

DYNAPROC CRUISE

Scientist responsibility :

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Parameters

The biological variables measured included the **numbers of bacteria, cyanobacteria, phototrophic and heterotrophic nanoflagellates, bacterial production and ectoaminopeptidase activity**. Biochemical variables were **POC and DOC**.

All these data are published in Van Wambeke et al (1998)

Sampling strategy

sampling site = Dyfamed station (43° 25 N, 07° 52 E, 2350 m depth)

time series : Following the drift of sediment traps during four cycles of 36 hours each (C1: May 11-12, C2: May 14-15, C3: May 27-28, C4: May 30-31, 1995). Time series observations were assembled from serial hydrocasts through the upper 500 m made every four hours, from 10:00 the first day until 22:00 the second day (UT+2).

For these biological parameters, samples were collected from Niskin bottles only at two depths: 5 and 30 m

vertical profiles

0-200m CTD casts as follows :

CTD number	date	time
108	11 May	14:00
117	12 May	14:00
129	14 May	14:00
139	15 May	14:00
266	29 May	18:00
279	31 May	14:00

POC - DOC

For particulate organic carbon (POC), 4 l of seawater sample were filtered onto pre-combusted (450°C, 6 hours) GF/F glass fiber filters. POC was determined using a LECO CHN 900 analyzer. CV inter-filter was within 2 -10 %.

Results are expressed in $\mu\text{M C}$

Samples for dissolved organic carbon (DOC) analyses were measured by high temperature combustion on a Shimadzu TOC 5000 Analyzer. Details of this procedure have been previously described (Yoro et al. 1999). A four point-calibration curve was performed daily

with standards (83-332 $\mu\text{M C}$) prepared by diluting a stock solution of potassium hydrogen phthalate in Milli-Q water. The instrument blank ranged from 6 to 9 $\mu\text{M C}$ and error between two duplicate samples ranged 3 to 10 %.

Results are expressed in $\mu\text{M C}$. In practise, Total organic carbon (from unfiltered samples) were analysed and then POC was subtracted to estimate DOC.

Bacteria, cyanobacteria, nanoflagellates: epifluorescence microscopy

Samples for the enumeration of bacteria and protozoa were fixed with 2% buffered formalin filtered through 0.2 μm . Between 6 and 15 ml were filtered within 12 h after sampling on black Nuclepore filters (0.2 μm) that were subsequently stained with DAPI (4,6-diamidino-2-phenylindole, final concentration 2500 $\mu\text{g l}^{-1}$). Filters were mounted in Olympus immersion oil on slides and stored frozen until examination. On the same filter, we counted bacteria by image-analyzed epifluorescence microscopy (Van Wambeke 1995), and cyanobacteria (CYAN), heterotrophic nanoflagellates (HNAN) and phototrophic nanoflagellates (PNAN) by manual epifluorescence microscopy. For bacteria, up to 40 fields containing 50-80 bacteria each were counted (CV inter-field, 6-16%). CYAN were counted under blue light excitation. About 60-100 flagellates were counted per filter in random 10 mm-strips (80 μm width). CV inter-strips was within 10-22%. HNAN were recognized as all eucaryotic non pigmented organisms less than 10 μm in size, and PNAN were identified as chlorophyll containing cells by their red autofluorescence.

Results are expressed in abundance of cells per ml

Bacterial production : thymidine technique

Bacterial production (BP) was estimated by the thymidine method (Fuhrman & Azam 1982). Duplicate samples (20-40 ml) and a blank (formalin-killed, 2% final concentration) were incubated with 20 nM [Methyl- ^3H]-thymidine ($^3\text{H-Tdr}$, 44 Ci mmol^{-1}) for 2 to 4 hours in sterile disposable plastic flasks at 15°C. Incubation was terminated with the addition of 2% formalin. Samples were filtered through 0.2 μm Nuclepore filters, extracted with ice cold 5% trichloroacetic acid (TCA) for 15 min, and finally rinsed three times with 5% TCA. We preliminarily checked the conditions for linear incorporation and isotope saturation.

Activity is expressed in terms of pmole thymidine incorporated in the cold TCA precipitate per liter per hour

Ectoaminopeptidase activity : MCA-leucine fluorogenic substrate

Hydrolysis of fluorogenic substrate was used to measure ectoaminopeptidase activity (Hoppe et al, 1988). The fluorescent analog L-leucine-4-methyl-7-coumarinylamide (MCA-leu) was added to 30 ml of water (1 μM final concentration) in sterile disposable plastic tubes and incubated for up to 5 hours. During the incubation, subsamples were transferred to the quartz cuvette and fluorescence (excitation 365 nm, emission 445 nm) was read with a Kontron SFM 23B spectrofluorometer calibrated with MCA. Blanks consisted of 0.2 μm filtered sea water which was boiled for 15 min. MCA-leu hydrolysis rates were calculated by subtracting the rate of change in fluorescence of the blanks from the average rate of change in fluorescence of the duplicates. Mean reproducibility within duplicates was 3% and rate of blanks ranged from 0.5 to 4% of the mean response of duplicates.

Activity was expressed in terms of nmole leu hydrolysed per liter per hour

References

Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic production in marine surface waters. Evaluation and field results. *Mar Biol* 66:109-120

Hoppe H, Kim S, Gocke K (1988) Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. *Appl Environ Microbiol* 54:784-790

Van Wambeke F (1995) Numération et taille des bactéries planctoniques au moyen de l'analyse d'images couplée à l'épifluorescence. *Océanis* 21:113-124

Yoro SC, Panagiotopoulos C, Sempéré R (1999) Dissolved organic carbon contamination induced by filters and storage bottles. *Water Res*, 33:1956-1959

How to read the data base.

Excel file

Sheet time serie

column A : depth (m)

column B : CTD number

column C : time of day - date of month (May, 1995)

column D : time expressed in decimal units of days

column E : Syncechococcus-like cyanobacteria (abundance per ml)

column F : Phototrophic nanoflagellate abundance (per ml)

column G : Heterotrophic nanoflagellate abundance (per ml)

column H : bacterial abundance (per ml)

column I : Thymidine incorporation rate (pM per hour)

column J : ectoaminopeptidase activity (n mole leu per liter per hour)

column K : dissolved organic carbon ($\mu\text{M C}$)

column L : particulate organic carbon ($\mu\text{M C}$)

Sheet profile

column A : CTD number

column B : depth (m)

column C : Syncechococcus-like cyanobacteria (abundance per ml)

column D : Phototrophic nanoflagellate abundance (per ml)

column E : Heterotrophic nanoflagellate abundance (per ml)

column F : bacterial abundance (per ml)

column G : Thymidine incorporation rate (pM per hour)

column H : ectoaminopeptidase activity (n mole leu per liter per hour)