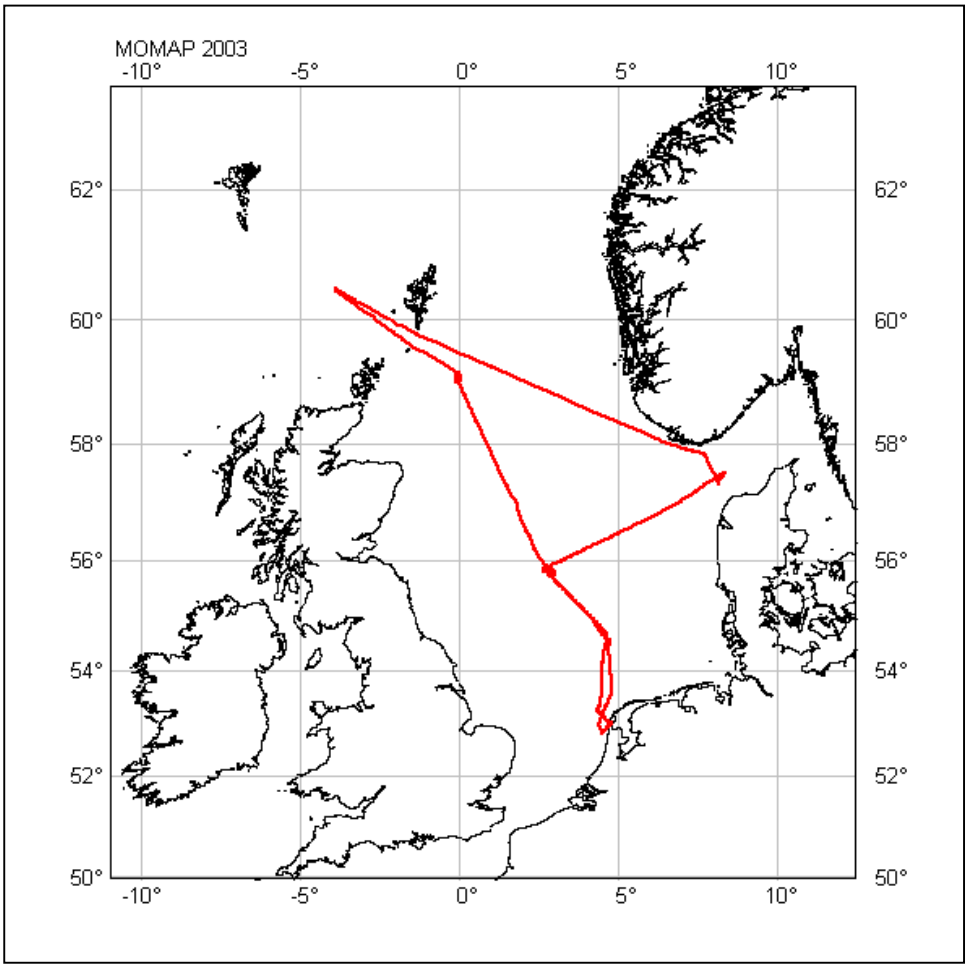
CRUISE REPORT

MOMAP-2

(64PE214 momap-2)

8 July – 20 July 2003



Ship	**: RV Pelagia**
Cruise Name	**: MOMAP-2 (MOrtality of MArine Phytoplankton)**
Cruise Number	**: 64PE214 momap-2**
Cruise Period	**: 8 July – 20 July 2003**
Port of departure	**: NIOZ - Texel (NL)**
Port of return	**: NIOZ - Texel (NL)**
Responsible Institute	**: Royal Netherlands Institute for Sea Research (NIOZ)** **Landsdiep 4, 1797 SZ 't Horntje, Texel, The Netherlands**
Chief Scientist	**: Dr. C. Brussaard** **Dept. Biological Oceanography**

Introduction

With phytoplankton forming the base of the marine food web, extensive research has been conducted on factors controlling the growth. Studies focussed mainly on the appearance, rather than on the disappearance of algal species. Traditional views of the dynamics of marine phytoplankton in natural ecosystems considered mortality due only to external forces such as grazing or sedimentation. In the past decade, phytoplankton cell lysis became also recognised as a potentially important loss factor. Studies measuring total phytoplankton cell lysis showed that the specific lysis rates of the algal community are variable over time and can be very high. The release of the cell content had a structuring impact on total marine pelagic food web by enhancing the activity of the microbial food web.

The scientific research on phytoplankton mortality has gained much interest the last years, for one due to the discovery of viruses being significant agents for algal cell death. Although long overlooked in aquatic ecosystems, we now know that viruses are very abundant in marine environments and that they can infect a large variety of important primary producers. Besides viruses and bacteria, phytoplankton can die due to physiological stress, or even show a certain intrinsic death rate that is independent of the growth-limiting condition. Some of the reported examples of physiological induced mortality suggest active participation of the cell in its own death (apoptosis).

Information on the mechanisms by which phytoplankton cells die is important since it can be expected that the amount and composition of the released dissolved organic matter (DOM) differs for the different mortality processes. Knowledge on processes leading to the production of DOM is crucial for a good understanding of the global carbon cycle. Whether phytoplankton sinks out, is grazed upon or dies due to cell lysis has major implications for the understanding of material and energy cycling in the marine pelagic food webs. Information on the importance of phytoplankton mortality in systems with contrasting trophic status, as well as the mechanisms behind algal cell death is also still largely lacking. Because of the very different food web structure the eutrophic ecosystems have as compared to the more oligotrophic ecosystems, knowledge on this topic is warranted.

Generally, in oligotrophic waters the import rate of the controlling nutrient is low and regeneration of the liming nutrient by members of the microbial food web is found important to sustain high productivity. Small algae dominate the photoautotrophic community due to their good competitive growth characteristics. As a result of rapid numerical response by heterotrophic nanoflagellates and microzooplankton, phytoplankton biomass is size-selective grazer controlled. Although this highly dynamic regenerative system is present in any photic zone, at increasing import rates of the controlling nutrient (eutotrophic waters) microzooplankton set limits to the biomass of the small algae. The primary effect is an increase in the biomass of larger algae escaping the size-selective grazing by microzooplankton. Control by larger grazers is relatively slow due to the relatively long generation times of these grazers. Furthermore, non-edible algal species will be able to increase in concentration, forming temporal algal blooms (e.g. diatoms, *Phaeocystis* spp.). Lysis of a single dominating algal population will affect population interactions (competition, commensalism, antagonism) and may lead to changes processes that drive the population dynamics in the system. The temperate eutrophic ecosystems are likely to be more controlled by viruses. Infection of the host organism with lytic viruses leads to cell death while releasing numerous progeny viruses. Infection with lytic viruses is expected to be most prominent in environments with high abundance of the algal host (e.g. algal blooms) because of the increased host-virus contact rates. Phytoplankton at low abundance are not expected to be easily infected, but they may contain temperate viruses instead.

The MOMAP-2 cruise

The MOMAP-2 cruise, 8th of July till 20rd of July 2003 from Texel to Texel, was undertaken as part of larger integrated study with the main merit of assessing the ecological role of phytoplankton cell mortality, with special emphasis on the quantitative importance of virus induced lysis during the summer conditions. The cruise track is shown in figure 1.

For that reason the phytoplankton blooms in different areas of the North Sea were sampled in summer (Table). Besides the eutrophic coastal zone of the Southern part of the North Sea, different areas in the North Sea, Skagerrak and North Atlantic Ocean (west of Shetland Islands) were compared. Except for the coastal station all other main stations showed a clear stratification allow a distinction between the surface and subsurface phytoplankton conditions.

Phytoplankton mortality (including viral induced cell death), abundance's and richness of the various groups of organisms will be determined in depth and over diel cycles.

These bloom dynamics were followed for a period of 24 to 48 hours in situ or using a large volume (1000 l) incubator. On regular intervals (4 hours) samples were taken. In case of direct field sample using the CTD-ROS covering the upper 65 m of the water column (6 water depths). In case of the incubator subsamples were taken directly from the incubator. To trace the water mass a drifter was deployed (7m water depth; kruis van Jan) equipped with radio bacon and radar reflector. Each morning and evening a 3 by 3 nm grid was sampled with the Scanfish to examine the hydrographic features of the sampling area.

Additional sampling was performed using the ships clean Aquaflow system for direct measurements of temperature, fluorescence and optical backscatter.

Although a great deal of measurements could be analysed on board like nutrients, chlorophyll and life counts of phytoplankton applying flow cytometry numerous samples were stored for later analysis at the laboratory.

Detailed description of the different scientific activities can be found in the following section.

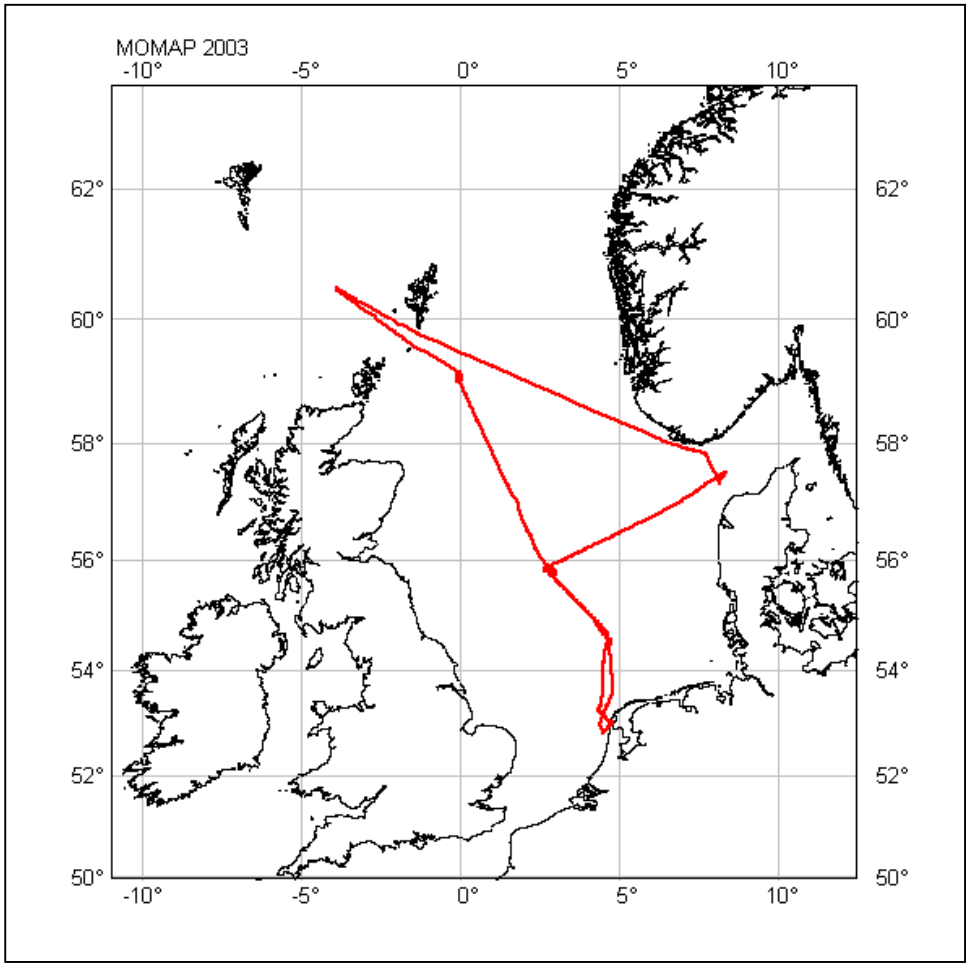


Fig. 1. Cruise track MOMAP-2

Research approach per participant:

Scanfish

- by Santiago R. Gonzalez

The Scanfish *MKII 1250* is an undulating scanning system designed for simultaneous measurements of variety oceanographic parameters down to 500 m depth at high horizontal resolution. The Scanfish undulates vertically through the water column while being towed behind the ship moving at 6-8 knots.

-*Surface component*, means Scanfish MKII presentation, logging software, PowCom-Power and communication unit for vehicle interface. The navigational data from DGPS is incorporated in the data stream using the NMEA-interface of the Seabird deckunit.
-*Underwater vehicle and sensors*: inboard control unit, cables, depth sensor, altimeter, Seabird 911 interface (CTD), Optical Batch Scatter (OBS), Transmissiometer (PAR) and Chelsea fluorometer.
-*Winch type Cormac 1500*, equipped with ca. 2300 m cable ∴8.3 mm, type 32-OHM COAX2-20.
In order to enhance the depth range of the Scanfish system, the winch is connected with the controller computer and by pay out/in of cable during ascent or descent the performance envelop from 5 to 500 m will be obtained.

Scanfish tracks MOMAP 2

In 3 stations the scanfish was used performing each morning and evening a grid of 3 by 3 nm around the drifter to examine the hydrographic features of the sampling area. Most of the tows were performed using the auto winch control, paying cable in/out at 0.6 m/sec. The ship's speed was kept approx. 8 knots. One additional tow was made in the area of the Frisian Front.

Phytoplankton dynamics in contrasting ecosystems in the North Sea

- by Anna Noordeloos and Marcel Veldhuis -

A variety of samples were taken to determine the phytoplankton species composition as well as net and gross growth rates of the major species or groups of phytoplankton present. Results thus obtained provide insight in the growth parameters of the phytoplankton community and will be compared with a variety of experiments designed to examine species specific due to grazing or viral infection.
Sampling was done *in situ*, close to a drifting buoy or using a 1000 litre large outdoor deck incubator. The species/group composition was characterised based on the cellular bio-optical properties (size, scatter and chlorophyll fluorescence) of algal cells applying flow cytometry. The dynamics of different groups were traced over a period of up to 36 hours. This included net changes in cell abundance but also samples were taken for gross growth rate measurement based on the DNA-cell-cycle method. The general physiological status of the phytoplankton cells was derived from the status of the plasma membrane using a green fluorescent DNA dye non-permeable in healthy cells and the general intracellular esterase activity.
The basic instrument applied in the single cell analysis of the phytoplankton community was a bench top flow cytometer (Coulter XL-MCL). This instrument is equipped with a 15mW laser (488

nm excitation) and emission bands in the green (FL1: 525 ∂ 20 nm); orange (FL2: 575 ∂ 20 nm) and red (FL3: >600 nm). In addition forward light scatter is collected as a fourth parameter. Phytoplankton is distinguished from other particles based on their chlorophyll fluorescence which is collected in the red detector (FL3). In its basic configuration the size range on the instrument ranged from 2 to ca. 30 σm.
The numerical abundance was sufficiently high to reduce the counting time to 5 minutes. This equals a sample volume of ca. 600 σl. Samples were analysed based on the presence of clearly distinguishable groups up to a maximum of 4 size classes or species. The presence of chain forming diatoms occasionally limited clear cut-off of the different cluster.

In total over 2000 samples were analysed and because of the variability in phytoplankton species distribution in the various regions samples the data have not been processed on board.
In general phytoplankton contained a continuos number of cells increasing in size, with partly overlapping regions. Phycoerythrin containing *Synechococcus* were found at virtually all stations.
As in most aquatic ecosystems there was a covariance of size and fluorescent properties of the phytoplankton.

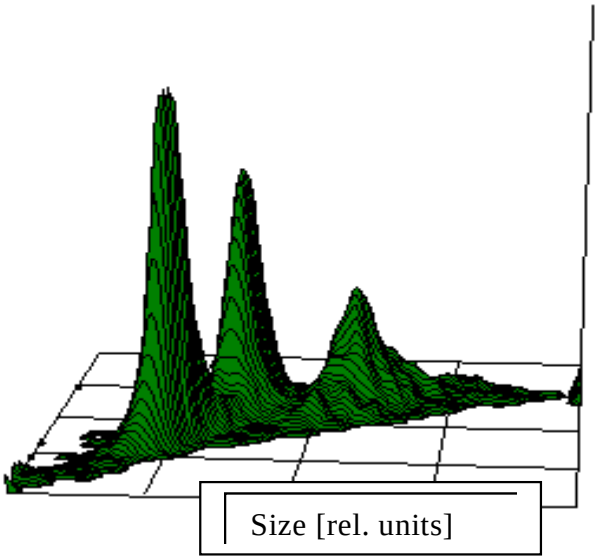


Figure 1 isometric projection of size versus chlorophyll fluorescence signals

Fig. 2. typical example of co variation in cellular size and chlorophyll distribution of phytoplankton populations

The genome size of phytoplankton will be estimated at the NIOZ, using preserved samples. To obtain the gross growth rates of the phytoplankton cells, the DNA content and distribution in G1, S and G2 phase will be analysed using the green nucleic acid stain PicoGreen dye (1:5 diluted stock solution).

Furthermore, different single cell activity assays measuring the viability of the algal cells were applied to determine the status of cell membrane integrity test the integrity of the plasma membrane or viability of the cells, SYTOX Green solution was added 1 ml of sample. Staining with SYTOX Green allows detection of algal cells with a compromised cell membrane (dead cells). Samples were incubated for 10 min in the dark prior to analysis. The increase in green fluorescence (FL1) was recorded and compared with the unstained cells and the DNA signal in paraformaldehyde fixed

cells. Low values indicate high degree of viability whereas FL1 signals closely matching that of full DNA signal are indicative of non–viable cells.
The general physiological status of the cell was tested using the intracellular esterase activity as an indicator (live active cells).
Enzyme activity was measured using fluoresceine diacetate (FDA) as substrate. FDA produces a green fluorescent compound after cleavage. In the case of FDA green fluorescence was measured after 5 minutes of incubation, whereas samples with Calcein-AM were incubated for 1 h at *in situ* temperature and light before analyses.

Phytoplankton primary production
- Jan Hegeman -

Phytoplankton primary productivity was measured at each main station using ^{14}C-labeled bicarbonate as inorganic tracer. In general samples were taken at down and incubated for 24 h period in a deck incubator. Light regime mimicked in the incubator ranged from 85 down to 0.6% of the surface incident irradiance.
To duplicate samples of 250 ml (polycarbonate bottles) a known amount of 14C-tracer was added. After incubation samples were gentle filtrated (GFF). Filters were fumed shortly (50 min) in fuming HCl. Amount of 14C-organic carbon produced will counted after adding counting solution in a liquid scintillation counter using the standard procedure.

Algal, bacterial and viral abundances, algal composition, and pigment sampling
- by Dedmer van der Waal -

Basic parameters such as algal, bacterial and viral abundances were sampled for on board and will be further analyzed at the NIOZ. Samples were taken from most stations and from different depths, about 140 samples per parameter in total.
For the algal composition study, Lugol-fixed samples (in brown glass bottle; 200 mL of sample and 4mL of lugol were taken and stored in the fridge. About 35 samples from the main stations were sampled and brought back for analysis.
For the phytoplankton abundance samples, sample was prescreened through a 50 σm pore-size sieve, after which 3.5 ml was fixed with formaline/hexamine (18/10%; final conc. in sample 0.5%). After fixation for 15-30 min. at 4∀C, samples were fast frozen in liquid nitrogen and stored at -80°C.
Virus and bacteria abundance was sampled for using cyrovials filled with 1.5 ml of sample, fixed with glutaraldehyde (25 % EM grade; final conc. 0.5 %), put in the fridge for 30 min., frozen in liquid nitrogen; after which stored at -80°C.
For pigment analyses, Chl *a* and HPLC samples were taken. The Chl *a* samples (typically 1.5 L) were filtered through a GF/F filter, after which they were stored at -80°C. About 150 samples were taken, to be brought back to the NIOZ for analyses. The HPLC samples were filtered (typically 2.5 L) through a GF/F filter, and stored at -80°C. About 60 samples were taken. Analysis will be performed at the NIOZ.

Variable fluorescence (F_v/F_m) measurements

- by Caroline Chenard and Anne-Claire Baudoux -

Variable fluorescence measures the potential for phytoplankton cells to perform photosynthesis and as such is a somewhat crude indicator of their state of health. Samples of phytoplankton were collected from cube containers or water sampling bottles in 13 mm glass culture tubes. The tubes were placed in darkness at close to *in situ* temperature for a minimum of 10 minutes to allow acclimation. Dark-acclimated fluorescence (F_o) was then measured in a Turner Designs 10AU fluorometer (excitation at 430 nm, emission at 680 nm, with a red-sensitive photomultiplier tube). Under these conditions, F_o also provides an index of chlorophyll *a* biomass. After this, a final concentration of 10 µM of the photosynthetic inhibitor dichlorodimethylurea (DCMU, added in 100% ethanol) was added, the tube mixed and re-read in the fluorometer. The inhibitor stops photosynthetic electron transport, and the energy that would have gone to photosynthesis is now dissipated as fluorescence. As a result, the fluorescence rises to a maximum (F_m). The difference between F_m and F_o (= F_v) is scaled by F_m, resulting in a ratio, F_v/F_m. Phytoplankton cells with Fv/Fm of 0.65 or greater are near the theoretical maximum for the ratio and thus show no evidence of nutrient or light stress.

Phytoplankton cell lysis

- by Mariek Bossink -

The most straightforward method used to estimate total phytoplankton cell lysis was originally developed in our laboratory, and is based on the measurement of dissolved esterase activity (DEA). Esterases are strictly cytoplasmatic enzymes, so their release into the surrounding water upon cell lysis can be used to as indicator of cell lysis (cell breakage or cell damage). In addition, their concentration in autotrophs is far greater then that in heterotrophs so that they provide a reliable tracer of phytoplankton cell contents.

Three fractions of seawater were gathered by filtering. The three fractions were the total fraction, the fraction <0.2µm and the <10kD fraction. To those fractions a small amount of buffer was added and a excess of substrate. Nonspecific esterase cleave of the acetates of fluorescein di-acetate (FDA), which is added as a substrate, yielding the fluorescent compound fluorescein. Fluorescein accumulates in samples as a product of the cleavage of FDA by esterases. By measuring the fluorescein concentration in the three fractions with a fluorimeter before and after incubating for about one hour at 20 ∀C, the lysis rate per day can be calculated. This is corrected for the breakdown on the enzyme over 24 hours.

Viral diversity

- by Caroline Chenard and Anne-Claire Baudoux -

Since viral lysis can be a factor of mortality for phytoplankton, samples for virus diversity were taken. Samples were collected from the CTD main stations, few depth profiles were performed and the shift up experiment in cube vessel was followed (24 hours basis). Samples were concentrated to

50 ml with a starting volume of 2 L seawater. This concentration step was done on board using a Vivaflowsystem (vivascience) with a cut-off of 30 KDa. Following the concentration, 37 σl of 10 % Tween 80 was added, the sample was cooled in the fridge for 30 minutes and was centrifuged for 30 minutes at 10000 x g to remove cellular and bacterial debris. Supernatant was stored at –80 ^{o}C until further analyses back in the lab using pulsed field gel electrophoresis (PFGE), allowing separation of the different viruses on the basis of their genome size.

Isolation of viruses, MPN
(Most Probable Number)
- By Caroline Chenard -

Different cultures from the algae collection were brought and grown on board (15ºC). Few of them were regularly screened for virus isolation. About 100 ml of natural seawater were gently filtered over 0.2 σm in order to remove the bacteria and phytoplankton from the viral community. A serial dilution of the viral community was performed (10^0 to 10^4) and 1 ml of these dilutions were added to 4 ml of algae culture. The fluorescence of the culture were measured at T=1, 5 and 10 days.

Estimation of the virus induced mortality on the algal component by measuring viral production
- by Anne-Claire Baudoux -

In order to determine the importance of viral induced mortality in the North Sea, the estimation of virus production on phytoplankton and bacteriophage productions were performed during this cruise, according to the method described by Suttle *et al.*. This experiment focused on the small size-phytoplankton ({ 2 σm, mainly cyanobacteria).

First, a filtration through (i) 0.8σm (cf Veronica Parada) or (ii) 2 σm was applied (PC filters) and, a concentration step by cross flow filtration was performed further. This cross flow filtration (0.2 σm cutoff), aimed to separate and concentrate the organisms larger than 0.2 σm (mostly cyanobacteria and bacteria) from the viruses. This concentrate was rediluted in particle free water (30kDa filtrated water, cross flow filtration), and incubated in temperature and light control container on a wheel.
The assays were sampled for viral and algal abundance's every 6-7 hours during +/- 24 hours. One should assume that each virus produced originates from infected algae and bacteria when the samples are exposed to the light, and from the bacteria when the sample is incubated in the dark.

Samples and treatment :

- { 2 σm communities: incubation in duplicate in light and dark condition
- { 0.8 σm communities: incubation in duplicate in light (dark condition done by Veronica)
- 20% of the natural community diluted in particle free water: incubation in duplicate in light and dark condition

Effect of UV on phytoplanktonic and viral communities
- Caroline Chenard and Anne-Claire Baudoux -

The removal or damage of phytoplankton and viruses due to UV exposure was estimated during this cruise.

Phytoplankton :

100 ml of natural phytoplankton was incubated on the front deck (temperature controlled incubator) and exposed to

- surface light condition
- surface light condition minus UV (thanks to Perspex UV filter)
- dark

Samples were incubated with and without light filter.

Samples were taken every 6-8 hours and followed for

- DNA damage (fixation with formaline:hexamine), further analysis in the lab
- Photosynthetic capacity (PAM measurement)
- Abundances
- Viral abundances

Viruses

Viral decay rate can be characterized by a lost of abundance and/or infectivity. Since we are unable to measure the infectivity of the natural viral community, a virus host system was brought on board (PgV c-P) and added to the natural sample as an "infectivity control".

Different samples were prepared :

- Natural viral community (0.2 σm filtrated seawater, PC filter)
- Natural viral community + PgV c-P
- Natural viral community + 30 kDa filtrated (and autoclaved) PgV
- PgV

These samples were exposed to

- surface light condition
- surface light condition minus UV (thanks to Persplex UV filter)
- dark

and followed for their infectivity (MPN at T=0 and T=24) and viral abundance (every 6-8 hours).

Dilution experiments to estimate algal grazing loss –rates and the loss-rates due to viral lysis

- by Bouwe Kuipers, Anne-Clair Beaudoult, Marcel Veldhuis, Govert van Noort, Corina Brussaard -

During MOMAP II grazing was measured according to the Landry and Hassett method. Two 15-bottle series of dilutions were always prepared simultaneously, using GFF-filtered water to dilute the one and ultra-filtration water (<30 kD) to dilute the other series. The series comprised the dilutions 100,70,40,20 and 10% in triplo). All T0 and T24 samples were taken simultaneously from both series and measured directly by Coulter XL-MCL flowcytometry. This way we were able to estimate the grazing on Cyanobacteria and 2 or sometimes 3 size categories of eukaryotes, whereby

especially the GFF-diluted series yielded a nice set of growth- and grazing rates, which will be compared with the results of the experiments with fluorescently labelled prey by Govert van Noort. The experiments with the cross-flow filtered water yielded less good results and the share of viral lysis in the algal loss-rates did not become clear by these dilution experiments.

MOMAP II: Grazing measurements with fluorescently labelled prey

- Govert van Noort -

Stations and casts from which the water was taken for the grazing experiments							
station	date	cast	bottle	diepte in m.			
2	9/7/2003	3	3	5			
3a	11/7/2003	2	3	5			
3b	12/7/2003	12	3	37			
5	14/7/2003	3	3	5			
6	15/7/2003	3	3	5			
10	17/7/2003	3	3	5			
13	18/7/2003	3	20	5			

Next to the Landry & Hassett type dilution experiments, an independent estimate of grazing pressure in the microbial food web was obtained by measuring in threefold the 24-h decrease-rates of three types of fluorescently labeled prey (FLB (bacteria), FLCY(Cyanobacteria) and FLA (*Prorocentrum*). At the stations and depths given above, the content of one CTD bottle was carefully distributed over 9 1-liter bottles, each containing 20 ml of fluorescent prey suspension that, once evenly distributed over the whole liter of natural seawater, would produce fluorescent prey densities of ca 10% of the natural counterparts. After taking 20-ml samples at T0, all bottles were attached to a rotating (1 r.p.m.) incubation wheel in a temperature controlled room under 75 µE m^{-2} sec^{-1} (from 07:00-21:00) until all bottles were sampled again at T24. Samples were fixed with a 2% (final concentration) buffered formalin solution and stored in the dark at 4^0 C until the fluorescently labeled particles will be enumerated with the flow cytometer at NIOZ.
At selected stations fluorescently labeled prey were also incubated (same sampling and incubation as the rest) in ultrafiltrated water in order to measure, as a blank, their spontaneous decrease over 24 hrs.

MOMAP II: Bacterial Bioassay

- Govert van Noort -

At each of the stations a small bacterial bioassay was performed to determine whether bacterial growth is limited by N, P or C shortage. Water from the CTD (5-m depth) was filtered (gravity) over 0.6 µm polycarbonate filters and distributed over 5 incubation bottles of 350 ml. The scheme of additions was: +C, +P, +PN, +PNC and one blank. The bottles were incubated together with the grazing incubations. Samples were taken every 6-8 hours from all bottles, over 48 hours. Samples were split into: a part that was frozen in liquid Nitrogen in cryovials and stored at -20^0 C for later FCM-analysis of bacterial numbers and cell-size and, a part that was pre-filtered through 0.2 µm for the determination of inorganic nutrient concentrations by Karel Bakker.

Dissolved organic carbon (DOC)

- by Santiago R. Gonzalez -

Water samples for DOC were taken from different depths using the NEOX bottles (CTD) directly into pre-cleaned 150 ml polycarbonate bottles. Duplicates samples were filtered through pre-rinsed 0.2 µm polycarbonate poretics filters using a glass filtration unit (47 mm filter diameter) and then transferred (8 ml) into combusted glass ampoules. The filtered seawater samples were acidified with 2 droplets 85 % phosphoric acid, sealed in 10 ml ampoules and stored at 5 ^{o}C.
Measurement of DOC will be performed at NIOZ using a Total Organic Carbon Analyzer, Model TOC-5000A (Shimadzu Corporation) where 4 injections of 100 σml sample solution is injected into a combustion tube (680 ^{o}C) containing the catalyst Platinised Alumina (Aluminium Oxide) and Silica (Quartz) Chips. The total carbon component in the sample is combusted to become CO_2. A stream of ultra pure oxygen carries the water vapour and combustion gasses trough a series of traps before entering a Li-Cor Model LI-6252 CO_2 analyzer. The signal generated by the non-dispersive infrared (NDIR) detection of CO_2 is quantified as peak area on a Shimadzu Model C-R6a integrator. The instrument is calibrated with a standard addition curve of Pottassium Phthalate in Milli-Q (0-20-50-100-200 µMC). DOC concentration in the samples can be calculated from the slope of the calibration line.

Diel cycles of viral and bacterial activity

- by Veronica Parada and Eva Sintes-

Samples from different depths were taken to determine viral and bacterial abundance, diversity and activity. The drifting stations were sampled every four-eight hours to determine the diel cycles, and other stations were sampled to evaluate the spatial variability.
To determine viral production in diel cycles 20 L of surface water (5 m depth) were processed via tangential flow filtration to obtain 200 ml of bacterial concentrate. This concentrate was added to 1900ml of filtrate particle free water and incubated for 24 hs in darkness at in situ temperature. The experiments were performed in duplicate and subsampled for bacterial and viral abundance every 6 hours and stored at –80°C to be counted later at the flow cytometer.
To evaluate the viral diversity produced after the incubation period, the 2.1 L were processed by 30KDa tangential flow filtration. The viral concentrate obtained was centrifuged for further concentration and stored frozen to perform later concentration steps at the laboratory. From these samples we plan to perform PFGE that explains the new viral diversity.The viral diversity produced after 24 hs of incubation will allow direct comparison with the natural viral diversity from the environment (cf Anne Claire Baudoux).
Samples for bacterial abundance were fixed and stored frozen at –80°C for later analysis by flow cytometry after Syto13 staining. 500 ml to 1 l water were filtered through 0.2 σm polycarbonate filters to determine the bacterial diversity by T-RFLP (terminal restriction fragment length polymorphism) analysis.
Different methods were used to assess bacterial activity: bacterial production (leucine-3H incorporation), Microfish and active cells (CTC method).
Bacterial production was measured via [3H]-leucine (10nM final conc.) incorporation into bacterial protein. All the samples were done in duplicate with one formaldehyde-killed (2% final conc.) blank. Incubation was performed during 5 h at in situ temperature. The samples were filtered on

0.45 σm cellulose filters and stored frozen. The incorporated activity will be measured at the laboratory.
Samples for Microfish were incubated in the presence of [3H]-leucine following the same procedure described above for bacterial production but filtered on 0.2 σm policarbonate filters and kept frozen. The groups that have incorporated [3H]-leucine will be identified by epifluorescence microscopy after adding different probes.
The enumeration of CTC active cells were performed adding CTC to duplicates with 2 killed blanks (10% paraformaldehyde) and 2 controls without CTC. The samples were incubated during 2 h at in situ temperature and stored frozen at -80°C for analysis by flow cytometry.

Nutrients

- by Karel Bakker -

Nutrients were analysed with a Technicon TRAACS 800 rapid flow autoanalyser. The sample rate was set at 60 samples per hour. Measurements were made simultaneously on four channels: phosphate, ammonia, nitrate and nitrite together, and nitrite separately. Silicate was analysed on a second Technicon TRAACS 800 rapid flow autoanalyser. All measurements were calibrated with standards diluted in low nutrient sea water. Data are presented in Table 6.
The samples were collected in 100ml poly-ethylene sample bottles, after first being rinsed three times with a small amount of the sample, taken directly from the CTD-rosette bottles. The samples were kept cool and dark, stored in a refrigerator. The samples were filtered over a 0.2 um Gelman acrodisc filter. Analyses were carried out using "pony-vials" and the vials, with a volume of 4 ml, were rinsed three times before filling with the samples. To avoid evaporation during the run all vials including the calibration standards used were sealed with "para-film" under tension, so that a sharpened sample needle easily penetrated through leaving a small hole in the film.

The photometric methods used:
- Phosphate: Ortho-phosphate is measured by formation of a blue reduced molybdophosphate-complex at pH 0.9-1.1 . Potassiumantimonyltartrate used as a catalyst and ascorbic acid as a reducing agent. The absorbency is measured at 880nm. Described by J.Murphy and J.Riley, 1962. Analytica Chim.Acta
- Silicate: Measured as a blue reduced silicomolybdenium-complex at 800nm.
Ascorbic acid is used as reducing agent and oxalic acid is used to prevent interference of phosphate. Described by Strickland and Parsons, 1972. A practical handbook of sea water analysis.
- Nitrite: Diazotation of nitrite with sulfanylamide and N-(1-naphtyl)-ethylene diammonium dichloride to form a pink dye measured at 550nm.
- Nitrate and nitrite: Nitrate is first reduced in a copperized cadmium-coil using imidazole as buffer and is then measured as nitrite at 550nm.
- Ammonium: Formation of the indo-phenolblue-complex by using phenol and sodiumhypochlorite at PH 10.5. Citrate is used as a buffer and complexant for calcium and magnesium at this PH. The colour is measured at 630nm. Described by Korolefff (1969) and optimised by W. Helder and R. de Vries , 1979. Neth. J. Sea Research 13(1): 154-156.

Calibration and Standards:
Nutrient primary stock standards were prepared at the lab and stored onboard in a refrigerator. For phosphate: by weighing potassium dihydrogen phosphate in a calibrated volumetric PP flask set to 1mM P. For silicate a certificated standard (Baker) was diluted until 1.667mM Si (stored at room

temperature). Nitrate by weighing in potassium nitrate set to 10mM N. Nitrite by weighing in sodium nitrite set to 1mM N.
The calibration standards were prepared daily by diluting the separate stock standards, using three electronic pipettes, into four volumetric 100ml PP flasks (calibrated at the lab) filled with low nutrient sea water LNSW. The values of the LNSW were added to the calibration values to get the absolute nutrient values.

Cocktail standard:
This standard acts as a reference and its use is described under "quality control". It is made in the lab containing phosphate, silicate and nitrate in a solution containing 40mg Hg2Cl2 per litre as an inhibitor. Every time it was used it was diluted 500 times with the same 1ml pipette and the same volumetric 250ml flask. For silicate the dilution factor was 250.

Quality Control:
Our standards have already been proven by intercalibration exercises like ICES and Quasimeme to be within the obtainable limits to the mean of the better laboratories.

Statistics:
At each run the cocktail standard was measured. The precision off one run is shown in the next table.

	Conc uM	std dev.	%
PO4	0.442 uM	0.004	0.90
SiO2	13.94 uM	0.02	0.14
NO3+NO2	6.79 uM	0.03	0.44
NH4	0.50 uM	0.01	2.0

Used measuring ranges:

For PO4 the range was 0.01-1.01 uM
For NH4 the range was 0.09-6.76 uM
For Nox the range was 0.01-11.54 uM For “coastal”values 0.01-21.61 uM
For NO2 the range was 0.01-1.51 uM
For SiO2 the range was 0.39-17.05 uM

Acknowledgements:

We like to thank the Captain and crew of the Pelagia, the technical assistance of DZT, and Theo Buisman for their excellent assistance. The cruise was supported by the Research Council for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organisation for Scientific Research (NWO).

Table listing:

MOMAP - 64PE214

Table 1 station list

Station/ Track	Cast/ Action	Type	Event	Datum/ Tijd	Lat	Lon	Depth	remark
1	1	CTD-ROS	Begin	7/8/03 14:53	52.83302	4.50063	23	
1	1	CTD-ROS	Bottom	7/8/03 14:54	52.83312	4.50052	23	
1	1	CTD-ROS	End	7/8/03 15:11	52.83285	4.5005	23	
1	2	Waterkist	Close	7/8/03 15:24	52.83258	4.49993	22	cleaning
1	3	Waterkist	Close	7/8/03 15:29	52.83178	4.50102	21	
2	1	CTD-ROS	Begin	7/9/03 2:07	54.52263	4.6885	46	
2	1	CTD-ROS	Bottom	7/9/03 2:08	54.52273	4.68842	46	
2	1	CTD-ROS	End	7/9/03 2:17	54.52258	4.68797	46	
2	2	Drift buoy deployment	Deployment	7/9/03 3:42	54.52302	4.69143	8	
2	3	CTD-ROS	Begin	7/9/03 4:09	54.52037	4.69812	45	CTD naast Drifte
2	3	CTD-ROS	Bottom	7/9/03 4:10	54.52025	4.69833	45	
2	3	CTD-ROS	End	7/9/03 4:15	54.51935	4.7005	45	
2	4	CTD-ROS	Begin	7/9/03 6:15	54.51057	4.71045	46	
2	4	CTD-ROS	Bottom	7/9/03 6:18	54.51027	4.71032	45	
2	4	CTD-ROS	End	7/9/03 6:28	54.50983	4.70913	46	
2	5	Scanfish	Begin track	7/9/03 6:49	54.51092	4.71687	45	
2	5	Scanfish	End track	7/9/03 9:58	54.49852	4.64598	45	
2	6	CTD-ROS	Begin	7/9/03 11:21	54.48893	4.64832	45	
2	6	CTD-ROS	Bottom	7/9/03 11:22	54.48903	4.64822	45	
2	6	CTD-ROS	End	7/9/03 11:31	54.489	4.64832	46	
2	7	CTD-ROS	Begin	7/9/03 14:00	54.49067	4.66045	46	
2	7	CTD-ROS	Bottom	7/9/03 14:01	54.49055	4.6606	46	
2	7	CTD-ROS	End	7/9/03 14:09	54.48993	4.66342	46	
2	8	CTD-ROS	Begin	7/9/03 18:03	54.4948	4.7074	45	
2	8	CTD-ROS	Bottom	7/9/03 18:07	54.49485	4.70808	45	
2	8	CTD-ROS	End	7/9/03 18:16	54.49502	4.70915	45	
2	9	Scanfish	Begin track	7/9/03 18:38	54.49415	4.71082	45	
2	9	Scanfish	End track	7/9/03 21:27	54.46102	4.65968	46	
2	10	CTD-ROS	Begin	7/9/03 22:14	54.49978	4.66537	45	
2	10	CTD-ROS	Bottom	7/9/03 22:15	54.49973	4.66512	45	
2	10	CTD-ROS	End	7/9/03 22:25	54.4995	4.66423	45	
2	11	CTD-ROS	Begin	7/10/03 2:01	54.49773	4.6574	45	
2	11	CTD-ROS	Bottom	7/10/03 2:02	54.49768	4.65778	46	
2	11	CTD-ROS	End	7/10/03 2:13	54.4982	4.65723	46	
2	12	CTD-ROS	Begin	7/10/03 6:00	54.50595	4.70607	46	
2	12	CTD-ROS	Bottom	7/10/03 6:04	54.50602	4.70562	45	
2	12	CTD-ROS	End	7/10/03 6:12	54.50662	4.70595	46	
2	13	CTD-ROS	Begin	7/10/03 11:23	54.51483	4.67492	45	
2	13	CTD-ROS	Bottom	7/10/03 11:24	54.51482	4.67473	45	
2	13	CTD-ROS	End	7/10/03 11:33	54.5147	4.67303	45	
2	14	CTD-ROS	Begin	7/10/03 14:05	54.52613	4.68473	46	
2	14	CTD-ROS	Bottom	7/10/03 14:05	54.52615	4.68502	46	
2	14	CTD-ROS	End	7/10/03 14:13	54.52647	4.6869	45	
2	15	Drift buoy recovery	Recovery	7/10/03 14:34	54.52585	4.69143	45	
3	1	CTD-ROS	Begin	7/11/03 2:08	55.83408	2.67153	81	
3	1	CTD-ROS	Bottom	7/11/03 2:08	55.8341	2.6715	81	
3	1	CTD-ROS	End	7/11/03 2:19	55.83447	2.6731	81	

3	2	Drift buoy deployment	Deployment	7/11/03 2:47	55.83542	2.67472	81	
3	3	CTD-ROS	Begin	7/11/03 4:02	55.83443	2.7029	82	
3	3	CTD-ROS	Bottom	7/11/03 4:04	55.83445	2.70362	82	
3	3	CTD-ROS	End	7/11/03 4:08	55.83445	2.7057	82	
3	4	CTD-ROS	Begin	7/11/03 6:02	55.82983	2.739	80	
3	4	CTD-ROS	Bottom	7/11/03 6:07	55.8297	2.74058	79	
3	4	CTD-ROS	End	7/11/03 6:17	55.83013	2.74657	80	
3	5	Scanfish	Begin track	7/11/03 6:29	55.83	2.751	79	
3	5	Scanfish	End track	7/11/03 9:52	55.81448	2.69912	81	
3	6	CTD-ROS	Begin	7/11/03 11:23	55.81053	2.7666	80	
3	6	CTD-ROS	Bottom	7/11/03 11:25	55.81055	2.76752	80	
3	6	CTD-ROS	End	7/11/03 11:36	55.81033	2.76825	80	
3	7	CTD-ROS	Begin	7/11/03 14:03	55.80487	2.792	78	
3	7	CTD-ROS	Bottom	7/11/03 14:14	55.80367	2.79327	78	
3	7	CTD-ROS	End	7/11/03 14:14	55.80367	2.79327	78	
3	8	CTD-ROS	Begin	7/11/03 18:01	55.80712	2.8645	76	
3	8	CTD-ROS	Bottom	7/11/03 18:04	55.80648	2.86588	76	
3	8	CTD-ROS	End	7/11/03 18:14	55.80777	2.86565	76	
3	9	Scanfish	Begin track	7/11/03 18:25	55.80768	2.87572	75	
3	9	Scanfish	End track	7/11/03 21:42	55.78928	2.8321	76	
3	10	CTD-ROS	Begin	7/11/03 22:16	55.79987	2.90602	9	
3	10	CTD-ROS	Bottom	7/11/03 22:24	55.79922	2.90428	75	
3	10	CTD-ROS	End	7/11/03 22:29	55.79895	2.90367	75	
3	11	CTD-ROS	Begin	7/12/03 2:08	55.7856	2.89448	76	
3	11	CTD-ROS	Bottom	7/12/03 2:09	55.78555	2.8946	75	
3	11	CTD-ROS	End	7/12/03 2:20	55.78435	2.89363	76	
3	12	Waterkist	Close	7/12/03 2:53	55.7842	2.89405	76	Diep
3	13	Waterkist	Close	7/12/03 3:08	55.78497	2.89517	11	Ondiep
3	14	CTD-ROS	Begin	7/12/03 4:09	55.78632	2.90948	75	
3	14	CTD-ROS	Bottom	7/12/03 4:10	55.78655	2.90978	8	
3	14	CTD-ROS	End	7/12/03 4:15	55.78667	2.91198	75	
3	15	CTD-ROS	Begin	7/12/03 6:01	55.79043	2.9424	74	
3	15	CTD-ROS	Bottom	7/12/03 6:03	55.79043	2.94303	74	
3	15	CTD-ROS	End	7/12/03 6:14	55.78945	2.94628	74	
3	16	Scanfish	Begin track	7/12/03 6:31	55.7905	2.96533	74	
3	16	Scanfish	End track	7/12/03 9:51	55.77462	2.90862	74	
3	17	CTD-ROS	Begin	7/12/03 11:19	55.78368	2.96775	73	
3	17	CTD-ROS	Bottom	7/12/03 11:21	55.78358	2.96752	73	
3	17	CTD-ROS	End	7/12/03 11:32	55.78277	2.96525	73	
3	18	CTD-ROS	Begin	7/12/03 14:03	55.77098	2.94875	73	
3	18	CTD-ROS	Bottom	7/12/03 14:03	55.77097	2.94862	73	
3	18	CTD-ROS	End	7/12/03 14:13	55.76968	2.94915	73	
3	19	CTD-ROS	Begin	7/12/03 18:03	55.76692	2.98358	71	
3	19	CTD-ROS	Bottom	7/12/03 18:05	55.76698	2.9836	71	
3	19	CTD-ROS	End	7/12/03 18:15	55.76738	2.9853	71	
3	20	Scanfish	Begin track	7/12/03 18:29	55.76703	2.9983	70	
3	20	Scanfish	End track	7/12/03 21:42	55.75068	2.94772	70	
3	21	CTD-ROS	Begin	7/12/03 22:09	55.77033	3.00688	70	
3	21	CTD-ROS	Bottom	7/12/03 22:10	55.77047	3.0069	70	
3	21	CTD-ROS	End	7/12/03 22:21	55.77153	3.00833	70	
3	22	CTD-ROS	Begin	7/13/03 2:00	55.75767	3.00417	70	
3	22	CTD-ROS	Bottom	7/13/03 2:04	55.7575	3.00433	70	
3	22	CTD-ROS	End	7/13/03 2:10	55.7575	3.0043	70	

3	23	Drift buoy recovery	Recovery	7/13/03 2:32	55.77283	3.00317	70	
4	1	CTD-ROS	Begin	7/13/03 11:21	56.88383	1.85005	92	
4	1	CTD-ROS	Bottom	7/13/03 11:23	56.88382	1.8502	92	
4	1	CTD-ROS	End	7/13/03 11:38	56.882	1.85103	7	
5	1	CTD-ROS	Begin	7/14/03 2:05	59.00072	-0.00128	131	
5	1	CTD-ROS	Bottom	7/14/03 2:11	59.00073	-0.00022	131	
5	1	CTD-ROS	End	7/14/03 2:22	59.00113	-0.00018	131	
5	2	Drift buoy deployment	Deployment	7/14/03 3:02	59.00182	-0.00597	131	
5	3	CTD-ROS	Begin	7/14/03 4:06	59.01665	-0.0166	131	
5	3	CTD-ROS	Bottom	7/14/03 4:09	59.01785	-0.0172	132	
5	3	CTD-ROS	End	7/14/03 4:14	59.01867	-0.01772	132	
5	4	Drift buoy deployment	Deployment	7/14/03 4:35	59.02512	-0.01953	132	Boei uitgehaald v controlle van het baken
5	5	CTD-ROS	Begin	7/14/03 6:03	59.05083	-0.02138	140	
5	5	CTD-ROS	Bottom	7/14/03 6:05	59.0514	-0.02127	140	
5	5	CTD-ROS	End	7/14/03 6:19	59.05505	-0.02162	139	
5	6	Scanfish	Begin track	7/14/03 6:33	59.0614	-0.01247	139	
5	6	Scanfish	End track	7/14/03 9:50	59.01422	-0.06777	130	
5	7	CTD-ROS	Begin	7/14/03 11:22	59.08413	0.02995	146	
5	7	CTD-ROS	Bottom	7/14/03 11:25	59.08365	0.03042	146	
5	7	CTD-ROS	End	7/14/03 11:40	59.0819	0.03248	146	
5	8	CTD-ROS	Begin	7/14/03 14:04	59.06482	0.02835	145	
5	8	CTD-ROS	Bottom	7/14/03 14:05	59.06492	0.02812	144	
5	8	CTD-ROS	End	7/14/03 14:13	59.06513	0.02695	145	
5	9	CTD-ROS	Begin	7/14/03 18:06	59.0991	-0.01595	144	
5	9	CTD-ROS	Bottom	7/14/03 18:08	59.09968	-0.01575	144	
5	9	CTD-ROS	End	7/14/03 18:22	59.10092	-0.01498	146	
5	10	Scanfish	Begin track	7/14/03 18:33	59.09552	-0.00818	145	
5	10	Scanfish	End track	7/14/03 21:33	59.0624	-0.00635	144	
5	11	CTD-ROS	Begin	7/14/03 22:17	59.13507	0.00027	146	
5	11	CTD-ROS	Bottom	7/14/03 22:23	59.13517	0.00278	145	
5	11	CTD-ROS	End	7/14/03 22:30	59.1356	0.00492	145	
5	12	CTD-ROS	Begin	7/15/03 2:02	59.12383	0.02467	145	
5	12	CTD-ROS	Bottom	7/15/03 2:05	59.124	0.0245	145	
5	12	CTD-ROS	End	7/15/03 2:21	59.12433	0.02583	144	
5	13	Drift buoy recovery	Recovery	7/15/03 2:38	59.12167	0.027	143	
6	1	CTD-ROS	Begin	7/15/03 17:05	60.46297	-3.88043	9	
6	1	CTD-ROS	Bottom	7/15/03 17:13	60.46378	-3.88205	50	
6	1	CTD-ROS	End	7/15/03 17:17	60.46483	-3.88212	50	
6	2	Waterkist	Close	7/15/03 17:44	60.47227	-3.88752	50	
6	3	CTD-ROS	Begin	7/15/03 18:24	60.48117	-3.88888	50	
6	3	CTD-ROS	Bottom	7/15/03 18:26	60.48157	-3.88848	50	
6	3	CTD-ROS	End	7/15/03 18:31	60.4831	-3.88728	50	
7	1	CTD-ROS	Bottom	7/16/03 6:16	59.58333	0.5	135	Handmatig ingev zie logsheet v/d b
8	1	CTD-ROS	Bottom	7/16/03 11:34	59.25	1	113	Handmatig ingev zie logsheet v/d b
9	1	CTD-ROS	Begin	7/17/03 6:07	58.00655	6.4987	137	
9	1	CTD-ROS	Bottom	7/17/03 6:12	58.00807	6.49987	362	
9	1	CTD-ROS	End	7/17/03 6:25	58.0099	6.49483	361	
10	1	CTD-ROS	Begin	7/17/03 11:20	57.82838	7.7042	503	
10	1	CTD-ROS	Bottom	7/17/03 11:23	57.82888	7.7039	266	
10	1	CTD-ROS	End	7/17/03 11:38	57.8317	7.70313	505	

10	2	Waterkist	Close	7/17/03	12:04	57.83367	7.69823	505
10	3	CTD-ROS	Begin	7/17/03	12:18	57.8353	7.6978	505
10	3	CTD-ROS	Bottom	7/17/03	12:23	57.83593	7.69667	88
10	3	CTD-ROS	End	7/17/03	12:35	57.83742	7.69605	203
11	1	CTD-ROS	Begin	7/17/03	16:39	57.30073	8.18455	39
11	1	CTD-ROS	Bottom	7/17/03	16:40	57.3008	8.18452	40
11	1	CTD-ROS	End	7/17/03	16:47	57.29995	8.18343	41
11	2	Boxcore	Bottom	7/17/03	17:09	57.29978	8.18302	40 Failed
11	3	Boxcore	Bottom	7/17/03	17:24	57.3002	8.1818	38 Failed
11	4	Boxcore	Bottom	7/17/03	17:49	57.32918	8.15728	43 Failed
11	5	Boxcore	Bottom	7/17/03	18:20	57.38285	8.13113	63 Stones
11	6	Boxcore	Bottom	7/17/03	19:14	57.4332	8.27443	72
11	7	Boxcore	Bottom	7/17/03	19:48	57.48967	8.34593	51
11	8	Boxcore	Bottom	7/17/03	20:05	57.49077	8.3471	91
11	9	Boxcore	Bottom	7/17/03	20:17	57.49063	8.3458	91
11	10	Boxcore	Bottom	7/17/03	20:28	57.49043	8.34448	92
11	11	Boxcore	Bottom	7/17/03	20:39	57.49062	8.34288	92
11	12	Boxcore	Bottom	7/17/03	20:50	57.49065	8.34185	92
11	13	CTD-ROS	Begin	7/17/03	21:17	57.49177	8.33438	93
11	13	CTD-ROS	Bottom	7/17/03	21:19	57.49152	8.33397	93
11	13	CTD-ROS	End	7/17/03	21:26	57.49112	8.33273	93
12	1	CTD-ROS	Begin	7/18/03	6:05	56.72908	6.00645	52
12	1	CTD-ROS	Bottom	7/18/03	6:06	56.72925	6.00617	52
12	1	CTD-ROS	End	7/18/03	6:21	56.72873	6.00667	52
13	1	CTD-ROS	Begin	7/18/03	16:41	56.01162	3.34228	78
13	1	CTD-ROS	Bottom	7/18/03	16:42	56.01162	3.34265	71
13	1	CTD-ROS	End	7/18/03	16:59	56.01105	3.3447	70
13	2	Waterkist	Close	7/18/03	17:32	56.01085	3.35305	71
13	3	CTD-ROS	Begin	7/18/03	17:52	56.01022	3.36017	71
13	3	CTD-ROS	Bottom	7/18/03	17:53	56.00995	3.3608	86
13	3	CTD-ROS	End	7/18/03	18:03	56.0105	3.3645	70
14	1	CTD-ROS	Begin	7/19/03	6:54	54.66642	4.66313	47
14	1	CTD-ROS	Bottom	7/19/03	6:55	54.66635	4.663	48
14	1	CTD-ROS	End	7/19/03	7:09	54.66552	4.66168	48
15	1	Scanfish	Begin track	7/19/03	11:23	54.07465	4.51618	46

Table 2 Participants listing with main research interest

Last name	first name	function	title	research subject	email
Brussaard	Corina	scientist	Dr.	viral ecology	corina.brussaard@nioz.nl
Baudoux	Anne-claire	Ph-D		viral ecology	baudoux@nioz.nl
Veldhuis	Marcel	scientist	Dr.	Phyt. Ecology	veldhuis@nioz.nl
Noordeloos	Anna	technician		phytoplankton	anna@nioz.nl
Bossink	Marieke	technician		phytoplankton	bossink@nioz.nl
Kuipers	Bouwe	scientist	Dr.	microzooplankton	kuipers@nioz.nl
Hegeman	Jan	technician		primary production	janh@nioz.nl
Gonzales	Santiago	technician		scanfish	gonzalez@nioz.nl
Van Noort	Govert	technician		microzooplankton	gvnoort@nioz.nl
Chenard	Caroline	student		phyto/virus	
Bakker	Karel	technician		nutrients	karelb@nioz.nl
van der Waal	Dedmer	student		phytoplankton	dedmerb@hotmail.com
Parada	Veronica	Ph-D		bacteria/virus	vparada@nioz.nl
Sintes	Eva	scientist	Dr.	bacteria/virus	esintes@nioz.nl
Laan	Martin	technician		CTD-operator	martin@nioz.nl

Table 3

not able to make PDF from this table. Information can be requested from Corina Brussaard, Email: corina.brussaard@nioz.nl