of small orange-fluorescing comma-shaped structures (1 µm diam., 5 µm length) occurred simultaneously. These structures were observed not only freely dispersed or in small clusters in the water, but also attached to naked D. speculum cells (see micrographs in 3.1). Highest concentrations (1.3 $X10^5$ structures ml^{-1}) were also detected on 18 May at 0 m.

Cyanobacteria maxima (Fig. 7) reached 14.4 and 13.7 X 10^4 cells ml^{-1} on 23 June 1988, and, 8.7 and 10.5 X 10^4 cells ml^{-1} on 9 June 1989, at 0 and 5 m respectively. In 1987, peak counts were observed on 21 October with 4.5 $X10^4$ cells ml^{-1} at 0m and 4.8 X 10^4 cells ml^{-1} at 5 m. During summer, cyanobacteria at 0 and 5 m averaged 5.2 and 5.5 $X10^4$ cells ml^{-1} in 1988 and, 4.1 and 4.7 X 10^4 cells ml^{-1} in 1989, respectively. Higher counts were generally detected in bottom waters.

Cyanobacteria observed at the IfM station were usually dispersed, orange-fluorescing small coccoid cells (≤ 1 µm diam.) resembling Synechococcus, which rarely appeared in colonies. Cells were divided into two size categories, namely a < 1 µm group (0.5-0.7 X 1-1.2 µm) with 0.3 $µm^3$ average volume and a 1 µm group (1 X 1.5 µm) with 0.8 $µm^3$ average volume. The large majority of cells counted at the IfM station belonged to the first group. During summer peak counts (June 1988 and 1989), cyanobacteria MCV averaged 0.35 $µm^3$ at both depths.

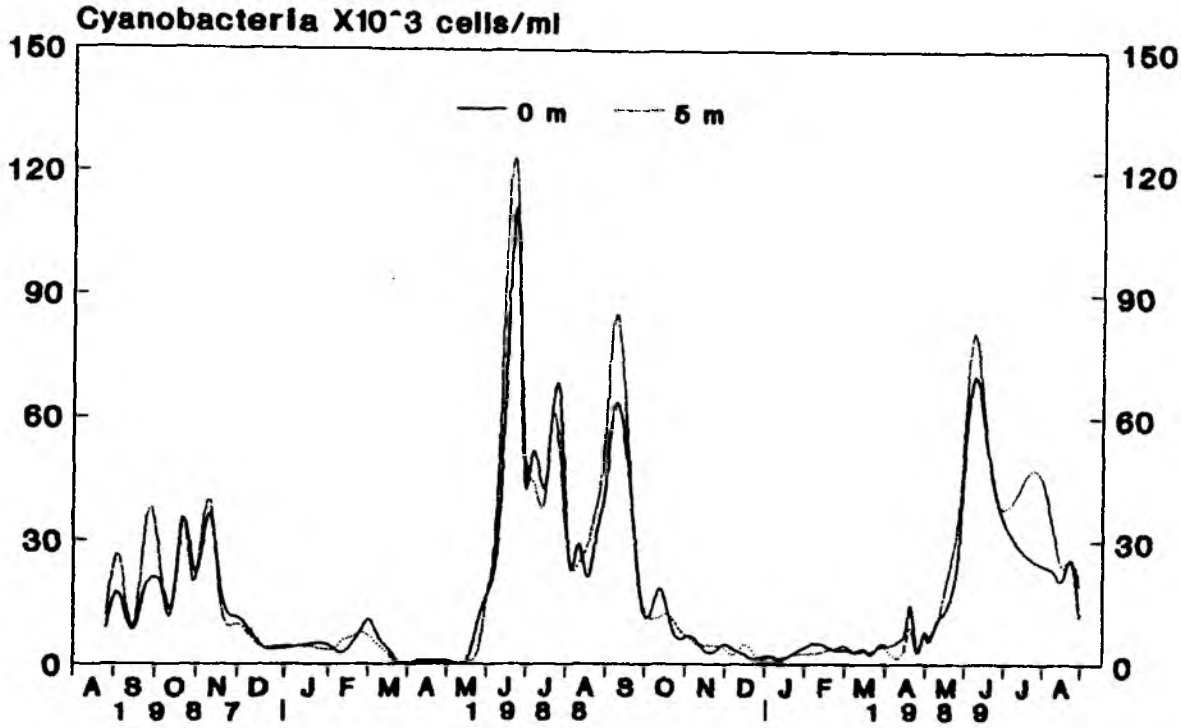


Figure 7. Cyanobacteria counts at the IfM station (curved lines).

c) Heterotrophic nanoplankton

HNF counts showed a more or less well-defined seasonality (Fig. 8) with maxima observed from late spring to early fall and minima during winter (mid-December to mid-February). Large fluctuations were observed in the spring and summer. In surface waters, peak numbers reached 16.5 X 10^3 cells ml^{-1} in 1987 (3 September) and 14.7 X 10^3 cells ml^{-1} on both 15 June 1988 and 3 May 1989. Cell numbers were usually higher at the surface. At 5 m, peak counts were 15.8 X 10^3 cells ml^{-1} on 3 September 1987, 17.2 X 10^3 cells ml^{-1} on 16 June 1988 and 10.5 X 10^3 cells ml^{-1} on 3 May 1989. In the winter, numbers remained generally in a low range of 1 to 2 X 10^3 cells ml^{-1}. An exception occurred on 11 May 1988 during the D. speculum bloom, when HNF abundance decreased to 0.3 X 10^3 cells ml^{-1}, which represented the lowest observed value for the entire study. HNF counts were not very strongly correlated with temperature with higher regression coefficient at 0 m (r^2 = 0.317, $P < 0.001$) than at 5 m (r^2 = 0.204, $P < 0.001$).

MCV and biomass of HNF (Fig. 9) also exhibited seasonal trends at both depths with generally higher values at the surface. Peak MCV and biomass values at 0 m reached 109 μm^3 and 164 μg C l^{-1} on 23 June 1988, and, 81 μm^3 and 155 μg C l^{-1} on 7

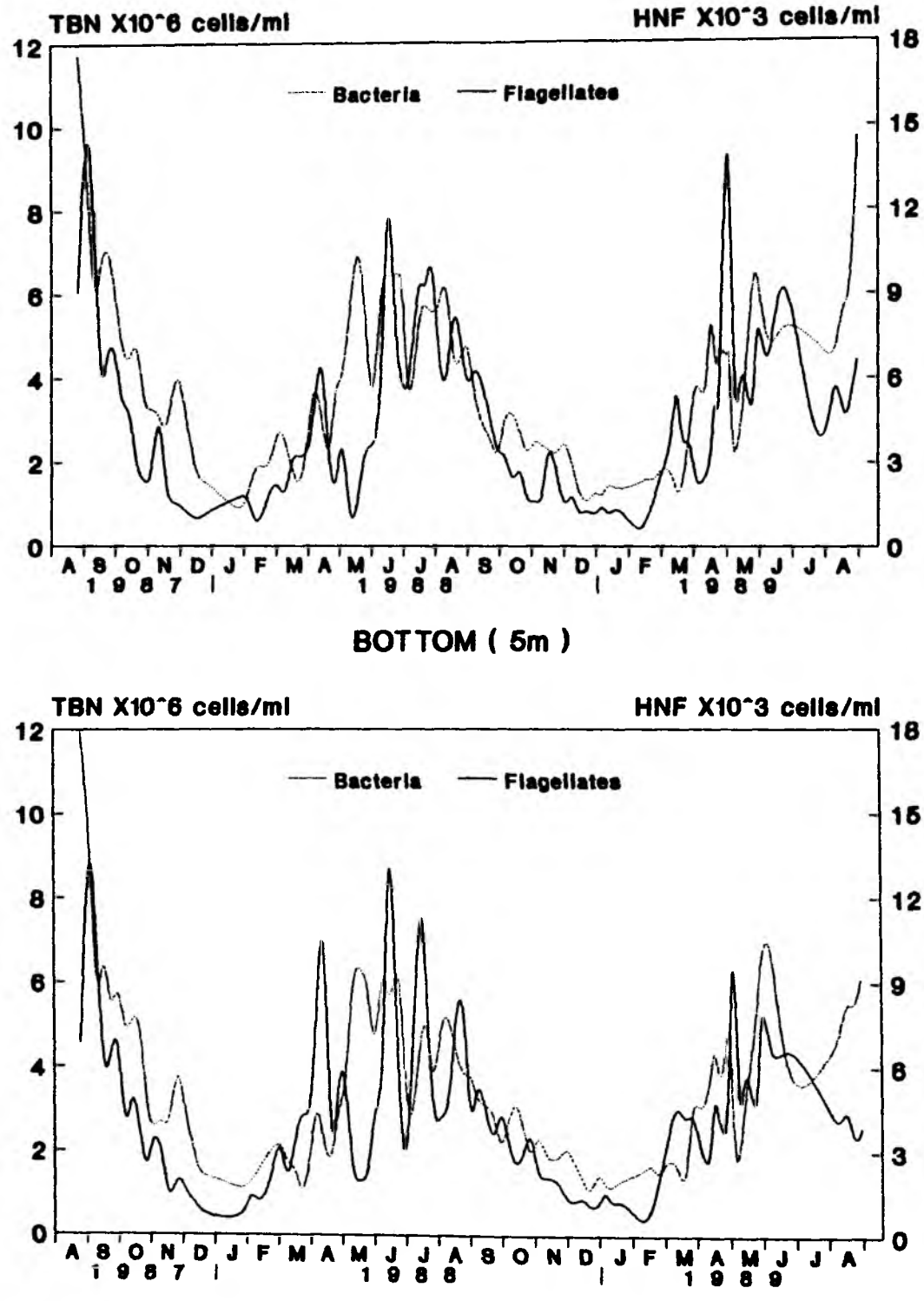


Figure 8. Total bacteria number (TBN) and heterotrophic nanoflagellates (HNF) at the IfM station (curved lines).

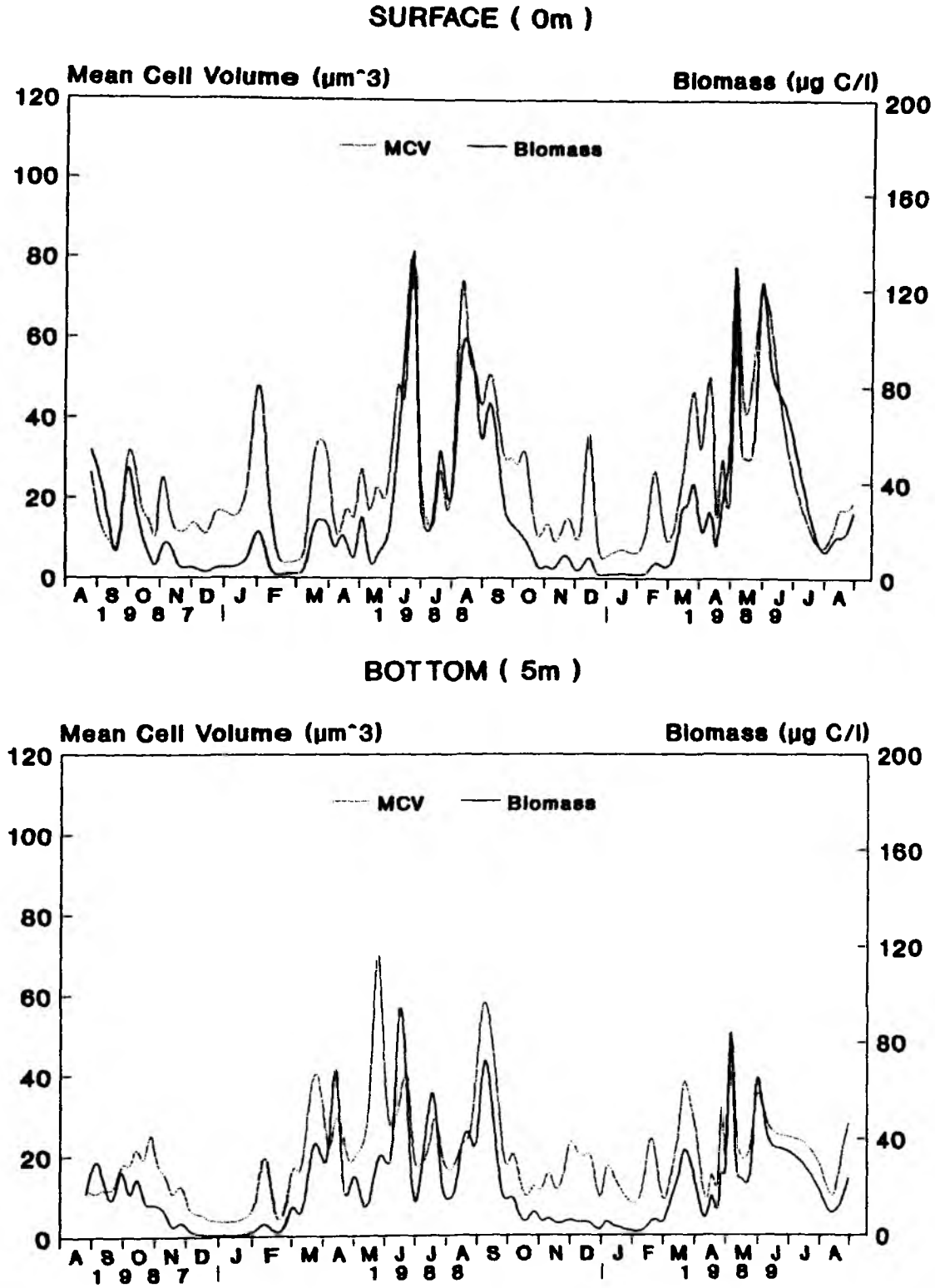
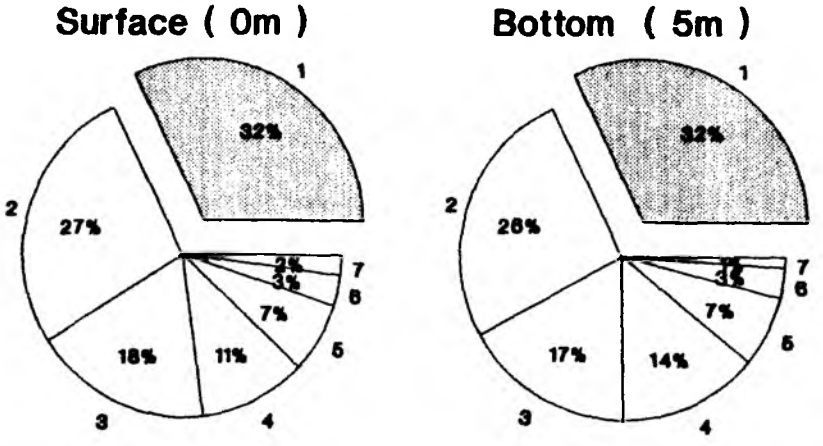


Figure 9. Mean cell volume (MCV) and biomass of heterotrophic flagellates at the IfM station (curved lines).

May 1989. Overall average MCV was 26 μm^3 at 0 m and 23 μm^3 at 5 m for the entire study. Overall mean biomass was also slightly higher at the surface (29 $\mu g\ C\ l^{-1}$) than near the bottom (23 $\mu g\ C\ l^{-1}$). For the entire study period, the most frequently encountered size class had the smallest average volume (class 1: 2 μm^3) with an overall mean frequency of 32 % at both depths (Fig. 10). It could also be seen that relative importance decreased with increasing size class.

It was noticed that HNF, mostly choanoflagellates (see micrographs in 3.1 and 3.2), started to increase in number and MCV at both depths at the beginning of March, prior to the spring phytoplankton bloom, which occurred sometime between the middle and end of March (see chlorophyll curves in Fig. 6). Choanoflagellates, mostly loricate forms, were the predominant group with an average frequency of 32% throughout March 1988 and 1989. Abundances decreased to background levels of ca. 1 X 10^3 cells ml^{-1} in April and May.



Average volume of HNF size-classes

Class 1: 2 μm^3 Class 2: 6 μm^3 Class 3: 14 μm^3 Class 4: 32 μm^3 Class 5: 68 μm^3 Class 6: 140 μm^3 Class 7: 288 μm^3

Figure 10. Average size-class distribution of heterotrophic nanoflagellates at the IfM station.

d) Heterotrophic bacterioplankton

TBN exhibited a better defined seasonality than HNF, although considerable variations were also present in the spring and summer (Fig. 8). Higher numbers were usually detected at the surface. At 0 m, TBN maxima were 11.8 X 10^6 cells ml^{-1} on 26 August 1987, 7.4 X 10^6 cells ml^{-1} on 18 May 1988 and 9.7 X 10^6 cells ml^{-1} on 30 August 1989. At 5 m, peak numbers reached 12.0 X 10^6 cells ml^{-1} on 26 August 1988, 6.9 X 10^6 cells ml^{-1} on 23 June 1988 and 7.3 X 10^6 cells ml^{-1} on 9 June 1989. Superimposed on seasonal trends, a certain degree of negative correlation on a short-term or weekly basis could be observed between HNF and TBN, particularly in surface waters (Fig. 8). The most striking example was detected in May 1988 during the D. speculum bloom when maxima in TBN and minima in HNF were simultaneously observed.

Temperature was the best predicting variable for TBN, given by linear regression with $r^2 = 0.515$ ($P < 0.0001$) at 0 m and $r^2 = 0.471$ ($P < 0.001$) at 5 m. A weaker positive correlation was found between TBN and HNF with regression coefficients of 0.38 at 0 m and 0.32 at 5 m ($n = 96$), when May cell counts were omitted. Multiple regression analyses with different variables revealed that the combination of temperature, HNF and chlorophyll gave the best prediction model for TBN with $r^2 = 0.63$ ($P < 0.0001$) at the surface and $r^2 = 0.62$ ($P < 0.0001$) at the bottom.

Bacteria MCV and biomass revealed opposite trends at both depths, whereby MCV tended to increase when biomass decreased (Fig. 11). The negative correlation between MCV and TBN was somewhat higher at 5m ($r = -0.55$) than at 0 m ($r = -0.51$) with $P < 0.0001$. Maximum MCV at the surface was observed at the end of winter, prior to the spring phytoplankton bloom. In surface waters, MCV reached 0.09 μm^3 on 24 February 1988 and 0.11 μm^3 on 2 March 1989. Near the bottom, largest MCV was measured on 13 April 1988 and on 10 March 1989 (0.10 μm^3). In 1988 and 1989, smallest MCV values occurred in the summer at both depths (0.04 - 0.05 μm^3). In 1987, smallest MCV was observed in the fall (0.03 μm^3) also at both depths. Seasonal averages of MCV were 0.06-0.07 μm^3 from mid-winter to early spring, and 0.04-0.05 μm^3 from late spring to early fall. For the entire study, MCV averaged 0.059 μm^3 at both depths.

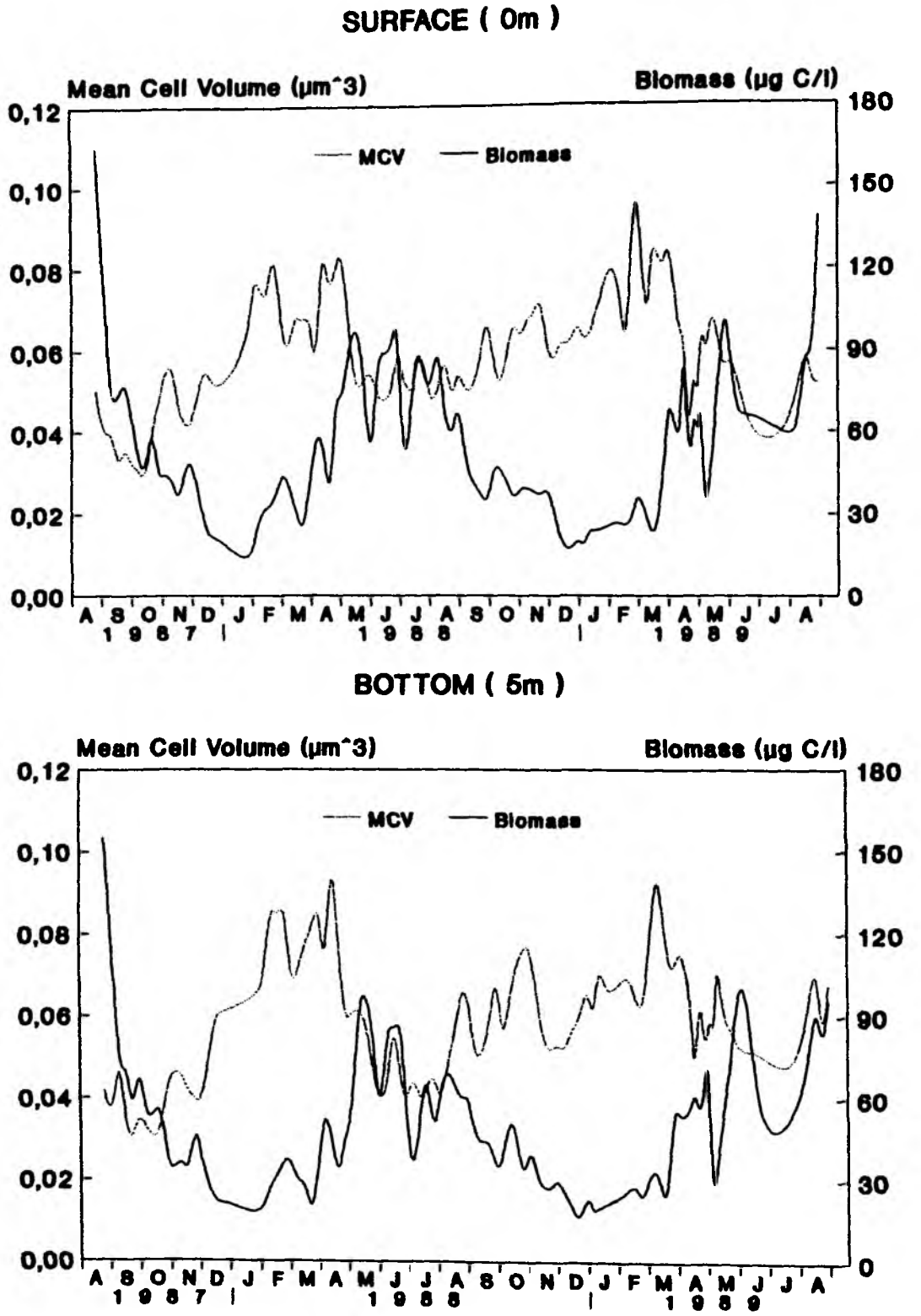
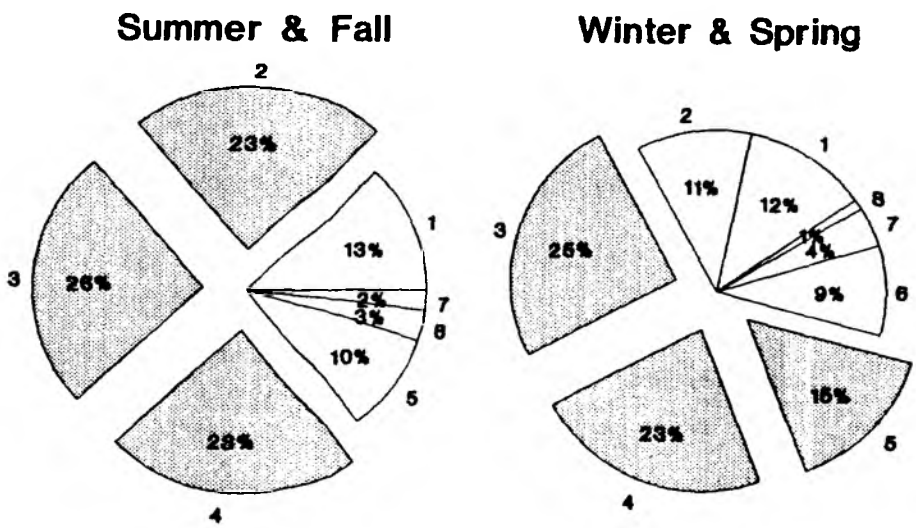


Figure 11. Bacterial mean cell volume (MCV) and biomass at the IfM station (curved lines).

Seasonal differences in bacteria size-class distribution are depicted in piecharts (Fig. 12). Descriptions of size-classes are given in Table 2. During winter and spring, classes 3, 4 and 5 predominated, whereas dominance was shifted to classes 2, 3 and 4 during summer and fall. Classes 6, 7 and 8 also increased in relative importance in the cold season. Overall mean frequencies of classes 1 to 8 were 13%, 12%, 26%, 24%, 13%, 8%, 3% and 1%, respectively. Therefore, mid-range sizes (3, 4 and 5) constituted more than 60% of all bacteria.



Average volume of bacteria size-classes

Class 1: 0.007 μm^3 Class 2: 0.012 μm^3 Class 3: 0.019 μm^3
Class 4: 0.047 μm^3 Class 5: 0.100 μm^3 Class 6: 0.195 μm^3
Class 7: 0.384 μm^3 Class 8: 0.684 μm^3

Figure 12. Seasonal differences in bacteria size-classes at the IfM station.

Time series analyses was performed on the variables TBN and MCV using a seasonal decomposition procedure (multiplicative method) to obtain an estimate of trends with the removal of short-term variability (Statgraphics, STSC Inc., 1986). Resulting moving averages (12 period length) are plotted in Figure 13 for the surface. Opposite long-term (seasonal) trends in MCV and TBN can be more clearly seen in these plots. Although the apex of the TBN curve coincided with the trough center of the MCV curve,

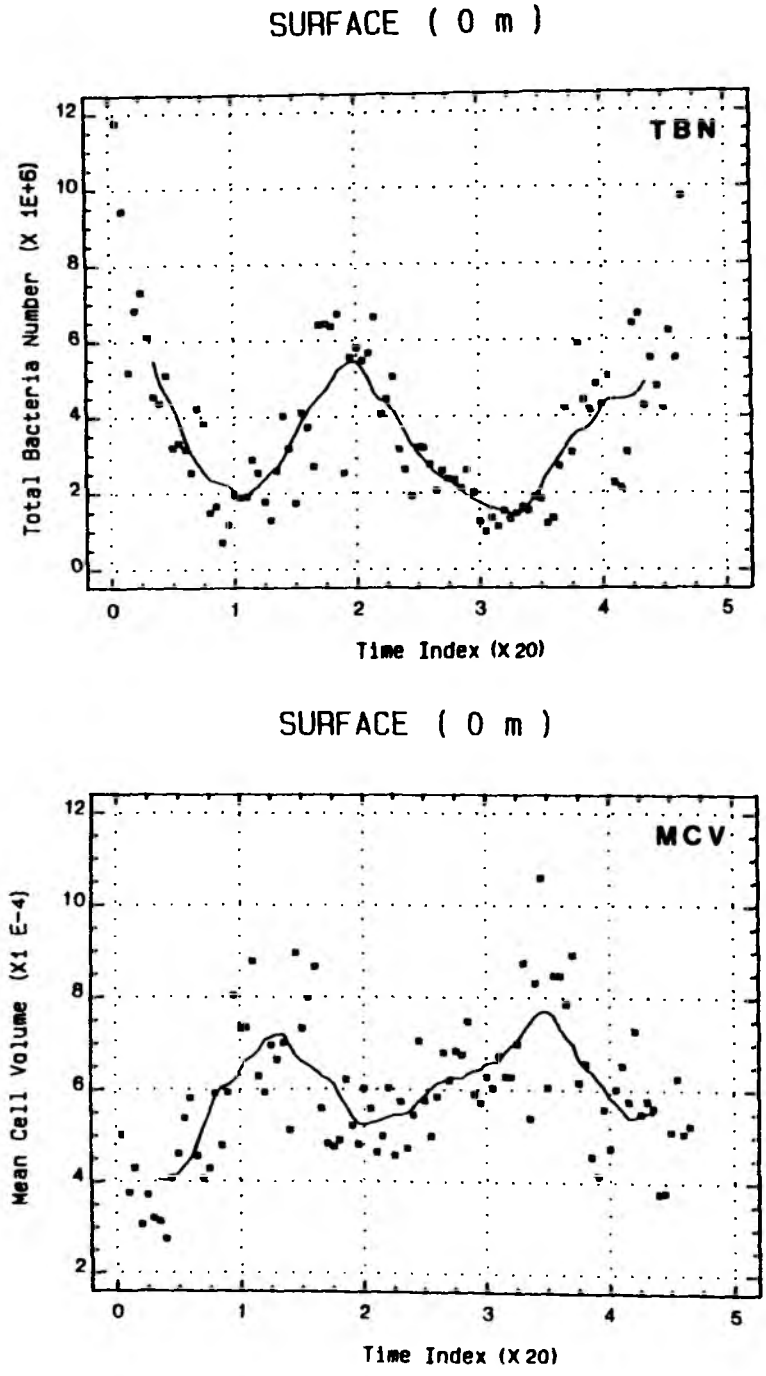


Figure 13. Time series analysis (seasonal decomposition) of total bacteria number (TBN) and mean cell volume (MCV) in surface waters at the IfM station. Time index intervals ca. 1 month.

MCV apices were offset by approximately one time index interval (ca. 1 month) from TBN trough centers. In other words, maximum numbers coincided with minimum cell size in the summer, whereas minimum numbers occurred in mid-winter and maximum cell size about one month later in early spring.

Biomass ratios of bacteria versus flagellates averaged 8.5 at 0 m and 6.9 at 5 m. Maximum ratio (124) was estimated on 11 May 1988 at the surface during the D. speculum bloom (see Fig. 14). Excluding this period, ratios averaged ca. 7 at both depths. Ratios tended to increase throughout fall and winter, as the HNF population was reduced to a few small cells, whereas bacteria decreased in number but increased in MCV, thus experiencing a smaller reduction in biomass relative to HNF during the cold season. Lowest ratios (≤ 1) were measured in the spring and summer at both depths, when HNF biomass often surpassed bacteria biomass.

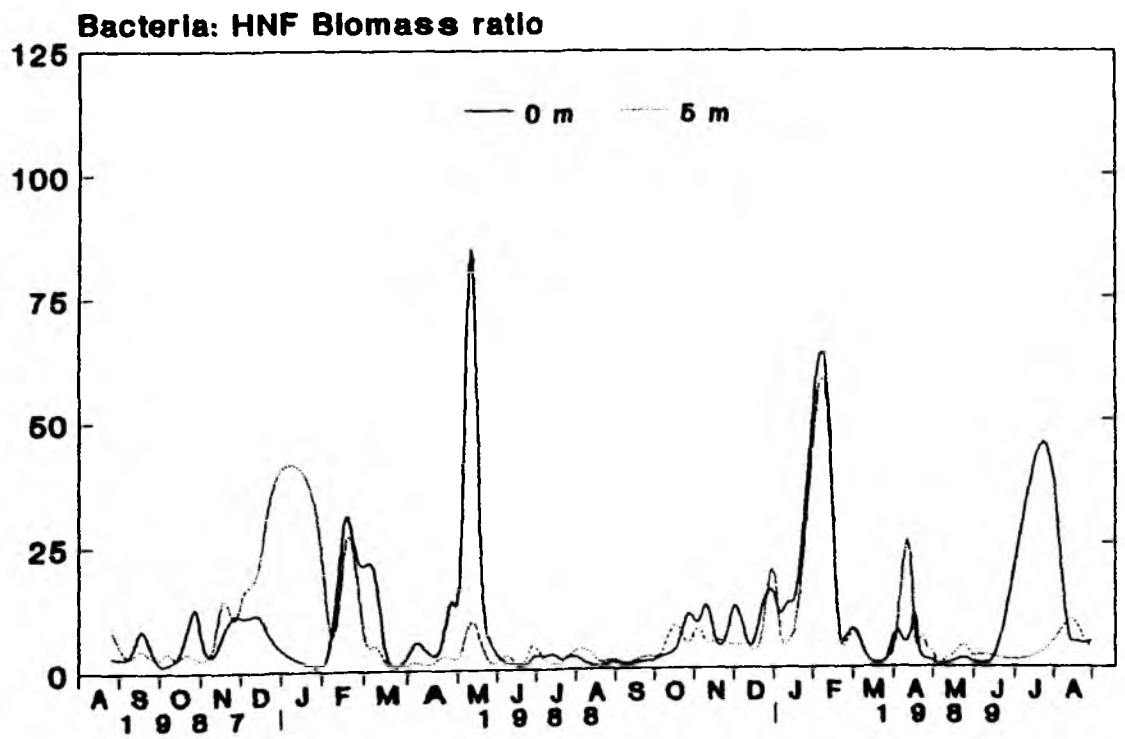


Figure 14. Biomass ratios of bacteria vs heterotrophic nanoflagellates at the IfM station (curved lines).

1.2 Western Baltic study

a) Basic environmental measurements

Water temperatures at three depths are plotted for the three Baltic monitoring (BMP) stations in Figure 15. These will be referred to as Kieler Bucht (KB), Boknis Eck (BE) and Fehmarn Belt (FB). Maximum surface temperatures in the summer were observed at KB in July (17.5 °C), at BE in June (16.4 °C) and at FE in August (16.4 °C). Minimum temperatures reached 2.7-2.8 °C in early March 1988 at the three stations. No significant differences in temperature regime were detected between KB and FB. BE revealed the largest divergence between surface and bottom temperatures in the summer.

Minimum salinities (data not shown) occurred in the spring at the three stations, namely, in surface waters, 1.9 S X 10^3 at KB in May 1988, 11.7 S X 10^3 at BE in May 1989 and 9.1 S X 10^3 at FB in April 1989. Maximum surface salinities (S X 10^3) reached 19.0 at KB in February 1989, 22.0 at BE in April 1988 and 18.3 at FB in January 1989. BE exhibited widest salinity fluctuations at the surface and FB the strongest stratification in the summer.

Chlorophyll a showed seasonal maxima in the fall during 1987 and 1988 (Fig. 15). Highest concentrations were measured in November at the three stations with FB showing generally lower levels than the other stations. Peak values reached 8 µg l^{-1} at KB, 12 µg C l^{-1} at BE and 5 µg C l^{-1} at FB. Chlorophyll was not correlated with either cyanobacteria or phytoflagellates ($r^2 < 0.05$, n = 35 to 44) at any of the stations.

b) Bacterioplankton

TBN showed a well-defined seasonality at the BMP stations, particularly at KB and BE (Fig. 16). Maximum TBN were counted in the spring and summer at all three depths. TBN in surface waters reached 4.7 X10^6 cells ml^{-1} at KB in June, 6.0 X10^6 cells ml^{-1} at BE in May and 3.0 X10^6 cells ml^{-1} at FB in August. Bacteria mean cell volume (Fig. 17) did not show any well-defined seasonal trends at any of the stations. Largest mean cell volumes in surface waters during 1988 were observed at KB in January (0.134 μm^3), at BE in February (0.111 μm^3) and at FB in August (0.070 μm^3). In contrast, bacteria biomass (Fig. 17) exhibited the same strong seasonal pattern as TBN with maximum values attained in late spring and summer at the three depths. In the surface layer

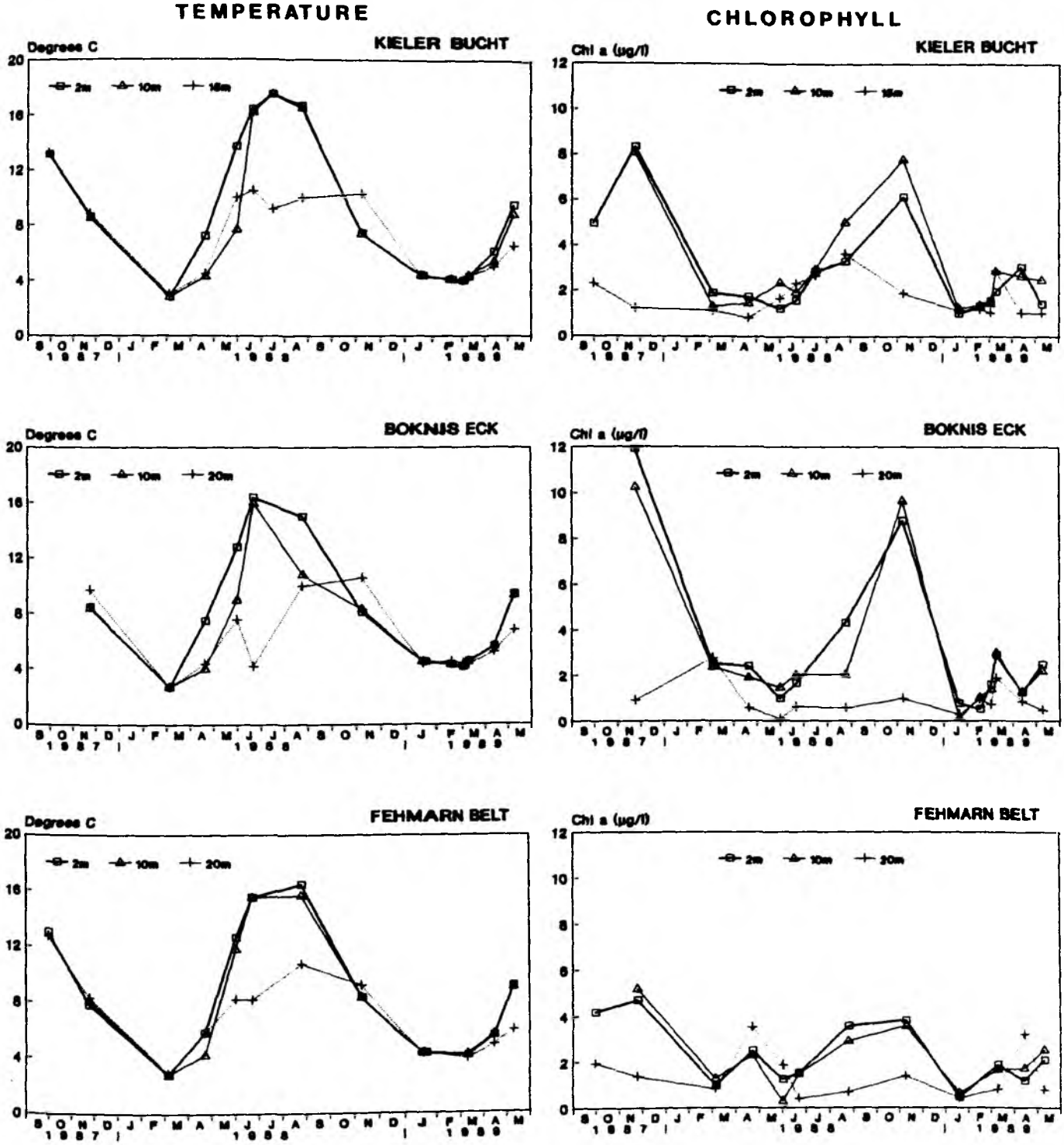


Figure 15. Water temperature and chlorophyll a at the three Western Baltic stations.

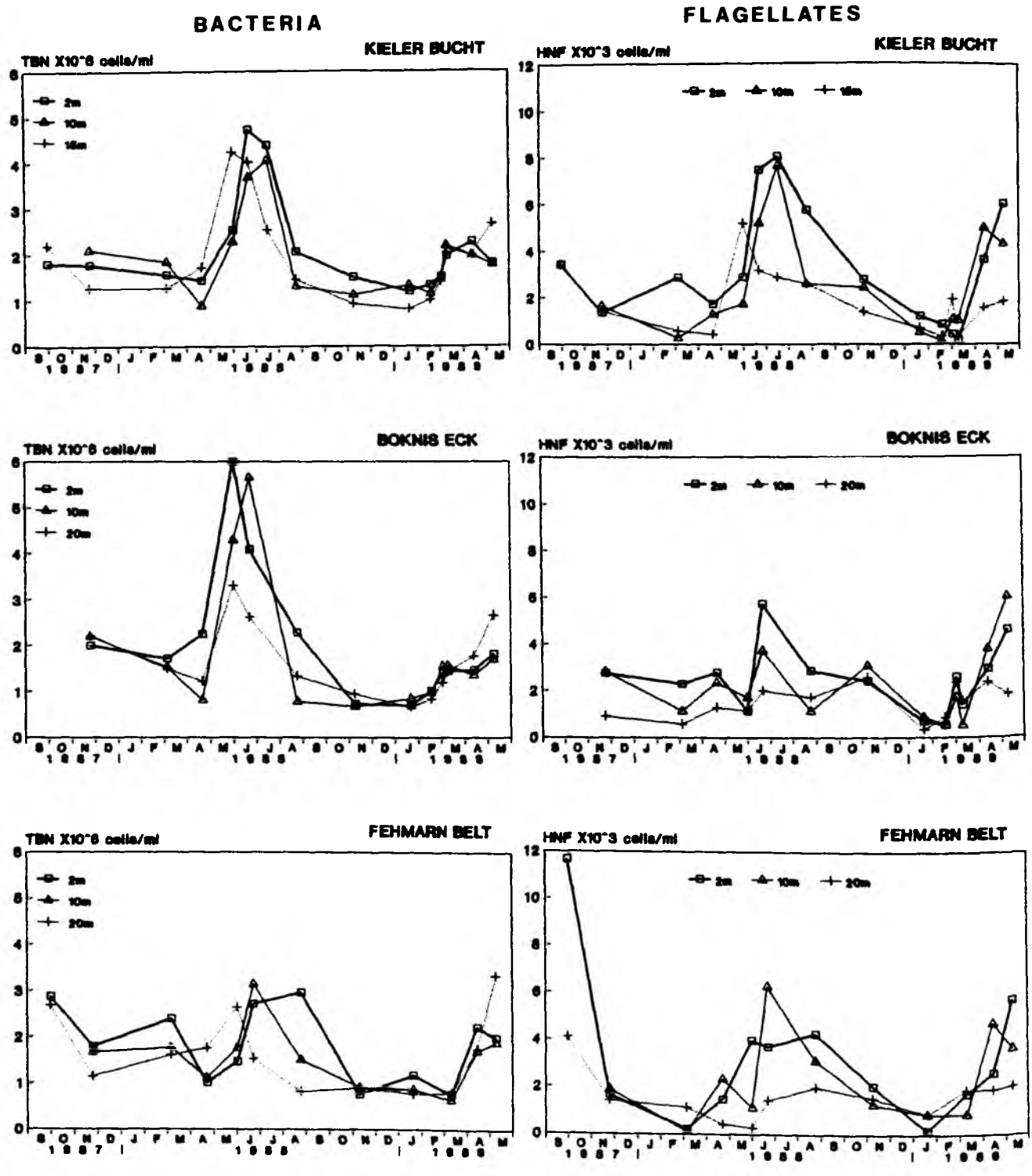


Figure 16. Total bacteria number (TBN) and heterotrophic nanoflagellates (HNF) at the three Western Baltic stations.

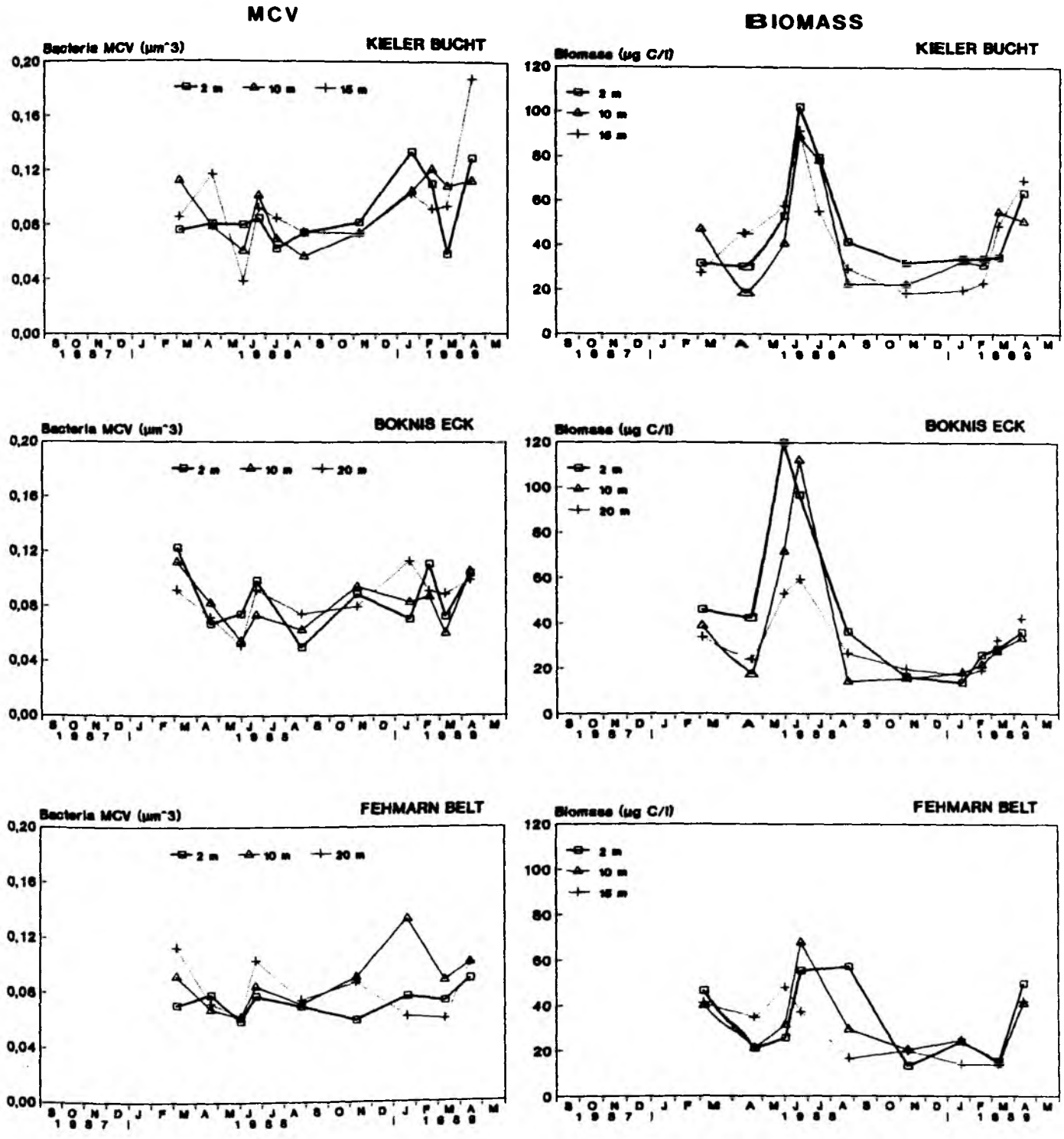


Figure 17. Bacterial mean cell volume (MCV) and biomass at the three Western Baltic stations.

during 1988, biomass reached 102 µg C l^{-1} at KB in June, 120 µg C l^{-1} at BE in May and 48 µg C l^{-1} in August at FB.

Cyanobacteria also exhibited strong seasonal trends similar to TBN, although blooms occurred one month later than heterotrophic bacteria (Fig. 18). BE showed generally lower concentrations than the other stations. Maximum abundances in the surface layer during 1988 reached 3.6 $X10^5$ cells ml^{-1} in July at KB, 2.2 $X10^5$ cells ml^{-1} at BE and 3.5 $X10^5$ cells ml^{-1} at FB in June (July not sampled).

These blooms were mostly constituted by red-fluorescing chroococcoid cells (≥ 1 µm diam.), resembling Microcystis sp., which formed large amorphous colonies. Synechococcus-type cells were also present, but with lower relative abundances than Microcystis. These colonies were typically invaded by numerous large rod-shaped heterotrophic bacteria, as well as by HNF. Cell sizes had a relatively wide spectrum, within which four groups were defined according to cell length: < 1 µm (0.3 $µm^3$ avg. volume), 1 µm (0.8 $µm^3$), > 1 µm (2.4 $µm^3$) and short filaments ca. 5 - 8 µm X 0.7 - 1 µm (4.3 $µm^3$). Large filamentous forms were rarely encountered. Mean cell volumes estimated at peak summer abundances at the surface (2 m) were 0.81 $µm^3$ at KB, 0.71 $µm^3$ at FB and 0.52 $µm^3$ at BE. Maximum primary production was measured during these blooms (see Berichte zum Biologischen Monitoring der Ostsee, 1987 and 1988).

TBN was better correlated with cyanobacteria than with water temperature at the three stations. A particularly strong linear relationship existed between cyanobacteria and TBN at BE (r^2 = 0.67, $P < 0.0001$), while weakest correlations were found at FB for TBN regressed either with temperature or with cyanobacteria ($r^2 < 0.3$, $n = 35$). Cyanobacteria combined with temperature predicted 75% of bacteria biomass variability at BE ($P < 0.0001$) and 30% at KB and FB ($P < 0.001$).

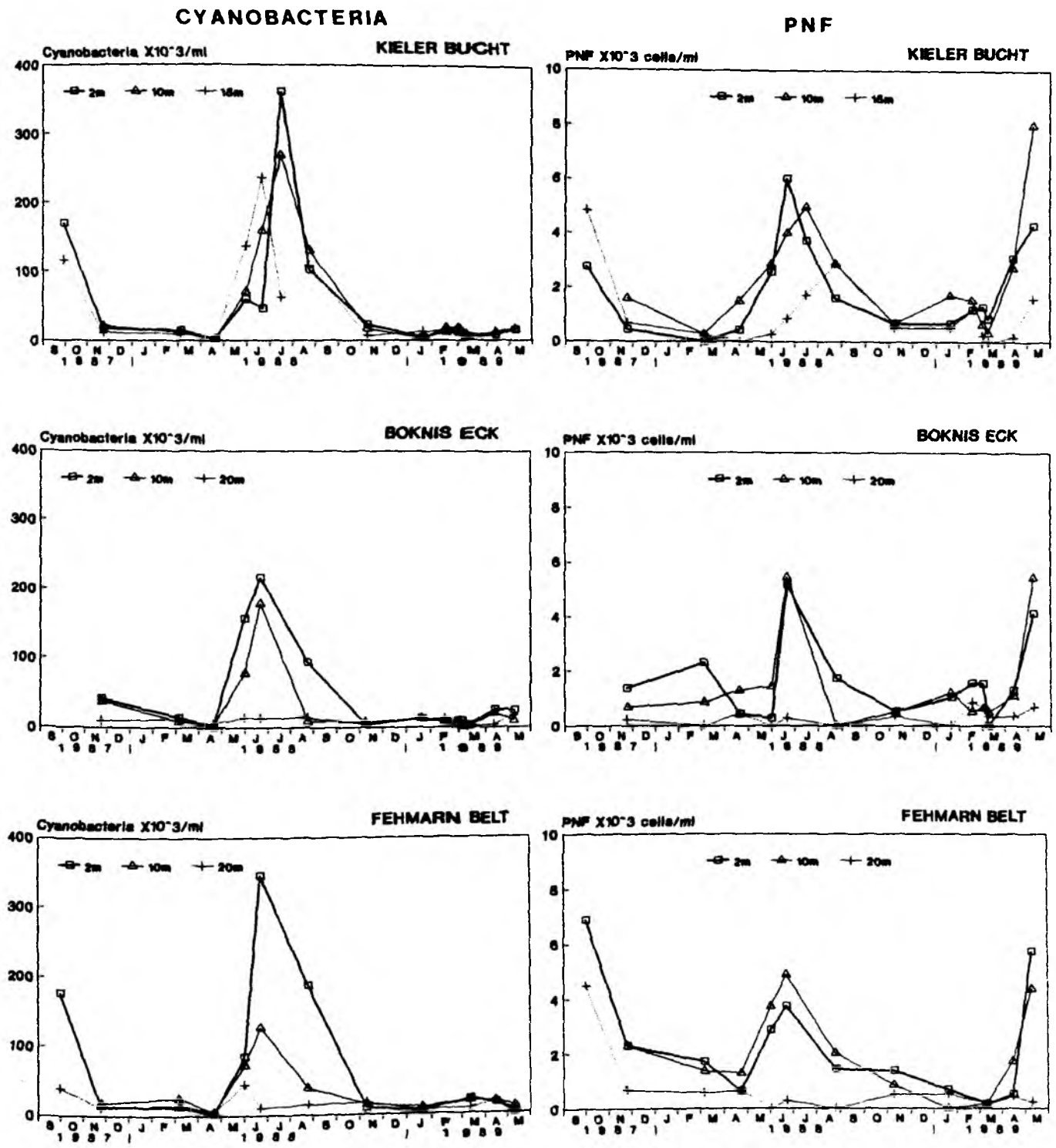


Figure 18. Cyanobacteria and pigmented nanoflagellates (PNF) at the three Western Baltic stations.

c) Nanoplankton

HNF counts (Fig. 16) revealed seasonal trends similar to bacterioplankton with maximum abundances in the summer. Excluding one exceptionally high value in October 1987 at FB (11.7 $X10^3$ cells ml^{-1}), KB showed higher abundances than the other stations. Counts reached 8.0 $X10^3$ cells ml^{-1} at KB (2 m) in July, 6.1 $X10^3$ cells ml^{-1} at BE (2 m) and 6.3 $X10^3$ cells ml^{-1} at FB (10 m) in June (July not sampled). Regression analyses confirmed stronger covariation with TBN at KB (r^2= 0.53, $P < 0.0001$) than at FB (r^2= 0. 23, $P < 0.005$) and BE ($r^2 < 0.1$, $P > 0.1$). Multiple regression analyses between TBN and HNF combined with temperature and cyanobacteria improved model fitting up to 58% ($P < 0.0001$) for KB and up to 28% for FB. HNF did not improve multiple regression at BE, where cyanobacteria remained the single best predicting variable for TBN.

HNF mean cell volume and biomass are plotted in Figure 19. MCV was generally higher in the spring and summer. Maximum MCV was measured during spring 1989 at the three stations, reaching in the surface layer 81 μm^3 at KB and 90 μm^3 at BE in May, and 78 μm^3 at FB in March. HNF cells tended to be larger at KB (overall mean 24 μm^3) than at the other stations (overall mean 18 μm^3). Similarly to the IfM station, smallest size class (2 μm^3) predominated with a mean frequency of 45% and relative importance decreased with increasing size class at the three stations (Fig. 20). In contrast to Kiel Fjord, choanoflagellates constituted less than 10% of the HNF population in the spring at these stations.

Overall means of bacteria:HNF biomass ratios were lower at BE (19) than at KB (55) and FB (81). Ratios were lowest near the bottom (20 m) at BE and FB, while KB had lower ratios at 2 m than at 15 m. During 1988, ratios in the surface layer decreased below 1 at FB in the spring and averaged 2 in the summer. In contrast, biomass ratios at the surface averaged 3-4 at KB and FB during summer and never decreased below 1 at these two stations.

Pigmented nanoflagellates (PNF) exhibited seasonal trends similar to HNF (Fig. 18). Maximum counts reached 8.0 $X10^3$ cells ml^{-1} at KB (10 m) in May 1989, 5.4 $X10^3$ cells ml^{-1} at BE (10 m) in June 1988 and 6.9 $X10^3$ cells ml^{-1} at FB (2 m) in September 1987. PNF were strongly correlated only with HNF at the three stations, particularly at FB (r^2 = 0.62, $P < 0.0001$). PNF were

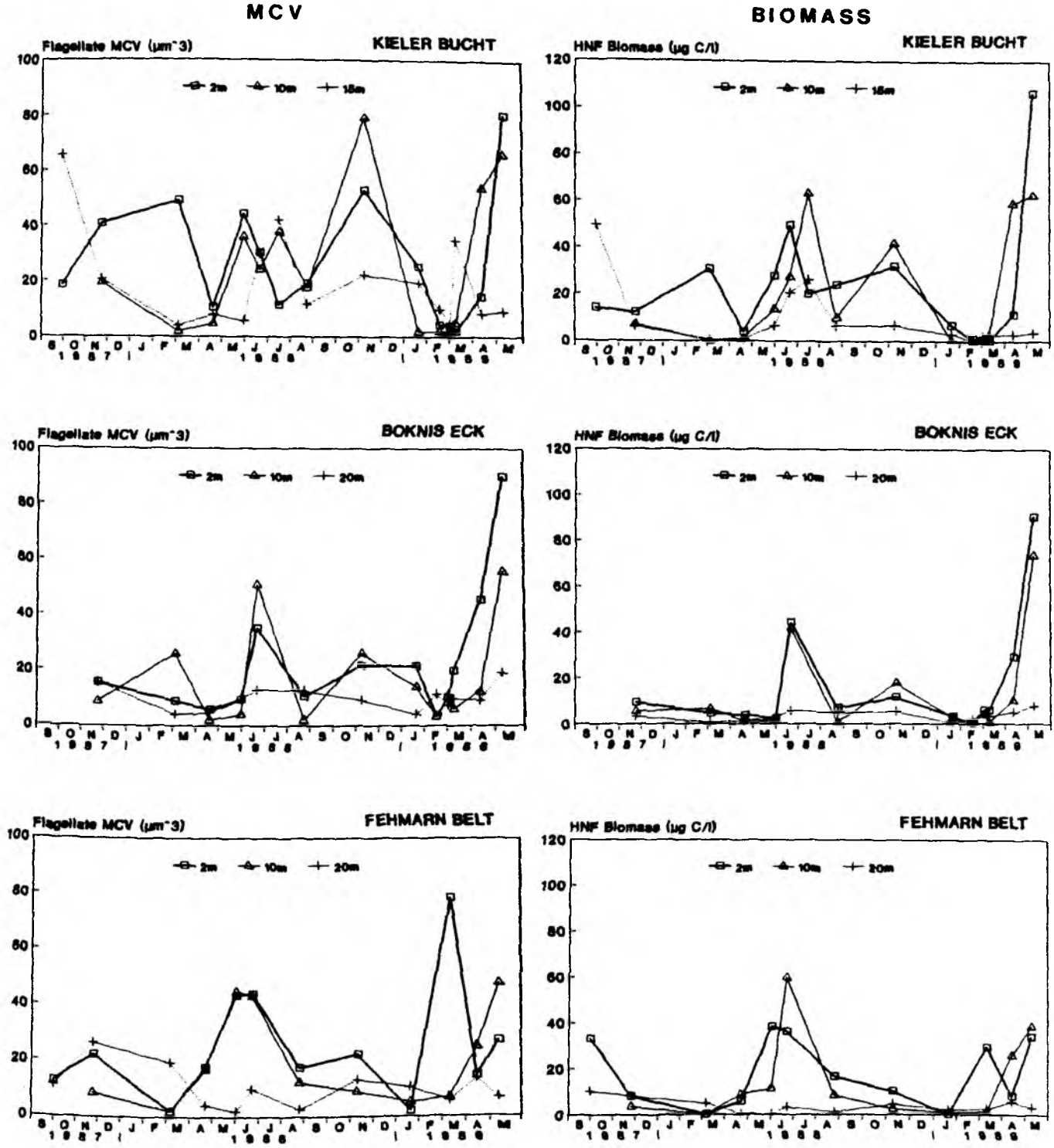
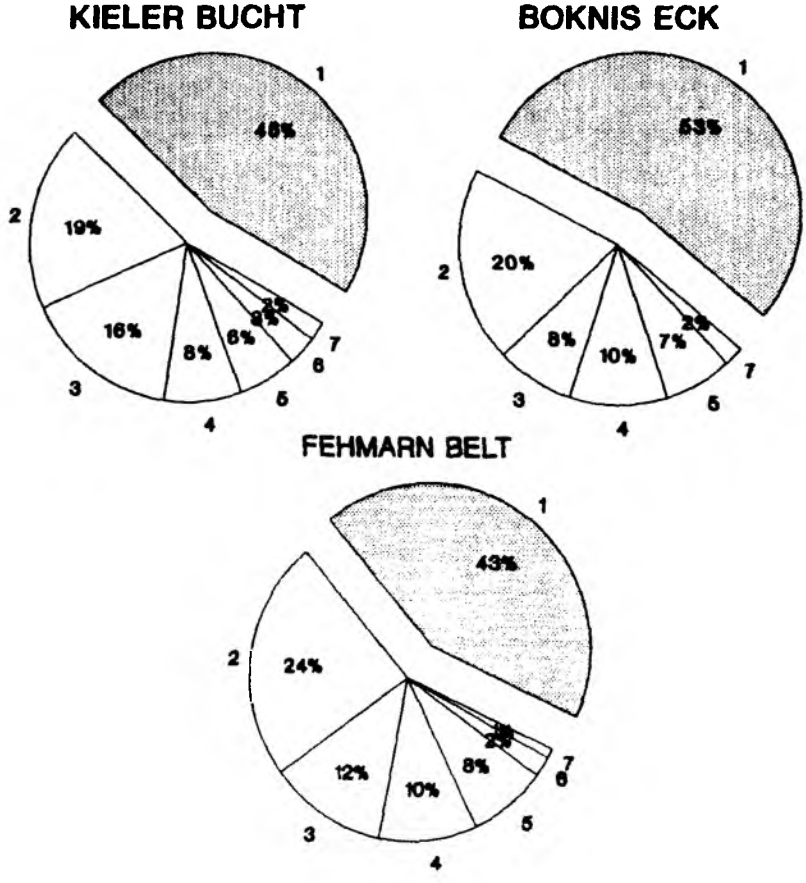


Figure 19. Mean cell volume (MCV) and biomass of heterotrophic flagellates at the three Western Baltic stations.

mostly represented by small cryptomonads (2-6 µm diam., 4-8 µm length). The D. speculum bloom of May 1988 was not observed at these stations, perhaps because both spring cruises 19 - 21 April and 30 - 31 May missed the bloom period, which was observed from 3 to 25 May inside the fjord.



Average volume of HNF size-classes

Class 1: 2 µm^3 Class 2: 6 µm^3 Class 3: 14 µm^3 Class 4: 32 µm^3 Class 5: 68 µm^3 Class 6: 140 µm^3 Class 7: 288 µm^3

Figure 20. Average size-class distribution of heterotrophic nanoflagellates (HNF) at the three Western Baltic stations.

2. Grazing Study

2.1 Temperature and salinity

Temperature and salinity ranges of ambient water at 1 m for each experiment are given in Table 3. Lowest water temperatures occurred in the March experiment (3 - 4 °C) and highest in July (14 - 17 °C). Temperature varied by < 1 °C in fall, winter and early spring experiments, and by 2-3 °C in late spring and summer. Maximum salinities were measured in August (19-20 S X 10^3) and minimum in May (12-13 S X 10^3). Widest fluctuations in temperature occurred during the July experiment (3.1 °C) and in salinity during March (3.2 S X 10^3).

Table 3. Temperature and salinity ranges of ambient water (1 m) during grazing experiments.

DATE	Temperature (° C)	Salinity (S X 10^3)
21OCT-26OCT87	11.0 - 11.6	17.4 - 18.5
26JAN- 1FEB88	4.8 - 5.3	17.2 - 19.2
18MAR-28MAR88	2.9 - 3.7	13.2 - 16.4
12MAY-20MAY88	10.7 - 12.9	11.7 - 13.3
12JUL-20JUL88	14.0 - 17.1	13.6 - 15.8
22AUG-29AUG88	13.2 - 14.9	19.2 - 20.3
12OCT-19OCT88	11.0 - 11.9	16.5 - 19.1

2.2 Direct cell counts

Means and ranges of heterotrophic bacteria and nanoflagellate counts in grazer chambers (c3, c4 and c5) are plotted in Figure 21. HNF always exhibited an initial lag period, during which numbers decreased and cells appeared senescent with faded cytoplasm and loss of flagella. This period varied from 1-2 days in late spring and summer, 3 days in the fall and 7 days in early spring. Although HNF growth in the March experiment was observed only after 7 days, some oscillations were detected within this period. Since the HNF population at this time was dominated by choanoflagellates, which tend to form aggregates and attach to surfaces, it is possible that growth occurred earlier without being detected. No HNF growth was observed in the January

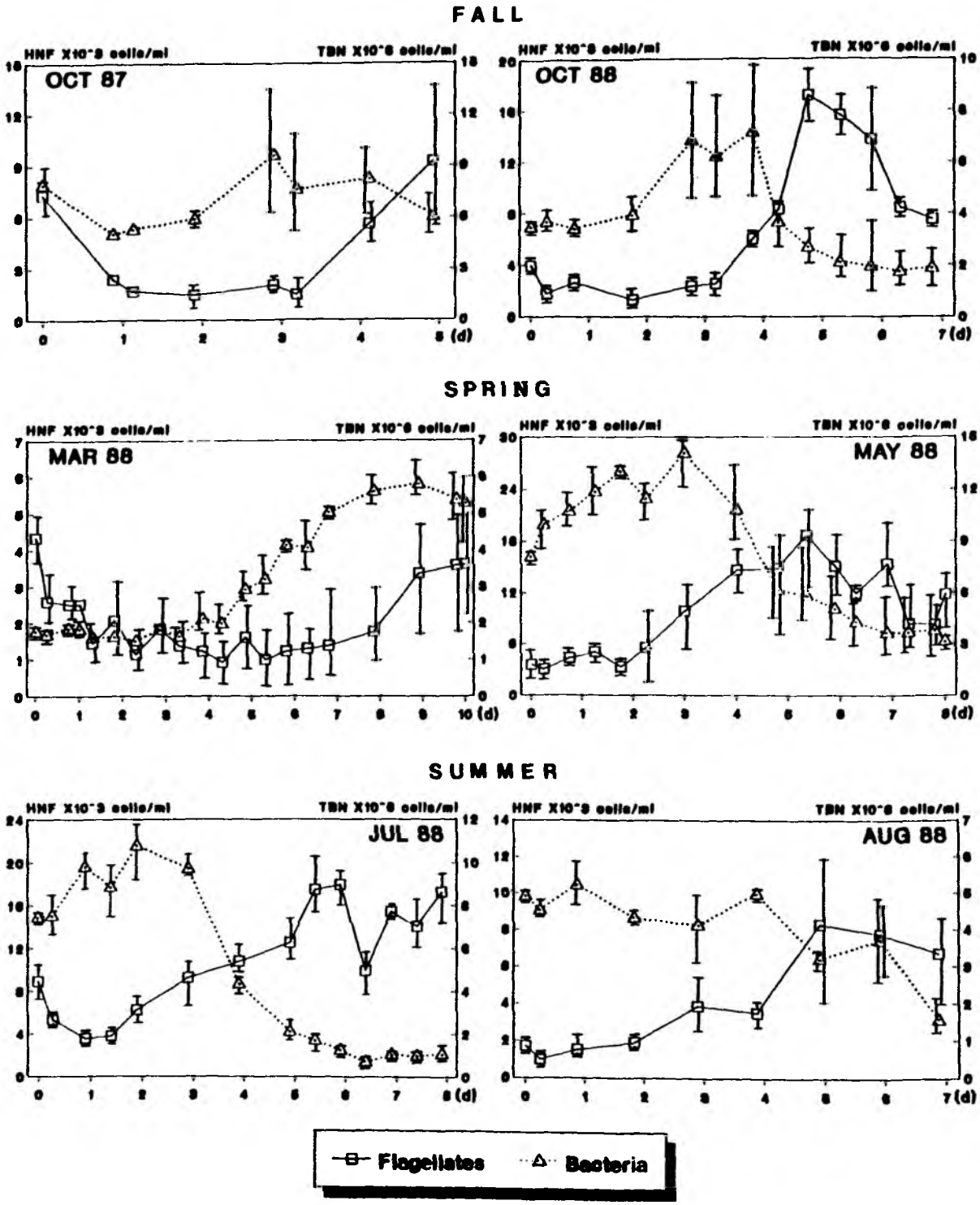


Figure 21. Mean concentration of heterotrophic nanoflagellates (HNF) and bacteria (TBN) in three grazer chambers in the course of different experiments. Bars depict maximum and minimum values.

experiment, during which HNF decreased from a starting concentration of 1.3 X 10^3 cells ml^{-1} to undetectable (< 100 cells ml^{-1}) and remained below detection limits at the end of 6 days, therefore only bacteria growth and biomass production will be reported for this experiment.

An inverse relationship between HNF and bacteria was present in May, July, August and October 1988 (Fig. 21). In these experiments, a sharp decrease in bacteria occurred after the third or fourth day, at least one day past the onset of HNF growth. Incubation periods in October 1987 and March 1988 probably needed to be extended for a clear grazing impact to be determined. After a period of sustained growth lasting 3-4 days, HNF decreased in number (May, July, August and October 1988) and exhibited oscillations in some cases (May and July). For comparison, ambient TBN and HNF counts for 1988 experiments are plotted in Figure 22. With the exception of March, grazer chambers exhibited considerable enrichment of HNF relative to ambient water in the course of incubation. It was noted that in July ambient HNF and TBN revealed parallel trends, whereas in August and October 88 some negative correlation was evident.

A bloom of small phytoflagellates (2 - 3.5 µm diam.) developed in unfiltered treatments during the second half of incubation in July and August (see micrographs in section 3.2). Cell counts reached 26 X 10^4 cells ml^{-1} in July and 5 X 10^4 cells ml^{-1} in August. Algae growth rates averaged 1.0 d^{-1} in July and 0.9 d^{-1} in August (ca. 1.4 div. d^{-1}). In the course of these blooms, many HNF cells were observed with ingested algae. Ciliates remained undetectable (< 100 cells ml^{-1}) throughout the experiments with the proflavin counting method.

At the end of each incubation period, control chambers (c1 and c2), containing GF/C filtered water, were checked for HNF development, since a few small cells could have escaped filtration and grown to significant numbers over prolonged incubation periods. In January, March and August, no significant HNF numbers could be detected in these chambers by the end of the incubation. However, in the other experiments, a bloom of small flagellates (size-classes 1 and 2) had developed, usually in one single chamber and within the last two days of incubation. Maximum numbers occurred in October 1988, when small HNF reached ca. 15 X 10^3 cells ml^{-1} in both controls at the final sampling.

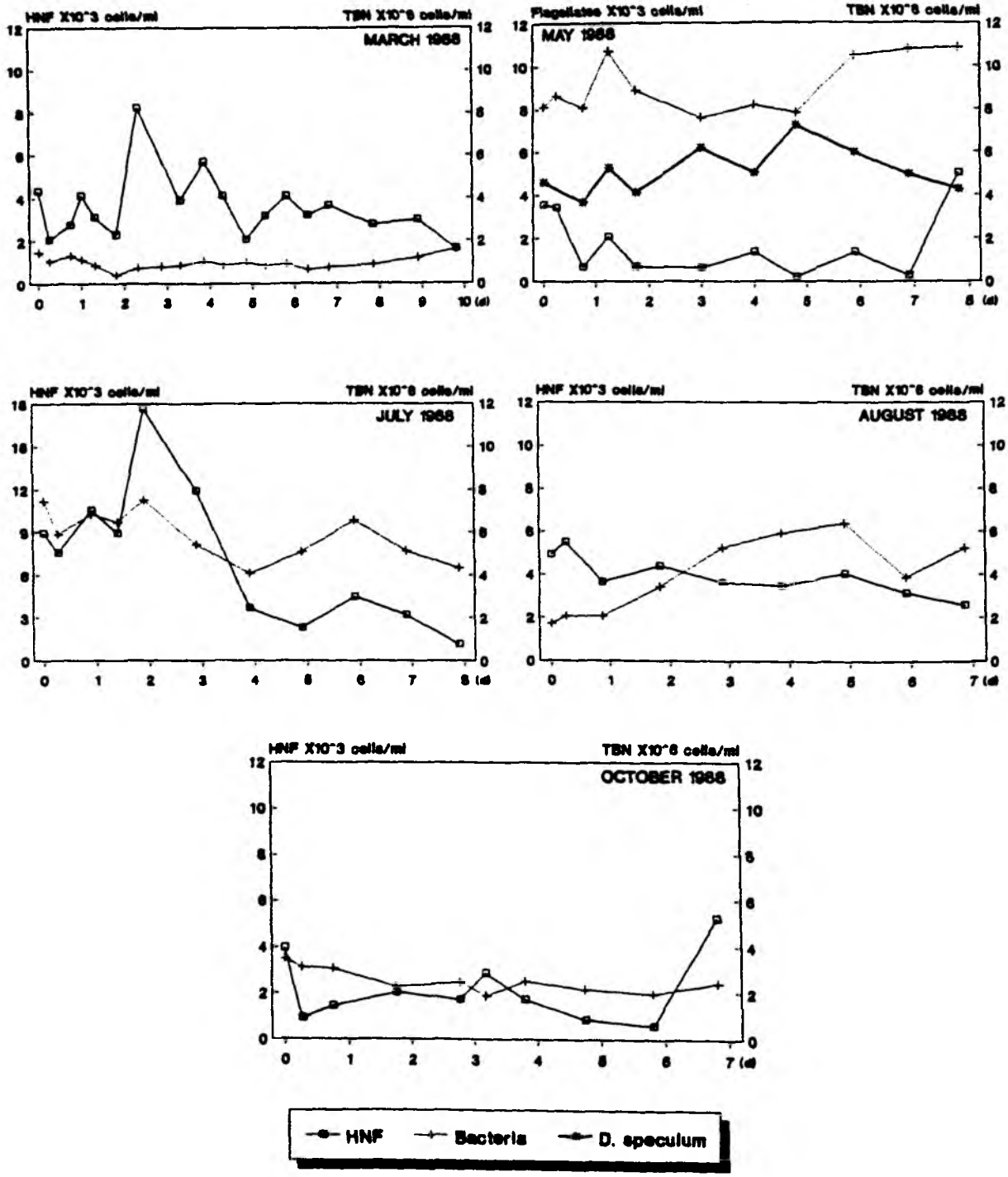


Figure 22. Total bacteria number (TBN) and heterotrophic nanoflagellates (HNF) in ambient water during grazing experiments.

Membrane fouling of diffusion chambers was checked at the end of experiments with AO stain. It was observed that, although in cold waters during January relatively little bacteria colonization was detected after six days, in warm summer waters many bacteria colonies with large rod-shaped cells had developed on membranes at the end of a week. It was also noted that the external surface of membranes exhibited significantly more colonization than internal surfaces. Few flagellate cells (mostly choanoflagellates) were observed attached to membranes, however, since membranes were rinsed with filtered BDW before AO staining, any loosely associated cells could have been washed away. In view of these experimental problems, both bacterial and HNF growth were estimated within the first half of incubation periods in late spring and summer experiments, whereas results of last two samplings were disregarded in control chambers for all experiments, except October 1987.

2.3 Flagellate mean cell volume and biomass

In October 1987 and March 1988, MCV of HNF in grazer chambers fluctuated during the incubation with a net increase in biomass detected only in the last 2 or 3 days (Fig. 23). In the other experiments, biomass started increasing within the second day, but fluctuations also occurred. The maximum rate of increase was observed in July, when biomass tripled between day 4 and 6, representing a flagellate production of 50 $\mu g\ C\ l^{-1}\ d^{-1}$ (Fig. 23). In all experiments, a decrease in flagellate MCV was detected within the period of active cell growth.

Changes in flagellate size classes during grazing experiments are depicted as bargraphs in Figure 24. In general, it could be seen that, in the course of incubation, class 1 (see descriptions Table 1) decreased in relative importance as larger sizes (class 3, 4 and 5) became more predominant. HNF populations in the March experiment, due to the presence of many large choanoflagellate cells, exhibited the widest size-class spectrum. The sharp increase in flagellate MCV at the end of the May experiment (Fig. 23) was due to the rapid development of large loricate choanoflagellates, which became the predominant type (36 - 49% of total HNF) in the last two days of incubation.

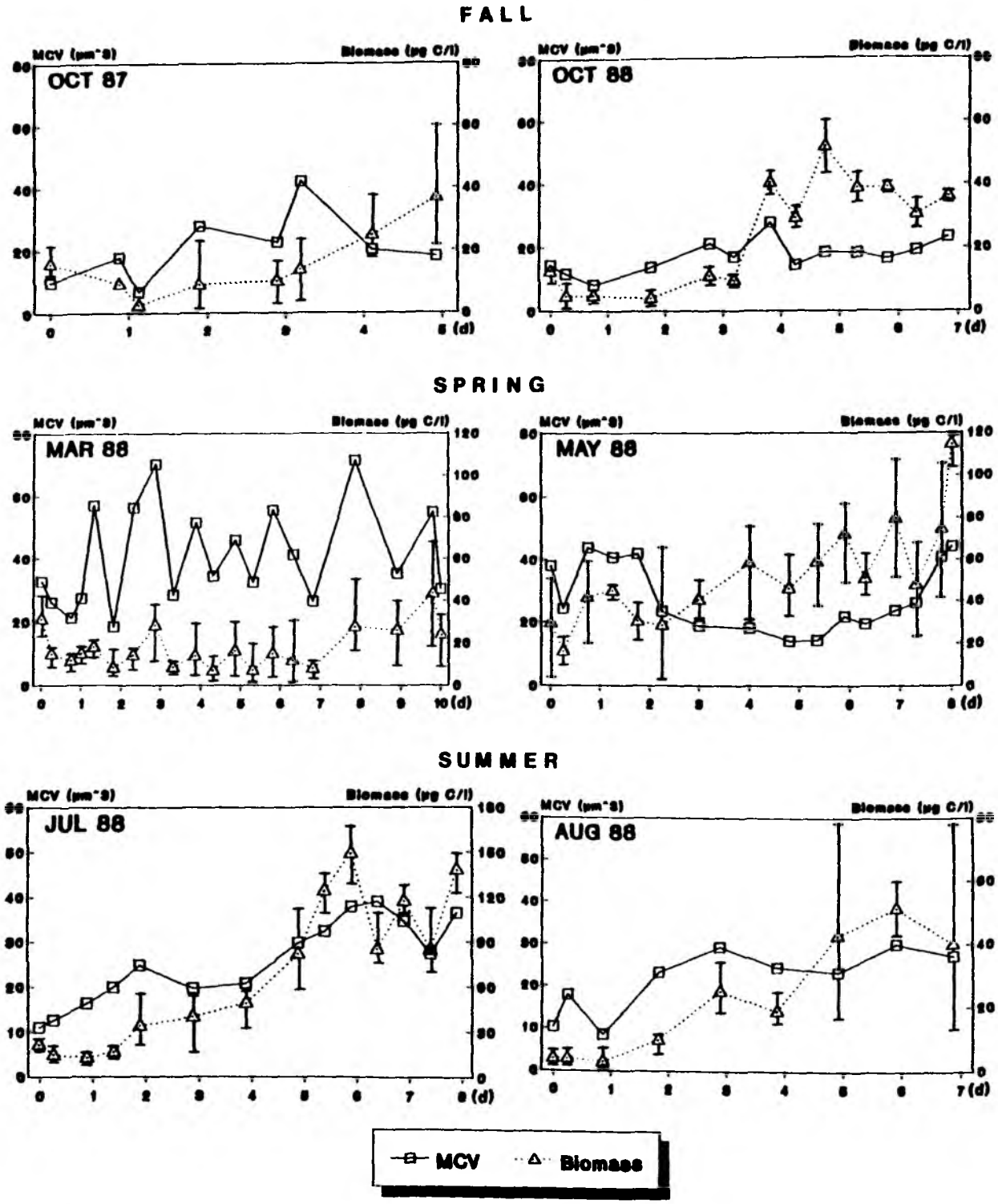


Figure 23. Average mean cell volume (MCV) and biomass of heterotrophic flagellates in three grazer chambers in the course of different experiments. Bars depict maximum and mininum values.

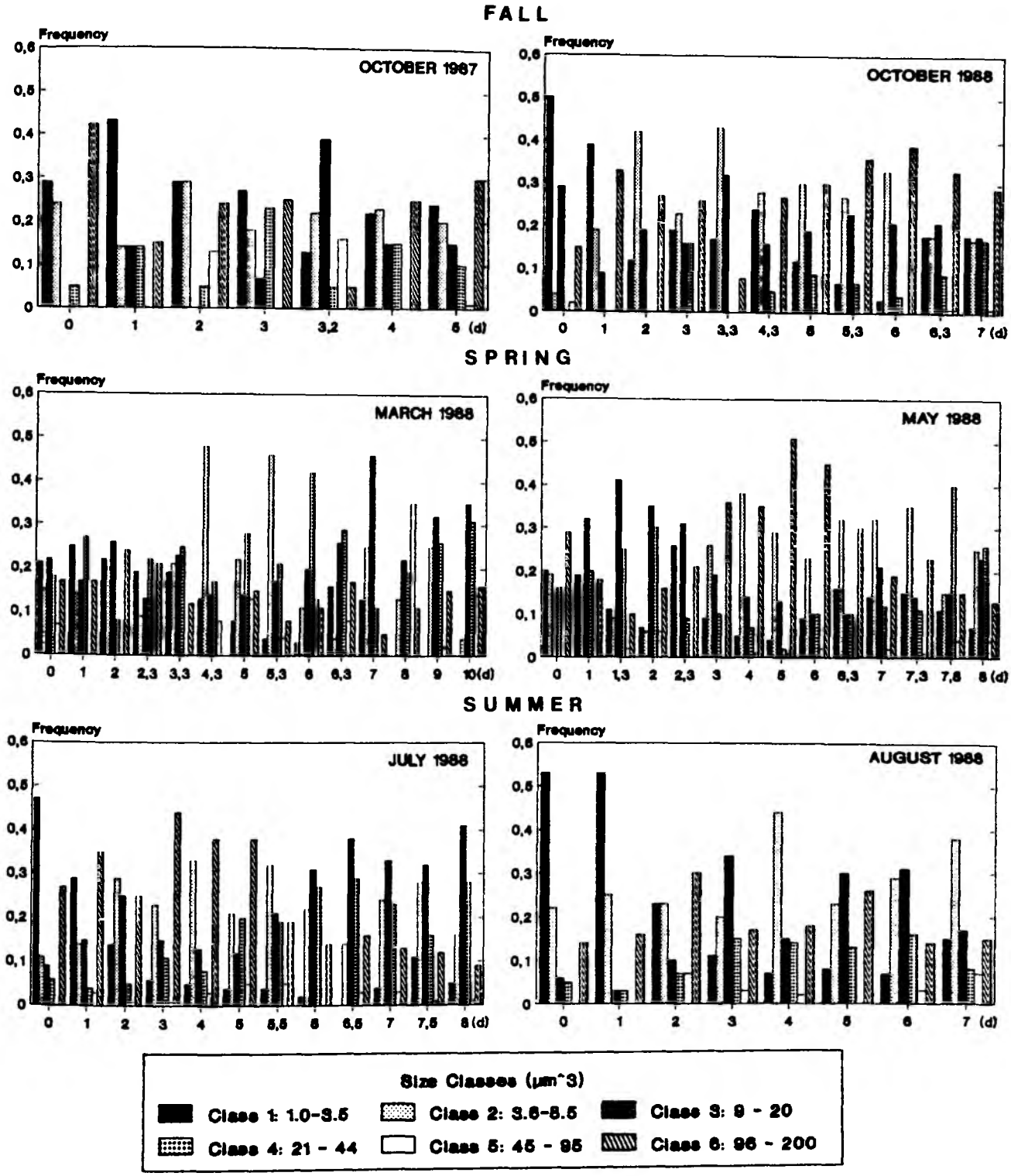


Figure 24. Changes in size-class distribution of heterotrophic flagellates in the course of grazing experiments.

2.4 Bacteria mean cell volume and biomass

Bacteria MCV and biomass in control and grazer chambers are plotted in Figures 25 and 26, respectively. Generally few significant differences in MCV could be detected between grazer and control chambers (Fig. 25). In October 1987, MCV and biomass in control chambers increased above grazer chambers only at the end of the incubation period. In March 1988, MCV_g (grazer chambers) remained larger than MCV_c (control chambers) throughout the incubation. A positive difference in biomass between control and grazed populations was detected only at the final sampling. In the May experiment, a negative difference between MCV_g and MCV_c developed after day 4 and lasted up to day 7. Maximum HNF counts were detected within this period (see Fig. 21). Large differences between control and grazed biomass were observed after day 4 with maximum difference occurring on day 6.

In both July and August experiments, MCV_c was higher than MCV_g during the first half of incubation (day 1 to day 4) and at the final sampling, while bacteria biomass in control chambers remained considerably larger than in grazer chambers after the first day. Maximum differences occurred on day 6 for the July experiment and on day 5 for the August experiment. In October 1988, MCV_c was significantly larger than MCV_g at the end of the incubation period, while biomass in control chambers became larger than in grazer chambers after day 4.

Biomass ratios (bacteria vs flagellates) in grazer chambers decreased to < 1 towards the end of May, July, August and October 1988 experiments. In July, final HNF biomass was X12 higher than bacteria. Biomass ratios in ambient water remained on the average higher than in chambers, with the exception of March, when HNF populations inside chambers surpassed ambient populations only in the last two days. An extreme case occurred in May during the D. speculum bloom, when ambient biomass ratios averaged 144 in contrast to chambers, which had a mean ratio of 5. It was noted that, although grazer chambers exhibited at first enrichment in bacteria biomass relative to ambient, HNF succeeded in grazing bacteria down to or below environmental levels, with the exception of March, by the end of experiments.

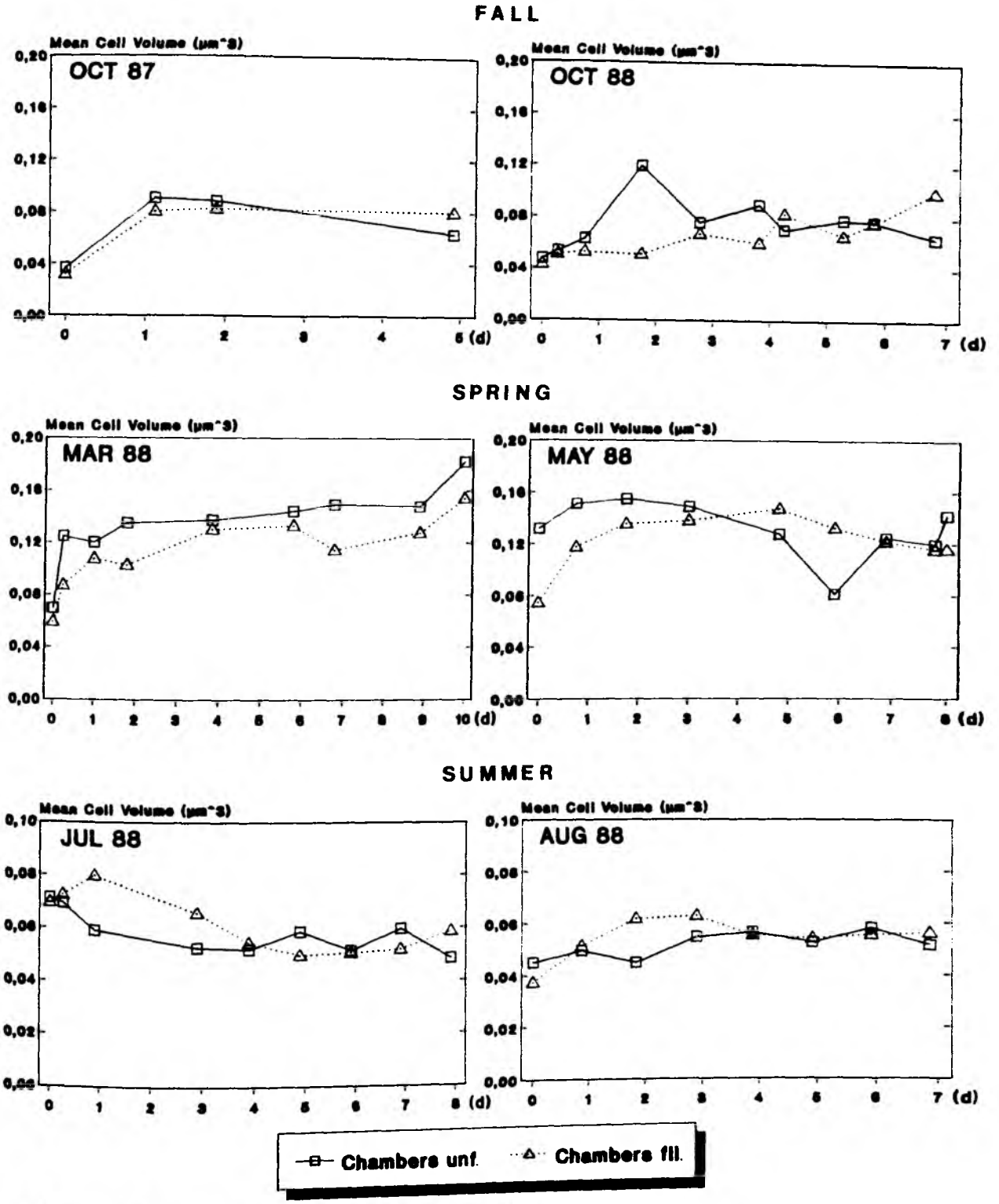


Figure 25. Average bacterial mean cell volume (MCV) in the two filtered and three unfiltered treatments in the course of grazing experiments.

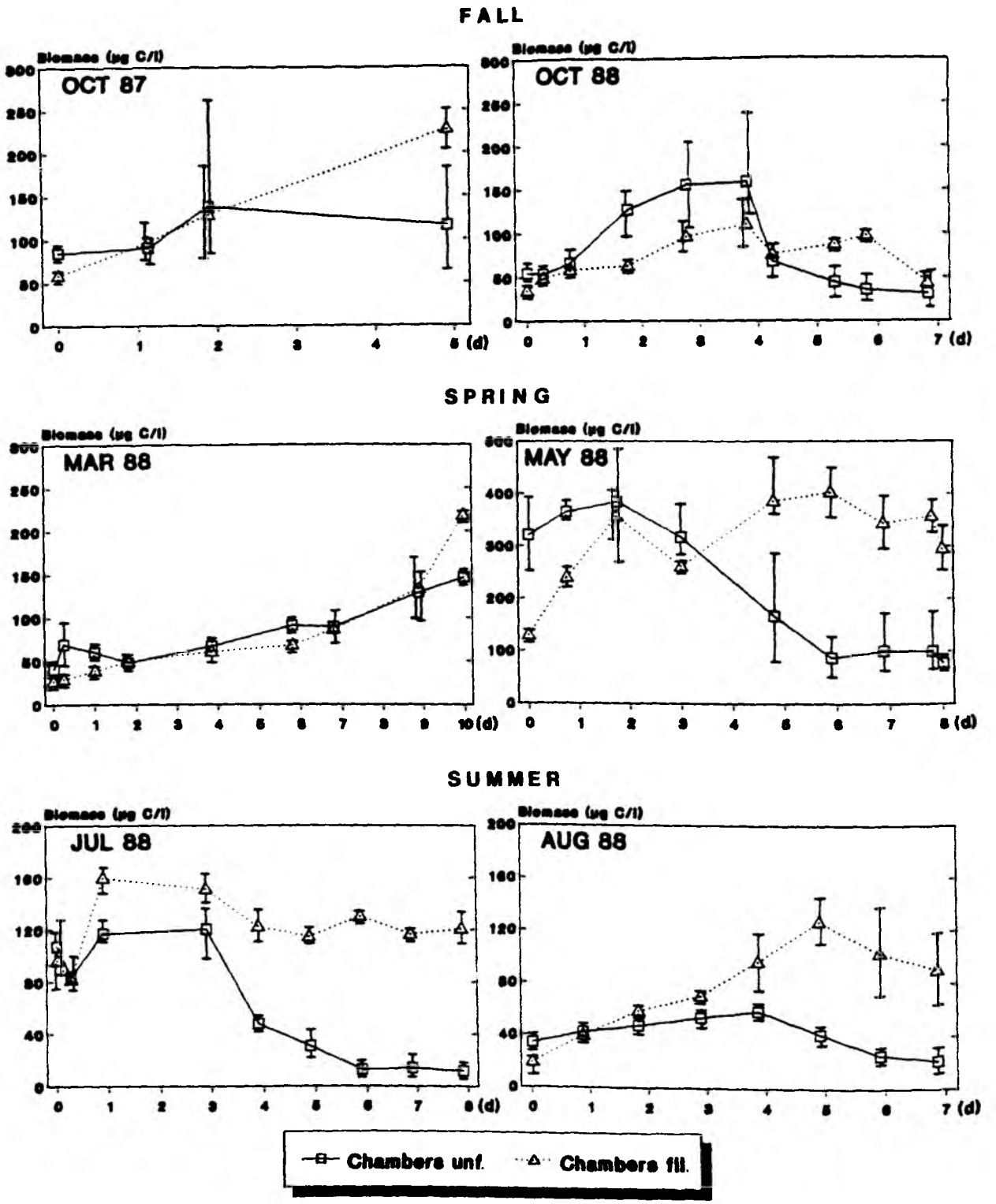


Figure 26. Average bacterial biomass in the two filtered and three unfiltered treatments in the course of grazing experiments. Bars depict maximum and minimum values.

2.5 Growth and grazing rates

A summary of growth and grazing rates as well as biomass production for both HNF and bacteria is given in Table 4. Omitting January when HNF remained at undetectable numbers inside grazer chambers, highest growth rate of HNF was observed in October 1987 (1.06 d^{-1}) and lowest in March 1988 (0.28 d^{-1}). Largest HNF biomass production was estimated in July (24 µg C l^{-1} d^{-1}) and smallest in May (10 µg C l^{-1} d^{-1}). HNF growth efficiency, calculated as the proportion of HNF biomass produced over bacteria biomass grazed for a given sampling interval, averaged 27% omitting the extreme case in July.

Highest ingestion rates (66 - 90 bacteria per flagellate per hour) were estimated in May, which amounted to 29.3 pg bacterial carbon grazed per flagellate per day. Lowest ingestion rate (13 - 14 bac. $flag.^{-1}$ h^{-1}) were calculated for July constituting 2.4 pg bacterial carbon grazed per flagellate per day. Since an unrealistic growth efficiency of 112% was also obtained, this indicated that bacteria were not the only food source for HNF during the July experiment. HNF consumed an average of 11 pg bacterial carbon per cell per day for the whole grazing study.

Table 4. Heterotrophic nanoflagellate and bacteria growth and grazing rates including estimates of biomass produced and grazed.

	FLAGELLATES					BACTERIA		
Expmt	µ (d^{-1})	Biomass Prod. (µg C/l/d)	Ingestion Rate* (bac/flag/h)	Biomass Grazed (pgC/flag/d)	Growth Eff. (%)	μ_c fil. (d^{-1})	μ_g unf. (d^{-1})	Biomass Prod.** (µg C/l/d)
OCT87	1.06	10.3	28 - 49	9.64	25.1	0.49	0.19	38.6
JAN88	< 0	< 0	0	0	0	--	0.02	0.8
MAR88	0.28	13.0	23 - 31	9.74	35.3	0.26	0.37	35.3
MAY88	0.43	10.0	66 - 90	29.32	11.9	0.66	0.55	129.9
JUL88	0.34	23.8	13 - 14	2.37	112	0.47	0.37	26.4
AUG88	0.45	13.6	12 - 18	6.92	39.8	0.34	0.08	21.8
OCT88	0.73	11.0	24 - 28	10.01	22.0	0.25	0.37	19.0
MEAN[b]	0.55	13.6	28 - 38	11.3	26.8[a]	0.41	0.32	45.2

* ingestion range calculated from bacteria growth rates in unfiltered (μ_g) and in filtered treatments (μ_c); μ_g estimated during initial lag phase of HNF.
** bacteria production calculated from biomass increase in filtered treatments.
a average growth efficiency calculated without July value.
b means calculated without January values.

The overall mean of bacterial growth rates, excluding January, was 0.41 d^{-1} in control chambers and 0.32 d^{-1} in grazer chambers, which represented about half a division per day. This was lower than mean HNF growth rate, estimated at 0.55 d^{-1} or 0.8 div. d^{-1}. Highest bacterial growth rates were detected in May (0.66 d^{-1} or 1.1 d doubling time) and lowest in October 1988 (0.25 d^{-1} or 2.8 d doubling time) for filtered treatments. Mean bacterial production for the whole study, calculated from biomass increase in control chambers, was ca. 3 times higher than mean HNF production, that is 45.2 and 13.6 µg C l^{-1} d^{-1}, respectively.

2.6 Bacteria size-classes

In both filtered and unfiltered treatments, frequencies of bacteria sizes maintained a normal distribution throughout incubations, whereby the majority of bacteria were included in middle size-classes. In both spring experiments, frequencies of classes 4, 5 and 6 totalled 72 - 75%, while 64 - 80% of bacteria were included in the class group 3, 4 and 5 in summer and fall experiments. For all experiments, class 4 consistently exhibited highest relative abundance in both treatments varying from 25% to 35%.

To analyse grazing impact on bacteria sizes, relative differences of size classes between grazer and control chambers are plotted over time for the 1988 experiments in Figure 27. On the three-dimensional plots, indentations below the 0 difference plane indicate negative differences in frequency between grazer and control classes, which were presumed due to grazing. In March, differences in relative importance existed only in class 4, which occurred in the first three days and became particularly visible from day 6 to 9, during the period of active HNF growth.

During May, two grazing impact events could be detected, on class 4 between days 1 and 2 and on classes 6 and 7 between days 5 and 7 (Fig. 27). This large difference coincided with the MCV decrease in unfiltered treatments observed on days 5 and 6 (Fig. 25). During the initial period of the July experiment (day 1 to 3), the negative difference between MCV_g and MCV_c (Fig. 25) concerned classes 3 and 6 (Fig. 27). In the second half of the incubation, grazing impact was particularly strong on classes 3 & 4 (days 4-7), then at the final sampling, negative differences had shifted to larger size classes (5 and 6), whereas class 3

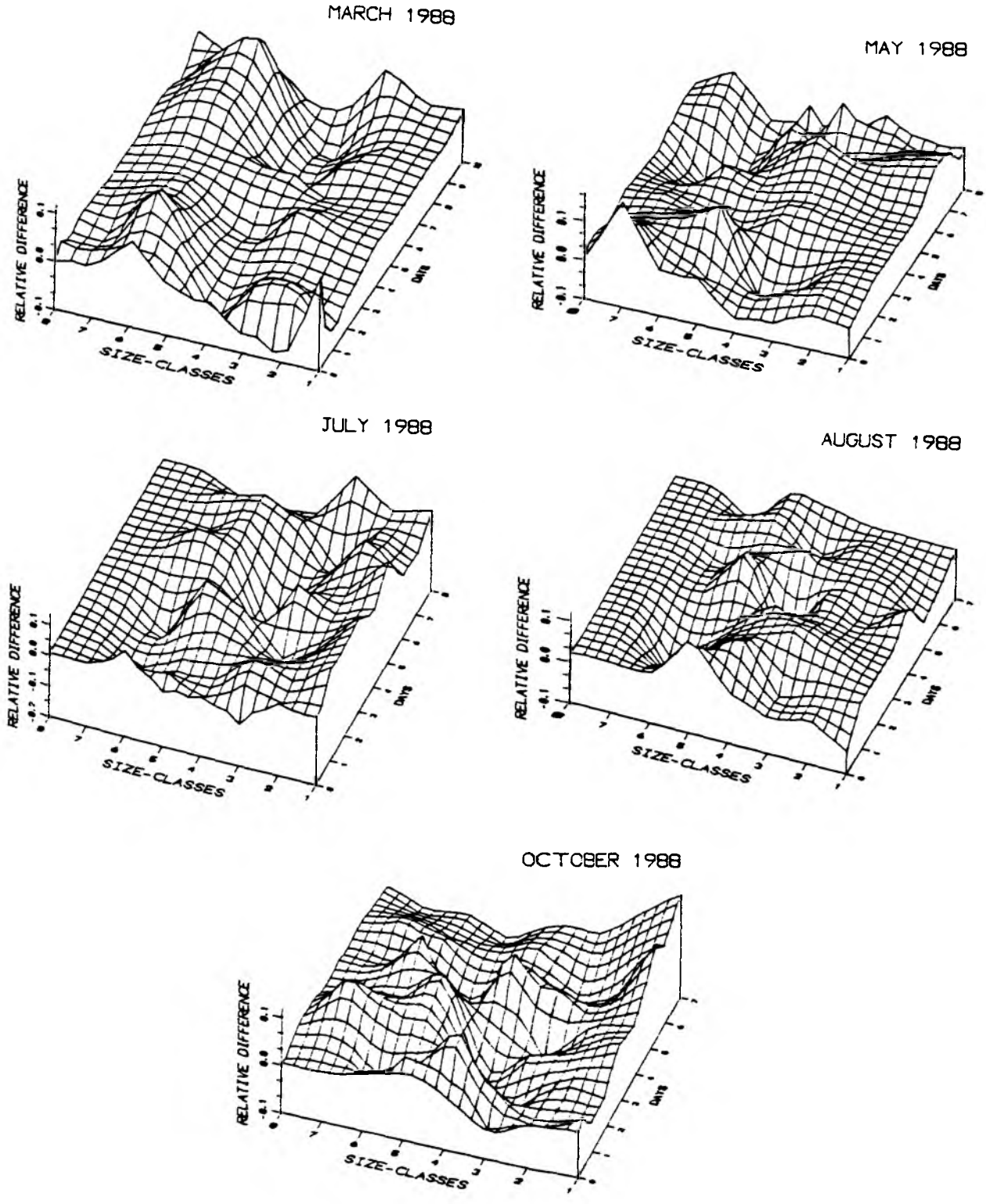


Figure 27. Relative differences of bacterial size-classes between grazer and control chambers in the course of experiments.

had increased in relative importance (Fig. 27).

The negative difference between MCV_g and MCV_c observed on days 2 and 3 (Fig. 25) of the August experiment was due to a decrease in relative importance of class 6. This was again observed at the end of the incubation. At mid-incubation (day 4), class 4 exhibited a sharp decrease in relative importance in grazer chambers (Fig. 27), although no net difference between MCV_g and MCV_c could be detected at this time (Fig. 25). Classes 3 and 5 exhibited negative differences in grazer chambers relative to controls during the HNF growth from day 3 to day 5 in the October 1988 experiment. The negative difference between MCV_g and MCV_c detected at the final sampling (Fig. 25) was mainly due to an increase in relative importance of smallest size-classes (1 and 2) in October 1988. In the same experiment, MCV_g reached a maximum on day 2 corresponding to a predominance of class 7 bacteria, after which MCV_g tended to decrease while class predominance shifted to smaller sizes (4, 5 and 6). In the first half of this experiment, classes 3 and 4 appeared to be removed from grazer chambers, whereas a large decrease in relative importance of class 5 occurred later on day 4 (Fig. 27).

2.7 Thymidine incorporation

Tritiated thymidine incorporation (TTI) for four grazing experiments is plotted in Figure 28. In October 1987, July and August 1988, TTI in control chambers increased considerably above grazer chambers towards the end of incubation periods. In October 1988, however, TTI in control chambers had decreased to the level of grazer chamber by the final sampling. The pronounced decrease in TTI for both filtered and unfiltered treatments on day 3 in July occurred during a sharp decline in both HNF and TBN observed between days 2 and 4 in ambient water (see Fig. 22). Since both a salinity increase (13.7 to 14.7 S X 10^3) and temperature decrease (16.5 to 14.0 °C) was observed during this period, this indicated that a different water mass had been transported to the site, thus affecting bacteria productivity inside chambers.

Specific activity (TTI/TBN) in July, August and October 1988 experiments was higher in grazer chambers than in control chambers at the end of incubation (Fig 29). Maximum specific activity in grazer chambers was observed at the final sampling in July and

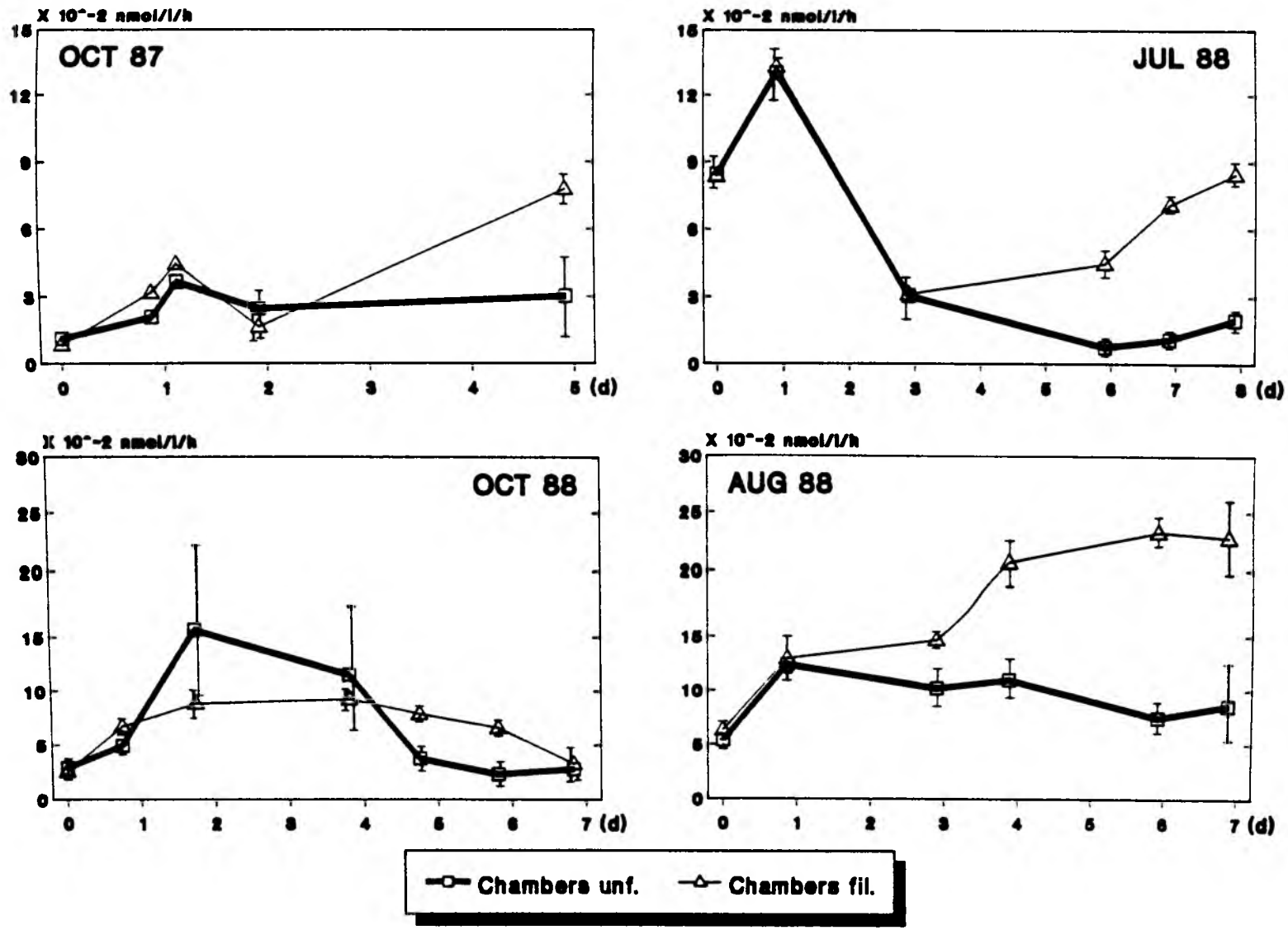


Figure 28. Mean tritiated thymidine incorporation in two filtered and three unfiltered treatments during several grazing experiments. Bars depict maximum and minimum values.

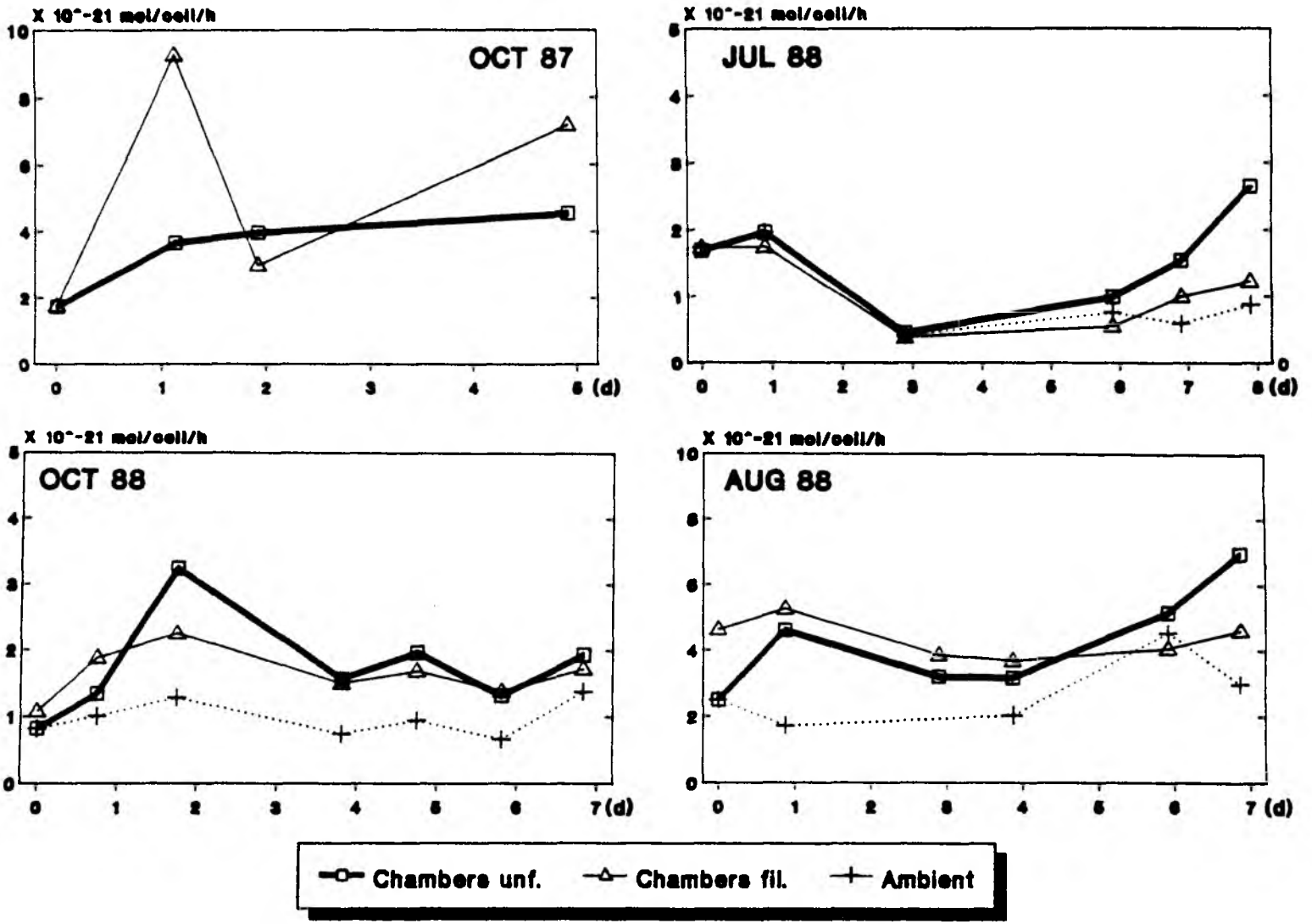


Figure 29. Mean specific activity (TTI/TBN) in chambers (unfiltered & filtered treatments) and in ambient water during several experiments.

August, whereas a maximum was reached on day 2 in October 1988. Specific activity in ambient water followed chamber trends in July and October 1988 (Fig. 29).

A comparison of bacterial production estimated from TTI and from biomass determination is compiled in Table 5. Production from thymidine uptake was calculated as an average of the first four experimental measurements with a conversion factor of 1.1 $X10^9$ cells $nmol^{-1}$ (Riemann et al., 1987). Biomass production was estimated from the point when maximum measurements were obtained within the first half of incubation periods. TTI gave a wider range with estimates from 15 to 60 µg C l^{-1} d^{-1}, compared to biomass determination, which varied from 6 to 39 µg C l^{-1} d^{-1}. Although production proportions of control vs grazer chambers obtained for each experiment were comparable, production measurements exhibited different patterns with each method. With TTI, production in control chambers was lowest in October 1987 and highest in August, whereas with biomass determination maximum estimates were obtained in October 1987 and mininum October 1988.

Table 5. Comparison of bacterial production estimated from tritiated thymidine incorporation (TTI) and from biomass determination.

Expmt.	TTI* Control (µg C/l/d)	TTI* Grazer (µg C/l/d)	Biomass Control (µg C/l/d)	Biomass Grazer (µg C/l/d)
OCT87	18.8	14.8	38.6	31.7
JUL88	37.6	30.1	26.4	13.9
AUG88	59.9	41.8	21.8	6.2
OCT88	29.9	53.3	19.0	32.1

* calculated with conversion factor of 1.1 $X10^9$ cells $nmol^{-1}$ (Riemann et al., 1987).

Grazing rates calculated from TTI were 3 to 7 X lower (5 - 6 bac. flag.$^{-1}$ h^{-1}) than from direct cell counts in October 1987, July and October 1988, but gave comparable estimates in August (22 bac/flag/h). To resolve these discrepancies, an attempt was made to extrapolate conversion factors from observed changes in TBN and TTI during the first half of incubation periods. Factors varied over a 15-fold range (0.22 - 3.33 X 10^9 cells $nmol^{-1}$) remaining substantially lower in unfiltered treatments. An

average of 0.5 X 10^9 cells $nmol^{-1}$ was obtained for grazer chambers and 1.7 X 10^9 cells $nmol^{-1}$ for control chambers. Using these experimentally determined average factors, grazing rates could be adjusted to a range in better agreement with cell-based estimates (10 - 61 bac. $flag.^{-1}$ h^{-1}).

3. Descriptive Study

3.1 Epifluorescence microscopy

Comparing DAPI (blue on the left) with proflavin (green-yellow on the right) images in the double-exposure micrograph (Fig. 30-A), it can be seen that both cytoplasm and flagella are more brightly fluorescent with proflavin, giving larger cell measurements than with DAPI. However, the nucleus (right side of cell) is better contrasted with DAPI. Two rows of defense ultra-structures (ejectosomes), appearing as a bright halo on the left side of the proflavin image, were not stained with DAPI. These differences are due to DAPI binding specifically to DNA, while proflavin is a more general protein stain.

It was also noticed that, owing to weaker fluorescence of DAPI, orange and red autofluorescence of photopigments was more visible than with proflavin. For example, chloroplasts of D. speculum (Fig. 30, part A, micrographs C, D & E) are only faintly visible due to bright yellow staining of cytoplasm by proflavin. However, orange autofluorescence of ultra-structures (arrows), which were not stained at all by proflavin, was very clear. For optimal determination of phototrophic cells stained with proflavin, the field has to be illuminated up to one minute for proflavin fluorescence to fade and autofluorescence to become more apparent. It is to be noted that much color definition was lost in photomicrographs (Part A - A, C to E) between original slides and color prints. Replicate HNF counts between DAPI and proflavin deviated by ca. 15% (n = 3). Due to problems encountered in frozen storage of DAPI preparations (i.e. loss of fluorescence), the double-stain was not systematically employed.

In the acridine orange (AO) preparations in Figure 30 (Part B), lorica of choanoflagellates is as brightly stained as flagella facilitating the identification of Acanthoecidae, in contrast to DAPI and proflavin, which stain both collar and

Part A

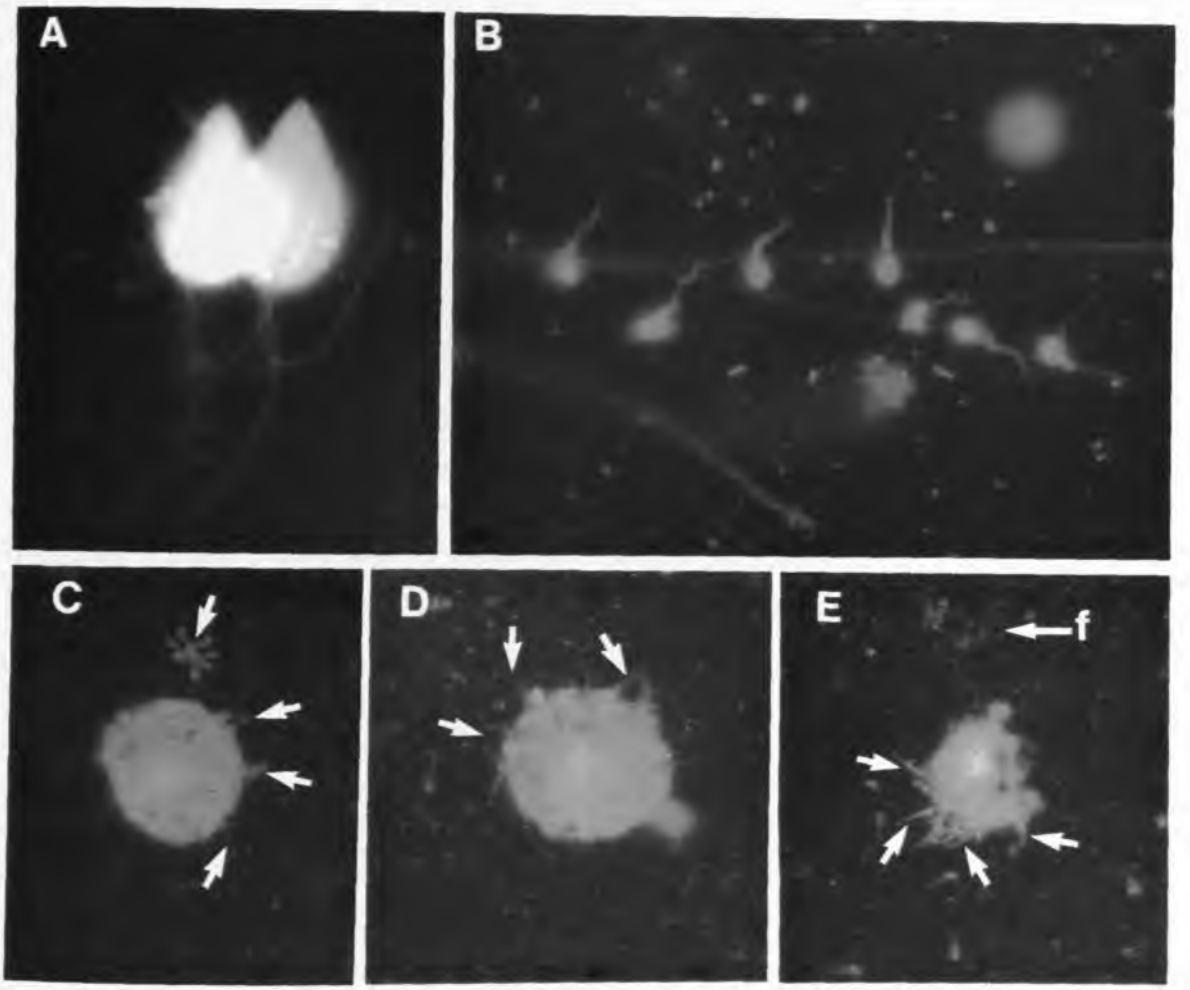
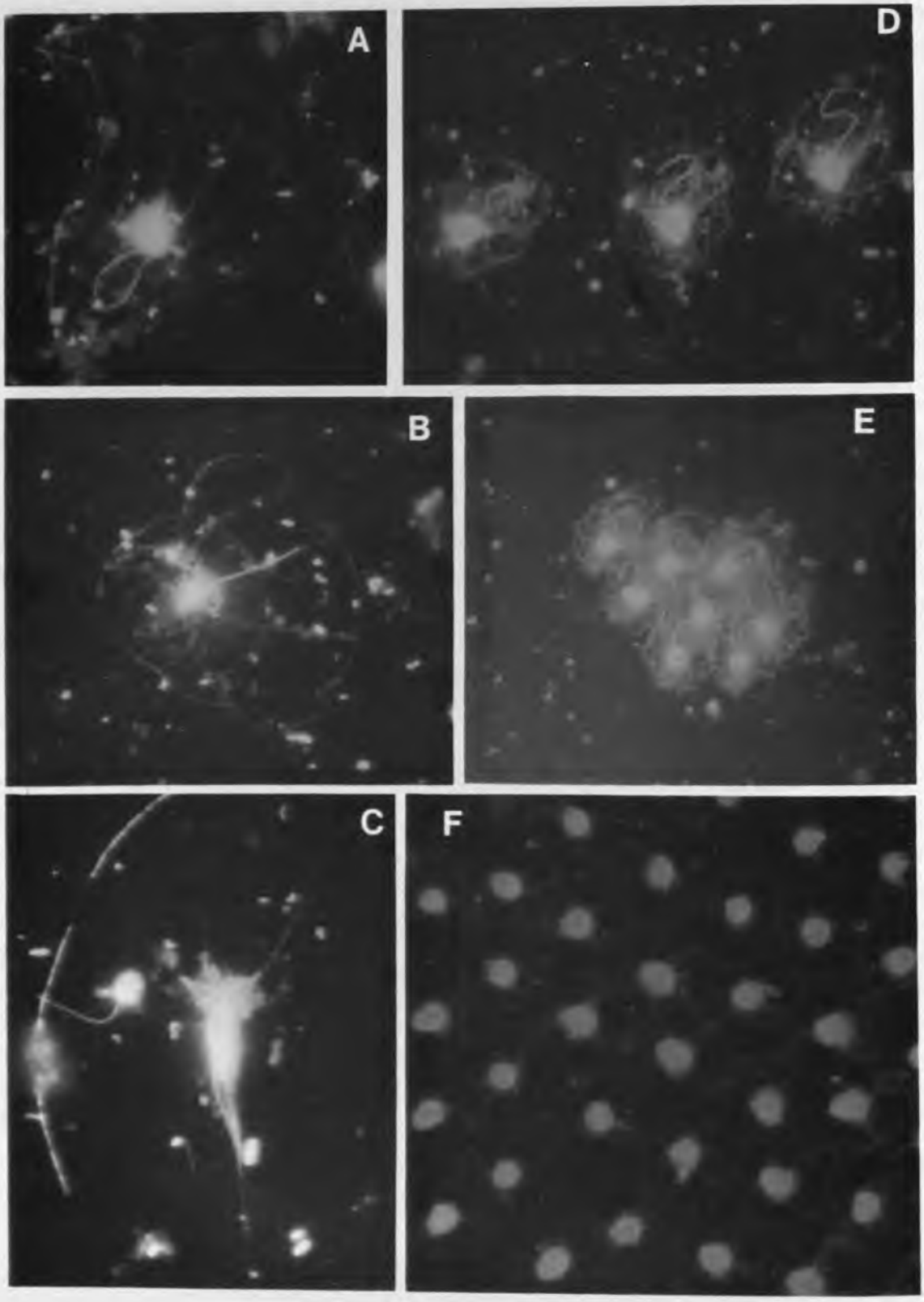
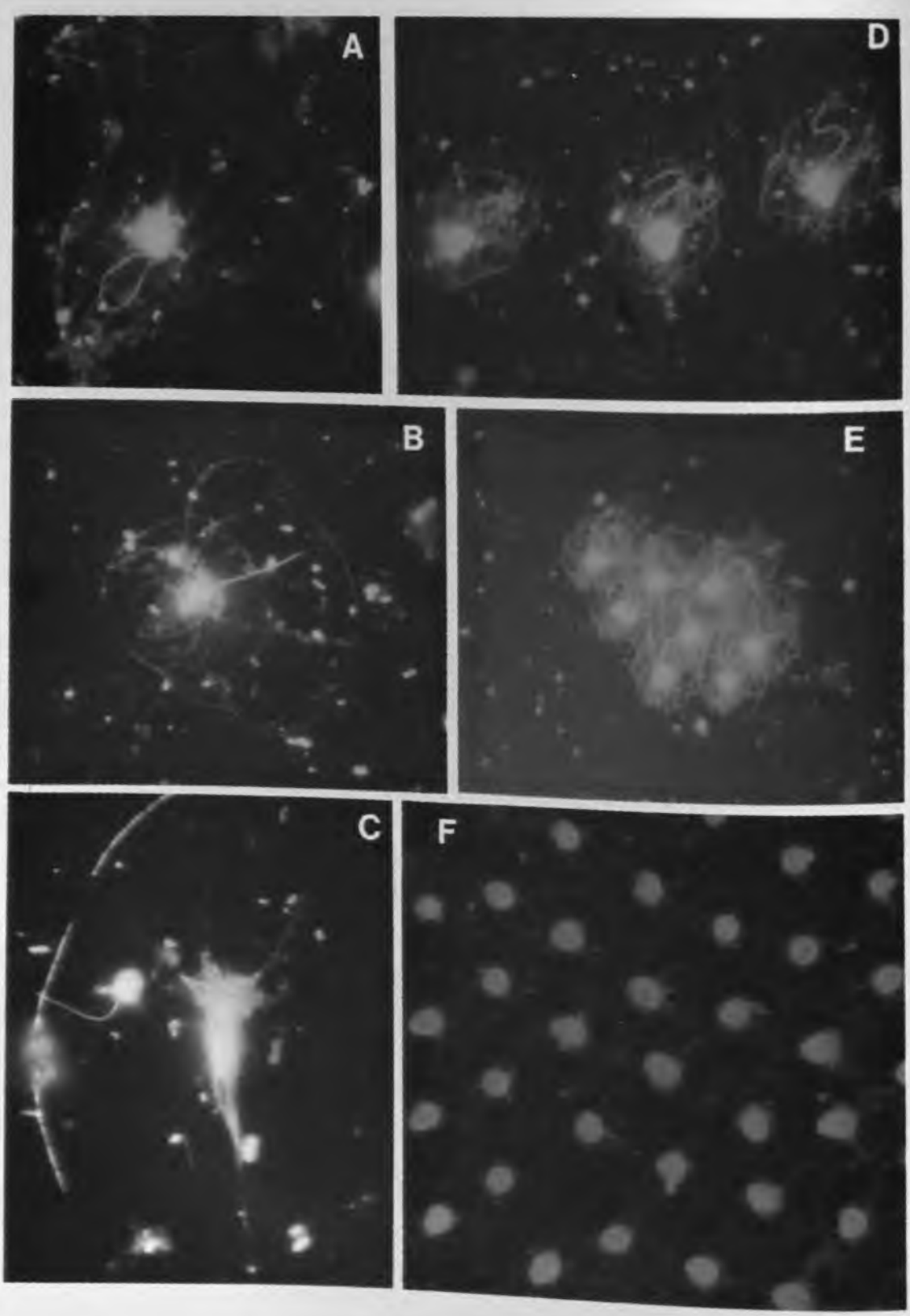


Figure 30. Part A: Epifluorescence micrographs taken at X1250 magnification of IfM monitoring samples from 26 November 1987 with DAPI-proflavin double stain in a shifted double exposure (A), from 23 March 1988 stained with acridine orange (B) and from 18 May 1988 with proflavin (C, D & E) portraying a large unpigmented cryptomonad, probably Leucocryptos (A), several small HNF with single flagella attached to diatom spines (B), and naked cells of Dictyocha speculum in (C, D & E) with short arrows pointing to orange-fluorescing ultra-structures ca. 1 µm wide and 5 µm long (f: flagellum).
Part B: Epifluorescence micrographs of AO-stained water samples from IfM monitoring on 18 March 1988 taken at X1000 (A, B, C) and on 23 March 1988 taken at X800 (D & E) and at X1250 (F), where different species of loricate choanoflagellates (Acanthoecidae) can be seen, Crinolina isef jordensis (A), Parvicorbicula circularis (B), Calliacantha natans (C), and different aggregates of Diaphanoeca sp. (D, E & F).

Part B



Part B



lorica often too faintly for recognition, thus causing underestimation of choanoflagellate counts. AO is a nucleic material stain, which also binds strongly to other organic material and appears to be much less specific than DAPI. Although particularly suitable for loricate choanoflagellates, AO staining cannot be applied for naked nanoflagellate enumeration, since autofluorescence of photopigments is completely masked by its orange fluorescence, so that autotrophic forms cannot be distinguished from heterotrophic forms. Another important feature of Acanthoecidae revealed in AO preparations was the frequent formation of aggregates or colonies with varying cell number, which often exhibited a geometric arrangement with cells equidistant from each other (Fig. 30, Part B, micrographs B, D & F). Cell number in these colonies varied from 4 (D) to 68 (F), so that accurate counting is hampered by such clustered distribution.

3.2 Scanning electron microscopy

A rough comparison can be made between AO epifluorescence microscopy (Part B - B to E) and SEM micrographs in Figure 31 (B to E). In most cases, AO staining revealed enough detail of lorica structure to identify different species. A web-like integument lining inner surface of lorica and attaching cell to lorica is visible joining different coastae in Figure 31 (A, D & G). Thin tentacles or pseudopods form a fan-like collar around the flagellum (Fig. 31 - A & B), which constitute an efficient filter-feeding apparatus retaining the smallest of bacteria in the intervals between pseudopods (0.1 - 0.2 μm, Fig. 31-A).

For naked (non-loricate) nanoflagellates, transmission electron microscopy (TEM) is needed for positive identification. SEM micrographs in Fig. 31 (H to K) reveal different types of flagellation, for example a single hispid flagellum (H) and paired smooth flagella (I). In Fig. 32 – M), a Pyramomonas sp. with four equal flagella shows geometric ultra-structure in the external surface of the cell, a characteristic of many autotrophic nanoflagellates. The small spherical slightly bilobed nanoflagellates in Figure 32 (M & N) formed a bloom in grazer chambers during the second half of incubation in July and August 1988. These cells, which were also characterized by two short

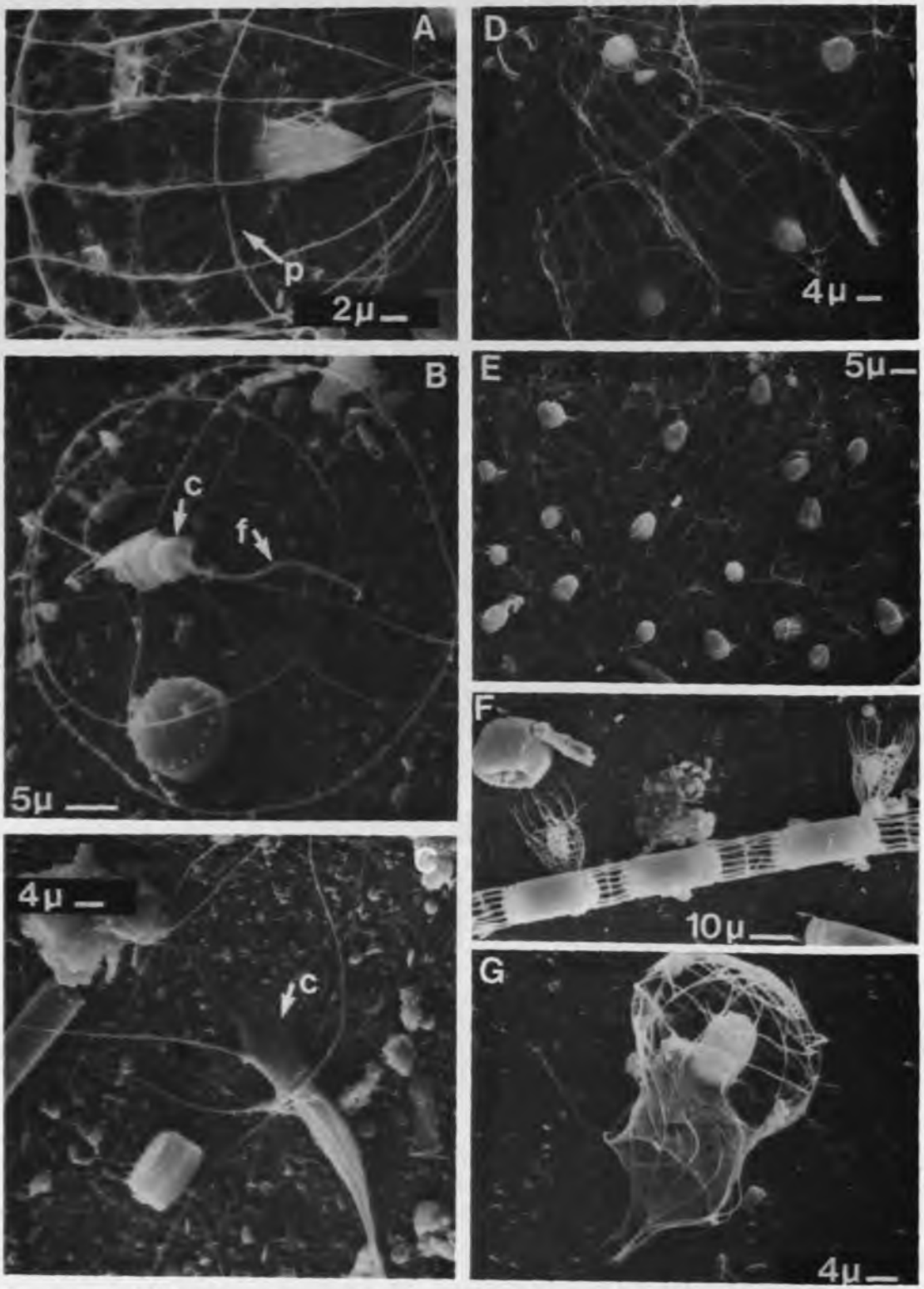


Figure 31. SEM micrographs of different species of loricate choanoflagellates present in IfM monitoring samples during spring 1988, Diaphanoeca sp. (A, D & E), Parvicorbicula circularis (B), Calliacantha natans (C), Acanthoecorbis unguilata attached to a Skeletonema chain (F), and Diaphanoeca grandis (G); arrows point to c: collar, f: flagellum, p: pseudopod.

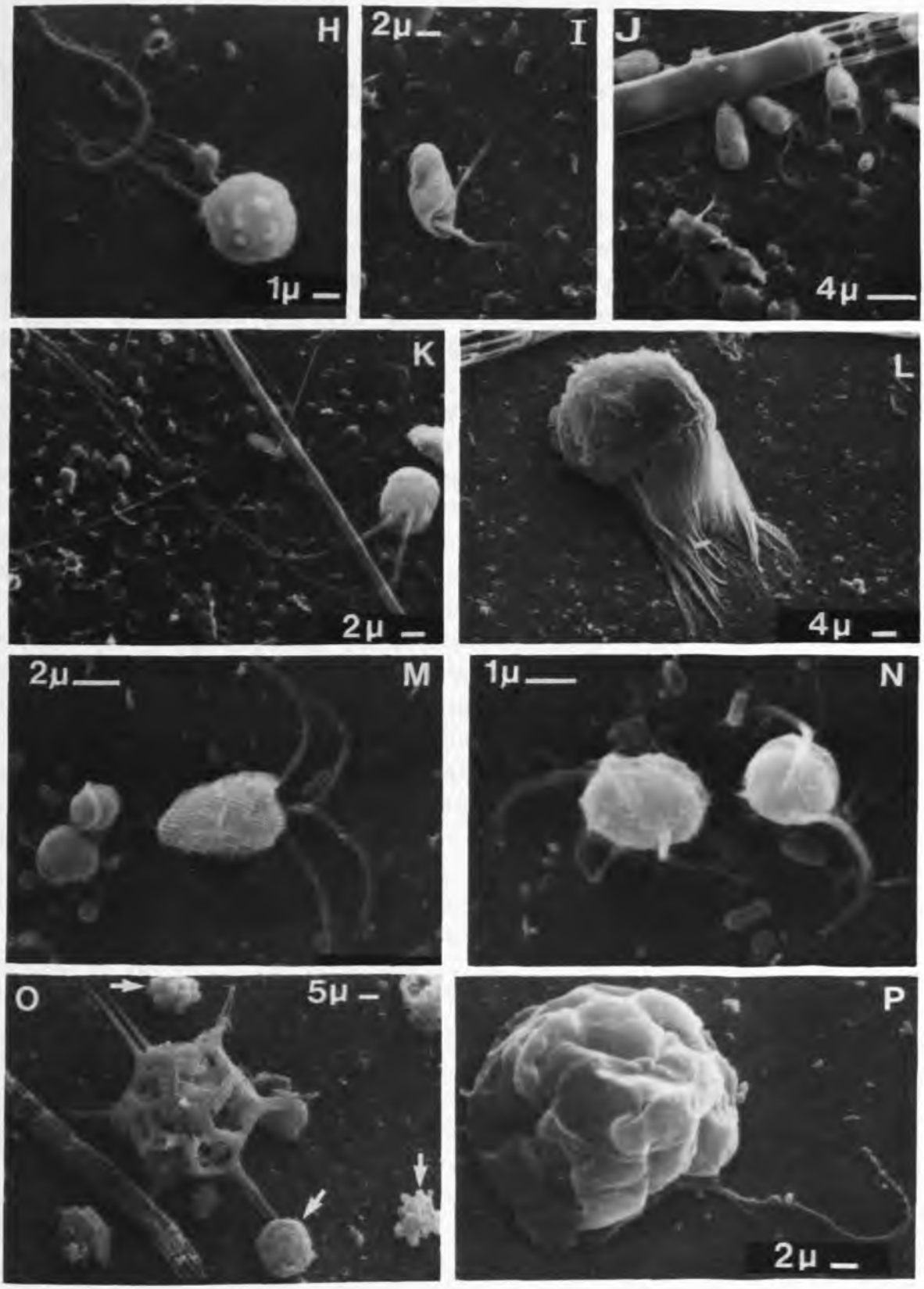


Figure 32. SEM micrographs of unpigmented nanoflagellates showing different types of flagellation (H to K), single flagellum with fine hairs or mastigonemes (H) and paired smooth flagella with unequal lengths (I & J), small oligotrich ciliate (L), autotrophic nanoflagellates present in grazer chambers during July and August 1988 with small spherical cells and paired flagella (M, N) and Pyramomonas with four equal flagella (M), silicoflagellate Dictyocha speculum in IfM water samples from 16 and 18 May 1988 (O & P) showing characteristic star-shaped exoskeleton and several naked cells (arrows in O; enlargement in P).

equal flagella, could be seen to contain two small weakly auto-fluorescent chloroplasts in proflavin preparations and, thus, were classified as pigmented nanoflagellates (PNF).

A small oligotrich ciliate can be seen in Figure 32 (L), which represented an additional component of heterotrophic nanoplankton ubiquitously present at concentrations 100 - 1000X lower than HNF. Since these small ciliates can squeeze trough 15 µm mesh size, they hamper the efficiency of pre-screening to eliminate HNF predators. Apart from causing damage to cells passing through the mesh, pre-screening also eliminates loricate choanoflagellate and HNF attached to diatoms (see Fig. 30, Part A - B) or other large particles. Thus, pre-screening was not applied to grazing experiments in the present study, since it was judged to cause too much disruption to the natural nanoplankton population.

Finally, SEM micrographs of naked cells of the silico-flagellate D. speculum in IfM water samples from 16 and 18 May 1988 (Fig. 32 - O & P) failed to reveal comma-shaped ultra-structures visible under epifluorescence microscopy (Fig. 30, Part A - C to E). This could be due to disintegration or `washing away' of extremely fragile structures during critical point drying when preparations are subject to strong turbulence inside high pressure chamber. TEM is needed to elucidate the origin and function of these ultra-structures.

DISCUSSION

1. Environmental Study

1.1 IfM monitoring

a) Bacterioplankton

One of the more interesting features in the seasonal development of bacteria revealed in this study was the inverse relationship between TBN and MCV, which was observed on both a long-term or seasonal scale and on a short-term or weekly scale. Recent studies in brackish water environments have detected similar seasonal trends in MCV (Coffin and Sharp, 1987; Bjørnsen et al., 1989). MCV ranges in these estuarine studies (0.02 - 0.16 μm^3) compared well with those at the IfM station (0.03 - 0.11 μm^3), even though different methods were used. Seasonal changes were attributed mainly to temperature (Bjørnsen et al. 1989) or to the combination of temperature and grazing pressure (Coffin and Sharp, 1987).

A decrease in bacterial MCV from spring to summer in both chemostat cultures and pelagic bacterioplankton in the northern Baltic was attributed to size-selective grazing by microflagellates, rather than to growth physiology (Andersson et al., 1986). In the Kiel Fjord, HNF exhibited on the average larger MCV in summer than in winter, so that it could be argued that large prey could be more often ingested during summer by a higher number of large predators, thus, effectively reducing the MCV of prey populations. However, opposite trends observed on a short-term basis could not be explained in terms of size-selective grazing of large bacteria. Since bacteria MCV had a stronger negative correlation with temperature ($r = -0.55$, $P < 0.0001$) than with HNF ($r = -0.33$, $P < 0.005$) at the IfM station, temperature probably played a more important role than grazing in regulating seasonal variations of bacterial sizes in the Kiel Fjord. Increased growth rates in warm but nutrient-depleted waters in the summer would produce large numbers of increasingly smaller cells.

Although TBN and biomass in a previous investigation of the Kiel area (1974-75) exhibited seasonal trends similar to the present study, no seasonality in MCV was reported (Zimmermann, 1977). This past work found no marked deviations in size distribution over a one-year period (March 1974 to March 1975) at different stations in the Kiel Fjord and Kiel Bight. More than 70% of all bacteria in the 1974 study were found to be smaller

than 0.4 μm (diam. and length) corresponding to a cell volume of 0.018 μm^3. This size range corresponded roughly to the combination of classes 1 to 3 (Table 2), which were found to constitute only 50% of TBN for the 1987-89 period. The largest size category with cell volume > 0.19 μm^3 (classes 7 and 8) had a mean frequency of 4% in both studies.

Owing to the degree of subjectivity in measuring very small cells with epifluorescence microscopy, it should be expected to find discrepancies between studies. In fact, it is rather a coincidence that an identical overall mean volume of 0.06 μm^3 was estimated in both studies of bacteria in the Kiel area. Sampling strategies performed in these studies were different, with monthly sampling of several stations in 1974 and weekly sampling of one station in the present study. Higher sampling frequency, considering the unstability of hydrographic conditions in the Kiel Fjord, could explain in part the higher degree of variability in TBN detected in 1987-89. Monthly sampling during the dynamic period of spring and summer does not seem adequate, particularly when describing the microbiological community, the short-term responsiveness of which has been well documented (Gocke, 1975; Meyer-Reil et al., 1979).

TBN in the surface layer (2 m) of the inner fjord varied from roughly 1 to 6 X 10^6 cells ml^{-1} during 1974 (Zimmermann, 1977). This was comparable to the current surface data from the IfM pier, which calculated as monthly means gave a range of 1.3 to 7.0 X10^6 cells ml^{-1}. Both studies detected somewhat lower numbers near the bottom. Surface biomass, however, estimated in the inner fjord during 1974 was considerably lower (5 to 24 μg C l^{-1}) than 1987-89 monthly means for the IfM station (22 to 94 μg C l^{-1}). This discrepancy was mainly due to the conversion factor used here, which was 3X higher (0.3 pg C μm^{-3}) for the average bacteria size (0.06 μm^3).

Even though an inverse relationship between TBN and MCV has not been previously reported for the Kiel area, it could be predicted from bacteria activity findings. Autoradiography of bacterioplankton in the Kiel area (Hoppe, 1977) demonstrated that the fraction of active bacteria increased six-fold from winter (9%) to summer (55%). Most of these actively metabolizing bacteria were contained in the 0.4-0.6 μm fraction in the innner fjord (Hoppe, 1977), corresponding roughly to size classes 3 and

4 (Table 2), which represented 55% and 52% of TBN during summer 1988 and 1989, respectively. Thus, extrapolating from both studies, the majority of bacteria in the Kiel Fjord during summer were small and active, whereas bacteria in the winter tended to be larger and relatively inactive.

Cyanobacteria standing stocks in the Kiel Fjord were very low relative to those of heterotrophic bacteria, HNF and phytoplankton. Rough estimates of cyanobacteria biomass with a conversion factor of 0.25 pg C μm^3 (Kana and Glibert, 1987) and MCV of 0.35 μm^3 gave a summer average of 5 μg C l^{-1} in 1988 and 4 μg C l^{-1} in 1989 at the IfM station for both depths. Total phytoplankton biomass at both depths, estimated conservatively with a carbon: chlorophyll ratio of 50, averaged 460 and 370 μg C l^{-1} during summer 1988 and 1989, respectively. Therefore, cyanobacteria constituted at most only 1% of the summer phytoplanton standing stock. These estimates were considerably lower than in a previous study of picophytoplankton (Jochem, 1988) which reported cyanobacteria biomass values up to 130 μg C l^{-1} in the fjord, contributing 12 and 32% to total phytoplankton biomass in July and August 1986. Since neither study performed exact measurements of cell sizes, very different MCV were obtained. Thus, exact estimates of cyanobacteria MCV are needed to assess biomass adequately. Unfortunately, such data are still lacking for the Baltic Sea in general.

b) Nanoplankton

Since the present work represents the first intensive study of heterotrophic nanoflagellates (HNF) in the Kiel area, comparison with previous results to assess long-term changes was not possible. Maximum HNF abundances in the warm season surpassed by 2-3X those reported for other brackish shallow water environments (Fenchel, 1982c; Sherr et al., 1984; Galvão, 1984), whereas TBN seasonal maxima were comparable. Wide fluctuations in numbers could be expected under the unstable hydrographic conditions in the fjord. These short-term oscillations would make eutrophication trends difficult to ascertain even for more prolonged study periods. In a recent one-year cycle investigation in a Danish eutrophic fjord (Limfjorden), HNF and TBN in the surface layer also showed pronounced oscillations in the spring and summer with a range of variation of 0.2 - 15.2 X 10^3 ml^{-1} for

flagellates and 0.5 - 15.2 X 10^6 ml^{-1} for bacteria (Andersen and Sørensen, 1986). The amplitude and frequency of variation was similar to the Kiel Fjord, while yearly means in Limfjorden were higher for bacteria (6.3 X 10^6 ml^{-1}) but lower for HNF (2.0 X 10^3 ml^{-1}) compared to overall means at the IfM station (see Table 6). Trophic coupling between bacteria and HNF populations were particularly clear during the warm season in Limfjorden.

Similarly, trends in HNF and TBN appeared coupled throughout the IfM study. Superimposed on long-term seasonal cycles, a short-term negative relationship could be frequently observed, in particular during spring and fall. An exceptionally good example occurred during the silicoflagellate bloom in May 1988. The HNF minima observed at this time, when environmental conditions seemed optimal to sustain large populations, suggested an antagonistic effect from the D. speculum bloom. One possible inhibitory mechanism could have been the release of a large number of defense organelles, such as the orange-fluorescing comma-shaped structures observed in association with D. speculum naked cells (see micrographs in Results, section 3.1), which resembled a large type of discharged discobolocyst (see descriptions in Lee, 1980).

Naked D. speculum blooms have been observed in the Kiel area since 1983 (Nöthig, 1984; Jochem and Babenerd, 1989), although this was the first reported incidence of large numbers of ultra-structures associated to naked cells. D. speculum counts at a mid-fjord station reported by Jochem and Babenerd (1989) on 18 May 1988, namely 5.5 X 10^3 naked cells ml^{-1}, were in good agreement with those found at the IfM station on the same date (5.8 X 10^3 naked cells ml^{-1}). Even though different types of projectiles are common in certain algae groups (eg. Dynophyceae, Cryptophyceae, Crysophyceae), such ultra-structures have not been described until now for the genus Dictyocha/Distephanus (van Valkenburg, 1971; Moestrup and Thomsen, in press). Fish mortality has been attributed to silicoflagellate blooms in Danish waters; however, antagonistic mechanisms of D. speculum towards other microorganisms are unknown. In any case, even if HNF minima during this period were not caused by D. speculum, the unusually large bacteria bloom observed at the time seemed a direct consequence of severely depressed grazing by HNF combined with high DOM production by the micro-algae.

Small cells (2 - 6 μm^3 avg. volume) represented the dominant size group (classes 1 and 2, Table 1) of HNF with a mean relative abundance of 58% for the entire study. This fraction increased to 72% of HNF during winter and decreased to 55% during spring and summer, when larger sizes (> 45 μm^3) became less rare. Seasonal averages of cell volumes varied from 17 μm^3 in the winter to 33 μm^3 in the summer, which constituted a smaller range of variation than could have been expected from the diversity of sizes observed (Table 1). This was due to the ubiquitous presence of very small cells, which represented the most constant group throughout the study. In a recent seasonal study in the northern Baltic, small flagellates were also observed to constitute the bulk of the population (overall MCV 16 μm^3) with a small range of variation in monthly MCV from 11 to 19 μm^3 (Kuosa and Kivi, 1989), which constituted a smaller seasonal range than in the Kiel Fjord.

The most distinct feature of HNF seasonal distribution in the Kiel Fjord was the predominance of choanoflagellates in early spring. The majority of choanoflagellates observed at this time possessed silica lorica and thus, had to compete with the spring phytoplankton bloom for dissolved silicates. During spring 1988 and 1989, silicate decreased from ca. 50 µM and 20 µM at the end of February, respectively, to ca. 10 µM and 3 µM at the end of March, according to Planktology Dept. data. For both spring periods, silicate surface to bottom ratios increased from an average of 1.0 during February to an average of 2.0 during March. In March 1988, not only was the monthly mean abundance of diatoms twice as high at the surface (6 X 10^3 cells ml^{-1}) than at the bottom (3 X 10^3 cells ml^{-1}), but also cells at 5 m appeared senescent with faded autofluorescence and many empty frustules were observed. Diatoms were also more abundant at the surface during March 1989. Loricate choanoflagellates in March 1988 and 1989 averaged 2 X 10^3 cells ml^{-1} (underestimated by proflavin, see Results, 3.1) at both depths with somewhat higher abundance at 5 m. This pattern indicated that choanoflagellates could effectively compete with diatoms for silicate, particularly below the surface layer.

In contrast to cyanobacteria, pigmented nanoflagellates (PNF) constituted a more predominant fraction of phytoplankton standing stock during summer 1988 and 1989. PNF biomass at the

surface, estimated with the same MCV and carbon conversion factor as for HNF, averaged 76 µg C l^{-1} during summer 1988 and 26 µg C l^{-1} during summer 1989, which represented respectively, 16% and 6% of the phytoplankton standing stock. On the other hand, HNF:PNF number ratios at 0 m averaged 1.3 and 1.8 in summer 1988 and 1989, with higher values in the spring (2.1-2.5). Ratios were higher at 5 m with ca. 2 in the summer and 3-5 in the spring. Thus, heterotrophic cells generally represented the dominant fraction of nanoplankton in the Kiel Fjord.

Other studies in tidal shallow water environments have reported an opposite trend, whereby PNF numbers tend to be higher than HNF during the warm season (Sherr et al., 1984; Galvão, 1984). A better correlation between HNF and PNF existed in the Kiel Fjord ($r = 0.52$, $n = 96$) than in the tidal environments. Furthermore, adding chlorophyll to multiple regression analyses improved the linear fit of temperature and TBN, such that roughly 50% of HNF variability was predicted at 0 m and 40% at 5 m by these three variables. These findings suggested that phytoplankton, particularly the smaller size fraction (< 20 µm) constituted mostly by PNF, could represent an important alternate food reservoir for HNF in the Kiel Fjord. Grazing on both bacteria and small micro-algae could sustain the large HNF standing stock present during spring and summer.

1.2 Western Baltic study

To enable a rough comparison of conditions in the Kiel Fjord with the three BMP stations, a summary of overall means for different variables is given in Table 6. It is to be noted that IfM means were calculated with approximately 4X more data points than the other three stations. Therefore, means at IfM are not statistically comparable with means at KB, BE and FB. Nevertheless, since generally less variability can be expected outside the fjord (Zimmermann, 1977), means at BMP stations can be considered to give a reasonable approximation of variable dimensions, even if calculated with fewer data points.

IfM showed enrichment relative to other stations in terms of chlorophyll, nanoplankton (pigmented and unpigmented) and heterotrophic bacteria. This was not surprising considering the higher temperature and nutrient regimes inside the fjord. At the end of winter, nitrate at the IfM station reached concentrations

typically 3-4X higher than at Fehmarn Belt and in the Kiel Bight in the surface layer (see Berichte zum Biologischen Monitoring der Ostsee 1987-1988). Although nitrate rapidly became limiting during summer 1988, concentrations of ≤ 1 µM were reached by the end of May at FB, but only in August at IfM, according to Planktology Dept. data. Surface ammonium remained < 1 µM at FB and 3-5 µM at IfM during summer 1988. Therefore, increased eutrophication in terms of nutrients translated into larger pico- and nanoplankton populations inside the fjord.

Table 6. Comparison of overall means of different variables at the four monitoring stations for all depths during the entire study.

Sta.	Temp. °C	Sal. SX10^3	Chl a µg/l	PNF X10^3	CYANOBAC. No. X10^3	/MCV* µm^3	/Biom* µgC/l	HET. BACTERIA No. X10^6	MCV µm^3	Biom. µgC/l	HET. FLAGELLATE No. X10^3	MCV µm^3	Biom. µgC/l
IfM[a]	9.8	15.7	6.86	3.33	17.4	0.35	4.1	3.63	0.059	52.5	4.40	24.2	25.7
KB	8.1	16.7	2.53	1.80	54.3	0.81	30.4	2.03	0.091	45.8	2.52	24.0	17.5
BE	7.0	18.2	2.36	1.15	28.8	0.52	20.1	1.86	0.084	38.6	2.09	16.6	11.0
FB	8.0	15.1	1.96	1.69	40.7	0.71	36.3	1.68	0.081	32.0	2.42	18.6	12.2

* Cyanobacteria mean cell volume (MCV) and biomass were estimated in the surface layer at peak summer abundances.

[a] IfM means calculated with ca. 4X more data points

N.B. Cell number (No.) for pigmented nanoflagellates (PNF), cyanobacteria, heterotrophic nanoflagellates and bacteria are given in X10^n cells ml^{-1}.

a) Phytoplankton

One exception to the previously mentioned trend was the cyanobacteria component, which exhibited considerably higher cell numbers and biomass outside the fjord (Table 6). Relative to total phytoplankton biomass, calculated as chlorophyll X 50, cyanobacteria during the summer represented an average of 24% at KB, 27% at BE and 42% at FB in the surface layer. Therefore, cyanobacteria constituted an important -if not dominant- fraction of summer phytoplanton outside the fjord. In fact, cyanobacteria could represent the main determinant for microbiological populations during the warm season in the Western Baltic.

Cyanobacteria were generally aggregated into amorphous colonies, which varied in size from only a few to several hundred

cells. Associated to these agglomerates, were numerous large heterotrophic bacteria and nanoflagellates, which seemed to constitute a specialized micro-community. It is possible that cyanobacteria sustained a major portion of the heterotrophic activity in these waters. Preliminary evidence for this was obtained from correlation analyses between variables. Cyanobacteria gave the single best linear fit for TBN at all three stations and depths. Furthermore, the combination of temperature, HNF and cyanobacteria counts gave the highest multiple regression coefficient, such that roughly 60% of TBN variability was predicted at KB and BE ($P < 0.0001$), and 30% at FB ($P < 0.005$). Mean cyanobacteria abundance increased with MCV following the order IfM, BE, FB and KB. In a previous study (Jochem, 1988), considerably higher counts were obtained inside the fjord (14×10^4 cells ml^{-1}) than outside (8×10^4 cells ml^{-1}) during the summmer maximum (July 1986). This was somewhat contrary to the observations of the current study, where maximum counts reached 36.1×10^4 cells ml^{-1} at KB (July 1988), but only 14.4×10^4 cells ml^{-1} at IfM (June 1988). In both studies, maximum counts were associated with the surface.

Lower counts at BE could be caused by higher surface salinity (mean: 17.3 S X 10^3) compared to KB (mean: 15.7 S X 10^3) and FB (mean: 12.8 S X 10^3). Cyanobacteria also revealed the strongest negative correlation with salinity ($r = -0.51$, $P < 0.001$) at BE. It is possible that species found in the Baltic are primarily brackish water and/or halotolerant freshwater forms, which start becoming inhibited at salinities > 16 S X 10^3. Similar Microcystis type cells were found at concentrations varying from 11 to 30 X 10^4 cells ml^{-1} in surface waters troughout a fall cruise (29 September to 11 October 1987) extending to the Gotland Basin (57°23'10 N, 19°56'10 E) with salinities as low as 7 - 8 S X 10^3. Furthermore, maximum summer abundance reaching 38 X 10^3 cells ml^{-1} was detected at Gotland Deep (57°20'00 N, 19°51'42 E) in July 1988 at 15 m with 6.9 S X 10^3 salinity (Galvão, unpublished data).

These various findings indicated that Baltic cyanobacteria were well-adapted to low salinities. Halotolerance, however, could not explain differences between KB and IfM stations, which had similar salinity regimes. More favourable hydrographic conditions at KB, such as prolonged periods of stratification

during summer, which would maintain algae at optimal light levels, coupled with decreased grazing pressure by microprotozoa, could perhaps account for the magnitude of cyanobacteria blooms outside the fjord. Quiescent conditions have been invoked to promote aggregation of Trichodesmium filaments lacking heterocysts, and consequently, increase the potential for nitrogen fixation through the stimulation of nitrogenase expression in oxygen depleted microzones inside aggregates (Pearl et al., 1989). Similarly, chroococcoid colonies in the Baltic may possess a large potential for nitrogen fixation, which would have important implications for nitrogen and new production budgets of this ecosystem.

PNF outside the fjord also represented an important constituent of phytoplankton in terms of biomass calculated from chlorophyll levels. During summer 1988, PNF contributed 42% at KB, 17% at BE and 32% at FB to phytoplankton biomass in surface waters. It is to be noted, however, that phytoplankton biomass calculated as chlorophyll X 50 gave reasonable estimates only when small cells predominated. During blooms of larger microalgae such as diatoms and dinoflagellates, this method severely underestimated phytoplankton biomass, according to microscopy measurements in Berichte zum Biologischen Monitoring der Ostsee. Due to low specific chlorophyll content, cyanobacteria and PNF counts correlated poorly with chlorophyll a levels for the whole study period at the three stations. Maximum chlorophyll a was detected in the fall during blooms of large dinoflagellates (eg. Ceratium sp.), when relatively few cells with a very high specific chlorophyll content were present. Species specific contents of chlorophyll a would improve the predictive function of chlorophyll a with phytoplankton abundance.

b) Heterotrophic pico- and nanoplankton

Higher MCV for heterotrophic bacteria measured outside the fjord (Table 6) may be due to inherent subjectivity of microscopic measurements, since MCV for the BMP stations was determined by other people. Nevertheless, there was a parallel trend between MCV and TBN decreasing from KB to FB, which might have reflected decreasing eutrophication conditions. Similarly to the IfM station, HNF constituted the dominant fraction of nanoplankton at KB, BE and FB. HNF:PNF number ratios averaged 2

at the surface (2 m) and 3-4 near the bottom (15/20 m). Seasonal shifts in predominance were detected. For example, during 1988, predominance of HNF over PNF at the surface was more marked in the spring than in the summer at KB and BE, but stronger in the summer at FB. On the other hand, PNF became predominant in spring 1989 at KB (2 m).

The pattern of HNF distribution followed that of cyanobacteria with lowest counts and MCV at BE. This could be a result of a predator:prey relationship between HNF and either cyanobacteria or heterotrophic bacteria associated to chroococcoid colonies. In the extended Baltic cruise in fall 1987, high numbers of HNF tended to be detected together with high numbers of cyanobacteria. This trend was also observed in a vertical profile at Gotland Deep in July 1988, where HNF numbers ($4.3 - 6.2 \times 10^3$ cells ml^{-1}) correlated with cyanobacteria ($1.9 - 3.8 \times 10^4$ cells ml^{-1}), rather than with TBN (Galvão, unpublished data). In a recent mass-balance model of the microbial food web in an oligotrophic marine ecosystem, where cyanobacteria were the dominant primary producers, it was calculated that 57% of primary production was grazed by bacterivores in the form of cyanobacteria (Hagström et al, 1988). Further investigations in the Baltic are needed to determine to what extent HNF actually graze cyanobacteria and/or associated heterotrophic bacteria. Besides yielding a better understanding of the ecosystem, this would enable the assessment of the role of microprotozoa in controlling cyanobacteria blooms.

2. Grazing Study

2.1 Population development

The pattern of HNF development showed some seasonality in terms of duration of the initial lag phase. Shorter lag periods lasting less than 2 days occurred at the beginning of late spring and summer experiments (May, July and August), whereas these periods extended from 3 to 6 days in early spring and fall (October 1987, March and October 1988). Since no HNF growth was observed after 6 days in January, the lag period in winter presumably lasted even longer. Water temperature alone could not explain these differences, considering that ranges in October 1987 and 1988 were similar to May 1988 (see Table 3). In fact,

ambient water in the first 2 days of incubation was slightly colder in May (10.7 - 10.8 °C) than in October 1987 (11.3 - 11.5 °C) and 1988 (11.7 - 11.9 °C). Therefore, other factors such as the type or state of HNF populations at the beginning of experiments were also at play. For example, in March choanoflagellates were predominant. Apart from attaching to surfaces and forming aggregates, which hamper accurate counting, these specialized forms build fragile silica lorica and are particularly susceptible to physico-chemical perturbations during experimental manipulation. These characteristics could explain the prolonged lag phase in the March experiment.

Lag periods in HNF growth have not always been reported in the literature. In Georgia coastal waters (Sherr et al., 1984) vigorous growth of HNF in pre-screened natural water samples was found in 24 h bottle incubations. In a shallow embayment in Hawaii, substantial HNF development also occurred in intact water samples incubated 24 h in diffusion chambers (Landry et al., 1984). On the other hand, a decrease in HNF number was reported in the initial 12 h with natural water samples from the Mediterranean and Pacific coast, incubated in large carboys (Hagström et al., 1988). Lag periods lasting up to 6 days were detected in mesocosm experiments in Finnish coastal waters (Progress Report 1988, Project PELAG 1988, Hanko, Finland). Although different incubation methods were used in these various reports, a regional trend seemed to exist in HNF development, whereby natural populations adapted to warmer waters seemed able to respond more quickly to incubation.

A pronounced decrease in bacteria numbers inside grazer chambers occurred usually after 3-4 days, when HNF increased beyond 6 - 8 X 10^3 cells ml^{-1}. This sharp decrease in bacteria lasted at most two days, afterwards bacteria populations generally decreased more gradually and stabilized at ca. 1 - 3 X 10^6 cells ml^{-1} before the end of the experiment. At this point, HNF no longer exhibited sustained growth and numbers either decreased or oscillated. This could be due to predation on HNF by ciliates and/or to a minimum threshold level of prey, below which grazing continued, but was no longer sufficient to sustain growth. If it is assumed that flagellate growth became limited by the carrying capacity of the environment, then this level varied in different experiments, since maximum concentrations attained

by HNF also varied. In May and October 1988, the threshold level for HNF growth was reached when biomass ratios of bacteria:HNF approached 1. In July and August, HNF growth was still observed when ratios had decreased well below 1. This was possible by supplementary grazing on small PNF, which formed a bloom inside grazer chambers during these experiments.

Even though shifts in relative abundance of different HNF size classes occurred, populations maintained a broad spectrum of shapes and sizes throughout incubation inside chambers. This morphological diversity, which also reflected to some degree species diversity, confirmed that diffusion chambers were able to approximate environmental conditions. If physico-chemical factors inside chambers had been considerably different from ambient, then the effects of strong selection would rapidly cause the dominance of one or two opportunistic species. This is often the case in artificial laboratory conditions, when unnaturally high amounts of food are given to stimulate HNF development and enables isolation of pure cultures of HNF.

Strong size/species selectivity occurred in preliminary experiments, when glucose was added to natural water samples to test diffusion. At the end of 2-4 day incubation (June 1987, see Fig. 4), very large populations of HNF had developed, which were dominated (83 - 93%) by two size classes (2 + 3) with similar morphology (rounded shape, paired unequal flagella). In parallel bottle incubations performed in May and July, HNF in unfiltered treatments exhibited a strongly skewed size distribution, where 70-90% of HNF were included in size classes 1 and 2 towards the end of incubations. At the same time MCV in bottles were ca. 2X smaller than in chambers, although numbers tended to be higher, so that no net increase in biomass occurred. This indicated that HNF growth in bottles became rapidly unbalanced in the course of incubation.

2.2 Growth and grazing rates

Means of bacterial growth rates for different grazing experiments were comparable for both filtered and unfiltered treatments giving an average doubling time of 45 h (see Table 4). The range of growth rates in control chambers in the summer (0.34 - 0.66 d^{-1}) was in good agreement with a previous Irish Sea study in July, which used comparable methodologies with *in situ* incuba-

tions in dialysis bags of filtered (3.0 µm) and unfiltered natural water, and reported similar ranges (0.37 - 0.73 d^{-1}) for both treatments (Turley and Lochte, 1986). On the other hand, bacterial growth in a temperate estuary in spring and summer was somewhat higher (0.48 - 0.86 d^{-1}) with 1:1 dilution of 1.0 µm filtered samples (Kirchman and Hoch, 1988). Rates were even higher (1.2 d^{-1}) in 1.0 µm filtered samples from the Hawaian coast incubated in diffusion chambers (Landry et al., 1984). These variations could be attributed to regional differences in water temperature regime rather than methodology.

The range of HNF growth rates in summer and fall (0.3 — 1.1 d^{-1}) was lower than in Georgia coastal waters (0.8 - 1.7 d^{-1}) for the same seasons (Sherr et al., 1984), whereas spring rates (0.28 - 0.43 d^{-1}) were higher than in Limfjorden, Denmark (0.19 - 0.26 d^{-1}) in May (Andersen and Sørensen, 1986). Growth rates reported for natural populations tend to be considerably lower than for pure cultures, which can grow at rates of 2 - 6 d^{-1} (Davis, 1982; Fenchel, 1982b; Caron et al., 1985). The fact that specific growth rates for HNF were higher than for bacteria in diffusion chambers, did not represent an unusual situation, since it has been previously observed in the natural environment (Landry et al., 1984) and has been postulated in ecological modelling (Fenchel, 1982c). Unfortunately, not enough information comparing HNF and bacteria specific growth rates is available in the literature to assess how realistic this situation was for the Kiel Fjord.

Ingestion rates of HNF (12 - 90 bac. flag.$^{-1}$ h^{-1}) were higher than those recently reported in natural community studies using fluorescently labelled bacteria (Sherr et al., 1987), fluorescent particles (McManus and Fuhrman, 1988) and TTI (Kuupo-Leinikki and Kuosa, 1988), which found rates varying from 2 to 27 prey flag.$^{-1}$ h^{-1}. It is possible that these reports underestimated ingestion rates owing to inherent artifacts of the methods, such as unpalatable food sources, which were egested or discriminated against, as well as invalid conversion factors for TTI. Using methodology similar to the present study, Landry et al. (1984) measured ingestion rates of 17 - 38 bac. flag. h^{-1} within a 24 h incubation period, which were in better agreement the range estimated here. On the other hand, pure HNF cultures (eg. Davis, 1982; Fenchel, 1982b; Caron et al., 1985) were able

to ingest bacteria at rates 1.5 - 3 X higher than the naturally mixed assemblages studied here. In conclusion, the average ingestion range of 28 - 38 bac. flag. h^{-1} calculated for the whole study was not inconsistent with previous investigations on natural populations. Very high rates observed in May 1988 (66 - 90 bac. flag.$^{-1}$ h^{-1}) were more characteristic of pure cultures and may have represented an extreme case caused by unusual conditions during the D. speculum bloom.

From high estimates for both growth and grazing in the May experiment, it was evident that HNF were not inhibited inside diffusion chambers, even though surrounding water contained high numbers of D. speculum (see Fig. 22), which averaged 4.7 X 10^3 cells ml^{-1} from 12 to 20 May 1988 reaching maximum abundance on May 17 and 18 (7.3 X 10^3 cells ml^{-1}). Naked silicoflagellates were observed to rapidly lose autofluorescence and cellular integrity inside grazer chambers within the first 3 days of incubation decreasing to < 0.2 X 10^3 cells ml^{-1} by May 15, probably due to unfavourable light conditions. More importantly, orange ultra-structures degraded even more rapidly and were undetectable by May 14. Since HNF developed rapidly in chambers, in contrast to ambient population, which remained at low numbers (see Fig. 22), this indicated that inhibitory effect on ambient HNF was not in the form of dissolved toxins. This would argue in favor of the hypothesis that ultra-structures were indeed some form of projectile, which could function as a disrupting or irritating mechanism for foreign cells. By limiting competition from other microorganisms, this mechanism would further ensure the success of D. speculum blooms. However, pending further investigation, this explanation remains entirely speculative.

2.3 Grazing impact on bacteria sizes

With the exception of the March experiment, grazing did not appear to continously remove one particular size, but rather varying sizes were affected at different times in the course of incubation. No grazing impact could be detected in the smallest and largest size classes (1, 2, 7 and 8), so that only medium sizes (3, 4, 5 and 6) appeared to be removed. Class 4 with an average volume of 0.05 μm^3 (see Table 2) suffered most frequently removal `events'. Therefore, if negative differences in class frequency between unfiltered and filtered treatments could indeed

be attributed to grazing impact, then the most frequently encountered sizes were also the most frequently grazed. Thus, grazing on bacteria seemed to follow a random encounter pattern, rather than true size selectivity.

Fenchel (1982a; 1987) postulated that filter-feeding flagellates would tend to capture all particles retained by the sieving meachanism (collar of pseudopodia in choanoflagellates, see micrographs in section 3.2) and would not discriminate against prey above a minimum size, compared to raptorial or interception feeders, which would tend to select larger prey due to physical factors regulating optimal relationships between prey and predator sizes. In two recent studies dealing with pure and quasi monospecific cultures of raptorial feeding HNF, size selectivity for larger bacteria was observed (Andersson et al., 1986; Lucas et al., 1987). Cocci and rods within a MCV range of 0.14 - 0.20 μm^3 were grazed preferentially by a HNF culture dominated by Pseudobodo sp., whereas cells larger than 0.67 μm^3 were not removed (Lucas et al., 1987). A pure culture of Ochromonas only grazed bacteria larger than 0.2 μm^3 with a preferred MCV of 0.32 μm^3 (Andersson et al., 1986). Bacteria in the Ochromonas cultures had a MCV range of 0.1 - 0.3 μm^3, while a shift in bacteria predominance from 0.14 to 0.67 μm^3 cells occurred with Pseudobodo.

In contrast, not only did bacteria MCV remain relatively low in diffusion chamber experiments (< 0.20 μm^3 in spring and < 0.10 μm^3 in summer and fall), but also a diverse population of HNF was maintained throughout the incubations. According to resource partitioning theory, different species of predators coexisting in mixed communities would be expected to have different prey selectivities to limit food competition. Therefore, if different HNF species effectively selected for different prey sizes, then the resulting grazed population would not be expected to exhibit removal of just one size class, but rather different sizes would be removed at different times following shifts of predominance in HNF species. Results from diffusion chambers tend to show this pattern, or rather lack thereof. Thus, conditions inside chambers seemed to constitute a better approximation of the natural environment. To obtain ecologically representative results in size selectivity, it is necessary to duplicate environmental conditions, where mixed populations of HNF are usually present

and where bacteria populations exhibit MCV typically smaller than 0.2 μm^3. Nevertheless, owing to methodological difficulties, this type of study is still rare in the literature.

2.4 Bacterial production

Thymidine incorporation (TTI) tended to overestimate bacterial production relative to net increase in biomass, but underestimated ingestion rates relative to observed changes in cell counts. These discrepancies could be partly resolved by adjusting conversion factors, which varied with different experiments and treatments, yet remained considerably lower in unfiltered treatments (unfiltered mean: 0.5×10^9 cells $nmol^{-1}$ vs filtered mean: 1.7×10^9 cells $nmol^{-1}$). Recalculating ingestion rates with experimentally determined factors, increased ingestion rates to 10 - 61 bac. $flag.^{-1}$ h^{-1}, which agreed better with cell-based estimates. These results question the validity of using a single empirical conversion factor for determining production and grazing rates from size fractionated treatments, since this assumes balanced growth in all samples. Evidence that growth was not continuously balanced in the course of incubation was given by varying specific activities, which also differed between treatments.

Conversion factors determined for various systems and with different techniques vary across at least a 10-fold range (Riemann et al., 1987; Scavia and Laird, 1987). In a recent study designed to investigate causes of variation, different treatments were applied to prevent bacterivory and stimulate bacterial growth (Coveney and Wetzel, 1988). The authors reported similar effects of increased conversion factors in filtered samples (1.0 and 3.0 μm) relative to intact samples and concluded that "much of the variation in cell number-based conversion factors was related to changes in apparent MCV of produced bacteria". To solve this apparent uncoupling between cell production and TTI caused by cellular growth cycles, characterized by DNA content varying as a function of MCV, the authors calculated a conversion factor based on biovolume rather than cell counts, which seemed to hold constant across a wide spectrum of treatments and bacterial growth rates. Since a high frequency of sampling was required during experiments to follow cell cycles, the validity of biovolume-based factors could not be ascertained in the

present study.

For comparable seasonal periods and using the same conversion factor, bacterial production estimated from TTI in diffusion chambers (1.3 - 2.9 µg C l^{-1} h^{-1}) was within the range reported in enclosure manipulations in a Danish eutrophic estuary (1 - 8 µg C l^{-1} h^{-1} in Bjørnsen et al., 1989), but higher than in the Skagerrak (0.2 - 1 µg C l^{-1} h^{-1} calculated from Kiørboe et al., 1990), and even more so in the more pristine surface waters of the Gulf of Finland (0.02 - 0.30 µg C l^{-1} h^{-1} in Kuosa and Kivi, 1989). Bjørnsen et al. (op. cit.) as well as Kuosa and Kivi (op. cit.) estimated a higher proportion of bacterial vs primary production in the summer (21 - 26%) than in the fall (2 - 16%). In contrast, bacterial production (TTI) in ambient water at IfM was only 3 - 4% of primary production (monthly means of Planct. Dept. data), during July and August 1988 experiments, but increased to 15% in October 1988.

2.5 Flagellate production

Flagellate production rates in grazer chambers showed a range of variation in agreement with other reports measuring increase in biomass of natural HNF assemblages (Sherr et al., 1984; Bratbak, 1987; Hagström et al., 1988). Sherr et al. (op. cit.) reported HNF productivities in a range of 6 - 14 µg C l^{-1} d^{-1} with 33% growth efficiency in Georgian coastal waters, where FDC based bacteria productivity was high (14 - 194 µg C l^{-1} d^{-1}). Productivities recalculated from gross production during 20-day incubations in Bratbak (op. cit.) were 5.5 - 9.5 µg C l^{-1} d^{-1} for HNF and 45.8 µg C l^{-1} d^{-1} for bacteria with a 24% HNF growth efficiency, which were very similar to the averages obtained from diffusion chamber experiments (see Table 4). In Hagström et al. (op. cit.), HNF production rates were estimated at 4.1 - 8.4 µg C l^{-1} d^{-1} with relatively low bacteria secondary production of 5.2 µg C l^{-1} d^{-1} (TTI), which was sustained by increased grazing on cyanobacteria (59%) relative to heterotrophic bacteria (11%) and assuming a relatively high growth efficiency for HNF (50%).

From chamber experiments, it was noted that the range of HNF production rates varied across a 2.4-fold range, whereas bacterial productivity varied 7-fold (see Table 4). In July, maximum HNF production was measured at rates nearly equal to bacteria, while grazing rates on bacteria were low. This resulted

in an unrealistically high growth efficiency for HNF (112%), indicating that a major fraction of HNF growth was being maintained by another food source, in this case small PNF. Concomitant grazing on PNF probably occurred in August as well, but to a lesser extent, since relatively high HNF growth efficiency (40%) was also measured at low ingestion rates (12 - 18 bac. flag.$^{-1}$ d^{-1}). These findings led to the conclusion that HNF were able to maintain relatively high production over a wide range of bacterial secondary production by switching to other food sources. An opportunistic grazing mode would maintain HNF production at maximum levels in a widely fluctuating environment, such as the Kiel Fjord.

2.6 Grazed bacterial production

HNF grazed a varying fraction of bacterial production depending on the state and size of populations in grazer chambers. HNF were capable of grazing more than 100% of bacterial production at maximum ingestion rates, as seen by the pronounced decrease in bacteria biomass during period of sustained HNF growth in grazer chambers. This period of intensified grazing pressure did not last longer than one or two days, after which bacteria standing stocks stabilized, when production tended to balance grazing. Therefore, it is difficult to compute an average for the amount of bacteria production grazed by HNF based on observed changes in biomass, since it varied substantially depending on HNF growth phase.

Based on TTI, HNF grazed a range of 61 to 85 % of bacterial production at maximum ingestion rates. The grazed fraction also varied in the course of incubation, although it never surpassed 100%. Therefore, means could be more easily computed with this method, giving for the second half of incubation periods an average of 40 % in October 87, 82% in July 88, 52% in August 88 and 45% in October 88. Recent studies using various methods reported varying fractions from 23% (McManus and Fuhrman, 1988) to 95% (Coffin and Sharp, 1987). In the northern Baltic, Kuosa and Kivi (1989) estimated a HNF carbon demand twice as high as bacterial production, thus, concluding that HNF utilized other food sources besides bacteria, such as pico- and nanoplanktonic algae. This was probably the case in the Kiel Fjord during the

warm season, when HNF: bacteria biomass ratios frequently approached 1 suggesting an `overload' in flagellate demand, which could be satisfied by grazing on an ample supply of pigmented nanoplankton.

2.7 Assessment of the microbial `loop'

An assessment of carbon fluxes in the microbial `loop' based on grazing experiments in the Kiel Fjord is compiled in Table 7. It is to be noted that the grazing study disregarded the role of ciliates, although their potential importance as bacterivores in the Baltic Sea has been demonstrated in laboratory experiments (Gast, 1985). However, since intact water samples were used in grazer chambers, the grazing effect of ciliates was included in the estimates of bacterial biomass grazed. If ciliates were able to compete effectively with HNF, then specific ingestion rates (Table 4) were overestimated, while `flagellate' carbon demand (Table 7) based on observed changes in bacterial biomass represent reasonable estimates of total `protozoan' demand. In view of other areas of uncertainty in determining these fluxes, the following discussion was intended to present a qualitative rather than quantitative analysis.

According to recent measurements of phytoplankton exudation, 11 - 16% of fixed carbon is lost in the form of DOM (Larsson and Hagström, 1982; Hagström et al., 1988). Consequently, a mid-range value of 13 % was used to correct primary production measured during experimental periods (means of 2-3 weekly surface measurements by Planktology Dept.) in Table 7. An average of 21 % of total primary production was required for bacterial growth (mean percentage from Table 7), so that exudation by phytoplankton might not satisfy it completely. Sloppy feeding by protozoa could release a large fraction of ingested carbon (ca. 50%, Hagström et al., 1988), which in turn could be reutilized by bacteria. With only 11 - 16% exudation by phytoplankton, then protozoa would have to furnish the remaining 5 - 10% DOM required by bacteria, either through incomplete assimilation of food particles or through mortality and cell lysis. However, these are average estimates and the importance of different pathways varied considerably, not only depending on the season, but also following daily and growth cycles, particularly in the highly dynamic environment of the Kiel Fjord. In surface waters in the Kiel

Fjord, large seasonal variations of up to 50% of primary production lost to exudates were found (Wolter, 1982). In enclosure manipulations, different algae released different amounts of exudates; PNF, in particular, produced significantly more exudates, which in turn were assimilated to a greater extent by bacteria (Wolter, op. cit.). With respect to seasonal variations in the present study, in fall 1987 and summer 1988, bacterial growth required a modest fraction of primary production (4 - 12%). This fraction increased in the spring, particularly in May 1988 during the D. speculum bloom (33%) and became predominant (> 50 %) only in October 1988. Therefore, a much greater contribution to the DOM pool had to come from protozoa (or from allochthonous sources) in October 88 than in August 88 to fulfill bacterial demand.

Table 7. Assessment of carbon fluxes in the microbial `loop' based on grazing experiments in the Kiel Fjord.

Expmt.	Primary Prod.* (µgC/l/h)	Bacterial C Demand** (µg C/l/h)	% of Prim. Prod.	Flagellate C Demand** (µg C/l/h)	% of Bac. Prod.
OCT87	22.43	2.68	11.9	1.71	106
JAN88	1.47	0.05	3.7	0	0
MAR88	10.28	2.45	23.8	1.53	104
MAY88	27.10	9.02	33.3	3.50	65
JUL88	14.89	1.83	12.3	1.00	91
AUG88	39.83	1.51	3.8	1.42	156
OCT88	4.10	2.23	54.4	2.08	155

* mean of Planct. Dept. measurements adjusted for exudation loss of 13% (Hagström et al., 1988; Larsson and Hagström, 1982).

** bacterial carbon demand calculated from biomass production in control chambers (Table 4) or from grazer chambers (Oct 88) assuming 60% growth efficiency (Button, 1985); flagellate carbon demand calculated from production and growth efficiency estimates given in Table 4.

Except during the D. speculum bloom, HNF carbon demand balanced or exceeded bacterial production (Table 7). This `overload' was particularly strong in late summer and fall 1988, when bacterial production could fulfill only 64% of HNF requirements. Concomitant grazing on small micro-algae (cyanobacteria and PNF) probably occurred not only during these

periods, but throughout spring and summer as well, when an ample supply was available. Results from the July experiment indicated that HNF were utilizing another carbon source besides bacteria, probably PNF, even though bacterial production was sufficient to sustain HNF growth. Direct measurements of micro-algae grazing would be particularly useful during this period. In August and October 1988, HNF required an additional 0.51 and 0.75 μg C l^{-1} h^{-1}. Assuming that this could be provided by grazing on phytoplankton, then at least 1.3 and 18.3% of primary production was consumed by HNF/protozoa. This amounted conservatively to 5 and 73% of primary production required by heterotrophic bacteria and protozoa.

These estimates lead to very different evaluations of the microbial 'loop'. In late summer, with high primary production and comparatively low heterotrophic demand by pico- and nanoplankton, the microbial loop cycled a small fraction of photosynthetically fixed carbon and therefore could not be considered as an important energy sink in the food web. Yet, in the fall, when primary production was low (in the absence of a fall bloom), but heterotrophic demand was moderately high, a much larger fraction of carbon was cycled through this loop, leaving a minor fraction available for larger heterotrophs, thus, rendering it a significant energy sink. These two seasonal periods constituted extreme cases and the microbial loop probably cycled moderate amounts of carbon (10 - 35 % of total primary production) at most times, based on the fraction of primary production required by bacteria in other experiments (Table 7). This was in agreement with a yearly study in 1986 in the northern Baltic, which reported an average of 35 % of primary production utilized by heterotrophic bacteria and flagellates (Kuosa and Kivi, 1989).

In Mediterranean waters, 57 % of total primary production (including exudation) was due to cyanobacteria, almost all of which was utilized by bacterivores (Hagström et al., 1988). By measuring only 11% exudation, the authors concluded that bacterial carbon demand (60% of primary production) must have been fulfilled for the most part by bacterivores, which contributed to the DOM pool through sloppy feeding and mortality. In their mass-balance model, 89 % of total primary production was grazed by protozoa and 94 % of photosyntetically fixed carbon was respired by heterotrophic activity within the microbial loop,

rendering it an effective energy sink (Hagström et al., op.cit.). Similar conditions probably prevailed outside the fjord in the warm season, when cyanobacteria dominated phytoplankton stocks and contributed an important fraction of primary production. Heterotrophic bacteria and protozoa in direct association to chroococcoid colonies have the opportunity to utilize a substantial fraction of carbon shortly upon fixation. By promoting quasi absolute coupling between photosynthetic and heterotrophic activity, these micro-aggregates could function as extremely effective mini-sinks, where most of the fixed carbon cycles rapidly and continuously. Thus, the microbial loop outside the Kiel Fjord could act continuously as a significant energy sink, whereas its role inside the fjord varied from modest to moderately high.

CONCLUSIONS

1. Environmental Study

1.1 Kiel Fjord

1.1.1 Total bacteria number (TBN) and MCV exhibited a negative relationship on both short-term and long-term scales at the IfM station. The seasonal cycle of small MCV in summer relative to winter could be explained by growth physiology with the production of increasingly smaller cells in warm but nutrient-depleted waters. Based on regression analyses with temperature and HNF, grazing probably played a secondary role in regulating bacteria sizes.

1.1.2 TBN and HNF showed similar well-defined seasonalities with increased incidence of oscillations in the spring and summer. These fluctuations seemed often to reveal trophic coupling. A particular striking example occurred in May 1988 when HNF minima and TBN maxima occurred simultaneously during a bloom of the silicoflagellate Dictyocha speculum. An antagonistic mechanism could have inhibited HNF in the form of large numbers of comma-shaped ultra-structures, which were observed in association to naked D. speculum cells and resembled discharged discobolocysts.

1.1.3 The most distinct features of HNF species distribution was a predominance of loricate choanoflagellates in early spring and the ubiquitous presence of very small cells. Choanoflagellates could compete effectively for silicates with the spring diatom bloom, particularly below the surface layer. Small HNF (2 μm^3 avg. volume) constituted the single most constant fraction of HNF populations and dampened variations in flagellate MCV.

1.2 Western Baltic

1.2.1 The pattern of bacteria and HNF populations reflected decreased eutrophication conditions with lower numbers and smaller MCV outside the Kiel Fjord. TBN and HNF exhibited stronger covariance and seasonality at KB than at the other stations.

1.2.2 Colony-forming cyanobacteria constituted a predominant fraction of phytoplankton standing stocks outside the fjord. Increased stability of the water column at the three Western Baltic stations probably favoured bloom formation by promoting cell aggregation. Many HNF and large heterotrophic bacteria were

associated to chroococcoid colonies, so that cyanobacteria seemed to sustain a large fraction of the heterotrophic activity during the warm season and represented the single best determinant for TBN. The potential for nitrogen fixation in oxygen-depleted microzones inside colonies could have important implications for the nitrogen budget and eutrophication processes in the Baltic Sea.

2. Grazing Study

2.1 Trophic coupling between HNF and TBN in diffusion chambers was particularly evident in late spring and summer experiments. HNF development also revealed seasonal differences with no growth in the winter and prolonged lag periods at the beginning of experiments in early spring and fall. The period of sustained growth lasted 3 to 4 days, after which HNF numbers decreased or oscillated due to ciliate predation and/or environmental carrying capacity.

2.2 Highest ingestion rate was measured together with maximum biomass production by bacteria in May 1988 reflecting unusual conditions during the D. speculum bloom. HNF development was not affected inside chambers, where rapid breakdown of silico-flagellate cells and associated ultra-structures was observed. This constituted evidence for an antagonistic mechanism against HNF stemming from ultra-structures, as opposed to dissolved toxins.

2.3 A HNF growth efficiency above 100% based on bacterial biomass grazed in July, combined with low ingestion rates, indicated another carbon source besides bacteria, which could be supplied by PNF. This probably occurred as well in the natural environment during the warm season, when bacteria to HNF biomass ratios often decreased below 1, thus, suggesting an `overload' in carbon demand.

2.4 Removal of bacteria in grazer chambers affected different size classes at different times, but was restricted to middle sizes. The lack of pattern could be explained by a mixed population of HNF undergoing shifts in predominance of different species with distinct selection strategies. However, the fact that the most frequently encountered bacteria sizes also

exhibited the most frequent removal `events' (e.g. class 4, 0.05 μm^3 avg. volume) suggested that HNF feeding followed a random encounter strategy, rather than true size-selectivity.

2.5 Thymidine incorporation tended to underestimate grazing relative to observed changes in cell counts, but overestimated bacterial production relative to net increase in biomass. These discrepancies were in part resolved by adjusting conversion factors, which varied with different experiments and treatments, remaining considerably lower in unfiltered treatments. Using a mean estimate of 0.5×10^9 cells $nmol^{-1}$ for unfiltered samples and 1.7×10^9 cells $nmol^{-1}$ for filtered samples, a better concordance with cell-based grazing rates was obtained.

2.6 Estimates of bacterial and flagellate carbon demand relative to primary and bacterial production indicated large variations in the importance of the microbial `loop' as an energy sink in the Kiel Fjord. Owing to the predominance of colony-forming cyanobacteria, the microbial loop could function as a more significant sink for photosynthetically fixed carbon outside the fjord.

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