

Size fractionated simultaneous Dissolved Inorganic Carbon and Phosphate (DIC and DIP)
uptakes measurements
Serial filtration followed by scintillation counting

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The method consist in adding ^{33}P and ^{14}C radioactive tracers in seawater samples to measure the transformation of dissolved P and C to particulate P and C. This mainly corresponds to planktonic community uptake. 250 ml of marine water samples are collected into acid-washed, Milli-Q water and sample-rinsed, polycarbonate bottles (Nalgen). The samples are inoculated with ^{33}P and ^{14}C working solutions respectively and then incubated in *in situ* simulated conditions for 4 to 5 hours. Boxes provide with light filters (nickel screens) were used to reproduce the light level at the sample depth. Incubations are stopped by KH_2PO_4 (10 mM) addition for DIP uptake and samples are kept in the dark to stop DIC uptake. 50 ml of each labelled sample are then filtered on 25 mm polycarbonate membranes (0.2 μm), placed on a GF/F filter soaked with saturated KH_2PO_4 . It is recommended not to exceed 0.2 bars. When all samples are filtered, pressure is increased to 0.6 bars during 5 seconds in order to eliminate non incorporated ^{33}P . Filters are placed in scintillation vials (Wheaton low-potassium 6 ml glass-clear vials with screw-cap foil liner) with 150 μl of HCl (0.5 M) in order to eliminate non incorporated ^{14}C . After 12 hours, 6 ml of scintillation liquid are added in each vial before count per minute (cpm) counting. Cpm counting has been carried out on a Packard Tri-Carb® 2100TR scintillation counter by using Ultimagold MV scintillation liquid (Packard).

The detailed protocol is currently submitted to L&O: Methods, **Application of a method using C and P dual isotope labelling to the study of simultaneous carbon and phosphate uptakes by plankton species in the open ocean**, by *Solange Duhamel, Florence Zeman, and Thierry Moutin*.

Calculation method:

1°) Dissolved Inorganic Phosphate uptake rate

$$V_{DIP} = \left[\frac{DPM_{^{33}\text{P}} - DPM_{\text{Blk}} * DIP}{Qi^{^{33}\text{P}}} \right] / t(h)$$

With :

- ✓ V_{DIP} = Dissolved Inorganic Phosphate uptake rate (nM h^{-1})
- ✓ $DPM_{^{33}\text{P}}$ = ^{33}P filter activity (disintegration per minute)
- ✓ DPM_{Blk} = blank activity (disintegration per minute)
- ✓ DIP = Dissolved Inorganic Phosphate concentration
- ✓ $Qi^{^{33}\text{P}}$ = ^{33}P initial activity (disintegration per minute)
- ✓ $t(h)$ = incubation duration (hour)

Daily rate is obtained by multiplying hourly rate by 24.

2°) Dissolved Inorganic Carbon uptake rate (primary production)

$$V_{DIC} = \left[\frac{DPM_{14C} - DPM_{Blk}}{Q_i^{14C}} * DIC \right] * \frac{1}{\tau}$$

With :

- ✓ V_{DIC} = Dissolved Inorganic Carbon uptake rate (nM J⁻¹)
- ✓ DPM_{14C} = ¹⁴C filter activity (disintegration per minute)
- ✓ DPM_{Blk} = blank activity (disintegration per minute)
- ✓ DIC = Dissolved Inorganic Carbon concentration
- ✓ Q_i^{14C} = ¹⁴C initial activity (disintegration per minute)

1/τ: is determined according to Moutin et al. (1999) with considering for each station: Latitude, Julian day, Dawn time (GMT), Incubation starting time (GMT), Time of the end of incubation (GMT) and the **measured solar radiation (compilation by D. Taillez)**.

3) Integrated primary production

Integrated primary production IPP* (mmol m⁻² d⁻¹) has been calculated with trapezium method assuming (1) that subsurface (50% of incident light) rates are identical to surface rates (not measured) and (2) that rates are zero at 20 m below the deepest sampled depth (1% of incident light).

Six depths corresponding to the six transmitted light levels (50 - 25 - 15 - 7 - 3 - 1%) have been sampled throughout the transect. These depths were determined on board according to Morel & Berthon (1989), using the relationships between the euphotic layer and the chlorophyll concentrations published in Morel & Maritorena (2001). The chlorophyll concentrations were deduced from the *in vivo* fluorescence measurements after calibration with considering the previous measurements (on board fluorimetric determination using methanol as extractant by Patrick Raimbault).

At the laboratory, the euphotic zone depths (Ze) were reevaluated with considering all measurements (Profileur Satlantic (Stan Hooker), Biospherical (Stan Hooker), Profileur LICOR (André Morel), Profileur PNF-Biospherical (André Morel)), Cf “Ze_Zm_readme-1_couche_euphotique.doc”).

Then, we give two results for the integrated primary production:

A: with depths determined on board

B: with depths calculated from the mean reevaluated euphotic zone depth.

References:

Morel, A., and S. Maritorena, Bio-optical properties of oceanic waters: A reappraisal, *Journal of Geophysical Research*, 106 (C4), 7163-7180, 2001.

Morel, A., and J.F. Berthon, Surface pigments, algal biomass profiles, and potential production of the euphotic layer: Relationships reinvestigated in view of remote-sensing applications, *Limnology and Oceanography*, 34 (8), 1545-1562, 1989

Moutin, T., P. Raimbault, and J.-C. Poggiale. 1999. Production primaire dans les eaux de surface de la Méditerranée occidentale. Calcul de la production journalière. *C. R. Académie des Sciences* 322:651-659.