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A dual-labeling method for the simultaneous measurement of dissolved inorganic carbon and phosphate uptake by marine planktonic species

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Abstract

The measurement of primary production is the foundation for aquatic biogeochemistry research. The biogeochemical cycles of phosphate (P) and other biolimiting elements are tightly linked to marine primary production. We have optimized an existing method of carbon (C) and P dual isotope labeling to study the simultaneous C and P uptake by plankton species in marine environments. The two main objectives of this study were (1) to test the preservation properties of the labeled samples and the calculation methods used in separating the signals of the different radionuclides, and (2) to adapt the method to marine environments. The procedure was successfully implemented in contrasting locations within the southeast Pacific (between 146.36°W and 72.49°W). The uptake rates determined using this method ranged from 14 to 900 nM h^{-1} for dissolved inorganic C and from 0.03 to 4.5 nM h^{-1} for dissolved inorganic P in surface water. The detection limit found in ultraoligotrophic surface water was 3.33 nM h^{-1} and 0.01 nM h^{-1} for C and P, respectively. C and P assimilation fluxes in low- and high-productivity open-ocean systems may be studied using this sensitive method. We outline a protocol for marine environments that is appropriate for use under oceanographic cruise conditions. Results from the application of this method will lead to a better understanding of the interplay between carbon and phosphate biogeochemical cycles in the upper ocean.

Introduction

Since the beginning of the industrial era, the atmospheric CO_2 content has dramatically increased, leading to a rise in temperature (Falkowski et al. 2000). Phytoplankton are able to consume inorganic carbon through primary production. These rates are necessary to assess the global carbon (C) cycle and to explore the possibility of oceanic absorption of excess CO_2 (Longhurst 1991). Thus, the determination of time-varying phytoplankton productivity in the ocean has been one of the main objectives of biogeochemical oceanography over the past century (Barber and Hilting 2002). Measurement of primary production alongside that of phosphate (P) uptake can assist in understanding the interactions between C and P cycles (Karl et al. 2001, Lampman et al. 2001). P is an essential nutrient in pelagic marine ecosystems. P cycling in the upper ocean is poorly understood, however, and few studies have directly investigated the biological use of this essential element (Benitez-Nelson and Buesseler 1999). P availability in the ocean is considered to have a predominant role in controlling planktonic biomass and production (Karl et al. 2001, Moutin et al. 2005).

The use of radionuclides for the study of dissolved inorganic carbon and phosphorus (DIC and DIP) uptake has been the most popular method employed to date (Steeman-Nielsen 1951, Robinson 1969). Although Robinson (1969) indicated that ^{33}P should be particularly useful in dual labeling, it has never been applied to samples taken from the natural marine environment. Measuring several processes in the same sample

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has the advantage of maintaining exactly the same experimental conditions. There are numerous examples of methods that use the simultaneous determination of different parameters in the same sample (Mykytiuk et al. 1980, Orret and Karl 1987, Slawyk and Raimbault 1995, Raimbault et al. 1999, Cobelo-Garcia et al. 2005). The dual-labeling technique using ^{33}P and ^{14}C was first applied by Lehman (1993) in freshwater experimental studies. Olsen et al. (2002) and Vadstein et al. (2003) have taken advantage of this technique for applications in microcosm experiments. To the best of our knowledge, this method has only been employed on laboratory-based, freshwater ecosystem experiments, and no detailed and tested protocol has been proposed for the marine system.

Here, we apply and optimize the dual-labeling protocol and demonstrate the accuracy of the results obtained using this technique. The procedure has been successfully implemented during an oceanographic cruise where we followed the combined assimilation rates of C and P in the different trophic regimes encountered. For the first time we show that it is possible to follow C and P uptake in the same sample taken from the marine environment and in extreme trophic regimes. The dual-labeling method provides a useful tool for studying C and P uptake rates in marine and freshwater ecosystems. The application of this method for studying the coupling/decoupling processes of carbon and phosphate uptake rates should give better insight into the deviations that regularly occur from the Redfield ratio. The significance of this on global biogeochemical cycles remains poorly understood (Christian et al. 1997, Christian 2005). More importantly, the dual-labeling method provides an easier technique for simultaneously obtaining both the C and P uptake parameters from a single sample incubation.

Materials and procedures

Analytical process—^{33}P and ^{14}C radioactive tracers are added to the seawater samples as dissolved inorganic P and C. The transformation to the particulate form is then measured, which mainly corresponds to planktonic community uptake. ^{14}C radioactive tracer permits the measurement of DIC uptake by autotrophs (i.e., primary production), whereas ^{33}P radioactive tracer permits the measurement of DIP uptake by both autotrophs and heterotrophs. Marine water samples (250 mL) are collected into acid-washed, Milli-Q water– and sample-rinsed polycarbonate bottles (Nalgene). The samples were inoculated with 100 and 50 µL of the ^{33}P and ^{14}C working solutions, respectively (see below). Samples were incubated under simulated light conditions for 4 to 5 h around noon. We used incubation boxes equipped with light filters (nickel screens) to mimic the light irradiances at appropriate sample depths. After incubation, 600 µL of a KH_2PO_4 solution (10 mM) was added to each container to stop ^{33}P incorporation (Thingstad et al. 1993, Moutin et al. 2002, Tanaka et al. 2004). The plankton cells use this nonradioactive P source in preference to the radioactive one because of its greater concentration. This addition dramatically reduces the incorporation of ^{33}P. The blank was not affected by this KH_2PO_4 addition (data not shown). The samples were filtered within an hour after the addition of cold phosphate; 50 mL of each labeled sample was filtered through 25-mm polycarbonate membranes (0.2 µm) that were placed onto GF/F filters soaked with saturated KH_2PO_4, to minimize the physical adsorption of ^{33}P on the filter. It is recommended that the vacuum does not exceed 0.2 bars, to avoid cell destruction. When all samples were filtered, pressure was increased to 0.6 bars for 5 s to eliminate unincorporated ^{33}P. Filters were placed into scintillation vials (Wheaton low-potassium 6-mL clear-glass vials with foil-lined screw-caps) with 150 µL HCl (0.5 M) to drive off any unincorporated ^{14}C. After 12 h of reaction, 6 mL of scintillation liquid (Ultimagold MV scintillation liquid; Packard) was added to each vial. The radioactive decay in the vials was then counted using a Packard Tri-Carb 2100TR scintillation counter.

Working solutions—A fresh working solution of ^{33}P-orthophosphate was prepared just before use by diluting 30 µL of the tracer (orthophosphate in dilute hydrochloric acid; Amersham BF 1003) (specific activity > 92.5 TBq mmol^{-1}; total activity 370 MBq mL^{-1}) in 1000 µL filtered Milli-Q water (0.22 µm, Sterivex-GV; Millipore). The commercial stock solution of ^{33}P was kept at –20°C. The commercial ^{14}C working solution was used (bicarbonate aqueous solution; Amersham CFA3) (specific activity 2.0 GBq mmol^{-1}; total activity 74 MBq mL^{-1}). Consistent with the France-JGOFS (1991) protocol, 740 kBq was used for a 320 mL sample (see Moutin and Raimbault 2002). The commercial solution of ^{14}C was placed into 50-µL aliquots and stored at –20°C.

Initial activity—After the addition of 50 µL ^{14}C working solution to the sample, the flask was mixed and 100-µL aliquots were placed into scintillation vials containing 6 mL scintillation liquid, to measure the initial activity of ^{14}C. To measure the initial activity of ^{33}P, 5-µL aliquots of the ^{33}P working solution were placed into scintillation vials with 6 mL scintillation liquid.

Blanks—Duplicate samples of surface water (250 mL) were incubated with 300 µL $HgCl_2$ (20 g L^{-1}) to act as a control for nonbiologic uptake (Kirkwood 1992). These samples were then treated in the same way as the other samples.

Dissolved inorganic phosphate was determined using the molybdenum blue reaction (Strickland and Parsons 1972). This method has a current detection limit of 30 nM and an accuracy of 30 nM (Rimmelin and Moutin 2005).

Calculation methods—There are 2 standard methods for determining the activity (disintegrations per minute, or dpm) of dual-labeled samples. The first method is a conventional dual-label dpm determination (A), and the second is the full-spectrum dpm technique (B).

Calculation method A uses the different half-lives of the two isotopes. The signal from the ^{14}C decay can be distinguished from those of ^{33}P, because these 2 radionuclides have widely different half-lives (5700 ± 30 years for ^{14}C and

25.383 ± 0.040 days for ^{33}P; Bé et al. 2005). This method requires the measurement of the activity of a sample containing ^{14}C and ^{33}P at two different times (see below). The activity of the ^{14}C is essentially constant during the experiment, so that any decrease in disintegration rate is attributable to the decrease in ^{33}P, whose initial activity (expressed as dpm) can be estimated as

$$dpm^{33}P(t_0) = \frac{\frac{cpm(t_0) - cpm(t_1)}{1 - e^{-\lambda(t_1 - t_0)}}}{E_{33_P}} \quad (1)$$

where $C(t_0)$ and $C(t_1)$ are total counts per minute (cpm) at the first (t_0) and the second (t_1) counting, respectively, λ is the radioactive decay rate constant for ^{33}P (λ = ln 2/$T_{1/2}$, $T_{1/2}$ is the half-life of ^{33}P), and E_{33_P} is the ^{33}P efficiency counting. The activity corresponding to ^{14}C is given by

$$dpm^{14}C(t_0) = \frac{C(t_0) - dpm^{33}P(t_0)}{E_{14_C}} \quad (2)$$

where E_{14_C} is the ^{14}C efficiency. Because of the relatively short half-life of ^{33}P, it is possible to wait for "all" the ^{33}P to be lost from the dual-labeled samples. When ^{33}P reaches an undetectable level, the activity of the sample is close to the ^{14}C activity at t_0 because only ^{14}C remains.

Calculation method B is based on the emission spectra of ^{33}P and ^{14}C. The emission spectrum of ^{14}C extends from 0 to 156 keV and that for ^{33}P emission ranges from 0 to 250 keV. As ~100% of the ^{14}C quenched spectrum extends from the 0- to 90-keV window, it is possible to discriminate between the two spectrums via the two counting windows: one between 0 and 90 keV (region 0-90) and one between 90 and 250 keV (region 90-250). In contrast to method A, the spectrum method has the advantage of providing instantaneous results. The activity attributable to each radionuclide can be calculated by

$$dpm_{33P} = E_{33P} \frac{cpm_{0-90} \times e_{14C_{90-250}} - cpm_{90-250} \times e_{14C_{0-90}}}{e_{33P_{0-90}} \times e_{14C_{90-250}} - e_{14C_{0-90}} \times e_{33P_{90-250}}} \quad (3)$$

$$dpm_{14C} = E_{14C} \frac{cpm_{0-90} \times e_{33P_{90-250}} - cpm_{90-250} \times e_{33P_{0-90}}}{e_{14C_{0-90}} \times e_{33P_{90-250}} - e_{33P_{0-90}} \times e_{14C_{90-250}}} \quad (4)$$

where dpm_{33P} and dpm_{14C} represent the dpm attributable to ^{33}P and ^{14}C activity, cpm_{0-90} and cpm_{90-250}, the cpm in the regions 0–90 and 90-250, and e_{33_P} and e_{14_C} the percentage of ^{33}P and ^{14}C signal in each region (0–90 and 90–250). As it is necessary to determine the percentage of the signal emitted by each radionuclide in each region, we have analyzed the spectral distribution from single-labeled samples. We used 21 ^{33}P single-labeled samples (range from 0 to 10,000 dpm) and 66 ^{14}C single-labeled samples (10,000 to 95,000 dpm) corresponding to working solutions of isotope diluted in filtered milli-Q water (0.22 µm; Sterivex-GV; Millipore). Most of the ^{14}C signal (99.6% ± 0.3%) is in the 0–90 region and only 0.4% ± 0.3% in the 90–250 region, whereas 88.2% ± 0.9% of the ^{33}P signal is in the 0–90 region and 11.8% ± 0.9% in the 90–250 region.

Uptake calculations—After the dpm determination, V_{DIC} and V_{DIP}, the C and P uptake rates (nmol L^{-1} h^{-1}), can be calculated as

$$V_{DIC} = \frac{R_{T^{14}C} \times [DIC]}{t_i} \quad (5)$$

$$V_{DIP} = \frac{R_{T^{33}P} \times [DIP]}{t_i} \quad (6)$$

with

$$R_i = \frac{dpm_S - dpm_B}{dpm_Q} \quad (7)$$

where [DIC] and [DIP] are dissolved inorganic carbon and phosphate concentrations of the sample, t_i is incubation time, R_i is the consumed fraction of added label (^{14}C or ^{33}P) after an incubation time t; dpm_S, dpm_B, and dpm_Q are the dpm attributable to the ^{14}C or ^{33}P activity, to the blank, and to the initial activity, respectively.

Note that counting efficiency (E) of ^{14}C and ^{33}P must be determined before each cruise. The relationship linking cpm to dpm is E (%) = cpm / dpm. The ^{14}C quench curve has been established from a standard Kit (^{14}C–W for aqueous samples, Perkin Elmer) containing 10 capsules of 102,100 dpm (theoretical value). The quenching range was obtained by the addition of increasing trichloroacetic acid volumes in vials containing a capsule and 10 mL of scintillation liquid. The ^{33}P count efficiency was calculated from samples of the working solution diluted in milli-Q water (dpm range: 0–111,000, n = 26). For a quenching range corresponding to the samples obtained during the BIogeochemistry and Optics SOuth Pacific Experiment (BIOSOPE) cruise, E_{14_C} was 94.50% ± 0.03% and E_{33_P} was 100.00% ± 0.04%.

Assessment

Sample preservation—As samples need to be preserved for long periods of time between counting procedures, tests are required to ensure signal preservation (e.g., no loss of radioactivity). Classic plastic scintillation vials are not leak-tight. We checked the preservation of different single-labeled samples over 21 months in glass scintillation vials with foil-lined screw-caps. Single-labeled samples correspond to (1) duplicate samples labeled with ^{14}C (20 × 10^3 dpm) and (2) duplicate samples labeled with ^{33}P (7.5 × 10^6, 7.5 × 10^5, or 7.5 × 10^3 dpm). Data are shown (Figure 1) for the samples of ^{14}C (20 × 10^3 dpm) and ^{33}P (7.5 × 10^6 dpm). The slope of the regression line obtained from the activity in the sample containing only ^{33}P indicates that λ is equal to 0.0271 ± 0.0001, which corresponds to a half-life of 25.55 ± 0.05 days. This result is close to the mean of the measured values of ^{33}P half-life reported in the literature (25.383 ± 0.040 days; Chisté and Bé 2003) and is consistent with the most recent measured value of 25.56 ± 0.07 (Lagoutine et al. 1972). Moreover, the natural logarithm of the activity of the sample containing only ^{14}C was constant (9.93 ± 0.04 dpm) over the experiment (21 months) (Figure 1). The liquid scintillation properties and the scintillation vials with foil-lined screw-caps thus enabled excellent sample preservation.

Radioactive decrease of a dual-labeled sample—In a dual-labeled sample with ^{33}P and ^{14}C, the activity measured at the

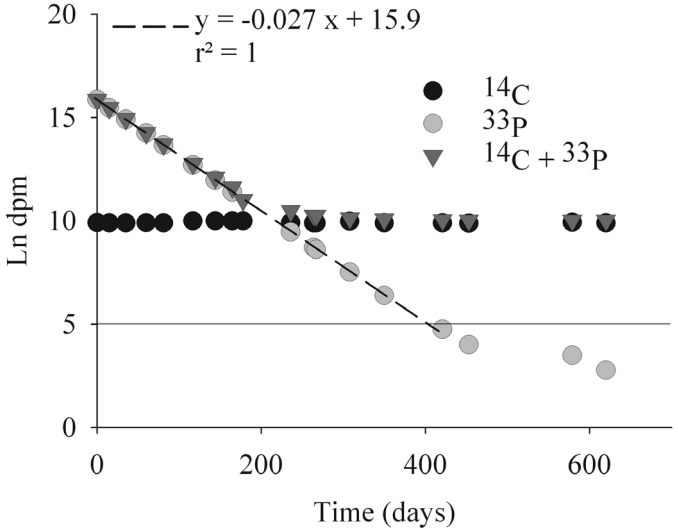

Fig. 1. Natural logarithm of the activity vs. time in a sample containing only ^{14}C (20×10^3 dpm), a sample containing only ^{33}P (7.5×10^6), and a sample containing ^{33}P and ^{14}C (20×10^3 dpm of ^{14}C plus 7.5×10^6 dpm of ^{33}P). The solid line represents the detection limit for ^{33}P activity measurement. The dotted line represent the regression line between activity and time from samples containing only ^{33}P, excluding the points under the detection limit.

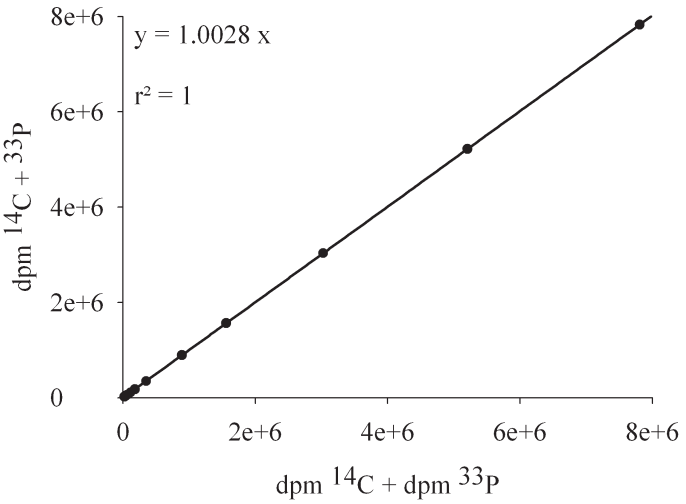

Fig. 2. Relationship between total disintegration per min (dpm) in samples containing ^{14}C and ^{33}P (dpm ^{14}C + ^{33}P), and the sum of counts in samples containing only ^{14}C and samples containing only ^{33}P (dpm ^{14}C + dpm ^{33}P).

beginning of the experiment, between 0 and 250 keV, was mainly ^{33}P activity. After some months, however, ^{33}P decayed and the activity in the sample was overwhelmingly from ^{14}C. Three duplicate samples containing a mixture of ^{14}C and ^{33}P (20×10^3 dpm of ^{14}C plus 7.5×10^6, 7.5×10^5, or 7.5×10^3 dpm of ^{33}P) were prepared in parallel with a duplicate sample labeled with ^{14}C (20×10^3 dpm) and three duplicate samples labeled with ^{33}P (7.5×10^6, 7.5×10^5, or 7.5×10^3 dpm). Regardless of the ^{33}P activity, the results were identical. Data are shown (Figure 1) for the samples of ^{14}C (20×10^3 dpm) and ^{33}P (7.5×10^6 dpm). The natural logarithm of the activity in the dual-labeled sample decreases from 15.9 to 10.03 ± 0.01 from day 0 to 200, and the same is observed in the ^{33}P single-labeled sample activity. From day 200 to 620, the natural logarithm for the dual-labeled sample activity was constant (9.93 ± 0.04) and followed the constant level of the ^{14}C single-labeled sample activity until the end of the experiment (620 days). In theory, the activity of a sample containing the two β-particle emitting isotopes is equal to the addition of the activities contained in single-labeled samples. We compared the dpm values obtained in the samples containing both ^{14}C (20×10^3 dpm) and ^{33}P (7.5×10^6 dpm), and the sum of the dpm values obtained in the single samples of ^{14}C (20×10^3 dpm) and ^{33}P (7.5×10^6 dpm). The signal emitted by a dual-labeled sample equaled the sum of the two single-labeled samples ($y = 1$; $r = 1$; $n = 36$) (Figure 2). Thus, the association of the two isotopes in the same flask did not effect the counting, even over a long period.

Tests on calculation methods—This investigation took place during the BIOSOPE cruise (see in situ application) where we applied the dual-labeling method. We compare the results obtained after different preservation times and the results obtained with the two methods.

Method A requires waiting for the ^{33}P to decay, so we checked the possibility of obtaining precise results following weeks or months of storage (Figure 3). We obtained a very good correlation between the dpm calculated according to method A over several weeks (t_1 = after 20–30 days) and several months (t_2 = after 5 months and t_3 = after a year) ($y = 1.00$, $r = 1.00$, $n = 266$ between t_1 and t_2 and between t_1 and t_3 for ^{14}C dpm; $y = 0.99$, $r = 1$, $n = 270$ between t_1 and t_2 and between t_1 and t_3 for ^{33}P dpm). We conclude that it is possible to get an excellent estimation of the final results only after several weeks of storage. These tests also enable us to conclude that it is possible to store samples for up to 1 year before the second count. The positive implication is the utility of this method on oceanographic cruises, where it is not always possible to have a second count on board due to time constraints, and sample transfer to the laboratory takes several months.

We compared the dpm values obtained when the percentages of ^{33}P and ^{14}C signal (e_{33_P} and e_{14_C}) in each region (0–90 and 90–250) vary according to the "e" standard deviation. Results shows that the dpm values can vary up to 25% ± 19% and 4.50% ± 0.35% for ^{14}C and ^{33}P, respectively ($n = 147$) (data not shown). Even if method B provides the advantage of instantaneous results, we must consider that the dpm values calculated with this method are only estimates.

Blanks and detection limits—We made 25 duplicate blanks (i.e., 50 total blanks) for the surface waters and 6 duplicate blanks (i.e., 12 total blanks) for samples from the 1% irradiance depth. There was no statistical difference between the blank values in the surface or at 1% depth (e.g., $U = 62$, df = 24, $P = 0.56$). Thus, the surface blank values are representative of the

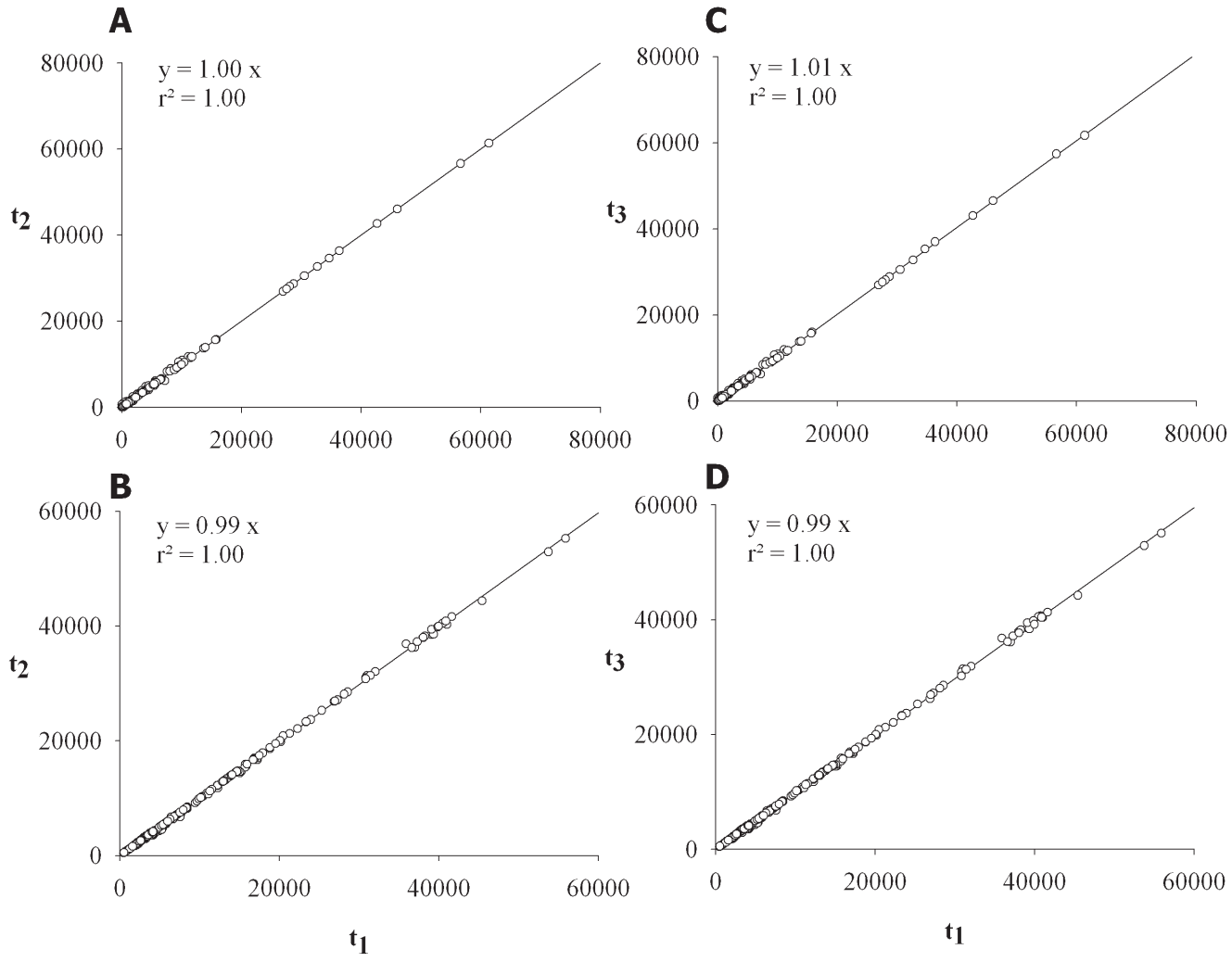

Fig. 3. Regression lines between activities determined from different times (t_1, t_2, and t_3), according to the calculation method A. Data from samples collected during the BIOSOPE cruise. Four regression lines are shown: (A) ^{14}C dpm at t_0 from counting at t_1 and t_2; (B) ^{33}P dpm at t_0 from counting at t_1 and t_2; (C) ^{14}C dpm at t_0 from counting at t_1 and t_3; (D) ^{33}P dpm at t_0 from counting at t_1 and t_3. t_1 matches with the data obtained from the recount on the ship (after 20-30 days), t_2 matches with the data obtained from the first recount in the laboratory (after 5 months), and t_3 matches with the data obtained from the last recount in the laboratory (after a year).

whole water column in the BIOSOPE area. The blank values vary according to the trophic level of the area. With our methodology (250-mL sample; 4–5 h of incubation), the mean value of the blanks obtained in the ultraoligotrophic gyre area were 1.11 ± 0.33 nM h^{-1} and 0.005 ± 0.005 nM h^{-1} for ^{14}C and ^{33}P, respectively, whereas in the eutrophic upwelling area they were 1.61 ± 0.75 nM h^{-1} and 0.03 ± 0.04 nM h^{-1} for ^{14}C and ^{33}P, respectively (Table 1). The ^{14}C blank values were on average 5.9% ± 3.9% and 1.6% ± 1.6% of the in situ values for oligotrophic and eutrophic areas, respectively, whereas the ^{33}P blank values were on average 6.9% ± 4.2% and 4.0% ± 3.7% of the in situ values for oligotrophic and eutrophic areas, respectively, and for surface water (50% irradiance) (Table 1). The poisoned "blank" samples are different in the 2 extreme trophic regimes we have shown as examples. Nevertheless, they represent a low percentage of the total uptake. This difference is probably due to the higher levels of suspended material, which leads to a higher physical adsorption in the upwelling area. The detection limit was set to be equal to three times the average blank value. The detection limit is thus 3.3 nM h^{-1} and 4.8 nM h^{-1} for C uptake in oligotrophic and eutrophic areas, respectively, and 0.01 nM h^{-1} and 0.10 nM h^{-1} for P uptake in oligotrophic and eutrophic areas, respectively.

Background and precision estimate—We checked that the background counts were low and stable relative to the total counts (data not shown).

The precision estimate of the dual-labeling method has been calculated from all the samples obtained during the

Table 1. Mean (M) blank values in nmol L^{-1} h^{-1} for ^{14}C (V_{DIC}) and ^{33}P (V_{DIP}) at time T_0 and percentage (%) of the blank values compared to the in situ values in surface water, in the gyre and in the upwelling area.

	Gyre				Upwelling			
	^{14}C t_0, nM h^{-1}		^{33}P t_0, nM h^{-1}		^{14}C t_0, nM h^{-1}		^{33}P t_0, nM h^{-1}	
	V_{DIC}	%	V_{DIP}	%	V_{DIC}	%	V_{DIP}	%
M	1.11	5.91	0.005	6.86	1.61	1.61	0.03	4.04
SD	0.33	3.93	0.005	4.16	0.75	1.55	0.04	3.71
n	28	26	28	26	14	14	14	14
DL	3.33	—	0.01	—	4.83	—	0.10	—

In this table are also represented the standard deviation (SD), the number of data (*n*), and the detection limit (DL) set to be equal to 3 times the average blank value.

BIOSOPE cruise (see in situ application). The relative error (ε_r) was calculated as

$$\varepsilon_r = \left| \frac{V_M - V_R}{V_M} \right|,$$

with V_M the average uptake rate value (2 measurements at each depth) and V_R the measured uptake rate value (each an independent measurement). The mean and the standard deviation of all relative errors is 5% ± 6% (n = 338 samples) for DIP uptake rates and 6% ± 7% (n = 338 samples) for the DIC uptake rates.

In situ application—The dual-labeling method was applied during the BIOSOPE cruise (for more details, see http://www.obs-vlfr.fr/proof/vt/op/ec/biosope/bio.htm) in the southeast Pacific between 146.36°W and 72.49°W. Six duplicate depths were sampled at each station, according to the irradiance (50, 25, 15, 7, 3, 1, and 0.3%). Seawater was collected in Niskin bottles attached to a rosette sampler. The samples were treated as described in the analytical section. First, two profiles of DIC and DIP uptake rates were presented, at noon, in two contrasting areas of the southeast Pacific Ocean. The ultraoligotrophic southeast Pacific gyre area (25.96°S 114.00°W) (Figure 4A and B) is characterized by low chlorophyll *a* concentrations (0.03 ± 0.01 µg l^{-1}; n = 3) and low DIP concentrations (121 ± 5 nM; n = 3) in the mixed depth layer. The Chilean upwelling area (34.54°S 72.41°W) (Figure 4C and D) is characterized by high chlorophyll *a* (1.11 ± 0.08 µg l^{-1}; n = 3) and DIP (1718 ± 98 nM; n = 3) concentrations in the mixed depth layer. The mixed depth layer corresponds to the depth at which the density gradient passes above 0.01 kg m^{-4}. Chlorophyll *a* concentration was determined by fluorometry using the methanol extraction procedure as described by Raimbault et al. (1988). All fluxes calculated during this experiment are above the detection limit, except for samples taken from the 0.3% light level, where DIC uptake rates were at the detection limit of the method. Uptake values of DIP and DIC were 150 and 60 times higher in the upwelling area than in the gyre area for surface waters. Moreover, there were large variations in uptake values with depth. The uptake values of DIP and DIC were 3.3 and 5.2 times higher in the surface waters (50% of transmitted light) than in the 0.3% irradiance depth waters in the gyre, whereas DIP and DIC uptake values were, respectively, 12.5 and 82 times higher in the surface waters than in the 0.3% irradiance depth waters in the upwelling zone. This method is well adapted to the extreme trophic regimes encountered in the southeast Pacific Ocean. We will provide more detailed, quantitative information on P and C fluxes in another paper.

C uptake rate is known to be light-dependent in phytoplankton, but various responses to the P uptake rate have been observed, ranging from significant stimulation, circadian rhythms, to no light effect (Nalewajko and Garside 1983, Moutin et al. 2002). We conducted an experiment in the eastern gyre region of the southeast Pacific (31.85°S 91.44°W) to investigate diel changes in C and P uptake rates (Figure 5). These waters were characterized by low chlorophyll *a* concentrations (0.08 ± 0.03 µg l^{-1}; n = 3) and low DIP concentrations (173 ± 5 nM; n = 3) in the mixed depth layer. High DIC (882 ± 69 nM h^{-1}) and DIP (3.25 ± 0.25 nM h^{-1}) uptake rates were coupled during the day (sunrise to sunset) and decoupled during the night (sunset to sunrise) with very low DIC uptake (13.50 ± 6.35 nM h^{-1}) and medium DIP uptake (1.45 ± 0.47 nM h^{-1}). Moutin et al. (2002) found that DIP turnover times measured every 3 h during a 24-h period, in the Ionian basin of the Mediterranean Sea, indicated no significant differences between light and dark measurements. The difference in night P uptake rates for the two studies highlights the necessity to quantify fluxes in different areas.

Discussion

Compared to using two separate instrumental analyses with two independent samples, our technique is advantageous, in that the water sampling effort is reduced. In this way, it is possible to build up more replicates and/or to sample more depths. It also reduces the volume sampled, and this is advantageous both at sea and in laboratory cultures where the user is often limited by the sample volume. This technique can also improve the integrity of subsequent samples drawn from the same Niskin bottle. The ability to follow simultaneous C and P assimilation avoids some of the difficulties encountered with interpretation, because the measurement is

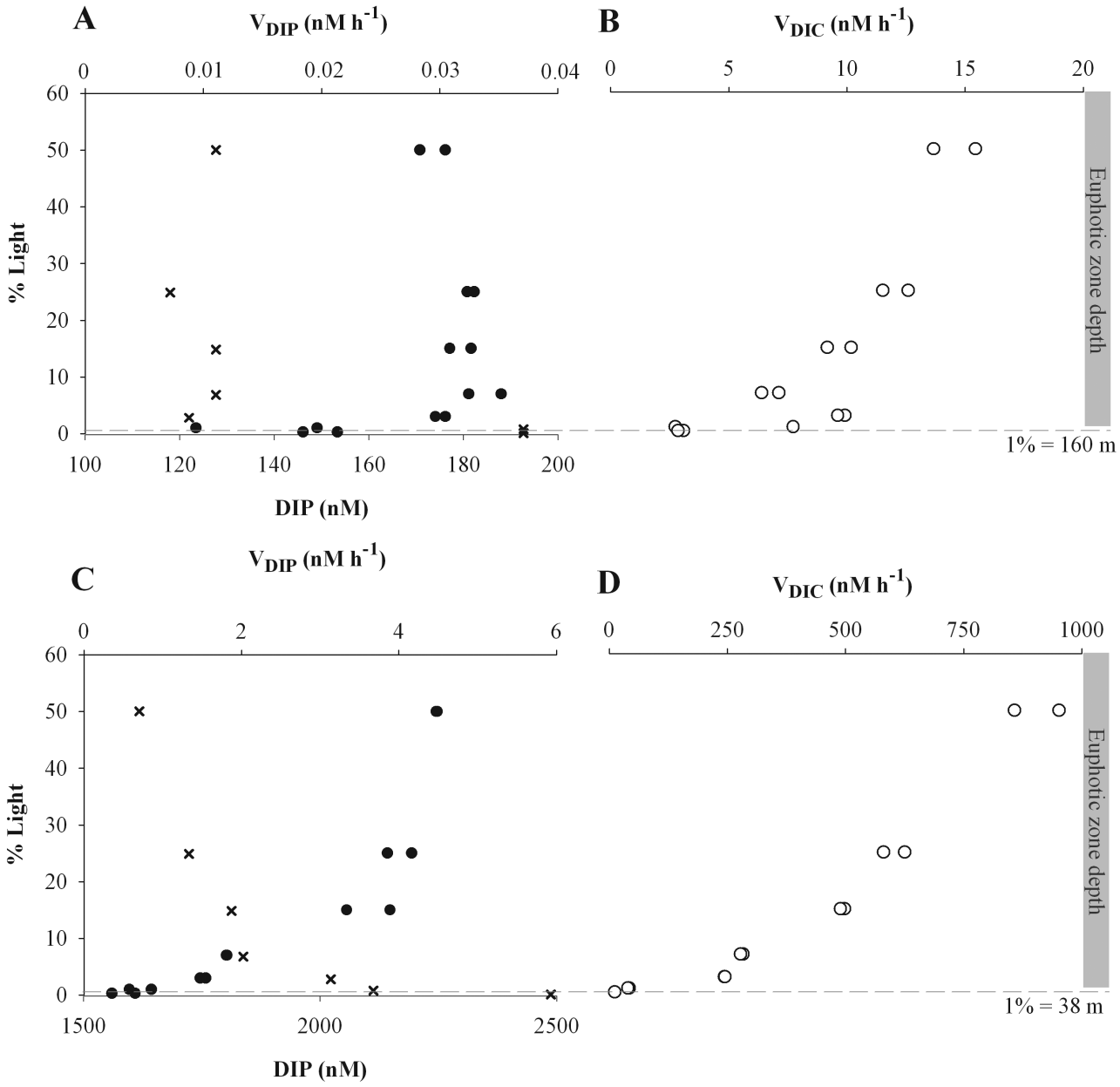

Fig. 4. DIP (●), DIC uptake rates (○) (nM h^{-1}) (duplicates), and DIP concentrations (×) (nM) in the south Pacific gyre (A and B) and in the Chilean upwelling (C and D). The data are presented according to the in situ light levels (%). The 1% depth is indicated by a gray dotted line.

carried out in the same sample. Moreover, simultaneous measurements are less time-consuming than separate ones. Finally, the dual-labeling method consumes less material, including vials, scintillation liquid, and filters.

Because the methodological problems associated with 24-h incubation experiments affect both carbon (Moutin et al. 1999, Maranon et al. 2005) and phosphate (Nalewajko and Garside 1983, Harrison and Harris 1986) uptake measurements, we decided to measure hourly rates, at noon. Losses remain negligible for short-term incubations (Nalewajko and Garside 1983, Harrison and Harris 1986, Moutin et al. 1999, Maranon et al. 2005) and consequently, these rates are close to gross rates. They represent a valuable parameter, and may be more representative than daily rates, in characterizing phytoplankton productivity. Daily rates are underestimated by 24-h incubation measurements, but extrapolation of results from shorter incubation times to daily rates is problematic (Moutin et al. 1999) (Figure 5). As a first approximation, the daily C uptake rates may be calculated using the method of Moutin et al. (1999), whereas daily P uptake rates may be calculated by simply multiplying the hourly rate by 24. Indeed, in several studies (Perry and Eppley 1981, Moutin et al. 2002),

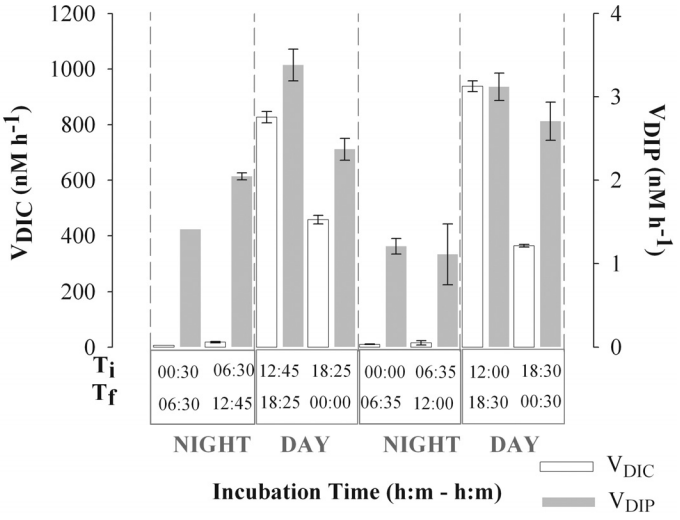

Fig. 5. Time course of average DIP and DIC uptake rates (nM h^{-1}) during 42 h, in the eastern gyre region of the southeast Pacific. Uptakes are presented in function of the incubation time (GMT) with T_i the beginning and T_f the end of the incubation. Error bars represent the maximum and the minimum value of the duplicate.

P uptake was shown to be constant over 24 h. More experiments are necessary to provide a better understanding of the relationship between hourly rates and daily rates for both carbon and phosphate uptake, and to study the nycthemeral variations of the C:P uptake ratio. The method proposed here is well adapted for this type of study.

Harrison (1983) and Harrison and Harris (1986) have shown that the dilution of the labeled substrate pool by the release of unlabeled substrate (isotope dilution) can be important in areas of low DIP concentration relative to P demand. Although this effect can cause an important error in the calculation of P uptake rates, the use of short incubation times relative to turnover times of DIP reduces its impact. In our experiments, incubation times were kept short (4–5 h, maximum) relative to the turnover time of DIP (7 days, minimum) in the sampled water mass. Isotope dilution should not, therefore, have been a problem, but it could bias results in P-depleted systems (see "Recommendations"). In the same way, recycling of P can be important in environments where algal biomass (and P demand) exceed DIP concentration, i.e., under very P-limited growth (Nalewajko and Lean 1978). The short incubation time of our method should reduce the errors linked to isotope dilution and recycling of P relative to results from longer incubations.

The first attempts at dual labeling with ^{33}P and ^{14}C had very different objectives. Lehman (1993) used this technique to measure the efficiency of element ingestion, retention, and assimilation by an invertebrate predator, *Bythotrephes cederstroemi*. Olsen et al. (2002) and Vadstein et al. (2003) applied the method to food web experiments in microcosms to measure the elemental content. We demonstrate that the C and P dual-isotope labeling method is a very useful tool to study C and P dynamics in oceanic waters and more particularly within the context of a cruise. But this method can also be very useful for process studies, in situ or in the laboratory.

Nutrient dynamics and their roles in determining the stoichiometry of organic matter pools are the central focus for biogeochemical studies. The study of stoichiometric C:P uptake and biomass ratios is a cornerstone of marine biogeochemistry (Redfield et al. 1963, Falkowski and Davis 2004). Because uptake may be uncoupled from growth, C:P ratios in uptake measurements on individual samples may theoretically be far from balanced (Thingstad et al. 1996). Dual labeling, thus, can be a very useful tool for studying C:P ratios and the coupling/decoupling between C and P fluxes.

The C and P cycles are linked through the stoichiometric composition of phytoplankton and bacterial biomass and their activity. There are still two unresolved central questions that need to be addressed to understand marine microbial food web functions: What fraction of organic carbon produced by primary production is channeled through the heterotrophic bacteria? (Cole et al. 1988), and What are the consequences for the marine microbial food web of the competition between heterotrophic bacteria and phytoplankton for DIP? (Thingstad et al. 2005). This method can thus be used in combination with size fractionations to help answer these questions.

Comments and recommendations

The analytical process detailed in this paper was adapted to the southeast Pacific study area. In other areas, optimal incubation times must be determined from a prior time-series experiment, as described in the "Materials and procedure" section. The concentration of the ^{33}P working solution should be calculated depending on the DIP turnover time (e.g., higher ^{33}P for short DIP turnover time). The detection limit of the method has been set to be equal to 3 times the blank value. This value corresponds to the limit under which the measurement is considered undetectable. Nevertheless, we recommend the sample counts to be at least 10 times greater than the blanks to work in optimum conditions. Less than 5% of the radioactivity in the samples should be consumed to avoid problems linked with recycling. Finally, incubation should not exceed several hours to minimize the increase in bacterial production caused by confinement. Indeed, during the BIOSOPE cruise, we have followed the bacterial production versus time over 24 h. The results show that leucine incorporation was not constant over time after 6 h of incubation (data not shown). DIC concentrations are relatively constant throughout the ocean but DIP concentrations are very variable. As a consequence, initial ^{14}C activity can remain the same whereas DIP concentrations need to be measured at each new site or season to adjust the initial ^{33}P activity in the experiments. We fixed initial conditions at the beginning of the BIOSOPE cruise and did not change them during the 8000-km transect. Our protocol can only be directly used when DIP concentrations are

measurable with the classical Strickland and Parsons (1972) procedure (DIP > 30 nM). Caution should be applied for measurements in very P-depleted systems, because ^{33}P assimilation is rapid and so incubation times must be shortened. In this case, it would be necessary to increase the specific ^{14}C activity to obtain significant dpm measurements.

^{32}P is the most commonly used radioisotope in molecular biology and biogeochemistry experiments. ^{33}P has a significantly longer half-life (25.4 and 14.3 days for ^{33}P and ^{32}P, respectively) and its specific activity is lower, but hazards are much reduced (maximum β emission energy, 0.249 and 1.709 MeV for ^{33}P and ^{32}P, respectively). It is possible to order and handle larger quantities of ^{33}P for regular, laboratory practice. Either isotope may be used, but ^{33}P is more convenient for security reasons. We recommend the use of ^{33}P.

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