Deep Chlorophyll Maximum 1996

Cruise Report

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The Deep Chlorophyll Maximum of the oceans:
persistence of the plankton community,
its biodiversity and its implication for carbon cycling

Hr. Ms. Tydeman
22 July to 31 August 1996

Contents

PREFACE

This cruise report is a summary of a wealth of data and information collected during a nearly 7 weeks cruise in the Atlantic Ocean. Although it may look like a long period the cruise it self is only a brief moment of true seagoing activity. The whole preparation of the DCM cruise took over 2 years. The idea of this project was even much older. It also will take several of the participants probably an other 2 years to analyse all collected samples and data.

The scientific reports and data incorporated in this cruise report should be considered as preliminary data and there will be an ongoing process of quality control. These data will be the backbone of the final data report. We would urge each participant to check and recheck your data before submitting them. Comments concerning the bottle files should be addressed to Margriet Hiehle.

On a regular base we will inquire about the status of your personal data set and the type of data you want to submit to the data report. The final version of the data report should be ready by the end of 1997.

When submitting data to the data report please contact Margriet Hiehle beforehand to make sure that the data are in the proper format. Essentially, the variables must be accompanied by the station, cast and bottle number. So, the fourth parameter is the variable of interest. e.g.

station	cast	bottle	chlorophyll
100	01	24	0.2

Within the next months there will be several small workshops with participants presenting data but there will be also emphasis on the interdisciplinary character of this cruise.

As far as the Dutch participants are concerned a hot-line is present. An email message for all can be mailed using the connection DCM@NIOZ.nl. A list of separate addresses is given in the Appendix.

Acknowledgements

We acknowledge a very good co-operation with the commander KTZ L. ter Haar and the crew of the Hydrographical Service of the Dutch Royal Navy. The service of the navy included a mail dropping by aircraft on 19/8. Other social highlights are described in the weekly reports (Appendix). The cruise and scientific program were supported by the Netherlands Geosciences Foundation (GOA) with financial aid from the Netherlands Organisation for Scientific Research (NWO) and additional funding of the Netherlands Institute for Sea Research (NIOZ).

INTRODUCTION

(H.G. Fransz and M.J.W. Veldhuis)

Scientific objectives of the Deep Chlorophyll Maximum Study

The cruise with RV Tydeman was devoted to study permanently stratified plankton systems in the (sub)tropical ocean, which are characterised by a deep chlorophyll peak between 80 and 150 m. To minimise lateral effects by horizontal transport of nutrients and organic matter from river outflow and upwelling regions, stations were selected in the middle of the North Atlantic Ocean between the continents of America and Africa. (5 - 35° N and 50 - 15° W). Here the vertical distributions of light and nutrients control the abundance and growth of autotrophic algae in the thermically stratified water column. This phytoplankton is numerically dominated by the prokaryotic picoplankters Synechococcus spp. and Prochlorococcus spp., which are smaller than 2 µm. The productivity of the 100 to 150 m deep euphotic zone can be high, because a high heterotrophic/autotrophic biomass ratio induces a rapid regeneration of nutrients and inorganic carbon. Primary grazers are mainly micro-organisms such as heterotrophic nannoflagellates and ciliates, which feed on the small algae and on bacteria. Heterotrophic bacteria can outnumber the autotrophic algae, because their number is related to the substrate pools of dissolved and particulate dead organic matter. These DOC and detritus pools reach equilibrium at a concentration, where the rate of their production (proportional to algal biomass) equals their mineralisation and sinking rate (proportional to the concentration and weight of POC and detritus). At a relatively low value of the weight-specific loss rates, the equilibrium concentration of these carbon pools and their load of bacteria can be high. The bacterial productivity is proportional to the mineralisation rate, which in a steady state can never be higher than the rate of primary production. Hence the ratio in turnover rate of bacteria and autotrophs tends to be reciprocally proportional to their biomass ratio.

In a microbial food web carbon and nutrients circulate between inorganic pools, autotrophs, bacteria, DOC and detritus, and the grazing microzooplankton. In (sub)tropical ecosystems changes in biomass tend to be small, but fluxes of carbon and nutrients between the various components occur to be high. Bacteria can stimulate primary production by mineralisation of DOC and detritus, but they also compete with the primary producers for nutrients. The microzooplankton reduce the number of algae and bacteria, but enhance their growth by regeneration of nutrients. This component, however, induces instability because it can graze down the algae to low levels and cause predator-prey oscillations.

The nutrient -algae-POC/detritus/bacteria complex by itself can reach a steady state when production and consumption of carbon and nutrients are in balance on a long term. In general these components, and changes therein, are considered to be mainly effected by the inverse light/nutrient (depth) gradient. However, evidence is increasing that the microbial food web shows diel production cycles as a consequence of diurnal fluctuations in light conditions (12light :12dark). But in a system with only nutrient limitation of production there will be no over-exploitation of resources. Where grazing keeps the algae below an equilibrium level, the system is potentially unstable. Stability will be improved when the grazing pressure on algae is released. This can be the case when either the microzooplankton feeds mainly on bacteria and detritus, or when it is kept low by larger predators in the mesozooplankton.

The mesozooplankton forms the link towards macrozooplankton and large marine invertebrates and vertebrates such as squids and fishes, which mainly at night rise to the euphotic zone to harvest the secondary production. Mesozooplankton can feed on phytoplankton, microzooplankton and the detritus/bacteria complex. Macro- and mesozooplankton are the only plankton components capable of active vertical migration. By predator-prey control at different trophic levels mesozooplankton can affect the stability of the system.

The main question to be answered is, why under rather stable environmental conditions in (sub)tropical seas and oceans the typical DCM structure occurs world-wide as a system with little temporal and spatial variation. What are the factors that prevent (large) oscillations in this global ecosystem? Once we understand the reasons for this stability, we are able to predict its response to global environmental changes and the role it plays with respect to carbon cycling in the ocean. The following aspects are studied:

1. What is the effect of the vertical light and nutrient gradients on species diversity within the euphotic zone?

2. What is the nature of the ecosystem stability in the DCM, how does it depend on phytoplankton, micro- and mesozooplankton and microbial trophic interactions, and how does it affect the carbon and nitrogen mass balance?

3. How does the conversion of phytoplankton-derived organic compounds by grazing activity of different size classes of zooplankton, microbial activity and vertical transport affect the export to the aphotic zone?

4. Is the diurnal rhythm in light conditions (12l:12d) responsible for a phasing in biological (growth/grazing) activity, and does this induce temporal variation in the different components?

To find some answers, five stations on a south-north transect were sampled to measure profiles of stocks and activities of the different system components and physical/chemical parameters of the environment to estimate state variables, transport rates and conversion rates. With this data a 1-dimensional ecosystem model will be implemented to study the consequences of adaptations and trophic interactions for the equilibrium and the mass balance of carbon and nitrogen.

Summary review of the cruise

Hr. Ms. Tydeman left Willemstad (Curaçao) on 22 July 1996 and, after conducting a test series for seagoing instruments on 25 July, a transect of CTD casts was carried out on the continental slope of Guyana on 26 and 27 July to study inorganic carbon exchange between the southern and northern Atlantic ocean at different water depths. A detailed study of the DCM was conducted at 5 main stations:

	geographic position	date
station 100	12N, 48W	29/7-02/8
station 200	14N, 40W	05/8-09/8
station 300	23N, 38W	11/8-15/8
station 400	34N, 35W	18/8-22/8
station 500	34N, 23W	24/8-28/8

Between the main stations a single CTD cast (400 m) and some optical measurements were made. The cruise track and station list are presented in the Appendix, as well as a list of scientific participants and the station programs.

Due to good weather conditions and the skilful assistance of Navy crew the cruise was very successful. All planned measurements and sampling schemes were carried out without any loss of material, equipment or sampling time.

Preliminary conclusions

A pronounced DCM was found at all 5 stations but each of them could be characterised by typical features (physical, chemical or biological) not present at the others. The first three stations (stations 100, 200 and 300) showed the DCM layer at a depth of 80, 100 and 130 m, respectively. Concurrently, the peak layer showed a decrease in the fluorescence signal and a broadening of the peak. In the following two stations (stations 400 and 500) sharp DCM peaks were found at a depth of 100 m.
With respect to the physical structure two processes were weakening the stability of the upper water column. The first was double diffusion prominently present at the first two stations. Internal waves, on the other hand, with an amplitude of over 30 meters were also found. Both these processes will effect the vertical flux of matter as well as the daily solar radiation of phytoplankton growing in the area of the critical depth. The optical profiles showed a pattern typical for open ocean water verifying the depth of the DCM. The highest abundance of particles however, was found above the fluorescence peak. Analysis of the particle distribution carried out with flow-cytometry confirmed this observation. Below a depth of 200 m the water column was extremely transparent indicating a low particle content.
As far as the nutrient distribution is concerned in the surface water layer, till the peak of DCM, NO3 was depleted. At the same time PO4 and NH4 were present in low but detectable concentrations. With depth the nutrient concentrations increased but not in an identical pattern for every station. Next to differences in absolute values also the N/P ratio varied. In the two Western Atlantic stations (100 and

200) and station 500 the N/P ratio was around 16. In the other two the ratio varied between 20 and 24. The phytoplankton abundance, as examined with flow-cytometry showed a numerical abundance of the prokaryotic phytoplankter Prochlorococcus in numbers up to 100,000 cells per ml. At all stations this species could be traced from the surface till a depth of 180 m. As a result of the decreasing light intensity pigmentation increased as could be derived from the increase in chlorophyll fluorescence. Synechococcus spp. were only dominant in the surface water layers. Typical populations of other pico-eukaryotes or larger phytoplankton were found around the DCM but dominated only over a limited depth range. Some of these algal groups were found below their critical depth since the fluorescence signal showed a decrease after an increase. This suggests that these cells have lost their ability to adapt to the prevailing light intensity and cells are about to metabolise their structural components (photopigments).
A high frequent sampling program showed that the Prochlorococcus cell numbers increased at the end of the day indicative of a diel rhythm. Phasing of the physiological activity was not restricted to phytoplankton only. Also bacteria showed daily changes in activity and growth characteristics. Time course measurements of bacteria to which different mixtures of organic carbon and nutrients (N and P) were added showed different growth characteristics. The organic carbon source was stimulating in particular bacteria growth in samples collected in the morning. This observation was supported by the fact that the incorporation of thymidine (indicative of DNA synthesis in bacteria) was pronounced in the early morning (04:00 AM to 07:30 AM). On the other hand the highest protein synthesis rate (leucine incorporation) was around noon. The different phasing of DNA and protein synthesis rates suggest an unbalance between activity and growth in bacteria as well. In some incubations bacteria stopped assimilating nutrient for several hours, but resumed this later. This daily unbalance of growth and physiological activity seems indicative for synchronisation of bacterial activity. A first impression of the grazing experiments showed only moderate grazing activity of microzooplankton (<200 (m) limited to the upper part of the DCM (range 0.1 to 0.2 per day). For the prochlorophytes these rates were in the upper part of the DCM equal to the growth rates of the species. At the peak and bottom of the DCM growth rates were 0 and grazing resulted in decrease in cell abundance.
A inventory of the larger organisms present (>50 (m) showed low numbers of net phytoplankton (few long diatom chains) but a relatively high number of mesozooplankton (200 to 1000 (m size class). These large grazers were mainly copepods in the upper 200 m (mean abundance about 0.2 per l). Next, also many protozooplankters we present.
Based on the respiration rates and fact that incubation experiments showed no significant grazing activity on picophytoplankton is must be concluded that the dominant food source for this mesozooplankton had to be microzooplankton rather than phytoplankton, although the exact nature of this source has to be determined..

CTD-profiles and Physical Oceanography

(C. Veth and M.A. Hiehle)

The CTD-rosette system is in a number of aspects an essential part of the DCM expedition. In the first place the CTD-rosette combination provides the water from different depths for biological and chemical studies and, secondly, the physical profiles determined with the CTD-system show the physical environment of the deep chlorophyll maximum.

The sensors attached to the CTD-system were for measuring conductivity, temperature and pressure (Seabird) , from which salinity and density were derived, and sensors for fluorescence (for the determination of chlorophyll concentration), for underwater PAR and for turbidity. The standard CTD-sensors worked very well during the whole cruise. It turned out, however, to be difficult to get enough samples for calibration of the salinity, because most cast were surface casts, not deeper than 400 m. The surface water is extremely inhomogeneous with strong vertical gradients and small steps. To overcome this problem of getting water for salinity calibration, a bottom cast was planned at each main station, but technical problems with the winch (cogwheel wear) made it necessary to go not too deep. Even at 2500 m most physical profiles were shown to be highly irregular and in fact unsuitable for salinity calibrations. The marginal salinity calibration, however, showed that the JGOFS recommendation for surface water was met. The fluorometer (Chelsea) used at the beginning of the cruise was replaced during the first main station, because of extremely bad performance. The turbidity meter turned out to be sensitive to temperature changes of the water and worked only well during the first 200 m of the downcast. An oxygen sensor was incorporated in the system.

On the rosette NOEX sampling bottles were used. These bottles are still an ergonomic disaster. Also continuous care is necessary to keep the system with all those tubes and balloons in good shape. Occasionally bottles seemed to have sampled the wrong type of water, even when the reversing pressure meter has given the right sample depth. Continuous determination of the macro-nutrient concentrations (nitrate, phosphate and occasionally silicate) in the samples was the best way to identify "wrong" bottles. It is clear that the NOEX bottles in the present state are not the final answer to the hydrographic sampling of water. The stepping motor of the rosette system also gave some problems that were solved in a satisfactory way. In the first part of the cruise strategically placed reversing thermometers and pressure meters offered the opportunity to reconstruct the tripping sequence of bottles.

First impressions on the Physical Oceanography during DCM

The DCM cruise started with a hydrographic section off the continental Shelf near Surinam. The salinity profiles showed very saline sub-surface water. Occasionally a salinity of almost 38 was found. The surface layer of a thickness of 10 to 20 m had a salinity that was much lower, even down to 32. This surface water apparently had a high concentration of river run-off from the South-American continent.

The backbone of the cruise were the five main stations in different biological provinces lasting 4 to 5 days with about 30 CTD casts per station. During the transfers between the stations one cast per day was done. Figure 1 and 2 show the profiles of a number of parameters of a, more or less representative, cast at each main station. The stations show large differences. Stations 1 and 2 are in the equatorial region with a clear rain surplus over the evaporation. In station 1 even some river water may be present. Although the surface salinity is high, the salinity maximum is near 120 m in both stations. In particular at station 1, weaker in the other stations, the step structure caused by double-diffusion processes was visible. The circumstances for these processes were clearly present at depths where increasing density caused by the increasing salinity towards the surface was compensated by the temperature distribution. Mixing processes related to double-diffusion may play an important role in the flux of nutrients from below the deep chlorophyll maximum. At station 3 the evaporation is apparently stronger than the precipitation. Casts taken during one whole day showed an enhancement of salinity in the upper meters in daytime that was mixed downward during the night by convective overturning caused by surface cooling. Stations 4 and 5 show gradually less saline profiles when going north. In the deep casts of stations 4 and 5 Mediterranean water was visible near 1000 m. The combination of all casts during the first one-and-a-half day at a station presents the relation between the vertical motions of the DCM and the physical structure of the water column showing internal waves (Figure 3). Amplitudes of several tens of meters have been observed and, although the number of casts per unit of time was not sufficient to resolve that, indications were found for breaking of the internal waves. These breaking waves will stimulate the vertical flux of matter.

Figures physical plots

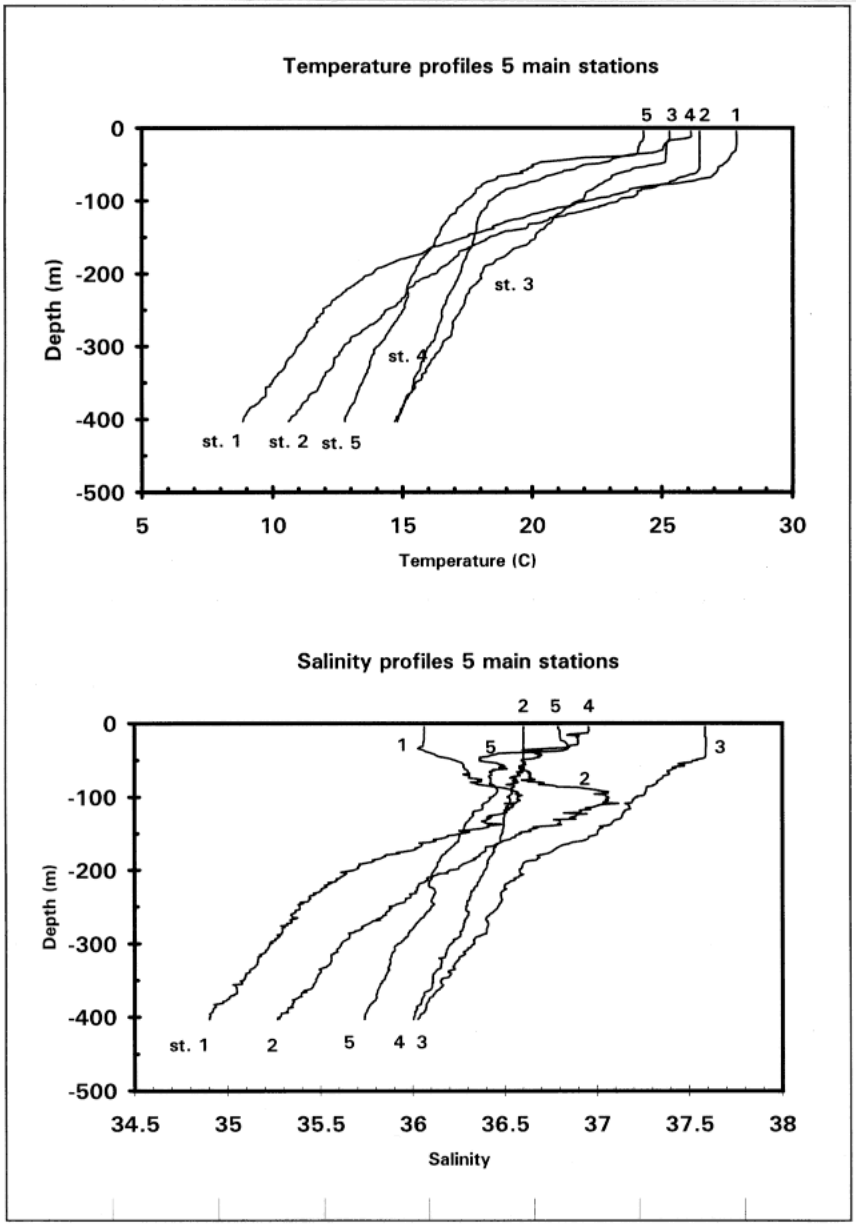


Fig. 1 Typical temperature and salinity profiles of 5 main stations of upper 400 m (numbers represent station numbers)

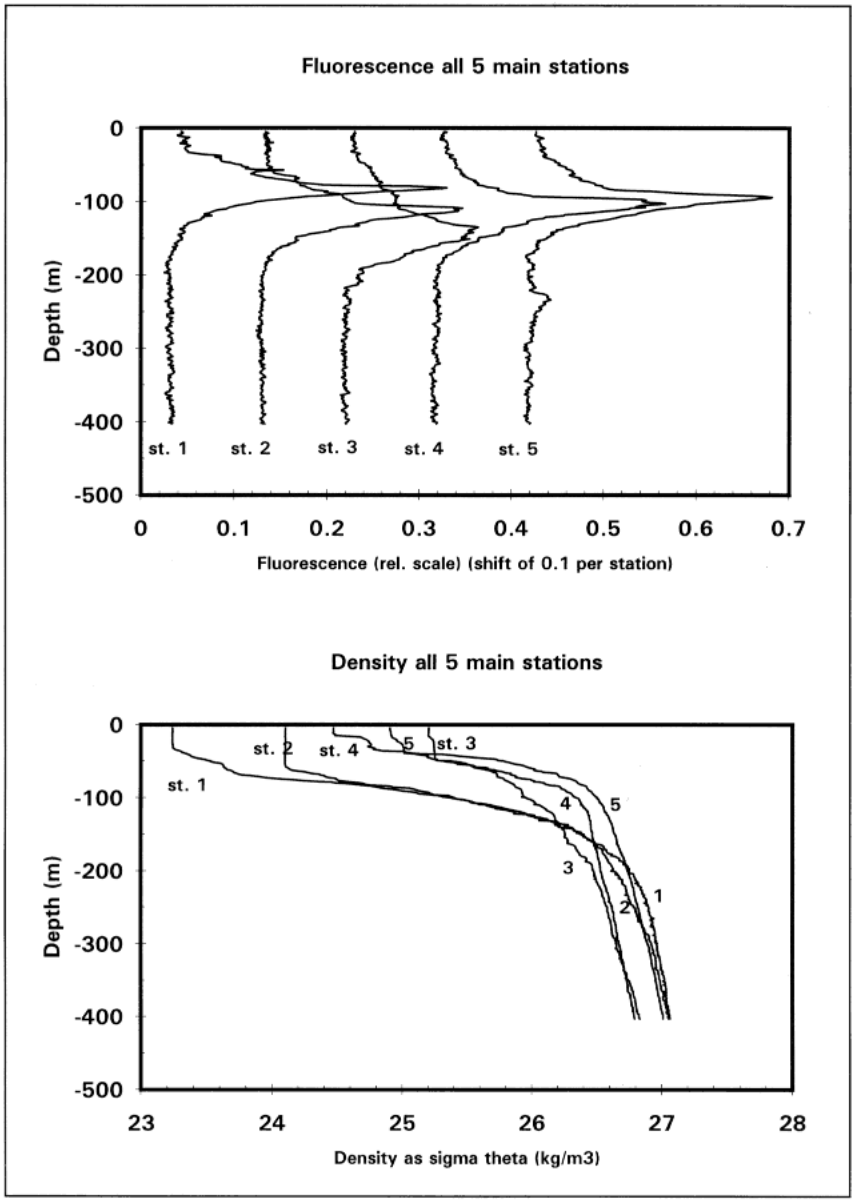
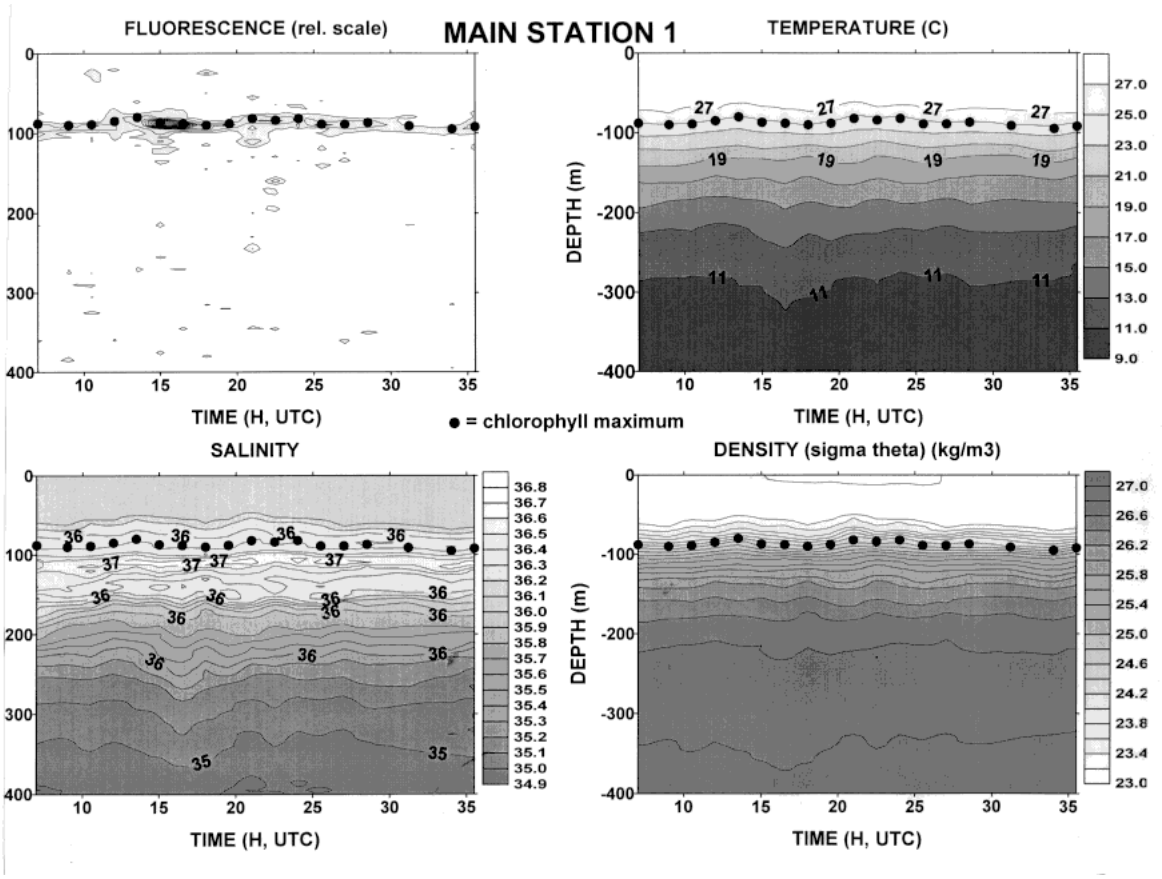
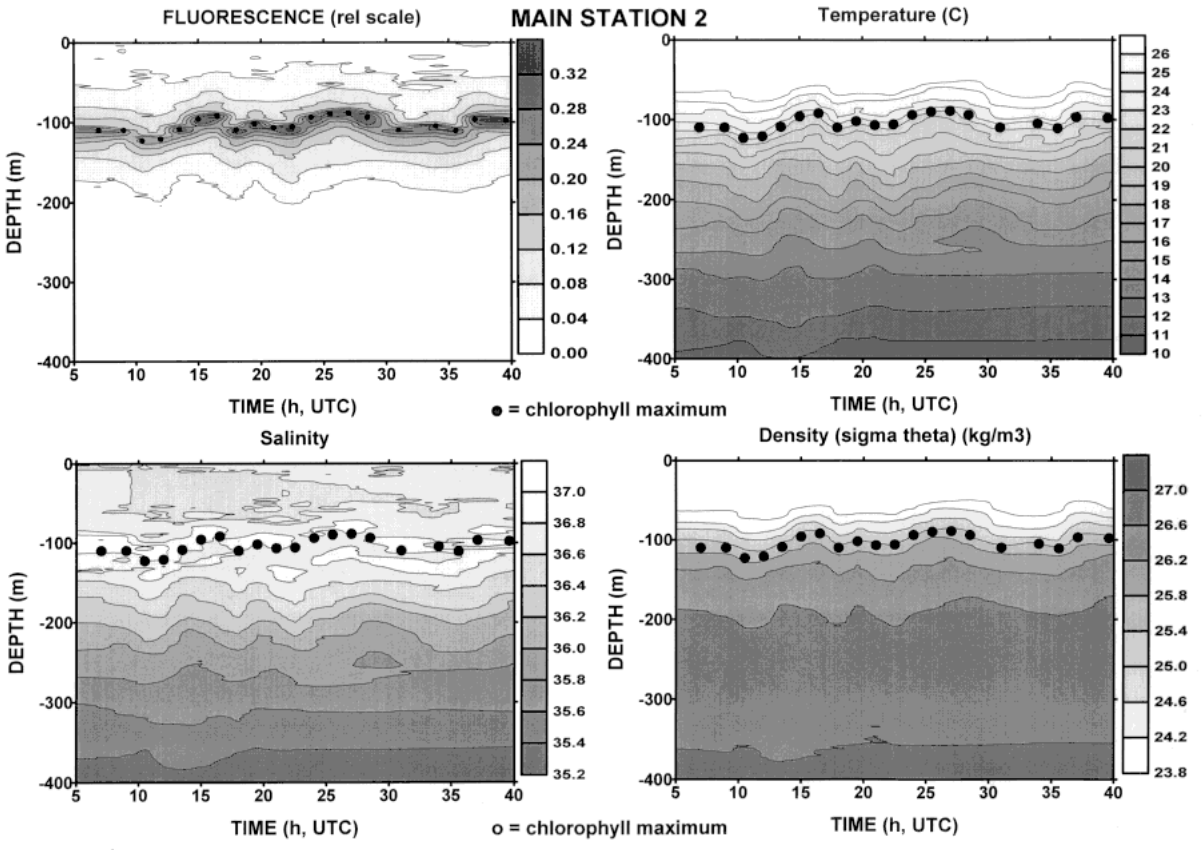
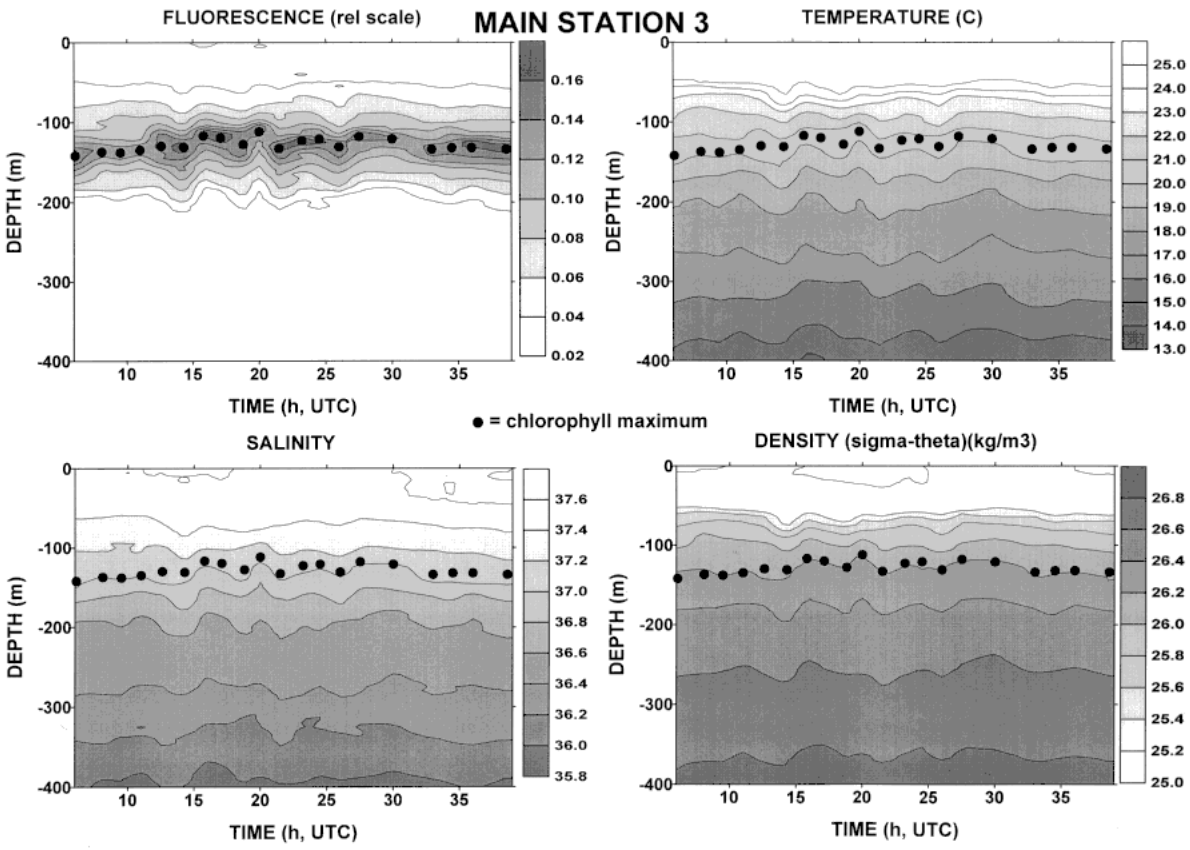
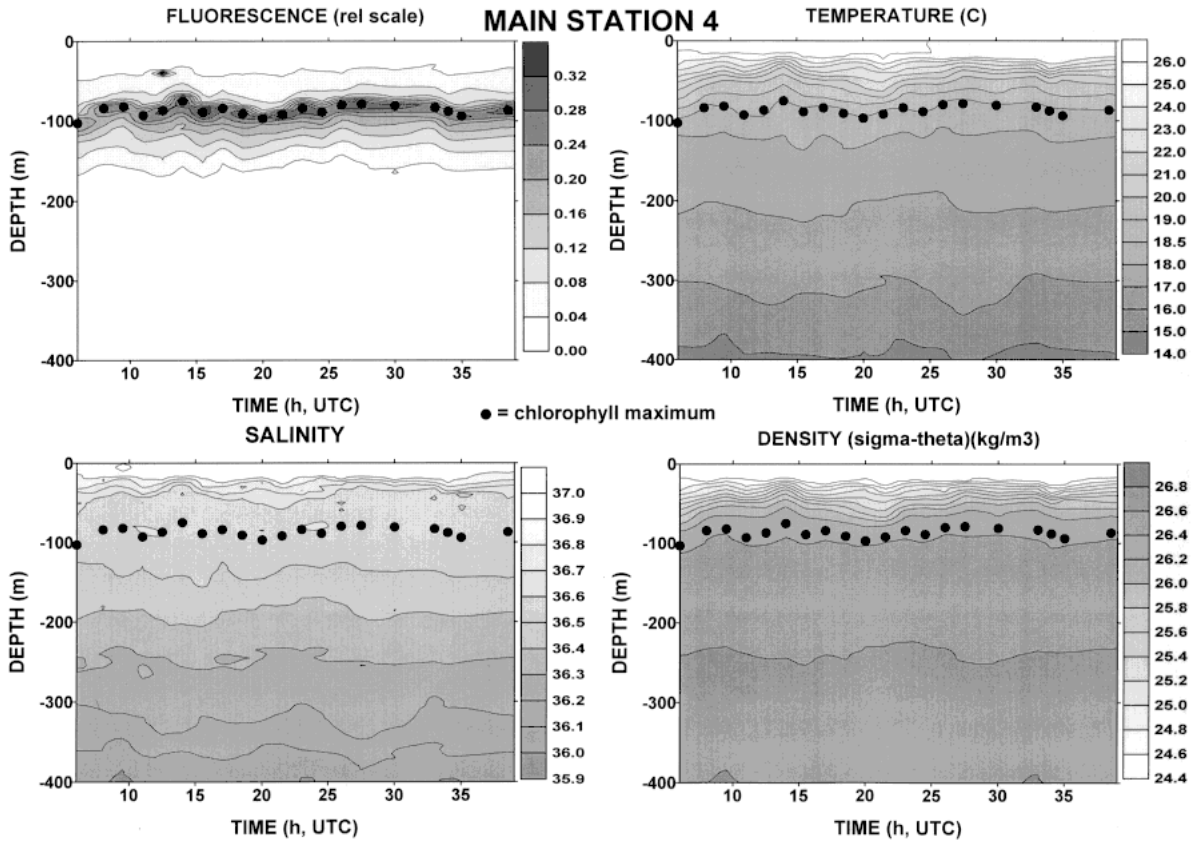


Fig. 2 as fig. 1 but for fluorescence and density.


FLUORESCENCE (rel. scale)
MAIN STATION 1
TEMPERATURE (C)
DEPTH (m)
TIME (H, UTC)
● = chlorophyll maximum
SALINITY
DENSITY (sigma theta) (kg/m3)


FLUORESCENCE (rel scale)
MAIN STATION 2
Temperature (C)
DEPTH (m)
TIME (h, UTC)
● = chlorophyll maximum
Salinity
Density (sigma theta) (kg/m3)
o = chlorophyll maximum


MAIN STATION 3
FLUORESCENCE (rel scale)
TEMPERATURE (C)
SALINITY
DENSITY (sigma-theta)(kg/m3)
DEPTH (m)
TIME (h, UTC)
● = chlorophyll maximum


MAIN STATION 4
FLUORESCENCE (rel scale)
TEMPERATURE (C)
SALINITY
DENSITY (sigma-theta)(kg/m3)
DEPTH (m)
TIME (h, UTC)
● = chlorophyll maximum

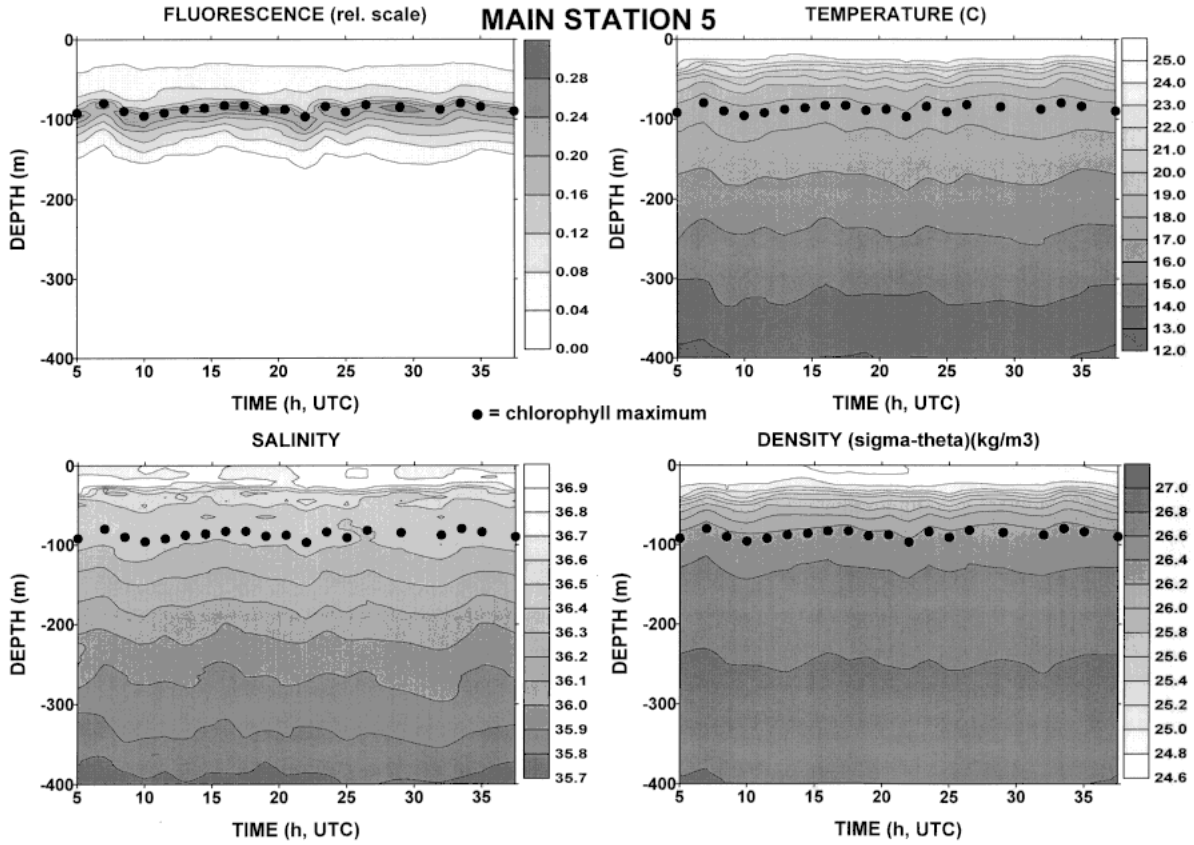


Fig. 3 A-E. Collection of CTD measurement (fluorescence, temperature, salinity and density) of first day of the main station. Isolines fitted to CTD casts taken with a frequency of 1.5 h over a period of 35 h. Dots indicate position of DCM peak.

Marine Optics en Remote Sensing

(Marcel R. Wernand)

Measurement of Inherent Optical Properties IOP's.

During all stations an in-situ absorption/transmissometer (AC-9) was deployed for the vertical tracing of i) pigmentlike matter and ii) all other particulate and dissolved matter. The first day of a station down cast and up cast profiles were made every 1.5 hours during 24 hours. The 4 following day's a single down and up cast profile was made. The instrument measures the absorption a in m-1 and the beam attenuation coefficient c in m-1 in 9 spectral bands in agreement with SeaWiFS (spaceborne ocean colour sensor) spectral bands. The instrument was recently developed by Wetlabs, Oregon, USA. Before this cruise the AC-9 was thoroughly tested during a 2 years program on the North Sea. For this cruise this instrument has been upgraded with a pump system and a depth sensor. The instrument generates direct in-situ profiles of a and c by means of a flow-through system. Profiles were measured throughout the euphotic zone up to 250 meters depth. It could clearly be seen that the chlorophyll maximum depth varied from 70 to 120 meters. The water mass below the depth of 200 meters was more transparent than the purest water that can be made at the laboratory nowadays (milliQ). Corrections for this phenomena will be applied in a later stage. Comparisons of the IOP's profiles will be made with Salinity profiles and with particle distribution determined with flow cytometry .

Fig. 4: Example of the down cast profile, station 304, of the AC9. The profile shows the absorption coefficient and beam-attenuation coefficient at 440 nm and 675 nm.

Measurement of Apparent Optical Properties AOP's.

During all stations from the second day until the fifth day at 13.00 GMT the water column was measured at different depth up to 200 meters with the Advanced Spectral IRradiance Meter (ASIR, NIOZ). Simultaneously this radiometer measures the down and up welling light (resp. Edown, Eup) between 400 and 720 nm in 22 spectral bands. Per depth the spectral diffuse attenuation coefficients Kdown, Kup and the spectral reflectance R- was then calculated. Underwater relationships between R and particulate and dissolved matter will be established.

Fig.5: Example of the down and up welling irradiance, the calculated Reflectance and the calculated diffuse attenuation coefficient by ASIR.

Above water ocean colour measurements were performed from the bridge of the ship 3 times a day including sailing days. Measurements of the down welling irradiance Ed+, upwelling radiance Lu+ and in some cases sky radiance Ld+ with the PR650 radiometer. The instrument measures between 380nm and 780 nm in 101 bands. The spectral R+ was then calculated. These measurements were specially performed for the validation of forthcoming ocean colour sensors. Together with the surface in situ values of total suspended matter and total pigment concentration ocean colour algorithms including primary production can be developed or given ones validated.

*Fig.6: Example of the down welling Irradiance, up welling radiance and the calculated reflectance R= 5*Lu/Ed above the water surface at station 300, cast 45 (by PR650).*

Ultraviolet radiation- its evaluation and some potential effects on the marine microbial community and its chemical environment

(Ingrid Obernosterer)

(1) - Evaluation of the underwater light regime with special concern to ultraviolet radiation
(2) - Spatial and diurnal dynamics of hydrogen peroxide
(3) - Measurements of the photochemical oxygen demand
(4) - DNA damage in phytoplankton and bacteria
(5) - DNA dosimeter
(6) - Sampling for the molecular analysis of the bacterial community above, in, and below the DCM

General Introduction

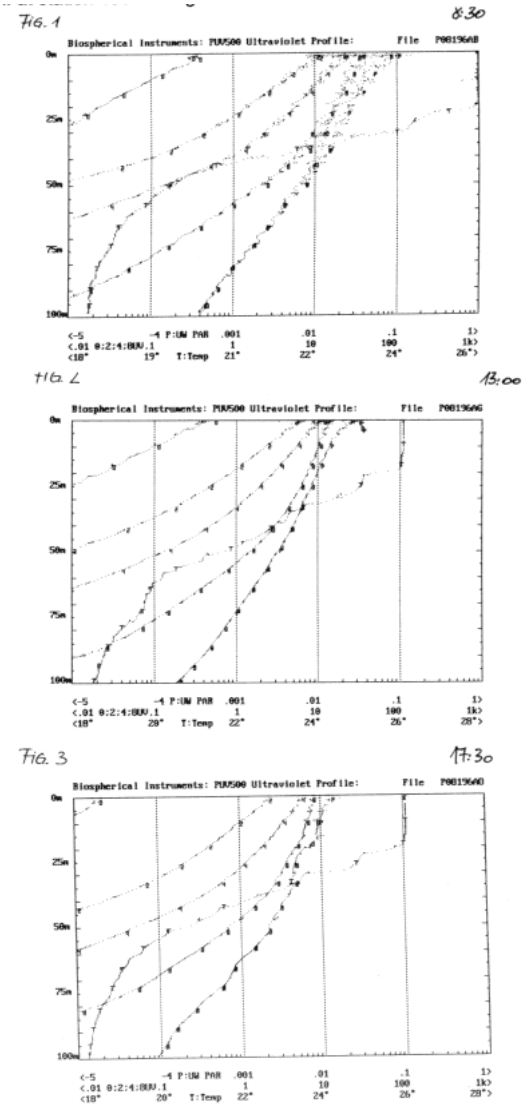
The increase in ultraviolet (UV)-radiation due to the depletion of stratospheric ozone has gained enormous attention during the past decade. The alteration of the chemical environment through the production of bioactive free radicals, such as hydrogen peroxide, the photode-composition of organic matter and thus the change in the availability of trace metals and inorganic nutrients can be regarded as processes affecting indirectly the biology of marine organisms. Among the direct potential effects of enhanced UV-B radiation on marine micro-organisms, DNA-damage through the production of thymidine dimers has been quantified recently. The goal of this cruise was to investigate the underwater irradiance spectrum with special concern to UV-radiation and to relate specific chemical and biological processes to the radiation environment.

(1) - Evaluation of the underwater light regime with special concern to ultraviolet radiation

A Profiling Ultraviolet Radiometer (PUV-500), consisting of a PUV-500 underwater unit and a PUV-510 surface reference sensor, has been used. The instrument measures the irradiance at 4 distinct wavelengths in the UV-B (305 nm, 320 nm) and UV-A (340 nm, 380 nm) regions as well as the integrated irradiance of the photosynthetic active radiation (PAR, 400 nm-700 nm).

(2) - Spatial and diurnal dynamics of hydrogen peroxide

Hydrogen peroxide (H2O2) is an important and seemingly ubiquitous photochemically generated compound. In natural surface waters, H2O2 is formed by secondary photochemical reactions involving light absorbing organic materials. Its stability relative to other compounds formed by solar radiation makes it a useful indicator of overall photochemical processes in the marine environment. Variations in H2O2 concentrations result from a kinetic balance of formative, destructive and input processes. Hydrogen peroxide has been measured most extensively in the gas phase and in rain and cloud vapour largely due to its role in atmospheric chemistry and in acid rain generation. Its distribution in surface waters is also of great interest and has recently received significant attention as an indicator of photochemical activity.
On this cruise, the spatial and temporal distribution of hydrogen peroxide was examined and related to UV-profiles in order to investigate the role of UV-radiation in the formation processes of H2O2. At each station, time series and vertical profiles of H2O2 were collected at 10 different depths (0 m-120 m) for 48 h every 1.5 h. Hydrogen peroxide concentrations were measured with a Jasco spectrofluorometer using the enzyme catalysed dimerization of p-hydroxyphenylacetic acid.
Preliminary results show that the average H2O2 concentration at noon is about 100 nM at 0 m, decreasing to 10 nM at 100 m depth. Time series have shown an increase in H2O2 concentration of up to 180 nM at 3:00 p.m. at 0 m, while the concentration increased from 40 nM at 7:30 a.m. to 70 nM at 3:00 p.m. at 40 m.



Figures 7A,B,C show typical depth profiles taken at 8:30 p.m., 1:00 a.m. and 5:30 p.m. at station 400 on August 19th.

(3) - Measurements of the photochemical oxygen demand

Sunlight photolyzes organic compounds in surface waters of the marine environment, destroying some substances and synthesising others. Such reactions may proceed at high rates and have biological, chemical and geological implications. The rates of photoreactivity are determined by the sunlight spectrum and intensity, the absorption spectrum of the chromophore and the efficiency with which chemical change results from excitation of the chromophore (quantum yield). It is proposed that photochemical processes have a net oxidative character in seawater, and that such reactions may provide one sink for organic molecules in the marine environment. Quartz-glass bottles were filled with 0.2 'm-filtered seawater from 10 m depth. In order to elucidate the role of UV-B versus UV-A radiation in photo-oxidation processes, cut-off filters (Mylar-D, 320 nm) were wrapped around the quartz-bottles. The incubation was done for 48 hours in an on-deck water bath at in-situ temperature. Simultaneously, 0.8 'm-filtered water was incubated in the dark.

Photochemical and biological oxygen consumption was then measured by the Winkler-method, applying the spectrophotometrical method to determine the oxygen concentration.

(4) - DNA damage in phytoplankton and bacteria

Intracellular absorption of UV-B radiation can be detrimental to bacteria, phytoplankton and other marine organisms. The goal of this experiment was to quantify UV-induced thymidine dimers in DNA using fluorescently-labeled antibodies targeted against thymidine dimers. Samples from two different fractions (bacteria: 0.2 - 1 (m, phytoplankton 1 - 10 (m) have been taken at several depths and times of the day. According to the UV-profiles, freshly collected seawater from 0, 5 and 15 m was filtered onto 10 (m, 1(m and 0.2 (m filters at 4:00 a.m., 1:00 p.m., 4:00 p.m. and 7:00 p.m. at each station. To avoid repair of the damaged DNA, the filtration was done as quickly as possible with the three different filters 'in line', followed by freezing the filters at -80 -C. The filters will be further processed according to a protocol developed at the Department of Marine Biology, University of Groningen.

(5) - DNA dosimeter

At each station, bare DNA was incubated in quartz tubes at several depths from sunrise to sunset. The amount of thymine dimers produced in the DNA correlates with the biologically effective dose received. According to the ultraviolet radiation levels, duplicate quartz tubes (10 cm) were incubated at 1, 2.5, 5, 15, 30 and 50 m depth. The incubation was performed on the same day as experiment (4).

Experiments (4) and (5) were conducted in co-operation with Anita Buma and Peter Boelen from the University of Groningen, Dept. of Marine Biology.

(6) - Sampling for the molecular analysis of the bacterial community above, in, and below the DCM

In order to determine variations in the composition of the bacterial community at different depths of the water column, samples were taken for PCR and RFLP analysis using a newly-developed fluorometric HPLC-technique to quantify the DNA fragments.
At each station, samples from 20, 200 m and, according to the CTD-profiles, from the deep chlorophyll maximum layer were taken.
The freshly collected water samples were immediately 0.8 'm-prefiltered, then filtered onto 0.2 (m filters which were frozen at -80oC.
The filters will be processed by Markus M"seneder at the Department of Marine Biology, University of Vienna.

Nutrient analysis:

(K. Bakker)

Some 3000 CTD samples were taken for nutrient analysis. The CTD-NOEX bottles were first sampled for nutrients before other samples were taken, because ammonia is sensitive for contamination. The samples were directly poured into polyethylene bottles, stored cool at 4º C and filtered within 6 hours over 0.2 µm pore size and immediately capped with a sheet of parafilm. The samples were analysed within 24 hours after filtration for ammonia, phosphate nitrate and nitrite, the last two in a medium and a high sensitive range (see statistics) because of depletion above the deep chlorophyll maximum layer.

Furthermore some 1500 samples were analysed from different bacteria experiments.

I want to thank Ruud Groenewegen for assisting with filtering at least 50% off the samples.

Methods:

All nutrients were measured on a Technicon Autoanalyzer system "TRAACS 800" using the following colorimetric methods:

Phosphate:

Phosphate was measured, as the blue colloid complex formed with ammonium molybdate using potassium antimonylartrate as a catalyst and reduced with ascorbic acid, at 880 nm.

Ammonia:

Ammonia was measured during the first part of the cruise (first two stations) by dialysing the NH3 as a gas, by adding NaOH in citrate medium (to prevent precipitation of Ca and Mg salts), through a dialysis-membrane to a second acidified-stream; where it is detected by the classic phenol-hypochlorite method. The colour is measured at 630 nm. During the second part of the cruise, problems with the dialysis necessitated to measure the NH4 directly with the phenol-hypochlorite method.

Nitrite and Nitrate:

Diazotation of nitrite with sulfanilamide and-(1-naphtyl)-ethylene diammonium dichloride to form a pink coloured dye measured at 550nm. Nitrate is first separately reduced in a copperized Cd-coil using imidazole as a buffer and is measured as nitrite.

Silicate:

Silicate is measured as the blue reduced silicomolybdenium complex at 810 nm. Ascorbic acid is used as the reductant and oxalic acid is used to prevent interference of the phosphate molybdenium complex.

Preliminary results:

Above the DCM, NO3 was depleted (below detection limit) while PO4 was still present (ca. 0.02 (M). NH4 was still available at all stations at a level off 0.15(M. At the peak of the DCM a NO2 peak was observed just 5 meters below this Below the DCM the nutrient values of stations 100 and 500 differed from the other three. Other changes were observed in the N/P ratio of the nutrient rich water below the DCM. A "normal" Redfield ratio for P:N is about 16 and was observed for the stations 100, 200 and 500 but 24 to 20 for station 300 and 400, respectively.

Dissolved oxygen by Winkler spectophotometry

(G.W.Kraay)

During this cruise 500 dissolved oxygen samples as duplicates or replicates have been taken in order to calibrate the O2sensor of the CTD. At the 24 hours day-stations every CTD have been sampled at two depths on the oxygen maximum and minimum. Further on most CTD cast's on the transfer between the stations; every oxygen production cast and also all the deep calibration cast. The analysing was according the method from Su-Cheng Pai(1993) modified by G.W Kraay and J v Bennekom.

We have chosen for this technique because of the analyse time (40 samples per hour) the precision; the reliability, and also the more independence of the bottle volume. The only thing you need is a high quality spectrophotometer equipped with a wide bore volume flowcell. We used the Hitatchi U1000 spectrophotometer with on the analog output a four digit voltmeter.
The calibration curve was made on the NIOZ lab and the empirical coefficients K was 0.0005455 $\mu mol_{-1} cm_{-l}$ for 456nm and 0.0126 $\mu mol_{-1} cm_{-l}$ for 355nm. The last one is used for measuring the seawater-blank and the reagents-blank.

Calculation:

(O_2)
µmol = mV*(1/(2*k*d))*(Vb+Vc)/(Vb-Vr)

mV = reading voltmeter.
K = empirical coefficient
Vb = bottle volume
Vc = volume sulphuric acid
Vr = volume Winkler reagents

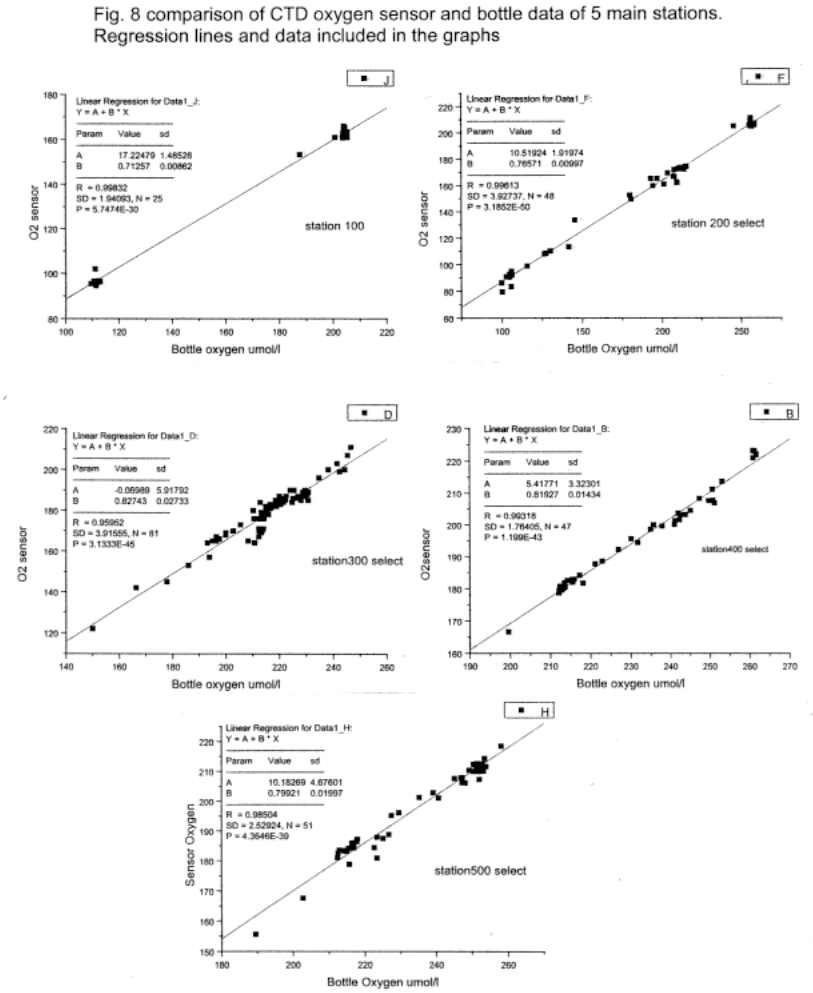
Data recorded in the data report are corrected by subtracting 1.05 (Mol/l for the oxygen in the pickling reagents. No correction was made

for the seawater and reagents-blanks.

Some results:

Reagents blank 0.32 µMol/l
Seawater blank 0.36±0.1 µMol/l for samples deeper then 100 m and 0.16±0.1 µMol/l for samples in the euphotic zone.
The reproducibility: 55% of the duplicates had a CV smaller than 0.1% and by 83% of all the samples was the CV smaller than 0.2%.

Fig. 8 comparison of CTD oxygen sensor and bottle data of 5 main stations.
Regression lines and data included in the graphs



Dissolved and particulate organic carbon

(Jan Hegeman)

On all the large stations, samples were taken for DOC and POC on different days and times. The samples for DOC were filtered over polycarbonate filters with a pore-size of 0.2 micron. The POC-samples were filtered over Whatman glassfibre filters with a pore-size of 0.7 micron. Initially the DOC-samples should be measured onboard the ship using an automatic instrument, but because the instrument did not perform well, possibly caused by power problems on the Tydeman, another method was used. The samples were transferred in triple to sealed ampoules with phosphoric-acid and persulphate, following the method of Menzel and Vaccaro, for later measurement at the institute on Texel. Furthermore extra samples were sealed with only phosphoric-acid for HTC-measurement. The filters of POC were also sealed in ampoules following the same method of Menzel and Vaccaro. Samples were always taken at six depths.

Sediment-traps:

(Jan Hegeman)

On all large stations where POC and DOC was sampled, free floating sediment-traps were released, suspending from buoys on two depths. One set of traps was deployed at 300 m, the other set just below the chlorophyll-maximum. After 24 hours the traps were taken on board and the sediment was collected on filters, which were stored deep frozen for analysis of organic carbon and nitrogen at the NIOZ on Texel. The traps were released again for the next 24 hours. This was continued for every day on the stations.

Phytoplankton pigments.

(G.W.Kraay, B. Kuipers and S Oosterhuis)

In order to estimate the algal pigments by HPLC large volumes (20 l were filtered over 47mm GF/F filters by over-pressure. On every station at the first day the water column was sampled from 180 to 10 m divided in about10 depths intervals.
The filters were immediately frozen by -80(C for analysis later at the laboratory. The extraction and separation will by done by the method described in Kraay et al. (J.Phycol.28:708-712)

Comparison of the Fluorometers used for the Chlora extraction method

(G.W.Kraay and B. Irwin)

Since two fluorometers were used on the cruise: one from the NIOZ and one from Bedford Institute both calibrated independently. Comparison of both instruments was necessary. At two occasions 1 litre and 0.1 litre of seawater was filtered and extracted in 10ml 90 % acetone and this solution was measured at the same time on both fluorometers.

Results:

no	vol (l)	chlora(NIOZ) (microg/l)	phaeo(NIOZ) (microg/l)	chlora(Bedford) (microg/l)	phaeo(Bedford) (microg/l)

1	1	0.191	0.261	0.189	0.196
2	1	0.126	0.208	0.122	0.164
3	0.1	0.113	0.288	0.138	0.193
4	0.1	0.153	0.205	0.174	0.131

Chlorophyll extraction.

(G.W.Kraay, H. Bouman, S. Oosterhuis, H. Witte and B.R. Kuipers)

Samples for the chlorophyll a extraction were taken of almost every CTD cast. Ten depths were chosen over the water column from 10 to 200m. One litre was filtered over a 45mm GF/F filter by low vacuum pressure. All the filters were stored in the min 800C freezer before analysing on board. Except for the filters from station 5 those are analysed on the lab two months later.

Extraction was done following the procedure from Holm-Hansen, et al. (J.Conseil, Conseil perm. Intern. Exploration Mer, 30: 3, 1965) and (Strickland and Parson: A practical handbook of seawater analysis. Fisheries Research Board of Canada).

The filter was extracted in 10 ml 01% acetone and measured twice first Rb and after adding one drop 10% HCL for the second time Ra.

Turner designs 10AU fluorometer was calibrated with HPLC pure Chlor a.

Concentrations were calculated by the following formula's:

(g chlorophyll a/l = Fd(af/af-1)(Rb-Ra)
(g phaeopigment/l =Fd(af/af-1)(af*Ra-Rb)

Where Fd is the calibration factor and af the acid factor.
Calibration February 1996: FD= 1.89
AF=2.31

Bacterial production, microbial diversity, and methanogenesis in the deep-chlorophyll maximum

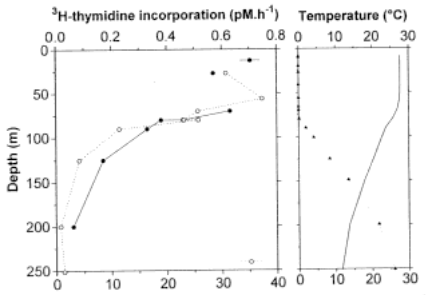
(M. J. E. C. van der Maarel)

During the 1996 Deep-Chlorophyll Maximum (DCM) research cruise on the central Atlantic Ocean three different aspect of the microbiology of the DCM has been studied. The first aspect was concerned with the bacterial production, based on the incorporation of radiolabelled thymidine, which is incorporated into newly synthesized DNA, and leucine, which is incorporated into newly synthesized proteins. By using conversion factors reported in the literature the amount of carbon produced by bacteria can be calculated. Samples from ten different depths were taken at every major station. Preliminary results show that the bacterial production ranged from 48.7 mg C/m2/day to 114.7 mg C/m2/day. At station 200 and 400 samples were taken at four different times of the day (04:00; 07:30; 12:30; and 19:30 local time) to see whether the bacterial production showed a diurnal cycle. Preliminary results indicate that thymidine incorporation was the highest between 04:00 and 07:30. Leucine incorporation, on the contrary, showed a maximum in the early afternoon. A similar experiment was done by incubation of a water sample, which was taken from the chlorophyll peak of the DCM, at a light intensity of 11% of the photosynthetic available radiation in the deck incubator and measuring leucine incorporation every 2 hours during a 24 hour period. The leucine incorporation in this experiment showed an increase starting at approximately 12:00 and was at its maximum at 22:00. A similar incubation in the dark did not show an increase in the leucine incorporation.

The second aspect of the microbiology of the DCM that was studied was the possible presence of a methane maximum in the top of the DCM and the possible presence of methanogenic archaei, the microorganisms responsible for the formation of methane. The underlying thought is that in many oceans a supersaturation of methane can be found at a certain depth that also shows a high concentration of oxygen. Since methanogenesis is an obligately anaerobic process this phenomenon has been called the oceanic methane paradox. To see whether active methanogenesis and a methane maximum exists in the DCM of the central Atlantic Ocean samples were taken to measure the concentration of methane and dimethylsulfide, a well known precursor of methanogenesis that is derived from the algal osmolyte dimethylsulfoniopropionate. These samples will be analyzed in the laboratory. Also enrichment cultures using seawater samples supplemented with vitamins, micro nutrient, and monomethylamine as a substrate were setup to look for the presence of methanogens. The seawater samples were taken above, in, and below the DCM at station 100, 300, and 500.

The third aspect is an investigation into the microbial diversity of the DCM, compared to the microbial diversity above and below the DCM. At every major station 200 l of seawater was sampled and subsequently filtered over 8.0 mm and 0.8 mm and finally concentrated using cross-flow filtration over a 0.1 mm filter (Durapore, Millipore). From these samples DNA and RNA will be extracted for further molecular analysis based of the microbial diversity with special emphasis on methanogens and marine archaea, a recently discovered group of microorganisms.

Fig. 9 Typical vertical profiles of Thymidine and leucine incorporation in bacterial communities of the 5 main stations (left). In the right part temperature and nitrate profiles are shown.



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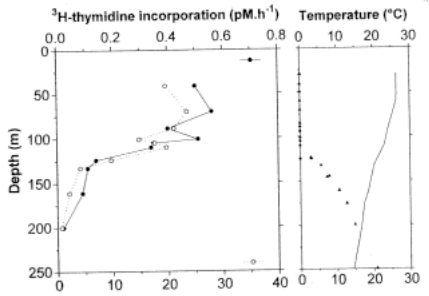

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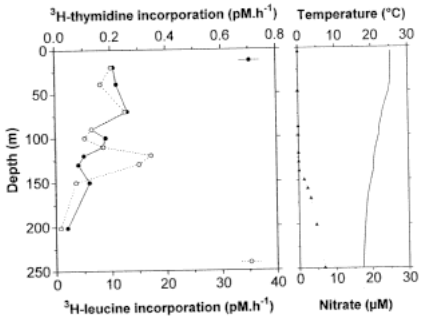

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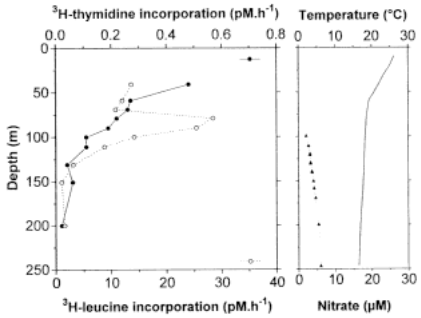

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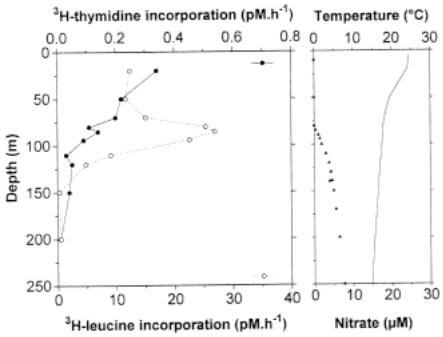


#500-06

Microbial biomass and activities at five stations in the Atlantic ocean during the Deep Chlorophyll Maximum cruise

(G.J. van Noort in co-operation with J.H. Vosjan)

Introduction

We studied the microbial biomass and activities in the Deep Chlorophyll Maximum layers at the five main stations in the Atlantic Ocean. The distribution of the bacteria and flagellates were analysed at five depth in the water column. The bacterial number in the upper water layers were estimated too.

We expected that the Deep Chlorophyll Maximum is in a P and N limited condition. In this situation bacteria, formerly thought to be mineralisers, can be N and P consumers if there is enough dissolved organic C as energy and C source. In this way bacteria could be competitors for P and N of phytoplankton. Only together with bacteriovores (heterotrophic protozoa) the N and P, incorporated in the bacteria, will be excreted to the environment and will become available again to phytoplankton and bacteria. That's why also bacterial growth and nutrient uptake have been studied at three depth (upper, middle and lower level) of the Deep Chlorophyll Maximum layers.

Methods

The microbial biomass is calculated from counted number of the micro-organisms and the measured volumes. The bacteria and flagellates have been filtered on a 0.2 µm nuclepore filter. They have been stained with acridine orange for bacteria and with proflavine for flagellates, and fixed on slides and stored in a deep freeze. Later on they will be counted and measured at the laboratory by the epifluorescence microscope technique. Incubation experiments have been executed with water samples from three depths at every station. The growth rate of bacteria and the uptake rate of nutrients like, ammonia, nitrate and phosphate have been measured in these experiments. The effect of additions of P, N and organic C is followed in incubation experiments with unfiltered and over 0.6 µm filters prefiltered seawater (to remove algae and protozoa). These bioassay experiments were executed to follow the effect of nutrient additions on the bacterial growth and uptake kinetics. Several hundreds of nutrient samples have been analysed by K. Bakker.

Preliminary results

Experiments with water from the D.C.M. layer (upper, middle and lower level) showed that addition of C,N and P immediately increased the uptake of the C,N and P and did increase the bacterial numbers. This occurred in the unfiltered and prefiltered (over 0.6 (m filter) samples. This directly proved that there is a shortage of degradable organic carbon and that the bacteria consume N and P. Sometimes a small increase of ammonia was seen at the end of the experiment when nitrate and phosphate had been consumed. Samples enriched with P and N showed only a small uptake of N and P from the water. This means that bacteria were limited by organic carbon in the water. This mostly happened only in the upper layer of the D.C.M. and when the experiment was started at 12 o'clock in the morning. When such an experiment at the same station was started at 6 o'clock in the morning, there was no response at all on the N and P additions. Even after 48 hours, as long as the incubations lasted, no effect was seen. This probably means that the organic carbon was almost taken up by the bacteria during the night. This happened at several stations.

In some experiments bacteria stopped taking up nutrients for several hours, but resumed this later. Perhaps a rhythm in bacterial growth, synchronic divisions or a period of lysis which submitted organic materials could be an explanation for this phenomenon. This rhythm of nutrient consumption through the day could be triggered by the limiting factor organic matter. This organic material is produced by the diel rhythm of primary producers. Dividing rhythms in bacteria, if they grow synchronously, can also cause periodic P and N uptake. These possibilities will be studied when the bacterial numbers and biomasses in the preserved samples have been analysed.

Phytoplankton dynamics

(Marcel J. W. Veldhuis & Gijsbert W. Kraay)

In order to study the phytoplankton dynamics (in situ and during incubations) on 24 h light/dark cycle different types of experiments were designed.

1) Tracing in situ cell abundance. This was done by a high frequent sampling strategy (14 times over 24 h light/dark period) of the upper 300 m of the water column. CTD data were collected to assay changes in the physical structure of the upper water column (internal waves). Bottle samples were collect for chlorophyll and species composition (flow cytometry). Since cell abundance is subject to drifting water mass's and therefore patchy distribution of the phytoplankton samples were also taken van DNA-cell-cycle analysis. The advantage of the DNA-cell-cycle method is that this assay estimates the growth rate independent of the cell numbers present and is therefore not sensitive to changing water masses.

2) Phytoplankton biomass (whole and species selective) will be estimated using either the cell size distribution, chlorophyll fluorescence or DNA cell concentration. These three independent parameters will be compared with total POC and chemical measured chlorophyll values. Besides phytoplankton bacterial biomass will be calculated based on microscopically- and/or flow cytometrically counts.

3) In a different set of experiments (deck incubator) changes in cell abundance (phyto- and bacterioplankton) were traced over a light dark period experiments which ran parallel to 14C primary productivity. These experiments are carried out at 7 depths mimicking the light regime of the whole euphotic zone. Primary productivity will be compared with changes in cell biomass. A comparison of both data sets will be used to estimate the carbon turnover rate and to calculate the actual amount of primary production converted in plant biomass. Since grazing activity in these small bottles (ca. 250 ml) is included this approach only gives a net change in the plankton biomass. In combination with the DNA-cell-cycle it is possible to calculate the gross growth rates. From these two parameters the grazing activity can be derived.

4) Simultaneously, changes in the cell fluorescence will be measured to trace adaptive response of the phytoplankton cell to day to day and light/dark cycle. Selective changes in light intensity (shift-up or shift down) of the samples is thought to give insight in rates of adaptation on the level of the photosynthetic activity as well as growth responses. Hence, changes in community structure within the time span of a single day. The optical properties will be compared plant pigment composition and spectral properties of the upper water column.

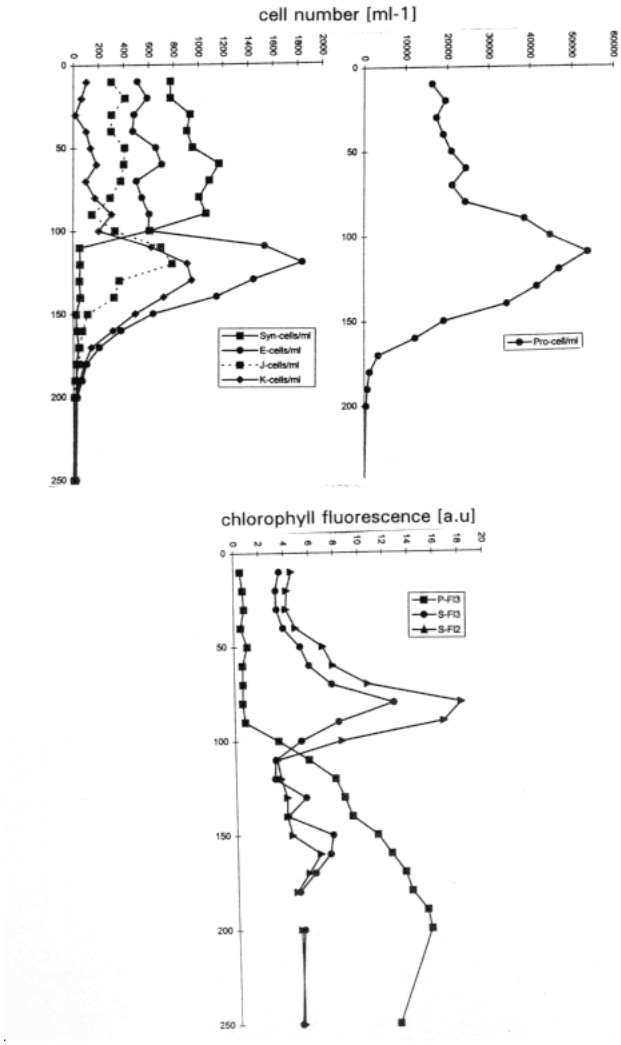


Fig. 10 Vertical profiles of cell numbers of different groups of phytoplankton (Prochlorococcus, Synechococcus, total eukaryotes (E), pico-eukaryotes (K) and larger phytoplankton (J), all number per ml. Changes in Chlorophyll fluorescence signal of phytoplankton with depth.

Flow-cytometry

(Marcel J.W. Veldhuis, Gijsbert W. Kraay, Harry Witte)

Algal Flow-Cytometry (AFC) was used as a technique for a rapid analysis of the phytoplankton composition and numbers using small sample volumes (<1 ml). After calibration the relative values for size, scatter and chlorophyll fluorescence can be determined of selective groups of phytoplankton.

The instrument used was a Coulter XL-MCL equipped with a carousel (32 vials). This standard bench top flow cytometer is equipped with a 15mW air cooled laser 488nm. Cell properties (scatter and autofluorescence) were measured with special detectors. Since the instrument in its standard configuration is not sensitive enough, the hard ware was upgraded. To enhance the sensitivity of the instrument the pressure of the sheath fluid was lowered. Next, sidescatter (SS) was measured in PMT1. PMT2 was used to detect the PE-fluorescence of Synechococcus type of cyanobacteria or PE-containing eukaryotes (575 nm BP). PMT3 was used to collect the chlorophyll fluorescence (>630 nm).

All parameter values were transformed in a logarithmic scale (4 decades). The instrument was optimised using monospherical beads (3.06 µm in diameter). The whole sample analysis was stored as a listmode file for post-processing at NIOZ. Instrumental sample flow rate was calibrated on a regular base but turned out to be remarkably constant (ca. 120-130 µl/min). Accuracy of counting: replicate measurements showed that variation in cell numbers was in the order of 2% (n=5)

The advantage of direct analysis is that insight of the phytoplankton composition can be given within a few minutes using whole untreated life samples. As a result set-ups for growth or grazing experiments can be adapted or changed if desired. Furthermore, the prokaryotic phytoplankter Prochlorococcus, in particular at surface waters, can only be detected in life samples. Preservation negatively effects fluorescence signals and causes cell rupture. Since this species was in particular abundant (often >80% of total cell numbers), on-line analysis turned out to be a wise decision.

Life samples were counted usually within 1 hour after sampling and kept on melting ice in the dark to prevent light damage or changes in cellular properties due to storage. Next to life samples, small volumes (2 ml) were preserved with paraformaldehyde (0.5% final solution) at -80º C for post-analysis of DNA of phytoplankton and bacteria at NIOZ.

The instrument was used to analyse phytoplankton samples of the CTD casts and experiments:

1) the continuous 24 hour CTD-rosette sampling (Day 1)
2) whole community incubations in the deck incubator subject to different incident irradiances (Day 2)
3) grazing experiments of microzooplankton (Day2, 4,5)
4) grazing experiment of copepods (Day 2, 4 and 5)

Primary production

(G.W.Kraay and S Oosterhuis)

During this cruise samples were taken for primary production at each station 3 times for the 14C production and one to two times for the oxygen community production and respiration. On the stations 4 and 5 additional samples were spiked with H2O18 to measure the real gross production.

Sampling was done at the first morning CTD cast at four o'clock, eight depths were sampled for the 14C method and six depths for the oxygen light-dark method. These depths were calculated from the optical PAR sensor measurements from the day before. The PAR sensor was mounted on the rosette sampler. In the sampling strategy special attention was given to the fluorescence peak

Duplicates of the acid cleaned 250ml 14C polycarbonate bottles were spiked with 5 to 15 (Ci (14C) bicarbonate, depending on expected production. For the oxygen method three to four replicates were taken each for the initial the dark and the light values. The H2O18 was done in 250ml oxygen glass bottles, two for the incubation and two for the initial isotope ratio.

All these kind of bottles were deployed on a free floating rig at about six o'clock local time, at the same depth of sampling. coolbox. At six o'clock in the afternoon, just before dark. The dark incubations and the night-time of the 24 hours incubations were stored the in a coolbox. The 14C samples were filtered over 47mm GFF filters at low overpressure. Overpressure was used because of the high depth of incubations, this gives often gas oversaturation in the water and increases the filtration time. Filters were fumed with damp of fuming HCL in order to remove inorganic radioactive bicarbonate and the possible calcification of some algae species. The filters were stored in scintilationflasks in the minus 80 freezer, for analysing later in the lab.
The oxygen bottles were analysed on board by the Winkler spectro- photometry method, described in an other paragraph. The samples for the H2O18 gross production were conserved with a iodide solution and stored under water in a coolbox for analysing later in the lab where the gases will be stripped from the seawater and the 18/16 ratio of the dissolved O2 can be measured with a isotope mass spectrometer.

The phytoplankton C14 production will be estimated using the following equation:

(Mol C/l= (((dpmL1+dpmL2)/2-dpmD)/added dpm)*1.05*2080.

Whereas dpmL1: radioactivity in terms of disintegration's per minute of light bottle 1
dpmL2: the same but for light bottle 2
dpmD: the same but for the dark bottle
1.05: the discrimination factor for C14/C12
2080: Concentration used for the total inorganic C in (Mol/l

For the oxygen community production and respiration: Net O2 production = the difference in the measured dissolved O2 concentration of seawater before and after the 12 hours incubation. Gross production = The difference in the measured dissolved O2 concentration in the light and the dark bottles.

O-18 Gross O2 will by calculated by the method M.L Bender et al.

Reference: The carbon balance during the 1989 spring bloom in the North Atlantic Ocean, 47oN,20o. Deep sea Research. Vol. 39, No 10. pp 1707-1725, 1992

Primary Production / New Production

(Brian Irwin and Heather Bouman)

PI Experiments

Discrete water samples were collected at pre-selected depths in the euphotic zone by the rosette sampler. At transit stations two depths in the mixed layer (10 m and 40 m) were sampled. On day 1 of each of the long stations water from a single depth was collected every 3 hours from 0400 to 1930 hours. Selected depths were 10m at station 100, 30m at station 200, 60m at station 300, 90 m (deep chlorophyll maximum) at station 400 and 70 m at station 500. On subsequent days on the long stations two samples at 10 m intervals were collected twice a day to get a complete profile of the euphotic zone.

Primary production was estimated by the 14C method. A total of 30 light bottles for each experiment were incubated for 3 hours in a light gradient. At the end of the experiment the phytoplankton was collected on GF/F glass fibre filters for later counting at the Bedford Institute of Oceanography (BIO).

For each PI experiment samples were also collected for chlorophyll, particulate carbon and nitrogen, pigments by HPLC, absorption spectra and inorganic nutrients. All samples except chlorophyll and nutrients will be analysed at BIO.

table of PI Experiments

Date	Station #	Cast #	Depth
26/07	002	01	10, 40
	004	01	10, 40
27/07	009	01	20, 50
	010	01	20, 50
28/07	013	01	10, 40
29/07	100	01	10
	100	06	10
	100	11	10
	100	15	10
	100	19	10
	100	23	10
30/07	100	33	5, 20
	100	41	30, 40
31/07	100	46	50, 60
	100	52	70, 80
01/08	100	59	90, 100
	100	65	110, 120
02/08	100	72	40, 90
03/08	104	02	10, 40
04/08	113	01	10,40
05/08	200	01	30
	200	06	30
05/08	200	11	30
	200	17	30
	200	21	30
	200	26	30
06/08	200	35	5, 20
	200	42	30,40

07/08	200	50	50, 60
	200	55	70, 80
08/08	200	64	90, 100
	200	66	110, 120
09/08	200	79	100, 130
10/08	202	02	10, 40
11/08	300	01	60
	300	06	60
	300	11	60
	300	17	60
	300	21	60
	300	26	60
12/08	300	35	5, 20
	300	43	30, 40
13/08	300	50	50, 60
	300	56	70, 80
14/08	300	61	90, 100
	300	69	120, 140
15/08	300	76	120, 160
16/08	304	02	10, 40
17/08	306	02	10, 40
18/08	400	01	90
	400	06	90
	400	10	90
	400	16	90
	400	20	90
	400	24	90
19/08	400	33	5, 20
	400	43	30, 40
20/08	400	52	50, 60
	400	58	70, 80
21/08	400	63	90, 100
	400	72	110, 120
22/08	400	81	70, 94
23/08	405	01	10, 40
24/08	500	01	70
	500	06	70
	500	10	70
	500	16	70
24/08	500	20	70
	500	24	70
25/08	500	32	5, 20
	500	43	30,40
26/08	500	52	50,60
	500	58	70,80
27/08	500	63	90, 100
	500	72	110, 120
28/08	500	81	50, 83

In Situ Experiments

Water samples were collected from 8 depths in the euphotic zone by the rosette sampler. Primary production was estimated by the addition of 13CO3 to 1L aliquots from each depth in 1L polycarbonate bottles. New production was estimated by the addition of 15NO3 and 15NH3 to each bottle. The bottles were incubated in situ at their respective depths from dawn to dusk. Phytoplankton were then filtered onto GF/F glass fibre filters for later analysis at BIO. These experiments were carried out on days 2, 3 and 4 of each of the long stations.

Table of In Situ Experiments

Date	Station #	Cast #	Depths
30/07	100	33	5, 10, 30, 45, 60, 75, 85, 95
31/07	100	46	5, 10, 30, 45, 60, 75, 85, 95
01/08	100	59	5, 15, 40, 55, 75, 85, 90, 105
06/08	200	35	10, 30, 60, 80, 100, 110, 120, 140
07/08	200	50	10, 30, 65, 82, 90, 100, 125, 135
08/08	200	64	10, 30, 65, 82, 90, 100, 125, 135
12/08	300	35	15, 35, 60, 80, 100, 115, 130, 150
13/08	300	50	15, 35, 60, 80, 100, 110, 120, 150
14/08	300	61	15, 35, 60, 80, 100, 110, 120, 150
19/08	400	33	10, 22, 43, 75, 88, 100, 120, 130
20/08	400	52	10, 22, 43, 75, 88, 100, 120, 130
21/08	400	63	10, 22, 43, 75, 88, 100, 120, 130

25/08	500	32	20, 40, 60, 70, 80, 90, 100, 110
26/08	500	52	20, 40, 60, 70, 80, 90, 110, 130
27/08	500	63	20, 40, 60, 70, 80, 90, 110, 130

Grazing of microzooplankton on picophytoplankton, nano-flagellates and heterotrophic bacteria in the tropical Atlantic DCM.

(B.R. Kuipers and H.J. Witte)

The chlorophyll-a maximum at low light intensities just above the nutricline at 70-120 m depth in the tropical Atlantic suggests a niche for a micrograzer community especially adapted to life in this surrounding. The most obvious function of the protozoan grazers is to effectuate (next to the heterotrophic bacteria) the local nutrient regeneration which allows for ongoing regenerated production in the DCM. At the same time the micrograzers function as the trophic link between the predominantly picoplanktonic DCM autotrophs and mesozooplankton. This leads to faecal pellet production and downward nutrient export from the DCM. Because modelling of the dynamics of the plankton community in the DCM as a function of light and nutrient availability along the vertical is one of the objectives of the present project, special attention was given to a quantitative field study of the DCM micrograzers. At all five stations of the tropical Atlantic DCM cruise, microzooplankton abundance and composition were studied in the vertical, whereas in situ and shipboard grazing measurements were made including size fractionation of grazers as well as prey.

For a quantitative description of the microscopic grazer population 100 ml microzooplankton samples were collected at a series of depth and preserved in 1.5 % acid lugol solution for later enumeration of the dominating 20 - 200 µm groups by inverted settlement microscopy. 5 ml glutaraldehyde preserved and proflavine stained samples were concentrated on Sudan black stained 0.2 or 0.4 µm polycarbonate filters for epifluorescence microscopically counting of the HNAN's and heterotrophic bacteria. 2 ml samples were preserved at -80oC in 4% paraformalin for later (experimental) flowcytrometrical counting of heterotrophic bacteria after staining (see Veldhuis and Kraay).

For the quantification of grazing on different components of the phytoplankton and especially its pico size-fraction, shipboard dilution series were incubated in fourfold in 300 ml polycarbonate bottles, starting with natural water from just below / in / just above the chlorophyll peak (as located during CTD downcast) and diluted with the same water filtered through GF/F and 0.2 µm filters to 100, 70, 40, 20 and 10 % of the natural concentration. The incubations (slowly rotating bottles in light and temperature conditions as naturally as possible) started at sunrise with T=0 sampling of total chlorophyll, microzooplankton and HNAN density in 100% bottles, from which T=0 values for al other dilution's were calculated. Flowcytometrical counting of cyanobacteria and prochlorophytes (quadruple) and bacterial sampling was done in all 100, 70. 40, 20 and 10 % incubation bottles at T=0.
Additional to this, a 100 and 40 % dilution experiment was done with natural water very carefully sieved (by gravity) through 10 µm, 5µm, 3 µ and 1 µm polycarbonate filters, in order to unravel the predator-prey size relationships. In this size fractionated set T=0 measurements comprised flow-cytometry, bacteria and HNAN's in all bottles and microzooplankton only in 100%. All measurements including the microzooplankton fixation were repeated at the end of the 24 hr light period; at T=12 all bottles were sampled for flow-cytometry. After analysis this set of data will provide estimates on gross growth rates (minus autotrophs respiration) and grazing rates for all size groups both for day and night.

Because estimation of in situ gross- and net growth rates (the principle of the applied Landry & Hasset dilution method) of the highly adapted picophytoplankton in the severely light limited DCM is most sensitive for the experimental light-regime, in situ incubations completed the grazing study. These were done in 80 ml polycarbonate bottles attached to the 14C in situ incubation string at the original depth of the water sample in order to ensure accurate low light conditions. These incubations were lowered before sunrise and recovered after sunset. Duplo incubations of 3, 2 and 1 µm sieved natural water diluted to 100, 50 and 20 % were made; flow-cytometrical measurements and bacterial and HNAN sampling were done at T=0 and T=24, flow-cytometry in all bottle at T=12.

Preliminary impressions of microzooplankton grazing could till now only be obtained from the shipboard flow-cytometry, which yielded prochlorophyte growth rates from the same incubations. Grazing rates on prochlorophytes were in the order of µ= 0.1 - 0.2 per 24 hrs, whereas prochlorophyte growth rates were 0 below and also in the chlorophyll peak, whereas they exceeded the grazing rates at the depth of maximal primary production (Oxygen-maximum) just above the Chl-a peak.Dilution had its most clear effect in the 3 µm filtered incubations; in the coarser samples there were obviously more trophic levels present, whereas under 1 µm only prochlorophytes and other prey remained. This means that the grazers feeding on prochlorophytes must be sought in the 1-3 µm size range and are most likely small heterotrophic flagellates (HNAN's). Grazing of microzooplankton on larger phytoplankton, HNAN's, bacteria, cyanobacteria and prochlorophytes, and grazing of HNAN's on bacteria and cyano's will be known after analysis of all samples. Grazing of microzooplankton by mesozooplankton was studied by Fransz and Gonzalez.

Mesozooplankton

(George Fransz and Santiago Gonzalez)

The animal plankton in the size range of 200 to 2000 µm can be separated from the usually smaller bacteria, algae, microzooplankton and detritus particles by filtering with a 200 µm screen. These animals feed on algae, microzooplankton and POC and can be important as participants in the food web and for the mineralisation and sedimentation (in fecal pellets) of particulate matter. The work on board was directed to obtain data on 1) abundance and vertical distribution of stocks and 2) on activities such as grazing, respiration and egg production in the different depth layers related to the DCM.. Sampling and incubation experiments were carried out at each of the 5 main stations around noon and around midnight to be able to study effects of diurnal migration and activity patterns.

Stocks

Two series of depth stratified samples were obtained per station with a Hydrobios multinet fitted with 5 nets with 50 µm mesh width. The series were obtained by oblique haul of the towed net. The first series started at 400 m depth and concerned the 5 discrete depth layers between 400 m - 300 m - 200 m - underside DCM - upper side DCM - 50 m - surface. A second series of 5 layers with a more detailed depth resolution in the biologically active zone started at the underside of the DCM and covered three zones in the DCM (lower, peak and upper zone), and two depth layers between upper side DCM - 25 m - surface. At each station both series were obtained once at noon and once at midnight. Because the depth sensor of the multinet did not function well, intervals of the temperature output were used to indicate the depth layers according to temperature profiles provided by the last CTD cast before the net tow. The CTD profiles of chlorophyll were used to indicate the position of the DCM.

The net samples were split with a Folsom plankton splitter. One half was preserved in 4% buffered formaline for numerical and species analysis. This half also includes organisms between 50 an 200 µm, mainly small sized cyclopoid copepods which can be predominant in tropical waters, and eggs and juvenile stages which can indicate the productivity of the species concerned. The other half was filtered over 2000 µm, 1000 µm and 200 µm screens to obtain the size classes 200 to 1000 and 1000 to 2000 µm. Both size classes were split into two halves. One of these halves was filtered over a tared GF/C glass fibre filter to determine AFDW (ash free dry weight), the other half was subsampled to adequate size and filtered on a small GF-F filter to determine carbon and nitrogen weight with a CHN analyser.

Throughout the cruise photographs were made with a microscope or macroscope camera of remarkably shaped organisms and predominant copepod species to prepare colour slides.

Activities

During each station a 200 l water sample was collected in the morning with the rosette sampler in three subzones of the DCM zone: at the chlorophyll peak, above the peak and below the peak. Per day one subzone was sampled and at station 500 the lower subzone could not be sampled due to lack of incubation time. Four 20 l glass jars were filled with the water and connected in such a way, that two jars could be sampled for oxygen with immediate replenishment with water from the other jars. In this way two couples of jars formed two set-ups for respiration experiments. To both jars of one of the couples the 200 to 2000 µm size fraction of mesozooplankton from 60 l of water was added, carefully concentrated by siphoning off through a 200 µm gauze. In this couple the mesozooplankton concentration was 4x the natural concentration. Both set-ups were placed in a container at ambient water temperature as controlled by air conditioning. Samples were collected 0h, 6h, 12h and 24h after incubation to measure oxygen, and 0h, 6h and 24h to measure chlorophyll, algal cell

number and microzooplankton concentration. Respiration and grazing rates can be estimated from relative decreases of these variables in the jars with added zooplankton. Oxygen was measured on board by a Winkler titration method developed by G. Kraay (see ....). Algal cells were counted by flow cytometry. The microzooplankton will be analysed later in the laboratory by methods used by B. Kuipers and H. Witte (see ....), while the chlorophyll will be measured later by spectroscopical methods. At the end of the experiments 5 to 10 l of water was filtered to count the zooplankton (mainly copepods). This sample was filtered subsequently on a GF-F filter for CHN analysis.

On each station the upper layer from the underside of the DCM to the surface was sampled with a 200µm WP-2 vertical net to collect alive zooplankton during 3 days at noon and at midnight. Adult females of predominant copepod species were picked out and placed in 1 l glass beakers to be incubated for 24 h. After incubation eggs and fecal pellets were counted. Gauze bottoms in the beakers prevented ingestion of eggs and pellets. Fecal pellets and females were filtered separately for CHN analysis.

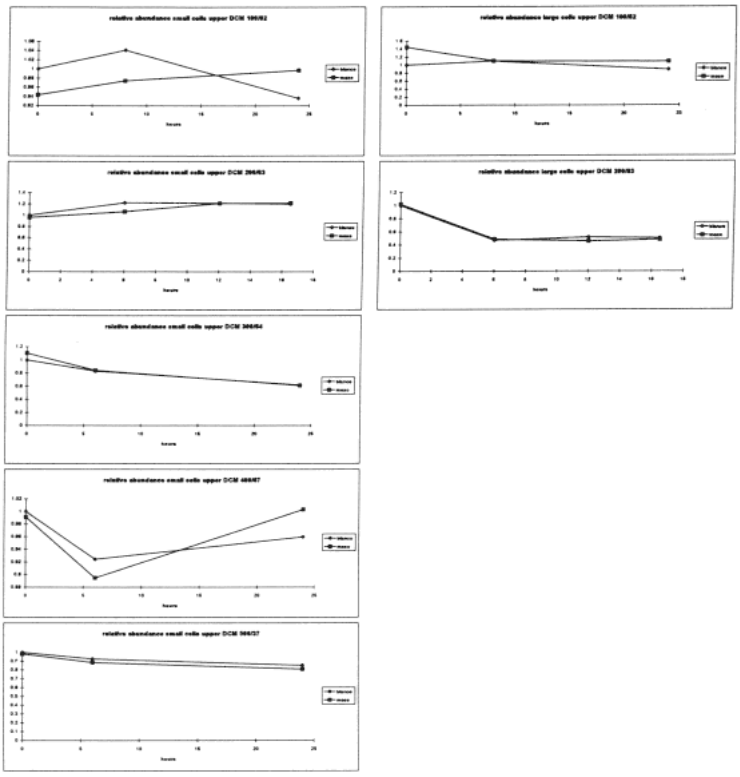
Preliminary results

Visual inspection of the filters for dry weight gave an impression of mesozooplankton abundance. The filters suggested that mesozooplankton was present at all stations with the highest abundance in the water layer between the surface and the peak of the DCM. Below the DCM the abundance was very low. Biomass seemed to be dominated by the 200 to 1000 µm size class, mainly consisting of copepods in a high species diversity. Quantitative data will be obtained by analysis of the samples in the home laboratory. The 50 µm net samples contained mainly zooplankton and only low numbers of net phytoplankton (mainly long diatom chains).

In the grazing and respiration experiments algal cell abundance was monitored on board with the flow cytometer and dissolved oxygen was measured with the Winkler method. Two clusters of algae were counted: small cells representing the predominant prochlorophytes and (if present as a separate cluster) a cluster of somewhat larger and more fluorescent cells (large cells). The following Figures present the changes of relative algal cell concentration (the concentration divided by the initial concentration in the untreated water) and dissolved oxygen concentration for the 5 stations in the upper, middle and lower part of the DCM. The variation in algal abundance was consistently similar in natural water (blank) and water with 4x increased density of mesozooplankton (meso). Hence there was no indication of increased grazing in the zooplankton enriched jars. The simplest explanation of this result is that the cells counted by the flow cytometer were not eaten by the mesozooplankton.

During most incubation experiments the oxygen concentration decreased faster in the jars with added zooplankton than in the control jars. This difference may be used to estimate weight specific respiration rates. The following table gives estimates of the oxygen consumption per day of the mesozooplankton.

Figures mesozooplankton



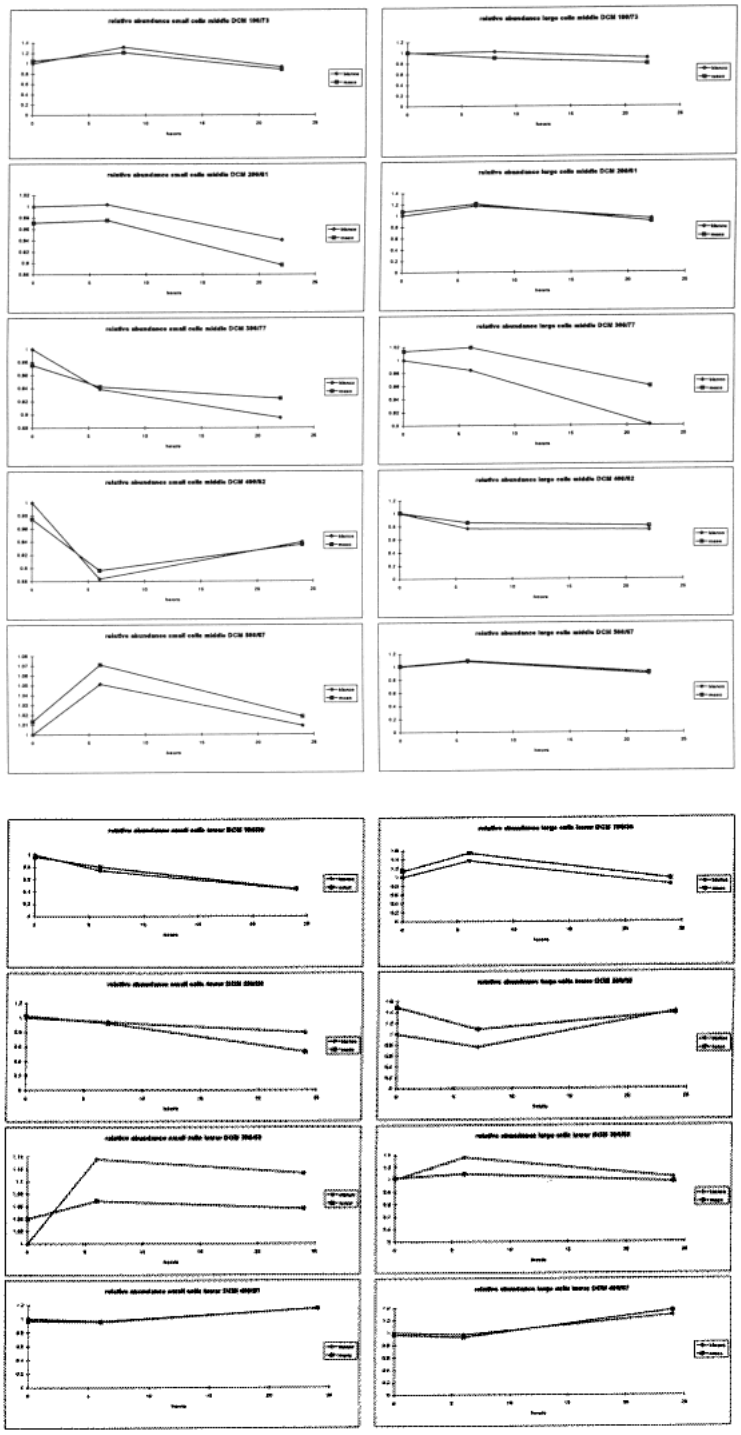


Fig. 11A,B,C Changes in cell numbers of selected groups of phytoplankton in incubations with increased number of mesozooplankton A: upper part of DCM, B: middle part of DCM (peak) and C: lower part of DCM.

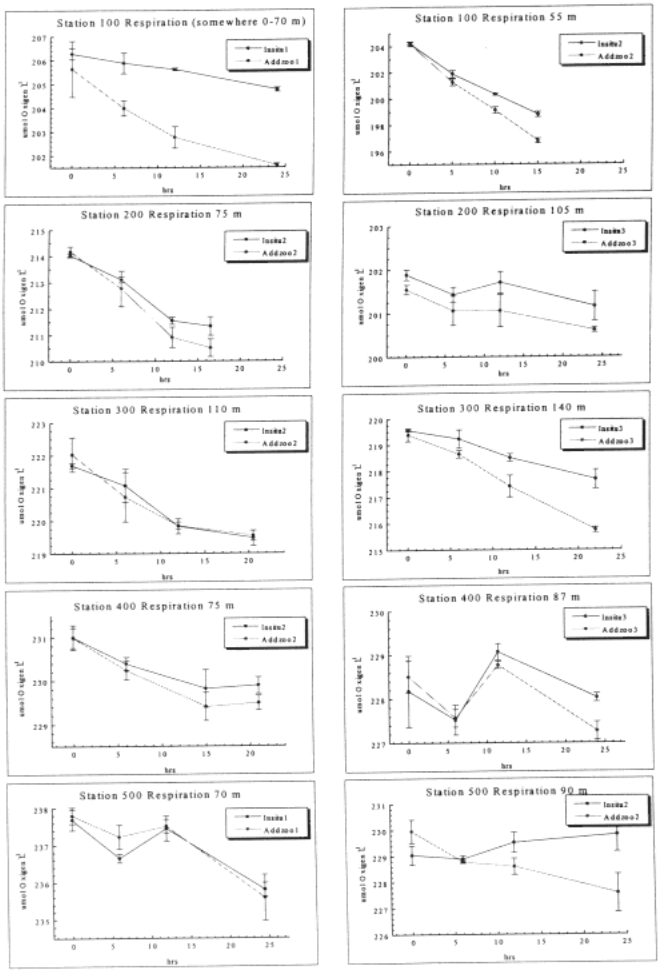


Fig. 12 A,B Time course measurements of oxygen respiration in samples with natural and increased number of mesozooplankton.

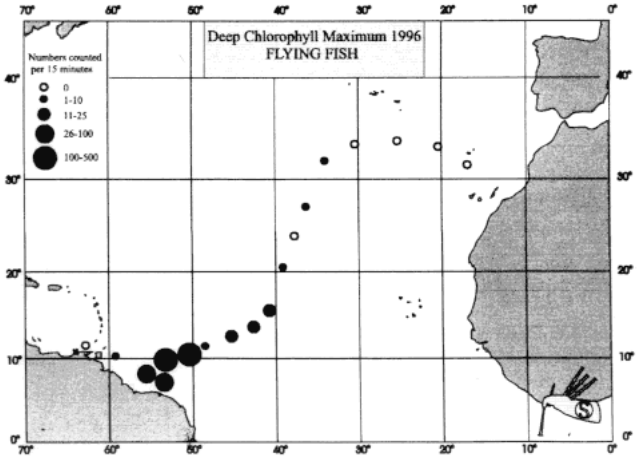
Flying fish observations

(Swier Oosterhuis)

During the DCM project 1996 flying fish was counted. Taxonomy was not performed since the fishes couldn't be caught. It was noticed that there were different species. The counting was done during a 15 minutes period on the days when the ship was in transit. The area observed was an area in front and on the side of the bow in an angle of 45 degree.

Date	time	position	number

23 July	11:00 hr	11 25 14 N 62 42 17 W	0
24 July	11:00 hr	10 11 00 N 59 12 30 W	3
25 July	17:30 hr	08 19 47 N 55 28 09 W	33
26 July	10:30 hr	07 32 28 N 56 48 28 W	65
27 July	16:00 hr	08 40 10 N 53 04 28 W	116
28 July	16:15 hr	10 04 29 N 51 15 12 W	360
2 August	16:00 hr	11 22 26 N 48 48 53 W	5
3 August	16:30 hr	12 44 22 N 45 19 27 W	12
4 August	14:45 hr	13 52 54 N 42 33 54 W	18
9 August	12:45 hr	15 40 00 N 40 49 00 W	14
10 August	17:00 hr	20 24 01 N 39 02 01 W	2
15 August	15:45 hr	23 58 06 N 37 38 38 W	0
16 August	11:45 hr	27 08 12 N 36 11 28 W	2
17 August	16:30 hr	31 57 08 N 33 57 40 W	2
22 August	16:30 hr	33 39 00 N 30 06 00 W	0
23 August	15:45 hr	33 51 41 N 25 12 41 W	0
28 August	15:30 hr	33 18 42 N 20 10 18 W	0
29 August	12:30 hr	31 37 28 N 16 48 29 W	0



Technical highlights during DCM96.

(NIOZ technicians Lorendz Boom, Eduard Bos and Ruud Groenewegen

The expedition was technically a success. The problems encountered were mainly minor and were well spread out over the duration of the cruise. The majority were solved or an appropriate alternative found. A brief selection:

CTD-winch: Due to excessive wear on the non-metal gearwheels the intended full ocean depth CTD-cast on each station was limited to 2000 meters only. Salt intrusion and human error led to some electrical problems with the cable. Restriction of the coolant flow for the hydraulic power packs diesel motor caused rapid overheating until cured.
In the last few days of the cruise the hydraulic brake system for the gantry failed and had to be replaced by a rope and pulley system to fix its position.

The airconditioners of two containers had broken down while on board when not being used. Both were repaired by a technician of the Tydeman crew, at great effort, but one kept losing its coolant and eventually had to be shut down.

A lot of equipment was having problems with the severe brown-outs of the ships power supply, caused by heavy machinery such as the active rudder. A spare motor-generator combination was put into service to bridge these power gaps by using the inertia of their rotors.

Use of the large volume water sampler (waterchest) had to be abandoned because the ships winch could not pay-out fast enough to ensure proper functioning of the chest.

A number of radio beacons got their antennas broken but there were enough spare parts to last the cruise. Given the antenna heights used, their practical range perfectly matched the theoretical 10 nautical miles.

The pressure sensor of the Multinet appeared unrepairable. This led to sampling based on the temperature profile provided by the CTD.

The UV-sensor got its cable entangled in the ships propeller and was rerouted through the cable used by ASIR and AC-9. This appeared to be an improvement in both handling the equipment and obtainable maximum depth.