

Smetacek, Victor; de Baar, Hein JW; Bathmann, Ulrich; Lochte, Karin; Rutgers van der Loeff, Michiel M (1997): Ecology and biogeochemistry of the Antarctic circumpolar current during austral spring: Southern Ocean JGOFS Cruise ANT X/6 of R.V. Polarstern. *Deep-Sea Research Part II-Topical Studies in Oceanography*, **44(1-2)**, 1-21, doi:10.1016/S0967-0645(96)00100-2

Documentation of Bottle data

2a. CTD, O₂-probe, fluorometry

Cees Veth, Sven Ober, Ronald de Koster (all NIOZ)

{CTD.press, CTD.temp, CTD.sal, CTD.O2.sens, CTD.Fluor, CTD.Trans}

2b. Underway salinity, temperature, fluorometry

Uli Bathmann (AWI)

{No data in this report}

3. Dissolved Oxygen

Rinus Manuels (NIOZ)

{Oxy1, Oxy2, Oxy, OxyT}

Oxygen samples have been collected and analyzed in duplicate according to the Winkler method, following recommendations of the Woce Hydrographic Program (WHP) except for the calibration of the sodiumthiosulphate solution which was done gravimetrically (rather than with pipettes) for better accuracy. The photometric high precision oxygen titrator provides reproducibility better than 0.1 percent, well exceeding the 0.5 % WHP requirements. Because of the very cold seawater in the Antarctic region gas bubbles were seen to escape from the samples after having been acidified under normal laboratory conditions. In order to avoid this outgassing all samples were stored and analyzed at 4.5 °C in a thermostatted waterbath.

Abbreviations:

Oxy1 = 1st replicate

Oxy2 = 2nd replicate

Oxy = Mean (excluding possible outliers)

OxyT = Sample temperature on closing bottle

Culberson, C.H. (1991) Dissolved Oxygen, chapter in: WHP Operations and methods - July 1991.

4. Nutrients

Karel Bakker (NIOZ), Peter Fritsche (IFM), Jacques Poncin (IEM)

Nitrate, nitrite, phosphoric acid, orthosilicic acid

Karel Bakker, Peter Fritsche

{Silicate, Nitrate, Nitrite, Phosphate}

Samples were collected by Niskin, NOEX or GoFlo bottles and as soon as possible taken for nutrients in polyethylene bottles. Measurements were carried out within 12 hours after collection; meanwhile samples were kept cool at 2 °C and dark in a refrigerator. Volumetric flasks for dilution of nutrients stocks were precalibrated giving linearity with correlation coefficients of at least 0.999 for 4 calibration points. Baseline water obtained by dissolving 34g NaCl / 1 demiwater (18 MOhm) is used as washwater between the samples and as a carrier for the diluted stocks in order to avoid matrix problems on an autoanalyzer. Blank measurements of this water obtained values of zero µM for phosphate, nitrate,

nitrite and silicate. For the CTD samples the maximum sample value is compared with the full scale standard value so to obtain the best resolution of the system. With every run of CTD samples we put in a stable nutrient-cocktail containing all the parameters as an independent check standard.

The samples were measured on a Technicon AA-II autoanalyzer with a sample rate of 30 hr⁻¹ using 80 seconds sample-time and 40 seconds washing-time to reach a steady state level. Calculations were done with an attached PC correcting for baseline and gain drift.

The chemical methods used for the various nutrients were:

Silicate: Measured as the reduced molybdenum blue complex at 660 nm, with ascorbic acid as reductant; using oxalic acid to eliminate the phosphate interference.

Ortho-phosphate: Formation of the reduced molybdo-phosphate complex at pH 0.9-1.1 whereby potassium-antimonyl tartrate is used as a catalyst and ascorbic acid as the reductant. The developed color is measured at 880 nm. Method described first by Murphy and Riley (1962). At the end of the cruise all hydrographic data were corrected upwards by multiplication with a factor 1.04 based on the overall offset observed through two months versus the absolute standard. Data provided for several shipboard experiments is to be corrected accordingly.

Nitrate and nitrite:

Method described by Grasshoff (1983). Nitrate is first reduced into nitrite using a copperized cadmium coil (reduction > 95%) with imidazole as buffer agent. Used are two channels, one for nitrate plus nitrite with the cadmium coil in the first stage sample line, and the other for nitrite alone using the same color reagent for both. The pink colour formed after diazotation with sulphanylamide and naphthylethylenediamine is measured at 550 nm for both channels nitrate is obtained by substracking the nitrite values from the first channel.

The overall statistics for this cruise were:

	detection limit [μM]	accuracy at typical value [μM]
silicate	0.4	0.8 at 100
o-phosphate	0.02	0.05 at 2
nitrite	0.005	0.01 at 0.5
nitrate	0.3	0.6 at 30

N.B. see also project 24. for separate measurements of orthosilicic acid.

Ammonia

Jacques Poncin

{NH₄}

Ammonia is determined by the manual method of Koroleff (1976). Standards are realized with freshly prepared deionized water. Blanks are determined by using seawater samples taken at 1000-1500 m depth.

Koroleff (1976). Determination of ammonia. In: Grasshoff, K. (ed.) Methods of seawater analysis. Verlag Chemie, Weinheim, 126-133.

6. Carbondioxide system

Dorothee Bakker , Joop Rommets, Michel Stoll, Hein de Baar (all NIOZ)

Partial pressure of CO₂ in atmosphere and ocean

Dorothee Bakker
{pCO₂.GC-cm}

The partial pressure of CO₂ of discrete samples.

Discrete samples of 600 ml were taken in glass bottles with a screw cap containing a rubber septum from 1500, 1000, 500, 300, 200, 150, 100, 80, 60, 40, 20 and 10 meter from the CTD at all whole degree stations. Also all depths in deep casts to the bottom were sampled. Samples were poisoned by adding 0.1 ml of a saturated mercury chloride solution and put in a waterbath of 4.5 to 5 C for a minimum of one hour. 20 ml of water in each bottle was replaced by calibration gas of 473 ppmv CO₂ in artificial dry air. After at least another hour in the waterbath the headspace of the sample was injected into the gaschromatograph. A GC-run typically consisted of one discrete sample and calibration gas of 473 ppmv CO₂ by volume. The temperature of the waterbath was registered continuously. The temperature correction of Copin-Montegut (1988, 1989) was applied. Results were checked by comparing them with measurements of alkalinity and total CO₂.

Copin-Montegut, C., 1988. A new formula for the effect of temperature on the partial pressure of CO₂ in seawater. *Marine Chemistry*, **25**, 29-37.

Copin-Montegut, C., 1989. Corrigendum. *Marine Chemistry*, **27**, 143-144.

Continuous measurements of the partial pressure of CO₂ in surface water and marine air.

Seawater was pumped continuously from 12 meter below sealevel to an equilibrator. The temperature difference between water at the intake and in the equilibrator was typically less than a degree. Every 10 minutes the CO₂ content of the headspace of the equilibrator was measured by a gaschromatograph. Marine air was pumped from 22 meter above sea level. Calibration gases of 259, 361 and 473 ppmv in artificial dry air by BOC, UK were used. Each GC-run consisted of two calibration gases, an equilibrator sample, followed by marine air and a second equilibrator sample. CO₂ was converted to methane by a nickel catalyst and detected by an FID-detector. The temperature correction of Copin-Montegut (1988, 1989) was used.

Files are per day with nomenclature CO2Dmdd.XLS and CO2Smdd.XLS

- Files containing the suffix D contain ONLINE data per 10 minute interval
- Files containing the suffix S contain ONLINE data and air-sea fluxes computed with different methods.
- mm stands for the month
- dd stand for the day

CO2D????	Explanation	Unit	Parameterisation	Skin temperature difference	Length wind interval	Length atmospheric pressure
Date	Date	m/d/yr	-	-	-	-
Time	Time	hh:mm	-	-	-	-
Wvel	wind velocity	m·s ⁻¹	-	-	-	-
AirTemp	air temperature	°C	-	-	-	-
Humidity	humidity	%	-	-	-	-
Latitude	Latitude	N+, S-	-	-	-	-

Longitude	Longitude	E+, W-	-	-	-	-
SpeedAh	speed ahead	m·s-1	-	-	-	-
Air pressure	atmospheric pressure	hPa	-	-	-	-
Glob rad	global radiation	W·m-2	-	-	-	-
Visibility	visibility	m	-	-	-	-
Cloud base	cloud base	m	-	-	-	-
Depth	water depth	m	-	-	-	-
DewPoint	dew point	°C	-	-	-	-
Wdir	wind direction	°	-	-	-	-
Chlorophyll	chlorophyll a content	mg·m-3	-	-	-	-

CO2S????	Explanation	Unit	Parameterisation	Skin temperature difference	Length wind interval	Length atmospheric pressure
Date	Date	m/d/yr	-	-	-	-
Wanted time	Time	hh:mm	-	-	-	-
xCO2DryAir	dry volume fraction of CO2 in dry air	µmol·mol-1	-	-	-	-
fCO2Air	fugacity of CO2 in with water saturated air	µatm	-	-	-	-
fCO2Eq	fugacity of CO2 in the equilibrator	µatm	-	-	-	-
fCO2w	fugacity of CO2 in water	µatm	-	-	-	-
Gamma eq	fugacity coefficient of CO2 for the equilibrator	-	-	-	-	-
K0_Air	solubility of CO2 at the sea surface	mol·kg-1·atm-1	-	-	-	-
K0_H2O	solubility of CO2 in bulk water	mol·kg-1·atm-1	-	-	-	-
Cair	concentration of CO2 at the sea surface	µmol·kg-1	-	-	-	-
Cwater	concentration of CO2 in bulk water	µmol·kg-1	-	-	-	-
Tempeq	temperature of the equilibrator	°C	-	-	-	-
TskTemp	Water temperature bow salinometer	°C	Bow salinometer	-	-	-
TPyro	Uncorrected Pyrometer signal	°C	Detected signal (before correction)	-	-	-
TPyroCorr 40°	Corrected pyrometer temperature 40°	°C	Detected and corrected to 40°	-	-	-
TskSal	Salinity bow salinometer	-	-	-	-	-
Density	Density of seawater	kg·m-3	-	-	-	-
TWVel	wind velocity	m·s-1	-	-	-	-
AirTemp	air temperature	°C	-	-	-	-
Humidity	humidity	%	-	-	-	-
PosLat	Latitude	N+, S-	-	-	-	-
PosLon	Longitude	E+, W-	-	-	-	-
SpeedAh	speed ahead	m·s-1	-	-	-	-
SpeedAc	speed across	m·s-1	-	-	-	-
Airpressure	atmospheric pressure	hPa	-	-	-	-
GIRad	global radiation	W·m-2	-	-	-	-
Vis	visibility	m	-	-	-	-
CloudBase	cloud base	m	-	-	-	-
SysDepth	water depth	m	-	-	-	-
DewPoint	dew point	°C	-	-	-	-
WDir	wind direction	°	-	-	-	-
Chloro	chlorophyll a content	mg·m-3	-	-	-	-
Flux LMno skin	CO2 air-sea flux	mmol/(m2·d)	Liss-Merlivat	none	10 min	10 min
Flux LM meas	CO2 air-sea flux	mmol/(m2·d)	Liss-Merlivat	detected	10 min	10 min
Flux LMskin02	CO2 air-sea flux	mmol/(m2·d)	Liss-Merlivat	0.2°C	10 min	10 min
Flux LM1002	CO2 air-sea flux	mmol/(m2·d)	Liss-Merlivat	none	10 min	6 weeks
Flux LMwind	CO2 air-sea flux	mmol/(m2·d)	Liss-Merlivat	none	6 weeks	10 min
Flux Wno skin	CO2 air-sea flux	mmol/(m2·d)	Wanninkhof	none	10 min	10 min
Flux W meas	CO2 air-sea flux	mmol/(m2·d)	Wanninkhof	detected (40°)	10 min	10 min
Flux Wskin02	CO2 air-sea flux	mmol/(m2·d)	Wanninkhof	0.2°C	10 min	10 min
Flux W1002	CO2 air-sea flux	mmol/(m2·d)	Wanninkhof	none	10 min	6 weeks
Flux Wwind	CO2 air-sea flux	mmol/(m2·d)	Wanninkhof	none	6 weeks	10 min

Flux Hasse	CO2 air-sea flux	mmol/(m ² ·d)	Wanninkhof	Skin effect, Hasse	10 min	10 min
Flux Saun8	CO2 air-sea flux	mmol/(m ² ·d)	Wanninkhof	Skin effect, Saunders, l=8	10 min	10 min
Flux Saun var	CO2 air-sea flux	mmol/(m ² ·d)	Wanninkhof	Skin effect, Saunders, l=variable	10 min	10 min
Flux Schnacht	CO2 air-sea flux	mmol/(m ² ·d)	Wanninkhof	Skin effect, Schlüssel et al, night	10 min	10 min
Flux Schtot	CO2 air-sea flux	mmol/(m ² ·d)	Wanninkhof	Skin effect, Schlüssel et al, day+night	10 min	10 min
Flux Soloviev	CO2 air-sea flux	mmol/(m ² ·d)	Wanninkhof	Skin effect, Soloviev and Schlüssel	10 min	10 min
dTsch-D	Skin temperature difference	°C	Model Schlüssel et al, day	-	-	-
dTsch-N2	Skin temperature difference	°C	Model Schlüssel et al, night	-	-	-
dTsch-tot	Skin temperature difference	°C	Model Schlüssel et al, day+night	-	-	-
dTsa, l=8	Skin temperature difference	°C	Model Saunders, labda =8	-	-	-
dTsa, l=var	Skin temperature difference	°C	Model Saunders, variable labda	-	-	-
dThasse	Skin temperature difference	°C	Model Hasse	-	-	-
dTso-tot	Skin temperature difference	°C	Model Soloviev and Schlüssel	-	-	-

Total Carbondioxide

Michel Stoll

{TCO2.C}

Total carbon dioxide in discrete samples was determined by the Coulometric method (Johnson et al., 1987). Samples were poisoned with 0.05 - 0.1 ml of saturated mercury(II) chloride solution to prevent changes due to biological activity. They were then analyzed with an automated extraction line. A subsample is acidified with 8.5% phosphoric acid and bubbled through with CO₂-free nitrogen gas. The released CO₂ gas is captured in ethanol-amine solution with an indicator which is photometrically backtitrated. Standards reference seawater as supplied by Dickson were determined regularly as a quality control check. Accuracy and precision amounts to $\pm 1.5 \mu\text{mol/kg}$.

Johnson K.M., P.J. LeB. Williams, L. Brändström and J. McN Sieburth (1987). Coulometric total carbon dioxide analysis for marine studies: Automatization and calibration. *Marine Chemistry*, **21**, 117-133.

Alkalinity

Joop Rommets

{Alk.A}

For the determination of the alkalinity 125 ml sea water samples were titrated at 20 °C with 0.1 M hydrochloric acid in a closed cell modified after Bradshaw and Brewer (1988). A Gran plot was made of the data points after the second equivalent point. For the calculation the constants of Goyet and Poisson (1989) were used. About five samples could be analysed in one hour with an accuracy of 1 micro-aequivalent per kg seawater. Most of the samples were taken at the full degree mesostations at 20, 40, 60, 80, 100, 150, 200, 300, 500, 1000 and 1500 metres, occasionally until the bottom.

Bradshaw, A.L. and P.G. Brewer (1988) *Marine Chemistry*, **34**, 155-162.

Goyet, C. and A. Poisson (1989) *Deep-Sea Research*, **36**(11), 1635-1654.

7. POC and PON

Uli Bathmann (AWI), Bernard Quéguiner (IEM), Laetitia Teissier (IEM), Jacques Poncin (IEM), Pascal David (CNRS)

Particulate Organic Carbon and Nitrogen

U. Bathmann

{POC, PON}

Shortly after taken water samples from CTD-rosette, 1 to 4 L of seawater water were filtered through a precombusted (550 °C, 12 hours) Whatman GF/F glasfibre filter and shortly rinsed with distilled water. Filters were stored frozen (-25°C) until measurement at the laboratory at AWI. Before measurements filters were exposed to fumed HCl for 24 hours, then rinsed shortly with distilled water and dried (60 °C, 2 hours). The measurements were done by means of an Perkin-Elmer CHN Analyzer. Values are given in mg POC / m³ or mg PON / m³.

Particulate Organic Carbon and Nitrogen (in relation to project 24.)

Bernard Quéguiner, Laetitia Teissier, Jacques Poncin, Pascal David.
{Biog.Si.POC, Biog.Si.PON}

2.5-l seawater were filtered through 25mm Whatman GF/F filters (precombusted at 450 °C) using Pyrex filter towers (Millipore). Filters were stored frozen in closed glass pill-boxes. POC and PON analyses were performed in the laboratory. After elimination of inorganic carbon remaining on the GF/F filters by fuming with concentrated HCl, POC and PON were measured by a combustion method (Strickland and Parsons, 1972), using a modified Carlo Erba model N 1500 analyser.

Strickland J.D.H. & Parsons T.R. 1972. A practical handbook of seawater analysis, 2nd edition. Bull. Fish. Res. Bd. Canada, 167 : 310 pp.

8. Dissolved Organic Carbon and Dissolved Organic Nitrogen

Avan Antia (SFB), Paul Kähler (SFB)
{DOC, DON}

The contents of dissolved organic carbon (DOC) and nitrogen (DON) are measured by High Temperature Catalytic Combustion similar to the method presented by Sugimura and Suzuki (1988). 10 ml of sample are filled into glass ampoules from the Niskin bottle, acidified with 60 µl concentrated phosphoric acid, and the ampoules sealed. Acidified samples may be stored for prolonged periods (months). Before measurement the water is sparged of (inorganic) CO₂ (i.e. all carbonates at the low pH) by bubbling with argon in the opened ampoules for 10 to 20 minutes. 100 µl of the sample is injected into a quartz tube containing 20 g of platinumized aluminum oxide beads (5% Pt content) covered with 2 g of Pt gauze and kept at a temperature of 900 °C. A stream of oxygen (or 10% oxygen in argon for combined DOC/DON measurements) carries the water vapor and combustion gases through several water traps (5 °C.-trap, ice bath, Mg-perchlorate tube) and adsorption traps for sulphur- and chlorine-containing gases (tin, zinc and bronze) to an infrared adsorption CO₂-detector. In one of three such setups a chemoluminescence detector for the measurement of NO (to measure total dissolved nitrogen compounds) is in line after the infrared detector. Calibration is against glucose and urea dissolved in seawater at appropriate concentrations. The obtained values of total organic carbon (TOC) and total dissolved nitrogen (TDN) are converted to DOC and DON by the subtraction of particulate C, particulate N and inorganic dissolved N-compounds respectively. We measure water column values of odd stations on board, even-numbered stations at home. Additionally, we measure DOC and DON in ice, porewater, and water from various experiments.

Values are given in µmol C and N per litre.

Sugimura, Y. and Suzuki, Y. (1988) A high temperature catalytic oxidation method of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Marine Chemistry*, **16**, 83-97.

9. Chlorophyll and Pigments

Uli Bathmann (AWI), Bärbel Bolt (FBB), Doris Meyerdierks (FBB), Bernard Quéguiner (IEM), Laetitia Teissier (IEM), Jacques Poncin (IEM), Pascal David (CNRS), Sylvie Mathot (ULB), Ilka Peeken (SFB), Maria van Leeuwe (NIOZ)

Determination of chlorophyll *a* and phaeopigments by fluorometric technique

U. Bathmann
{Chlorophyll}

Shortly after taken water samples from CTD-rosette, 0.5 to 2 L of seawater water was filtered through a Whatman GF/F glasfibre filter. Filters were stored frozen (-25 °C) until extraction which was normally performed within one day by adding 10 ml of 90% acetone/water (v/v) and grinding (plankton and filters) with glass beads. The extract was measured for chlorophyll *a* and phaeopigments (the latter after adding two drops of 1M HCl) with a Turner Design fluorometer according to the method described by Evans et al. 1982. The fluorometer was calibrated by using pure chlorophyll extract obtained from Sigma Chemical Company and checked by the photometric method described by Strickland and Parsons (1972). Values are given in mg chl. *a*/ meter³ or µg chl.*a* /l.

Chlorophyll *a* and phaeopigments (in relation to project 24).

Bernard Quéguiner, Laetitia Teissier, Jacques Poncin, Pascal David
{Biog.Si.Chla}

1-l samples are filtered through 25mm Whatman GF/F filters. Filters are placed in Pyrex tubes and kept frozen until analysis in the laboratory. Analysis is performed using the fluorometric method (Neveux, 1976).

Neveux J. 1976. Dosage de la chlorophylle *a* et de la phéophytine par fluorométrie. *Ann. Inst. Océanogr.*, **52**, 165-174.

Chlorophyll *a* (in relation to project 27.)

Doris Meyerdierks, Bärbel Bolt
{DMSP.Chla}

After prefiltration through a 200 µm nylon net to remove larger zooplankton, phytoplankton was concentrated by filtration (< 150 mbar) of 1 - 4 L seawater onto glass fibre filters (Whatman GF/F, 47 mm diameter). For chlorophyll *a* analysis, filters were stored frozen (-25 °C) in polyethylene tubes until extraction within the next two days. They were then homogenized in 5 ml of 90 % acetone, centrifuged and the supernatant was determined fluorometrically using a Chlorophyll-Fluorometer (biosens Hannover, Germany) calibrated against chlorophyll *a* standard (Sigma Chemicals) which was checked photometrically according to Jeffrey and Humphrey (1975).

Chlorophyll *a* (in relation to projects 12. and 13.)

Sylvie Mathot
{Data available upon request}

For below study on "Phytoplankton: chlorophyll, proteins, lipids, carbohydrates" (see section 12.) a small dataset of Chl *a* values was produced. This is not in the database but available upon request.

Pigments

Ilka Peeken

{Pigments, 19-but, 19-hex, allox, chla, chla/allo, chla/epi, chlb, chlc1+2, chlc3, chllda, diadino, fuco, lut/zeax, peridin, phorba, phphyta, prasinox, pyrophorb, pyrphyta}

Seawater samples (2-8 l) were filtered onto 25 mm Whatman GF/F filters with a pressure of less than 120 mbar. After filtration, the filters were folded and stored in 2 ml micro centrifuge tubes (Eppendorf cups) at -30 °C until analysis.

Samples from transects 2, 3, 5, 6 and 7 were measured on board by means of a HPLC-system, equipped with a Perkin Elmer pump (series 400), an LS1 fluorescence detector, a spectroflow 757 UV-Vis detector and a Waters integration system. Samples from transect 11 were measured in the laboratory 4 months after the cruise, where the UV-Vis detector was replaced by a diode array spectrophotometric detector (Waters 995).

For analytical preparation, 50 µl internal standard (canthaxanthin) and 2 ml acetone were added to each filter sample and then homogenised for 3 minutes in a cell mill. After centrifugation, the supernatant liquid was placed in Eppendorf cups and stored at -30 °C until analysis within the next 12 hours. Just prior to analysis, an aliquot (100-200 µl) of the sample was premixed with water (HPLC-grade) in the ratio 1:1 (v/v) and injected onto the HPLC-system. The pigments were analysed by reverse-phase HPLC, using a C₁₈ spherisorb ODS 3 µm (4.0x125mm) Pharmacia column and HPLC-grade solvents (Biomol). Solvent A consisted of 80% methanol and 20% 0.5 M ammonium acetate and solvent B contained 80% methanol and 20% acetone. The gradient was run from A to B in 30 minutes and then held for 10 minutes at B, with a continuous flowrate of 1.5 ml min⁻¹. Eluting pigments were detected by absorbance (436 nm) and fluorescence (Ex: 410 nm, Em: > 600 nm).

Pigments were identified by comparing their retention times with those of pure standards and algal extracts. Additional confirmation for each pigment was done with the remaining extracts of representative samples from all transects using on-line diode array absorbance spectra between 390-750 nm. Pigment concentrations were quantified based on peak areas of external standards, which were spectrophotometrically calibrated using extinction coefficients published by BIDIGARE (1991). For correction of experimental losses and volume changes, the concentrations of the pigments were normalised to the internal standard canthaxanthin.

Bidigare, R. R. (1991). Analysis of algal chlorophylls and carotenoids. In *Marine particles: Analysis and Characterisation*, vol. Geophysical Monograph (ed. D. C. Hurd und D. W. Spencer), pp. 119-123: American Geophysical Union.

Pigments (in relation to project 25.)

Maria van Leeuwe

{Var3.Pigments}

Seawater was collected typically at 80m and 40m depths as to coincide with the sampling depths for particulate trace metals (see below 25). At least 10 l of sea water was filtered over glass fibre filters (Whatman GF/F). Filters were immediately placed into a dewar-container holding liquid nitrogen, in which they will be stored until HPLC-analysis at home.

10. Bacteria biomass and production

Karin Lochte (AWI), Peter Bjørnsen (MBL), Anke Weber (UOS/IFM), (Hanna Giesenhagen, IFM), Alexandra Nielsen (MBL), Sylvie Becquevort (ULB).

Bacterial abundance

Anke Weber (Hanna Giesenhagen)

{Bact.CellNum.AO}

5-10 ml of formalin fixed sample filtered onto Irgalanblack pre-stained 0.2 μm Nuclepore filters, a second membrane filter was placed underneath to facilitate dispersion of the bacteria over the filter. After four minutes of staining by acridine orange, the counts were made under blue-light excitation using a Zeiss epifluorescence microscope (1000x) with ICS-optics. The shipboard dataset will be written up in collaboration with H. Giesenhagen (IFM).

Bacterial Biomass

Anke Weber (Hanna Giesenhagen)

{Bact.BM.AO}

Mean bacterial cellular biomass was estimated by measuring 50 randomly selected cells (from filters prepared as described above) and calculating mean bacterial biovolume by comparison to a New Portion grid which was calibrated by comparison with standard size fluorescent beads. Calculation of the mean bacterial cellular carbon content was done according to Simon & Azam, Mar. Ecol. Prog. Ser. 51, 201-213, 1989. Bacterial biomass was calculated by multiplying cell numbers and mean cellular carbon content. No correction was made for possible shrinkage as a result of sample preparation. The shipboard dataset will be written up in collaboration with H. Giesenhagen (IFM).

Bacterial biomass

S. Becquevort, Th. De Henau

{Bact.CellNum.DAPI, Bact.BM.DAPI}

Bacteria were enumerated by epifluorescence microscopy after 4',6-diamidino-2-phenylindole (DAPI) staining (Porter & Feig, 1980) and by flow cytometry after DAPI staining (De Henau, 1992). Biovolumes were estimated on enlargements of microphotographs. Conversion into carbon biomass was done using the biovolume dependent C/ biovolume ratio proposed by Simon & Azam (1989).

Bacterial production

Peter K. Bjørnsen, Karin Lochte

{Bact.TTI, Bact.LEU}

Production of bacterial biomass is determined by incorporation of [^3H]-labelled thymidine and leucine into macromolecules which are precipitated by cold trichloroacetic acid (TCA) as described below. The dependence of substrate incorporation on incubation time and concentration of the respective substrate was investigated in different water bodies throughout the cruise. The data in the database are the basic (and most reliable) data of substrate incorporation given in pmol thymidine $\text{l}^{-1} \text{day}^{-1}$ or in pmol leucine $\text{l}^{-1} \text{day}^{-1}$, respectively. The conversion factors to convert these incorporation rates to production of bacterial cells or biomass carbon are established in experiments with 0.8 μm filtered water samples, in which the production of new cells is monitored parallel to [^3H]-thymidine and [^3H]-leucine incorporation.

In order to convert the substrate incorporation into bacterial production the following conversion factors were determined in the experiments: 1 pmol of tritiated thymidine incorporation (TTI)

corresponds to a bacterial production of 1×10^6 cells; for leucine (LEU) 1 pmol leucine incorporated corresponds to a net carbon production of 3 ngC. Carbon content of the bacterial cells was determined as 1.87×10^{-5} ngC cell⁻¹. Carbon conversion efficiency of bacteria was assumed as 30%.

Incubations were carried out at a fixed temperature of 0 °C. Experiments showed that temperature has an effect of up to 15 % per degree C on incorporation rates. Corrected incorporation rates which take into account in situ temperatures will be supplied later.

Thymidine:

Four 10 ml aliquots of water sample are dispensed into plastic vials; one of these subsamples is fixed by addition of 100 µl 39% formalin amended with cold thymidine and serves as a blank. Each sample receives methyl-[³H]-thymidine, specific activity 3.11 TBq/mmol (Amersham), to a final concentration of 2nM. The samples are incubated for 2 to 3 hours at 0 °C and the incubation is stopped by addition of 100 µl 39% formalin amended with cold thymidine. The samples are filtered through 0.22 µm poresize cellulose acetate filters, pre-soaked in a cold thymidine solution, and rinsed 10 times with 2 ml 5% ice cold TCA. The filters are placed in 5 ml plastic scintillation vials and 4.5 ml scintillation cocktail (Lumagel SB, Baker Chemicals) are added. The radioactivity incorporated in the cold TCA precipitable material on the filter is measured on board by a Packard Liquid Scintillation Counter.

Leucine:

The procedure is identical to the thymidine incorporation method described above except for the following differences: The samples receive a final concentration of 10 nM L-[4,5-³H]-leucine (Amersham), specific activity 0.522 TBq/mmol. After incubation the samples are filtered through 0.2 µm poresize polycarbonate filters (Nuclepore).

12. Microplankton abundance; autotrophs, heterotrophs; incl. nanoplankton

Dick Crawford (AWI), Friedel Hinz (AWI), Renate Scharek (AWI), Christine Klaas (AWI), Andrea Detmer (IFM), Sylvie Becquevort (ULB), Sylvie Mathot (ULB), Thierry de Henau (ULB), Frank Jochem (IFM), Peter Bjørnsen (MBL), Alexandra Nielsen (MBL), Santiago Gonzalez (NIOZ), Bouwe Kuipers (NIOZ)

Microplankton & nanoplankton, mostly flagellates

S. Becquevort, Th. De Henau
{Auto.Flag.}

Nanoplanktonic auto- as well as heteroflagellates were enumerated by epifluorescence microscopy after DAPI staining (Porter & Feig, 1980). The lengths and widths of 100 flagellates were determined visually by comparison with an ocular micrometer. Cell volumes were estimated using the equation of a prolate spheroid. Nanoplankton biomass was calculated assuming a carbon density of 0.11 pgC µm⁻³ (Edler 1979).

Phytoplankton and protozoa enumeration

Sylvie Mathot
{muPhyt.CellNum, muPhyt.BM} (no data)

Several water sub-samples were taken for microscopical examination of the planktonic communities and preserved with appropriate (i.e. for reliable identification and abundance determination) fixatives immediately after collection. Thus samples for phytoplankton (mainly diatoms and dinoflagellates) and protozoan (ciliates and dinoflagellates) carbon biomass calculation were preserved either with glutaraldehyde-25% (final conc. 0.5%) or with a glutaraldehyde-lugol cocktail (35

o/oo, v/v; final conc. 1%). Carbon biomass was calculated from cell counts and cellular biovolume measurements under an inverted microscope (Utermöhl technique), by using appropriate carbon/cell biovolume conversion factors of $0.11 \text{ pgC} \cdot \mu\text{m}^{-3}$ (Edler, 1979) for dinoflagellates (auto- and heterotrophic) and diatoms, and $0.08 \text{ pgC} \cdot \mu\text{m}^{-3}$ (Beers and Stewart, 1970) for ciliates. Autotrophs were discriminated from heterotrophs by their red chlorophyll autofluorescence. Details concerning this procedure are extensively described in Becquevort et al. (1992).

Becquevort, S., Mathot, S. and Lancelot, C. (1992) Interactions in the microbial community of the marginal ice zone of the northwestern Weddell Sea through size distribution analysis. *Polar Biol.*, **12**: 211-218.

Phytoplankton: chlorophyll, proteins, lipids, carbohydrates

Sylvie Mathot

{Cell.Const.Proteins, Cell.Const.Lipids, Cell.Const.CarbHydr.RES,
Cell.Const.CarbHydr.TOT}

These measurements were done in conjunction with the project on photosynthetic assimilation rates (see below 13.), only at selected stations. Water samples were collected for chlorophyll a measurements, for phytoplankton biochemical determination (i.e. proteins, total and reserve carbohydrates, lipids) and for cell enumeration. All these variables will be measured in the home laboratory.

Chlorophyll a measurement:

1 to 2 liters of water were filtered onto 47 mm diameter Whatman GF/F glass-fiber filters. Filters are kept frozen in the dark until analysis. Chlorophyll a will be determined following either the spectrophotometric method of Lorenzen (1967) or the fluorometric method of Yentsch & Menzel (1963). The dataset is quite small and not part of the database, yet available upon request.

Phytoplankton biochemical composition:

For stocks determination, 1 to 2 liters of water were filtered on pre-ashed (512 °C) 47 mm diameter Whatman GF/F glass-fiber filters. Phytoplankton cellular constituents were estimated by regression analysis of measurements of particulate proteins, carbohydrates and lipids on Chl-a concentrations as proposed by Lancelot-Van Beveren (1980). Thus quantitative measurements of proteins in solution were performed with the Folin-Ciocalteu reagent (Lowry et al, 1951) following the experimental procedure developed by Hewitt (1958). For total carbohydrates, a modified form of the phenol-sulphuric acid method of Dubois et al (1956) was used. Total lipids were extracted with a chloroform-methanol solution, carbonized with concentrated sulphuric acid, and the amount of carbon was spectrophotometrically measured (Marsh & Weinstein, 1966). Experimental procedures are extensively described in Lancelot-Van Beveren (1982).

Lancelot-Van Beveren, C. (1982) Etude ecophysiologique du phytoplankton de la zone côtière belge. These de doctorat, Université Libre de Bruxelles, 221p.

Autotrophic Pico- and Nanoplankton Abundance

Andrea Detmer

{PPML.ANP.C, PPML.UNID.C, PPML.ANP.M, PPML.APP.M.}

Autotrophic pico- and nanoplankton cell numbers (ANP.C) and numbers of unidentified cells/particles (UNID.C) as estimated by flow cytometry under blue-light excitation (430-490 nm) on a Fluvo II cytometer, triggered for red autofluorescence normally characteristic for chlorophyll. Chlorophyll-containing as well as phycoerythrin-containing particles were observed. Also 50-100 ml of

sample fixed with glutaraldehyde (1%) were filtered onto Irgalanblack pre-stained 0.2 µm Nuclepore filters, stained by Proflavine and stored frozen (-28 °C) for counting with epifluorescence microscopy (ANP.M, APP.M). These methods also apply to observation of cyanobacteria (JGOFS activity 16.)

Heterotrophic Pico- and Nanoflagellate Abundance

Frank Jochem

{PPML.HNF10-20, PPML.HNF<10, PPML.HPF, PPML.HPP}

Cell numbers of heterotrophic nanoflagellates 10-20 µm (HNF10-20) and less than 10 µm (HNF<10) in size, and heterotrophic picoflagellates (HPF) less than 2 µm. 50-100 ml of sample filtered onto Irgalanblack pre-stained 0.2 µm Nuclepore filters, stained by Proflavine and Hoechst 33342, counted under blue-light excitation on a Zeiss epifluorescence microscope (200x and 1000x) with ICS-optics; suspect cells checked for nucleus presence under UV-excitation using Hoechst stain. Relying on these methods cyanobacteria were also observed (JGOFS activity 16.).

Abundance and biomass of heterotrophic dinoflagellates smaller than 20 µ

(in relation to experiments on growth potential)

Peter K. Bjørnsen and Alexandra C. Nielsen

{Bact.SHD.Cellnum and Bact.SHD.BM}

Ten ml samples were fixed by 150 µl of 25% glutar aldehyde, stained with proflavin hemisulphate (10 ppm final concentration) and filtered onto black polycarbonate filters of 0.2 µm pore size. More than 50 small heterotrophic dinoflagellates (SHD) were counted and sized under an epifluorescence microscope at 600 x magnification and blue excitation. Only dinoflagellates smaller than 20 µm were included in these counts. Biovolume was converted into biomass assuming a carbon density of 0.12 pg C per µm³.

Diatoms

Dick Crawford, Friedel Hinz

{Diatoms}

Apstein net (20µm mesh) from surface 20m and Multinet (64µm mesh) from 5 depths down to 300m were examined live for floristic assesment. Samples were preserved and prepared for a permanent slide collection to provide a detailed taxonomic account and relative frequency figures for the floral composition.

Microplankton and nanoplankton

Renate Scharek, Christine Klaas

{muPhyt.Prot.CellNum, muPhyt.Prot.Phyto, muPhyt.Protozoa, muPhyt.Protists}

Species composition and biomass of the mikroplankton assemblage are obtained by counting fixed material with the Utermöhl technique on:

- a) Water bottle 200ml samples.
- b) 10 l water bottle samples concentrated to 200ml by reverse filtration through 2.0 µm filters.
- c) Multinet samples (64 µm mesh).

Abundances will be given in cells/l. Biomass are given in nanograms/l for protistan carbon (PRC), and comprises a phytoplankton carbon fraction (PPC) and protozooplankton carbon (PZC).

Video recordings were taken for identification of living heterotrophic protozoans.

Microzooplankton

Bouwe Kuipers, Santiago Gonzalez
{Microzoo}

At all meso- and most microstations samples were taken from 20, 40, 80, 200 and 300 m. Niskin or NOEX bottles (three stations above and two under the usual thermocline) and preserved immediately in 2 % Lugol-A solution. At NIOZ numbers per ml. of tintinnids, other ciliates, large heterotrophic flagellates, rotifers and other groups will be counted by settlement inverted microscopy for size-categories <20, 20-60, 60-100 and >100 μm . The purpose is to check whether there are -similar to recent North Sea observations -significant numerical responses in these small grazers at the onset of blooms. Diatom numbers counted in the same samples could illustrate how because of microzooplankton size selective grazing larger algae can become dominating during blooms notwithstanding the much higher $\mu\text{-max}$ of the smaller microzooplankton algal prey-species.

13. Primary production by 14C

Sylvie Mathot (ULB), Frank Jochem (IFM), Bernard Quéguiner (IEM)

Primary Production

Frank Jochem

{PPML.TOTPP, PPML.PP>20, PPML.PP5-20, PPML.PP2-5, PPML.PP<2}

Size-fractionated primary production from 12 hrs. in-situ incubation (stn 885 only) or 24 hrs. in-situ simulated deck incubation using neutral density filters in a seawater-cooled deck incubator (see below description by S. Mathot, ULB Bruxelles). Added activity = 50 μCi per 250 ml polycarbonate bottle; subsamples of 45 ml for measurements in size fractions of "total" (untreated), "<20" (net gauze), "<5" and "<2" (by 5.0 μm and 2.0 μm Nuclepore filters, respectively), fractionated subsamples filtered onto 0.2 μm membrane filters, dissolved in Lumagel SB and measured in a Beckman LS-1800 liquid scintillation counter. Two bottles treated with 1.5×10^{-5} nM DCMU were used as «dark bottles» and subtracted from the light bottle values. The unfractionated value {PPML.TOTPP} is the JGOFS variable and compatible with below {PP.Prim.Prod}.

Primary production

Sylvie Mathot

{PP.Prim.Prod}

Assessments according to JGOFS protocols with the exception of using deck incubators rather than in situ incubation. 250 ml samples were collected from depths closer to 100%, 70%, 45%, 22%, 10%, 4.5%, 1.5%, and 0.5% (neutral density screens) of incoming PAR. An additional sample was taken and kept in the dark as «dark bottle» for which then the value was subtracted from the values of the light bottles. Samples were incubated for 24 hours in the "*in-situ* simulated deck-incubator", in polycarbonate bottles with $\text{NaH}^{14}\text{CO}_3$ at a rate of 10 μCi per 100 ml sample (Amersham, specific activity = 56 mCi.mmol^{-1}).

Samples were filtered on Whatman GF/F filters at the end of incubation time. Two drops of HCl 0.5N were added to the filters to release unassimilated $^{14}\text{CO}_2$. Scintillation cocktail was added to the filters prior to their radioactivity assaying in a Packard 1900CA Tri-Carb Liquid Scintillation Counter. Occasionally, size-fractionation was also performed onto 10 μm and 0.8 μm Poretics filters.

Photosynthetic assimilation rate, phytoplankton growth and respiration.

Sylvie Mathot

{PP.AssimRT, Phyto.GrowthRT}

This project was done in relation with above "Phytoplankton: chlorophyll, proteins, lipids, carbohydrates", see section 12. The experimental determination of physiological parameters characteristic of phytoplankton involved two kind of tracer experiments conducted in parallel under simulated *in-situ* conditions. For all these incubations, 100 to 250 ml seawater sample, which amount was chosen according to phytoplankton biomass, were incubated in polycarbonate bottles with $\text{NaH}^{14}\text{CO}_3$ at a rate of $10\mu\text{Ci}$ per 100 ml sample (Amersham, specific activity = 56 mCi.mmol^{-1}).

Experimental determination of photosynthetic parameters involved short-term (4 hours) ^{14}C incubations (based on the Steemann-Nielsen standard method), performed at various fractions of light intensity, either in an "*in-situ* simulated inside incubator" (0, 1, 4, 6, 10, 15, 20, 30, 40, 60, 80, 100%; Philips 500W halogen lamps) or in an "*in-situ* simulated deck-incubator" (0, 0.5, 1.5, 4.5, 10, 22, 45, 70, 100% of incoming PAR), both cooled by running seawater. Filters were treated as described above. Photosynthetic parameters K_{max} , a , and b were calculated by mathematical fitting of the data relative to the photosynthesis-light relationship using Platt et al.'s equation (1980).

Experimental determination of phytoplankton growth (net primary production) and respiration parameters was performed through long-term (24 hours) light-dark kinetics of ^{14}C assimilation into 4 pools of cellular constituents easily separable by biochemical procedure: small metabolites (composed of monomeric precursors for the synthesis of macromolecular compounds), lipids and polysaccharides (constituting together the reserve products of the phytoplankton cell), and proteins. Incubations were conducted at *in-situ* temperature under saturating illumination ($100\text{-}170\ \mu\text{E.m}^{-2}.\text{s}^{-1}$). The light-dark cycle was fixed at 14:10 to simulate environmental conditions. Two drops of HCl 0.5N were added to the filters to release unassimilated $^{14}\text{CO}_2$. Scintillation cocktail was added to the filters prior to their radioactivity assaying in a Packard 1900CA Tri-Carb Liquid Scintillation Counter. Occasionally, size-fractionation was also performed onto $10\mu\text{m}$ and $0.8\mu\text{m}$ Poretics filters. Filters for biochemical fractionations were kept frozen until analysis in the home laboratory. Details on experimental procedure and biochemical fractionation are described in Lancelot and Mathot (1985). Phytoplankton growth and respiration parameters were estimated by mathematical fitting of the data relative to the kinetics assimilation of ^{14}C into proteins and storage products, using the equations described in Lancelot et al. (1991).

Lancelot, C. and Mathot, S. (1985) Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short- and long-term incubation with ^{14}C -bicarbonate. I. Mixed diatom population. *Mar.Biol.*, 86(3): 219-226.

Lancelot, C., Veth, C., and Mathot, S. (1991) Modelling ice edge phytoplankton bloom in the Scotia-Weddell Sea sector of the Southern Ocean during spring 1988. *J.Mar.Syst.*, 2: 333-346.

Carbon primary production (in relation to project 24.)

Bernard Quéguiner

{Biog.Si.PP}

250-ml samples were collected from depths closer to 100%, 25%, 10%, 3%, 1% and 0,1% incident PAR. Samples were spiked with $10\mu\text{Ci}$ (370 kBq) $\text{NaH}^{14}\text{CO}_3$ and incubated during 24-h in a deck incubator maintained at sea surface temperature. Just after spiking, 100 ml is removed and immediately counted by liquid scintillation to determine the specific activity of each sample. After incubation samples were filtered onto $0.4\ \mu\text{m}$ Nuclepore filters. Non-incorporated $\text{NaH}^{14}\text{CO}_3$ remaining

on the filter is eliminated by adding a few ml of 0.01 N HCl in 0.4 µm-filtered seawater. Filters were then counted by liquid scintillation method.

15. New Production by ^{15}N and Export Production by ^{234}Th

Marita Wunsch (SFB), (Wolfgang Koeve, SFB), Frank Dehairs (VUB), (Leo Goeyens, VUB), Michiel van der Loeff (AWI)

Nitrate assimilation

Marita Wunsch, (Wolfgang Koeve)
{ $^{15}\text{NO}_3$.Uptake}, no data.

New Production here is defined as phytoplankton growth based on the assimilation of nitrogen in the form of NO_3 as opposed to Regenerated Production, which is based on ammonia uptake (EPPLEY etc. 19..). We measure NO_3 uptake with $^{15}\text{NO}_3$ as a tracer.

4.5-litre samples of seawater from various depths (corresponding to 66, 52, 13 and 1% light levels) are filled into transparent polycarbonate bottles, spiked with $^{15}\text{NO}_3$ at a concentration of about 10% of the ambient nitrate concentration and incubated on deck at surface water temperature for 24 hours. The respective light levels are attained by covering the bottles with appropriate light absorbing foils. After this incubation two to four litres (depending on Chl.-a content) of the water are filtered over GF/F-filters (200 bar suction) which are stored frozen then dried at 60 °C and later analyzed for particulate ^{15}N by mass spectrometry.

NO_3 uptake of various size classes is determined by additional sequential filtration over 20, 5, and 2 µm pore-size filters respectively. In these cases, sample depths and incubation light levels are 100, 30, and 1% of the surface light level

Values are given in $\mu\text{mol NO}_3$ uptake per day and m^2 .

New Production by ^{15}N

Frank Dehairs, (Leo Goeyens)
{ $^{15}\text{NH}_4$.Uptake, $^{15}\text{NO}_2$.Uptake, $^{15}\text{NH}_4$.Mineralisation}

Uptake of ammonium

Sampling and spiking:

4.5 l seawater was sampled in a Nalgene bottle. Spike solution (1.7 ml $^{15}\text{N-NH}_4\text{Cl}$ solution: 11.6 mg l^{-1} ; 99% ^{15}N) was added. The bottle was gently shaken and subsampled for the initial ammonium content. The spike addition was calculated to increase an original ammonium content of 1 $\mu\text{mol l}^{-1}$ by about 10%. Since during ANT X/6 natural ammonium concentrations never reached that level, spike additions were generally \gg 10% of the original content.

Incubations: During the transects samples from -10 and -30m were incubated for 24 hours in the on-deck incubator, respectively at 100% and 30% of incoming radiation using a neutral density screen.

During the drift station (from October 22 to 24), day incubations (06.00 to 18.00) and night incubations (18.00 to 06.00) were done. Day incubations concerned samples from -7, -20, -40, -60 and -80 m depth. The first day of the drift station experiment these samples were set out in-situ. On day 2 and day 3 the samples were incubated in the on-deck incubator, respectively at 100%, 66%, 30%, 13% and 1% of incoming light, using neutral density screens.

Night incubations were done on samples from -10 and -60m. These samples were incubated in the dark in the cold room at 1 °C or in the on-deck incubator.

At the end of the incubation period, subsamples were taken for determination of the final ammonium content. Another subsample is taken for assessment of the ammonium mineralisation rate (see point 2 below).

For cases where Chl-a content $\geq 0.3 \mu\text{g l}^{-1}$, one fraction of the seawater sample was passed over a $20 \mu\text{m}$ screen to assess ammonium uptake by the $\leq 20 \mu\text{m}$ sized phytoplankton. The other fraction was directly filtered (approx. 0.2 bar underpressure) on precombusted ($450 \text{ }^\circ\text{C}$) Whatman GF/F filters to assess ammonium uptake by the total phytoplankton population.

For situations with low Chl-a content ($< 0.3 \mu\text{g l}^{-1}$) no size fractionation was performed.

GF/F filters are oven dried ($50 \text{ }^\circ\text{C}$) and sealed in plastic petri dishes for later analysis in the home laboratory.

Mineralisation of ammonium

Mineralisation of ammonium is assessed during the same experiment as ammonium uptake. After the incubation and before the filtration (see point 1 above), 10 ml of the sample are transferred to a serum vial, and spiked with 500 μl of an unlabeled ammonium carrier solution (NH_4Cl : 54.15 mg l^{-1}) for the entrainment of ^{15}N -ammonium. Ammonium from this solution is extracted by adding 500 μl of strong base (KOH: 50%) and the produced ammonia is captured on aluminium oxide beads coated with sulphuric acid (0.25 M) and suspended in a tin cup above the solution. After ≥ 48 hours the tin cup is retrieved and sealed in a plastic envelope for later analysis in the home laboratory.

Uptake of nitrite

Sampling and spiking:

Sampling of the seawater from -10 m, in a 4.5 l Nalgene bottle. Addition of 2 ml spike solution ($^{15}\text{N-KNO}_2$: 4.8 mg l^{-1} ; 99% ^{15}N). The bottle is gently shaken and subsampled for the initial nitrite concentration.

Incubation:

The sample is incubated for 24 hours in the on-deck incubator at 100% of incoming light. After the incubation a subsample is taken for the determination of the final nitrite concentration. The remaining solution is filtered on precombusted Whatman GF/F and oven dried at $50 \text{ }^\circ\text{C}$. Samples are sealed in plastic petri dishes for later analysis in the home laboratory.

Determination of ^{15}N abundancy (home laboratory)

The ^{15}N abundancy is determined by emission spectrometry (JASCO NIA- ^{15}N analyzer) after conversion of particulate nitrogen and extracted ammonia into dinitrogen by means of an oxidation with CuO. This conversion is performed in quartz discharge tubes at $750 \text{ }^\circ\text{C}$. Specific and absolute uptake rates are computed using mathematical expressions based on the isotope dilution law and on a constant transport model (the latter assumes no significant biomass build up during the incubation)

16. Autotrophic pico/nano-plankton (including cyanobacteria)

Andrea Detmer, Frank Jochem (both IFM)
{PPML.SYN}

See above core activity 12., for our methods for autotrophic and heterotrophic pico- and nanoplankton.

18. Grazing by Microplankton

Christine Klaas (AWI), Sven Reitmeier (SFB), Sylvie Becquevort (ULB), Thierry de Henau (ULB)

Grazing by microplankton & nanoplankton

S. Becquevort, Th. De Henau
{NanoZoo.Grazing}

Protozoan grazing on phytoplankton and bacteria specifically were measured by the method proposed by Sherr et al (1987) based on the uptake of fluorescent - labelled prey (FLA *fluorescent - labelled algae*, Rublee & Gallegos 1989; FLB *fluorescent - labelled bacteria*, Sherr et al 1987).

Grazing

Christine Klaas
{Dinoflag}

From abundance's of dinoflagellates (see above 12.) it is conceivable to derive an estimate of grazing rates, in which case these rates will also be brought into the database.

Microzooplankton Grazing

Sven Reitmeier
{Var2.k, Var2.g, Var2.Regr, Var2.Init.Chla, Var2.PICG, Var2.ChlADoubl, Var2.CG}

Microzooplankton Grazing (MZP-grazing) is measured by a procedure similar to that described by LANDRY and HASSETT (1982). Diluting a water sample with filtered seawater will also reduce the grazing pressure on planktonic algae, while the phytoplankton growth rate is not affected. Water from the Niskin bottle (or Gerard Water Sampler) is pre-screened over a 100 µm gauze and then diluted to 20, 40, and 70% with 0.2 µm-filtered seawater. 2.5 litres of each of these dilutions, and of undiluted seawater, are incubated in transparent polycarbonate flasks in triplicate at ambient temperature on deck for 24 hours, the light level of the sampling depth being attained by a cover with an appropriate light absorbing foil.

Chlorophyll-a is determined fluorimetrically before and after incubation for each dilution after filtration of 1-2 litres onto a GF/F filter, and acetone extraction. Before and after incubations there are also samples taken for phytoplankton and microzooplankton microscopic analysis. These samples are fixed with Lugol's solution and analysed by the Utermöhl technique. The natural logarithms (ln) of the quotient between initial and final Chl.-a values (Phytoplankton apparant growth rate, AGR) are plotted against the dilution (fractions of 1), the grazing coefficient ("g") is read from the slope of the line and "k" (y-axis intercept) is the phytoplankton growth coefficient.

Values of "g" and "k" are given (dimensionless), describing the function of the linear regression like: $y=k+gx$. Once the C/Chl. ratio is established from the analysis of POM, the grazing rates can also be expressed in carbon units.

Additionally, samples were taken for later pigment analysis by HPLC in order to obtain information on the selectivity of MZP-grazing.

24. Silica biogeochemical cycling

Bernard Quéguiner (IEM), Laetitia Teissier (IEM), Jacques Poncin (IEM), Pascal David (CNRS)
{Biog.Si.BM, Biog.Si.Prod, Biog.Si.Silicate}

Biogenic silica biomass

1-l seawater is filtered onto 47 mm Nuclepore membranes (0.4 mm at each depth and size-fractionation : 0.4-10 mm at selected depths). Filters are dried for at least 24h at 60 °C and stored in

plastic Petri dishes. Biogenic silicate [$\text{mmol Si}\cdot\text{m}^{-3}$] analyses are performed in the laboratory using the NaOH digestion method of Paasche (1973) as modified by Nelson et al. (1989).

Nelson D.M., Smith W.O., Muench R.D., Gordon L.I., Sullivan C.W. & Husby D.M. 1989. Particulate matter and nutrient distributions in the ice-edge zone of the Weddell Sea : relationship to hydrography during late summer. *Deep-Sea Res.*, **36**, 191-209.

Paasche E. 1973. Silicon and the ecology of marine plankton diatoms. 1. *Thalassiosira pseudonana* (*Cyclotella nana*) grown in a chemostat with silicate as the limiting nutrient. *Mar. Biol.*, **19**, 117-126.

Biogenic silica production

PSi is determined by the ^{30}Si stable isotope method of Nelson and Goering (1977). The tracer solutions had been passed through Chelex resin before the cruise to limit possible trace metal contamination. 1-l samples are collected from depths closer to 100%, 25%, 10%, 3%, 1% and 0,1% incident PAR. Samples are then drawn in 1-l acid (HCl 0.1 N)-cleaned polycarbonate bottles that had been previously covered with neutral-density nickel screens. Under a laminar flow hood, samples are spiked with 20 μmoles of $\text{Na}_2^{30}\text{SiO}_3$ and placed in a plexiglas incubator maintained at sea surface temperature by constant flow of surface water. After a 24-h incubation the samples are filtered under a laminar flow hood through 47mm Nuclepore membranes (0.4 μm at each depth and size-fractionation : 0.4-10 μm at 100% and 0.1% depths). Filters are dried for at least 24h at 60°C and stored in plastic Petri dishes. The determination of the rates of orthosilicic acid uptake (PSi) is performed in the laboratory by mass spectrometry.

Nelson D.M. & Goering J.J. 1977. A stable isotope tracer method to measure silicic acid uptake by marine phytoplankton. *Anal. Biochem.*, **78**, 139-147.

Biogenic silica dissolution

At some stations, Diss Si is determined in parallel to PSi by the ^{30}Si stable isotope method of Nelson et al. (1991). 1.6-l samples are collected from depths closest to 100%, 25%, 10%, 3%, 1% and 0,1% incident PAR. Under a laminar flow hood, samples are spiked with 32 μmoles of $\text{Na}_2^{30}\text{SiO}_3$. 1-l of each sample is immediately transferred into acid-cleaned polycarbonate bottles and allowed to incubate during 24-h in a deck incubator maintained at sea surface temperature. 0.6-l remaining are immediately filtered through 0.4 μm Nuclepore filter. The filter is retained for BSi analysis and the filtrate treated to collect the dissolved silicic acid for isotopic analysis. 37.5 ml of Sephadex-cleaned ammonium molybdate/hydrochloric acid reagent are added to the filtrate. The silicomolybdate complex is then extracted on a Sephadex column. After 24-h incubation the 1-l sample is filtered onto 0.4 μm Nuclepore filter. The filter is retained for PSi analysis and the filtrate is treated as described above. $^{28}\text{Si}/^{30}\text{Si}$ ratios are determined in the laboratory by mass spectrometry. The difference between $^{28}\text{Si}/^{30}\text{Si}$ ratios in the dissolved phase before and after incubation allows to calculate the rate of dissolution of biogenic silica.

Nelson D.M., Ahern J.A. & Herlihy L.J. 1991. Cycling of biogenic silica within the upper water column of the Ross Sea. *Mar. Chem.*, **35** : 461-476.

Orthosilicic acid concentrations

$\text{Si}(\text{OH})_4$ concentrations are determined using a Technicon autoanalyzer. The analytical procedure is based on the reduction of silicomolybdate in acid solution to molybdenum blue by a

methyaminophenol sulfate (Metol) and sodium sulfite solution (Mullin & Riley, 1975 ; Strickland & Parsons, 1972).

Mullin J.B. & Riley J.P. 1955. The spectrophotometric determination of silicate-silicon in natural waters with special reference to sea water. *Anal. Chim. Acta*, 12 , 162-170.

Strickland J.D.H. & Parsons T.R. 1972. A practical handbook of seawater analysis, 2nd edition. *Bull. Fish. Res. Bd. Canada*, 167 : 310 pp.

N.B. This is independent from the orthosilicic acid measurements done for the hydrographic work (core activity 4).

27. Ecophysiology of ice algae: Dimethylsulfoniumpropionate (DMSP) content during ice melt

Doris Meyerdierks and Bärbel Bolt (FBB)

{DMSP.DMSP, DMSP.Chla}

After prefiltration through a 200 µm nylon net to remove larger zooplankton, phytoplankton was concentrated by filtration (< 150 mbar) of 1 - 4 L seawater onto glass fibre filters (Whatman GF/F, 47 mm diameter). The particulate DMSP was determined as gaseous Dimethylsulfid (DMS) using the specific reaction that cleaves DMSP 1:1 into DMS and acrylic acid upon the addition of a strong base. After base (25 % NaOH) was added the filters were incubated in gastight vials and headspace gas analysis was performed at least 4 hours later, after complete liberation of DMS, using a gas chromatograph (Shimadzu 8A) with flame photometric detector. For calibration, DMSP standards (Research Plus, Bayonne, NJ, USA) were treated and analysed in the same way.

28. Biogeochemistry of Barium

Frank Dehairs (VUB)

{diss.Ba, Part.Ba.Part.Ba, Part.Ba.Part.Ca, Part.Ba.Part.Al, Part.Ba.Part.Si, Part.Ba.Part.Sr}

Particulate Barium

During the north-south transects along 6° W total suspended matter samples were taken at every 2 degrees of latitude for particulate Ba-barite determination. The upper 600m of water column were sampled to document on the mesopelagic accumulation of Ba-barite.

Sampling

Depths were -10, -50, -100, -150, -200, -250, -300, -350, -425, -500 and -600m. Between 10 and 20 l seawater were filtered under pressure on Nuclepore membranes of 0.4 µm porosity. After filtration filters were rinsed with about 10 ml of Milli-Q type water and dried at 50 °C. They were then stored in plastic petri dishes at room temperature for further analysis in the home laboratory.

Determination of Barium

Filter samples are transferred to platinum crucibles. After careful combustion of the Nuclepore substrate and of the organic matter at ~ 400 °C, the remaining particulate matter is fused for 1 hour at 1100 °C with LiBO₂ as the flux. The fused pearl is redissolved in 4% hot nitric acid. This solution is brought to 10 ml volume. The analysis is carried out by inductively coupled plasma optical emission spectrometry (ICP-OES). Other elements such as Ca, Sr, Si and Al are analysed simultaneously.

29. ^{13}C in surface water particulate organic matter

Frank Dehairs (VUB)

{Part.Ba.13C}

When sampling for particulate barium, during the north-south transects along 6° W, the -10 and -50m depths were sampled for ^{13}C determination in POM. Occasionally samples were also taken from 20m and 60m depths sampled with the Gerard bottles. About 20 l of seawater are filtered under pressure on precombusted (450°C) Whatman GF/F filters. Filters are dried at 50°C and stored at room temperature till later analysis in the home laboratory.

Analysis of ^{13}C natural abundance

Filters are left for 1 hour in HCl vapour for elimination of carbonates. Subsamples are combusted in a CN analyzer (Carlo Erba NA 1500) and the produced CO_2 is automatically trapped in a trapping-box (Finnigan-Mat) for preconditioning the gas sample before introduction into the on-line mass spectrometer (Delta-E, Finnigan Mat). CO_2 mass measurements are relative to that of CO_2 reference gas (from marble standards, standardized relative to NBS CaCO_3 standards). Final results are expressed as ^{13}C abundance (^{13}C) relative to the PDB reference.