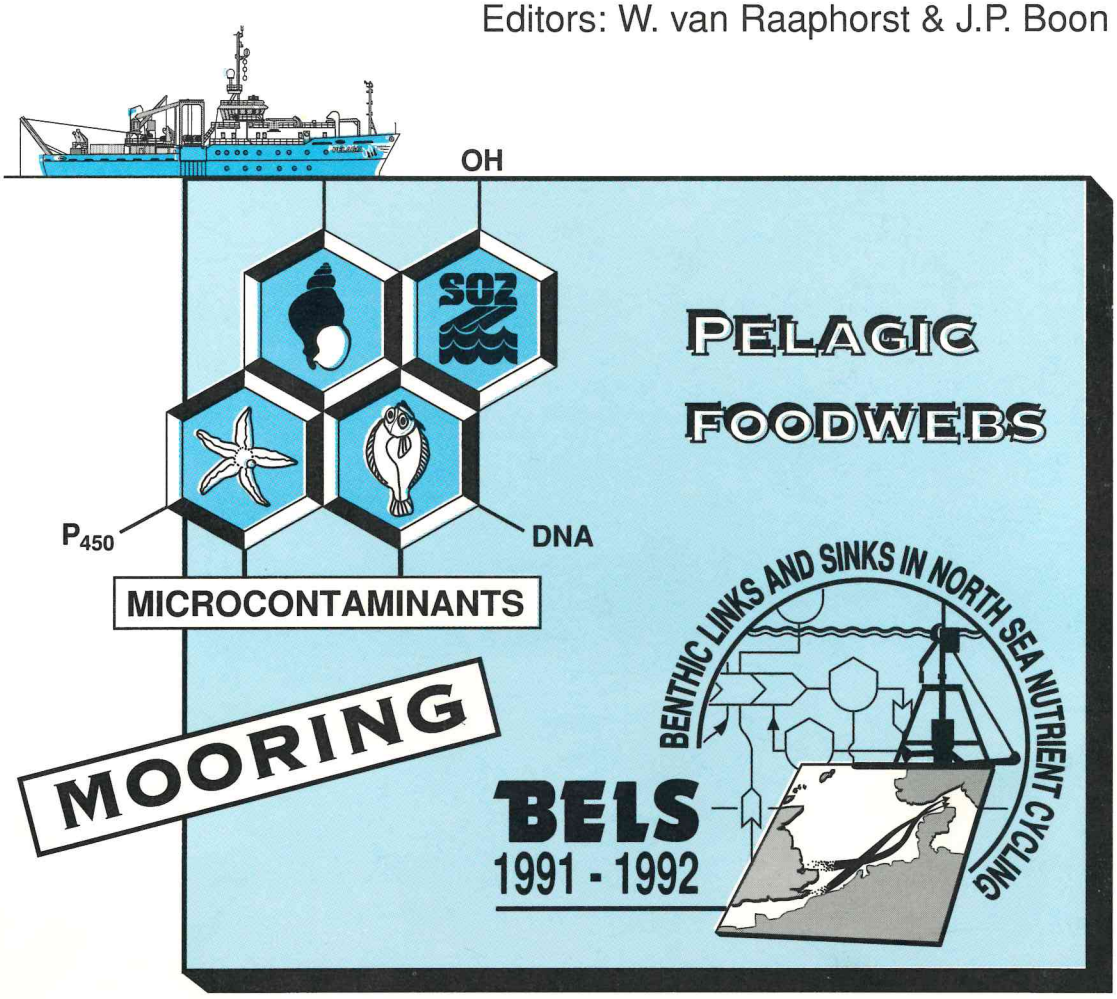
NIOZ-RAPPORT 1993 - 9

THE INTEGRATED NORTH SEA PROGRAMME 1991-1992

PRELIMINARY RESULTS

Editors: W. van Raaphorst & J.P. Boon



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NETHERLANDS INSTITUTE FOR SEA RESEARCH

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CONTENTS

2. SUMMARY RESULTS

2A. THEME I: MOORING

Participants:
J.J.M. VAN HAREN, H. RIDDERINKHOF, C. VETH, R. MANUELS (*NIOZ*); B. WETSTEYN, W. ZEVENBOOM (*RIJKSWATERSTAAT*); Y. VAN DEN BERG (*OCN*); D. MILLS (*MAFF, UK*); S. FLODERUS (*UNIV. UPPSALA, SWEDEN*)

The main objectives of the INP-mooring program are the quantitative determination of short term variability in phytoplankton biomass and activity in the North Sea, and to develop a monitoring mooring site with continuous measurements on physical and biological parameters. A relationship between phytoplankton dynamics and physical constraints is sought. This is to be determined from simultaneous measurements of physical and biological parameters that will be combined in a coupled one-dimensional model for the lower trophic levels in (seasonally) stratified areas under atmospheric forcing. The focus will be on diapycnal mixing events and associated (short-term bursts of) fluxes of nutrients and phytoplankton across a pycnocline separating nutrient-rich bottom and light abundant surface waters (Fig. 1.).

With the advent of recording fluorometers, high frequency (every hour) sampling for prolonged periods of time (a month or longer) provides the adequate means for the study of chl-a variability. Between July-October in 1991 and 1992 we have obtained a reasonably consistent data set from a single site in the Oyster Grounds (54 25 N, 4 02 E; TS 135 in Fig. 2.). This site is located generally away from frontal zones in a seasonally stratified part of the North Sea. The waterdepth, 45 m, is such that it is likely that entrainment of water from the bottom mixed layer occurs during periods of deepening of the surface mixed layer.

First analyses of the data show occasional sharp increases in fluorescence, both above and below the pycnocline (Fig. 3). Daily variability is apparent at times, probably related to photo inhibition. We focus, however, on the periods of increased fluorescence that last 2-10 days (for example around days 230, 235, 250 and 260). Although both records do not provide absolute values (data should first be calibrated), the occurrence of bursts can be used for comparison with other data sets. The surface layer record shows more variability than the bottom layer record, indicating that the two layers are governed by different dynamics, as expected. The light abundant zone is influenced by the, probably, increased phytoplankton and nutrient levels just below the pycnocline and atmospherically controlled mixing events, whereas the bottom layer is more dependent on sedimentation (from the upper layer) and turbulence (causing resuspension) generated at the sea floor.

Surface layer mixing is related to kinetic energy input via wind stress and chances in potential energy levels due to heating and cooling. As a first indicator for periods of cooling in which a deepening of the pycnocline is expected to occur, temperature records obtained in both layers are considered (Fig. 4). Clearly, decreases in surface water temperature at days 228 and 247 (and to a lesser extent near days 235 and 260) correspond to the period of enhanced surface fluorescence. During all of these periods a pycnocline is still apparent, indicating a separation between two layers, though it's magnitude varies over time (note day 235). Further analysis should distinguish between the deepening of the well-mixed surface layer and the passage of a front, that may generate a signal like the one occurring at day 235.

The combined plot of the air temperature (Ta) and surface water temperature (Tw) versus time also provides an indication of cooling events (when Ta-Tw < 0, see Fig. 5). These start to occur occasionally, around day 230 for instance, before Ta reaches a maximum (at day 245) and happen frequently during early autumn with a noteworthy start at day 245. The correspondence with periods of increased surface fluorescence levels is striking. This result stimulates further analysis on the precise establishment of times of correspondence through data analysis and numerical modelling, that might provide a distinction between fluxes of nutrients and phytoplankton.

Wind seems to be a minor agent generating surface layer mixing. A comparison between Fig. 6 and the foregoing figures shows that the period of increased fluorescence levels near day 250 occurred during a period of calm weather.

Data from other instruments (transmissometer, sediment trap (Fig. 7)) also correspond to some extent to the fluorescence data. All in all, we feel that these preliminary results from time series

measurements obtained in late summer/early autumn provide an interesting coherent view on the short term variability of phytoplankton in the watercolumn. During near-future analyses we will single out these events for a proper (budgettary) description by performing proper calibration on the data and by ruling out some other possible biological and physical causes through intercomparison of the complete data set.

This project will continue through 1993 and 1994 to obtain measurements from the winter and spring periods as well. We hope to incorporate essential instrumentation on nutrients (in situ auto analyzers) and on the vertical current structure (acoustic Doppler current profiler) that we sofar did not have. The latter, combined with a 'fast' thermistor chain might give 'direct' information on the vertical mixing rates.

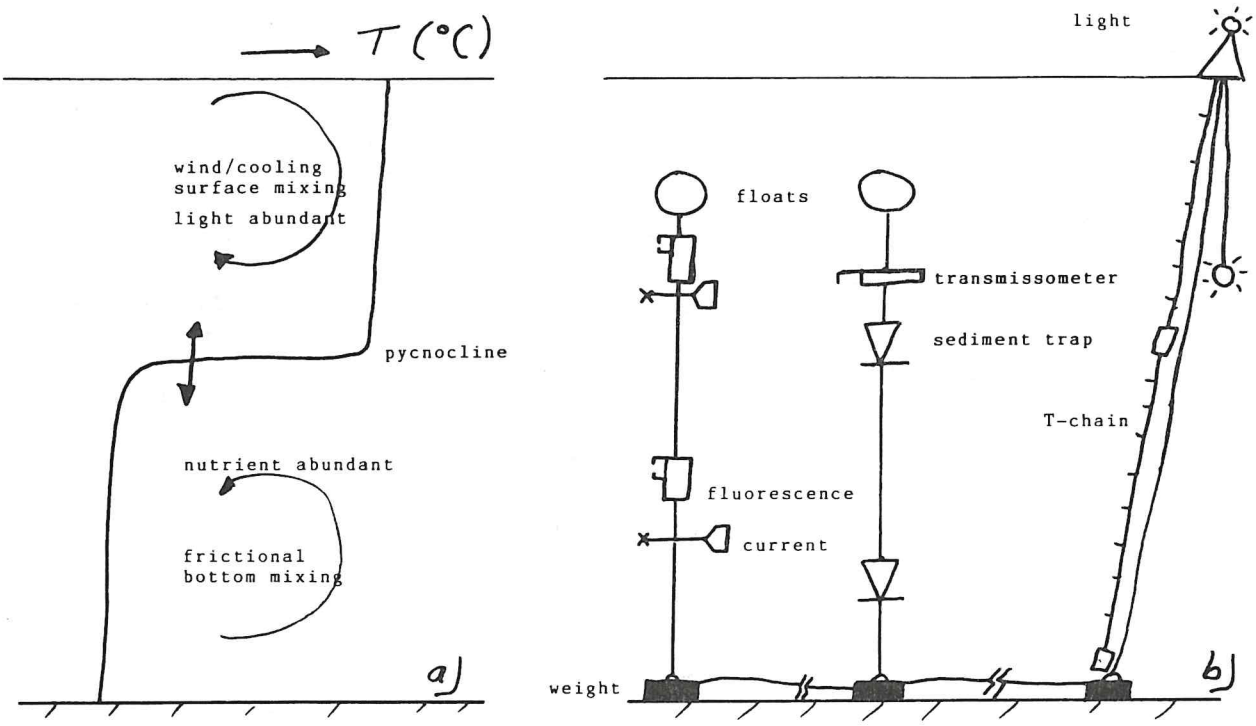

Fig. 1. a. Typical temperature profile of a 'two-layer sea'. Two different physical and biological regimes are separated by a thermocline, i.e. the sharp temperature jump with depth.
b. Schematic of a mooring system to study the system in Fig. 1a.

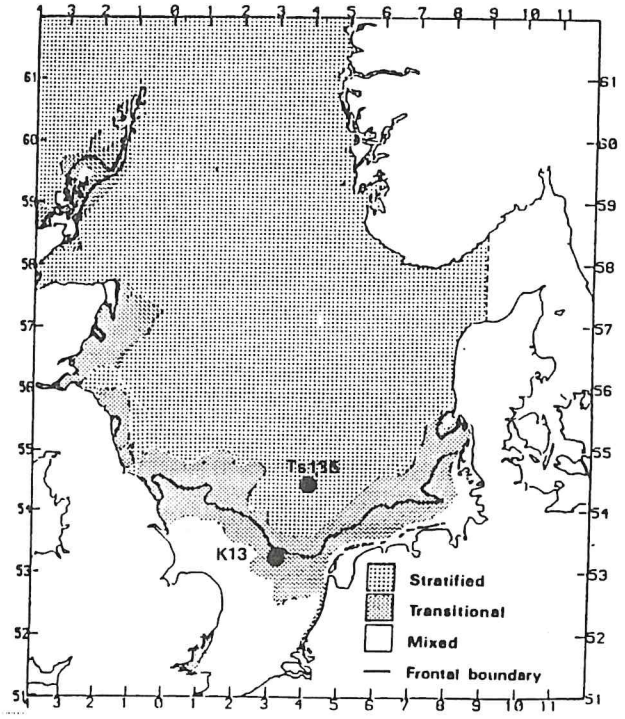

Fig. 2. Location of the INP mooring site (TS 135) and the meteo-station (K 13) with respect to the summer stratification rate.

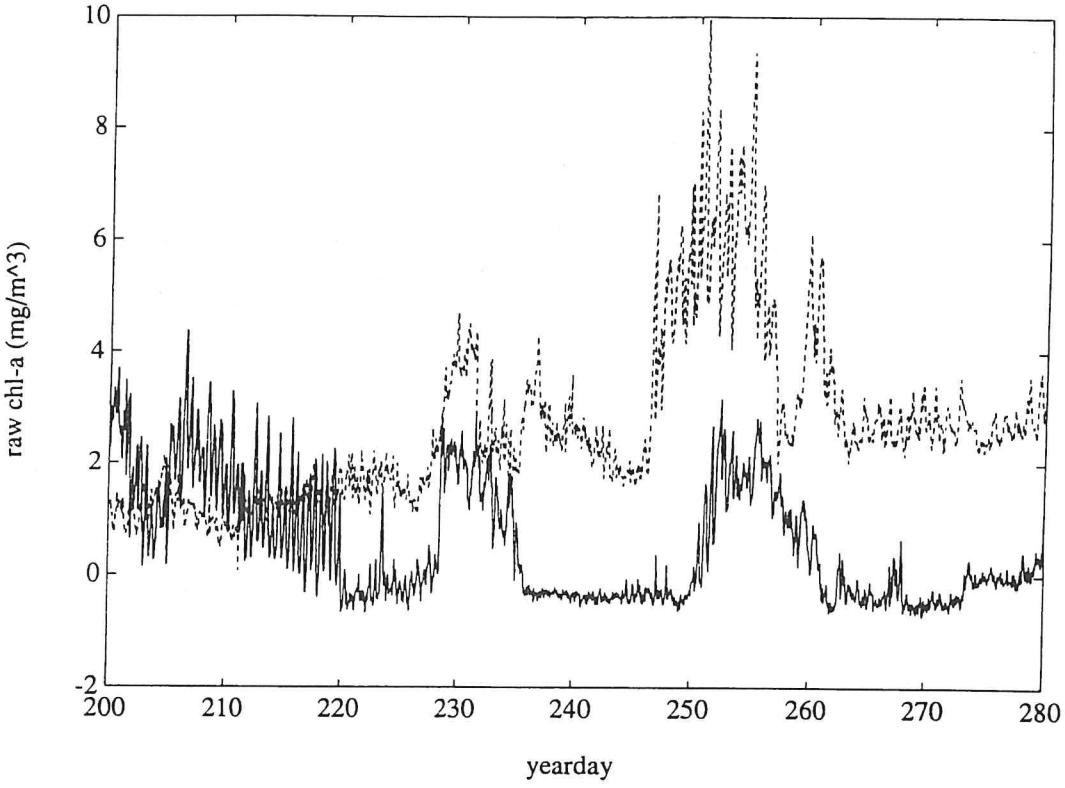

Fig. 3. Timeseries of uncalibrated fluorescence data (linear with chl-a content) obtained by fluorometer at 12 m depth (dashed) and 34 m (solid) in 1991. (day 200 = 19 July, the waterdepth is 45 m)

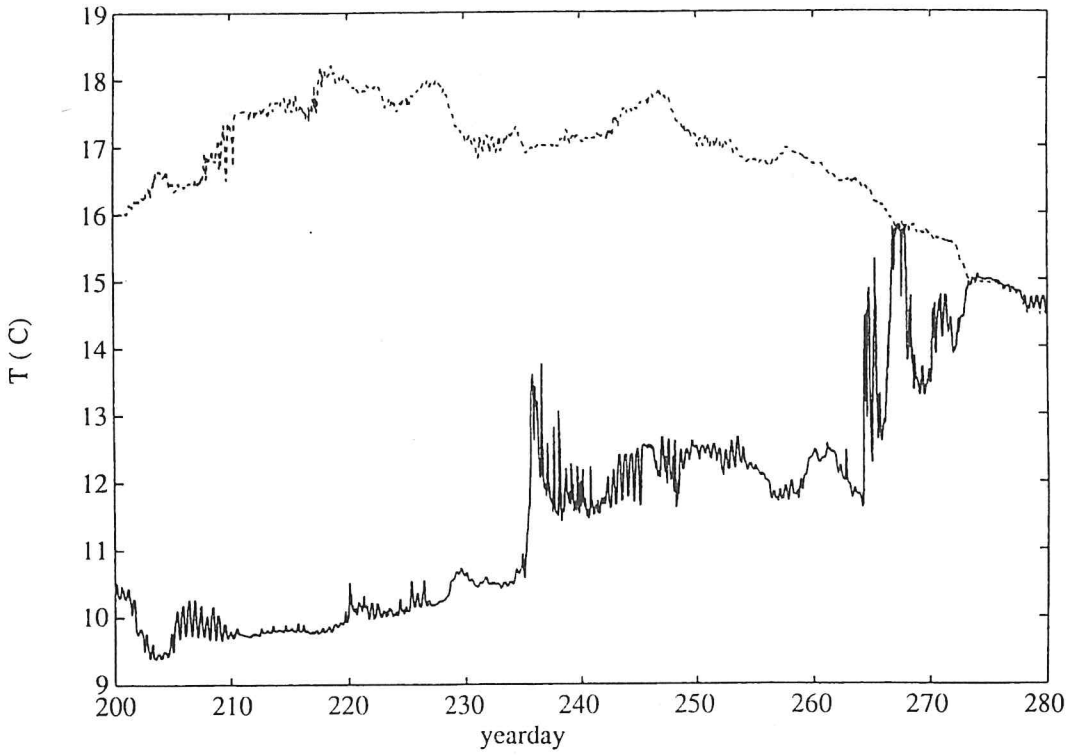

Fig. 4. Temperature records measured at 11 m depth (dashed) and 34 m depth (solid) in 1991.

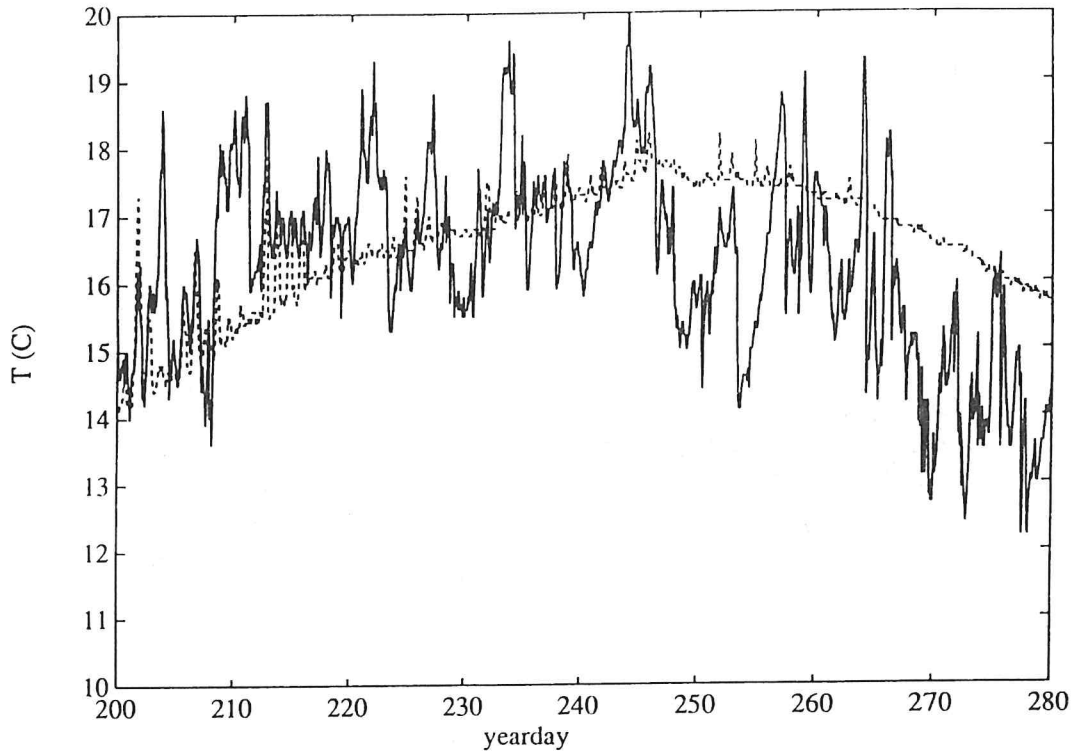

Fig. 5. Sea surface temperature (dashed) and air temperature (solid) measured at K13 in 1991.

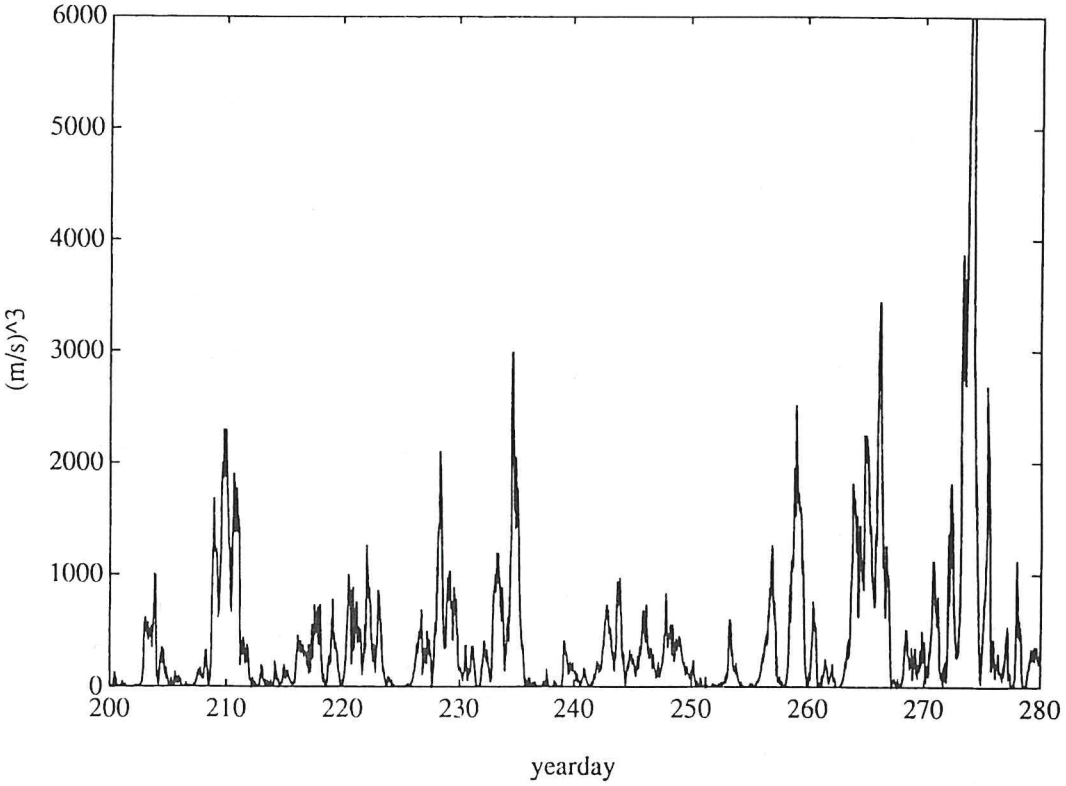

Fig. 6. Windspeed cubed calculated from data measured at K13 in 1991. The windspeed has been corrected to the standard height of 10 m above the sea surface.

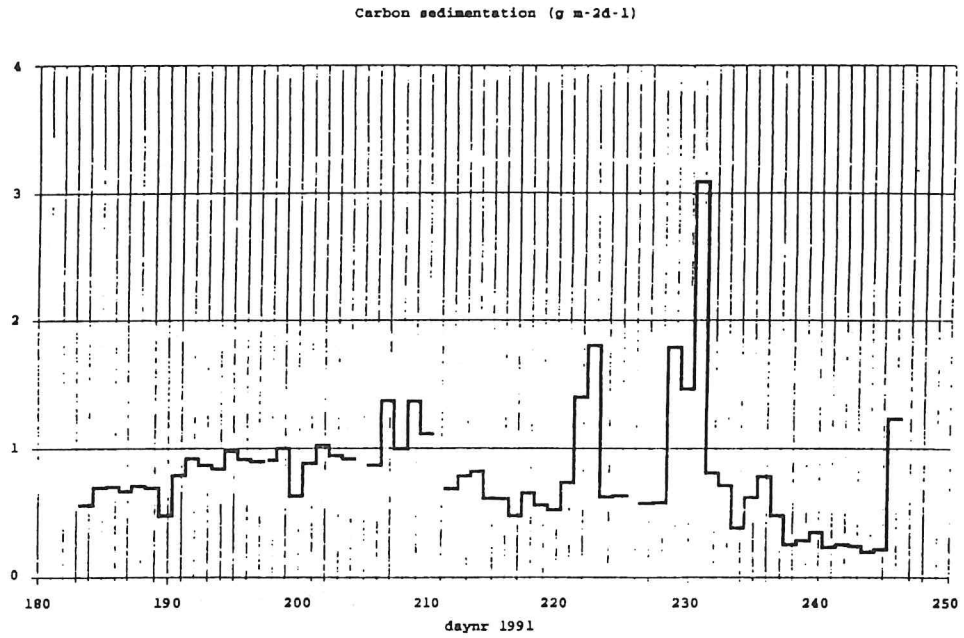

Fig. 7. Carbon sedimentation rate (in g/m /day) from sediment trap data at 39 m in 1991 (note the different length of the horizontal axis).

2B. THEME II: STRUCTURE PELAGIC FOODWEBS

Participants:
M. VAN LEEUWEN, J. DE JONG, B. KUIPERS, G. FRANSZ, S. GONZALEZ, R. RIEGMAN (*NIOZ*)

The impact of resource competition and selective grazing on the plankton structure is studied during four different seasons along a transect from the Dogger Bank towards the Shetland Islands. The results from the November '91 cruise were discussed. a typical winter situation was observed: the concentrations of Fe, Ni, Co, Cu, Cd and the dissolved inorganic macronutrients Si, N, P were found well above the typical summer levels. Algal biomass was between 0.2 and 0.7 µg/l with the highest values near the Dogger bank. 50-75% of the chlorophyll-a was present in particles smaller than 8 µm. Algal growth rates were nearly zero and also microzooplankton grazing was found to be neglible. Mesozooplankton biomasses were between 1 and 4 µg C/l and showed some correlation with the pattern in chlorophyll-a. Most copepod species were filled with lipid granules and the egg production was found to be zero for all the investigated areas (*T. longicornis, C. typicus, A. clausi, C. sp., M. lucens* and *O. similis*). The only exception was *T. longicornis*, which at one station at the Dogger Bank had an egg production of one egg/day per female.

In April and June '94 the same transect will be studied with special emphasis on resource competition and selective grazing on phytoplankton. Additionally there will be proces studies at the Mooring site at the Oyster Grounds (Theme I) during one week in addition to the continuous data recording at this site. For the measurement of size selective grazing on phytoplankton, attempts will be made to develop a suitable method.

2C. THEME III: BENTHIC LINKS AND SINKS IN NORTH SEA NUTRIENT CYCLING (BELS)

WIM VAN RAAPHORST *(NIOZ)*

INTRODUCTION

The BELS programme was part of the INP 1991-1992 and was dedicated to the sedimentary cycling of nutrients in the North Sea. The cruises (leg 1 in August 1991 and leg 2 in February 1992) were executed under the auspices of the Netherlands Marine Research Foundation (NWO-SOZ) and navigated with the RV Pelagia. Participants of the cruises were from NIOZ (Texel), the NIOO-CEMO (Yerseke) and from the Free University of Brussels. Cooperation was established with programmes from the UK on nutrients and primary production in the North Sea (PML, Plymouth) and on nutrient fluxes in UK rivers, estuaries and coastal waters (JoNUS programme MAFF, Lowestoft). The PML programme was executed in the same periods in 1991 and 1992 as BELS, the JoNUS progamme included stations that were also visited during BELS. Preliminary results of both UK programmes and of the BELS cruises may be found in Appendix A. Cooperation was also established with the EC-MAST project ERSEM on North Sea ecosystem modelling. Several of the results of the BELS cruises appear in the benthic nutrient submodel of ERSEM (RUARDIJ & VAN RAAPHORST, 1994).

The first objective of BELS was to study wether sediments in the North Sea act as nutrient "links or sinks", i.e. to quantify how much of the nutrients (N, P, Si) mineralized in the sediment is released to the water column and how much is retained in the sediment or removed from the system (e.g. by denitrification). As benthic nutrient cycling is controlled by interactions of (micro)biological, (geo)-chemical and physical processes, we focussed on several of these processes and their interrelations. The second objective thus reads: How do groups of benthic organisms (bacteria, protozoa, meio- and macrobenthos, demersal fish) interfere with geo-chemical processes and how do they affect sedimentary nutrient cycling.

STATIONS

The BELS cruises included 15 stations in the southern and eastern North Sea (Fig. 8). Station 3 was visited only during the cruise in August 1991. The stations were selected to cover coastal areas directly influenced by the nutrient inputs from the main rivers as well as some off-shore sediments. At the same time, the stations are situated along the main residual transport route of organic matter from the southern North Sea towards the permanent depocenter in the Skagerrak. Thus, the stations also represent erosional, 'neutral' and depositional sediments along the transport route. Station 5 in the Oysterground is the mooring position of theme I (this report). Stations 1 (Broad Fourteens) and 16 (Frisian Front) were subject to intensive benthic research at NIOZ during the previous years, including mesocosm studies (e.g. DE GEE *et al.*, 1991; VAN DUYL, 1991, 1992). The stations in the Skagerrak (9, 10) were very close to the sampling sites of CANFIELD *et al.* (1993) where they studied pathways of sedimentary oxidation of organic matter. Part of the stations in the German Bight (13, 14) and the stations 6 (Weiss Bank) and 9 in the Skagerrak were close to the sites for which JØRGENSEN (1989) presented sulfate reduction rates. Finally, sediment-water exchange of oxygen and nutrients and sulfate reduction rates were measured at comparable sites in the southern North Sea during the British NERC programme in 1989-1990 (NEDWELL *et al.* 1993).

RESULTS

In this report we present the preliminary results of the BELS cruises. For details and list of contributors see Appendices A1 to A11. Part of the results were already published in the scientific literature during the preparation of this report (LOHSE *et al.*, 1993; SLOMP & VAN RAAPHORST, 1993). Some other papers are presently submitted or in press (HONDEVELD *et al.*, 1994; LOHSE *et al.*, 1994; VAN DUYL *et al.*, 1994).

The data obtained during the PML cruises (WOODWARD *et al.*, A1) show high nitrate concentrations near the outflows of the main rivers and very low concentrations in the central North Sea. Summer chlorophyll and primary production were highest in the German Bight and along the UK and continental coastlines. Several areas in the North Sea exhibited urea concentrations in the water column at ecologically significant levels, particularly at the main river mouths. There were, however, also elevated urea concentrations near the Doggerbank, the Frisian Front and in the Fair Isle channel. The data from the JoNUS programme presented in this report (MALCOLM *et al.*, A2) focus on the joint station 2 of BELS in the Southern North Sea. Their measured sediment-water exchanges of nitrate and silicate agree well with the BELS data for this station. It is concluded that the sediments act as an important store and source of nitrogen to support phytoplankton growth during summer. The 'buffer' capacity of the sediments seems, however, a short-term property without substantial storage over the season.

The cycling of phosphorus, nitrogen and silicon in North Sea sediments was studied by SLOMP & VAN RAAPHORST (A3), LOHSE & VAN RAAPHORST (A4), MALSCHAERT & VAN RAAPHORST (A5) and GEHLEN (A6), respectively. Exceptionally high pore water concentrations and sediment-water exchange rates were measured at station 13 inbetween Helgoland and the mouth of the Elbe. Lowest values were found at the offshore stations and normally the fluxes of nutrients to the overlying water were lower in winter than in summer. Phosphate sorption by the oxic upper sediment layers is a rapid process by which phosphate concentrations in the pore water are strongly buffered. The nitrogen cycling is dominated by ammonification and ammonium release to the overlying water, particularly in the German Bight. Here, only 5% and 41% of the produced ammonium was nitrified in February and August, respectively and the bulk of the resulting nitrate was also released to the water column. At the offshore stations nitrification was more important consuming 80% of the produced ammonium in February and 50% in August. Denitrification seemed of minor importance in the sedimentary N budget, particularly in summer. Adsorption of ammonium is characterized by non-linear isotherms with the highest adsorption coefficient at low ammonium concentrations as observed in the upper sediment layers. The relative composition of the total pool of ammonium in the upper cm of the sediments was similar in winter and summer: approximately 61% was adsorbed to the sediment particles, 22% was incorporated in microbial cells and only 17% was dissolved in the pore water. The total pool was, however, twice as large in summer as in winter.

Winter fluxes of Si were between 2 and 8 times lower than summer fluxes. For the Skagerrak stations (9, 10) no seasonal differences were observed. Specific experiments indicated that particularly in summer the Si fluxes were largely enhanced by the activity of the benthic fauna.

Components of the benthic small food webb were investigated by VAN DUYL *et al.* (A7), HONDEVELD *et al.* (A8), MOODLEY (A9) and HEIP & SANDEE (A10) on bacteria, heterotrophic nanoflagellates, benthic foraminefera and meiofauna, respectively. Results on the bacterial ecology include seasonal and spatial variations of biomass and production (^{3}H-leucine incorporation method) and the effects of the benthic bacterial populations on nitrogen cycling. Benthic bacterial production in summer exceeded production in winter, particularly in the upper 3 mm of the sediments. This observation could largely be explained by differences in temperature and phyto-pigment contents of the sediment. Spatial variation of bacterial production seems related to organic matter chlorophyll a and pheopigment contents of the sediments, indicating that phytodetritus is an important food source for benthic bacteria. Bacterial production could not be stimulated by the the addition of ammonium to the sediments. Benthic flagellate densities were mostly higher in summer than in winter, particularly in the sandy sediments. Flagellate densities in the upper 3 mm of the sediment were 2-4 times higher than at 3 and 6 cm depth. Exceptionally high numbers were observed in the coars sandy station near Esbjerg (12). Grazing experiments showed similar grazing rates in winter and summer between 1 and 44 bacteria per flagellate per hour. Using these rates, it is concluded that flagellates consume less than 25% of the daily bacterial production in summer and upto 400% in winter when bacterial production is low. Only six of the BELS stations (4, 5, 7, 10, 13 and 17) were sampled for foramineferal studies in August. Maximum numbers were found in the Skagerrak (st. 10) whereas the eutrophic station in the German Bight (13) has relatively low numbers. This is explained by the potentially high grazing pressure in the latter station where a maximum number of macrozoobenthos was observed (see below). In spite of the limited penetration of oxygen in most sediments benthic foramineferal activity extended down to at least 10 cm. The meiofauna was dominated by nematodes and there was no clear correlation between nematode abundance and grain size distribution of the sediments. Lowest numbers were, however, found in the coarsest sediments. Total benthic oxygen consumption decreased with with grain size but did not correlate with nematode abundance, at least not in summer. From the coherence of the meiofauna with e.g. high correlations between nematode and copepod abundances it is concluded that the meiofauna is regulated by station-dependent characteristics.

Macrofauna and demersal fish were examined by Van der Veer et al. (A11). More then 6700 individuals per m^2 of macrozoobenthos were found at station 13 in the German Bight. Minimum densities were 250 individuals per m^2 at stations 3 and 11. Highest densities of demersal fish were observed also in the German Bight (st. 12, 13, 14) and only very low numbers in the Skagerrak. Flatfish density was positively correlated with macrofauna numbers, and the latter was positively related with bacterial production rates and even with the phosphate and inorganic nitrogen releases from the sediments.

GENERAL ANALYSIS

Major conclusions with respect to the main objectives of BELS may be found in the contributions presented in Appendix A1 to A11. Some additional conclusions emerge, however, from the combined set of results.

All sedimentary processes studied during BELS are controlled by the amount and quality of organic matter deposited on the seafloor. Nutrient releases are driven by mineralization of organic compounds and so is the cycling of redox elements in the sediments. Biomass and production rates of benthic organisms are determined by food availability. We conclude from the present data and from data obtained from the literature (e.g. JØRGENSEN, 1989; DE GEE *et al.*, 1991; VAN DUYL, 1991, 1992; CANFIELD *et al.*, 1993; NEDWELL *et al.*, 1993) that the most important factor controlling spatial variability of benthic processes, is the interplay between eutrophication induced by the main rivers and the residual transport route of organic matter in the North Sea. Due to the river inputs, the largest primary production occurs along the Dutch coast and in the German Bight. Here, freshly produced labile organic matter can reach the bottom and provide a suitable food source for benthic organisms during the productive season. Nutrients are probably

readily released from the labile organic materials. This explains the high activities and fluxes observed even in the coarse sandy sediments near the continental coast in August.

Hydrodynamical conditions prevent permanent deposition and preservation of organic matter in large parts of particularly the Southern Bight of the North Sea, including sediments along the Dutch Coast and probably also station 12 near Esbjerg in the German Bight. Organic matter is resuspended from these sediments within hours to days and further transported in northerly direction along the residual current. As a consequence, there is almost no supply of organic matter to the sandy stations in the Southern Bight out of the productive season, thus explaining the low activities observed in February. Several deposition-resuspension events likely occur before the materials finally settle in the Skagerrak. Underway the organic matter may be trapped for relatively longer times in temporary depocenters as the Frisian Front and particularly in the 'mud hole' in the Helgoland Bight (st. 13). These depocenters collect materials originating from a larger area of the southern North Sea, building up higher contents of sedimentary organic matter consisting largely of less labile compounds. The combination of settlement of these older materials together with locally produced fresh an labile organic matter in the German Bight probably explains the exceptionally high nutrient fluxes and biological activities measured at particularly station 13 in August. The Skagerrak stations, however, collect mainly older and more refractory materials which have undergone substantial mineralization during transport from the production zones towards the Skagerrak. KEMPE *et al.* (1988) suggest that it may take 10 to 15 years for fine grained particles to travel from the Elbe Estuary to the Skagerrak. This means that the quality of the organic matter deposited in the Skagerrak is poor in terms of nutrient contents and as a food source for benthic organisms, particularly when compared to the materials reaching the bottom in the production zones. Thus, a large and more or less constant pool of poorly degradable organic matter controlls the availability of organic substrates to the benthos in the Skagerrak. This explains the relatively minor differences in fluxes and activities in February and August at stations 9 and 10.

The final conclusion suggested by this analysis is that transport of organic matter from the production zones in the southern North Sea towards the permanent depocenter in the Skagerrak, including repeated sedimentation-resuspension events and the mineralization processes underway, is an important factor in controlling benthic nutrient cycling and benthic biology in the North Sea.

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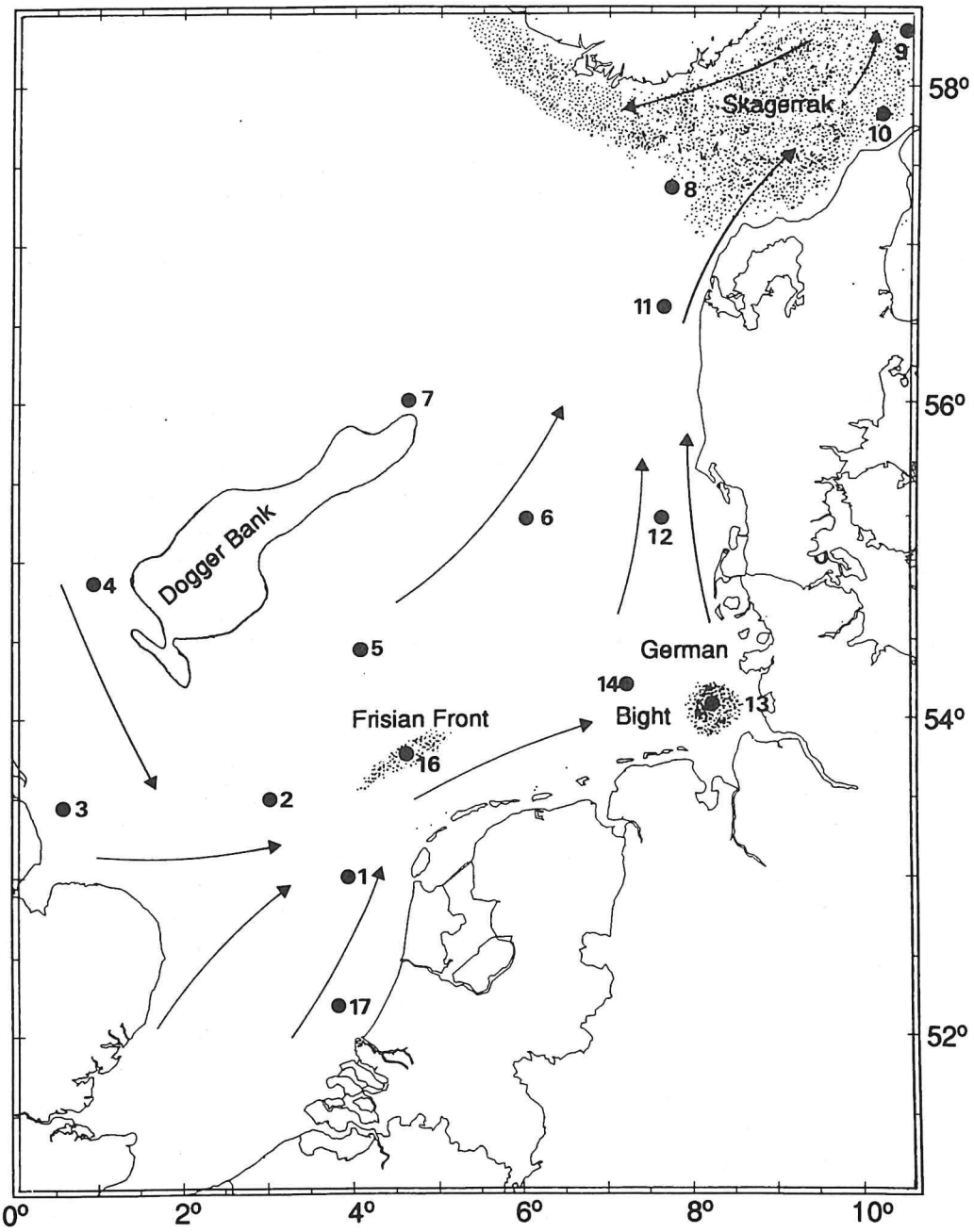

Fig. 8. Stations visited during the BELS cruises in August 1991 and February 1992. Arrows indicate the main transport routes of water and organic matter in the North Sea. St. 1: Broad Fourteens, 2: UK-NL Boundary, 3: Sliverpit, 4: Doggerbank West, 5: Oystergrounds, 6: Weiss Bank, 7: Tail End, 8: Skagerrak West, 9: Skagen, 10: Hirtshals, 11: Jutland, 12: Esbjerg, 13: Helgoland Bight, 14: Elbe Rinne, 16: Frisian Front, 17 H. of Holland.

2D. THEME IV: MICROCONTAMINANTS. AN OVERVIEW OF THE PRELIMINARY RESULTS.

JAN P. BOON, CRUISE-LEADER (*NIOZ*)
For participants see Appendix B.

The theme 'Microcontaminants' of the Integrated North Sea Programme (INP-MICON) has tried to connect increased environmental concentrations of selected polyaromatic microcontaminants with the occurrence of certain effects at the molecular, physiological, cellular and organ level for a decreased health of fish and invertebrates that live at the bottom of the North Sea. Such indications can serve as early-warning signals for more grave and probably irreversible effects that occur at a later stage, and may affect whole populations or ecosystems.

For this purpose, contaminant concentrations of polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and their metabolites, polychlorinated dibenzodioxins and -furans (PCDDs and PCDFs) were measured in the most suitable environmental compartment from the series sediment, the flatfish species dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*), the gadoid species whiting (*Merlangius merlangus*) and two invertebrate species: the seastar *Asterias rubens* and the whelk *Buccinum undatum*. Dab and cod are also chosen for monitoring programmes of the Joint Monitoring Group of the OSPARCOM and the North Sea Task Force (NSTF) of the International Council for the Exploration of the Sea (ICES).

The present report is based on the data known at the beginning of January 1993. The gradient of PCB concentrations in biota perpendicular to the Dutch coast has become less steep compared to the last decade (Contributions of BOON *et al.* and EVERAARTS *et al.*). This appears to be particularly due to lowered concentrations at coastal stations as a consequense of lower riverine inputs. No systematic differences in lipid-based concentrations occurred between seastars and dab from the same area.

Patterns of PAHs in sediments identified oil (alkylated compounds) as well as combustion processes (unsubstituted compounds) as sources (Contribution KLUNGSØYR and WILHELMSEN). Concentrations in total sediments are closely related to their TOC contents and thus concentrations at the Frisian Front and the Oyster Grounds are highest. PAH concentrations in dab muscle were very low due to enzymatic transformation reactions. One of the products of such reactions however, 1-hydroxypyrene in bile, was used to describe the exposure of fish to PAHs. This yielded consistent pictures for both cruises (Contribution ARIESE).

The activity of the enzyme system responsible for this type of transformations, the hepatic cytochrome P450-dependent monooxygenase system, correlated more with changes in water temperature between stations than with gradients in contaminant concentrations in case of dab (Contributions SLEIDERINK *et al.* and BEYER *et al.*), but not in plaice (Contribution EGGENS and BERGMAN) and whiting. The causal relationship between water temperature and EROD-activity was proven in a laboratory experiment with male dab.

DNA-aberrations in dab liver were more prominent in the Southern Bight than in the area near the Dogger Bank (Contribution HALBROOK *et al.*). In the seastar differences in DNA integrity occurred between stations, but these could not be clearly related to measured concentrations of contaminants (Contribution EVERAARTS *et al.*).

Of the histopathological changes in dab, the frequency of splenic melanitic macrophage centres was depressed along the Dutch Coast. The frequency of hepatic neoplastic lesions differed clearly between stations, but without a clear relation to measured contaminant levels (Contribution VETHAAK).

The frequency of imposex in whelks was much higher at stations located in or next to the deep-water traffic separation system than in the Northern part near the Dogger Bank. This might be due to the use of tributyltin-based anti-fouling paints on ship hulls (Contribution TEN HALLERS and BOON).

Research was supported by the Netherlands Marine Research Foundation (SOZ) of the Netherlands Organization for Scientific Research (NWO). Both cruises were navigated with the research vessel 'Pelagia' of the Netherlands Institute for Sea Research.

APPENDIX A

INP WORKSHOP. NIOZ. JANUARY. 1993.
PRIMARY PRODUCTION AND NUTRIENTS IN THE NORTH SEA.

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The other members of the nutrient cycling group at Plymouth are:
NICK OWENS, ANDY REES and DUNCAN PLUMMER.

The nutrient cycling group at Plymouth Marine Laboratory have carried out nine cruises in the North Sea since the summer of 1986. The aims of these cruises have been to study nutrient cycling processes in the sea area, and to investigate the influence of the major riverine inputs upon the phytoplankton in the coastal zones and hence to evaluate possible eutrophication processes in these shallow nearshore environments. Particular emphasis was placed on the roles of the reduced nitrogen species, ammonia and urea. The final two cruises in this programme were carried out on the RRS Challenger, and in parallel to the BELS cruises in August 1991 and February 1992, on the RVPelagia .

The research is mainly carried out in the water column, using on-line analysis for the major nutrient species, (nitrate, nitrite, phosphate, silicate, ammonia, and urea.), and the physical variables, with discrete samples being taken for a large suite of variables including primary production, 15N assimilation rates, and bacterial thymidine incorporation studies.

Results from the summer cruises showed the consistently high nitrate inputs from the major rivers, (eg: Rhine, Scheldt, Weser, Elbe, Humber, etc.), but central and northern offshore regions of the North Sea were found to be severely depleted in nitrate, (as low as 6 nmoles/litre), which is characteristic of an oligotrophic oceanic system. We found that the phytoplankton production was potentially phosphate limited in the nutrient rich coastal areas , but was potentially nitrate limited in the offshore areas. Surface urea concentrations were determined, and figure 1 shows the results from august 1991, for the whole of the north sea. Several areas are seen to exhibit urea concentrations at ecologically significant levels. Concentrations of >1 umole/litre were found at the mouths of the Ems, Humber, and Rhine/Scheldt. These are probably directly associated with anthropogenic inputs of sewage, and will undoubtedly significantly contribute to the nitrogen assimilation by the phytoplankton. These inputs may lead to an elevated biomass of phytoplankton, above that due to the assimilation of the other nitrogen species nitrate and ammonia. It can be seen that there are elevated urea levels associated with the Dogger bank and the Fresian Front. Also high concentrations were found associated with a high chlorophyll feature in the Fair Isle channel, which was also an area of elevated nitrate due to the upwelling effects of the Shetlands. In general there was a good correlation between urea and chlorophyll.

Summer surface chlorophyll fluorescence was elevated in the shallow nutrient rich waters of the southern North Sea, with the highest levels found in the German Bight and near shore areas. The nutrient deplete central and northern North Sea were low in chlorophyll .
Figure 2 shows the primary production from august 1991. These rates shown are for the entire phytoplankton community of >0.2 um. These results are in line with what would be predicted from the chlorophyll data, with the highest production rates along the UK and continental coastlines, particularly in the German Bight, of the Rhine, and at the Flamborough Front. The observed values varied from less than 0.5 mmoles C $m^{-3}d^{-1}$ in the northern North Sea, to over 50 off the Rhine. From size fractionation studies we found that over large areas of the North Sea much of the production was due to organisms smaller than 5 um. Overall, the large phytoplankton dominatedthe nutrient rich coastal zones whereas in the nutrient deplete offshore areas the small (< 5um) phytoplankton dominated. If we compare the primary production to the nitrate concentration, there is a significant correlation in the summer nutrient deplete regions between nitrate and the >5 um size fraction, but not with the smaller size fraction. Nitrate is important to the large phytoplankton species, but the production of the small phytoplankton is apparently not influenced by the nitrate concentration. Nitrogen assimilation rate studies using ^{15}N, showed that there was a co-variation of these rates with chlorophyll and primary production rates, with the highest rates off the German Bight and the Rhine. High ammonia assimilation rates were found to correlate with high dissolved ammonia concentrations, but this was not observed for nitrate. Ammonia assimilation accounted for between 45% and 99% of the total, and ammonia was consistently found to be assimilated in preference to nitrate, even when there were high nitrate concentrations.

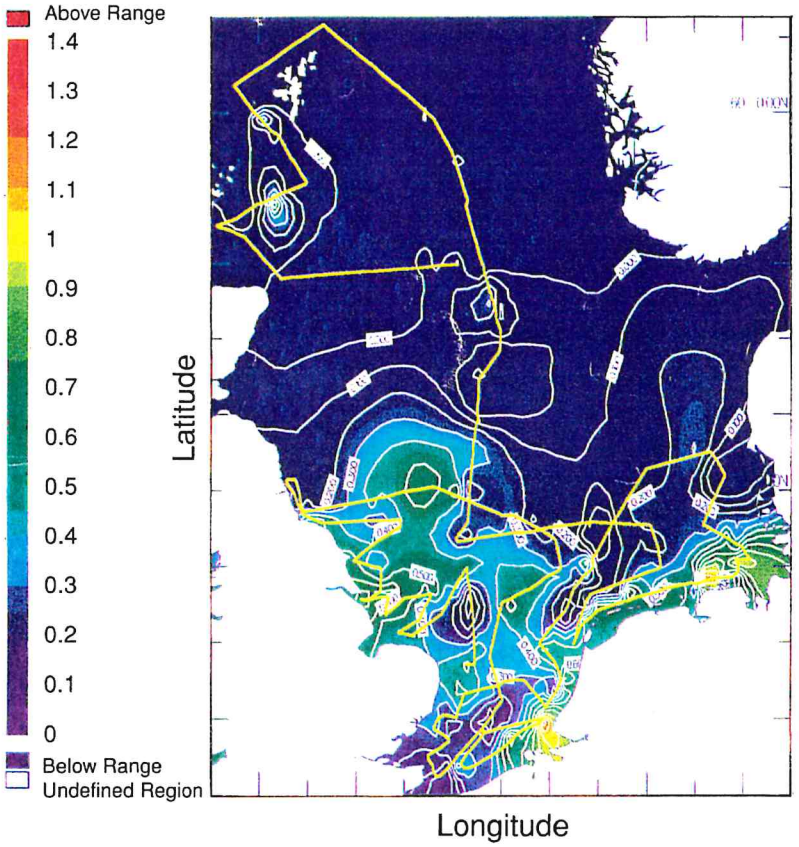

Fig. 1. Surface urea concentrations (umoles urea-N/litre) for the North Sea, measured during August 1991.

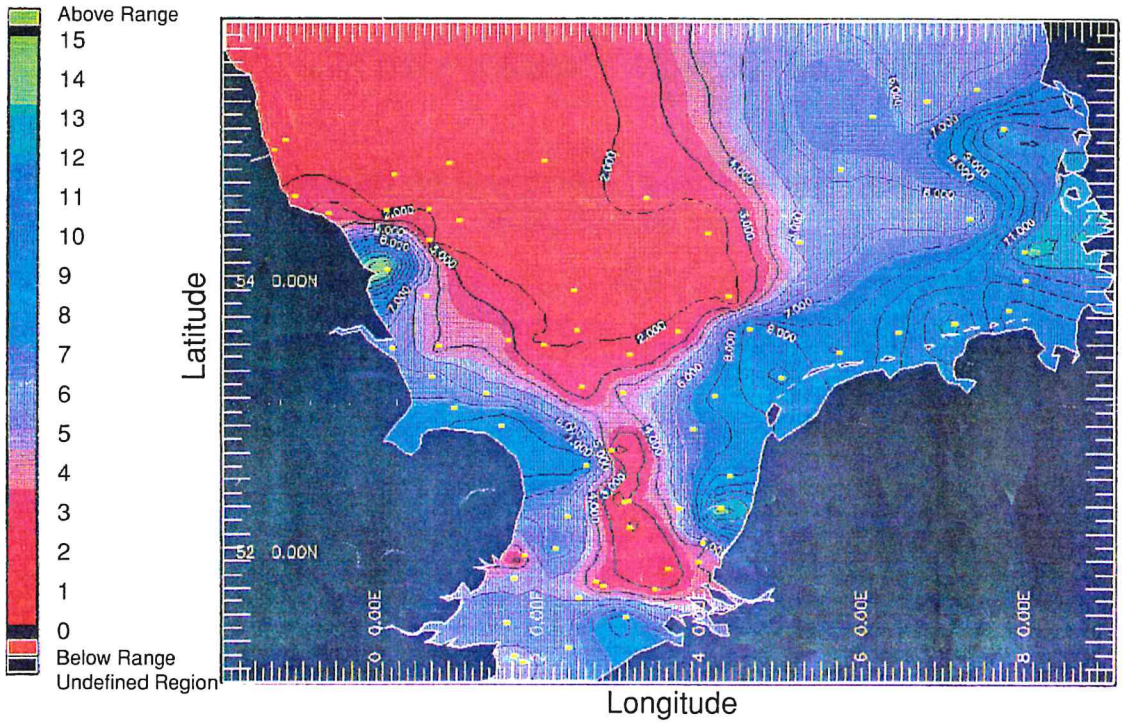

Fig. 2. Total primary production measurements for the southern North Sea during August 1991 (mmoles C m-3d-1). Data is for entire phytoplankton community (< 2 um).

SEDIMENT/WATER INTERFACE FLUXES OF NITRATE AND SILICATE AT A MUDDY SAND SITE IN THE SOUTHERN NORTH SEA

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A component of the JoNuS project, a multi-insitute study of the inputs of nutrients from the estuaries of eastern England to the soutern bight of the North Sea, is to study benthic nutrient cycling in the major estuaries and also in the southern North Sea. A collaborative link was established with the INP/BELS programme. Samples were collected from one site, BELS2, in September 1991 and February 1992. The samples were collected for the determination of fluxes across the sediment/water interface by core incubation and interstitial water chemistry. The interstitial water was extracted using a sipping system.

The interstitial water profiles of nitrate (Fig. 1) show marked variability between replicates and differences with season. During summer nitrate concentration increases with depth, due to nitrification, reaching a peak before reduction of nitrate commences. In winter high concentration of nitrate occurs throughout the sediment.

Direct measurement of the fluxes of nitrate (made using intact, undisturbed sediment cores) while showing large variation between replicates show clear differences between summer and winter (Table 1). There is a nitrate flux into the water column in summer, when nitrate concentration is low due to biological uptake, and unmeasureable in winter when the water column nitrate concentration was high. Nitrate is clearly responsive to the seasonal biological cycles in the sea.

The sediment act as an important store and source of nitrogen to support phytoplankton growth during the summer. The 'buffer' capacity of the sediments is important but is a seasonal, short-term, process. Further work will be required to reconcile the direct flux measurements and the potential fluxes due to the measured interstitial water profiles.

In contrast, there is no difference in the measured flux of silicate from the sediment into the water column in summer and winter. This is due to the abiological, long-term, regeneration of silica within the sediment.

To understand the potential problems that can result from the input of nutrients to coastal seas it is clearly necessary to know that each nutrient behaves independantly and is subject to different seasonal cycles. Interpretation of the JoNuS project data is currently at an early stage.

TABLE 1

Direct measured fluxes of nitrate and silicate at BELS2 ($\mu mol.m^{-2}.h^{-1}$)

	Summer (September 1991)	Winter (February 1992)
Nitrate	5-9	0-1
Silicate	10-20	10-20

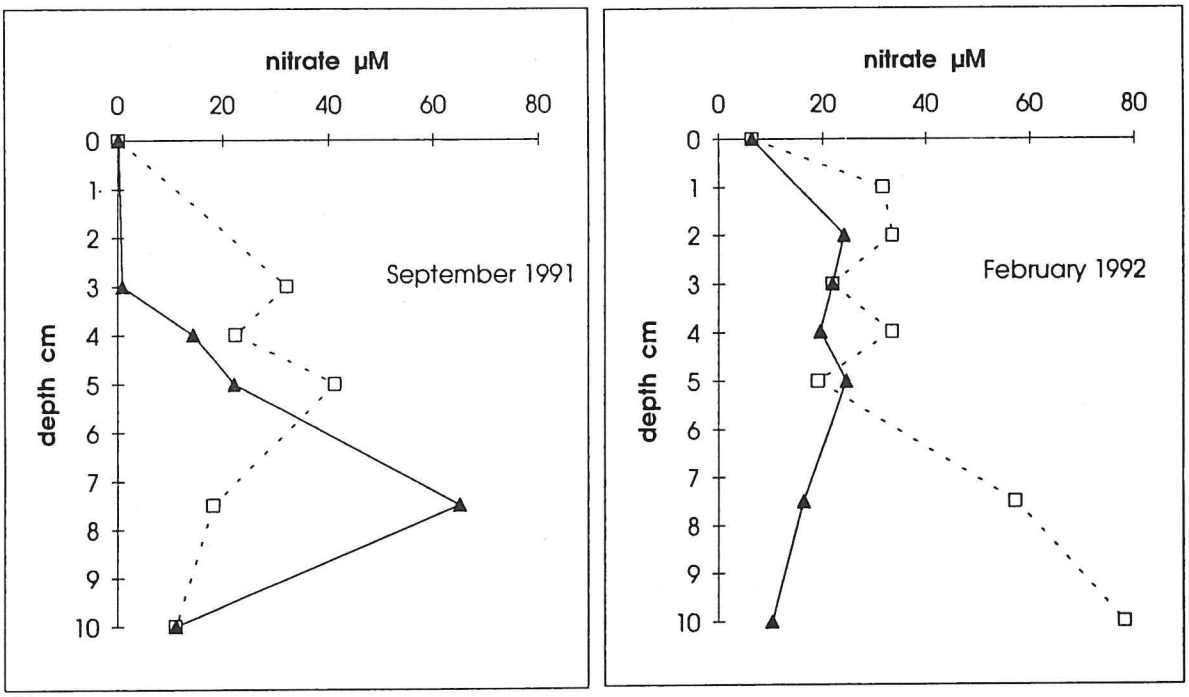

Fig. 1. Interstitial water profiles of NO_3^- at st. BELS-2 in August 1991 and February 1992. Interstitial water was obtained using a sipping system. Lines indicate replicate cores.

Forms of phosphorus in North Sea sediments and fluxes across the sediment-water interface

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Benthic phosphorus regeneration can strongly influence water column chemistry in shallow marine systems. Binding of phosphorus in the sediment may cause a time lag between mineralization of organic matter and actual release of phosphorus to the water column. Quantitative information on the role of sediments in phosphorus recycling and removal is still very limited.

During two INP/BELS cruises in August 1991 and February 1992 field results were obtained on phosphorus in sediments at 16 stations in the southern, central and eastern North Sea. Large seasonal and spatial differences in sediment-water exchange rates of PO_4 (measured using intact sediment cores) were found (Figure 1). In general, the flux to the overlying water was lower in winter than in summer: rates varied from ca. +20 (Weiss Bank; St. 6) to ca. +1700 (Helgoland Bight; St. 13) $\mu mol.m^{-2}.d^{-1}$ in August, and from ca. -30 (Elbe Rinne; St. 14) to ca. +40 (Hirtshals; St. 10) $\mu mol.m^{-2}.d^{-1}$ in February. The largest seasonal variation was found in the German Bight area. Low ratios of the sediment-water exchange rates of PO_4 and O_2 when compared to those expected due to the aerobic decomposition of sediment organic matter were found, indicating binding of phosphorus in the sediment at almost all stations, especially in February. The large seasonal effects are explained by lower PO_4 regeneration rates and a higher phosphorus binding capacity of the sediment due to an increase in depth of the oxidized sediment layer in winter.

Generally, ca. 10-25% of the inorganic sediment phosphorus in the upper sediment layer (0-5mm) was bound to the surface of Fe (oxyhydr-) oxides. The importance of these Fe forms in the oxidized layer for the binding of phosphorus is illustrated for the Elbe Rinne station (St. 14) in Figure 2. The decrease in Fe oxides with depth coincides with a decrease in P bound to Fe oxide surfaces and an increase of Fe and PO_4 in the pore water.

Results from oxic sorption experiments (isotherms and kinetics) performed at 8 stations indicate that (1) the adsorption of PO_4 by the oxic upper sediment layer (0-5 mm) is a rapid process (minutes to hours) and that (2) phosphate concentrations are strongly buffered by these sediments (mostly to ca. 1-3 µmol/l) as shown for the Oystergrounds (St. 5) and the Helgoland Bight stations (St. 13) in Figure 3. The slope of the isotherm (Kads) at the PO_4 concentration where $[PO_4]initial = [PO_4]final$, is a measure for the buffer intensity of the sediment at this concentration. The value of Kads is linearly correlated with sediment grain size, porosity and Fe oxide content.

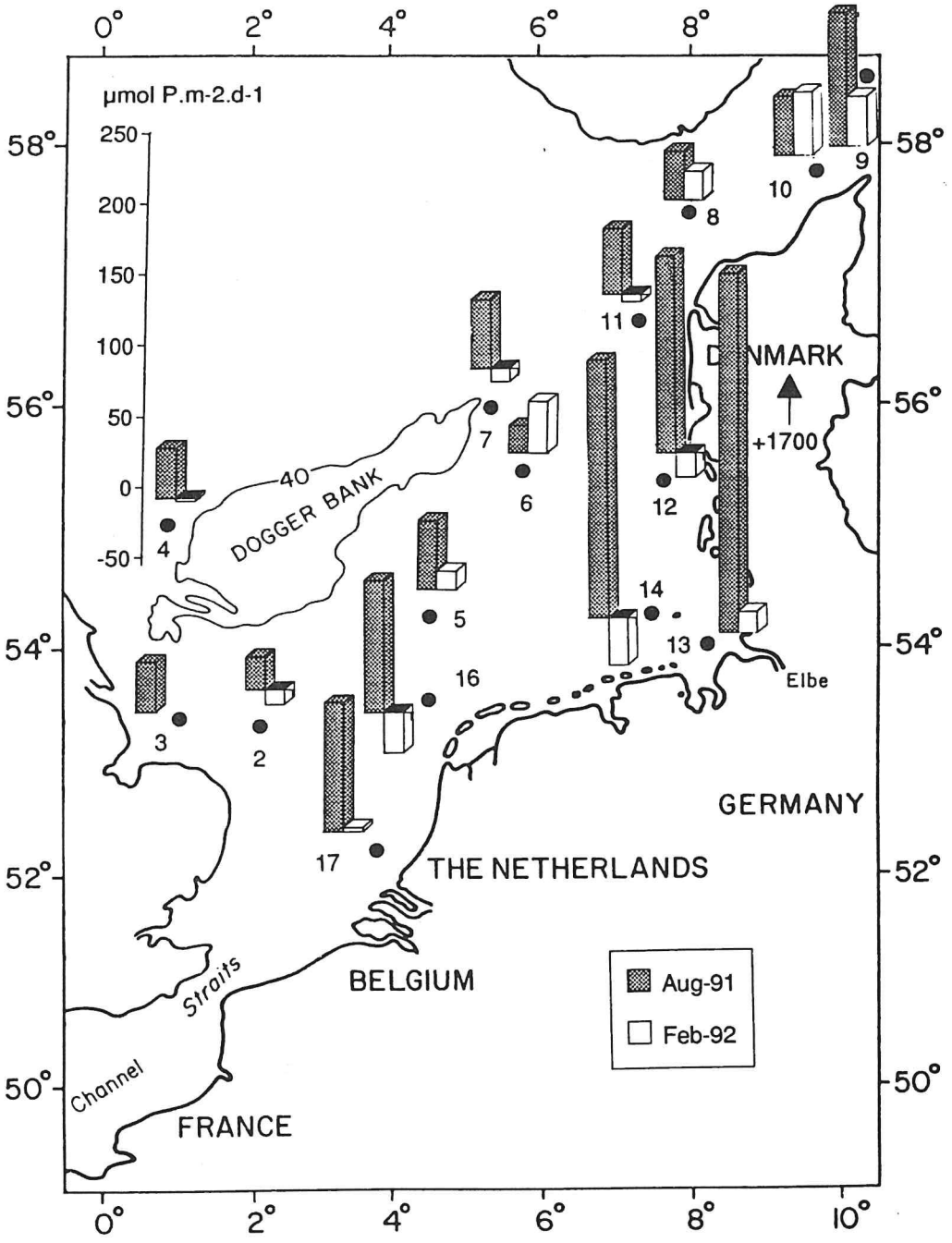

Figure 1. Sediment-water exchange rates of PO_4 ($\mu mol.m^{-2}.d^{-1}$) in August 1991 and February 1992.

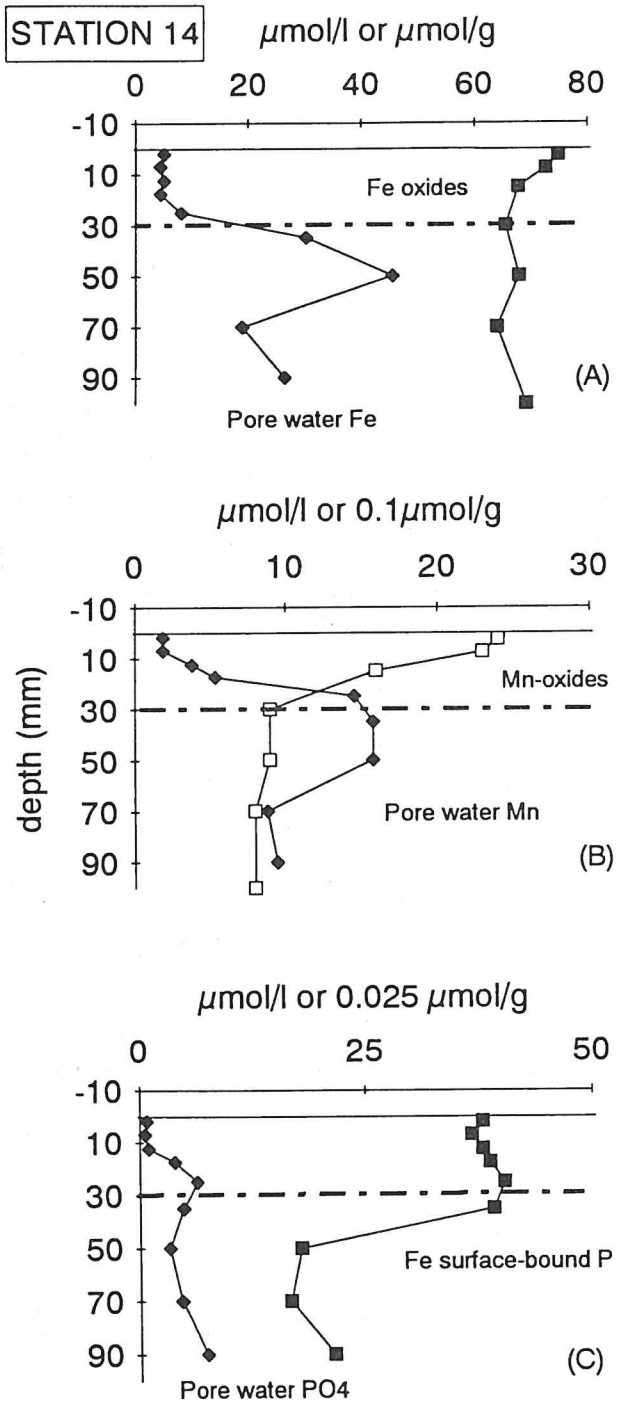

Figure 2. Profiles of (A) Fe-oxides and pore water Fe, (B) Mn-oxides and pore water Mn, (C) Fe surface-bound P and pore water PO_4 at the Elbe Rinne station (St. 14) in February 1992. The dashed line represents the boundary between the oxidized and reduced sediment zones.

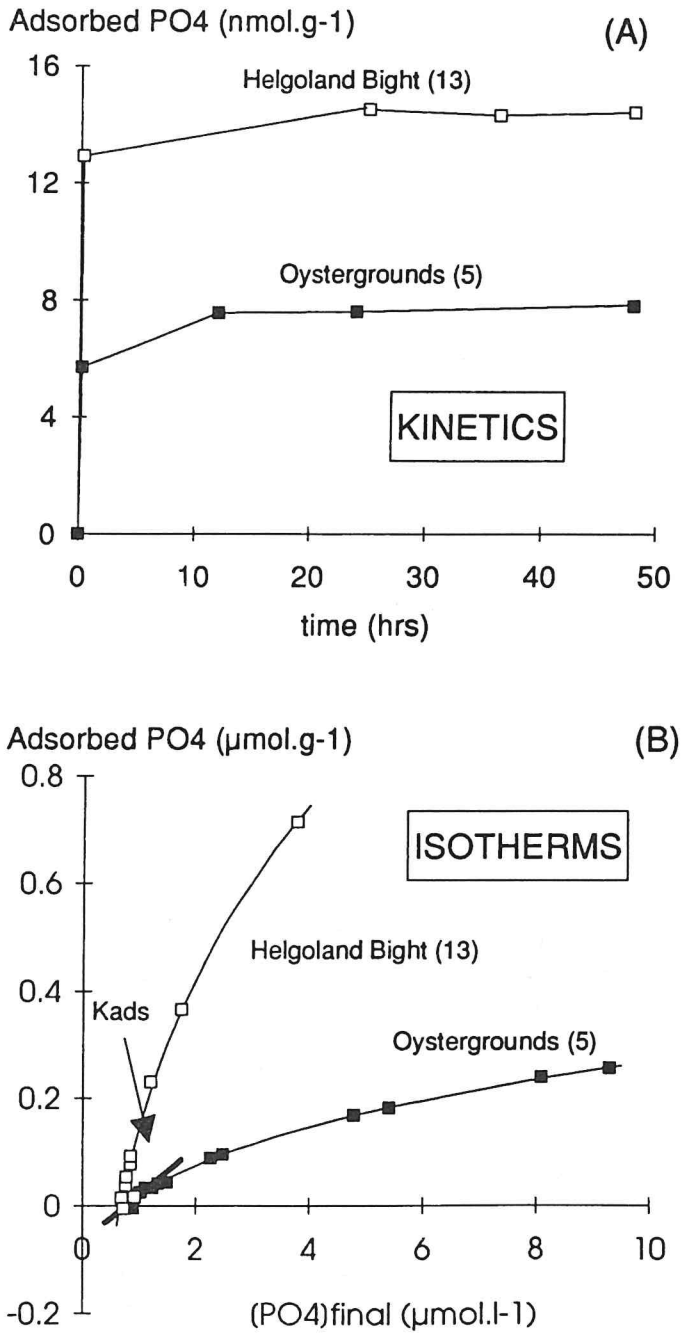

Figure 3. (A) Kinetics of PO_4 adsorption, (B) Adsorption isotherms (Freundlich equation) for the Oystergrounds (St.5) and Helgoland Bight (St.13) stations.

"Benthic nitrogen cycling in North Sea sediments"

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Results of two BELS (Benthic Links and Sinks in North Sea Nutrient Cycling) cruises carried out in August 1991 and February 1992 were presented. Independent whole core techniques were used to estimate denitrification, nitrification, sediment-water exchange of inorganic nitrogen compounds and oxygen microprofiles. Data were presented for the German Bight and more offshore areas.

Clear seasonal and spatial differences of the benthic nitrogen cycle were mirrored by productivity in the water column, transport of organic matter and sedimentation. During August, oxygen penetrated only a few millimetre into the sediments and large anoxic areas were found in the deposition area of the inner German Bight. The absence of an oxic layer in the latter area prevents nitrification, which is depending on oxygen. The exclusive source for denitrification was therefore the overlying water. This finding was confirmed by nitrate uptake of the sediment. Although nitrification recovered by the build-up of an oxic layer in February, the nitrate flux was still directed into the sediment. All other stations were characterized by higher nitrification rates in August in spite of thinner oxic layers. This was confirmed by elevated nitrate releases in that period. Denitrification did not correspond to these higher nitrification rates. During August rates of an average 1 μmol N m^{-2} h^{-1} were measured, and these rates had increased 3 to 4-fold in February. The only explanation of the discrepancy between the patterns of nitrification and denitrification is, that nitrate is produced in a very thin oxic layer at the sediment surface and is rapidly released to the (-nitrate depleted-) water column instead of being denitrified in deeper sediment layers. This situation occurred predominantly in August. In February when oxygen penetrated deeper into the sediments the nitrification zone moved downwards as well. Additionally the nitrate concentration in the overlying water had raised resulting in less steeper diffusion gradients at the sediment-water interface. Consequently, more nitrate became available for denitrification. From the total amount of nitrate produced, on average only 5% in August and 25% in February were denitrified

The overall nitrogen mineralisation was estimated by assuming that ammonification is the sum ammonium flux and nitrification. We conclude, that nitrogen cycling predominantly took place through ammonification, followed by NH_4^+ release to the water column, particularly in the German Bight. (Fig. 1 and 2). Here, the total ammonification was about 7 times higher than at offshore stations during August. Only 5% and 41% were nitrified in August and February, respectively. Because the bulk of nitrate was released to the water column as well (Fig. 1), the total DIN fluxes were in August almost identical to ammonification in August. At the offshore stations approximately 50% of the produced ammonium was consumed by nitrification. This value increased to more than 80% in February. Denitrification played a substantial role only in February, particularly at the German Bight.

To estimate the contribution of benthic DIN - fluxes to the requirements of phytoplankton, an annual primary production of 250 g C m^{-2} yr^{-1} and a molar C/N ratio in phytoplankton of 6.6 was assumed. It appeared that on an annual base 4 to 8 % of the nitrogen requirements of phytoplankton could be delivered by benthic nitrogen mineralisation. This value increased at the Helgoland Bight station to more than 50 % due to much higher nitrogen fluxes accompanied with an elevated primary production of 300 g C m^{-2} yr^{-1}. The role of denitrification as nitrogen sink was estimated from the assumption that the anthropogenic nitrogen input to the continental coastal water (comprising an area of 65000 km^2) is 971000 t N yr^{-1} (Nelissen and Stefels, 1988). Our average denitrification rate of 0.4 g N m^{-2} h^{-1} would result in an removal of 26000 t N yr^{-1}, which corresponds to 2 to 3 % of the estimated input of nitrogen. This value is lower than previous estimates made by Brockmann (1988, 20 %) and Law and Owens (1990, 8 to 12 %). The difference is partly caused by the fact hat our calculation is based on a restricted area directly affected by anthropogenic inputs, while other authors compared the total North Sea sediment surface area with anthropogenic nitrogen inputs.

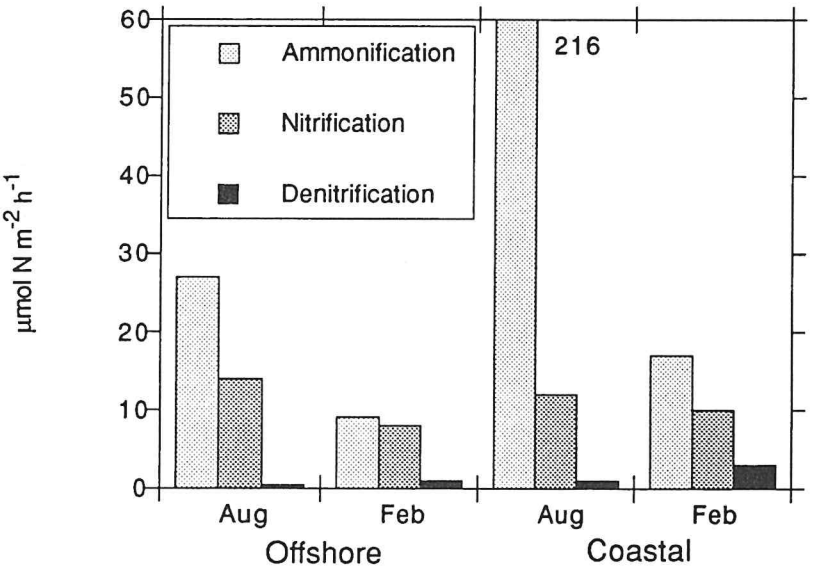

Fig. Total mineralisation (= ammonification), nitrification and denitrification at offshore and German Bight stations in August 1991 and February 1992

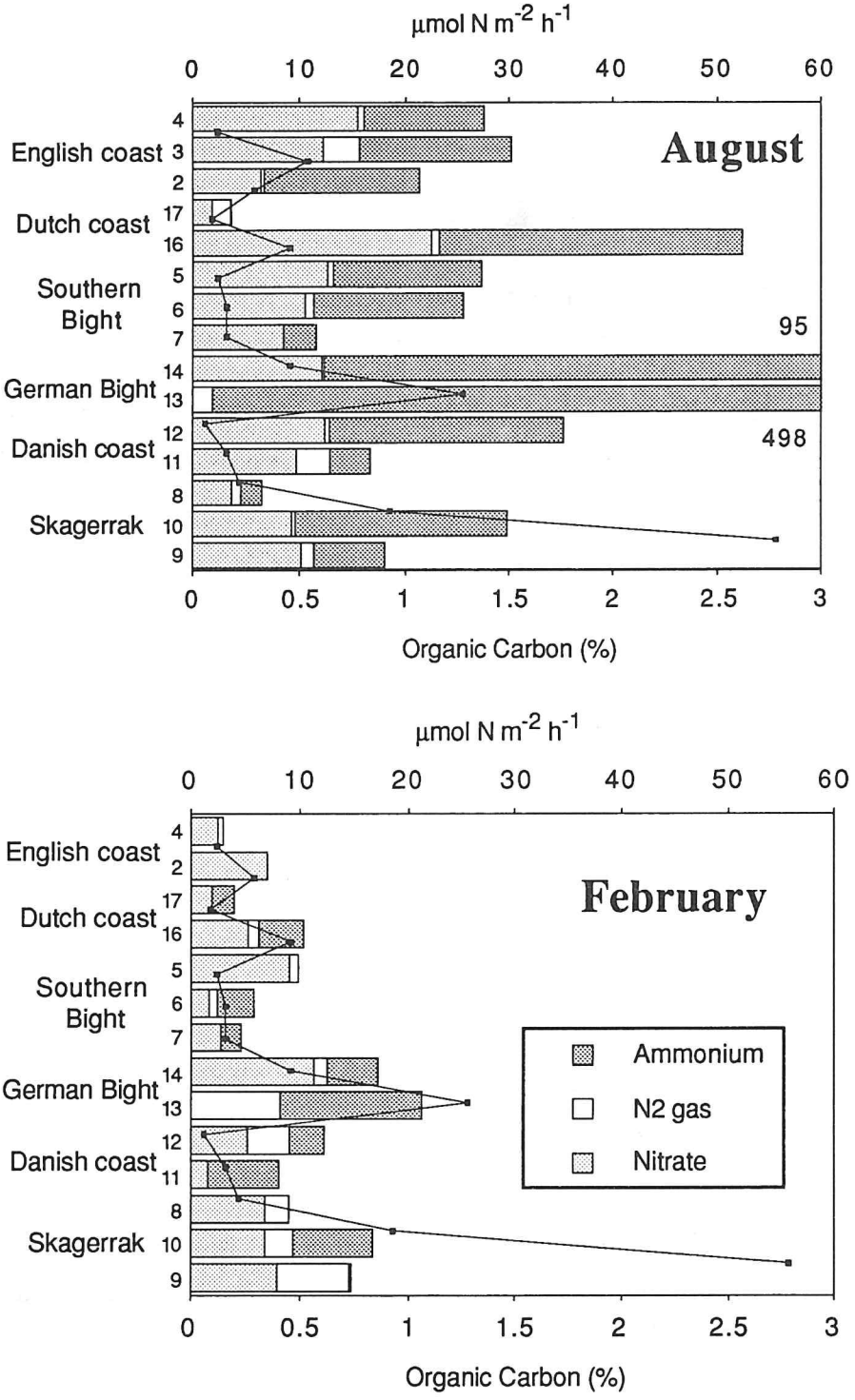

Fig. 2: Cumulative DIN Fluxes in August '91 and February '92 plotted according the general transport route of suspended matter from the southwestern to the northeastern North Sea. Solid line indicates org. carbon content of the sediment. Numbers at the horizontal scale indicate station numbers.

NORTH SEA NUTRIENT CYCLING: BENTHIC POOLS OF AMMONIUM

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Eutrophication of the North Sea has been an important issue during the last decades. Several studies have dealt with long-term trends in external nutrient inputs and effects of eutrophication for the North Sea ecosystem. Much less attention has been paid to the internal mechanisms potentially buffering the external inputs. Most of these mechanisms are situated in the upper layers of the sediments where nutrients can either be regenerated to the water column or retained.

In this study different ammonium pools were measured in the upper 10 cm of 15 south-eastern North Sea sediments . Data were obtained during 2 BELS cruises (Benthic Links and Sinks in North Sea Nutrient Cycling, part of the Integrated North Sea Programme) in August 1991 and February 1992. Results include vertical distributions of dissolved ammonium in interstitial waters and vertical distributions of amounts of KCl-exchangeable ammonium sorbed to sediment particles. The amounts of free ammonium in microbial cells was determined only in the 1 cm toplayer. In addition ammonium sorption-desorption experiments with natural sediments and seawater were carried out on six stations.

The equilibrium between dissolved and exchangeable ammonium is described by the partition coefficient K. This dimensionless coefficient K follows from the ratio exchangeable NH4:dissolved NH4 corrected for the in situ solid:solution ratio. The calculated coefficients from the field data showed that the adsorption of ammonium is strong (K=2.5 - 113) in the upper 3 cm and much weaker (K=1.5-6.0) below 3 cm. There seems to be a seasonal variation in the adsorption capacity of the upper layer (figure 1), with a relatively stronger adsorption in winter.

Highest K values (> 10) were found at low dissolved ammonium concentrations. This is in agreement with the measured adsorption isotherms (figure 2) showing that adsorption is non-linear with steep slopes at low dissolved ammonium concentrations (< 30 uM). The differences in the measured isotherms for the various stations are largely explained by the organic carbon content of the sediments. From this it is concluded that organic carbon is a major sorbent for ammonium.

Field data showed interstitial water profiles with large spatial differences and extremely high ammonium concentrations in the deposition area of the German Bight (1000-2500 µM). These stations have a larger pool (~1.3 x) of adsorbed ammonium than the open sea stations.

In general, the total pool of ammonium in the upper layer in summer was nearly twice as large as that in winter (figure 3). The composition of the total pool was equal; approximately 61 % adsorbed, 22 % microbial and 17 % dissolved ammonium. It seems that microbial ammonium forms a substantial part of the total pool in the upper cm particularly in the open sea stations.

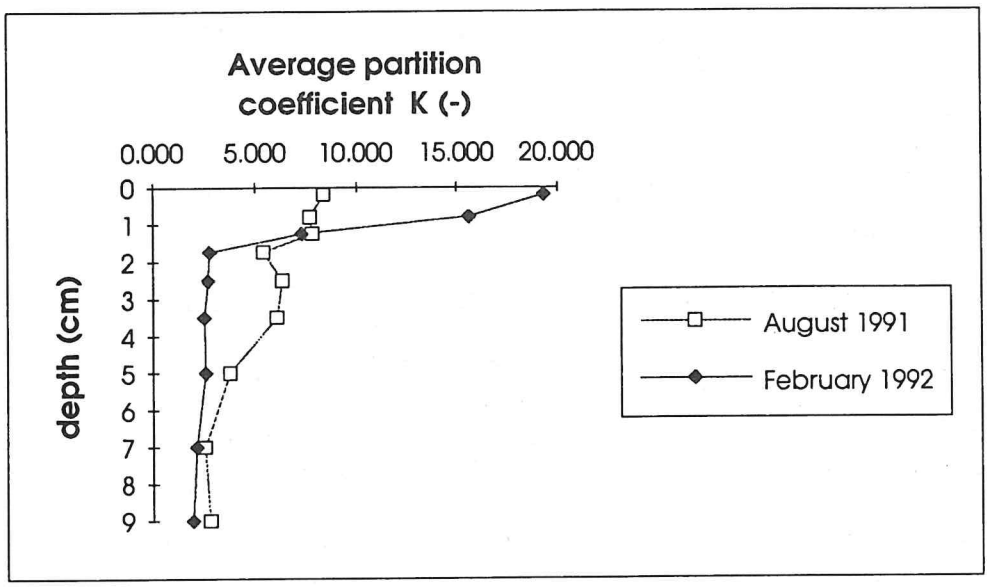

Figure 1. The partition coefficients (K) versus depth for 15 stations in the North Sea. The values represents average values for each depth layer in respectively 1991 and 1992.

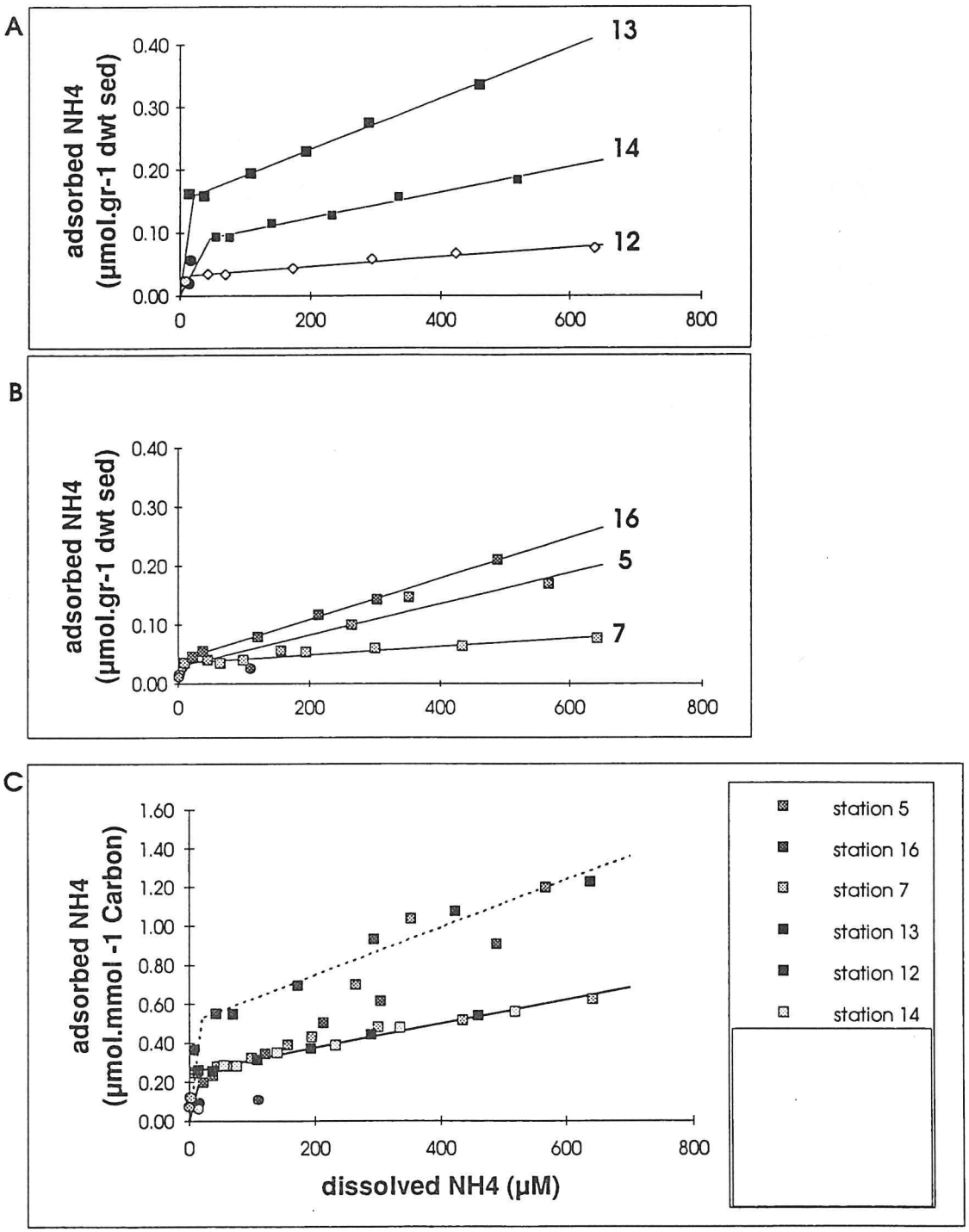

Figure 2 Adsorption isotherms of ammonium for six North Sea stations in february 1992 (A and B) and (C) normalized isotherms for organic carbon contents. Squares are experimentally measured and circles are field data (explanation see text).

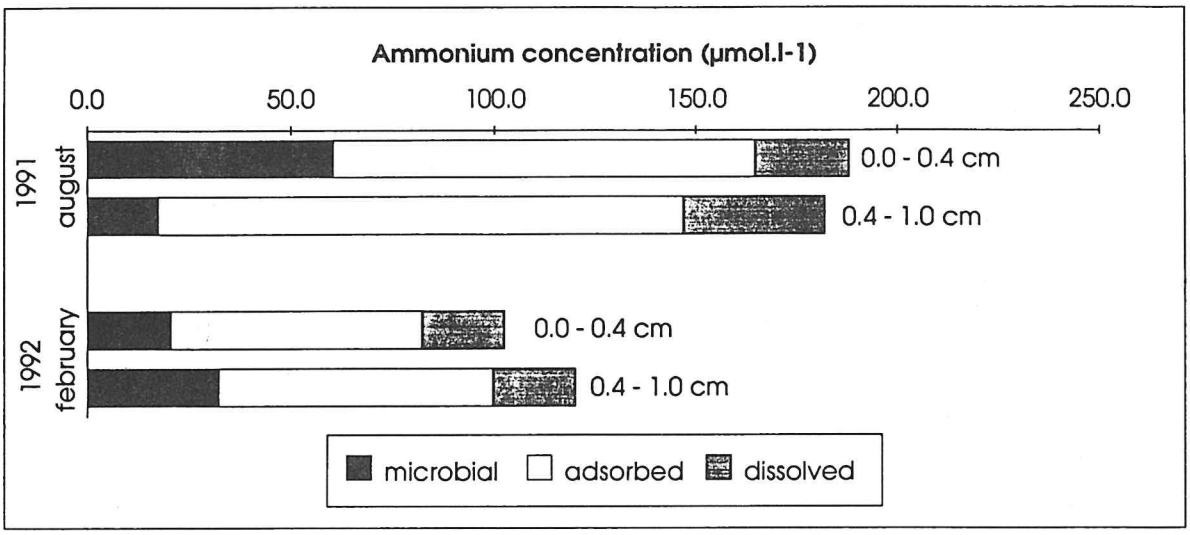

Figure 3. Exchangeable ammonium pools in the toplayer (0-1 cm) of 15 stations in the North Sea.

Fluxes of dissolved silica across the sediment-water interface.

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Fluxes of dissolved silica at the sediment-water interface have been estimated for 16 different stations in the eastern part of the North Sea (Fig.1) using on-deck whole-core incubation techniques. Biological enhancement of exchanges at the sediment-water interface was assessed by an asphyxiation technique using N_2-flushing and by comparing experimental fluxes to those calculated from pore water profiles assuming molecular diffusion. The oxygen concentration within the chambers was continously monitored.

In August, fluxes of dissolved Si ranged from 193.9 $\mu mol*d^{-1}*m^{-2}$ for st. 11 (Jutland) to 8899.4 $\mu mol*d^{-1}*m^{-2}$ for st.13 (German Bight). Winter fluxes are in general between 2 and 8 times lower than summer fluxes. For sts. 9 and 10, both located in the Skagerrak no significant seasonal difference was observed. Those two stations showed the highest fluxes during winter with respectively 3548.1 $\mu mol*d^{-1}*m^{-2}$ and 4404.1 $\mu mol*d^{-1}*m^{-2}$. After N_2-flushing, fluxes were decreased by a factor ranging from 1.1 (st.16, Frisian front) to 3.8 (st.6, Weissbank) during summer. During winter, inactivation of fauna lowered the exchanges at the sediment-water interface in average by a factor of 1.6. Comparing fluxes measured after flushing and those derived from pore water profiles indicates a much higher biological enhancement of exchanges at the sediment water interface. According to Tab.1, fluxes are increased between 6.8 (st. 10) and 140 (st. 5) times during summer as compared to fluxes calculated assuminmg molecular diffusion only. During winter, effective fluxes are between 9.3 (st. 10) and 65.9 (st. 12) times higher then the molecular ones. Since pore water profiles were evaluated independently from the incubation experiments, one would expect differences between the calculated molecular and the measured anoxic fluxes. Moreover the experimental fluxes were determined under artificial flow conditions. The high discrepancy especially during summer may however indicate, that the benthic organisms have not been completely inactivated.

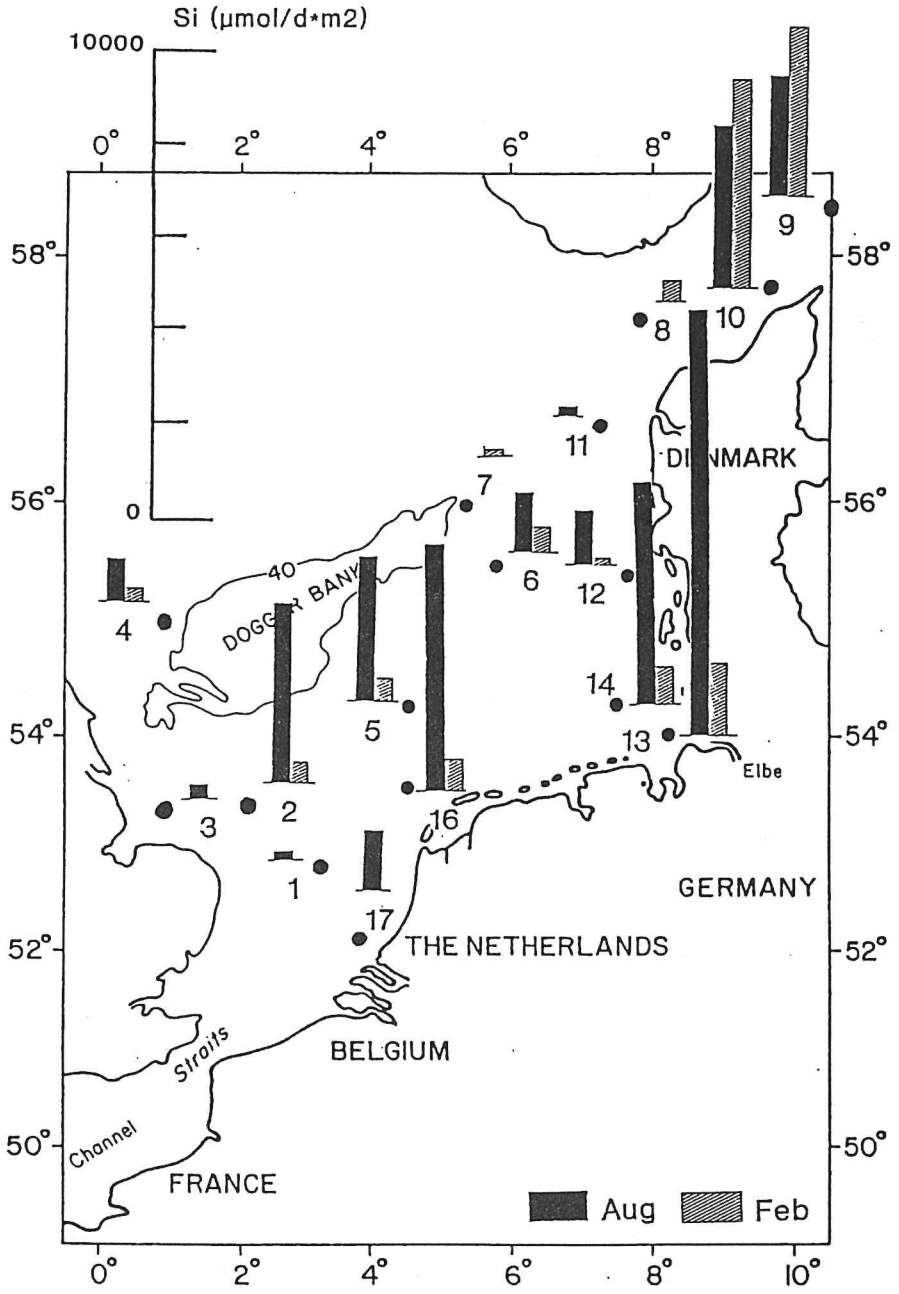
Looking at spatial and seasonal differences, high fluxes during summer combined with a strong seasonal signal appeared to be related to stations allowing for the transitional deposition

of fresh material (sts. 2, 5, 16, 13, 14). The general decrease of fluxes during winter is consistent with the strong temperature dependency of the first-order dissolution reaction of silica. The lack of significant seasonal changes for sts. 9 and 10 in the Skagerrak area, would indicate steady supply of material to this deposition area.

Tab.1: Comparison of average experimental and calculated fluxes ($\mu mol*d^{-1}*m^{-2}$).

St.	August 1992 oxic	anoxic	Ratio of oxic/ anoxic	calc.	Ratio of oxic/calc.
4	907.7 (n=3)	1357.1 (n=3)	0.7	11.6	78.0
5	3002.6 (n=3)	1933.3 (n=2)	1.6	21.3	140.0
6	1262.1 (n=3)	335.3 (n=2)	3.8	71.2	17.7
9	2534.9 (n=2)	1843.9 (n=3)	1.4	310.0	8.2
10	3418.4 (n=4)	2933.0 (n=3)	1.2	502.4	6.8
12	1136.5 (n=4)	915.3 (n=3)	1.2	31.4	36.2
13	8899.4 (n=2)	4241.5 (n=2)	2.1	896.6	9.9
14	4657.8 (n=3)	1620.3 (n=2)	2.9	407.8	22.0
16	5106.0 (n=4)	4726.9 (n=3)	1.1	228.2	34.3
	Febuary 1993				
4	286.3 (n=4)	204.1 (n=3)	1.4	25.0	11.4
5	473.5 (n=3)	396.6 (n=3)	1.2	13.1	36.2
6	534.0 (n=4)	276.7 (n=3)	1.9	27.4	19.5
8	428.7 (n=4)	213.6 (n=3)	2.0	13.4	32.1
9	3848.1 (n=2)	944.5 (n=2)	4.1	262.3	14.7
10	4404.1 (n=3)	3328.1 (n=3)	1.3	475.3	9.3
12	140.3 (n=2)	198.8 (n=1)	0.7	2.1	65.9
13	1474.4 (n=1)	933.5 (n=1)	1.6	23.9	61.8
14	768.2 (n=3)	756.3 (n=1)	1.0	25.7	29.8
16	657.7 (n=4)	1574.9 (n=1)	0.4	19.3	33.3

Fig.1: Seasonal trends in experimental fluxes (µmolSi*d^{-1}*m^{-2}) measured under oxic conditions.



The benthic small food web. Seasonal and spatial variations, trophic and nutrient dynamics

Fleur C. van Duyl, Bea J.M. Hondeveld & Arjen J. Kop

Introduction

Factors related to summer-winter variations in heterotrophic bacterial production and biomass in North Sea sediments and the role of bacteria in sediment nutrient cycling were studied during the BELS (benthic links and sinks in nutrient cycling) cruises in 1991 and 1992. The aim of the research was to increase our understanding of the key factors determining variation in benthic bacterial production and biomass. In addition we intend to gain a better insight in the sink or source function of benthic bacteria in nutrient cycling. It was hypothesized that benthic bacteria are sinks of N and P releasing nutrients only upon death by grazing.

The sample grid conformed to the course of the reststream from the Southern Bight of the North Sea along the continental coast to the Skagerrak (Norway) with a few reference stations in the Dogger Bank region. The sample stations were chosen to cover a wide range in the benthic biological activity and mineralization. The idea was that the degradability of the bulk of organic matter in the sediment varied along this reach with the ultimate sink of refractory material in the deep waters of the Skagerrak. In addition it was also meant to cover a wide range of sediment types with respect to grain size composition.

Measurements

At 16 stations intact sediment cores were sampled. Benthic bacterial production (^{3}H-leucine method) and biomass (acridine orange direct counts and biovolume estimates) were measured at 3 depths in the sediment and related to temperature, and benthic variables such as sediment organic matter, chlorophyll a, pheopigment and grain size composition.

Nutrient sediment-water exchange of N and P was measured in relation to bacterial production in the presence and absence of grazing by benthic eucaryotic organisms (e.g. protists). After reducing the grazing activity of benthic protists on bacteria by adding the eucaryotic inhibitor cycloheximide to intact sediment cores the sediment water fluxes of dissolved inorganic N were monitored for up to 6 h.

By ammonia additions to sediment slurries, it was assessed if bacterial production was stimulated by NH_4^+. In addition the effect of Nserve (inhibitor of nitrification) on bacterial production was assessed.

Results and discussion

Seasonal variations

There were no significant differences between the bacterial biomass in summer and winter (Fig. 1a, b). At several stations (st. 1, 8, 9, 16) the biomass at all depths in the sediment was higher in winter than in summer. A completely different pattern was found for bacterial production (Fig. 2a, b). In summer the benthic bacterial production exceeded bacterial production at all depths in winter. The largest differences between summer and winter values were found in the sediment surface layer (0-3 mm). Temperature and phytopigment content of the sediment appeared to be the best predictors of seasonal variations in bacterial production in sediment surface layers. Like the temperature, the phytopigment content of the sediment was in winter significantly lower than in summer. The bulk of sediment organic matter does not vary seasonally, but the labile part may indeed be larger in summer than in winter.

Spatial variations

In table 1 an overview is given of the depth integrated values of bacterial variables and sediment characteristics. Highest bacterial productions, 2-4.8 $gC.m^{-2}.d^{-1}$ (integrated over 63 mm depth), were restricted to continental coastal stations with well mixed water columns. These Stns may be strongly influenced by effects of local eutrophication (river outflows and Kattegat). We distinguished two groups in these stations: one group with high sediment organic matter and phytopigment content (Stns 10, 13, 14) and one group with low sediment organic matter and phytopigment content (Stns 1, 12, 17). This last group showed generation times of less than 1 day. Apparently a high sediment organic matter content is not a prerequisite for high bacterial production. This was also nicely illustrated by the bacterial production rates at Stns 3 and 9 (Wash/Silverpit, Skagen), which were remarkably low in relation to the sediment organic matter content (table 1). The organic matter at the Stns 3 and 9 is probably predominantly refractory material. After exclusion of the stations 1, 12, 17, 3 and 9 from the data analysis a significant positive relation was present between sediment organic matter (C) and bacterial production (Spearman rank r_s,=0.72, , n=11, $p<0.02$) in accordance with Cole et al. (1988). In addition significant relations were found between chlorophyll a, pheopigment and bacterial production after excluding the Stns 1, 12 and 17 (r_s,=0.70 (chl-a), r_s,=0.71 ((pheo.) n=13 $p<0.01$). These relations suggest that sedimentated phytodetritus is an important food source for benthic bacteria at

most of the Stns. Spatial variability in bacterial production might indeed be related to variability in phytodetrital input (van Duyl et al. 1993). At the Stns 1, 12 and 17 resuspension of sedimentated organic matter and rapid turnover of substrates, related to their location in shallow (c. 25 m), turbulent coastal waters, may mask the relations between bacterial variables and substrate indicators as found for the other stations. Physicochemical disturbances have been suggested as regulating factors of bacterial biomass under physically highly variable circumstances (Alongi 1988). Biomass at the Stns 1, 12 and 17 is also low in relation to the bacterial production and did not conform to the significant relation between bacterial production and biomass (r_s,=0.70 n=13 $p<0.01$) detemined for the other stations.

Bacterial biomass was significantly related to chlorophyll a and pheopigment in summer and in winter (Fig 3a, b). In winter bacterial biomass was significantly related to sediment organic C and N. The sediment grainsize composition appears to set an upper limit to the bacterial biomass. The finer the sediment, the larger the ranges in bacterial biomass can be.

Benthic mineralization by protists

At 3 out of the 10 Stns sampled in August, the sediment water efflux of DIN (dissolved inorganic nitrogen) decreased as a consequence of the cycloheximide treatment. This may imply that at those Stns reduction in grazing pressure by protists resulted in a decrease of the efflux of DIN. Subtle increase in bacterial biomass (immobilization of N) in the absence of grazing in the sediment surface layer may have been responsible for the change in sediment-water exchange of DIN. Bacterial production in the cycloheximide-treated cores and in the control cores was comparable suggesting that the inhibitor had no effect on bacterial growth. These results point to an important role of protists in benthic mineralization. However, at 7 of the 10 Stns no such effects could be determined. A closer examination of the results and additional experimental work is needed to solve this problem.

N-limitation of bacterial production

Bacterial production in sediment slurries was not stimulated by NH_4^+ additions in August. During the 24h incubations the bacterial production in the control and the experimental vial (50µM NH_4^+ addition) either increased together or remained constant. In the second cruise in February 400µM NH_4^+ was added. Bacterial production was stimulated at Stn 14 by the addition, but no effect was assessed by the other Stns. In August additions of NH_4^+ may

have been too low to stimulate bacterial production. In August when inorganic nutrients concentrations in the water phase are relatively low N-limitation of bacterial production in sediment surface layers is more likely than in February when the inorganic nutrient concentrations are relatively high in the water column. The experimental set-up (sediment slurries on a rolling device) may have disturbed the physicochemical characteristics of the sediment and the functioning of the microbial community.

Experiments with Nserve (inhibitor of nitrification) showed that bacterial production was inhibited by Nserve at most stations sampled (n=6). This has implications for the use of Nserve.

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Table 1. Overview of the 0-63 mm depth integrated values of bacterial production, biomass and other sediment characteristics

		depth	porosity	temperature		bacterial production		bacterial biomass		chlorophyll-a		pheopigment		organic C		organic N	
		(m)	(v/v)	(°C)		(gC.m-2.d-1)		(gC.m-2)		(mg.m-2)		(mg.m-2)		(gC.m-2)		(gN.m-2)	
Station	number			August	February	August	February	August	February	August	February	August	February	August	February	August	February
Broad Fourteens	1	26.5	0.38	16.7	6.4	2.44	0.31	2.7	4.2	18.5	5.3	105.6	35.6	52.2	55.2	9.0	17.9
UK/NL boundary	2	32.5	0.45	16.5	5.8	1.28	0.37	9.1	8.5	94.3	53.1	467.0	217.3	241.5	267.4	32.6	33.5
Silverpit	3	84.0	0.40	15.0		0.48		1.7		16.1		97.8		560.8		27.2	
Dogger Bank W.	4	55.5	0.39	7.0	6.6	0.63	0.31	4.9	4.9	18.4	12.3	267.6	103.3	107.1	128.5	19.7	21.2
Oystergrounds	5	47.0	0.45	10.7	6.4	0.54	0.15	5.3	3.5	35.3	15.7	311.2	159.0	49.2	172.9	10.2	28.1
Weiss bank	6	49.0	0.45	12.3	5.8	0.56	0.21	4.1	3.9	30.8	22.2	246.3	219.9	109.5	192.9	17.0	25.7
Tail end	7	49.5	0.40	9.2	6.1	0.41	0.21	5.6	5.7	47.3	18.9	306.8	194.1	133.8	153.8	22.6	26.1
Skagerrak W.	8	128.0	0.42	7.2	6.4	0.22	0.14	2.8	4.6	28.4	30.4	336.7	269.6	202.2	229.2	23.7	29.4
Skagen	9	331.0	0.85	6.8	7.0	0.38	0.17	4.6	6.5	41.6	57.5	565.7	567.4	657.5	691.2	83.7	80.7
Hirtshals	10	63.0	0.66	12.2	6.8	2.80	0.31	7.7	8.3	88.7	71.9	472.0	466.9	472.4	547.0	60.7	65.2
Jutland	11	41.0	0.37	10.0	6.3	0.51	0.04	1.2	1.0	11.4	0.4	43.9	6.1	235.6	33.4	30.5	12.3
Esbjerg	12	23.0	0.38	17.3	4.8	4.84	0.05	8.0	8.0	26.6	16.4	303.1	128.9	75.1	100.9	14.4	18.1
Helgoland Bight	13	20.5	0.68	18.7	4.5	2.41	0.24	11.4	13.8	145.9	97.8	1282.7	673.3	540.3	801.5	79.8	91.7
Elbe Rinne	14	39.5	0.48	17.0	5.3	2.01	0.29	11.4	12.9	21.7	26.0	515.0	285.2	251.0	250.6	55.1	33.8
Frisian Front	16	38.0	0.53	17.4	6.3	1.48	0.18	5.0	10.9	86.2	66.9	331.0	319.9	343.1	371.7	38.1	42.5
Hoek v Holland	17	24.8	0.37	18.6	5.8	3.70	0.07	3.4	2.4	19.3	4.0	111.1	7.8	53.1	81.3	5.9	10.0

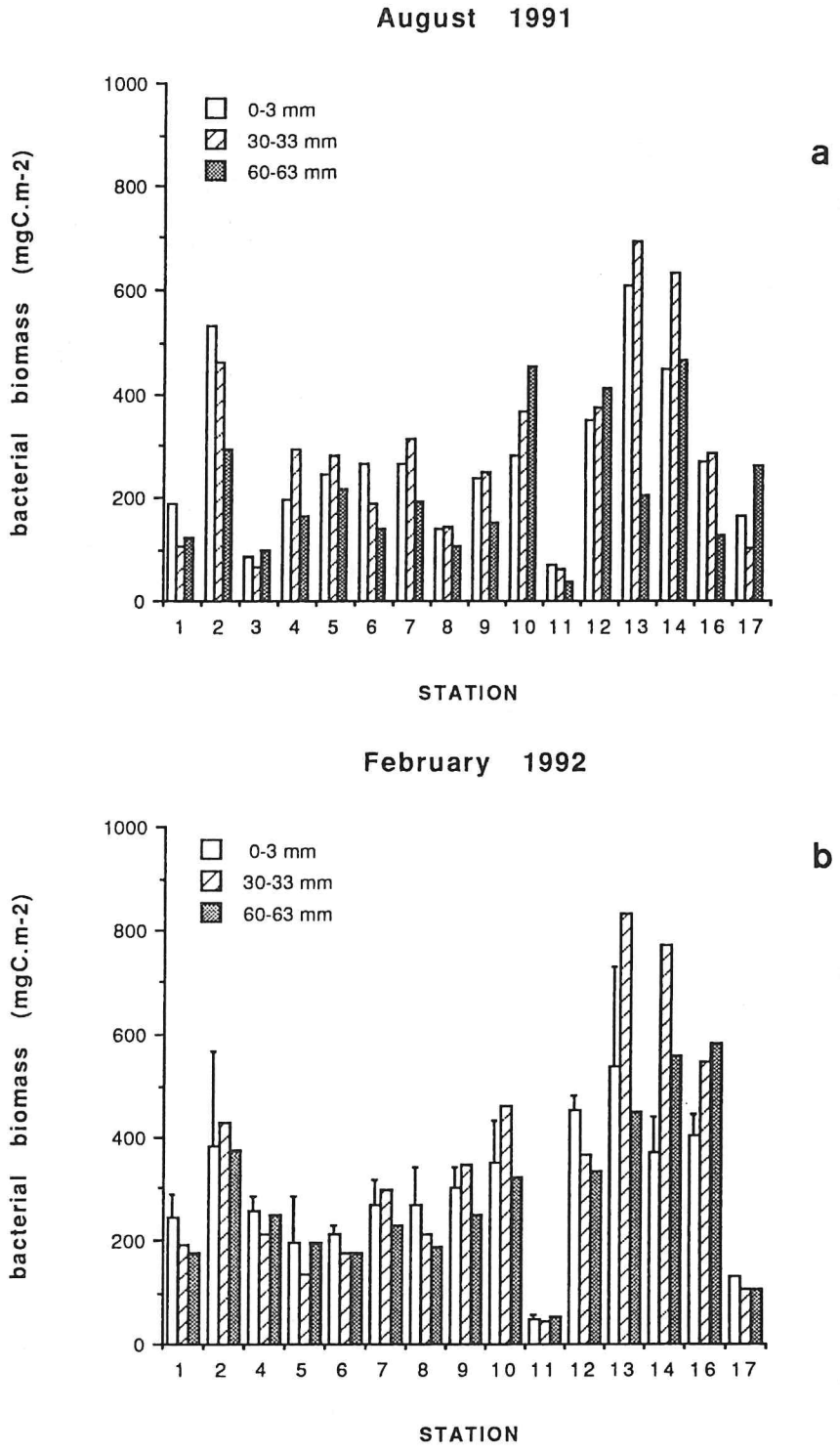

Fig. 1. Depth profiles of benthic bacterial biomass for the stations 1-17. a. August 1991 b. February 1992

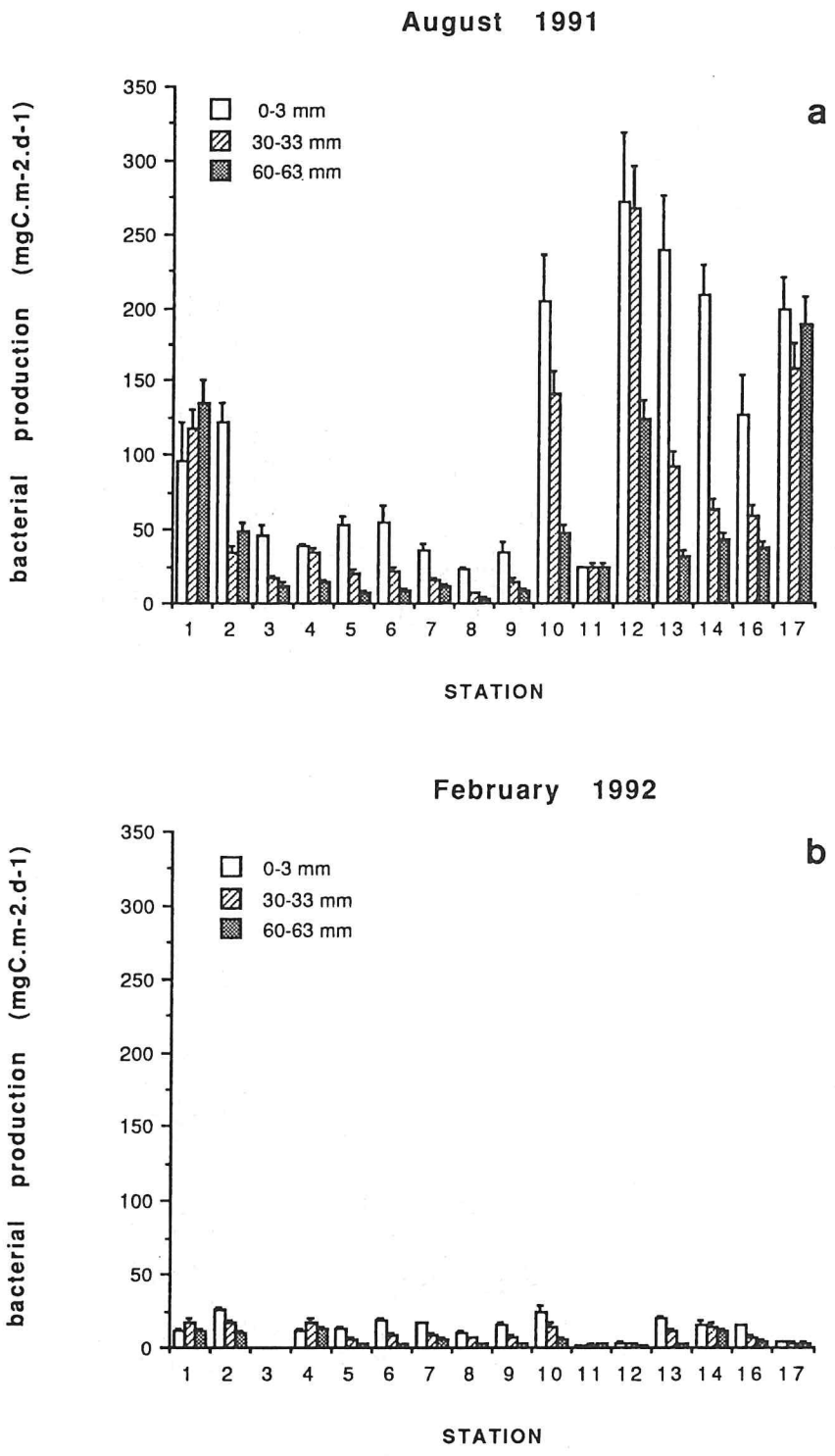

Fig. 2. Depth profiles of benthic bacterial production for the stations 1-17, a. August 1991 b. February 1992

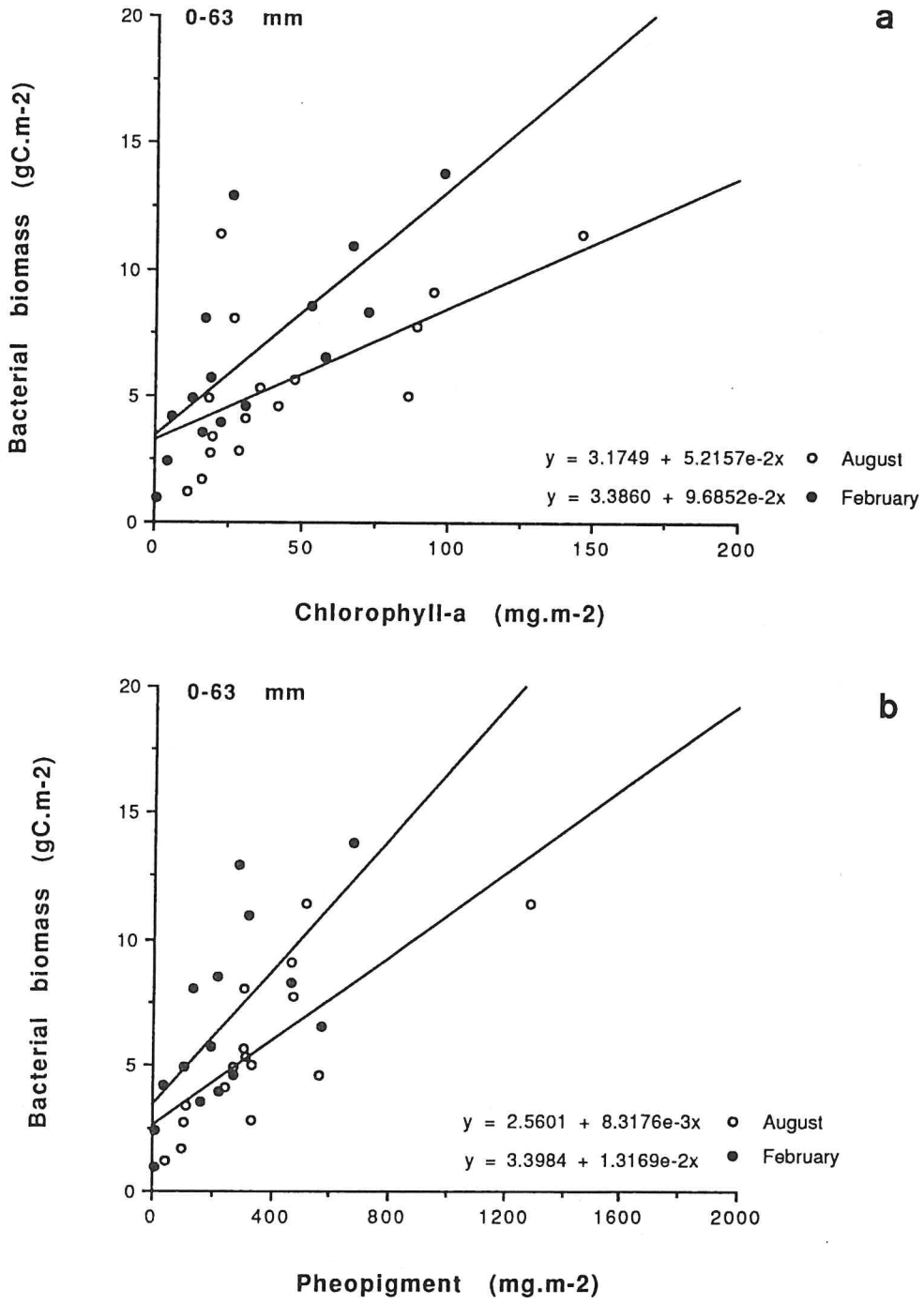

Fig. 3. Significant relations between benthic bacterial biomass and a. sediment chlorophyll a in August and February (Spearman correlation r_s=0.68, r_s=0.80 n=16/15 p<0.01) and b. sediment pheopigment content in August and February (r_s=0.75, r_s=0.74 n=16/15 p<0.01). Data represent results of the depth integration of 0 to 63 mm

Heterotrophic nanoflagellate abundance in North Sea sediments and their role in benthic small food web dynamics

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Introduction

Protozoa, especially heterotrophic nanoflagellates (diameter 2 to 20 µm), are recognized as major consumers of bacteria in pelagic ecosystems (Fenchel 1982; Azam et al. 1983).

The role that nanoflagellates play in nutrient and energy fluxes in benthic ecosystems is largely unknown. It has been shown that nanoflagellates occur in high and fluctuating densities in marine sediments and a correlation between flagellate densities and bacterial production has been suggested (Bak & Nieuwland 1989; van Duyl & Kop 1990). It seems reasonable to assume that benthic flagellates are as important as consumers of bacterial production as their pelagic counterpart. The impact of flagellate bacterivory on bacterial production is a function of flagellate density and grazing rate.

The aim of our research was therefore: (1) to study summer-winter variation in benthic nanoflagellate densities in North Sea sediments and to determine which physical, chemical and biological factors can influence distribution and abundance, (2) to measure short term flagellate grazing through the ingestion rate of monodispersed, fluorescently labeled bacteria (FLB; Sherr et al. 1987; Hondeveld et al. 1992).

Material and Methods

The BELS (BEnthic Links and Sinks in North Sea nutrient cycling) expeditions were carried out in August 1991 and February 1992. These periods of investigation were selected to study two clearly distinguishable situations. The experimental program was performed in the southeastern and central parts of the North Sea. The stations covered a wide range of sediment types with respect to grain size, organic matter content, benthic biological activity and mineralization.

Sediment cores were sampled at 15 stations for densities of heterotrophic nanoflagellates. Each core was subsampled at three depths: 0-3, 30-33 and 60-63 mm. In 5 replicate cores flagellates were counted and measured using epifluorescence microscopy after staining with proflavine (procedure: Bak &

Nieuwland 1989, Bak et al. 1991). These data on flagellate densities were related to bacterial production, abundance, biomass and specific growth rate, grain size, carbon and nitrogen content, chlorophyll a, phaeopigment, nematode abundance and temperature.

Experiments to establish grazing rates of benthic flagellates on bacteria were performed at each station. Fluorescently labeled bacteria (FLB) were added to sediment samples at densities that would give a final FLB concentration of 25% of total bacterial abundance. After mixing, samples were incubated for half an hour in the dark at *in situ* temperature. After incubation the flagellates were extracted from the sediment, stained with primulin and collected on 1.0 µm pore size Nuclepore filters. Slides were scanned with the epifluorescence microscope (1250x) to locate nanoflagellates. Subsequently these flagellates were examined to score numbers of flagellates containing FLB and to enumerate ingested FLB per flagellate. We calculated average FLB ingestion rates by two methods 1) A calculation based on total number of FLB ingested divided by total number of flagellates examined, possibly giving minimum values. 2) A calculation based on total number of FLB ingested divided by total number of flagellates containing FLB, possibly representing maximum estimates (Hondeveld et al. 1992).

Results and discussion

Benthic flagellate densities in summer ranged from 7 to 859 x 10^3 and in winter from 9 to 190 x 10^3 cells·cm^{-3} (fig. 1 a,b). These values are comparable to values reported previously for marine habitats (Alongi 1986, 1990; Bak & Nieuwland 1989; Bak et al. 1991; Epstein & Shiaris 1992; Hondeveld et al. 1992). The effect of season on densities was different between stations, although at 10 out of 15 stations summer values were significantly higher than winter values. The differences between summer and winter densities were larger in sandy sediments than in muddy sediments. Flagellate densities in the surface layer were 2 to 4 times higher than in the two deeper layers. The effect of season on flagellate densities was the same at all three depths. Remarkable were the enormous amounts of flagellates near Esbjerg (Denmark) in the surface layer: in summer 859 x 10^3 cells·cm^{-3} and in the two deeper layers during winter 1100 x 10^3 cells·cm^{-3}.

Grouping winter and summer data, flagellate densities in the sediment surface layer correlated positively with bacterial production and bacterial specific growth rate. This

explained 20% and 30%, respectively, of the variance. Pelagic studies suggest that protozoa are important in maintaining the bacterial assemblage in a state of 'physiological youth' (Fenchel & Harrison 1976; Sherr et al. 1982). Bacterial specific growth rate only depends on the physiological state of bacteria and the positive correlation obtained in our study supports the above mentioned hypothesis. There appeared to be a difference in factors correlating with flagellate abundance between summer and winter. In summer a positive correlation existed with bacterial specific growth rate and with grain size, together explaining 53% of the variance. In winter there were significant correlations with bacterial biomass and abundance accounting for 59% and 33%, respectively, of the variance. It is postulated that bacterial biomass and abundance determined minimum values to flagellate densities during winter. Increase in bacterial production was probably responsible for generally higher summer densities although grain size could become a limiting factor in silty sediments during summer.

The grazing experiments showed grazing rates, bacteria per flagellate per hour, to be the same in winter and summer. Using calculation method 1 grazing rates ranged from 9 to 44 bacteria $flag^{-1}$ $hour^{-1}$. Minimum values (calculation method 2) varied between 1 and 5 bacteria $flag^{-1}$ $hour^{-1}$. The percentages of flagellates consuming FLB were higher in sandy sediments than in silty sediments and were lower in winter than in summer.

When grazing rates and flagellate densities were related to the observed rates of benthic bacterial production, it appeared that the percentage bacterial production consumed varied between 0.2 and 23.5% in summer and between 1.0 and 415% in winter. The impact of flagellate bacterivory on benthic bacterial production is higher in winter than in summer probably due to the large decrease of bacterial production rates from summer to winter.

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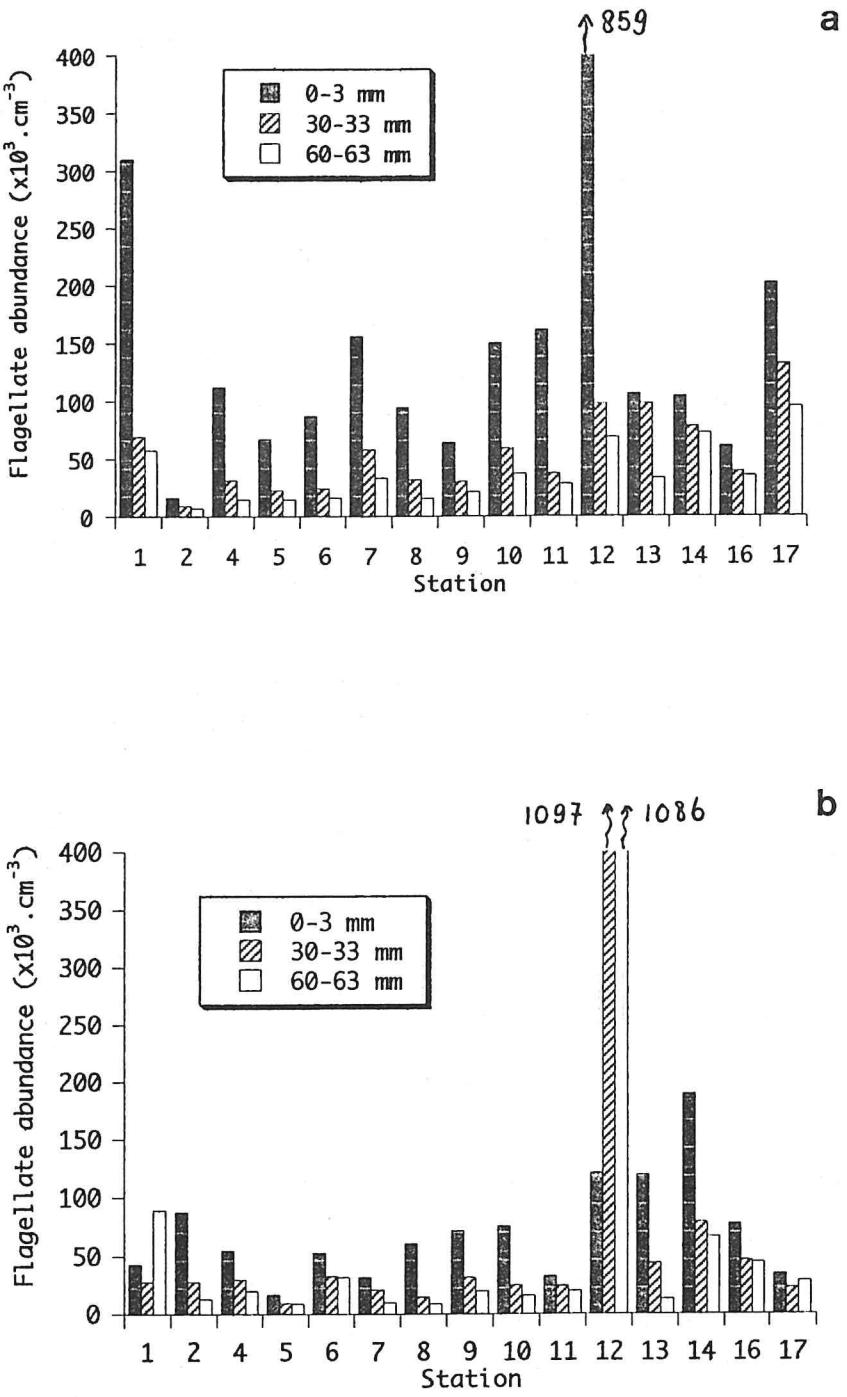

Figure 1: Heterotrophic nanoflagellate densities at 15 stations in the North Sea at 3 depths in the sediment in (a) August 1991 and (b) February 1992

BENTHIC FORAMINIFERA IN *BELS* SEDIMENT

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Six stations were sampled for foraminiferal studies during the first BELS expedition (August 1991). Sediment samples were analysed 16 months after collection. Unfortunately, all samples showed signs of dissolution which was probably due to syndepostional dissolution, as earlier reported for some parts of the southern North Sea, and/or a result of insufficient preservative added to the samples. Stations 4, 5, and 7 were most effected by dissolution. However, certain trends are evident but have to be interpreted with caution. This report is based on the analysis of foraminifera (> 63 μm) that were wet picked from a known split.

The upper 2 cm of the sediment is generally utilized for foraminiferal studies with the emphasizes on foraminifera that have a solid test (either calcareous of arenaceous). Maximum number of living (Rose bengal stained) foraminifera were found at station 10 (Fig. 1). Station 13 that also has a relatively higher percent organic carbon content (Fig. 7) has relatively much lower numbers (Fig. 1). This is probably not only a seasonal effect for the production of foraminiferal tests over a longer period of time (*i.e.*, the number of dead tests) is also much lower at station 13 when compared to station 10 (Fig. 2). Foraminiferal densities are generally controlled by a combination of two factors: the amount of food available and the effects of biological interaction (e.g., predation). At this stage, its interesting to note that the maximum number of macrozoobenthos is found at station 13 (6775 per m^2); station 10 supported < 2000 specimens of macrozoobenthos per m^2 (van der Veer *et al.*, this report). Station 10 which is the deepest station (62 m) also supports the largest number of foraminiferal species and probably reflects more stable conditions when compared to station 13. At station 13, the shallowest station (19 m), only one species was encountered living.

Densities of soft-shelled foraminifera, including species that have an agglutinated test that easily disintegrates upon death or when sediment samples are dried, sometime exceed those of calcareous species, e.g. station 4 and 5 (Fig. 3). This picture may be partly due to the dissolution of calcareous species.

Living foraminifera were encountered down to at least 10 cm in the sediment. The density pattern over a depth of 10 cm (Fig. 4) resembles that of the surface layers (Fig. 1). This is probably due to the fact that, in these samples the maximum numbers occured in the 0-2 cm inteval (Figs. 5 and 6). In spite of the limited penetration of oxygen in the sediment (0-5 mm, with the exception of station 17, > 15 mm; Lohse *et al.*, this report) benthic activity extends down to at least 10 cm. Station 17 that has the lowest number of foraminifera, both living and dead (Figs. 1, 2, and 4), is probably carbon limited.

Fig. 1. Number (#/10 cm^2) of live "hard-shelled" foraminifera in the upper 2 cm of the sediment at the different stations. The numbers in boxes above each bar = number of species.

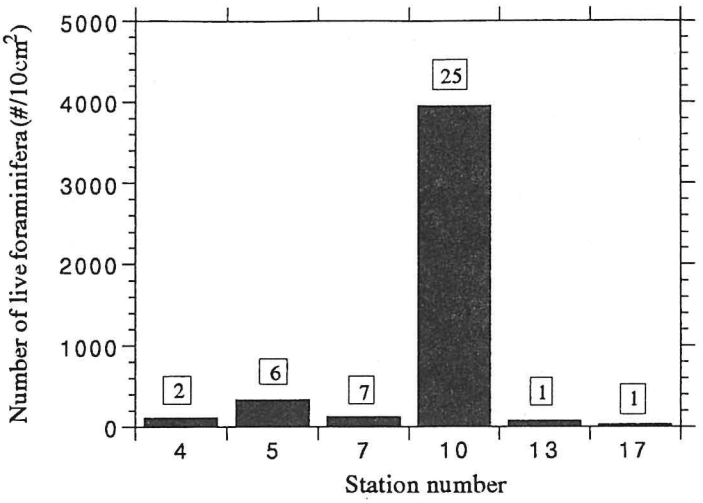


Fig. 2. Number (#/10 cm^2) of dead foraminifera in the upper 2 cm of the sediment at the different stations.

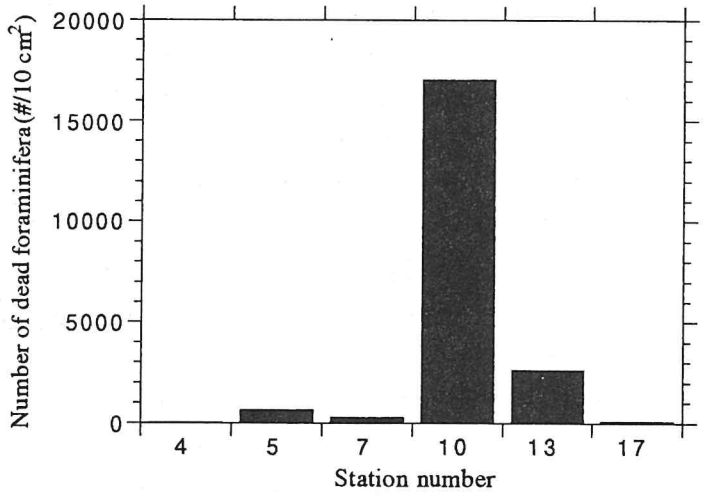


Fig. 3. Number (#/10 cm^2) of live "soft-shelled" foraminifera in the upper 2 cm of the sediment at the different stations.

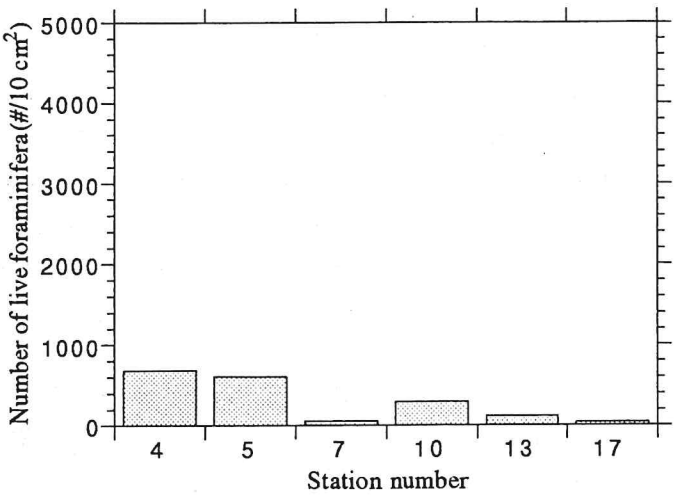


Fig. 4. Total number (#/10 cm^2) of live foraminifera over a depth of 10 cm in the sediment. Black bars and dotted bars represent respectively, the number of "hard-shelled" and "soft-shelled" foraminifera .

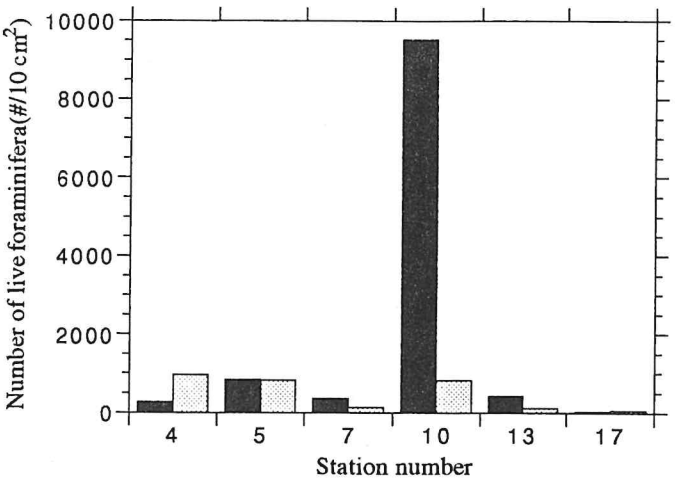


Fig. 5. Total number (#/10 cm^2) of live foraminifera at different depth intervals in the sediment at stations 4, 5, 7, 13 and 17.

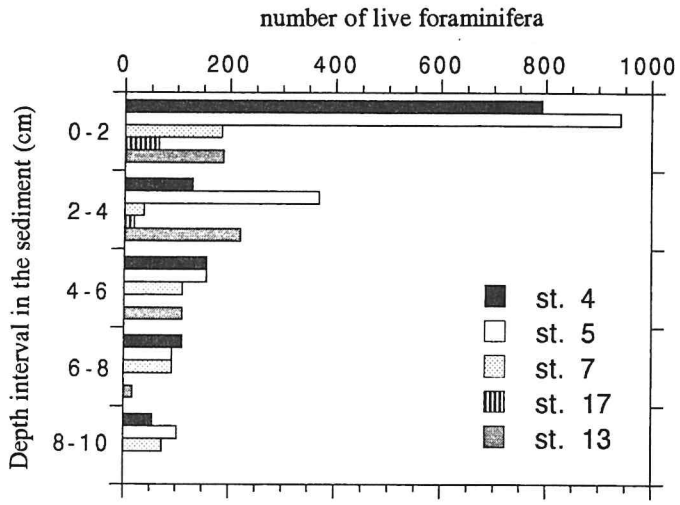
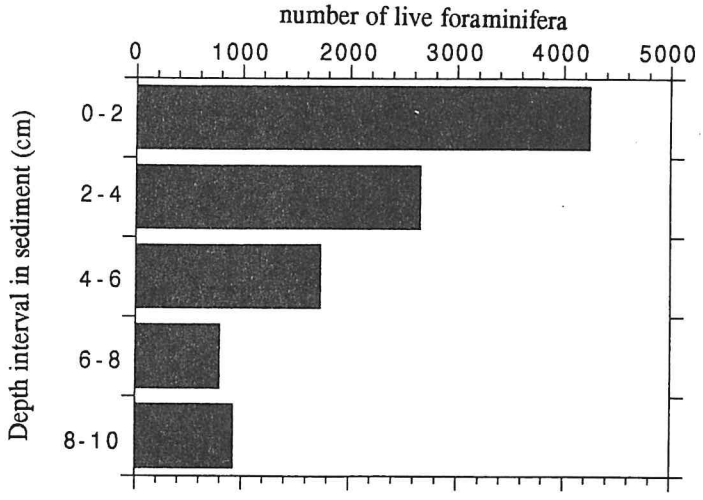


Fig. 6. Total number (#/10 cm^2) of live foraminifera at different depth intervals in the sediment at station number 10.



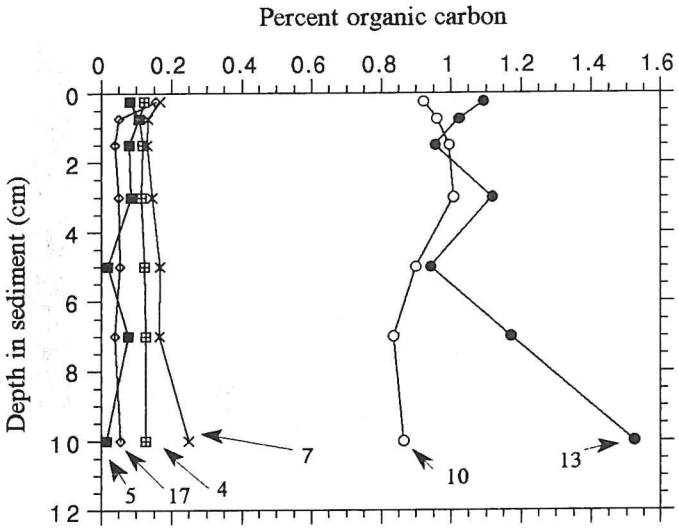


Fig. 7. Percent organic carbon at different depths in the sediment for August 1991 (Courtesy of J.F.P. Malschaert). Number at the end of arrows indicate station number.

Benthic Links and Sinks in North Sea Nutrient Cycling: The Meiofauna

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Abstract

The meiofauna of sixteen stations of the BELS project was sampled during two cruises in August 1991 and February 1992 (only 15 stations) with the aim of studying the correlation between meiofauna abundance and benthic processes. The sixteen stations represented a wide range of sedimentary conditions.

Meiofauna numbers varied between 100 and 6000 individuals per 10 cm^2 and the meiofauna was dominated by nematodes. There was no clear correlation between nematode abundance and grain size in general, but the lowest values were found in coarsest sediments. Total benthic oxygen consumption decreased with grain size and was not correlated with nematode abundance, indicating that nematodes do not contribute significantly to benthic metabolism directly, at least not in Summer. In winter the correlation coefficient was higher.

Copepod numbers varied between 17 and 390 ind.per 10 cm^2 and tended to be higher in coarser sediments. No correlation whatsoever between copepod abundance and benthic processes was found.

Despite the low correlation between meiofauna abundance and rates of benthic processes, the coherence of the meiofauna is larger than that of any other variable measured, with a high correlation between nematode and copepod abundance in Winter and in Summer. This shows that meiofauna abundance is regulated by station-dependent characteristics.

The role of macrozoobenthos in the benthic system

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1. INTRODUCTION

The macrozoobenthic community in the North Sea plays an important role, as well as food source for the demersal predators, and as a factor influencing all kinds of processes in the benthic system. The sampling grid of the two BELS expeditions was designed in such a way that transport and degradation of organic matter could be followed more or less from the southern part of the North Sea towards the sedimentation areas in the Skagerrak area. The macrozoobenthic community was studied, because they were thought to represent a key factor within the benthic system. In this contribution, the macrozoobenthic characteristics of the various stations are presented together with data of selected groups of demersal predators, and analysis of interactions with other processes or functional groups within the benthic system.

2. MATERIAL AND METHODS

Macrozoobenthos. At each station 1 to 4 intact box cores (diameter 31 cm) were taken and incubated for oxygen consumption measurements during 12 to 24 h. After finishing the measurements, the cores were sieved over 1 mm mesh size and the remaining material and animals were stored on a 4% formaline-seawater solution and sorted out in the laboratory within 6 mo. All animals collected were analysed to species level and of each species found the number of animals per core was determined. For each station, per species and for all species in total, the arithmetic mean number of animals per m^2 was estimated.

Demersal fish fauna. Normally 3 hauls were made at each station at the end of the benthic sampling programme. Fishing was done with a 5.5-m beam-trawl with 2 tickler chains and 25 mm mesh size in the tail-end of the net. The duration was on average 15 min, and in this way tracks of between 1000 and 2700 m were fished. The exact distance was determined by Decca position system. After sorting of the sample, all flatfishes were measured into 0.5 cm size classes and for all species numbers caught were converted into densities per 10^4 m^2. for each station the arithmetic mean density was estimated.

3. RESULTS

Macrozoobenthos. The mean numbers per station in August 1991 varied considerably (Fig.1) between about 250 individuals per m^2 at station 3 and 11 and 6775

per m^2 at station 13 in the German Bight. Due to the relative stability in macrozoobenthos, the same pattern was assumed for the February 1992 cruise.

Demersal fish fauna. The distribution of flatfish showed about a similar pattern in August 1991 and February 1992 (Fig.2). Highest densities were observed in the German Bight (station 12, 13 and 14), while low numbers were found in or near the Skagerrak area (station 8, 9, 10 and 11). The differences between August 1991 and February 1992 are due partly to sampling variability and furthermore to some seasonal migration movements.

Interactions. interactions can only be studied for the August 1991 cruise. Flatfish density showed a positive relationship with macrozoobenthos biomass (Fig.3). Also bacterial production was positively related to macrozoobenthos density (Fig.4). Even the $PO_4^-$and the total-N flux out of the sediment suggested some positive relationship with the density of the macrozoobenthic community (Fig.5).

4. DISCUSSION

The sampling grid was designed in such a way that transport and degradation of organic matter could be followed more or less from the southern part of the North Sea towards the sedimentation areas in the Skagerrak area. Due to tidal action, wind stress and other local hydrographic factors, sedimentation and resuspension at the various sampling stations will be a rather variable and fluctuating phenomena. In our opinion, in the benthic system, the macro-zoobenthic community is most stable and it forms the best indication of the overall sedimentation processes and organic carbon supply of the bottom.

The central position of the macrozoobenthic community is further illustrated by its relationship with various characteristic bottom parameters. The relationship with the bacterial production and the nutrient fluxes out of the sediment at least suggests some interactions that seems worth a further analysis. On the other hand, the top predators, by means of the flatfish density, also was directly related to the macrozoobenthic richness. This latter relationship supports results obtained in coastal areas such as the Wadden Sea

In conclusion, the statement that the macrozoobenthic community plays an essential role in the benthic system appears to be verified by the BELS expedition.

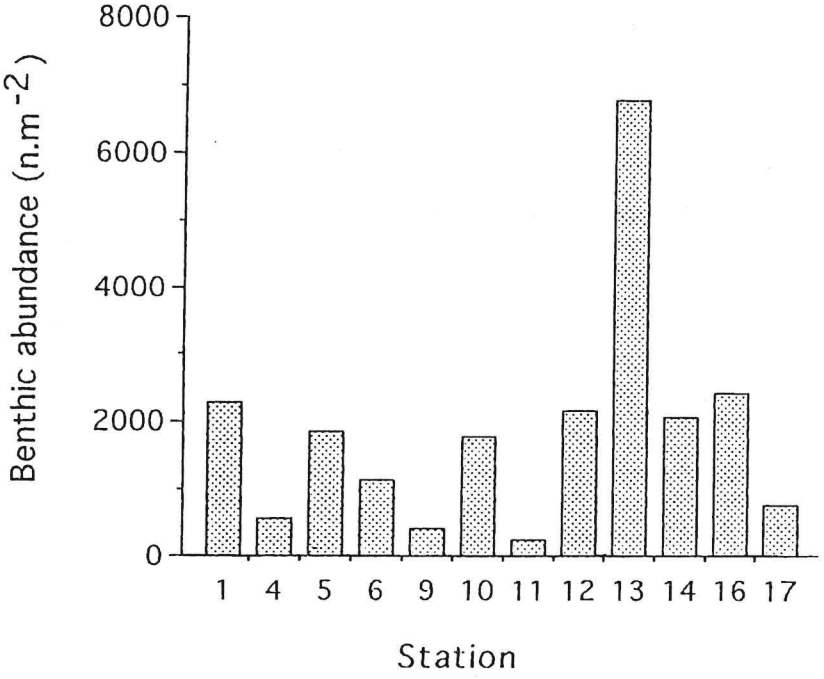


Figure 1. Mean number (n.m^{-2}) of macrozoobenthos at the various sampling stations during the BELS expedition in August 1991

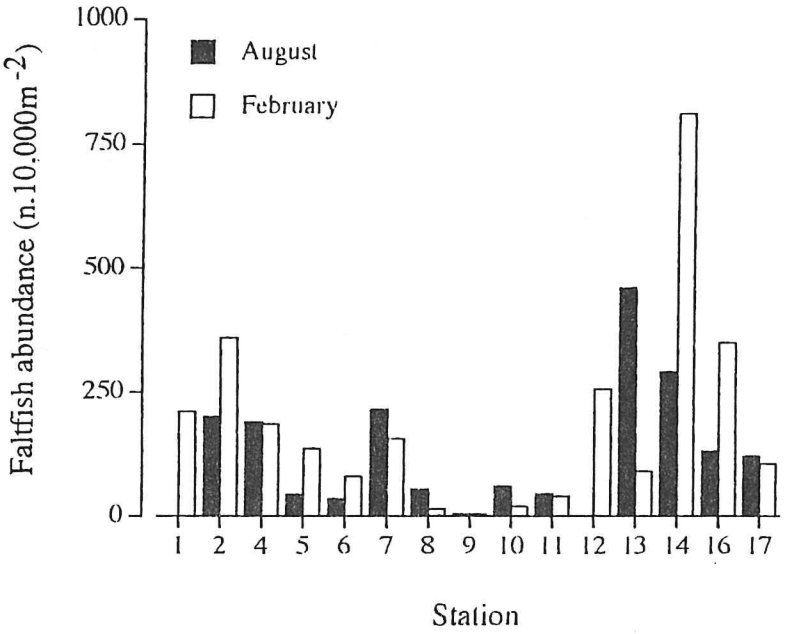


Figure 2. Mean number (n.10000m^{-2}) of flatfish at the various sampling stations during the BELS expedition in August 1991 and February 1992

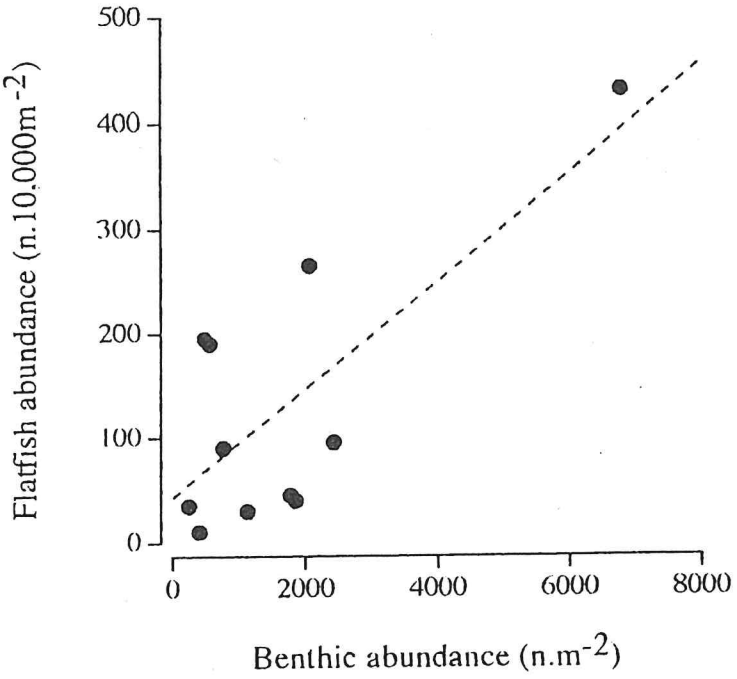


Figure 3. Relationship between density of macrozoobenthos ($n.m^{-2}$) and abundance of flatfish ($n.10000m^{-2}$) during the BELS expedition in August 1991

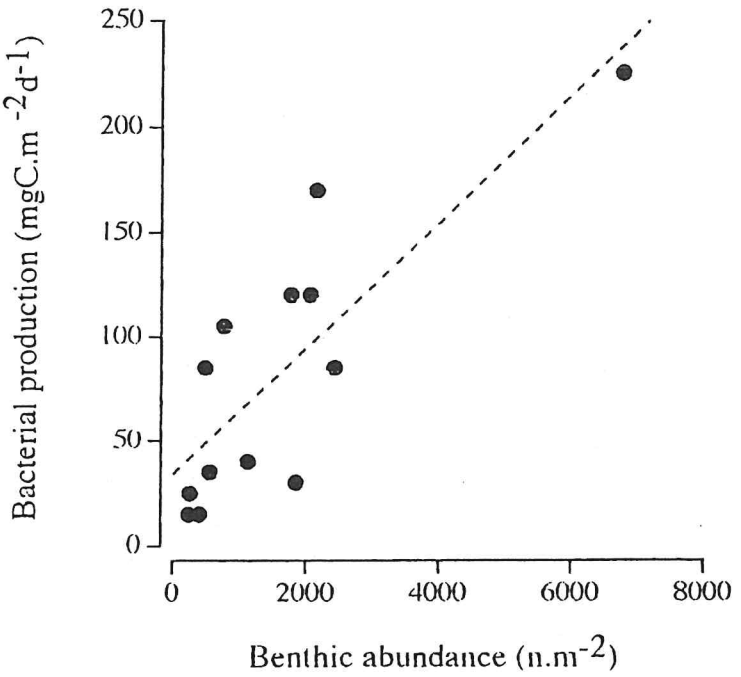
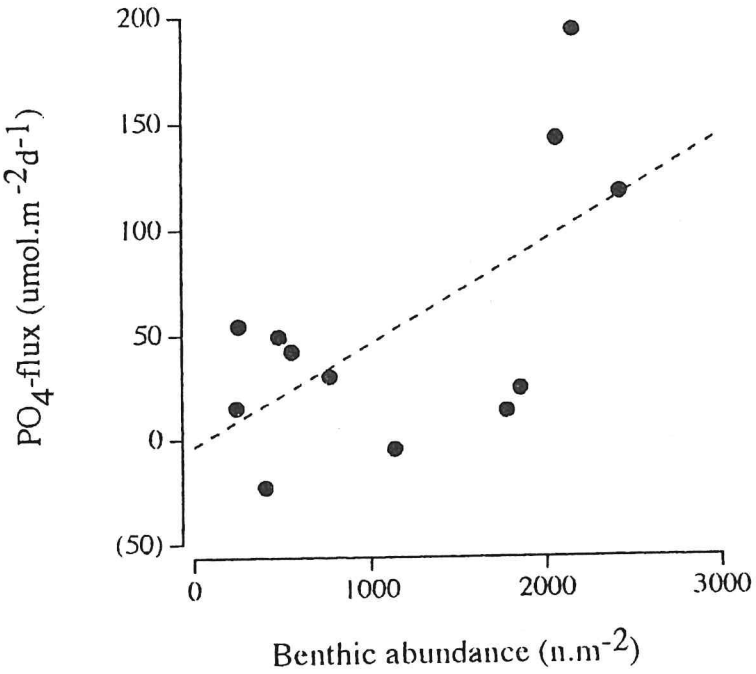


Figure 4. Relationship between density of macrozoobenthos ($n.m^{-2}$) and bacterial production ($mgC.m^{-2}.d^{-1}$) during the BELS expedition in August 1991



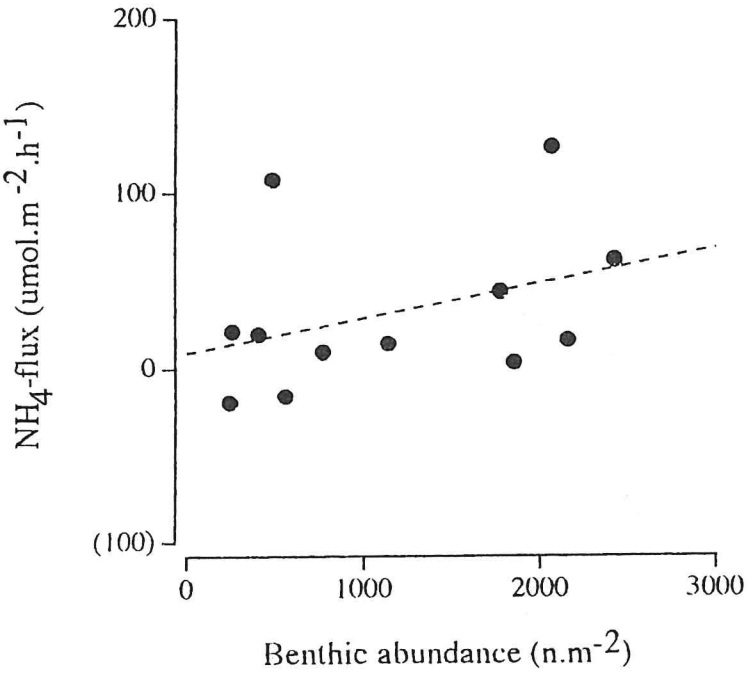


Figure 5. Relationship between density of macrozoobenthos (n.m^{-2}) and flux of nutrients during the BELS expedition in August 1991

APPENDIX B

INP-MICON PROGRAMME 1991-1992: CONCENTRATIONS OF PAHs AND PCBs IN TOTAL SEDIMENT.

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Abstract: Samples of total sediment (<2mm) collected from 30 stations in the southern North Sea in 1991 and 1992, were analyzed for selected polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Capillary gas chromatography/mass-spectrometry (GC/MS) and gas chromatography with electron capture detection (GC-ECD) were used in the analysis. The aim of the project was to study the spatial distribution of PAHs and PCBs in coastal and offshore areas and provide support for the measurements on biomarkers of biological effects.

Especially the dry-weight based concentrations of PAHs reflected the sediment characteristics with low levels in coarse sandy sediments and higher levels in silty sediments with higher contents of organic material (fig.1). The highest concentrations of PAHs were found at an offshore station (st. 8).

The concentrations of PCBs were highest near the mouth of the Western Scheldt (st. 17).

Based on total organic carbon, coarse coastal sediments contained higher levels of especially PCBs than similar sediments from offshore areas.

The pattern of PAHs at stations 4, 8 and 17 is given in fig. 2.

The INP-MICON programme was supported by the Netherlands Marine Research Foundation (SOZ) of the Netherlands Organization for Scientific Research (NWO).

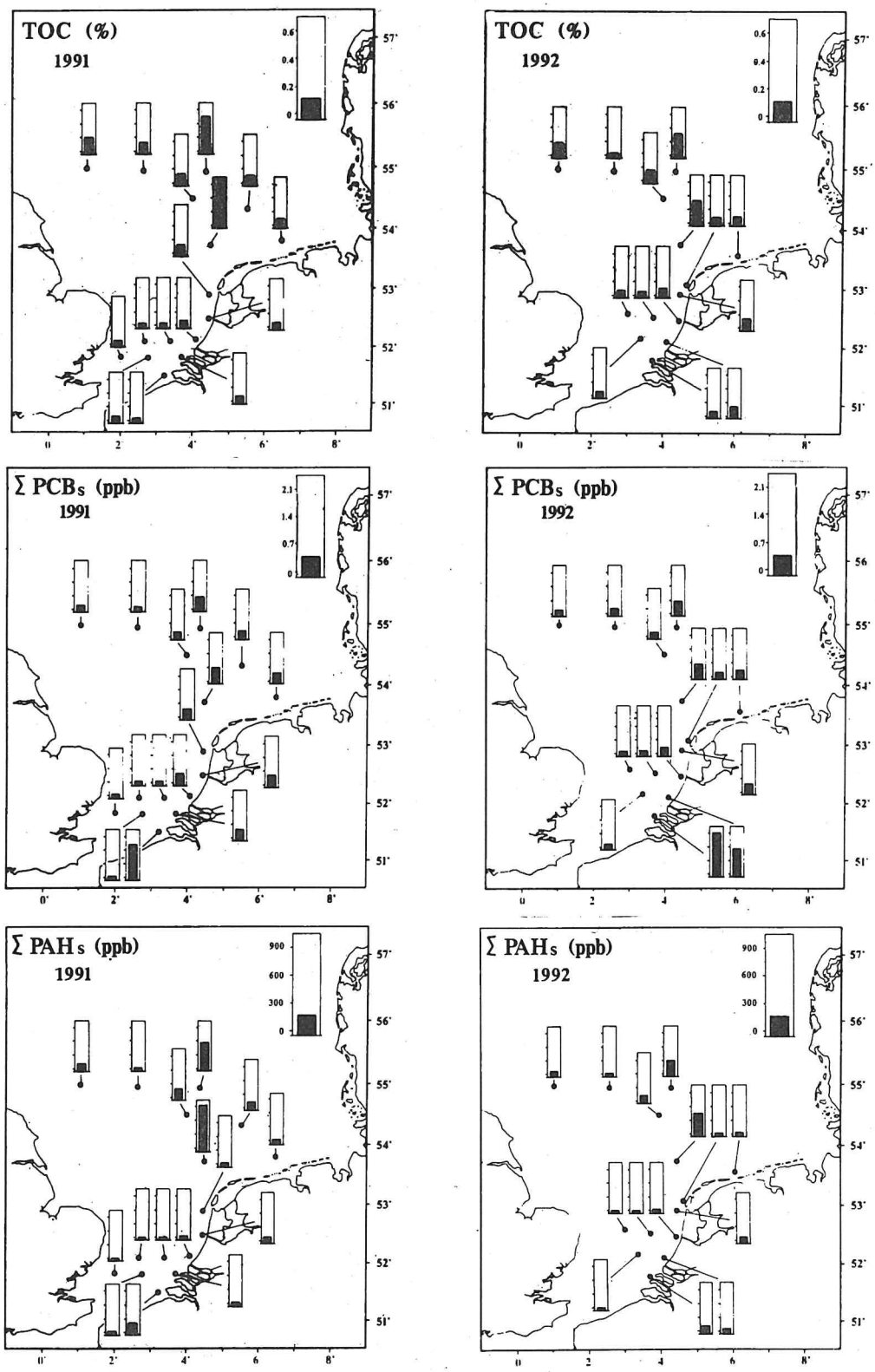

Figure 1. TOC (%), sum of aromatic hydrocarbons and PCBs in sediments. Concentrations are given in ng g^{-1} dry weight

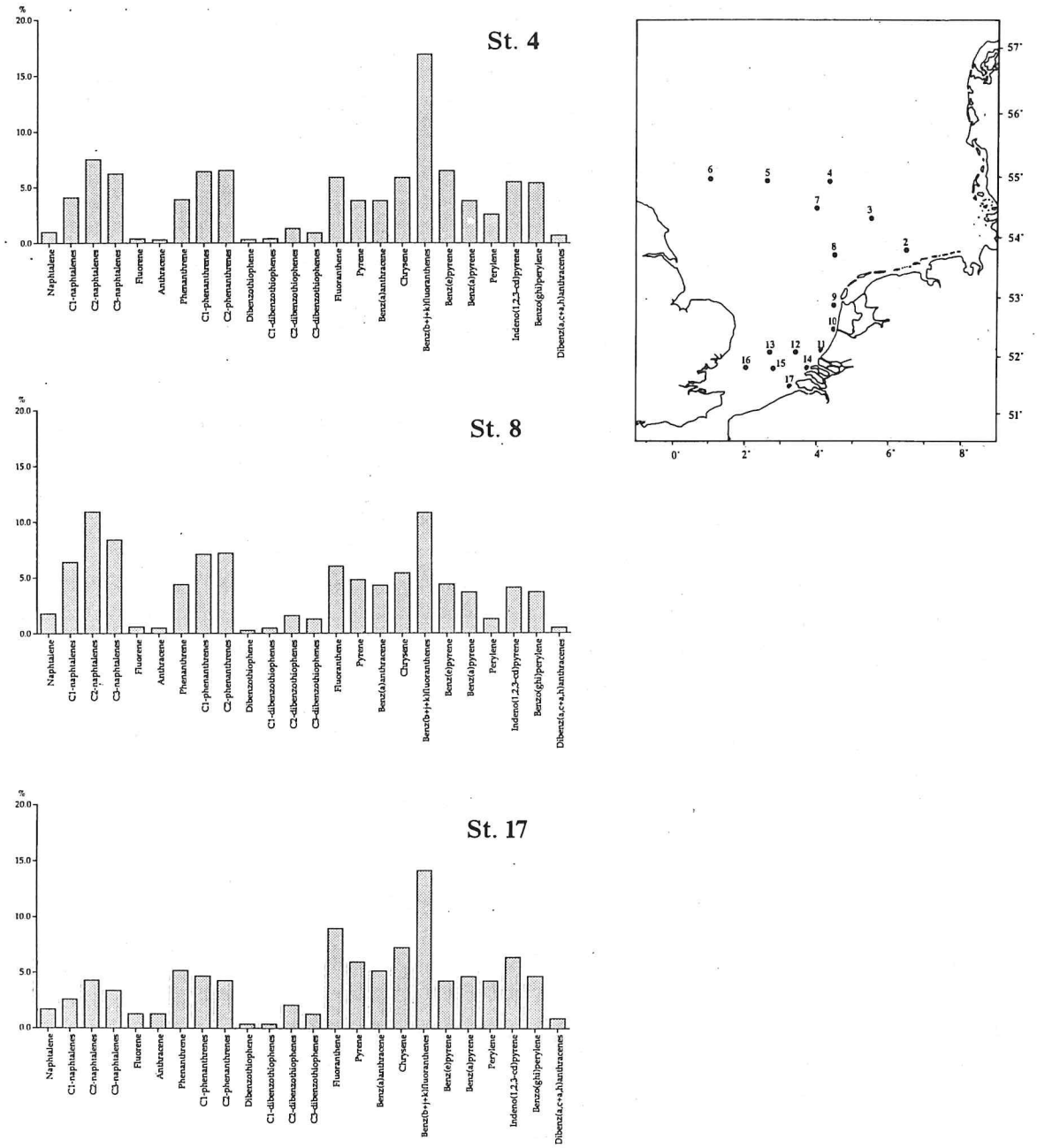

Figure 2. Relative abundance (%) 1991 of aromatic hydrocarbons at St. 4, 8 and 17 (see map)

SPATIAL AND TEMPORAL TRENDS OF EROD-ACTIVITY IN PLAICE (*PLEURONECTES PLATESSA*) AND FLOUNDER (*PLATICHTHYS FLESUS*).

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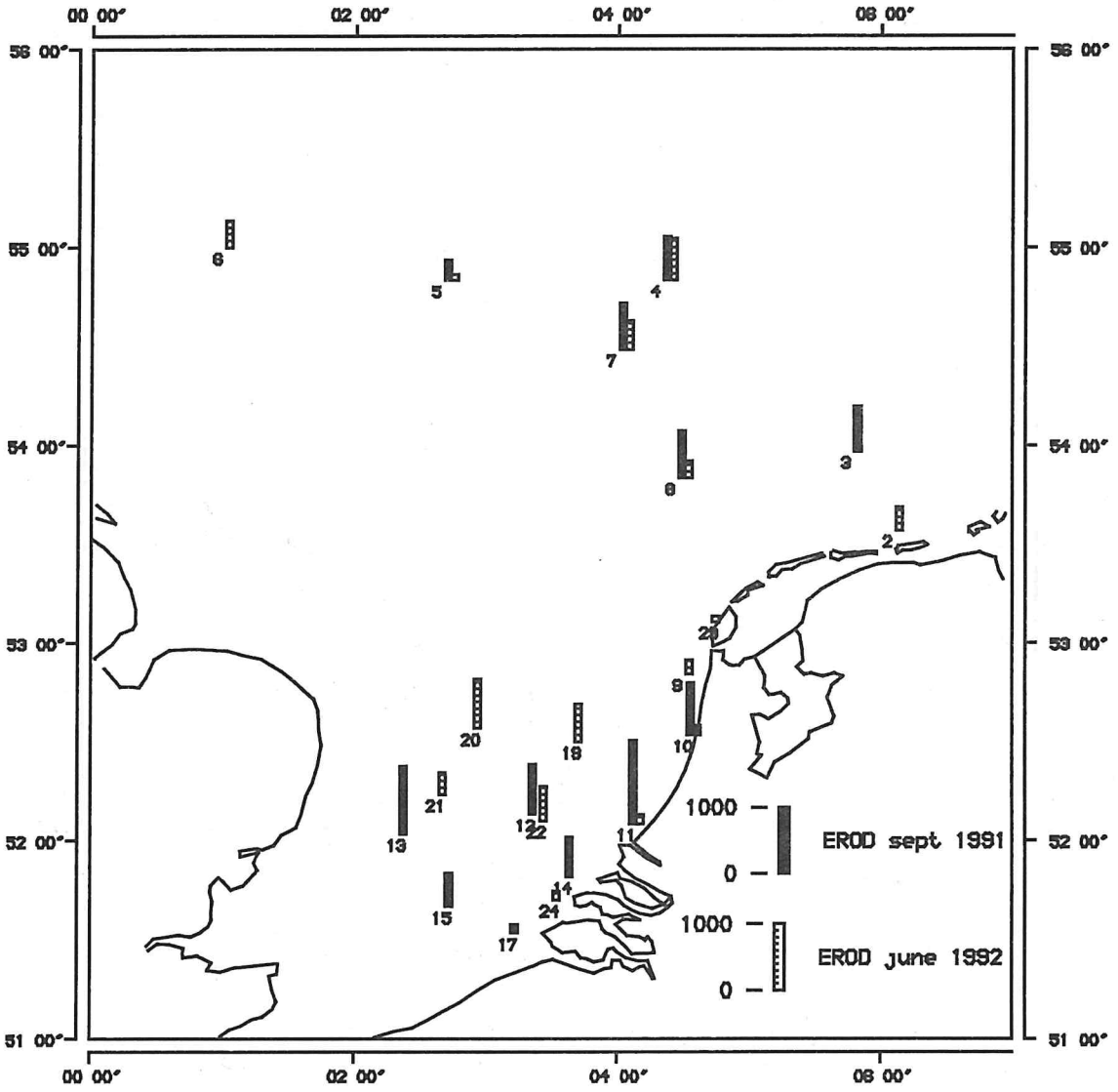
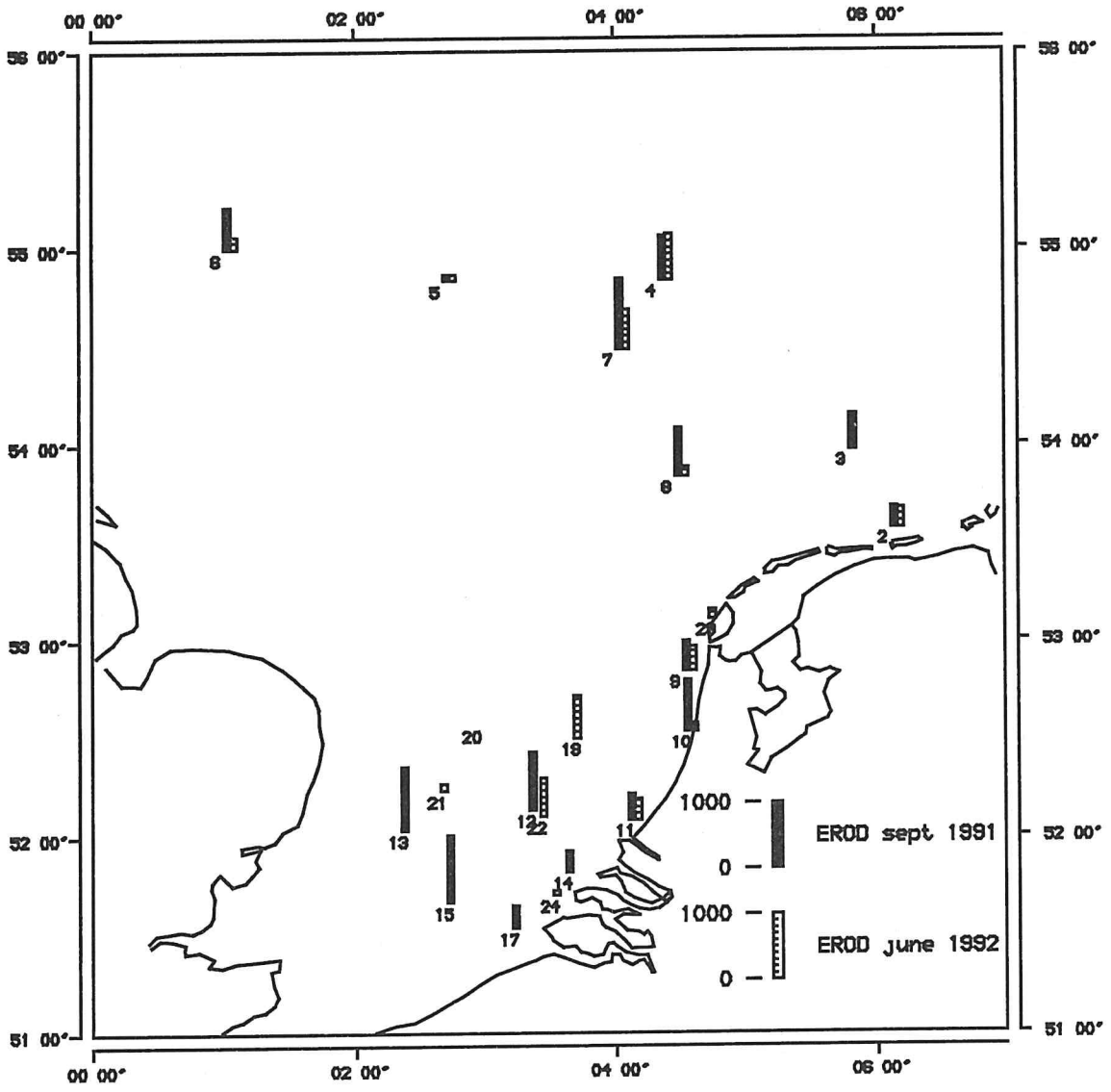
The objective of the study was to investigate the spatial and temporal trends of hepatic 7-ethoxyresorufin O-deethylation (EROD) in plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*)in the southern North Sea.
EROD is a model reaction of the cytochrome P-450IA (P450IA) dependent monooxygenase (MO) system. P450IA can be induced by compounds with dioxin like toxicity, i.e. polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins (PCDD) and polycyclic aromatic hydrocarbons (PAH).
Sampling of fish livers was concucted at 22 stations during two cruises (september 1991 and june 1992) of the INP programme (Integrated North Sea Programme).
EROD activities in plaice were generally lower at the coastal stations than at most off-shore stations. This trend was consequent for both cruises. No consistent correlations could be detected of hepatic EROD in plaice with OH-pyrene concentrations in bile nor with depth, salinity and temperature.
Flounder could only be collected at the coastal stations. A general correspondence of EROD in this species with the OH-pyrene concentration in bile was found.
The findings are discussed with special reference to the use of EROD activity in these species as a biomarker in environmental effect monitoring studies.

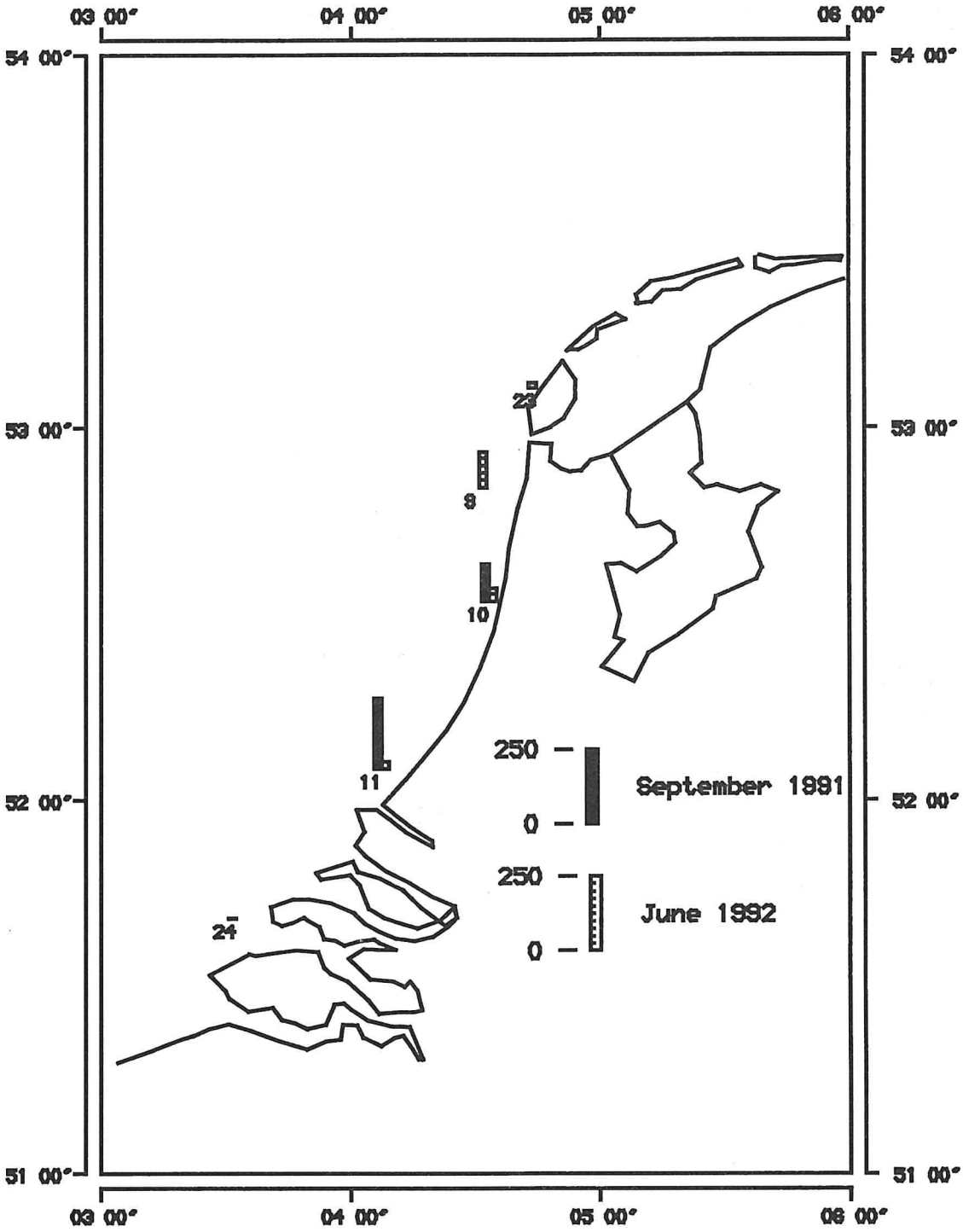
EROD activity in male plaice (Pleuronectes platessa)

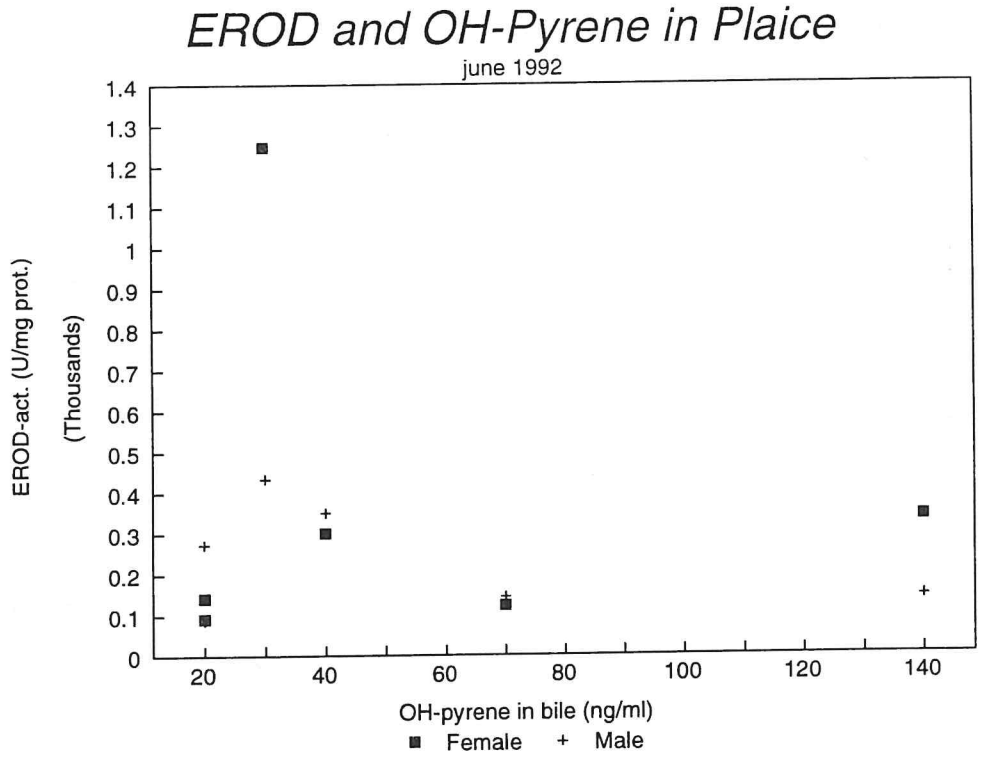


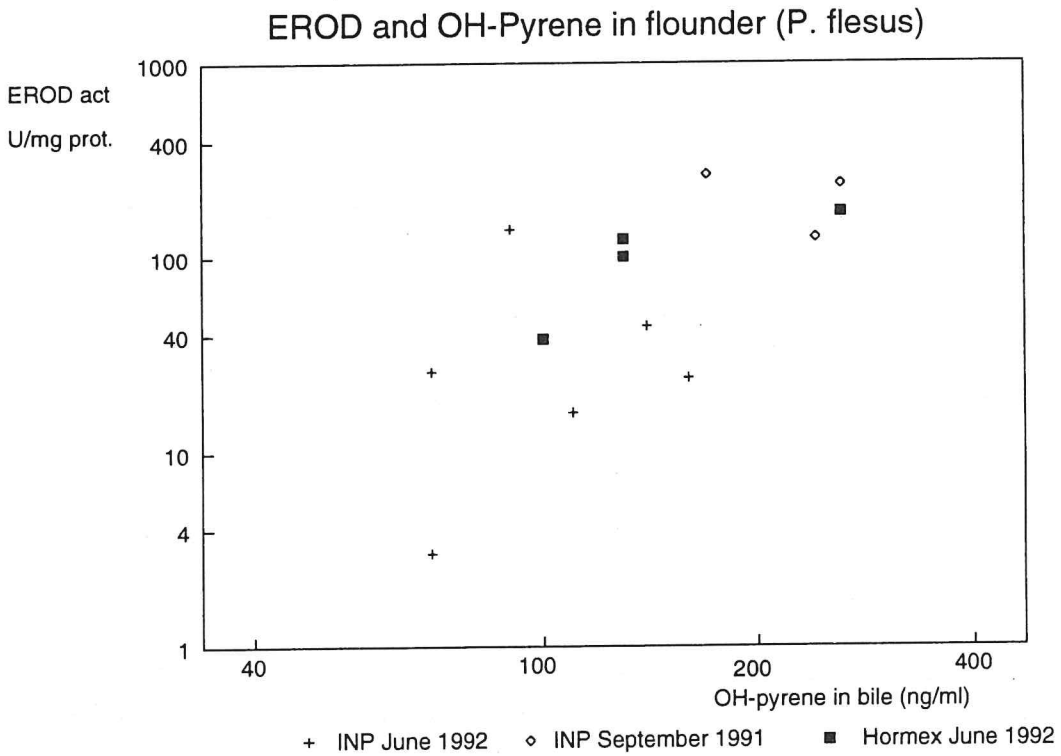
EROD activity in female plaice (Pleuronectes platessa)



EROD activity in European flounder (Platichthys flesus)




EROD and OH-Pyrene in Plaice
june 1992
EROD-act. (U/mg prot.)
(Thousands)
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
120
140
OH-pyrene in bile (ng/ml)
Female
Male


EROD and OH-Pyrene in flounder (P. flesus)
EROD act
U/mg prot.
1000
400
100
40
10
4
1
40
100
200
400
OH-pyrene in bile (ng/ml)
INP June 1992
INP September 1991
Hormex June 1992

DNA strand-breaks, cytochrome P450 dependent monooxygenase enzyme activity and levels of chlorinated biphenyl congeners in the pyloric caeca of the seastar (*Asterias rubens*) from the North Sea.

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Seastars (*Asterias rubens*) were collected at sampling locations in different areas along transects radiating into the southern North Sea, representing areas impacted by contaminants to different degrees. Strand breakage in DNA isolated from tissue of the pyloric caeca was measured by the alkaline unwinding assay, modified to allow for the isolation of intact highly polymerized DNA and the subsequent strand-breaks. Interpretation of results is based on the incidence of DNA strand-breaks (expressed as the [double:total] DNA ration or *F*-value, indicating the percentage of double-strandedness). Cytochrome P450 concentration and benzo[a]pyrene (BaP) hydroxylase activity were measured in microsomal fractions of the pyloric caeca. The chlorinated biphenyl (CB) congeners were determined by temperature-programmed, gas-chromatography, with a CPSil8 capillary column as stationary phase, hydrogen as carrier gas and ^{63}Ni-electron capture detection.

Areas where seastars showed different DNA integrity could be described (Figure 1); Highest integrity ($0.75 < F < 0.85$) was found in off-shore reference sites of the Dogger Bank and Southern Bight. The average percentage double-stranded DNA in seastars from most sampling location varied between 55 and 75 %. Lowest DNA integrity ($0.35 < F < 0.55$) was found in specimens obtained from sampling locations near the river Rhine delta, along the Dutch coast and at two expected uncontaminated off-shore areas.

BaP hydroxylase activity was relatively high near the mouth of the rivers Rhine and Scheldt, but also at a supposedly clean site near the Doggersbank (Figure 2).

The concentration of CB-congeners in the pyloric caeca of seastars decreased along transects radiating into the southern North Sea from the coastal areas of the Netherlands; highest concentrations in the nearby coastal areas and lowest in the open sea sampling locations (Figure 3a and 3b).

The data indicate that there might be a relationship between pollution from the rivers Rhine, Meuse and Scheldt and the incidence of DNA strand-breaks and/or BaP hydroxylase activity. Selected CB-congeners served as markers for increased exposure to riverine influence in coastal waters.

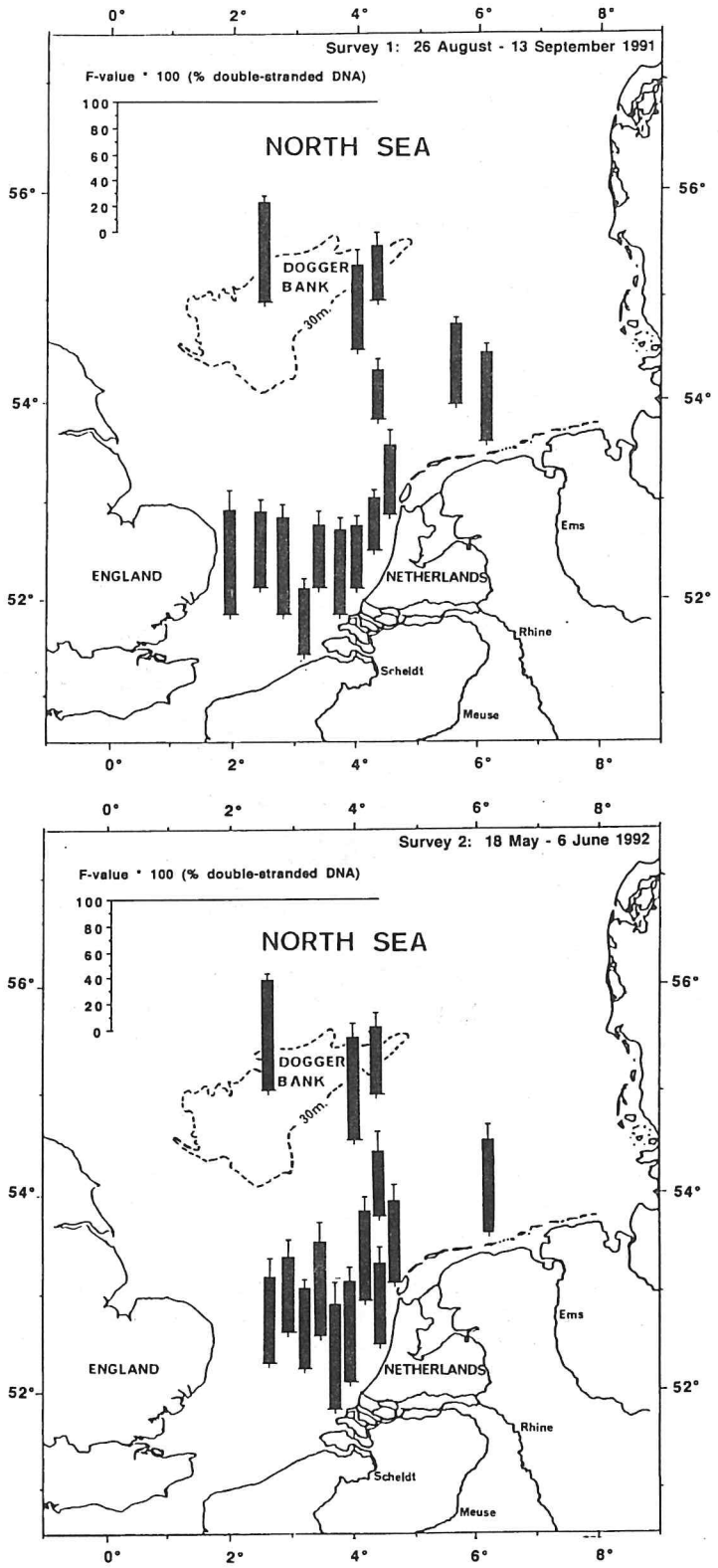

Fig. 1. DNA integrity, expressed as fraction of double-stranded DNA, in the pyloric caeca of the seastar (*Asterias rubens* L).

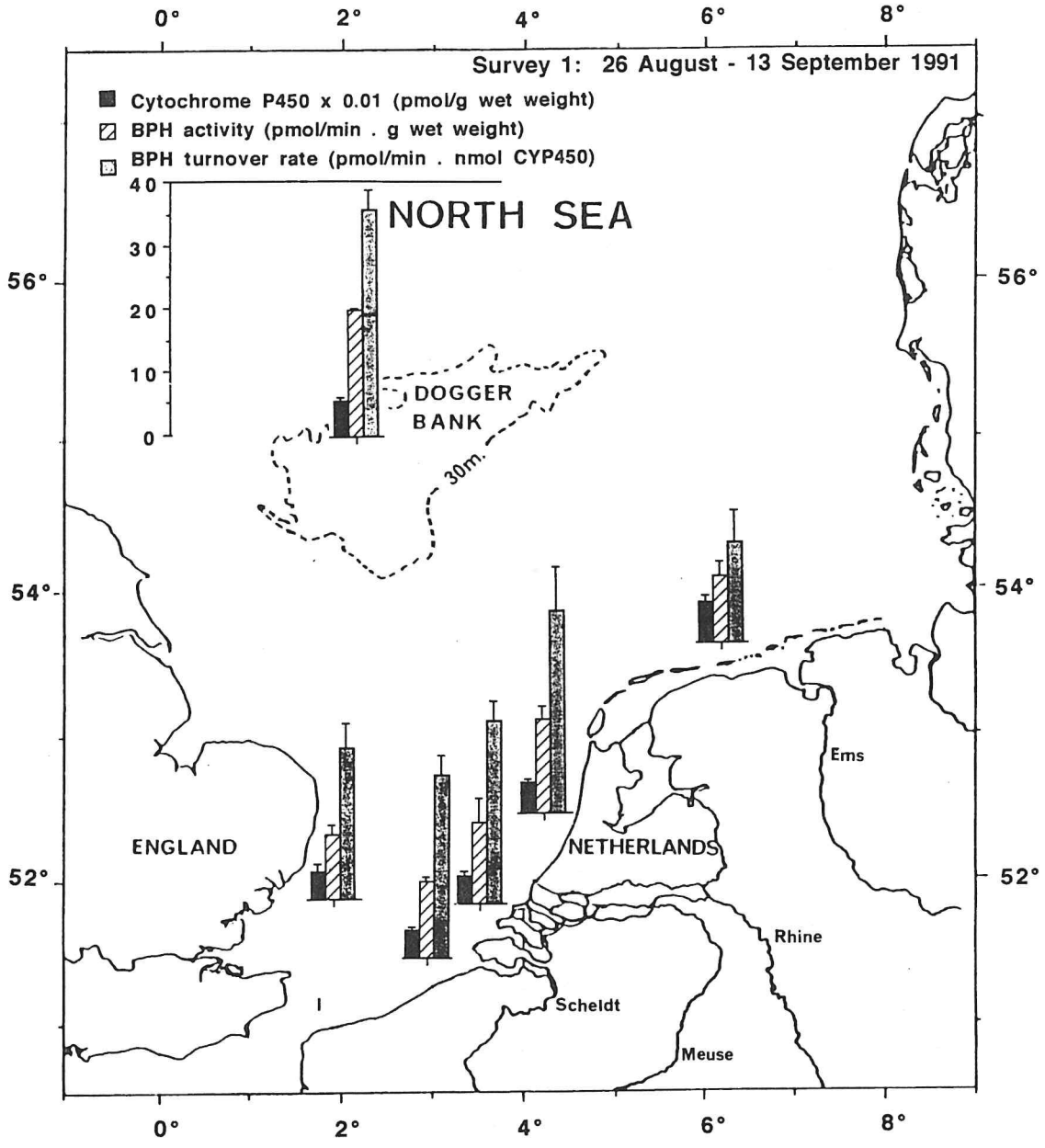

Fig. 2. Induction of the cytochrome P450 dependent monooxygenase system, in the microsomal fraction of pyloric caeca of the seastar (*Asterias rubens* L), and expressed as cytochrome P450 concentration, benzo[a]pyrene (BaP) hydroxylase activity and BPH turnover rate (i.e. the amount of BaP hydroxylated per unit of time and per unit of cytochrome P450).

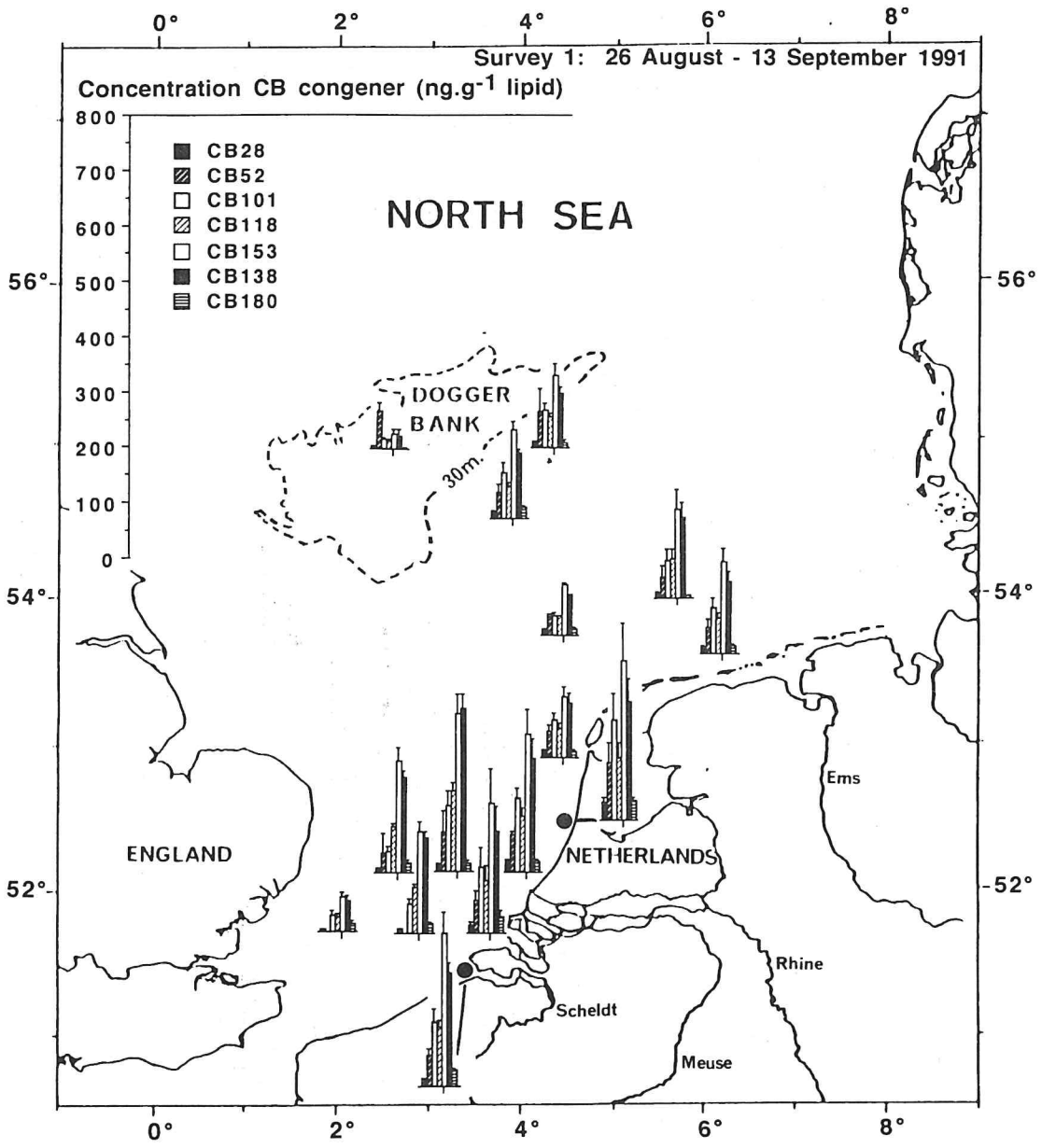

Fig. 3a. The concentration (mean of three duplicates with S.D. in ng/g lipid) of seven chlorinated biphenyl congeners in the pyloric caeca of the seastar (*Asterias rubens* L).

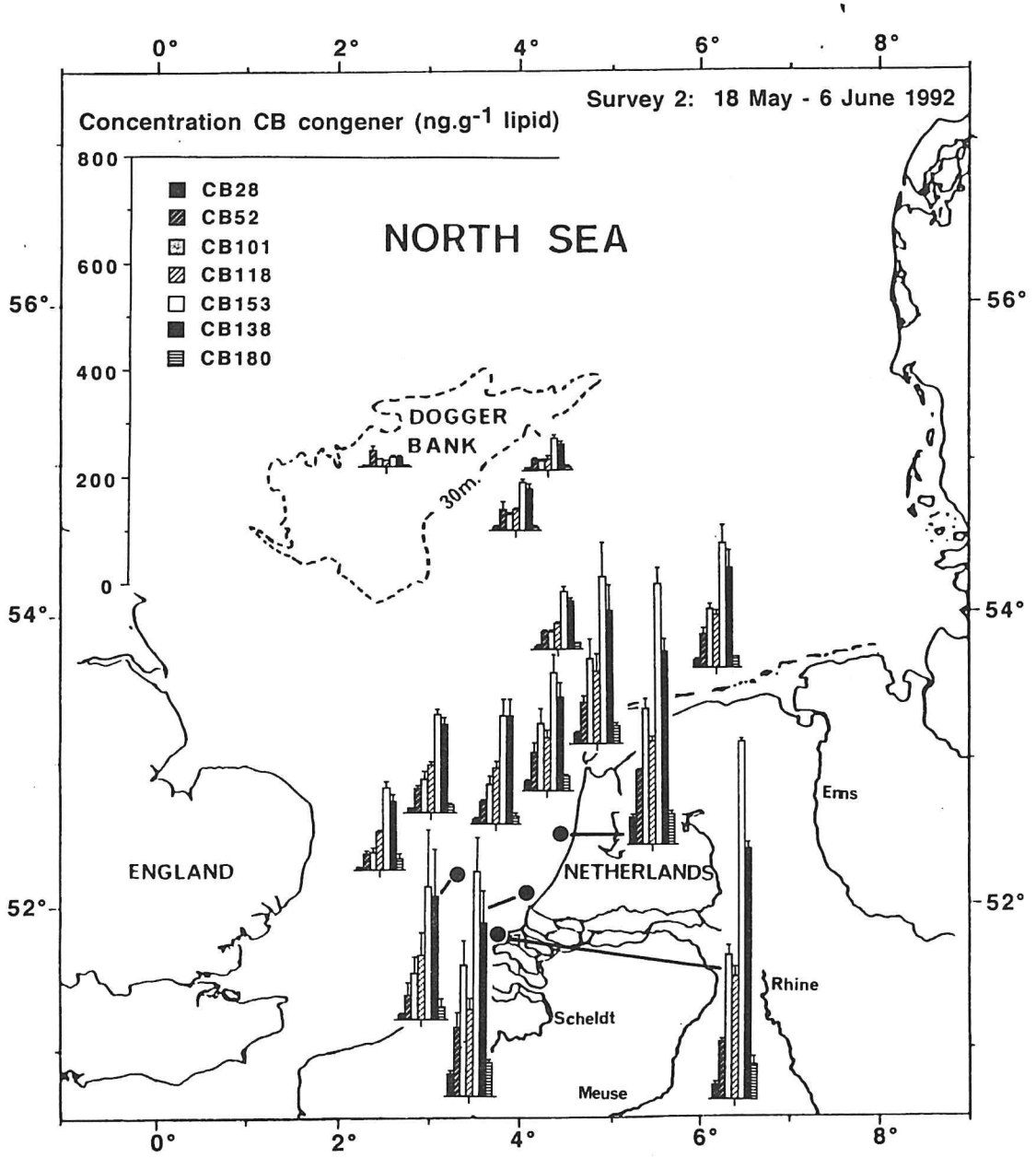

Fig. 3b. The concentration (mean of three duplicates with S.D. in ng/g lipid) of seven chlorinated biphenyl congeners in the pyloric caeca of the seastar (*Asterias rubens* L).

ANALYSIS OF 1-HYDROXY PYRENE IN FISH BILE BIOMONITORING OF PAH POLLUTION IN THE NORTH SEA

INP MICROCONTAMINANTS PROGRAM
1st part: Aug 26-Sept 14, 1991
2nd part: May 18-June 6, 1992

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Introduction

Pollution of the marine environment with Polycyclic Aromatic Hydrocarbons (PAHs) continues to be a matter of great concern. Some fish diseases, such as liver anomalies, are thought to be related to PAH pollution [1]. Fish may be exposed to PAHs through the water, the food chain, ingestion of particle-bound PAHs or via direct skin/gill contact with polluted sediment. The total daily burden is difficult to assess; it is, of course, related to the PAH-content of the sediments, but it also strongly depends on other factors such as bioavailability. After uptake, most PAHs are rapidly hydroxylated by liver enzyme systems (cytochrome P450 family) and temporarily stored in the gall bladder prior to excretion. Analysis of PAH metabolites in bile provides direct insight into the actual PAH uptake [2].

For monitoring purposes, it may not be necessary to perform a complete analysis of all PAH metabolites. Comparing bile samples from flounder (*Platichtys flesus*) and eel (*Anguila anguila*), we found that the PAH metabolite profile is rather constant (between individuals, between locations and between species), and dominated by one compound:1-hydroxy pyrene. Its dominancy is caused by the relatively high abundancy and bioavailability of pyrene in aquous systems [3], combined with the fact that pyrene is metabolized into virtually one single compound [2]. We developed a fast and simple analytical technique for 1-OH pyrene employing synchronous scanning fluorimetry of diluted bile [4] and suggested that it could be used for the biomonitoring of PAH pollution in the aquous environment. Good results were obtained during the course of a mesocosm experiment in which flounders were exposed to sediments with different degrees of PAH pollution [5]. Late summer 1991, the method was first tested in the field as the research vessel Pelagia from the Netherlands Institute for Sea Research (NIOZ) visited 16 locations at the North Sea and the Dutch coastal waters. Most stations, as well as some new locations, were sampled again during a second cruise in late spring 1992.

Bile collection

Bottom-dwelling fish were caught by trawl for chemical, biochemical and histopathological analysis. Four species were selected: dab (schar; *Limanda limanda*); whiting (wijting; *Merlangius merlangus* ; first cruise only); plaice (schol; *Pleuronectes platessa*) and flounder (bot; *Platichtys flesus*). Whiting was usually not caught in sufficient numbers; flounder was only found close to the Dutch coast. If the fish had at least a partly filled gall bladder, bile was collected by means of a syringe and stored in 2 ml Nalgene vials or Eppendorf cups on ice. At the end of the day all bile samples were frozen to -20 C and stored until further use.

For fluorimetric analysis 10 µl of bile is needed. Such a quantity could usually be collected from the larger fish (plaice, flounder, typically 100-400 µl), but most dabs from the length class selected

for monitoring (15-20 cm) did not possess sufficient bile. The data presented here on dab are mainly obtained from the larger individuals collected for histopathology. Since the gall bladder is emptied during digestion, only small volumes of (diluted) bile are present if the fish has just been feeding. Larger and more concentrated bile samples can usually be collected from fish with an empty stomach (best yield before sunrise).

Influence of feeding status

During the first cruise, at locations 6 and 12, a seperate experiment was carried out to investigate the accumulation of metabolites in bile. A number of plaice was kept in flow-through tanks on board (without food) and sacrficed after 1/2, one and two days of starvation. Some sediment from the same location was added to the tanks. Figure 1 shows the steady increase of the metabolite concentration in time (PAHs are continuously being transformed into hydroxylated metabolites while the total gall bladder volume remains approximately constant as water is being reabsorbed [6,7]). Obviously, the statistical variation between individual fish increases if there is not a constant time delay between the last feeding and section. In a field situation we do not know the feeding history of each fish. A short starvation period (two days) on board would remove some of that bias, while at the same time the analyte concentrations increase. Of course, contamination with PAHs on board should be avoided. This method would lead to a lower biological variability and better measurement precision, but unfortunately it cannot be combined with liver enzyme analysis, as the latter should be performed on freshly caught animals. On the other hand, direct section upon capture has some logistic advantages, but sufficient bile is not always available, and the feeding history can only be estimated on the basis of the stomach/intestine contents.

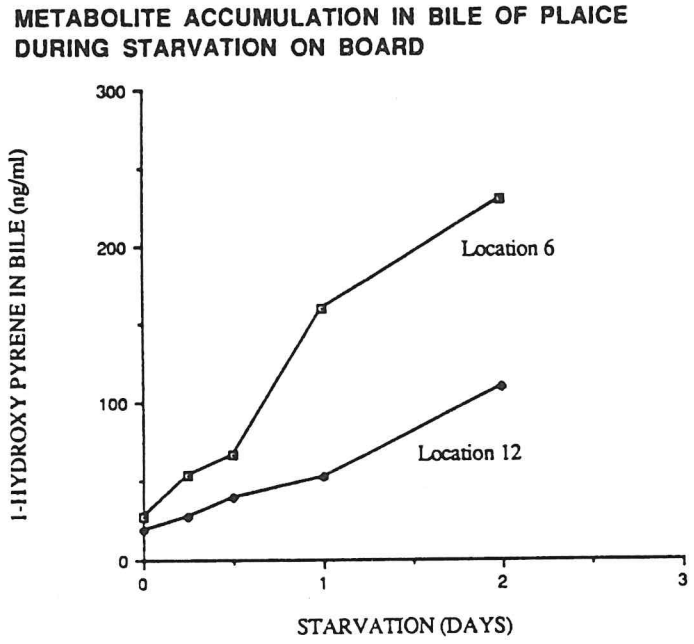

Figure 1: Accumulation of 1-OH pyrene in bile of plaice during starvation on board; point t = 0 represents fish freshly caught with a full stomach; point t = 1/4 is arbitrarily attributed to freshly caught fish with an empty stomach and partly filled intestinal tract.

Experimental

Bile samples, stored at -20 C, were defrosted in an ultrasonic bath. 10 µl of bile was diluted to 5 ml with ethanol/water (1:1 v/v). For the bile samples from the second cruise, a dilution factor of 200 was preferred because of the lower metabolite contents.The synchronous scanning fluorescence spectra of the samples were recorded using either a Spex Fluorolog (1st cruise) or a Perkin Elmer LS 50 spectrofluorimeter (2nd cruise). Excitation and emission bandwidths were 5 nm; emission offset was 37 nm. For quantitation the peak area from 335-356 nm (conjugated 1-OH pyrene) was compared to a calibration curve of free 1-OH pyrene in ethanol/water (peak area from 340/361 nm) A correction factor is needed for the fact that the fluorescence quantum yield of the conjugated metabolite is 2.2 times higher than that of the free 1-OH pyrene. The detection limit was 10 ng/ml. To validate the method, a limited number of samples was also enzymatically hydrolysed, extracted and analysed by means of HPLC/fluorescence [4]. The latter method is more time-consuming, but offers better selectivity and sensitivity. Both techniques yielded comparable results. Before starting the analysis of cruise 2, some samples from the first cruise that had been stored at -20 C were analysed for a second time to check for interlaboratory consistency. No systematic errors that may have resulted from the use of different instruments or different calibration standards were observed.

Table 1: CONCENTRATIONS OF 1-HYDROXY PYRENE IN FISH BILE (FIRST CRUISE)

Loc	Dab (LL)	Whiting (MM)	Flounder (PF)	Plaice (PP)	Combined
2	130 ± 60 (6)		170 ± 70 (11)		160 ± 70 (17)
3	50 ± 30 (7)	40 ± 40 (5)		80 ± 40 (9)	60 ± 40 (21)
4	40 ± 20 (4)	130 ± 60 (5)		90 ± 30 (8)	90 ± 50 (17)
5	~10 (6)			~20 (3)	~10 (9)
6	20 ± 10 (8)	40 (2)		40 ± 20 (14)	30 ± 20 (24)
7	150 ± 60 (3)			120 ± 40 (10)	130 ± 50 (13)
8	60 ± 10 (3)	120 ± 50 (4)		120 ± 40 (5)	110 ± 40 (12)
9	20 ± 10 (7)		20 ± 10 (4)	60 ± 50 (15)	40 ± 40 (26)
10			240 (1)	120 ±130 (6)	140 ±130 (7)
11	170 ± 70 (6)		260 ±100 (9)	260 ±140 (6)	240 ±110 (21)
12	20 ± 20 (3)			20 ± 10 (16)	20 ± 10 (19)
13	~10 (6)	20 (2)		60 ± 40 (18)	50 ± 40 (26)
14	70 ± 60 (3)	20 (1)	120 ± 10 (3)	120 ± 70 (3)	90 ± 50 (10)
15	~10 (2)			30 ± 10 (10)	30 ± 10 (12)
16		20 (2)		30 ± 20 (3)	30 ± 20 (5)
17	50 ± 10 (3)	40 (1)	30 (2)	80 ± 40 (11)	70 ± 40 (17)

Concentrations in ppb (nanogram /ml bile)
Data are expressed as the arithmatic mean ± standard deviation (number of samples)

Table 2: CONCENTRATIONS OF 1-HYDROXY PYRENE IN FISH BILE (SECOND CRUISE)

Loc	Dab (LL)	Flounder (PF)	Plaice (PP)	Combined
2	~30 (n = 26)	70 ± 40 (n = 20)	40 ± 10 (n = 14)	50 ± 30 (n = 60)
4	20 ± 10 (n = 15)		30 ± 10 (n = 12)	30 ± 10 (n = 27)
5	~10 (n = 26)		~20 (n = 9)	~10 (n = 35)
6	~20 (n = 8)		20 ± 10 (n = 11)	20 ± 10 (n = 19)
7	20 ± 20 (n = 15)		30 ± 10 (n = 12)	30 ± 20 (n = 27)
8	30 ± 10 (n = 12)		40 ± 10 (n = 6)	30 ± 10 (n = 18)
9	40 ± 10 (n = 9)	90 ± 40 (n = 12)	30 ± 10 (n = 9)	60 ± 40 (n = 30)
10	70 ± 20 (n = 21)	140 ± 50 (n = 11)	70 ± 20 (n = 12)	90 ± 40 (n = 44)
11	100 ± 30 (n = 15)	160 ± 40 (n = 12)	140 ± 40 (n = 14)	130 ± 40 (n = 41)
19	~10 (n = 4)		~20 (n = 9)	~20 (n = 13)
20	~10 (n = 6)		~10 (n = 12)	~10 (n = 18)
21	~10 (n = 11)		30 ± 10 (n = 15)	20 ± 10 (n = 26)
22	~10 (n = 9)		~10 (n = 12)	~10 (n = 21)
23	~20 (n = 13)	110 ± 40 (n = 20)	20 ± 10 (n = 6)	70 ± 50 (n = 39)
24	30 ± 10 (n = 11)	70 ± 20 (n = 12)	80 ± 20 (n = 12)	60 ± 30 (n = 35)

Concentrations in ppb (nanogram /ml bile)
Data are expressed as the arithmatic mean ± standard deviation (number of samples)

Discussion

The 1-hydroxy pyrene content of several hundred bile samples was analysed by means of synchronous scanning fluorimetry. The method is very rapid (3 minutes per sample); no sample preparation is needed except for a dilution step. At some clean stations at open sea (e.g. #5, 12) the metabolite concentrations were very close to the detection limit and quantitation was hampered by spectral overlap with the (low) native fluorescence of other bile consituents. (Spectral interference was mainly observed in dab bile.) If precise quantitation of low concentrations is needed, reversed phase HPLC of hydrolysed bile with fluorescence detection is a very good, but somewhat more tedious alternative. Synchronous scanning fluorimetry performs well for higher 1-OH pyrene concentrations, that is, if bile is collected after two days of starvation or at more polluted locations.

Table 1 lists the mean concentrations of 1-OH pyrene in bile at the 16 stations visited during the first cruise. At only a few locations (e.g. #4), there appears to be some interspecies difference, but that is not generally observed at other stations. In fact, it rather seems that the daily PAH burden is more or less the same for all four bottom-dwelling species investigated, indicating the general applicability of the method. The last column of Table 1 lists the pyrene metabolite concentration averaged over all fish captured at a given location. These average concentrations are also displayed in Fig. 2.

The average 1-OH pyrene concentrations determined in bile from the second cruise are presented in table 2. Again, no significant differences are observed between dab and plaice, but 1-OH pyrene concentrations in flounder bile are clearly higher at some locations (#2, 9, 10, 23). This difference could be caused by different feeding habits, but could also be the result of migration: since flounder is a typical coastal flatfish that is frequently encountered in brackish or even freshwater areas, the flounders caught at these locations may have been exposed to higher levels of pollution (Wadden Sea, harbours of Den Helder or IJmuiden) shortly before capture.

Some patterns are clear from Figures 2 and 3: High PAH exposure levels are found in the coastal area, especially around the harbours of Rotterdam (#11) and IJmuiden (#10), and near to the Western Scheldt (#17, 14, 24); elevated pyrene uptake is also observed north of the Wadden Sea and at the Frisian Front (#8, deposition area of polluted Rhine sediment or local sources ?). Much lower PAH metabolite levels are found around the Dogger Bank (#5 and 6) and also the area close to the English Channel seems relatively clean, as it is constantly flushed with relatively clean water from the Atlantic Ocean. It seems that the PAH distribution at the North Sea is mainly gouverned by river output of polluted sediment, which is subsequently transported to the north by the prevailing currents. Atmospheric deposition would probably result in a much more diffuse pattern. At present, we are waiting for the data from Bergen to see to what extent the 1-OH pyrene concentrations in bile are correlated to the PAH content of the sediments.

Interestingly, although figures 2 and 3 show the same trends, the absolute values determined in the samples from cruise 2 are lower by a factor of 2-4. At present, the most reasonable explanation

seems to be a seasonal effect: During cruise 2 in late spring, the average water temperature was 5-6 degrees lower than during cruise 1. Jimenez et al demonstrated that the uptake of benzo(a)pyrene by the bluegill sunfish (*Lepomis macrochirus*) is almost 6-fold slower when the fish are kept at 13 °C instead of 23 °C [8]. I would be pleased to hear if there are any other biological or physico-chemical effects that may explain the observed phenomenon. Of course, the metabolite content in fish bile should be regularly monitored during several years or a laboratory experiment should be set up to find out whether the PAH uptake rate is indeed a reproducible function of the water temperature.

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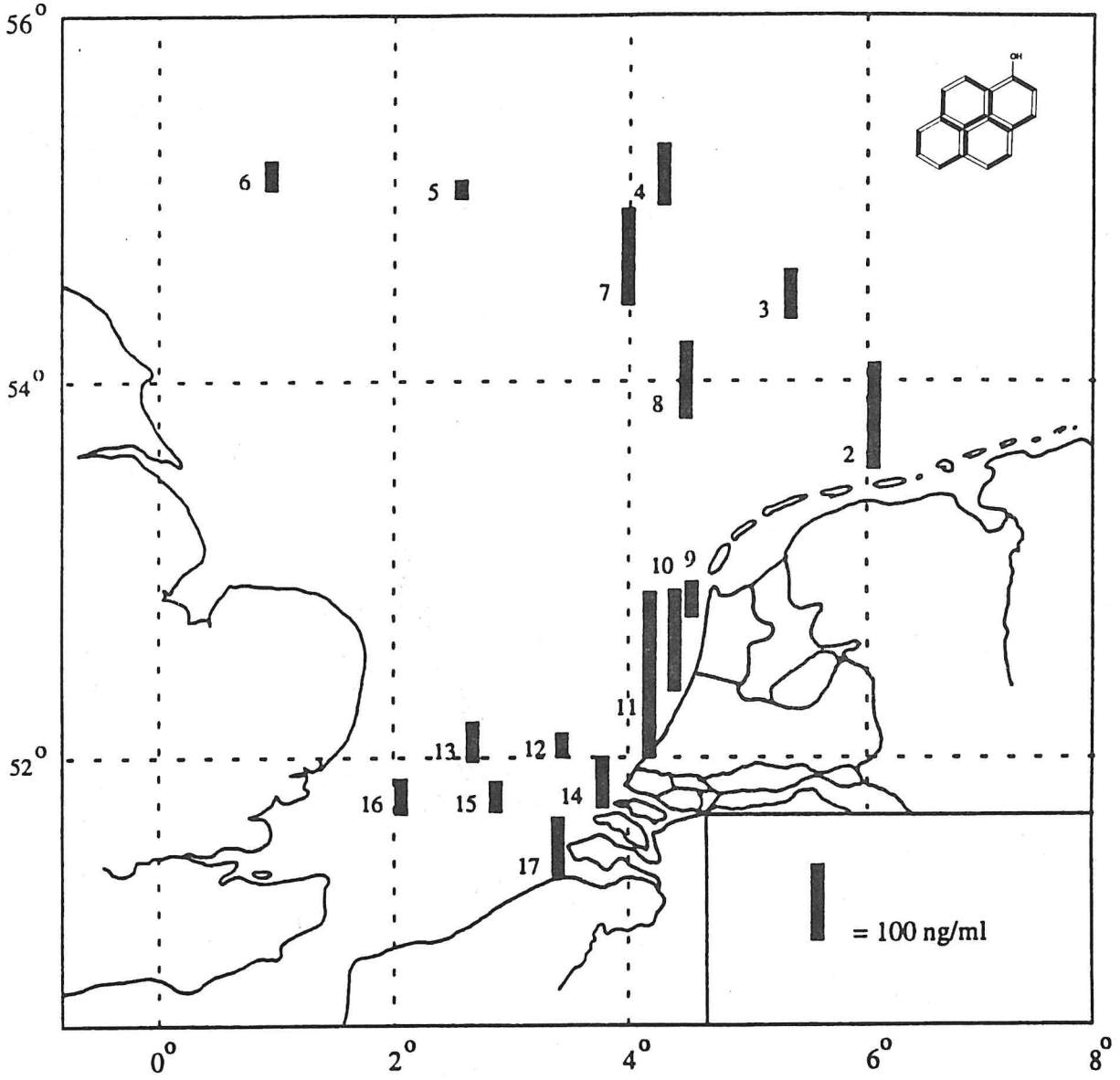

Fig. 2 Sampling stations at the North Sea and average 1-OH pyrene concentrations in bile of bottom-dwelling fish; first cruise , Aug-Sept 1991.

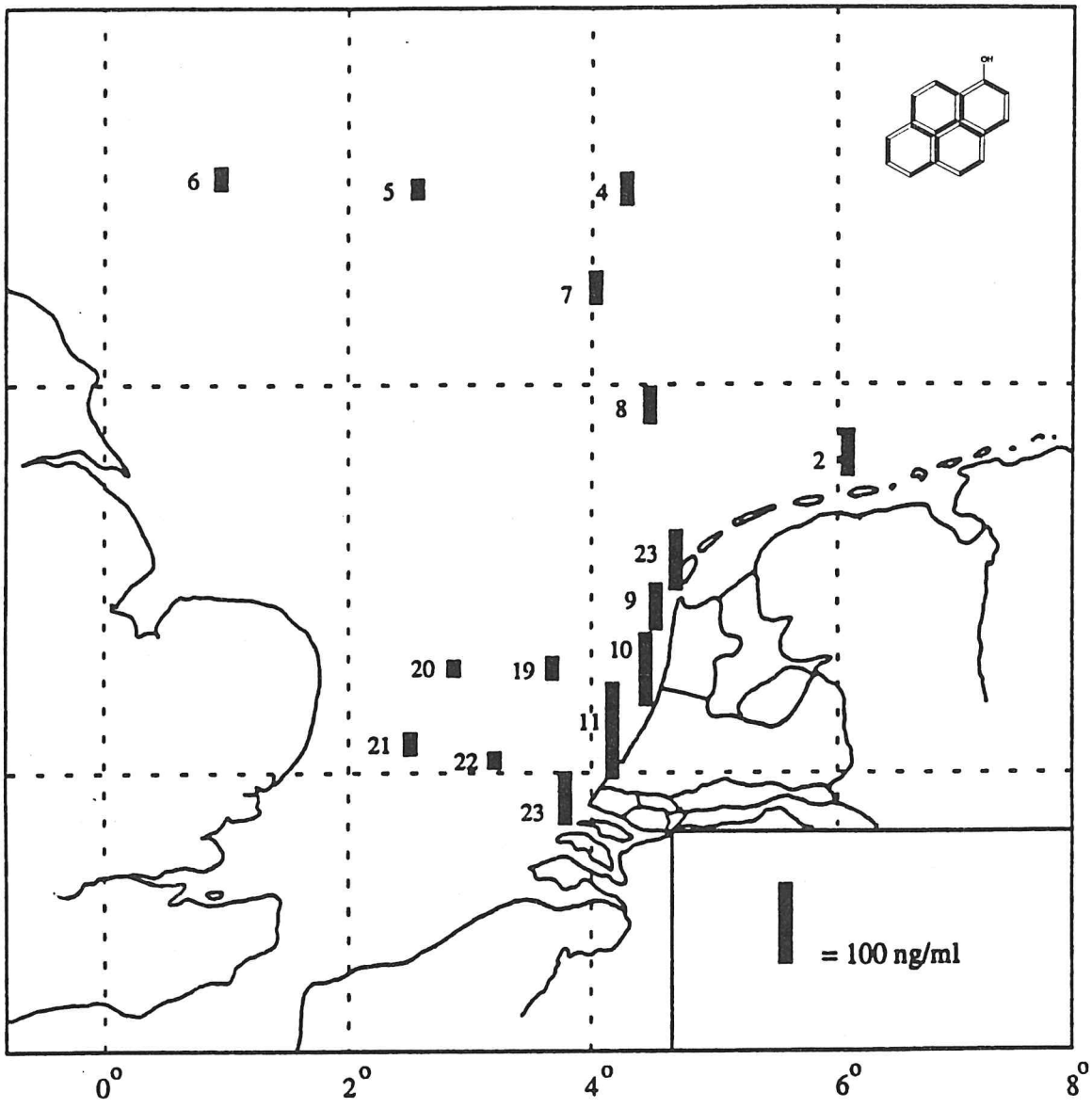

Fig. 3 Sampling stations at the North Sea and average 1-OH pyrene concentrations in bile of bottom-dwelling flatfish; second cruise , May-June 1992.

P450 1A1 ELISA measurements in dab (*Limanda limanda*). INP-MICON cruises 1991-92.

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Abstract

Liver 10.000 g supernatants of dab caught in Dutch coastal waters and the southern part of the North Sea during the 1991-92 INP-MICON cruises (Boon, this volume) were analyzed for levels of cytochrome P450 1A1 by a semiquantitative ELISA (fig. 1) using polyclonal anti-cod-P450 1A1 according to Goksøyr (1991). P450 1A1 in fish is used as a biomarker for exposure to PAH-, PCB- and dioxin-like pollutants (Goksøyr and Förlin, 1992). The INP sampling scheme at stations were 25 male dab, size 15-20 cm, caught 5 by 5 and without any visible signs of disease.

Differences between stations in terms of hepatic P450 1A1 level in the dab were observed at both cruises (fig. 2). High P450 1A1 levels were observed at stations 4 and 6, located far off-shore and designated as reference stations with regard to contaminant levels. These locations had low temperature in water at bottom depth due to stratification of the water column. Biplots fitting water temperature at bottom depth to mean levels of P450 1A1 in livers showed that cold stations were generally high and warm stations generally low in P450 1A1 levels (fig. 3), suggesting a temperature compensatory response. Influence on regulation of the P450 system in fish by temperature are reported earlier (Stegeman 1979; Sakai, Kawatsu et al. 1983; Blanck, Lindström-Seppä et al. 1989). The results presented here demonstrate that habitat temperature indeed is a factor of crucial importance when using P450 1A1 in fish as a biomarker in field studies.

Nevertheless, indications of P450 1A1 induction in fish at polluted sites were found. At the 92-cruise, dab from the coastal stations 2, 19, 20, 21 and 22 displayed significantly elevated P450 1A1 levels compared to dab from the reference location 5 at Dogger Bank (fig. 4), even though site 5 was the coldest of these stations. P450 1A1 observations will also be correlated to chemical data of dab and sediments before further conclusive statements are made.

P450 1A1 dependent 7-ethoxyresorufine-O-deetylase activity (EROD) was measured in the same samples on board the ship (Sleiderink et al., this volume). Statistical analyses displayed significant correlations ($p<0.0001$) between individual EROD and ELISA results both from the 91- and 92-cruise, $r=0.85$ and $r=0.82$, respectively (or $r=0.59$ and $r=0.90$ when using $\log_{10}$ transformed data). From the analyses it is clear that the two methods for studying P450 1A1 levels (catalytic and immunochemical) support and verify each other.

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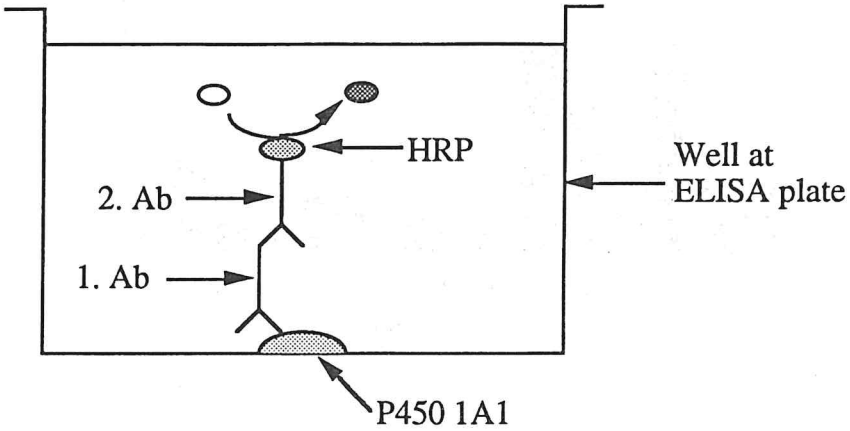

Figure 1: The P450 1A1 enzyme-linked immunosorbent assay (P450 1A1 ELISA) schematically shown. The wells of the ELISA-plate were coated with 1µg protein of the 10.000 g supernatant (100 µl coating solution). After incubations with primary and secondary antibodies (polyclonal rabbit-anti-cod P450 1A1 IgG and HRP-conjugated goat-anti-rabbit IgG respectively), a colour reaction semiquantitatively gives the level of P450 1A1 in the sample. HRP= horseradish peroxidase.

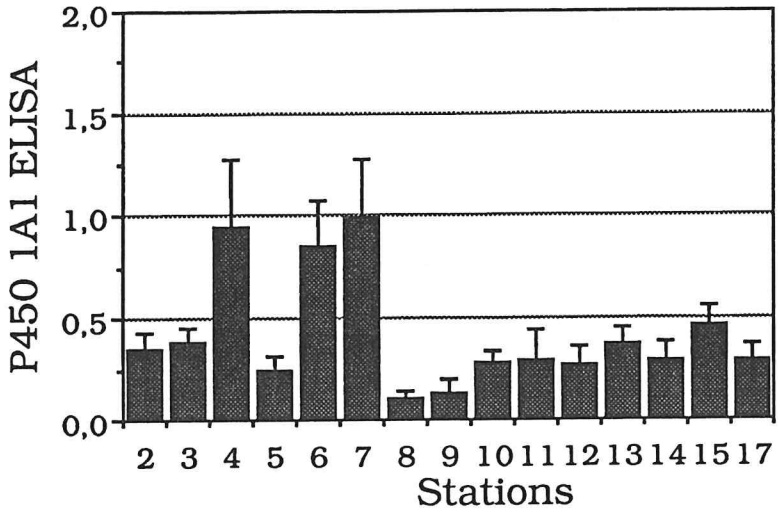

Figure 2A

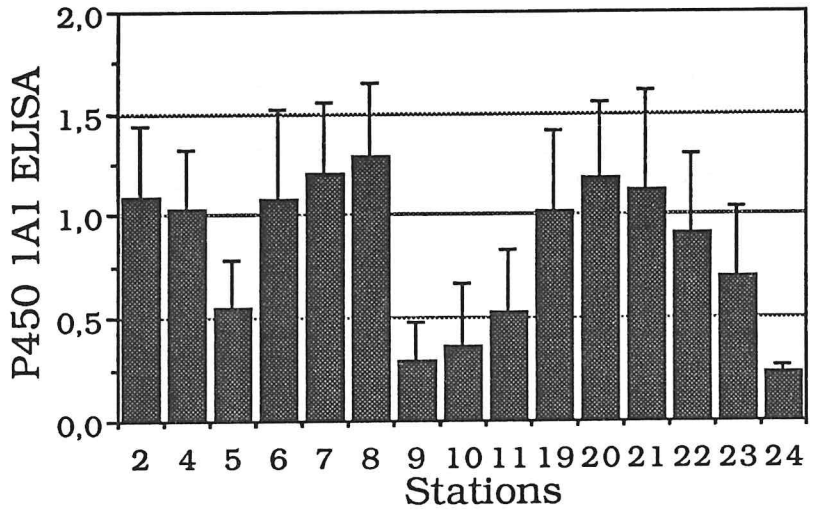

Figure 2B

Figure 2: Mean and standard deviations of P450 1A1 ELISA (absorbances at 492 nm) in liver 10.000 g supernatants of male 15-20 cm dab. Fig 2A: INP-MICON 1991 cruise. Fig 2B: INP-MICON 1992 cruise. 25 individuals were sampled (N=25) at all stations except at 1991 cruise stations 10, 11, 13, 15, and 17 where N=4, 24, 14, 15, 15 respectively and at 1992 cruise station 24 where N=15. Station maps of the 1991 and 1992 cruises can be found elsewhere in this volume.

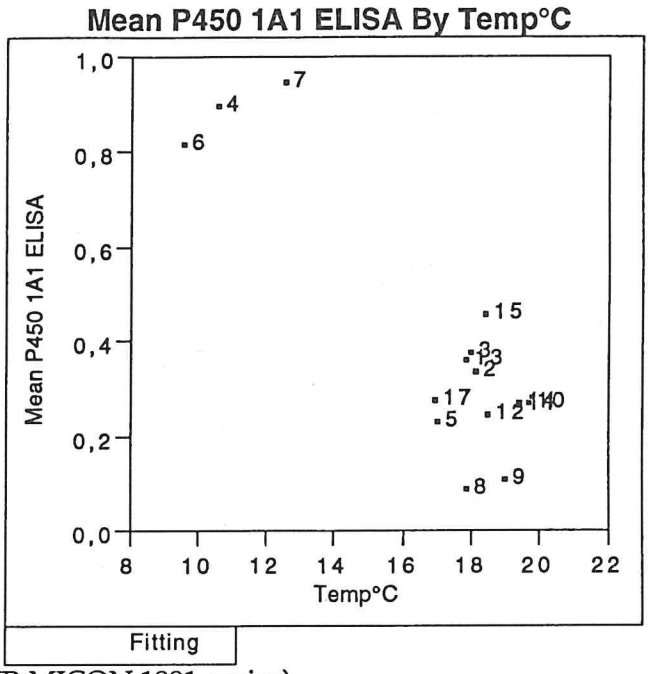

Figure 3A (INP-MICON 1991 cruise)

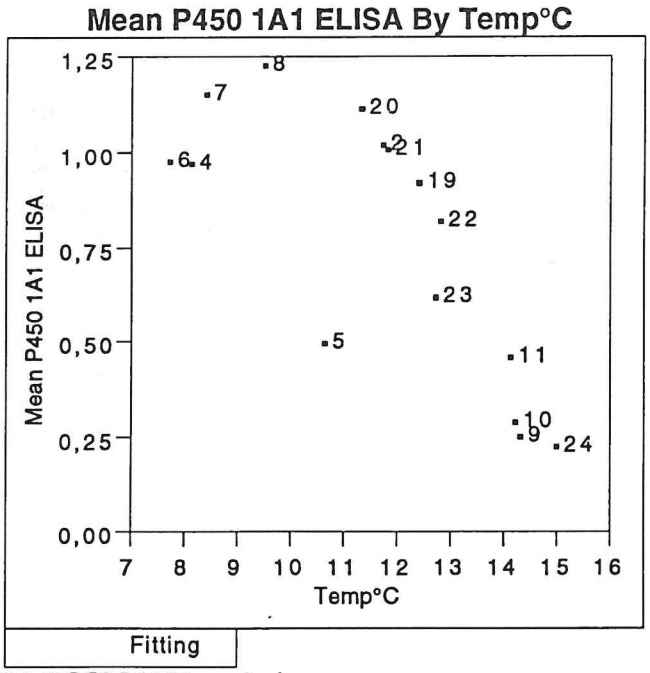

Figure 3B (INP-MICON 1992 cruise)

Figure. 3: Biplots fitting temperature in bottom water by mean P450 1A1 ELISA absorbances in male dab liver 10.000 g supernatants. Sampling stations are referred as numbers on the figures. To avoid contribution of outliners in the data set, calculation of the mean values was done with $\log_{10}$ transformed data. The mean log values were then transformed (10^{x}) back to normal form.

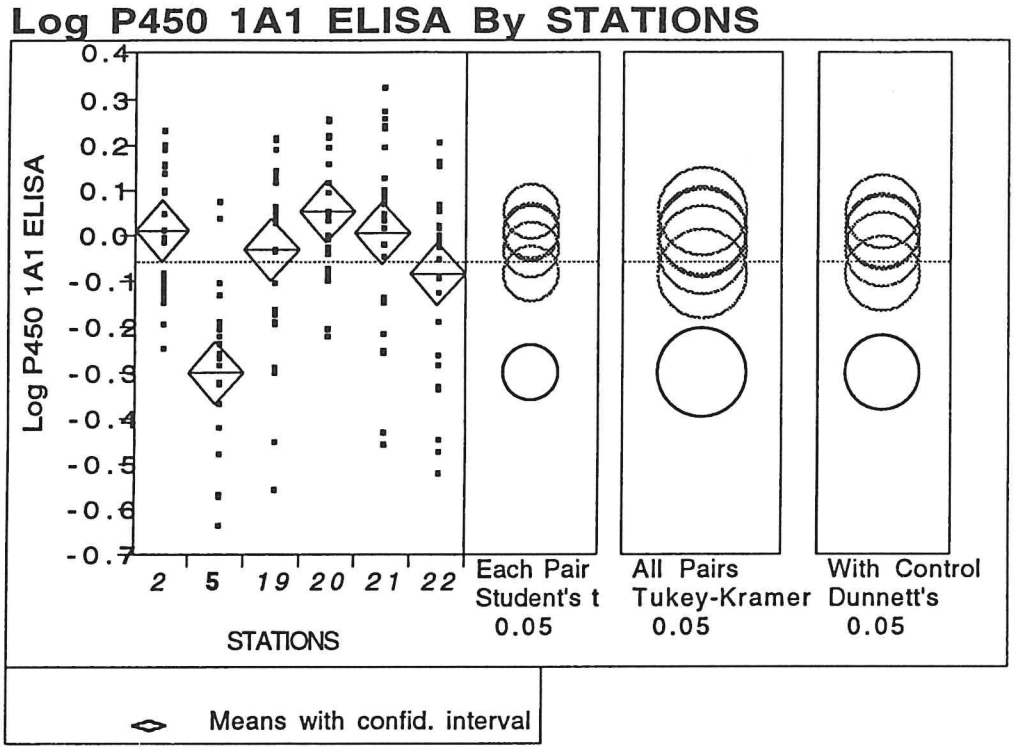

Figure 4: Statistical tests in JMP software of P450 1A1 levels in liver 10.000 g supernatants of dab from six selected stations of the INP-MICON 1992 cruise. The data were $\log_{10}$ transformed prior to testing. The normality of the data was tested by a Shapiro-Wilk W-test. Due to lack of normality, data of station 19 was tested by a non-parametric Wilcoxon/Kruskal -Wallis test. Stations significantly different ($p<0.05$) from reference station 5 (Dogger Bank) are shown in the figure with *italic style* on station names and grey circles at the right in figure. The test shows significant increase of the hepatic P450 1A1 level in dab at all coastal stations compared to the reference site even though station 5 was the coldest one of the six tested.

INP-MICON PROGRAMME 1991-1992: DNA STRAND-BREAKS IN LIVER OF DAB (*LIMANDA LIMANDA*)

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Abstract: Male dab (*Limanda limanda*) were collected at sampling locations along transects radiating into the southern North Sea from coastal areas of the Netherlands. The results of cruise 1 (August 26-September 14, 1991) are reported. Strand breakage in DNA isolated from liver tissue was measured by alkaline unwinding assay. Interpretation of results is based on the incidence of DNA strand breaks (expressed as the percentage of double stranded DNA:total DNA (ds DNA)), site-specific variance (expressed as coefficient of variation (CV)), and resistance of DNA to unwinding under standard alkaline assay conditions (resist DNA).

The stations could be separated into four levels (fig. 1):
Level I: All liver samples from dab from station 11, located near the combined mouth of the rivers Rhine and Meuse, contained resist DNA. The significance of this observation is under study; however, resistance of DNA to alkaline unwinding was previously observed in liver DNA from Japanese medaka (*Oryzias latipes*) exposed in the laboratory to benzo[a]pyrene. The presence of resist DNA appears to be species dependent and may reflect a basic defensive mechanism against contaminants.
Level II: Stations 7, 9, 12, 14 and 17 were characterized by an average of 74% double stranded DNA, a large CV, while 33-50% of the samples contained resist DNA. The large CV at this level may reflect differences in individual sensitivity towards genotoxic insult.
Level III: Stations 4, 13 and 15 were characterized by samples with < 75% ds DNA, still a large CV, while 20% of the samples contained resist DNA.
Level IV: Dab livers from stations 2,3 and 10 were characterized by <70% ds DNA, a small CV, and no samples with resist DNA. These characteristics are typical for animals exposed to lower concentrations of environmental genotoxins.

The above supposition relies to a large extent on the resistance of DNA to unwind under alkaline conditions, a phenomena not previously reported. The importance of this phenomenon as a biomarker of genotoxic exposure/effects is speculative at this time; therefore caution is advised regarding the interpretation pending additional study.

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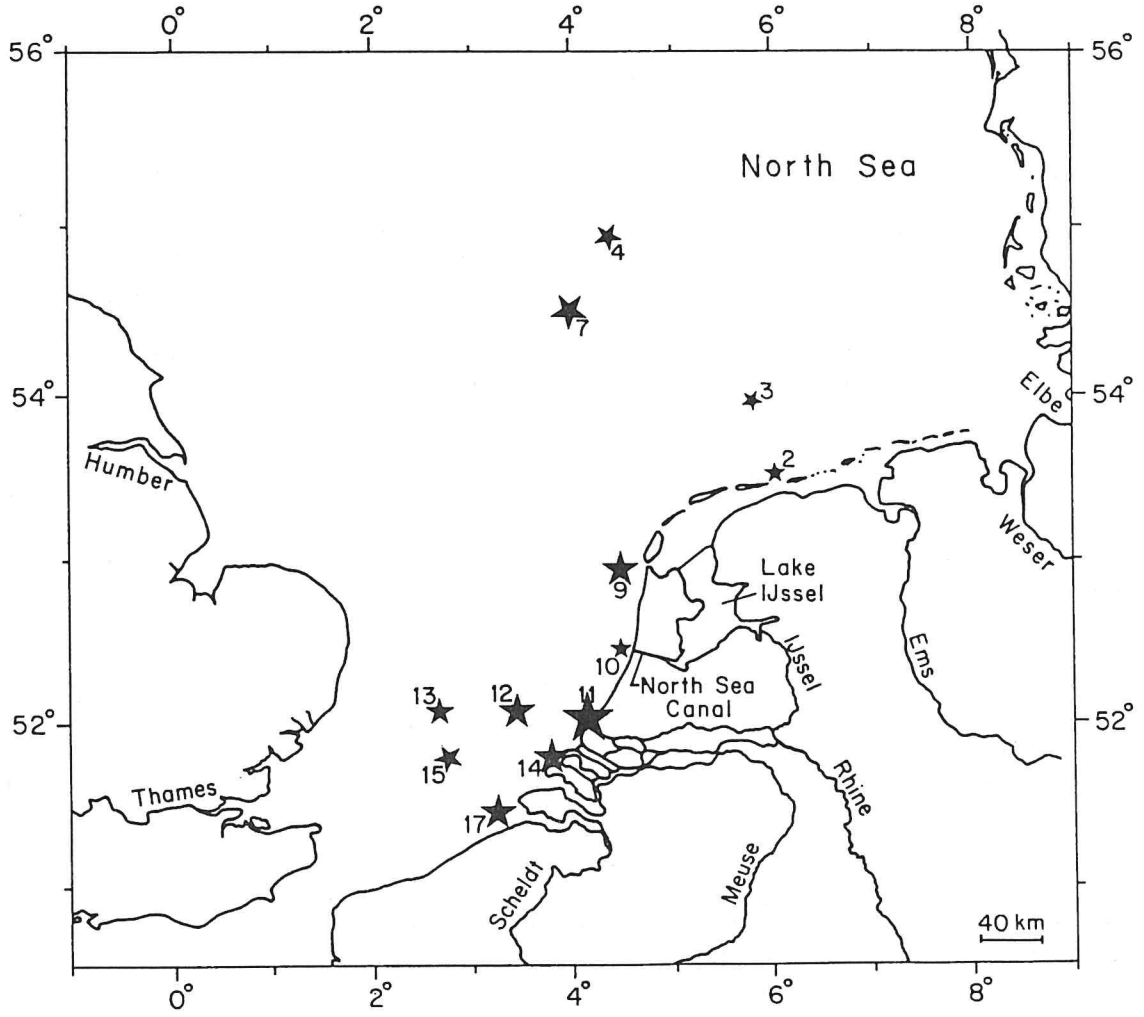
DNA strand break data in dab (*Limanda limanda*) collected during cruise I of the INP-MICON programme, August 26-September 14, 1991 (preliminary results Halbrook, Shugart & Everaarts).

Site	F-value	CV	% Resistance	Symbol
11	-	-	100	★
7,9,12,14,17	59-86	0.15-0.59	33-50	★
4,13,15	55-74	0.65-0.95	20	★
2,3,10	64-70	0.13-0.21	0	★

F-value = Percent double-stranded DNA of total DNA
CV = Coefficient of Variation
% Resistance = Percent of samples with DNA that resisted unwinding



BUCCINUM UNDATUM L IN THE NORTH SEA, STATE OF WHELKS AND IMPOSEX PHENOMENA.

Cato C. ten Hallers-Tjabbes* en Jan P. Boon**

During the Integrated North Sea Programme - Microcontaminants (INP-MICON, September-October 1991, May-June 1992) the state of the whelk, Buccinum undatum L was investigated. B. undatum is a predatory gastropod that mainly preys on bivalves. The animal lives in and on top of the sediment, is relatively long-living and confined in area, the more so as the eggs, deposited on hard-substrate objects on the sea floor, hatch into young snails. Distribution of whelks at the investigated locations and phenomena of imposex were used as indicators for the state of the whelk. Whelks were also collected for neurosensory experiments that are now in a pilot stage.

No live whelks could be caught at the stations close to shore. At most of these locations many empty and hermit-inhabited whelks were caught, indicating that live whelks had dwelled here in the past, an observation that is supported by data from earlier inventories. Live whelks, when caught, were more abundant at the Northern stations and at the Westernmost stations in the central North Sea than at stations closer to shore.

Imposex phenomena did occur in some female whelks, which showed tiny or somewhat bigger penis homologues. At some Northernmost stations no imposex phenomena occurred. At the other Northern stations the phenomena were smaller and the incidence was lower than in the central and Southern North Sea. The highest rates of imposex in the central and Southern North Sea coincided with the vicinity of shipping lanes. The imposex rates were higher in the more densely shipped routes than in the less busy North-South deep water route. The station Brucey's Garden, a supposedly reference area in the Northern North Sea, that showed somewhat elevated incidence of imposex, appeared to be situated in a relatively more densely used shipping area than the Dogger bank, where only one minor imposex phenomenon was found.

The levels of tributyltin (TBT) and its metabolites, dibutyltin (DBT), and monobutyltin (MBT), were measured in whelks from the first leg of the cruise. TBT was not detectable, but DBT and MBT were, albeit at low levels. At the only station where all females showed imposex, DBT and MBT levels were twice as high as in the other stations where DBT and MBT were detectable. Metabolizing of TBT in Buccinum undatum may differ from the one in Nucella lapillus, the snail in which imposex could clearly be related to elevated levels of TBT in animals and environment.

Laboratory experiments to try and link the whelks imposex phenomena to the presence of TBT and metabolites from anti-fouling paints are under way.

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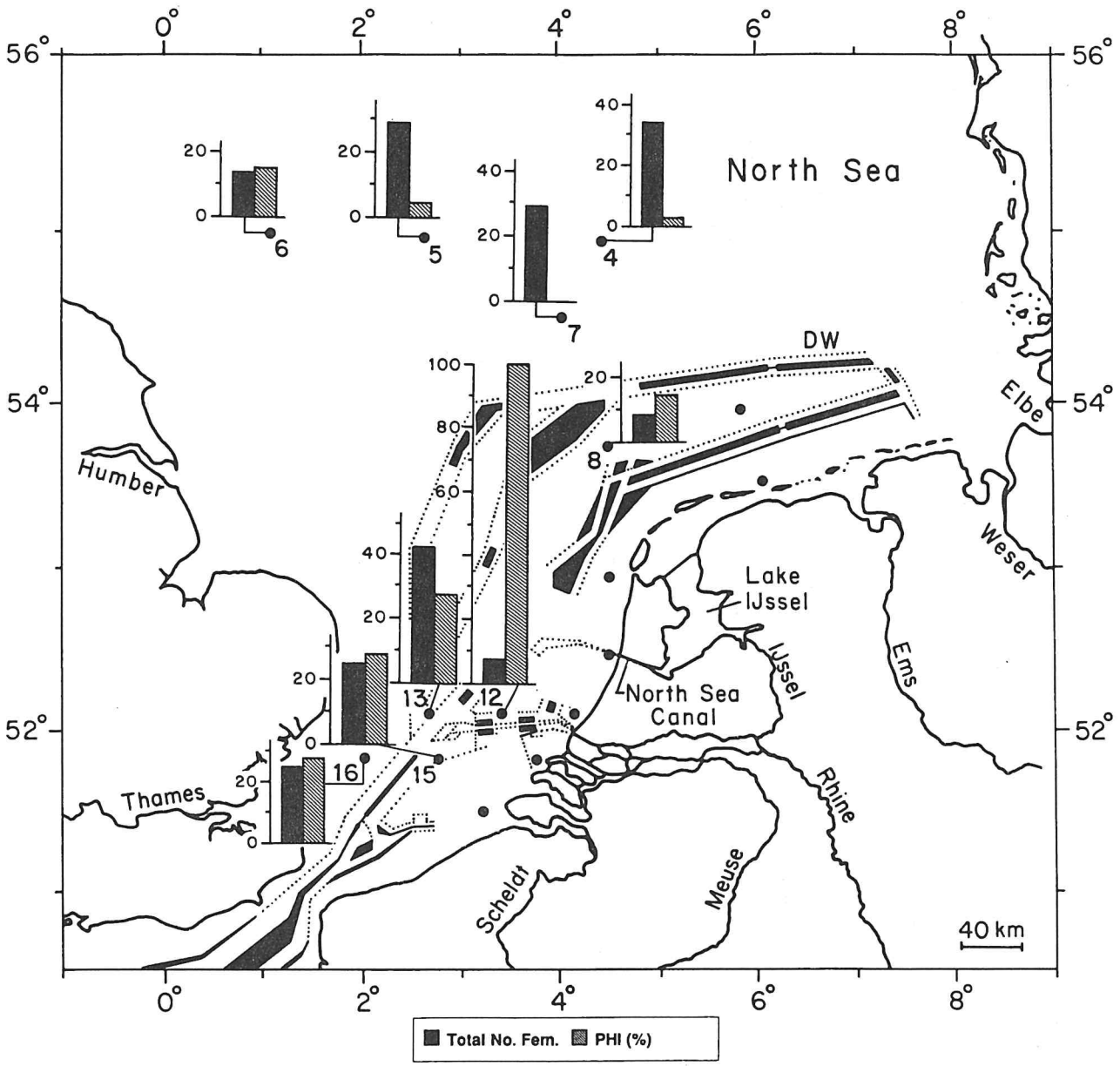

Figure 1. Nr of females investigated and imposex incidence during the first INP-MICON cruise (1991). Total No.Fem = Total number of females. PHI (%) = Percentage of females with a penis homologue.

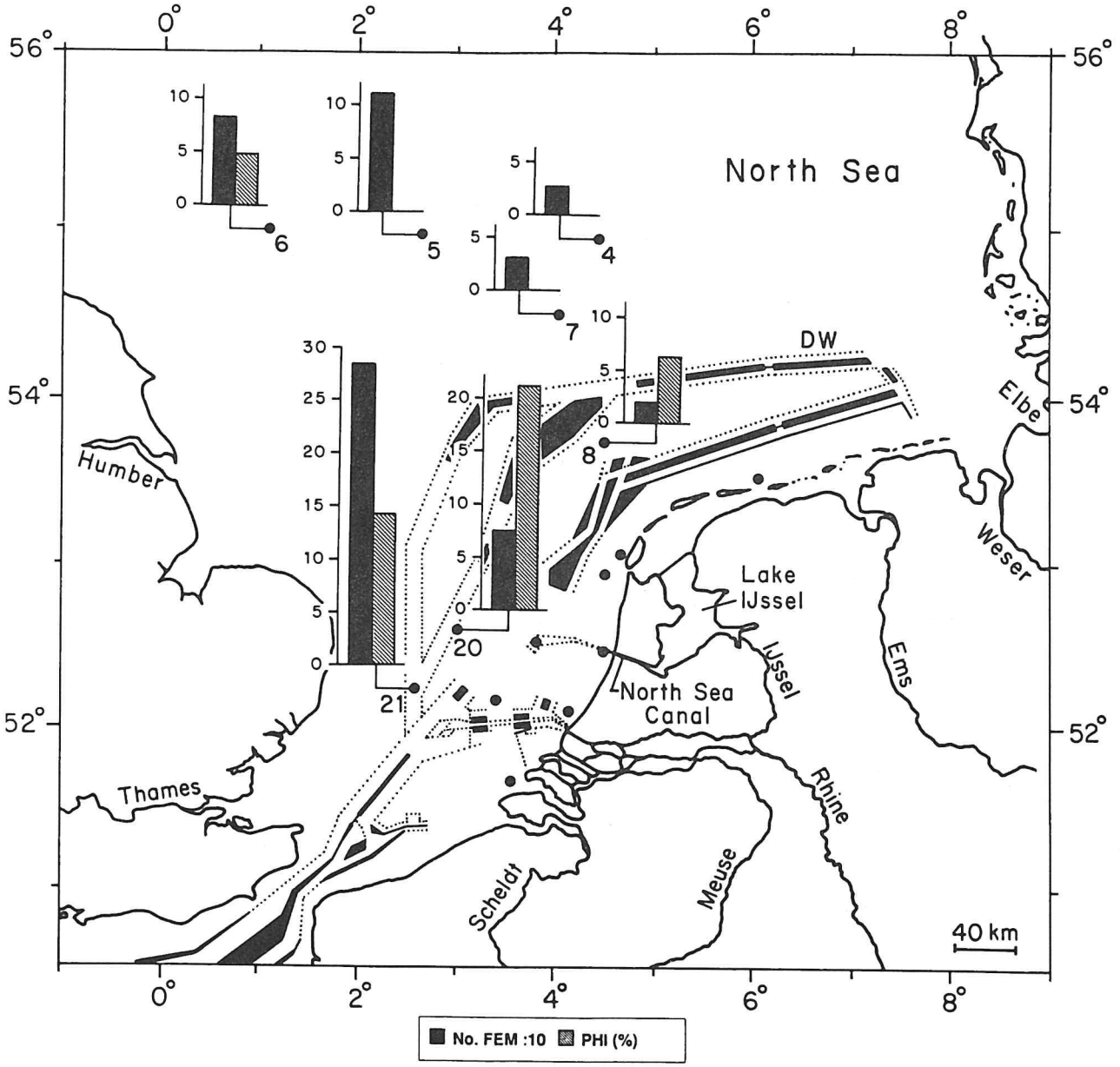

Figure 2. Nr of females investigated and imposex incidence during the second INP-MICON cruise (1992). No.Fem:10 = Total number of females divided by 10. PHI (%) = Percentage of females with a penis homologue.

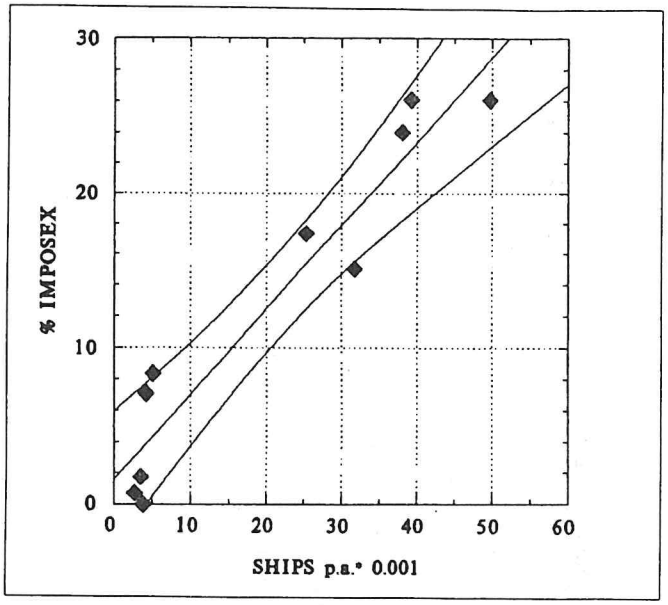

Figure 3. Correlation between shipping intensities (1982 data) and imposex incidence for stations where more than 20 female specimens were sampled. r = 0.96.

ABSTRACT

PATTERNS OF OCCURRENCE OF HISTOLOGICAL CHANGES OF HEPATO-SPLENIC ORGANS IN FLAT FISH FROM THE SOUTHERN NORTH SEA

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This study describes histological variability in livers and spleens from dab *Limanda limanda* and plaice *Pleuronectes platessa* sampled during two cruises of RV Pelagia in September 1991 and May-June 1992. Samples were taken in the southern part of the North Sea along several transects, starting in Dutch coastal waters and extending as far as the English coast and Dogger Bank area.

The following histopathological changes were observed in the livers of both species: focal and diffuse mono-nuclear inflammation, focal necrosis, parasitic cysts, foci of cellular alteration (considered to represent pre-neoplastic lesions), and neoplasia.

Neoplastic and putative pre-neoplastic lesions were observed in the livers of both species over the entire study area, including the Dogger Bank.

In addition to the above, the degree of vacuolization of the hepatocytes, assumed to represent storage of fat or glygogen and thus providing an index of nutritional status, was quantitatively assessed. In general, the extent of vacuolization was greater in coastal waters than in offshore waters.

The presence of melano-macrophage centres was noted as a possible index of non-specific immune function. These centres were particularly apparent in the spleen, where a semi-quantitative assessment of their occurrence showed lower values in coastal waters than in offshore waters.

In this paper, spatial and seasonal variability in the prevalences of observed pathologies, and in the values of the histological indices, are discussed in relation to host and habitat characteristics, environmental concentrations of organic contaminants and biochemical indices measured in the same fish by other participants.

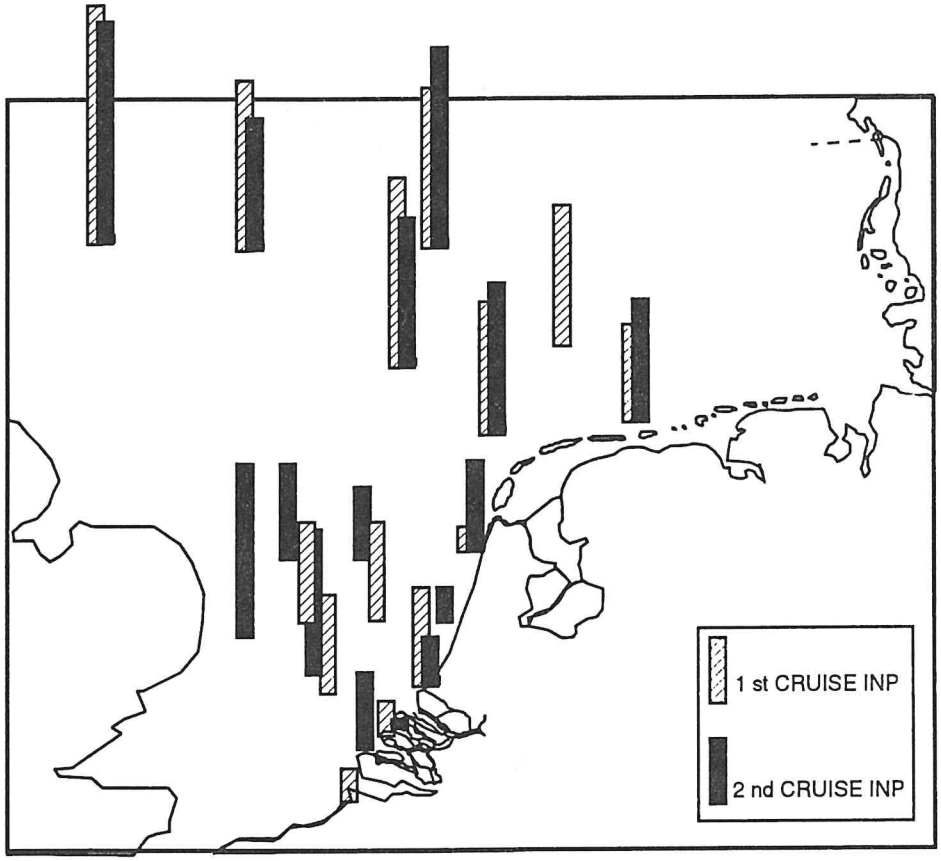

Fig. 1: Semi-quantitative assessment of the occurrence of melano-macrophage centres in the spleen of male dab (length 15-19 cm) in September 1991 (1st cruise) and May-June 1992 (2nd cruise) (Vethaak unpublished data).

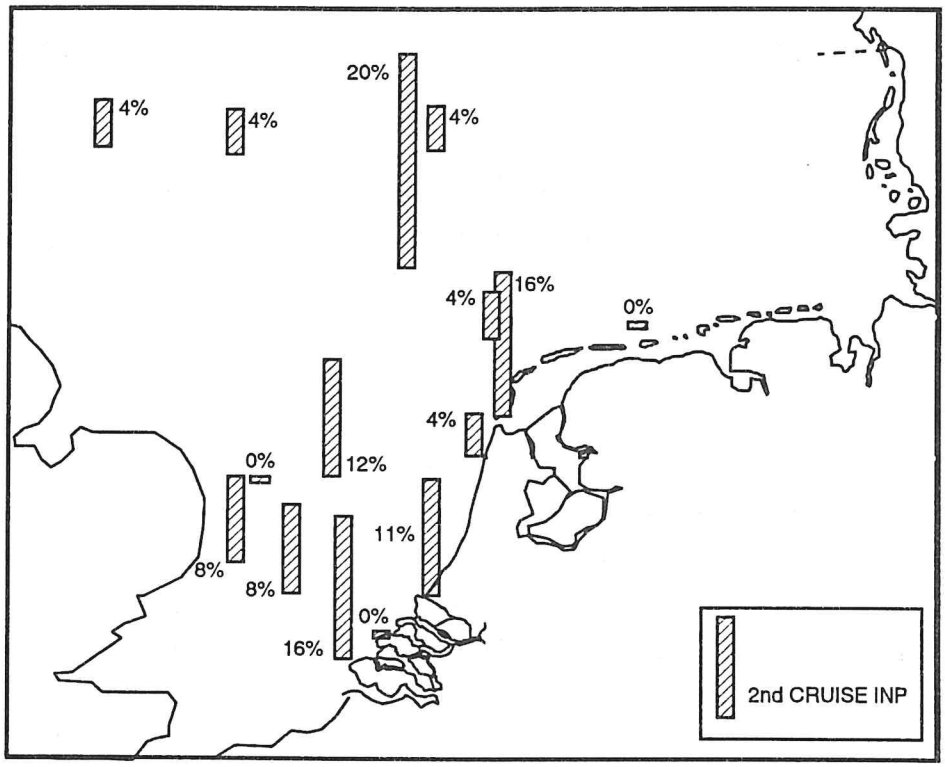

Fig. 2: Crude prevalences of foci of cellular alteration (considered to represent pre-neoplastic lesions) in the liver of male dab (length 15-19 cm) in May-June 1992 (2nd cruise). Prevalence estimates are based on 25 fish per station (Vethaak unpublished data).

ETHOXYRESORUFIN O-DEETHYLASE IN DAB (*LIMANDA LIMANDA*) FROM THE SOUTHERN NORTH SEA : RESULTS FROM A FIELD SURVEY.

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Problems in detecting impacts on marine organisms at population and community levels of biological organisation suggest that changes in various biochemical parameters may be useful indicators in identifying pollutant effects well before effects at the population level may be detected (1). The hepatic cytochrome P450-dependent monooxygenase (MO) enzyme system can be such an 'early warning' signal for certain classes of planar aromatic microcontaminants. In fish, the activity of ethoxyresorufin O-deethylase (EROD) is often used as a measure for the induction of cytochrome P4501A by those contaminants. Apart from the induction by xenobiotics, the activity and induction of P450 enzymes in fish can be influenced by a large array of abiotic and biotic factors (2,3).

For the present study mature specimens of male dab (*Limanda limanda*) were collected from several stations in the southern North Sea. The ethoxyresorufin O-deethylase (EROD) activity was determined in liver tissue. EROD and protein assays were performed with a microplate reader directly onboard the research vessel in the 13,000xg supernatants. The samples were taken during two surveys of the R.V. Pelagia (26 August-13 September 1991 and 18 May-6 June 1992) as a part of the Integrated North Sea Programme , theme Microcontaminants (INP-MICON).

The results of EROD activity in dab obtained in this study (Figure 1A and 1B) did not correspond to the known PCB distribution patterns where high contaminant levels are present in sediment and biota along the dutch coast with decreasing concentrations perpendicular to the coast (4,5). During both surveys highest levels of EROD activity were found at relatively clean off-shore stations with low bottom water temperatures due to stratification. Considerably lower EROD values were found at stations along the Dutch coast with relatively high bottom water temperatures. The inverse relationship between water temperature and EROD activity was confirmed in a laboratory study, dabs were acclimated to 8, 12 and 16°C during four weeks. A three-fold increase in EROD activity in the group acclimated to 8°C compared to the group acclimated to 16°C was observed (Figure 2).

Furthermore, the nutritional status of dabs from the coastal and off-shore stations differed, which was reflected in differences in condition factors. Thus differences in water temperature and nutritional status of dabs between sampling locations probably interfered with the effects of contamination, making the interpretation of the results more difficult.

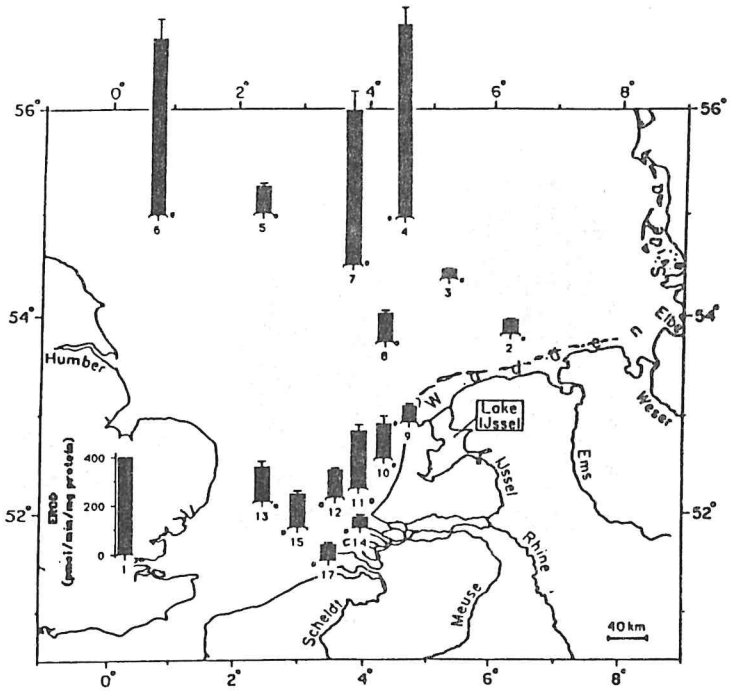

Figure 1A. Mean hepatic ethoxyresorufin O-deethylase activity (pmol/min/mg protein) of dab (*Limanda limanda*) measured at 15 stations in the southern North Sea during 1st survey. Bars represent mean values ± standard error of 25 livers, except station 10 (n=4), station 13 (n=14), station 9 (n=24) and stations 15 and 17 (n=15).

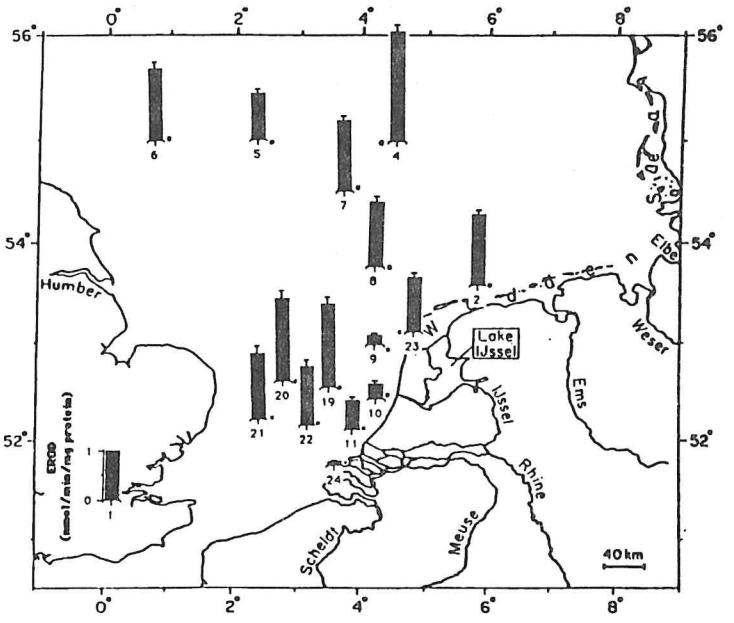

Figure 1B. Mean hepatic ethoxyresorufin O-deethylase activity (nmol/min/mg protein) of dab (*Limanda limanda*) measured at 15 stations in the southern North Sea during 2nd survey. Bars represent mean values ± standard error of 25 livers, except station 24 (n=15).

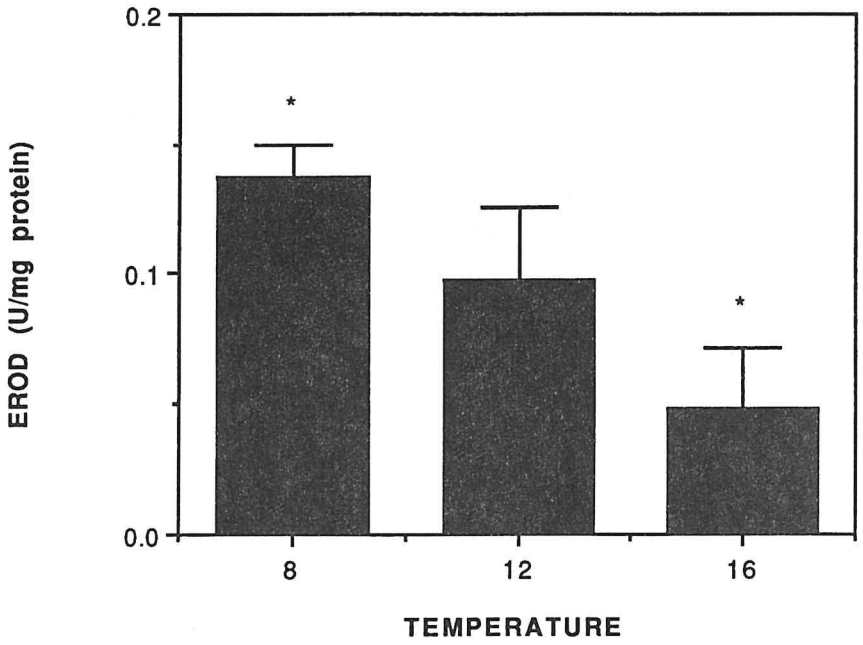

Figure 2. Hepatic ethoxyresorufin O-deethylase activity (U/mg protein) in dab (*Limanda limanda*) from the Doggerbank, kept at different temperatures in the laboratory, * significantly different from each other, $p<0.05$.

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INP-MICON PROGRAMME 1991-1992: CONCENTRATIONS OF PCBs AND PAHs IN DAB MUSCLE.

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Abstract: Specimens of 15-20 cm male dab (*Limanda limanda*) were collected by beam trawling at sampling locations along transects radiating into the North Sea from coastal areas of the Netherlands. Muscle tissue of the same specimens of dab that were used for measurements of biomarkers of biological effects was used for the analysis of PCBs and PAHs. When the numbers of dabs caught permitted, each sample contained muscle of five specimens from a single haul. Five of such samples were taken at each station. Based on former studies with flatfish, it was assumed that concentrations on a lipid basis would be highly similar in different tissues of each specimen.

The results of the first cruise of 1991 generally showed a much less steep gradient of PCB concentrations than expected on the basis of previous data in the polychaete worm *Nephtys spp.*. The concentrations of four congeners are reported (figs. 1-4). CB-153 was the dominant congener in all samples except those of station 17, which were enriched in tetra- and pentachlorobiphenyls. The concentrations of CB-153 were highest in the immediate vicinity of the Dutch coast near the 'Nieuwe Waterweg', the entrance to the harbour area of Rotterdam and part of the Rhine-Meuse estuary. The concentrations of three mono-*ortho* substituted CB congeners with a 'dioxin-type' toxicity could be determined on the microbore CPSil 19 column used for analysis: The highest concentrations of these CBs -105, 118 and -156 were found at station 17, located at the mouth of the Western Scheldt. The term 'dioxin-type toxicity' refers to the induction of cytochrome P4501A (CYP1A) via binding to the cytosolic Ah receptor protein. Induction of CYP1A was measured with the EROD-assay (Sleiderink *et al.*) and by ELISA (Beyer *et al.*) The lowest concentrations of CBs were found at stations 5 and 6 in the area of the Dogger bank.

The concentrations of several unsubstituted and alkylated PAHs were usually just above or below the limit of detection, probably due to biotransformation.

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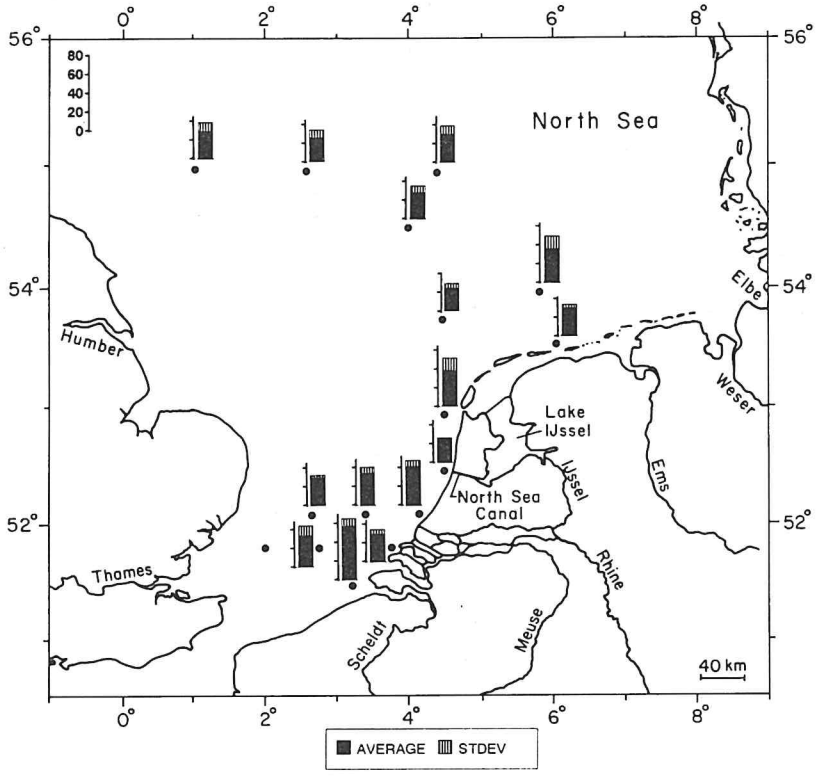
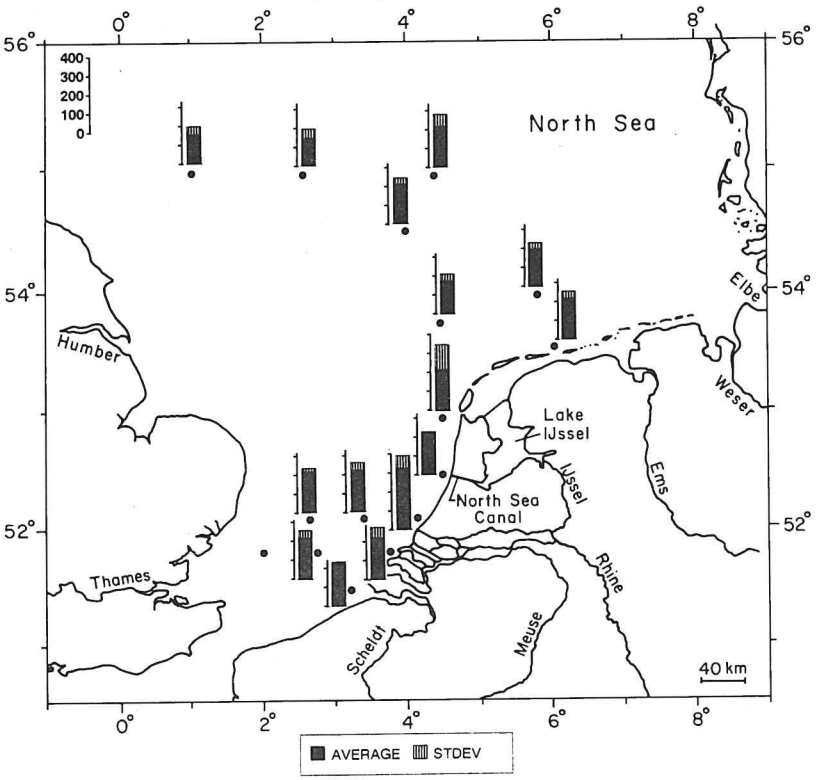
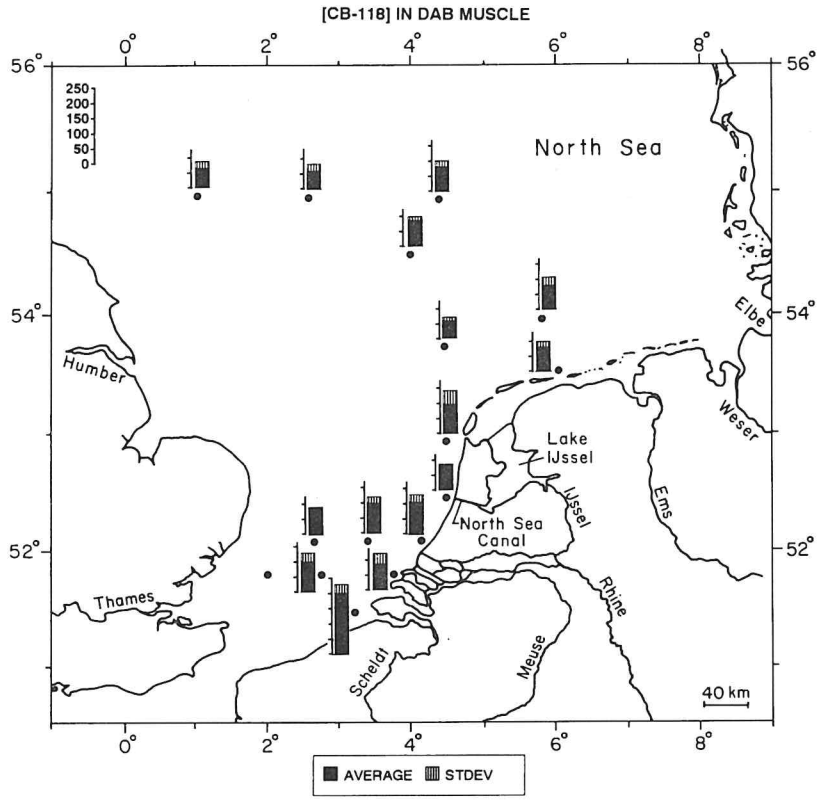
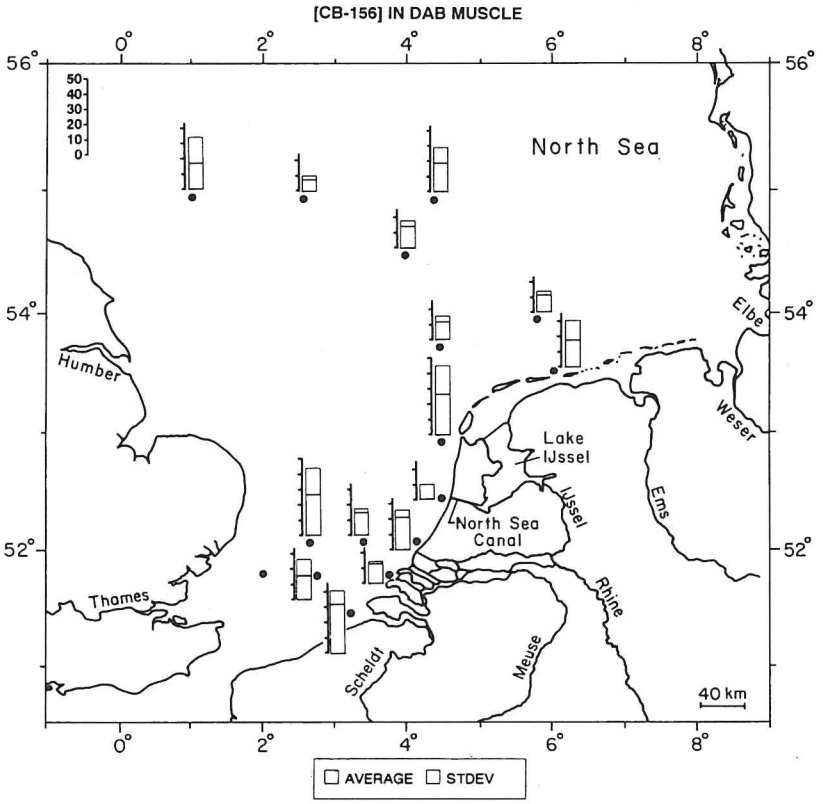
[CB-105] IN DAB MUSCLE



[CB-153] IN DAB MUSCLE




[CB-118] IN DAB MUSCLE
North Sea
Humber
Thames
Scheldt
Meuse
Rhine
Ems
Weser
Elbe
Lake IJssel
IJssel
North Sea Canal
40 km
AVERAGE
STDEV


[CB-156] IN DAB MUSCLE
North Sea
Humber
Thames
Scheldt
Meuse
Rhine
Ems
Weser
Elbe
Lake IJssel
IJssel
North Sea Canal
40 km
AVERAGE
STDEV

CONTENTS