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Temporal and spatial variations in heterotrophic nanoflagellate abundance in North Sea sediments

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ABSTRACT: Summer-winter variations in marine nanoflagellate densities at 3 depths in North Sea sediments (0–3, 30–33 and 60–63 mm) were studied using epifluorescence microscopy. Benthic flagellate densities in summer ranged from 7 to 859×10^3 and in winter from 9 to 1100×10^3 cells cm^{-3}. The effect of season on flagellate densities was different among stations. At 10 out of 15 stations summer values were significantly higher than winter values. The effect of season on flagellate densities was the same at all 3 depths. Flagellate densities in the sediment surface layer (0–3 mm) were in general 2 to 4 times higher than in the 2 deeper sampled layers (30–33, 60–63 mm). The extraordinary high flagellate densities near Esbjerg (Denmark) were remarkable: 859×10^3 cells cm^{-3} in the surface layer in summer and 1100×10^3 cells cm^{-3} in the 2 deeper layers during winter. In both seasons, at all depths and all stations most cells (50 to 75%) occurred in the 2 to 5 µm size class. Few flagellates were larger than 10 µm or smaller than 2 µm. Pooled winter and summer data of flagellate densities in the sediment surface layer showed a positive correlation with bacterial production and bacterial specific growth rate, explaining 20 and 30% respectively of the variance. In summer a positive correlation existed between flagellate density and bacterial specific growth rate and grain size, together explaining 53% of the variance. In winter nanoflagellate densities were significantly correlated with bacterial biomass and abundance accounting for 59% and 33%, respectively, of the variance. The data suggest that bacterial biomass/abundance during winter sets limits to flagellate densities. Increased bacterial production was probably responsible for generally higher summer flagellate densities although grain size could become a limiting factor for flagellate densities in silty sediments during summer

KEY WORDS: Heterotrophic nanoflagellates · Sediments · Abundance · Seasonality · Epifluorescence microscopy

INTRODUCTION

The significance of protozoa in pelagic food webs has been established during the past decade (Fenchel 1982b, Azam et al. 1983, Wikner & Hagström 1988, Sherr et al. 1989). The growing awareness of the importance of protozoa in pelagic microbial food webs and the fact that all components of the microbial loop are also abundantly present in the benthic habitat make it reasonable to assume that protozoa play an equally important role in the benthic microbial food-web (Kemp 1990 and references therein, Alongi 1991).

Studies on heterotrophic protozoa in marine sediments are few and most information concerns ciliates, because of their relative ease of extraction (Fenchel 1967, 1968, 1969, 1975, Kemp 1988, Sich 1990, 1991). First quantitative estimates of marine benthic flagellates were provided by Mare (1942) and Lighthart (1969). These early studies already pointed out that protozoa are sufficiently numerous to be potentially important bacterivores. Problems in extraction and enumeration of benthic flagellates limited subsequent progress; only recently has a new start been made to collect data on benthic heterotrophic nanoflagellates (Alongi 1986, 1990, Bak & Nieuwland 1989, 1993, Novitsky 1990, Bak et al. 1991, Cunningham & Ustach 1992). Knowledge of the abundance and distribution of benthic flagellates and of the forcing factors is indispensable if we are to understand the role of these protozoa in benthic microbial food-web dynamics.

Little is known about the relationship between benthic flagellates and physicochemical factors. Gradients

in e.g. temperature, Eh, oxygen availability, physical disturbance due to water movement, salinity, grain size, nutrient concentrations determine, to some extent, other benthic biota. It is assumed that the same applies to the temporal and spatial variations and species composition of benthic flagellate communities (see Fenchel 1969, reviews by Patterson et al. 1989, Alongi 1991). In addition, biological factors including predation, competition and food resources can be of regulating importance.

We studied summer-winter variations in benthic nanoflagellate densities in North Sea sediments and used concurrently measured physicochemical and biological factors to examine what influenced flagellate distribution and abundance.

MATERIAL AND METHODS

Study site and sampling. We studied benthic nanoflagellate densities during cruises in August 1991 and February 1992. Two periods of investigation were chosen to study clearly distinguishable situations. During summer, primary production and temperature were relatively high and at some stations temperature stratification occurred. In contrast, during winter primary production and temperature were low and there was almost no stratification.

A total of 15 stations distributed over the Southern Bight, German Bight and the Skagerrak were visited (Fig. 1). The stations covered a wide range of sediment types with respect to grain size, organic matter content, benthic biological activity and mineralization. Salinity in August ranged from 33 to 35‰ and in February from 31 to 35‰. Additional features of the stations are in Table 1. The depths of the stations varied between 19 and 62 m, with 2 deeper stations (Stns 8 and 9) in the Skagerrak. Temperature differences of the bottom waters were related to the depth of the stations. The shallowest stations (Stns 1, 2, 12 to 17) showed pronounced differences up to 14 °C between August and February, whereas seasonal differences varied from 3 to 7 °C at deeper stations (Stns 4 to 11).

Sediment cores were collected from on board the RV 'Pelagia' with a cylindrical box corer (i.d. 31 cm). This corer enclosed a 30 to 50 cm sediment column together with 15 to 25 l of overlying bottom water. Disturbance of the sediment-water interface during collection was prevented by a closing lid.

Flagellate enumerations. For heterotrophic nanoflagellate enumerations, 5 replicate samples were taken from the box core with polymethylmethacrylate tubes (26 mm i.d.) to a depth of 10 to 15 cm. Samples were stored at *in situ* temperature and processed within 3 h. Each sample was subsampled at 3 depths: 0–3, 30–33 and 60–63 mm. At these depths, layers of sediment were cut off with a 3 mm thick mould. The sediment samples (1.59 cm^3) were fixed, washed and stained with 1% glutaraldehyde/sterile seawater and 1 ml proflavine, total volume 25 ml. After gentle shaking, this was allowed to stand for 1 h. A plastic syringe, distal part cut off, was used to take a subsample of the supernatant at the centre of the 12 cm high column. This was filtered at low vacuum over a 0.2 µm Nuclepore filter stained with Sudan Black. Flagellates were counted using a Zeiss Axiophot epifluorescence microscope with a HBO 50 W bulb and filter set BP 450/490,

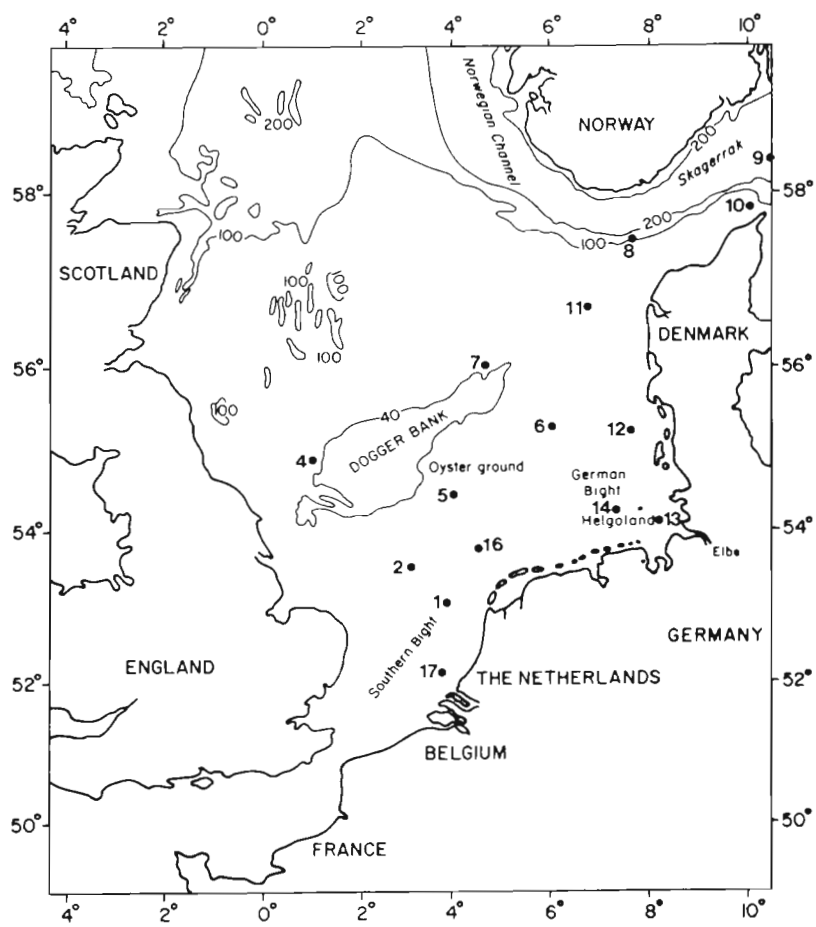

Fig. 1. Map of the North Sea showing stations visited during cruises in August 1991 and February 1992

Table 1. General features of the stations visited during August 1991 and February 1992. nm: not measured

Station	No.	Water depth (m)	Median grain size (µm)	Bottom temperature (°C) August	Bottom temperature (°C) February	Stratification August	Stratification February	Oxygen penetration depth (mm) August	Oxygen penetration depth (mm) February
Broad Fourteens	1	27	265	16.8	6.3	–	–	>50.0	>50.0
UK/NL boundary	2	32	138	16.8	5.8	–	–	2.5	nm
Doggerbank W.	4	55	287	8.2	6.4	+	–	3.0	40.0
Oyster Grounds	5	46	106	10.2	6.4	+	–	4.3	nm
Weiss Bank	6	49	85	12.2	5.8	+	–	3.0	5.8
Tail End	7	49	159	9.5	6.1	+	–	3.1	7.5
Skagerrak W.	8	126	158	7.3	6.4	+	Slightly	8.0	8.0
Skagen	9	330	11	6.9	7.0	+	+	12.0	10.0
Hirtshals	10	62	25	12.2	6.8	+	Slightly	3.0	9.0
Jutland	11	41	287	10.0	5.2	+	–	>17.0	>150.0
Esbjerg	12	23	175	17.7	4.8	–	–	<0.5	26.0
Helgoland Bight	13	19	24	18.7	4.4	–	–	<0.2	5.2
Elbe Rinne	14	39	97	16.5	5.4	–	Slightly	<0.2	5.1
Frisian Front	16	37	60	17.4	6.3	–	–	4.5	18.5
Hoek van Holland	17	28	338	18.5	5.8	–	–	>14.0	>14.0

FT 510, LP 520. At least 75 fields per filter were observed (magnification 1250×). All enumerations were done aboard within 2 d after processing using a model TXM Stabletop microscope table. Flagellates were classified by their longest linear dimension into 4 different size categories: <2, 2–5, 5–10 and 10–20 µm (Bak & Nieuwland 1989, Bak et al. 1991). Biovolumes were estimated assuming cells to have geometrical shapes (spheres, rotation ellipsoids) and were converted to carbon biomass assuming a conversion factor of 200 fg C μm^{-3} (Fenchel 1982a, Børsheim & Bratbak 1987).

Other biotic data. To enumerate benthic bacteria 5 sediment cores were taken. The top 3 mm sediment layer of each core was cut off (volume 1.59 cm^3). These samples were further processed according to the method described by van Duyl & Kop (1990). After staining with acridine orange, epifluorescence microscopy was used to enumerate at least 200 bacteria in 10 to 20 fields (magnification 1250×). The sizes of at least 100 bacteria were ranked in 13 size classes in order to estimate bacterial biovolumes. These data were converted to bacterial biomass using a carbon conversion factor of 2.2×10^{-10} mg C μm^{-3} (Bratbak & Dundas 1984).

Benthic bacterial production was measured by ^{3}H-L-leucine incorporation into protein. The procedure and a comparison with [methyl-^{3}H]thymidine incorporation into DNA is described by van Duyl & Kop (1994).

Bacterial specific growth rate was calculated by dividing bacterial production by bacterial biomass. Meiofauna was sieved out of the sediment and counted.

Physicochemical data. Grain size was analyzed using the Malvern particle sizer. Three fractions were distinguished; 10, 50 and 90% fractions. We used the 10% fraction, i.e. 10% of all particles were smaller than the given value (µm). Organic carbon and nitrogen contents were measured on a Carlo Erba NA 1500-2 elemental analyzer following the procedure of Verardo et al. (1990). Concentrations of chlorophyll *a* and phaeopigments (degradation products of chlorophyll *a*) were measured using spectrophotometry (Lorenzen 1967, Lorenzen & Jeffrey 1980, Daemen 1986). Oxygen profiles were measured using Clark type microelectrodes provided with an internal reference electrode (Lohse et al. 1993).

Data analysis. Variations in flagellate numbers among stations, sediment depths and seasons were examined by 3-factor analysis of variance (ANOVA). Because season and depth are fixed factors and station is a random factor, a mixed model ANOVA was used (Sokal & Rohlf 1981). Flagellate numbers were related to the physicochemical and biological factors using the simple regression model: $\log y = a + b \log x$. In particular cases we also calculated multiple regressions to determine if additional independent variables improved the model to predict flagellate abundance. Values for most properties were log-transformed [chlorophyll *a*, phaeopigments, bacterial specific growth rate and biomass were log $(x+1)$ transformed and temperature was not transformed] prior to ANOVA or regression analysis so as to stabilize the variance and meet the normality assumptions. Normality was confirmed by inspection of normal probability plots. All statistical analyses in this study were performed with Systat (Systat, Inc., Evanston).

RESULTS

Nanoflagellate numbers exhibited large variations with station, sediment depth and season (Fig. 2a to c). In summer at most stations, densities ranged between 7 and 310 × 10^3 cells cm^{-3}. Most densities in winter varied between 9 and 190 × 10^3 cm^{-3}. There was 1 exception, flagellate abundance at Stn 12 near Esbjerg (Denmark): in the surface layer in summer (859 × 10^3 cells cm^{-3}) and in the 2 deeper layers in winter (on average 1100 × 10^3 cells cm^{-3}). The enormous densities in winter were patchy and occurred only in 2 (30–33 mm layer, SE = 978 × 10^3 cells cm^{-3}) or 3 (60–63 mm layer, SE = 650 × 10^3 cells cm^{-3}) out of 5 cores, while in the other cores, densities were low. Microscopical examination of the slides showed that the extreme abundances were caused by the explosive growth of 1 flagellate species (Fig. 3).

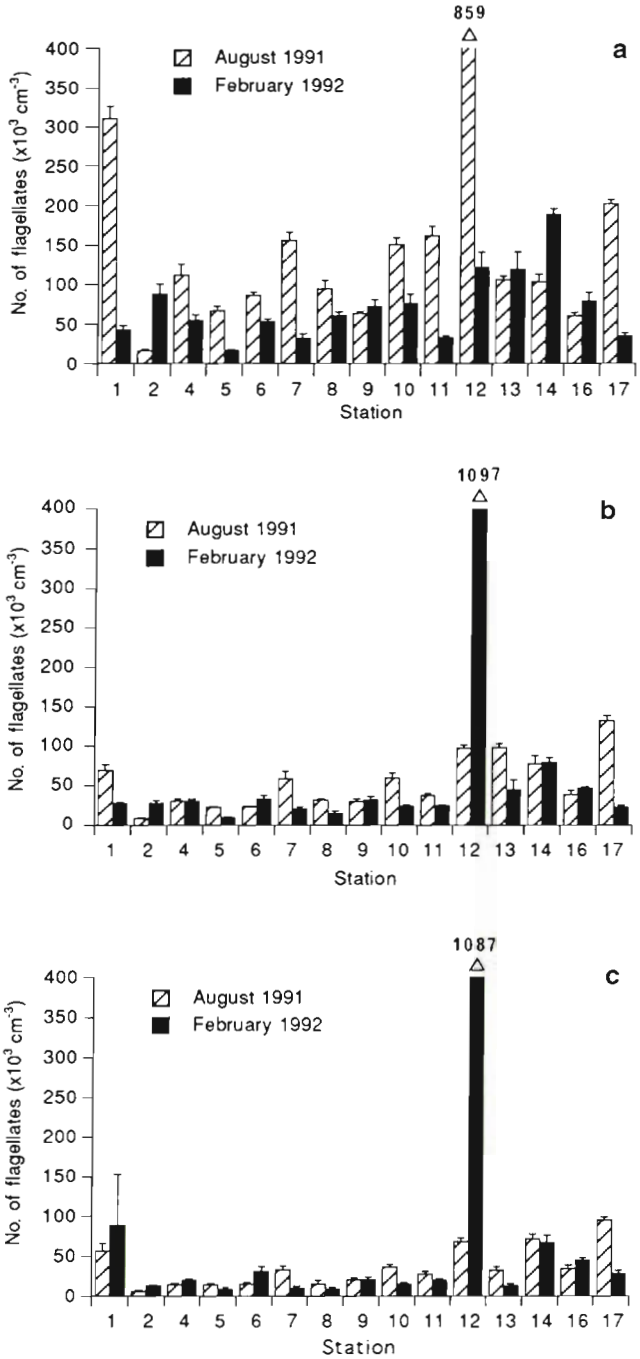

Fig. 2. Heterotrophic nanoflagellate abundances at 15 stations in the North Sea at (a) 0–3, (b) 30–33 and (c) 60–63 mm depth in the sediment

Flagellate densities showed significant differences with depth and station but also significant season × station and depth × station interaction effects, i.e. the effects of season and depth on flagellate numbers were significantly different among stations (Table 2).

At 3 stations (Stns 9, 13 and 16: *t*-test, $p = 0.460$, 1.000, and 0.175 respectively) no differences between summer and winter densities occurred. At Stns 2 and 14 winter densities were significantly higher than summer values ($p < 0.001$), while at the other 10 stations, summer values were higher than winter values (Stn 8: $p < 0.05$; all other stations: $p < 0.001$).

The above-mentioned differences in seasonal effects among stations were responsible for a non-significant seasonal main effect.

Flagellate densities decreased significantly with increasing sediment depth (a main effect), but the relative differences among the 3 layers were not the same for all stations. This significant interaction effect is illustrated in Fig. 4a, b by the crossings of the lines representing the decline of flagellate numbers with increasing depth. Generally, the differences between the 2 deeper layers were small, densities in the 30–33 mm layer being slightly higher than in the 60–63 mm layer. Densities in the sediment surface layer in winter were approximately 2 times and in summer 3 to 4 times as high as in the 2 deeper layers.

The season × depth interaction was not significant, i.e. the effect of season on flagellate densities was the same at all 3 depths.

In terms of carbon, flagellates varied between 0.07 and 5.11 µg C cm^{-3} in summer and between 0.05 and 2.75 µg C cm^{-3} in winter (Esbjerg values not included).

There were consistent differences in the numbers of flagellates in the different size classes. At all stations, depths and seasons, 50 to 75% of all flagellates occurred in the 2 to 5 µm size class, while 10 to 37% were in the 5 to 10 µm size class. Only a few percent of all flagellates occurred in the <2 and 10 to 20 µm size classes.

Pooled summer and winter data of flagellate densities in the sediment surface layer showed a significant positive correlation with bacterial production (Fig. 5a: $n = 30$, $R^2 = 0.21$, $p = 0.01$) and with bacterial specific

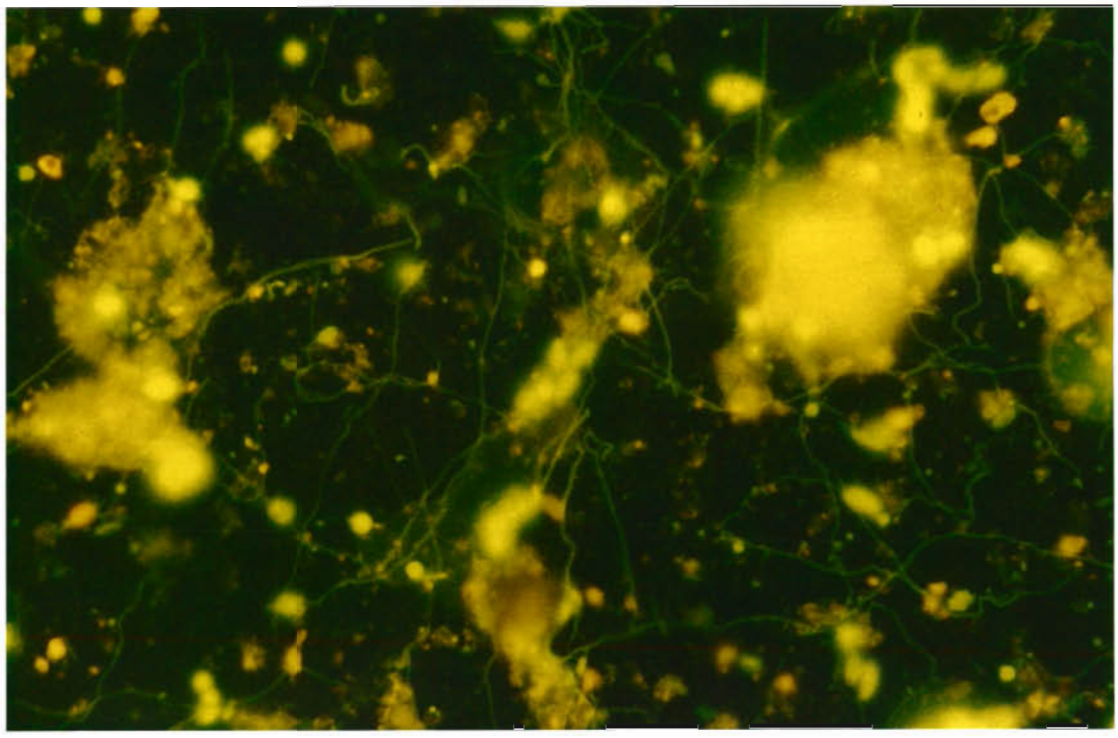

Fig. 3. Photomicrograph using epifluorescence microscopy of the abundant flagellate species near Esbjerg (Denmark) in winter

growth rate (Fig. 5b: $n = 30$, $R^2 = 0.30$, $p < 0.01$). When adding grain size the explained variance increased slightly to 34%. Adding other variables measured either did not improve the model to predict flagellate abundance or was not allowed, due to intercorrelation of the predictor variables.

There appeared to be a difference in factors correlating with flagellate abundance between summer and winter. In summer, flagellate abundance correlated significantly with grain size (Fig. 6: $n = 15$, $R^2 = 0.34$, $p < 0.05$) and bacterial specific growth rate ($n = 15$, $R^2 = 0.29$, $p < 0.05$). A multiple regression was used to provide improved predictions of flagellate densities in summer: log (flagellate abundance) = 4.344 + 0.281 log (grain size) + 2.693 log (bacterial specific growth rate), which explained 53% of the variance. Both variables contributed equally to the explained variance. In contrast, in winter grain size did not correlate significantly with flagellate densities. The differences between summer and winter flagellate densities were larger in sandy sediments than in muddy sediments (Fig. 6). However, in winter significant positive correlations with bacterial biomass (Fig. 7: $n = 15$, $R^2 = 0.59$, $p = 0.001$) and bacterial abundance ($n = 15$, $R^2 = 0.33$, $p < 0.05$) existed. Such correlations were absent in August. None of the other independent variables (temperature, chlorophyll *a*, phaeopigments, nematode abundance, organic carbon and nitrogen contents) correlated with flagellate abundances.

DISCUSSION

Benthic nanoflagellate densities varied between 7 and 1100 × 10^3 cells cm^{-3} and were highest at the coastal stations (Broad Fourteens, Esbjerg and Hoek van Holland). With the exception of the densities near

Table 2. Summary of the 3-factorial ANOVA flagellate abundance due to station location, season and depth in the sediment. •••$p < 0.001$; ns: not significant

Source	SS	df	Mean square	*F*-ratio	
Season	3.387	1	3.387	4.38	ns
Depth	20.410	2	10.205	105.21	•••
Station	29.582	14	2.113	55.60	•••
Season × depth	0.829	2	0.415	2.31	ns
Season × station	10.835	14	0.774	20.37	•••
Depth × station	2.724	28	0.097	2.55	•••
Season × depth × station	5.030	28	0.180	4.74	•••
Error	13.742	360	0.038		

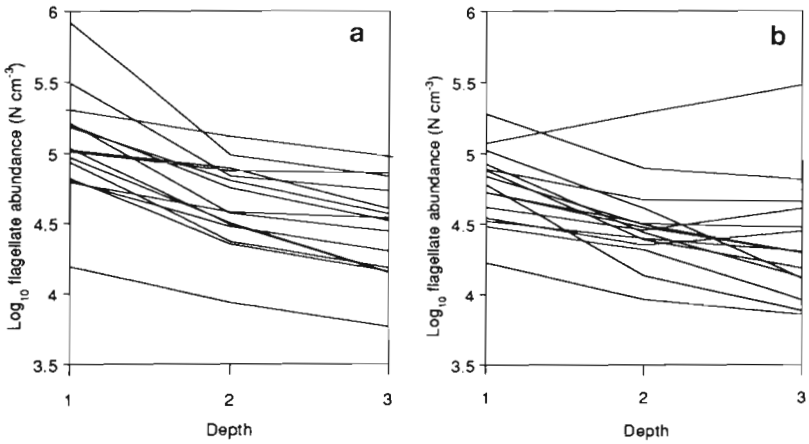

Fig. 4. Flagellate abundance versus depth in the sediment illustrating the significant depth × station interaction in (a) August 1991 and (b) February 1992. Each line represents a station. Depths: (1) 0–3 mm, (2) 30–33 mm, (3) 60–63 mm

Esbjerg, values were within the range of values reported previously in the literature. High densities (50 to 300 × 10^3 cells cm^{-3}) occurred in temperate sandy tidal flats, in sandy North Sea sediments, in sandy sediments in a coastal upwelling region and in an arctic tundra pond (Fenchel 1975, Bak & Nieuwland 1989, 1993, Bak et al. 1991, Hondeveld et al. 1992). In contrast, densities in muddy North Sea sediments and in a temperate muddy tidal flat were much lower, 5 to 45 cells × 10^3 cm^{-3} (Bak et al. 1991, Epstein & Shiaris 1992). In tropical habitats such as mangroves and reefs, Alongi (1986, 1990) estimated numbers of nanoflagellates as being in the range 8.5 to 260.5 × 10^3 cm^{-3}, with most esti-

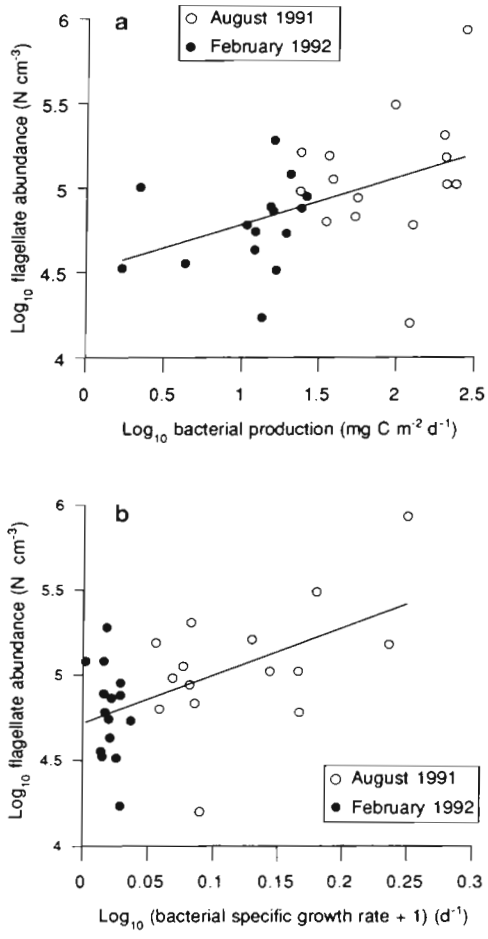

Fig. 5. Relationships between benthic nanoflagellate abundance and (a) bacterial production, regression: $y = 4.51 + 0.28x$ (R = 0.45), (b) bacterial specific growth rate, regression: $y = 4.72 + 2.81x$ (R = 0.55). Regression lines are based on pooled summer and winter data

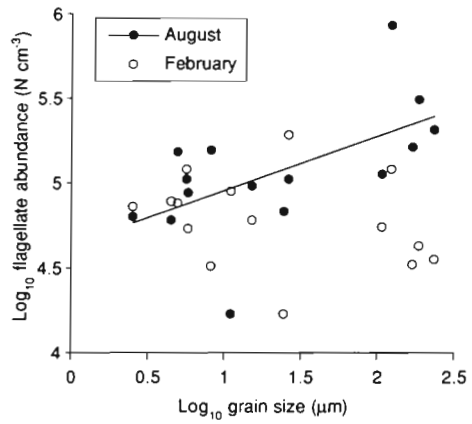

Fig. 6. Relationship between benthic nanoflagellate abundance and grain size. Regression line is based on August data: $y = 4.64 + 0.32x$ (R = 0.58)

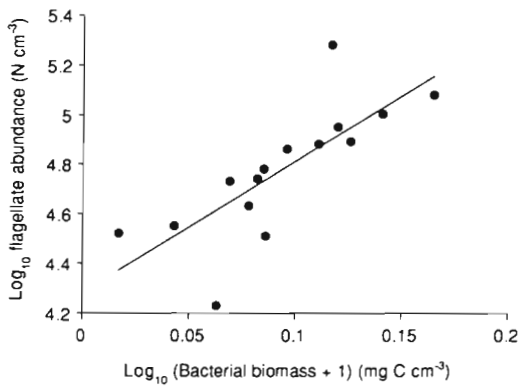

Fig. 7. Relationship between flagellate abundance and bacterial biomass in February 1992. Regression: $y = 4.28 + 5.30x$ (R = 0.77)

mates being lower than flagellate numbers in temperate habitats.

The physical/chemical/biotic data do not explain the extraordinary densities near Esbjerg. Remarkable was that these record densities all occurred under anoxic conditions, while it is generally stated that flagellate abundance is low when oxygen concentrations are low (Fenchel 1975, Alongi 1991). However, some flagellate species prefer reduced conditions (Patterson et al. 1989).

With the exception of winter values near Esbjerg, flagellate densities decreased with increasing depth in the sediment, which is in agreement with previous studies (Fenchel 1975, Bak & Nieuwland 1989, 1993, Alongi 1990, Novitsky 1990).

To understand the role of nanoflagellates in benthic microbial food webs it is necessary to know which factors influence flagellate abundance and distribution. An important question is then whether resource limitation (bottom-up) or predation pressure (top-down) is important in controlling flagellate numbers and growth. Bottom-up and top-down control are not mutually exclusive alternatives but may act simultaneously or alternately. An example is the pelagic population dynamics of heterotrophic nanoflagellates in a temperate freshwater lake (Weisse 1991).

Small flagellates are assumed to be bacterivorous. Consequently, fluctuations in bacterial variables are potentially regulating factors for flagellate densities. We found a difference between the 2 seasons, summer and winter, with respect to factors correlating with flagellate abundance. In February there was a significant relationship between bacterial and flagellate abundance explaining 33% of the variance. In addition there was a positive correlation with bacterial biomass accounting for almost 60% of the variance. These positive correlations between nanoflagellate abundance and bacterial abundance/biomass suggest either that bacterial abundance/biomass sets limits to flagellate densities during winter or that flagellates grazed bacteria until threshold values.

In August no correlation between flagellate and bacterial abundance or biomass was found. Three explanations for deviations from a numerical relationship between flagellate and bacterial abundance in the pelagic (1 flagellate : 1000 bacteria; Sanders et al. 1992) are as follows. (1) Grazing activity of other consumers and/or other loss factors (i.e. viruses). In benthic environments ciliates, foraminifera and meiofauna compete with flagellates for bacteria (Kemp 1988, Johnson et al. 1989, Bott & Kaplan 1990, Grossmann & Reichardt 1991, Bernhard & Bowser 1992, Epstein & Shiaris 1992, Epstein et al. 1992). (2) Top-down control on flagellates can occur which prevents flagellates from reaching the abundances that resources could potentially support (Gasol & Vaqué 1993, M. Starink pers. comm.). Potential predators of flagellates are nematodes, copepods, turbellarians, polychaetes, ciliates, crustaceans, echinoderms and molluscs. In our study area nematodes and harpacticoid copepods were most numerous but no correlation existed between nematode and flagellate abundances. (3) Flagellates may use sources of carbon other than bacteria (Gasol & Vaqué 1993, González & Suttle 1993, Tranvik et al. 1993). In enriched benthic environments osmotrophic nutrition is possible (Sanders et al. 1992). In summer the labile part of the sediment organic matter content may be larger than in winter (van Duyl & Kop 1994). Consequently, during summer flagellates grew abundantly due to a large amount of food.

Another potentially important bacterial variable for flagellate abundance is bacterial production. High flagellate densities corresponded with high bacterial production (Fig. 5a). Variations in bacterial production in summer were related to temperature and phytopigment content (van Duyl & Kop 1994). High bacterial production in summer was probably responsible for the pattern of generally higher summer flagellate densities compared to winter. Low winter and high summer densities due to increasing bacterial production in summer are also reported for temperate, intertidal sandy flats in the Wadden Sea (Bak & Nieuwland 1989, van Duyl & Kop 1990). Because of the limited size of the separate data sets, a correlation between bacterial production and flagellate abundance was absent.

Between flagellate numbers and bacterial specific growth rate a positive correlation existed. Because bacterial biomass did not differ significantly between the 2 seasons (van Duyl & Kop 1994) changes in bacterial production are directly reflected in bacterial specific growth rates.

The correlations between flagellate abundance and bacterial production and specific growth rate explained only 21 and 30% respectively of the variance, suggesting more factors are important for flagellate abundance and distribution.

Grain size can be of regulating importance, flagellate abundance being higher in sandy sediments than in muddy sediments (Alongi 1986, Bak et al. 1991, Bak & Nieuwland 1993). Protozoa seem to require a certain minimum pore space in which to live. Thus, low flagellate numbers may be related to a finer texture (i.e. higher silt content) of the sediments (Sinclair & Ghiorse 1987 and references therein). In our study in sediments consisting of medium to very fine sand, flagellate densities increased significantly in summer. In medium to fine silt sediments flagellate densities did not increase during summer. In winter no correlation between flagellate abundance and grain size existed, while in summer this correlation was significant. This suggests

that when flagellate densities were low, grain size had no influence, while with increasing flagellate numbers, grain size became a limiting factor for flagellate abundance.

The larger flagellate decrease from summer to winter densities in sandy sediments compared to muddy sediments is probably due to the relatively large decrease in bacterial production in the sandy sediments (van Duyl & Kop 1994). Another factor of potentially regulating importance is physical stress. Most sandy sediments occur in shallow regions where physical stress on the bottom, caused by tidal currents and storm waves, is prominent. In these shallow, turbulent waters of the North Sea, physical disturbances (storms) regulate macrofauna, allowing high numbers in summer and generally lowering numbers after the storm season (Duineveld et al. 1991). Physical stress also regulates the dynamics of bacteria in tropical, shallow water deposits (Alongi 1992). Protozoans in surface sediments are frequently disturbed by storm events (Cunningham & Ustach 1992).

Only part of the variance in flagellate abundance is explained by bacterial production, bacterial biomass and grain size. All other physical/chemical variables did not correlate with flagellate abundances. Because temperature and phytopigment accounted for up to 88% of the seasonal and spatial variations in bacterial production (van Duyl & Kop 1994), these factors indirectly affect flagellates. Only harsh environmental conditions (e.g. extreme low temperatures, storms) may result in flagellate densities being regulated mainly from outside the biotic system, as observed in temperate tidal flats in the Wadden Sea (Bak & Nieuwland 1989) and in shallow surface sediments (Cunningham & Ustach 1992).

In conclusion, our observations indicate bacterial production, bacterial biomass and grain size to be important predictors for determining flagellate abundance. Because variances were only partly explained by correlation with bacterial production, biomass and grain size, other factors, not included in our measurements, have to be important. Biological interactions of benthic organisms can be so numerous that single relationships are not easily discernible. Consequently, topics such as top-down control cannot be addressed because we have no data on ciliates and macrofauna. Since food web interactions are complex, the changing significance of specific factors and seasonal patterns of control mechanisms need more experimental research.

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