BACTERIOPLANKTON BIOMASS AND PRODUCTION DURING EUMELI 3 CRUISE

J.-P. TORRETON[1] and Ph. DUFOUR[2]

1. Sample collection

Water samples were collected with acid washed Go-Flo bottles from the surface to 4000, 3000 and 2200 m at the oligotrophic, mesotrophic and eutrophic site, respectively.

2. Bacterial enumeration

Bacterial enumeration were performed on samples filtered on 0.2 µm Nuclepore membranes, after staining with DAPI (Porter and Feig, 1980). An epifluorescence microscope suspended on a vibration isolation table (Technical Manufacturing Corporation, Peabody, Massachusetts) allowed bacterial enumeration on board, less than two hours after sampling or, if delayed, on frozen preparations (-20°C). More than 400 cells, on at least 20 fields were enumerated. Attached bacteria were systematically distinguished from free cells.

3. Bacterial biovolumes and carbon

Bacterial volumes were computed from bacterial dimensions estimated on enlarged photographs (Lee and Fuhrman, 1987). Bacterial carbon (BC, pgC.cell^{-1}) was deduced from bacterial volumes (V, µm^3) following the allometric model of Simon and Azam (1989) [$BC = 0.09V^{0.60}$], modified by Norland (1993) [$BC = 0.12V^{0.72}$].

4. Active bacteria

Active bacteria were defined as cells able to reduce 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) in formazan (Zimmerman *et al*., 1978) using a modification of the original method (Dufour and Colon, 1992). Duplicate 20 to 100 ml subsamples were amended with INT (0.02% final concentration) and incubated in the dark at ±1°C *in situ* temperature for 0.5 hour in surface waters to 3 hours in deep waters. Incubation was terminated by addition of buffered formalin (2% final concentration). Bacteria were then stained with DAPI, collected onto 0.1 µm pore size cellulose nitrate membranes and observed under epifluorescence and transmission light as described in Dufour and Colon (1992). INT reducing bacteria, presenting dense intracellular deposits of INT-formazan, were enumerated under the microscope. At least 100 INT reducing cells were enumerated on at least 20 fields.

5. Bacterial production of biomass from TdR incorporation

5.1. TdR incorporation in cold TCA precipitate (TdR-TCA)

5.1.1. 500 m deep and above

On 500 m deep samples and at upper levels, TdR incorporation was assayed by amending duplicate 20 to 100 ml subsamples with 20 nM [*methyl*-^{3}H]thymidine (final concentration, Amersham, 1.74 TBq/mmol).

After 0.5 to 3 h incubation with the label at ±1°C *in situ* temperature at atmospheric pressure, the duplicates were chilled in a 2°C water bath for 10 min. Samples were then filtered onto 0.2 µm

[1]IRD, PO Box A5, 98 848 Nouméa Cedex, New Caledonia E.mail : torreton@noumea.ird.nc
[2] IRD - Station d'Hydrobiologie lacustre de l'INRA, 74203 Thonon, France E. mail : dufour@ird.fr

Nuclepore polycarbonate membranes and rinsed with 5 ml of 0.2 µm filtered seawater. The vacuum was disconnected and filters received 15 ml ice-cold 5% TCA. After 15 min, vacuum was reapplied and the membranes were rinsed 3 times with 5 ml of ice-cold 5% TCA.

Linearity of incorporation over the periods of incubation was verified regularly and TdR incorporation rates always saturated at less than 20 nM TdR (checked twice at every site).

5.1.2. Below 500 m

Below 500 m, TdR incorporation was performed by incubating single 1-L sub-samples with 5 nM [*methyl*-^{3}H]thymidine at ±1°C *in situ* temperature at atmospheric pressure. A total of five 160 ml subsamples were retrieved from the incubation bottle every 6 hours from 0 to 24 hours.

Linearity of incorporation. The incorporation rate into cold-TCA precipitate was always linear over the 24h incubation ($r^2 > 0.87$).

5.2. TdR incorporation in DNA (TdR-DNA)

Labeled DNA was extracted enzymatically following a modification (Torréton & Bouvy, 1989) of Wicks & Robart's (1987) procedure. Radioactivity was determined after quench correction using external standards. Incorporation was calculated after subtracting a zero time blank.

Below 500 m, DNA was extracted enzymatically from an additional 160 ml subsample at the end of the incubation period (24h).

5.3. Conversion into cell or carbon production

The median value of literature (2×10^{18} cells mol^{-1}, Ducklow and Carlson, 1992) was used to convert ^{3}H-thymidine incorporation in cold TCA precipitate into bacterial cell production. Cell production was converted into carbon production by multiplying by bacterial cell carbon content estimated at the same level (see **3**.)

6. Turnover time for the whole bacterioplankton community

Turnover time (TT, day) was computed from bacterial biomass (BB, $fgC.l^{-1}$) and production (BP, $fgC.l^{-1}.h^{-1}$) using : $TT = BB \times Ln(2) / (BP \times 24)$

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