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The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean

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Abstract

Based on a long-term set of observations and measurements at a station in the subtropical North Pacific Ocean, it now appears that contemporaneous rates of primary production in low-nutrient open ocean regions and perhaps in the ocean as a whole may be greater than had been considered in field studies conducted in previous decades. Data collected at the Hawaii Ocean Time-Series (HOT) Station ALOHA from October 1988 to July 1997 indicate that daytime particulate organic carbon (POC) production, based on 12-h ^{14}C in situ incubations, averages 472 mg C m^{-2} d^{-1} (SD = 125 mg C m^{-2} d^{-1}; n = 70). This carbon production rate is two- to three-fold greater than most of the pre-1980 estimates. We present evidence that particulate production rates may have been overestimated by up to 30% as a result of ^{14}C-labeled dissolved organic carbon (^{14}C-DOC) adsorption onto glass fiber filters. More importantly, when one considers the ^{14}C-DOC that is produced but not adsorbed onto the filters, gross primary production rates (^{14}C-POC plus ^{14}C-DOC) in the subtropical North Pacific Ocean may approach 1 g C m^{-2} d^{-1}. We hypothesize that the large flux of ^{14}C-DOC may be a manifestation of decade-scale habitat changes resulting from variations in climate. The balance between POC and DOC production will ultimately influence the structure of the food web, especially the interactions of phytoplankton and heterotrophic bacterial populations, and the mechanisms and rates of carbon sequestration by the biological pump.

Since the early 1950's, marine primary production has generally been estimated using the radiocarbon (^{14}C) technique (Steemann Nielsen 1951, 1952). Even modern satellite-based models use empirical relationships between chlorophyll *a* (Chl *a*)- and ^{14}C-based field measurements to derive primary production from estimates of ocean color (Bidigare et al. 1992). Unfortunately, the ^{14}C method, despite a sound theoretical basis and seemingly straightforward methodology, has been a difficult and sometimes unreliable technique in practice. The method suffers from both conceptual (Williams 1993) and procedural (Peterson 1980) failures, some of which greatly interfere with the goal of estimating rates of global primary production. For example, the ^{14}C method was initially thought to measure a rate between net and gross production (Steemann Nielsen 1952), possibly closer to gross production because the respired carbon would be derived from a cellular pool with a lower average specific radioactivity than the newly synthesized compounds. Kinetic models of C incorporation and recycling indicate that during an initial linear period of C uptake, the rate of ^{14}C fixation approximates gross photosynthesis (Marra et al. 1981; Dring and Jewson 1982). Once ^{14}C isotopic equilibrium has been achieved, the longer term rate of ^{14}C incorporation should approximate net photosynthesis. Nevertheless, gross primary production (G), net primary production (G minus photoautotrophic respiration), and net community production (G minus both photoautotrophic and heterotrophic respiration) are very different ecosystem properties (Williams 1993). Like many other field methods that are used routinely in oceanography, the accuracy of the ^{14}C method is not known and can only be constrained by the use of redundant or complementary techniques (Laws et al. 1984; Bender et al. 1987).

One of the standard procedures used to process ^{14}C incubation samples consists of filtration onto glass fiber filters (typically Whatman GF/F grade or equivalent). The interpretation of field results requires at least two assumptions: (1) that negligible amounts of ^{14}C-labeled dissolved organic carbon (^{14}C-DOC) are produced during the incubation period and (2) that only ^{14}C-labeled particulate organic carbon (^{14}C-POC) is retained by the glass fiber filters.

Reasons to support the first assumption were presented by Sharp (1977). He critically reviewed the literature and reported that little excretion of organic matter should be expected for marine phytoplankton growth under a range of nutrient conditions. Furthermore, Fogg (1966) previously suggested that most of the ^{14}C-DOC produced by phytoplankton would be readily taken up by bacteria and converted to ^{14}C-POC or respired. However, there is increasing evidence over the last two decades suggesting that under certain environmental conditions ^{14}C-DOC production may represent a significant fraction of total phytoplankton production (Myklestad 1977; Williams 1990; Malinsky-Rushansky and Legrand 1996). Furthermore, Maske and Garcia-Mendoza (1994) presented convincing evidence indicating

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that glass fiber filters may adsorb significant amounts of ^{14}C-DOC, resulting in an overestimation of ^{14}C-POC and an underestimation of ^{14}C-DOC in a primary productivity measurement.

Herein, we report data from an extensive set of field experiments conducted in the subtropical North Pacific Ocean near Hawaii that identify a new methodological uncertainty regarding the accurate determination of in situ primary productivity. Our results suggest that rates of gross and net primary production in our study region have been significantly underestimated by perhaps as much as 30–50% and 15–30%, respectively, because of the production of ^{14}C-DOC during the standard 12-h incubation periods. We hypothesize that temporal changes in the physiological ecology of photoautotrophic populations in response to a shift from an N-limited to a P-limited system at Station ALOHA (Karl et al. 1995, 1997) have resulted in fundamental changes in the flow of carbon in the oceanic ecosystem. If our results from Station ALOHA accurately reflect ecological conditions in the subtropical North Pacific as a whole, then we may need to revise upward the current estimates of global marine production.

Materials and methods

Sample collections—The majority of the measurements and experiments were conducted at Station ALOHA (22.75°N, 158°W), an oligotrophic North Pacific Ocean site (Karl and Lukas 1996). Water samples were collected on 21 Hawaii Ocean Time-Series (HOT) program cruises between July 1994 and Sept 1997 (Table 1), although selected data sets derive from core measurements collected on 87 cruises since October 1988. During each cruise, a single approximately 12-hr in situ primary production incubation experiment was performed. Additional shipboard experiments were also conducted on selected cruises (Table 1). Complementary hydrographical, chemical, and biological data were also routinely obtained (Karl and Lukas 1996). A limited number of additional experiments were conducted at Station CLIMAX (28°N, 155.4°W), the site of an extensive investigation of plankton growth rates and processes during 1968–1985 (Hayward et al. 1983; Hayward 1987; Venrick et al. 1987). Station CLIMAX was also the site of the 1985 interdisciplinary PRPOOS (Plankton Rate Processes in Oligotrophic Oceans) primary production measurement program (Laws et al. 1987).

The "standard" primary production procedure—Water samples were collected from eight depths (5, 25, 45, 75, 100, 125, 150, and 175 m) and were subsampled directly into clean 500-ml polycarbonate bottles. To minimize metal contamination, the sampling and incubation procedures followed the recommendations of Fitzwater et al. (1982; *see also* Karl et al. 1996; Letelier et al. 1996). Typically, six separate 500-ml polycarbonate bottles were prepared from each depth, three for incubation in the light and three for incubation in the dark. Each bottle was spiked with ^{14}C-bicarbonate ($H^{14}CO_3^-$; cat. 17441H, ICN Biomedicals) to yield a final radioactivity of approximately 1.9–3.7 MBq liter^{-1}. Stock solutions of the radiotracer were routinely assayed for ^{14}C-organic contamination by counting replicate 10-μl aliquots before and after acidification and venting. Total radioactivity for each sample bottle was measured by removing a 250-μl subsample for liquid scintillation counting. In this procedure, β-phenylethylamine was used as an inorganic carbon trapping agent and Aquasol-II was used as the liquid scintillation cocktail. The ^{14}C activities of the samples were counted using a Packard model 4640 liquid scintillation counter, and sample quench was determined using the spectral index of external standard estimation. Total dissolved inorganic carbon (DIC) was measured using CO_2 coulometry (Winn et al. 1994) for calculation of the DIC specific radioactivity (^{14}C/^{12}C; MBq mol^{-1}).

Following in situ incubation, subsamples (100–500 ml) were filtered onto 25-mm-diameter Whatman glass fiber (Whatman-GF/F) filters, and each filter was placed into a glass vial for subsequent measurement of ^{14}C incorporation into particulate matter. The sample vials were stored for 2–3 days at −20°C until processed at our shore-based laboratories. Sample processing commenced by direct addition of HCl (1 ml, 2 M) to each vial. The samples were vented for 24 h prior to the addition of 10 ml of Aquasol-II in preparation for liquid scintillation counting. Samples were counted immediately and again after approximately 30 days. For selected cruises, the samples were counted several times during the first month of the storage period. Between repeated

Table 1. Summary of field experiments that were conducted to obtain data on the role of ^{14}C-DOC in the carbon budget of the subtropical North Pacific Ocean.

Cruise	Date	Experiments*
HOT-55	Jul 1994	1
HOT-56	Aug 1994	1
HOT-58	Oct 1994	1
HOT-60	Feb 1995	1
HOT-61	Mar 1995	1
HOT-62	Apr 1995	1
HOT-63	May 1995	1
HOT-64	Jul 1995	1
HOT-66	Sep 1995	1
HOT-71	Apr 1996	1, 4
HOT-72	May 1996	3, 4
HOT-74	Jul 1996	1, 3, 4
HOT-75	Aug 1996	2, 3
ALOHA-CLIMAX I	Jul 1996	1, 2
HOT-78	Dec 1996	1, 3, 4
HOT-80	Feb 1997	1, 3, 4
HOT-81	Mar 1997	3, 4
HOT-82	Apr 1997	4
HOT-83	May 1997	3
HOT-84	June 1997	1, 3
ALOHA-CLIMAX II	June 1997	2, 3
HOT-86	Jul 1997	3, 4
HOT-87	Sep 1997	3, 4

* 1 = in situ primary production with postincubation processing using Whatman-GF/F and Nuclepore-PC filters; 2 = comparisons of Whatman-GF/F and Nuclepore-PC filters with Millipore-HA filters or Anopore filters; 3 = direct measurements of ^{14}C-DOC by the direct acidification, MAGIC, or persulfate oxidation/CO_2 distillation methods; 4 = ^{14}C-DOC production and utilization, ^{14}C-DOC characterization, ^{14}C-DOC adsorption, and other experiments.

counts, the vials were kept in the dark at room temperature. The final (30 day) ^{14}C activity, which was generally significantly greater than the initial ^{14}C activity, was used in all productivity calculations. The euphotic zone primary productivity values (mg C m^{-2} d^{-1}) were calculated using the trapezoid rule and were routinely integrated to 200 m to include net incorporation occasionally detected at the deepest reference depth (175 m; Letelier et al. 1996). For these calculations, light-dependent assimilation of ^{14}C was assumed to be zero at 200 m. The error estimate for each depth-integrated primary production experiment was estimated by making a linear combination of variables.

Direct filter comparisons—Water samples collected and incubated in situ using ^{14}C-bicarbonate as above were filtered through either Whatman-GF/F (nominal 0.7-μm pore size) filters or Nuclepore-polycarbonate (Nuclepore-PC) filters for a direct comparison of the particulate ^{14}C activity in selected field experiments (Table 1). In one experiment, we tested other filters (Table 1). Several ^{14}C-DOC adsorption experiments were also conducted.

Direct measurements of ^{14}C-DOC—Two methods were employed to measure ^{14}C-DOC. The first method was similar to the whole water acidification technique of Schindler et al. (1972). After incubation, a 1.5-ml subsample was acidified with HCl (1 M final concentration) and allowed to vent passively for 24 h. Following removal of excess ^{14}C-DIC, 10 ml of Aquasol II was added and the samples were counted in a liquid scintillation counter. This estimate of total nonvolatile ^{14}C includes both particulate and dissolved ^{14}C-labeled products. The activity of ^{14}C-DOC was estimated by the difference between the total seawater and a filtered (Whatman-GF/F or Nuclepore-PC) subsample. Because this method is inherently less sensitive than our standard primary production procedure because of small sample size (i.e., 1.5 ml vs. 500 ml), it was necessary to add an order of magnitude more ^{14}C-bicarbonate to achieve reliable results.

A more sensitive assay system for ^{14}C-DOC was developed based on the oxidation of ^{14}C-DOC to ^{14}C-CO_2 with subsequent distillation into an alkaline trap. Following incubation and filtration, a 250-ml subsample of the Nuclepore-PC filtrate was placed into a polyethylene separatory funnel. The sample was acidified with 1 ml of H_2SO_4 (2 M), mixed thoroughly, and vigorously bubbled for 2–3 h with air (flow $\approx$1–2 liters min^{-1}) to remove ^{14}C-CO_2. At this point, the sample was split and processed for total ^{14}C-DOC and, separately, for an assessment of magnesium induced coprecipitation (MAGIC) ^{14}C-DOC (Karl and Tien 1992).

For total ^{14}C-DOC, duplicate 70-ml aliquots were transferred into 100-ml glass serum bottles containing 1 ml of NaOH (2 M) and 10 ml of oxidation reagent containing 0.37 M $K_2S_2O_8$ in 1 M NaOH (100 g $K_2S_2O_8$ per liter of 1 M NaOH). The bottles were sealed with 20-mm rubber stoppers and an aluminum crimp seal cap to provide gas-tight closure and heated in an autoclave for 200 min at 126°C. This high-temperature oxidation treatment converts ^{14}C-DOC to ^{14}C-DIC, and the alkaline pH of the solution prevents subsequent loss of ^{14}C-DIC when the samples are subsequently opened. After the oxidized samples cooled to room temperature, the bottles were uncapped and refitted with a gas-tight 20-mm rubber sleeve stopper and plastic center well assembly. Prior to closure, a 2- × 2-cm piece of fluted chromatographic filter paper (Whatman 2) soaked with 0.2 ml of β-phenylethylamine was placed into the plastic center well. After closure, 4 ml of H_2SO_4 (4.5 M) was added through the gas-tight stopper using a glass syringe and stainless steel needle, and the samples were stored at room temperature for 72 h to effect a passive distillation of the oxidized ^{14}C-DOC (now present as ^{14}C-CO_2 gas in solution and in the headspace) from the acidified mixture to the alkaline-filter paper CO_2 trap. Following ^{14}C-CO_2 gas distillation, the bottles were opened and the filter paper wick and plastic cup were placed into a liquid scintillation vial containing 10 ml Aquasol-II and counted for ^{14}C activity, as above.

Prior to routine use with Station ALOHA seawater samples, the reliability of the various steps in this ^{14}C-DOC method was evaluated. The initial acidification–air purging procedure removed >99.99% of added ^{14}C-bicarbonate during the 2–3-h period. The alkaline-persulfate autoclaving treatment resulted in >98% oxidation of ^{14}C-labeled glucose and ^{14}C-labeled amino acid mixtures to ^{14}C-CO_2, and the final passive distillation was an efficient means of concentrating the ^{14}C-CO_2 prior to liquid scintillation counting. These results are consistent with those presented by Sharp (1977) and Schindler et al. (1972) for the efficiency of ^{14}C-CO_2 removal by purging, by Raimbault and Slawyk (1991) and Libby and Wheeler (1994) for the efficiency of alkaline-persulfate oxidation of organic matter, and by Hobbie and Crawford (1969) for the efficiency of the ^{14}C-CO_2 passive distillation procedures. We also routinely assessed the efficiency of the initial ^{14}C-CO_2 purging and the combined efficiencies of the two subsequent steps by comparing the total ^{14}C activites in selected samples before and after purging, and in the postoxidation flasks before and after ^{14}C-CO_2 distillation. The final solutions are consistently at or near background, indicating that both the ^{14}C-DOC oxidation and ^{14}C-CO_2 distillation steps are effective.

The MAGIC method was originally developed for the measurement of soluble reactive phosphorus in seawater (Karl and Tien 1992) but also removed approximately 10–30% of the total seawater DOC, depending upon the sample. This method can easily effect a 100-fold concentration of those ^{14}C-DOC compound classes that can be coprecipitated, so we routinely performed an in vitro $Mg(OH)_2$ coprecipitation step as an independent measurement of that portion of the total ^{14}C-DOC pool that we operationally defined as MAGIC-DOC. Duplicate subsamples (40 ml each) of the ^{14}C-DIC purged acidified solutions were placed into clean disposable 50-ml polypropylene centrifuge tubes. One milliliter of NaOH (3.75 M) was added to each sample, and the tubes were capped and mixed thoroughly; a milky white precipitate (brucite; $Mg(OH)_2$) formed immediately. The samples were centrifuged (1,000 × g for 15 min), and the clear supernatant was aspirated with a Pasteur pipette attached to a vacuum line and then discarded. The $Mg(OH)_2$ pellet, containing the coprecipitated ^{14}C-DOC was dissolved in 0.5 ml of concentrated H_2SO_4 (18 M), and the entire sample was transferred to a liquid scintillation vial containing 10 ml of Aquasol-II and counted for ^{14}C activity.

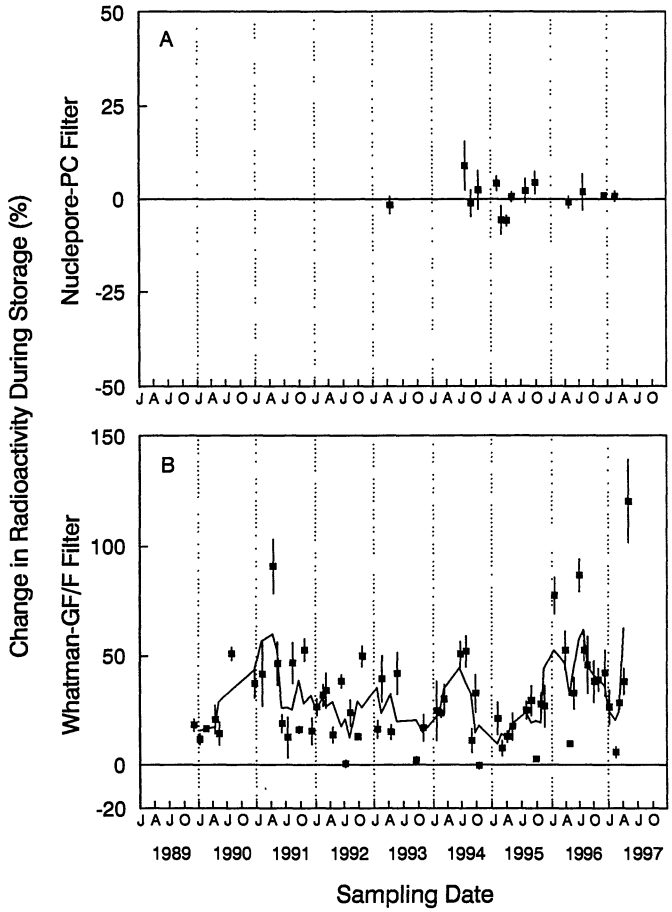


Fig. 1. Change in ^{14}C activity during Nuclepore-PC (A) and Whatman-GF/F (B) filter storage for 30 d in Aquasol II cocktail. Values shown are the mean differences, expressed as percentages (DPM [30 d] − DPM [0 d] ÷ DPM [0 d] × 100%) ± 1 standard deviation of the mean value for all 0–100-m depth samples (n = 10–15) from a given cruise. For some samples, the standard deviation bars are smaller than the symbols used. The solid line for the Whatman-GF/F data set is the 3-point running mean. The overall mean percentage change (±1 SD) in ^{14}C radioactivity during filter storage for the full data sets are 0.8% (±3.4%) for Nuclepore-PC and 31% (±22%) for Whatman GF/F.

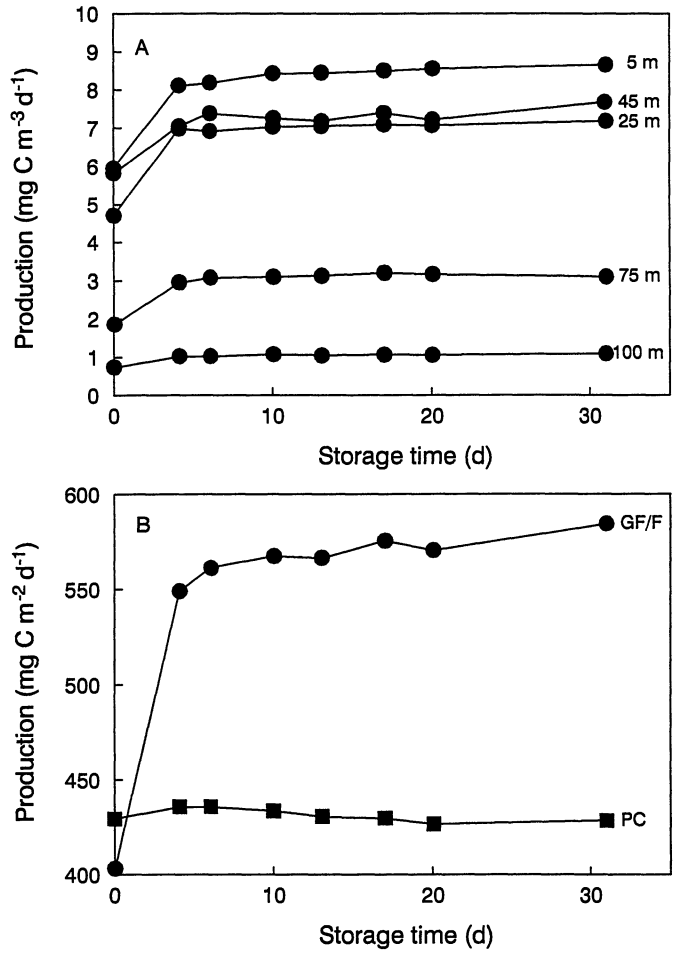


Fig. 2. Effects of storage on the measured ^{14}C activity and hence on calculated C production for standard in situ ^{14}C primary production experiments conducted on HOT-55 (July 1994). The Whatman-GF/F filters containing particulate and adsorbed DOM were acidified, vented, mixed with Aquasol II, and counted (day 0). The vials were then stored in the dark at room temperature and counted periodically over the next 31 days. Primary production was calculated from ^{14}C activity measured for water samples collected and incubated at various depths (indicated on the right of each curve) and plotted against storage time in days (A). Total euphotic zone (0–200 m) depth-integrated primary production was determined for HOT-55 water samples processed with Whatman-GF/F and Nuclepore-PC filters (B). Note the significant change in primary production following storage for the Whatman-GF/F filters compared with the temporal constancy of ^{14}C activity in Nuclepore-PC filters.

Results

"Standard" primary production protocol—When Whatman GF/F filters are stored in liquid scintillation counting cocktail, there is a significant but variable increase (typically 5–40%) in total ^{14}C activity over the first 15–20 days. Nevertheless, the absolute increase (e.g., disintegrations per minute (DPM) per filter), the relative percentage increase, and the time-rate of change in ^{14}C activity all differed across cruises. The percentage of ^{14}C activity increase was not correlated with total ^{14}C activity on the filter or with sample depth; on occasion, no increase in ^{14}C activity during Whatman-GF/F filter storage in Aquasol II was observed. By comparison to the results obtained with glass fiber filters, the ^{14}C activity measured using polycarbonate filters was stable over time in the presence of Aquasol II (Figs. 1, 2).

The increased ^{14}C activity with Whatman-GF/F filters during storage time had a dramatic impact on estimates of primary production (Fig. 2). The overall mean (±SD) percentage increase in radioactivity during 1 month of storage for 1989–1997 was 31% (±22%; n = 1,121). The range in cruise mean (±SD) values was −0.3% (±1.5%) for HOT-58 (October 1994) to 127% (±19%) for HOT-83 (May 1997). For HOT-38 (July 1992), HOT-49 (September 1993), HOT-58 (October 1994), and HOT-61 (March 1995), there were no detectable changes in DPM during filter storage. Since 1 January 1996, the cruise mean percentage increase

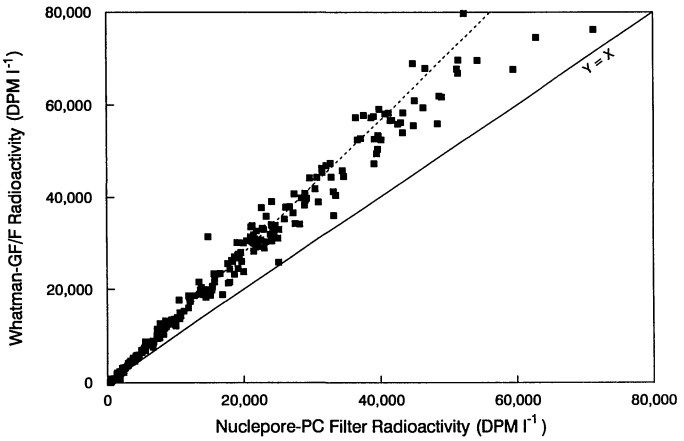


Fig. 3. Comparison of ^{14}C activity measured for paired water samples processed using either Whatman-GF/F or Nuclepore-PC filters. Each data point represents a single incubation bottle from 14 separate in situ primary productivity experiments at Station ALOHA. The solid line is the 1 : 1 relationship, and the dashed line is the model II linear regression: Whatman-GF/F activity (DPM liter^{-1}) = 1.44 (±0.007) Nuclepore-PC activity (DPM liter^{-1}) − 1132 (±3848) (r^2 = 0.99; n = 305).

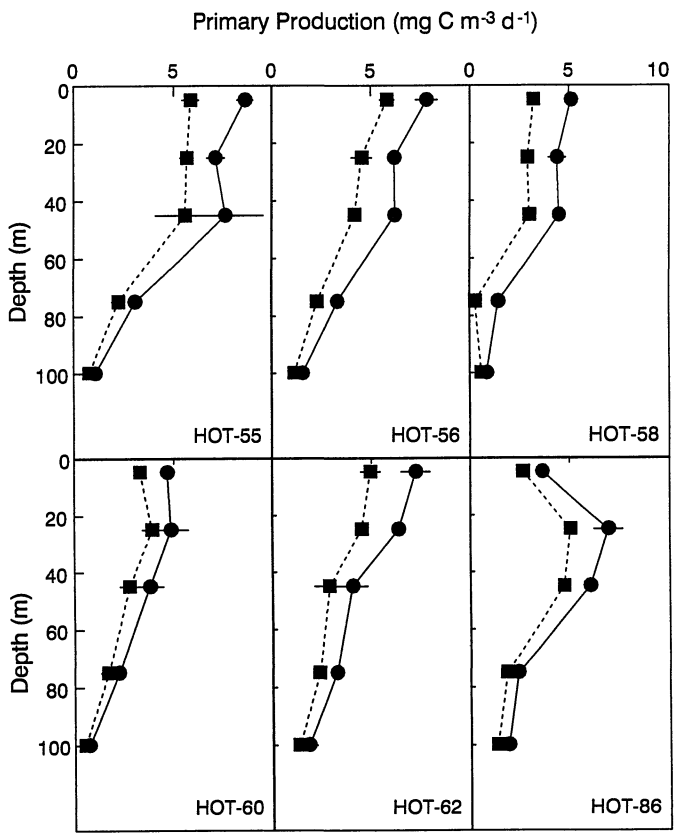


Fig. 4. Selected water column profiles (0–100 m) of in situ primary production rates calculated from triplicate water samples processed using either Whatman-GF/F (●, solid lines) or Nuclepore-PC (■, dashed lines) filters for a variety of HOT cruises, as indicated. The depth-integrated primary production calculated using the Whatman-GF/F filters was significantly greater than that measured using Nuclepore-PC filters (*see Fig. 5*).

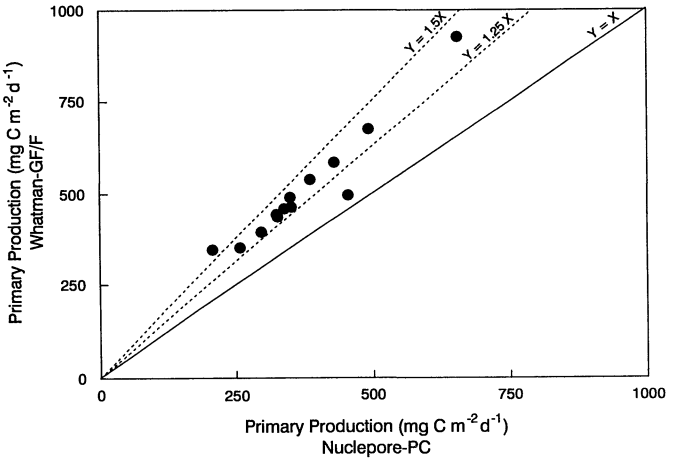


Fig. 5. Comparison of total euphotic zone (0–200 m) in situ primary production measured at Station ALOHA for paired samples processed using either Whatman-GF/F or Nuclepore-PC filters. The solid line is the 1 : 1 relationship, and the dashed lines represent lines of constant GF/F-PC productivities of 125 and 150%. Each data point is from a single in situ primary production experiment based on triplicate subsamples collected from eight sample depths. For most cruises, the standard deviation of the mean calculated for the depth-integrated production estimate is smaller than the size of the symbol.

in ^{14}C activity during storage was 46% (±30%), a value that was nearly twice as large as the mean value of 24% (±14%) observed during the prior 4-yr period (1992–1995). Coincident with the change in DPM vs. time was an increase in sample counting efficiency, a result attributed to clearing of the initially opaque Whatman-GF/F filters during storage in Aquasol-II. However, this effect by itself cannot account for our observations and especially cannot explain the large variations that were observed among individual cruises.

Because of the significantly lower retention of Chl *a* by Whatman-GF/F filters compared to Nuclepore-PC filters, as reported by Dickson and Wheeler (1993), we decided to compare these two filter types in our ^{14}C primary production protocols. For 13 separate comparisons conducted over a 3-yr period, we observed a significantly higher particulate ^{14}C activity with the Whatman-GF/F filters (Figs. 3–5). The absolute difference, expressed as DPM liter^{-1}, for the two filter types was highly correlated with total ^{14}C activity (Fig. 3) and was detected throughout the water column. On average, there was 44% more ^{14}C activity collected onto Whatman-GF/F filters (Fig. 3), which translated directly into higher rates of total euphotic zone depth-integrated primary production (Fig. 5).

Because the Nuclepore-PC filters should theoretically retain at least as much if not more particulate ^{14}C activity than the Whatman-GF/F filters (0.2 μm for PC vs. 0.7 μm nominal porosity for GF/F), we hypothesized that the significant positive bias in total ^{14}C activities for the Whatman-GF/F filters is the result of selective adsorption of ^{14}C-DOC onto Whatman-GF/F filters. Based on the physical properties of the filters (Table 2) and their relative ^{14}C retention characteristics (Tables 3, 4), we concluded that the Nuclepore-PC filters are more inert than the Whatman-GF/F filters. Based

Table 2. Physical and chemical characteristics of the three filters used in this study.

Filter	Pore size (μm)	Chemical composition	Thickness (μm)	Approximate weight* (mg)	Water retention (μg mm^{-2} surface area)	Protein binding (μg C m^{-2})§	Comments
Whatman GF/F	0.7	borosilicate	300	37.5(±0.4)	240†	ND‡	high flow rate, high loading, tortuous water path
Millipore HA	0.45	mixed cellulose ester	125	24.0(±0.2)	9†	150	high flow rate, surface retention of particles
Nuclepore PC	0.2	polycarbonate	10	5.2(±0.2)	0.3†	3	low flow rate, low loading, surface retention of particles
Anopore Anodisk	0.2	aluminum oxide	80	30.1(±1.7)	19.1(±2.3)	ND	low flow rates, low loading, surface retention of particles

* Mean (±1 SD).
† From Lean and Burnison (1979).
‡ Not determined.
§ From manufacturer's specifications.

on a limited number of field experiments comparing other filters, we observed that Millipore-HA (cellulose acetate) filters behaved like Whatman-GF/F filters both in terms of the extent of ^{14}C-DOC adsorption and the percentage increase in DPM following sample storage in Aquasol II. The aluminum oxide Anopore filters (ANO), however, were similar to the Nuclepore-PC filters with regard to ^{14}C-DOC interactions. Consequently, the calculated depth-integrated rates of primary production followed the expected trends: (1) GF/F $\approx$ HA, (2) PC $\approx$ ANO, and (3) GF/F or HA $>$ PC or ANO (Table 4).

The available data set on changes in DPM with sample storage does not permit us to distinguish fluctuations in ^{14}C-DOC production rates from changes in the nature of the produced ^{14}C-dissolved organic matter (DOM) pools. Both may affect changes in DPM during storage. For example, during HOT-58 (October 1994) there was a significant difference between the Whatman-GF/F and Nuclepore-PC filter treatments (Fig. 4), but there was no significant change in DPM during Whatman-GF/F filter storage (Fig. 1B).

^{14}C-DOC adsorption and measurement—We conducted several sets of experiments designed to quantify the amount of ^{14}C-DOC that is accumulated during a dawn-to-dusk primary production experiment and to assess the characteristics of the ^{14}C-DOC with regard to filter adsorption and other properties, including production and turnover rates. When particle-free filtrate was subsequently filtered through a Whatman-GF/F filter, ^{14}C activity well in excess of any time zero ^{14}C-DOC that might be present in the stock solutions was detected (Fig. 6). When this absorbed ^{14}C-DOC activity was then compared to the observed difference in DPM between filter types measured for a given water sample, there was good agreement between the two values, suggesting that the increased ^{14}C activity observed for the Whatman-GF/F filters is a result of ^{14}C-DOC adsorption. The amount of ^{14}C-DOC retained by the Whatman-GF/F filters is initially dependent upon the volume filtered, but saturation is eventually reached (Fig. 6). The loading capacity of the Whatman GF/F filter for ^{14}C-DOC was reduced by soaking the filter in a solution of yeast extract and peptone before use, a result that is consistent with adsorption being the primary removal mechanism (Fig. 6).

An experiment was conducted to characterize the nature of the ^{14}C-DOC removed from solution by attempted selective desorption of ^{14}C-DOC from the Whatman-GF/F filters using a variety of extractants (4°C and 85°C H_2O, 1 M HCl, 1 M NaOH; 4°C ether, ethanol, acetone, and dimethylsulfoxide [DMSO]). Of the solvents tested only DMSO was ef-

Table 3. Changes in ^{14}C activity during filter storage in Aquasol II of samples collected on Nuclepore-PC and Whatman-GF/F filters following specified treatments.

Treatment*	^{14}C activity† (Bq ml^{-1})†		% change‡
	21 Mar 1997	21 Apr 1997	
Nuclepore-PC	2,541(±39)	2,542(±41)	+0.02(±0.8)
Whatman-GF/F	3,136(±240)	3,605(±47)	+15.5(±7.5)
Stacked Whatman GF/F	2,933(±648)	3,669(±72)	+30.7(±28.2)

* A 5-m water sample was collected during HOT-81 (March 1997), spiked with ^{14}C-bicarbonate, and incubated for a 12-h light period in an on-deck incubator. Following incubation, samples were filtered through either single Nuclepore-PC and Whatman-GF/F filters or through two Whatman-GF/F filters layered together.
† Data presented are mean (±1 SD) values for six replicate filters.
‡ Final ^{14}C-activity ÷ initial ^{14}C-activity × 100%; mean (±1 SD) for six replicate filters.

Table 4. Comparison of Whatman-GF/F, Nuclepore-PC, Millipore-HA, and Anopore (ANO) filters for measurements of ^{14}C-primary production in the oligotrophic North Pacific Ocean.

Cruise (station)	Date	Incubation period (h)*	Filter type	Estimated primary production (mg C m^{-2} h^{-1})†
AC-I-2	Jul 1996	6	GF/F	27±0.5
(22°45′N, 158°W)			HA	30±0.6
		12	GF/F	38±0.5
			HA	38±0.4
AC-I-5	Jul 1996	6	GF/F	32±4.5
(28°N, 155°20′W)			HA	40±8.8
			PC	25±3.9
		12	GF/F	33±0.2
			HA	34±0.5
AC-II-2	Jun 1997	6	GF/F	26±2.0
(22°45′N, 158°W)			HA	26±2.6
			PC	20±2.4
AC-II-5	Jun 1997	6	GF/F	15±1.0
(28°N, 155°20′W)			HA	16±1.3
			PC	12±1.3
HOT-86	Aug 1997	12	GF/F	36±0.6
(22°45′N, 158°W)			PC	27±0.4
			ANO	29±0.8

* 6-h incubations were performed in deck incubators from noon to dusk; 12-h incubations were performed in situ from dawn to dusk.
† Mean ±1 SD.

fective at partial (~50%) ^{14}C-DOC desorption following the brief exposure periods (2 min) used in this experiment.

Direct determinations of total ^{14}C-DOC in Nuclepore-PC and Whatman-GF/F filtrates using either direct acidification or the MAGIC-DOC and persulfate oxidation methods demonstrated that adsorption onto the GF/F filters removed some but not all of the ^{14}C-DOC present in the primary production incubation bottles (Tables 5, 6). For example, on HOT-84 (June 1997) approximately 54% (49–61%) of the ^{14}C-DOC measured in the Nuclepore-PC filtrates was present in the paired Whatman-GF/F filtrates from common in situ incubations (Table 5). Likewise, measurements using either the MAGIC or the large volume oxidation-distillation methods independently documented the presence of significant concentrations of ^{14}C-DOC in the Nuclepore-PC filtrates (Table 6, Fig. 6). The percentage of the total ^{14}C-DOC pool that was detected by MAGIC varied considerably with depth, suggesting differences in the pool composition.

^{14}C-DOC production—During HOT-74 (July 1996), we observed a significant production of ^{14}C-DOC, which was nearly 50% of the 0–100-m depth-integrated ^{14}C-POC production, as determined by Nuclepore-PC sample processing (Fig. 7).

Discussion

The introduction of the ^{14}C technique for the measurement of primary production provided oceanographers with a sensitive method for quantitative investigations of the open ocean carbon cycle. However, several major criticisms have been raised over the years and most have not yet been resolved (Peterson 1980; Dring and Jewson 1982). For example, it is not certain whether the common dawn-to-dusk ^{14}C incubations measure a value closer to gross or to net primary production or to what extent the ^{14}C that is incorporated into organic matter is subsequently recycled (i.e., ^{14}C-DIC → ^{14}C-POC → ^{14}C-DIC) during the incubation period. A related matter concerns the role of DOM cycling (i.e., ^{14}C-DIC → ^{14}C-DOC → [^{14}C-POC + ^{14}C-DIC]) and, with regard to organic matter release, what actually controls the flux of ^{14}C-DOC from cells.

Issues raised by our results range from the mechanics of processing water samples for the most accurate routine measurements of in situ primary production to the ecological implications of a large DIC-to-DOC flux. Our results may also be viewed in the broader context of ocean habitat variability and climate change. We have reported elsewhere (Karl et al. 1995, 1997) evidence for a major change in the

Table 5. HOT-84 (June 1997) ^{14}C activity mass balance for dissolved and particulate pools of carbon following an in situ 12-h light period incubation, as determined by the direct acidification and filtration methods.

	Particulate pools			Dissolved pools			^{14}C-DOC/
Sample source	Filter type	^{14}C activity* (DPM ml^{-1})	GF/F ÷ PC (%)	Filtrate type	^{14}C activity (DPM ml^{-1})	GF/F ÷ PC (%)	^{14}C-POC (%)
5 m	GF/F	469	120	GF/F	80	52	17.1
	PC	390		PC	154		39.5
25 m	GF/F	458	116	GF/F	80	50	17.5
	PC	394		PC	161		40.9
45 m	GF/F	336	129	GF/F	52	59	15.5
	PC	260		PC	88		33.8
75 m	GF/F	126	137	GF/F	23	49	18.3
	PC	92		PC	47		51.1
100 m	GF/F	121	149	GF/F	17	61	14.0
	PC	81		PC	28		34.6

* Net (time zero and background corrected) 12-h light values for each sample treatment using the direct acidification method. The mean ±1 SD net ^{14}C activities for 12-h dark controls of paired samples collected and incubated at 5, 25, 45, 75, and 100 m were 13.8 ± 3.4 DPM ml^{-1} for Whatman-GF/F filters, 10.6 ± 5.4 DPM ml^{-1} for Nuclepore-PC filters, 12.4 ± 2.6 DPM ml^{-1} for GF/F filtrates, and 18.3 ± 2.8 DPM ml^{-1} for PC filtrates.

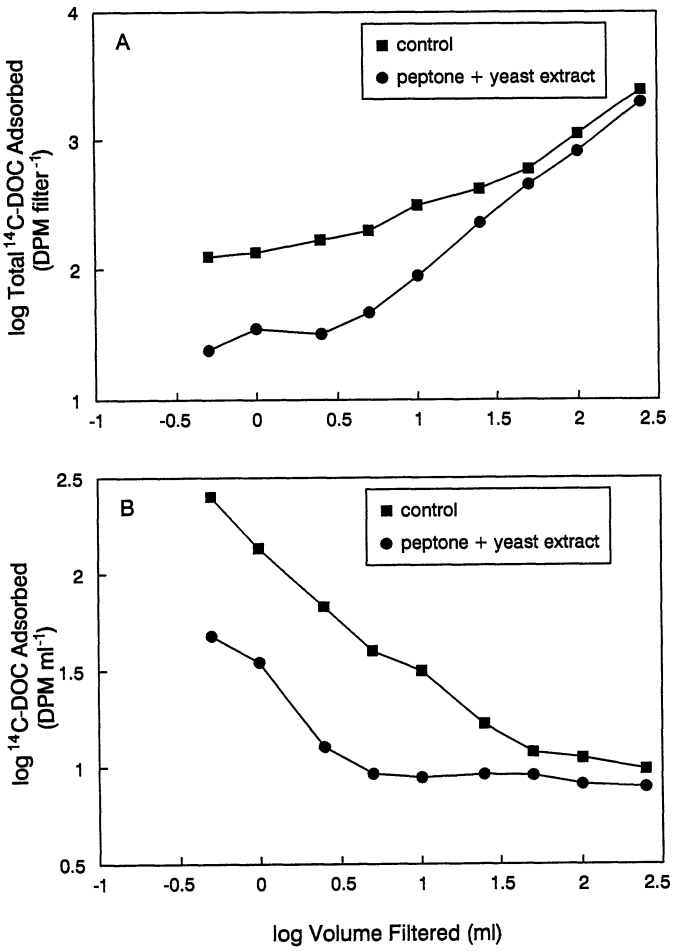


Fig. 6. Volume dependence of ^{14}C-DOC adsorption onto Whatman-GF/F filters (■) for regular filters (control, ■) and filters soaked in a solution of peptone plus yeast extract for 10 min before use (●). The ^{14}C-DOC used in this experiment was prepared by incubating a 5-m water sample from HOT-85 (August 1997) for 12 h in the light followed by filtration through a Nuclepore-PC filter. The log total ^{14}C-DOC adsorbed is expressed as DPM per Whatman-GF/F filter as a function of log volume filtered (A), and the log ^{14}C-DOC adsorbed is expressed as DPM ml^{-1} filtered as a function of log volume filtered (B). The observed decrease in ^{14}C-DOC removed (expressed as DPM ml^{-1}) with increasing volume filtered for both treatments and the consistently lower amount of ^{14}C-DOC removed onto filters previously exposed to a high concentration of DOM are both consistent with adsorption as the primary mechanism of ^{14}C-DOC retention by Whatman-GF/F filters.

physiological state of the subtropical North Pacific microbial assemblages, and we believe that enhanced exudation of photosynthetically derived DOC may be another response consistent with this hypothesized habitat change.

Methodological considerations—Brock (1983) stated "Although filtration appears to be a simple process, it is actually very complex." For ^{14}C primary production measurements, samples are generally concentrated onto filters prior to acidification and liquid scintillation counting. Although alternative procedures do exist, e.g. the direct acidification-bubbling method (Schindler et al. 1972; Riemann and Jensen 1991), they lack the sensitivity required for measurments in low biomass habitats. Furthermore, for questions regarding carbon cycling, including ecosystem modeling applications, it is desirable to have a separate accounting of both the ^{14}C-POC and ^{14}C-DOC pools. This is not possible using only the direct acidification procedure.

Table 6. HOT-74 (July 1996) ^{14}C activities for dissolved and particulate pools of carbon following an in situ 12-h light period incubation as determined by the MAGIC ^{14}C-DOC and alkaline-persulfate ^{14}C-DOC methods.

		Particulate pools		Dissolved pool		
Sample source	Filter type	^{14}C activity* (DPM liter^{-1})	GF/F ÷ PC (%)	MAGIC (DPM liter^{-1})	Persulfate (DPM liter^{-1})	MAGIC/ persulfate (%)
5 m	GF/F	75,453(±1,191)	112	9,140	13,229	69
	PC	66,994(±5,945)				
25 m	GF/F	62,656(±4,796)	126	6,060	6,000	101
	PC	49,584(±9,444)				
45 m	GF/F	37,841(±1,524)	122	5,380	5,571	97
	PC	30,993(±2,112)				
75 m	GF/F	19,414(±6,715)	142	2,580	7,400	35
	PC	13,686(±4,328)				
100 m	GF/F	7,221(±36)	137	1,780	5,329	33
	PC	5,282(±155)				

* Net (time zero and background corrected) 12-h light values for each sample treatment. Values are mean ± 1 SD (n = 3).

In our study, we used the common protocol that employs a microfine glass fiber filter (Whatman-GF/F) to concentrate ^{14}C-POC at the end of a 12-h in situ incubation experiment. The use of GF/F filters is preferred because of relatively high flow rates, high particle loading, and compatability with other ancillary measurements. The process of particulate matter concentration onto a Whatman-GF/F filter also provides a mechanism, albeit an arbitrary one, for the separation of dissolved and particulate matter. The validity of the entire filtration procedure lies with the characteristics of the filter type selected, including porosity, chemical compatability, and dissolved matter retention characteristics. The ideal membrane is manufactured from an inert matrix, retains all particles larger than the stated pore size and none that are smaller, and does not adsorb dissolved matter (Brock 1983). Unfortunately, this perfect filter does not exist, so all attempts to effect an unique separation of dissolved from particulate matter are compromised from the start.

We have documented at least two previously undisclosed potential problems with the use of Whatman-GF/F filters for processing ^{14}C primary productivity samples: variable adsorption of ^{14}C-DOC that is produced during the incubation procedure and a perhaps related problem with inefficient liquid scintillation detection of the collected ^{14}C activity. Our results with Whatman-GF/F filters would also apply to other microfine glass fiber filters (e.g., Whatman-GF/C, Reeve Angel-984H, Poretics GF-75) and to other membrane filters (e.g., Millipore-HA) and are consistent with results on adsorption of ^{14}C-DOC onto membrane filters (Maske and Garcia-Mendoza 1994).

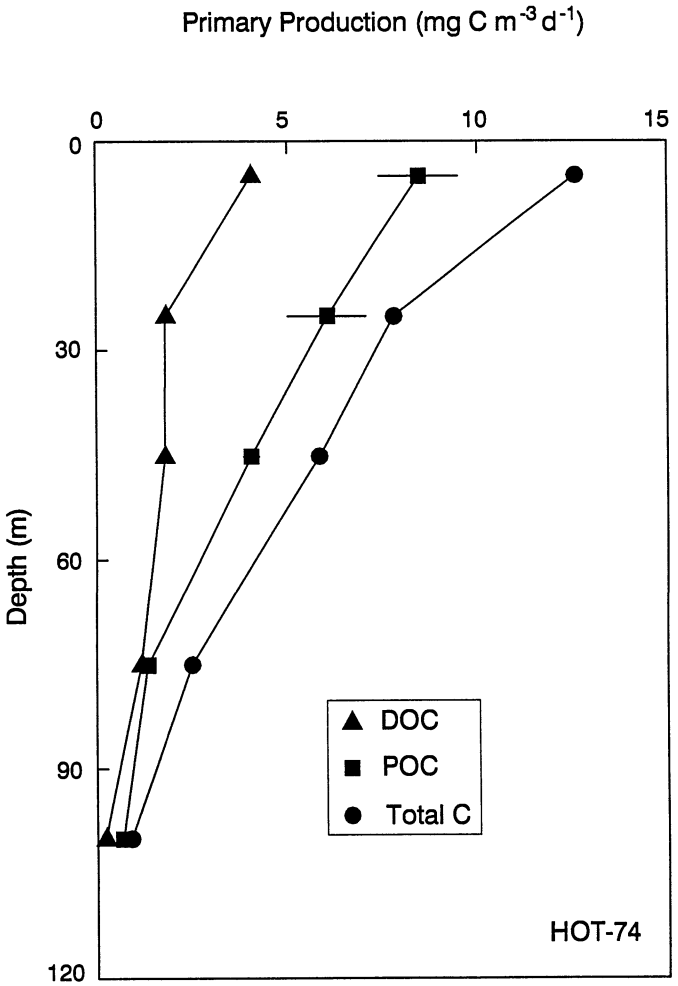


Fig. 7. Vertical profile of particulate (Nuclepore-PC; mean ± 1 SD, $n = 3$), dissolved (alkaline-persulfate oxidation of Nuclepore-PC filtrate), and total (sum of particulate and dissolved) organic matter production for the upper 100 m of the water column at Station ALOHA during HOT-74 (July 1996). The 0–100-m depth-integrated values for this cruise are 394 (POC), 177 (DOC), and 571 (total) mg C m^{-2} d^{-1}.

Previous researchers have pointed out the potential for overestimating particulate ^{14}C activity in primary productivity experiments as a result of adsorption of both ^{14}C-DIC and ^{14}C-DOC onto membrane filters. The sorbed ^{14}C-DIC is readily removed by acidification of the filter and venting, which is generally a routine part of the ^{14}C primary production procedure (McMahon 1973; Hitchcock 1986). However, the sorbed ^{14}C-DOC is a more fundamental concern both methodologically and in the overall interpretation of the field data. For example, Nalewajko and Lean (1972) showed that Millipore-HA (0.45 μm) filters retain ^{14}C-glycollate and other ^{14}C-labeled organic compounds that routinely accumulate in solution during primary production experiments with algal cultures and various freshwater habitats. Likewise, Jayakumar and Barnes (1983) reported the adsorption of large amounts of sorbitol and methylamine onto Millipore-HA filters, and between one and two orders of magnitude, respectively, lower adsorption onto Nuclepore-PC filters. Based on filter characteristics and the field results from Maske and Garcia-Mendoza (1994), one should anticipate a high adsorption of ^{14}C-DOC onto Whatman-GF/F filters relative to that observed with Nuclepore-PC filters (Table 2). This prediction was supported by our field measurements (Tables 3, 5; Fig. 3). Grande et al. (1989) had previously reported that ^{14}C activities from primary production experiments conducted at 28°N, 155°W collected using 0.2-μm Nuclepore-PC were, on average, 25% less than the ^{14}C activities on the larger porosity 0.45-μm Millipore-HA filters, a result that is identical to that reported here. Unfortunately, they were unable to determine the cause of this discrepancy, and because of a high ^{14}C-DOC background in their ^{14}C stock solutions, they were unable to measure ^{14}C-DOC fluxes or to distinguish plankton respiration from plankton excretion (Grande et al. 1989).

Our observation of increased ^{14}C activity in Whatman-GF/F filter preparations following a 30-d storage in Aquasol II scintillation cocktail was at first an enigmatic result that seemed different from results of experiments conducted elsewhere in the world ocean. Pugh (1973) reported a potential problem of self-absorption of ^{14}C by algal cells collected onto filters, which is caused by penetration of cells into the matrix of the solid support. Quench curves based on external standard counts will not correct for ^{14}C losses by self-absorption (Bransome and Grower 1970). Over time, the Whatman-GF/F filters clear, and in theory, this would reduce but not eliminate the apparent self-adsorption. We have also demonstrated that a variable portion of the ^{14}C-DOC pool is retained by Whatman-GF/F filters. The counting efficiency of these compounds would also be reduced by self-absorption of the ^{14}C activity. We interpret the increase in ^{14}C activity during Whatman-GF/F filter storage in Aquasol II to be caused by the combined time-dependent effects of filter clearing and release (extraction) of adsorbed ^{14}C-DOC. Both effects would cause an increase in total ^{14}C activity as a result of the higher counting efficiency but for different reasons. Because of the variability in the increased ^{14}C activity during storage observed among cruises (Fig. 1), we suggest that this increase is mainly the result of the extraction of variable amounts of absorbed ^{14}C-DOC into solution.

A legitimate question arises: What percentage of the ^{14}C activity is ^{14}C-DOC? One might argue that the Nuclepore-PC filter treatments can be used as reliable estimates of ^{14}C-POC and that the amount of ^{14}C-DOC adsorbed to the Whatman-GF/F filters can therefore be estimated as [^{14}C-GF/F] minus [^{14}C-PC], assuming that (1) the particle retention characteristics of the Nuclepore-PC and Whatman-GF/F filters are identical and (2) there is no ^{14}C-DOC adsorption onto the Nuclepore-PC filters. However, neither assumption is probably correct. For example, Altabet (1990) reported that up to 40% of the total POC collected on ANO filters (0.2 μm; aluminum oxide matrix) at a station off Bermuda passed a Whatman-GF/F. In our study, ANO and Nuclepore-PC returned similar ^{14}C-POC activities (Table 4). Consequently, the percentage of total fixed carbon present in the DOC fraction must be considered a minimum estimate.

Quantitative determination of ^{14}C-DOC—A major analytical challenge is the accurate measurement of the relatively

low ^{14}C-DOC activities in the presence of relatively high ^{14}C-DIC activities; generally ^{14}C-DIC activity is five to six orders of magnitude greater than ^{14}C-DOC activity. In our experiments, we used a commercially available ^{14}C-bicarbonate stock prepared by ^{14}C-CO_2 generation from barium carbonate and neutralization with NaOH (cat. 17441H, ICN Pharmaceuticals). The time-zero non-acid-volatile ^{14}C blank, which we interpret to be ^{14}C-DOC in the ^{14}C-bicarbonate stock, was typically 3–8 DPM ml^{-1} of seawater above background in our standard in situ primary production experiments (50–100 μCi ^{14}C stock added per liter of sample). This represents less than about 50 DPM μCi^{-1} of ^{14}C stock solution. With a typical precision of approximately 10% for triplicate subsamples, our ^{14}C-DOC oxidation procedure is, therefore, capable of detecting a net ^{14}C-DOC accumulation of approximately 1 μg C liter^{-1} using the standard primary production protocols (Karl et al. 1996; Letelier et al. 1996). The sensitivity could be scaled up by using a larger spike of ^{14}C-bicarbonate or by processing a larger volume of filtrate, or by doing both. If necessary, the ^{14}C-bicarbonate stock solution could also be recrystallized or photo-oxidized (Williams et al. 1972) to eliminate the residual ^{14}C-DOC contamination, thereby rendering a more sensitive ^{14}C-DOC production assay protocol.

The sensitivity and reproducibility of the ^{14}C-DOC alkaline-persulfate oxidation method proposed here makes it especially useful for oligotrophic ocean studies. The method employed is adapted from protocols previously used for particulate organic matter oxidation (Raimbault and Slawyk 1991; Libby and Wheeler 1994), which appears to perform well for DOM oxidation, too. Nevertheless, alkaline-persulfate oxidation procedures in general have been criticized in recent years because of potential problems related to inefficient oxidation (Hedges and Lee 1993). In our hands, the alkaline-persulfate method yielded essentially complete oxidation of the ^{14}C-DOC compounds that accumulate under natural incubation conditions, as determined by the direct measurements of residual ^{14}C activities in solution following the alkaline-persulfate oxidation and $^{14}CO_2$ distillation steps of the proposed ^{14}C-DOC procedure. It is conceivable, even likely, that the chemical composition of the ^{14}C-DOC that accumulates during a primary production incubation (which accounts for much less than 5% of the total DOC pool) is more susceptible to persulfate-based oxidation than the more refractory pool of total DOC in seawater. Although one could, in theory, use a more efficient high-temperature catalytic oxidation method, it is not obvious how one could achieve the necessary sensitivity required for ^{14}C-DOC measurements in oligotrophic waters. The alkaline-persulfate method that we endorse effectively concentrates the oxidized ^{14}C-DOC from 70 ml of seawater onto a single filter paper wick and can, if necessary, be scaled up to accommodate larger sample volumes. High-temperature catalytic oxidation methods typically handle only 0.1–0.25 ml per injection (Tupas et al. 1994) and are not easily scaled up to the volumes that would be required for routine ^{14}C-DOC measurements.

Extracellular products of photosynthesis—The observed production of significant amounts of ^{14}C-DOC during 12-h in situ primary production incubation experiments at Station ALOHA requires that we develop a mechanistic understanding of the procedural, physiological, and ecological processes involved if we hope to achieve a comprehensive understanding of the carbon cycle in the subtropical North Pacific Ocean. There is presently a fairly large and somewhat conflicting body of literature (cf. Sharp 1977; Fogg 1983) on the quantitative significance of ^{14}C-DOC in primary production experiments conducted in aquatic ecosystems, including the potential mechanisms of DOC formation. Nevertheless there appears to be a credible physiological basis for DOC production by photosynthetic microorganisms and overwhelming evidence to suggest that extracellular release is a normal function of healthy photoautotrophic cells (Hellebust 1974; Mague et al. 1980; Fogg 1983; Williams 1990; Wood and van Valen 1990). However, the actual mechanisms and rates of release are poorly understood. For example, ^{14}C-DOC could accumulate in an incubation flask as a direct result of inadvertent release (e.g., diffusion of low-molecular-weight compounds through the cell membrane) or by active excretion of specific molecules including both low-molecular-weight compounds and polymeric substances (e.g., polysaccharides and proteins). The list of specific compounds that are liberated by growing algae is large (Fogg 1966; Hellebust 1974), and it is likely that the process of extracellular release is very important in microbial ecology.

Alternatively, the release of ^{14}C-DOC could be an indirect effect of cell lysis (either naturally or during sample processing; e.g., Goldman and Dennett 1985) or could result from the activities of predator (grazer) or parasite (virus) populations. However, these indirect processes probably are not significant during a 12-h in situ incubation procedure. Processes that influence DOC release from whole cell fractions would be expected to produce DOC with a lower specific radioactivity than processes releasing recently produced photosynthate, so their relative importance would need to be much larger than that of direct release for an equivalent flux of ^{14}C-DOC. Nevertheless, each of these different pathways would produce a different suite of ^{14}C-labeled organic compounds so knowledge of the physiological and ecological processes is critical. Any process that inhibits cell growth and division but still permits carbon assimilation (e.g., high light, low nutrients) will result in a higher percentage of photoassimilated C release (Hellebust 1974; Mague et al. 1980; Wood and Van Valen 1990).

If phytoplankton have evolved an overflow mechanism for the release of fixed C when nutrients become depleted, they could effectively stimulate their bacterial competitors and exacerbate the nutrient stress (Bjørnsen 1988). However, Bjørnsen (1988) argued that the passive loss of organic compounds through the cell membrane would select for permeable compounds. This mechanism would also result in an enhanced loss from small cells because of higher surface-to-volume ratios and enhanced loss in oligotrophic seas where external organic compound concentrations are low and intracellular-to-extracellular diffusion gradients are large. Baines and Pace (1991) summarized much of the data that were then available for rates of extracellular release in marine and freshwater ecosystems. On average, extracellular release was 13% of the total ^{14}C fixation into particulate matter, and in the sea, extracellular release increased linearly with primary

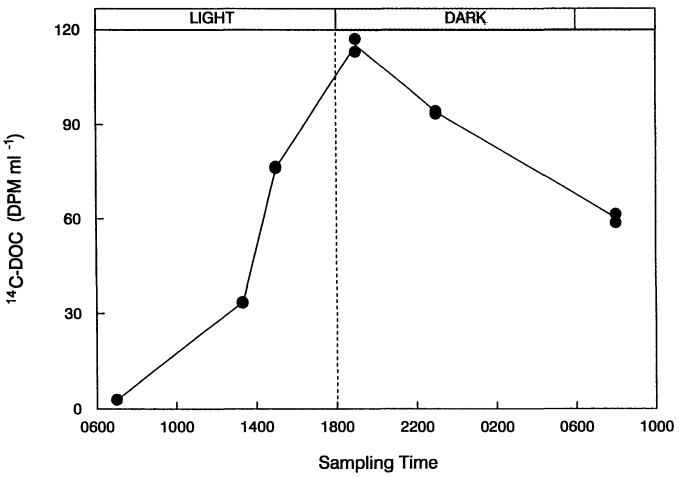


Fig. 8. Time course of ^{14}C-DOC production for a surface water sample collected during HOT-78 (December 1996) over a 24-h incubation period. At each time point, duplicate subsamples were removed and filtered through a Nuclepore-PC filter, and the filtrate was processed for ^{14}C-DOC by the alkaline-persulfate oxidation method. The individual determinations are shown; for the first three time points they are essentially identical.

production and was not related to phytoplankton biomass (estimated by Chl *a*). This observation is inconsistent with the passive diffusion model.

In our time-course ^{14}C-DOC production experiment (Fig. 8), the lack of a lag in the appearance of ^{14}C-DOC suggests that the organic compounds produced are probably in isotopic equilibrium with the recent products of photosynthesis (Marra et al. 1981; Dring and Jewson 1982). The rapid transition from net accumulation to net removal following the termination of photosynthesis at dusk (Fig. 8) also suggests that the ambient pool of these organic compounds must be small and that the gross production must be larger than the net accumulation measured during daylight hours because of the probable simultaneous production and uptake.

Station ALOHA primary production—Since 1989, we have been measuring the rates of in situ primary production and other ecosystem parameters at the open ocean Station ALOHA approximately every month (Fig. 9). Several features have emerged from this long-term data set. First, the mean rate of total euphotic zone primary production (mean = 472, SD = 125, n = 70) is higher, by about a factor of 2 or 3, than most earlier measurements in this region (Karl et al. 1996). We hypothesized that primary production in the subtropical North Pacific Ocean has probably increased significantly over the past 20–30 yrs and that the mechanisms of organic matter production, export, and remineralization may have also changed (Karl et al. unpubl.).

Primary productivity data at Station ALOHA (using Whatman-GF/F filters) is highest in summer (July–September) and lowest in winter (November–January). The typical seasonal amplitude (approximately 250 mg C m^{-2} d^{-1}; Fig. 9) is about 50% of the mean value. However, we now know that the Whatman-GF/F filter measurements include ^{14}C-POC plus a variable percentage of the total ^{14}C-DOC. We

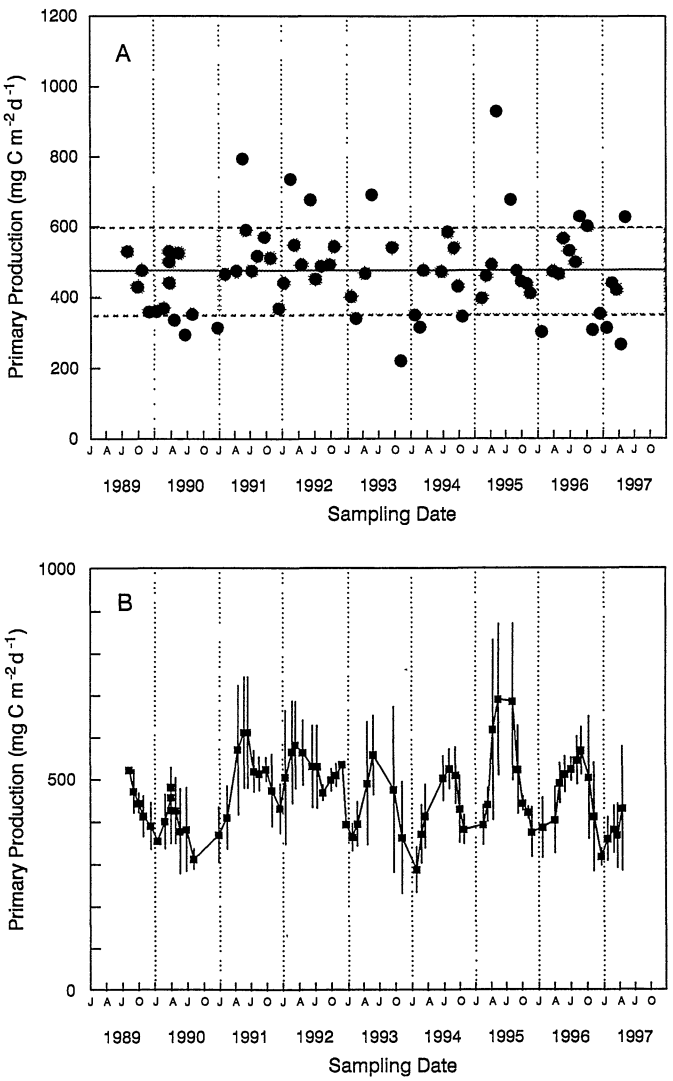


Fig. 9. Temporal variability in primary production for Station ALOHA. (A) Each data point represents the euphotic zone (0–200 m) depth-integral value for trace-metal-clean in situ ^{14}C-bicarbonate uptake at eight reference depths and processing using Whatman-GF/F filters. The solid line is the mean for the full data set and the shaded portion is ±1 SD (mean = 472, SD = 125, n = 70). (B) The data shown are the 3-point running mean values (±1 SD of the running mean) documenting a coherent seasonal trend of maximum rates of primary production each summer and minimum rates of primary production each winter, with some interannual variability in the respective maximum and minimum values.

are unable to determine, from the currently available data, an accurate estimate for gross primary production because of the presently unresolved magnitude and dynamics of the apparently large ^{14}C-DOC component. At the very least, primary production in the subtropical North Pacific gyre measured by the present protocols may be underestimated by 30–50%. If summertime gross primary production in these oligotrophic habitats is scaled accordingly, it may approach 1 g C m^{-2} d^{-1} (83 mmol C m^{-2} d^{-1}). If these results from Station ALOHA are typical of larger regions of the subtropical North Pacific habitat as a whole, as the observations of

Grande et al. (1989) at 28°N, 155°W seem to imply, then our present models of biogeochemical cycling in these low-nutrient, low-biomass habitats are probably in need of revision (*see* Doney 1997; Emerson et al. 1997).

Photosynthetic assimilation numbers—The light-saturated rates of carbon fixation normalized to chlorophyll (i.e., referred to as the assimilation number, productivity index, P^B_{max}, or photoperiod assimilation ratio usually reported in units of grams of C h^{-1} per gram of Chl a^{-1} is a measure of the physiological state of the photoautotrophic microbial assemblage under in situ conditions. This parameter reflects both the maximum photosynthetic efficiency and the maximum specific growth rate (Falkowski 1981). Based on theoretical considerations, including the maximum turnover time for a photosynthetic unit (PSU) of 1 ms, Falkowski (1981) suggested that 1 g of Chl *a* could potentially fix 25 g C h^{-1} at light saturation or a theoretical maximum assimilation number of about 25. Assimilation numbers measured for natural populations of light-saturated phytoplankton are typically only 10–50% of this theoretical value, suggesting slower PSU turnover times because of limitations of light or nutrient availability, or temperature. The photoautotrophic plankton communities in the warm (annual temperature fluctuation at Station ALOHA is 23–27°C; Bingham and Lukas 1996) upper water column (0–25 m) of the subtropical North Pacific Ocean are not likely to be temperature or light limited (Eppley et al. 1973; Letelier et al. 1996). Changes in the assimilation number may, therefore, be a diagnostic indicator of nutrient limitation or species succession, or both. Consequently, the low assimilation numbers previously reported from oligotrophic regimes (Table 7) have been interpreted as evidence for nutrient (generally N) limitation. Because chlorophyll contains N and is also generally bound in vivo to protein, which also contains N (Falkowski 1981), inorganic N limitation is predicted to lead to low assimilation numbers and low rates of total primary production.

In contrast to this traditional view of the oligotrophic North Pacific Ocean, we have recently presented evidence consistent with the hypothesis that a major ecosystem change has occurred during the past decade, resulting in a shift from a primarily N-limited to a primarily P-limited habitat with attendant changes in biogeochemical cycling pathways and rates (Karl et al. 1995, 1997). These changes are believed to be a result, in part, of a selection for N_2-fixing microorganisms due to El Niño-southern oscillation–induced changes in ocean circulation and mixing. It appears that N_2 fixation may presently be responsible for up to 50% of the N required to support contemporaneous new production at Station ALOHA and that this additional source of new N for the epipelagic ecosystem has resulted in a systematic drawdown of the ambient soluble reactive phosphorus pool (Karl and Tien 1997; Karl et al. 1997). We believe that the ^{14}C-DOC production results presented here, especially the large and increasingly important role of ^{14}C-DOC fluxes and the high and increasingly larger assimilation numbers observed at Station ALOHA (Figs. 1, 10; Table 7) are direct consequences of these ecosystem changes.

Several algal culture studies (Ignatiades and Fogg 1973; Myklestad 1977; de Madariaga 1992) indicate that the production of extracellular carbon (usually as carbohydrates) increases as the N : P ratio of the medium increases. Under extreme P limitation, extracellular polysaccharide production may constitute the main photosynthetic activity in selected species of marine diatoms (Myklestad 1977). More recently, Obernosterer and Herndl (1995) confirmed these earlier results and further demonstrated that a complex spectrum of dissolved organic compounds, in addition to mono- and polysaccharides, are released under P limitation. Under extreme P limitation, bacterial activity may be suppressed, allowing DOM to accumulate and eventually to form large aggregates (Obernosterer and Herndl 1995). Although the subtropical North Pacific gyre probably has not yet reached the point where DOM aggregation is occurring, we have observed over the last few years an enigmatic accumulation of DOM in the euphotic zone (Karl et al. 1995, 1997), a feature that we believe is also a consequence of the shift to P limitation. The extremely low rates of microbial β-glucosidase activity measured for surface waters at Station ALOHA (i.e., approximately three to four orders of magnitude lower than β-glucosidase activities measured at the equator; Christian and Karl 1995) is consistent with end-product inhibition by monosaccharides and P-limited conditions and is identical to environmental conditions previously reported for the Northern Adriatic Sea during summer (Obernosterer and Herndl 1995). Consequently, the formation of DOM during photosynthesis may have a negative feedback on the balanced growth of bacteria rather than the intuitive stimulatory effect.

Ecological implications—There are several potential implications of our results on DOC fluxes at Station ALOHA. First, to the extent that the ^{14}C-DOC produced during our in situ primary production experiments is not routinely accounted for, the previously reported rates (Karl et al. 1996; Letelier et al. 1996) must be considered minimum estimates of net and gross primary production but an overestimation of net photoautotrophic POC production. From the limited data on direct measurement of ^{14}C-DOC presented here, we estimate that gross primary production may have been underestimated by at least 30–50%. If our already relatively large estimates of 170 g C m^{-2} yr^{-1} are increased to account for the rates of ^{14}C-DOC production, then contemporaneous rates of primary production in the subtropical North Pacific Ocean may equal or exceed 250 g C m^{-2} yr^{-1}. If our results at Station ALOHA are characteristic of the gyre as a whole, then global production estimates would need to be revised upward accordingly.

The relatively large rates of ^{14}C-DOC production that we have measured at Station ALOHA may be a manifestation of the changing nutrient conditions in the North Pacific Ocean. If our hypothesis regarding an alternation of N and P limitation is correct (Karl et al. 1995, 1997), then these unusually high rates of primary production may be a consequence of changes in biodiversity (Karl et al. unpubl.). These potential ecosystem changes make it very difficult to develop meaningful biogeochemical models based on static steady state assumptions.

Because gross primary production at Station ALOHA is dominated by the oxygenic phototrophic bacterium *Pro-*

Table 7. Compilation of data on phytoplankton biomass, primary production, and assimilation numbers for the near surface waters of the North Pacific Ocean.

Date	Location and date	Water depth (m)	Primary production (mg C m^{-3} h^{-1})/filter*†	Chlorophyll concentration (mg Chl *a* m^{-3})/filter*	Assimilation number (g C g Chl a^{-1} h^{-1})	Reference
Jun 1966	Sta. A (29°12′N, 161°30′W)	10	0.46 HA	0.1 HA	4.6	Glooschenko and Curl (1971)
Aug 1971	5 oceanic stations 10–20 km from Hawaiian Is.	surface	0.13±0.03 HA	0.05±0.01 GF/C	2.9±0.6	Gilmartin and Revelante (1974)
Nov 1971	3 stations near 30°N, 140°W	0–60	0.18±0.08 GF/C	0.069±0.02 GF/C	2.71±1.07	Eppley et al. (1973)
Jun 1972, Mar 1974	28°N, 155°W	0–80	 GF/C	0.076±0.36 GF/C	1.3±0.8	Sharp et al. (1980)
Aug 1972	Sta. C (21°22′N, 159°12′W)	0–50	0.28±0.25 HA	0.16±0.12	1.65±0.65	Gundersen et al. (1976)
Feb 1973, Jun 1973, Feb 1974	28°N, 155°W	0–60	0.09±0.04† GF/C	0.077±0.040 GF/C	1.43±0.90	Eppley et al. (1977)
Jun 1972, Feb 1973, Mar 1974	28°N, 155°W	0–60	 GF/C	 GF/C	range = 1.4–3.6	Eppley and Sharp (1975)
July 1975	Sta. 2 (19°50′N, 164°15′W) Sta. 3 (18°20′N, 170°05′W)	10	0.07 0.046±0.002	0.05 0.047±0.006	1.4 0.99±0.10	Bienfang and Gundersen (1977)
Oct. 1978; Apr, Jun, Aug, Oct, Dec 1979	Ke-ahole Pt, 19°55′N, 156°10′W	0–30	 GN-6	 GN-6	1.63±1.30	Bienfang and Szyper (1981)
May, Apr, Nov 1980; Jan, Mar, May 1981	Kahe Pt., 2 stations near 21°20′N, 158°15′W	0–44	0.05–0.17 HA	0.08–0.12 HA	range = 0.11–2.38	Bienfang et al. (1984)
Sep 1982	Kahe Pt. 21°20′N, 158°12′W	surface	"clean" 0.96±0.02 "dirty" 0.90±0.04 GF/F	0.07 GF/F	"clean" = 13.7 "dirty" = 12.8	Marra and Heinemann (1984)
Aug–Sep 1985	28°N, 155°W	30	 GF/F	 GF/F	9.5±1.4	Laws et al. (1987)
		0–50	 HA	 HA	7.4	Marra and Heinemann (1987)
Mar–Apr 1986	26°N, 155°W	0–40	 GF/F	 GF/F	10.2±2.6 (*n* = 6) range = 6.6–13.9	DiTullio and Laws (1991)
Jul 1996	4 oceanic stations on a transect from Sta. ALOHA (22°45′N, 158°W) to CLIMAX (28°N, 155°W)	5	 GF/F	 GF/F	range = 3.5–9.9	Ondrusek and Bidigare (1997)
Jan 1990–Jun 1997	Sta. ALOHA (22°45′N, 158°W)	5 25	0.47±0.05 0.45±0.04 GF/F	0.092±0.007 0.092±0.08 GF/F	6.0±0.7 5.6±0.6 0–25-m range = 1.9–14.1	This study

* Individual values or as mean ± 1 SD of the mean. Also shown is the type of filter used in each study when given. Filter types: GF/C = Whatman glass fiber filter, GF/F = Whatman microfine glass fiber filter, HA = Millipore (0.45 μm), GN-6 = Gelman (0.45 μm).

† Per hour rates estimated from reported daily rates, assuming 12 h of daylight.

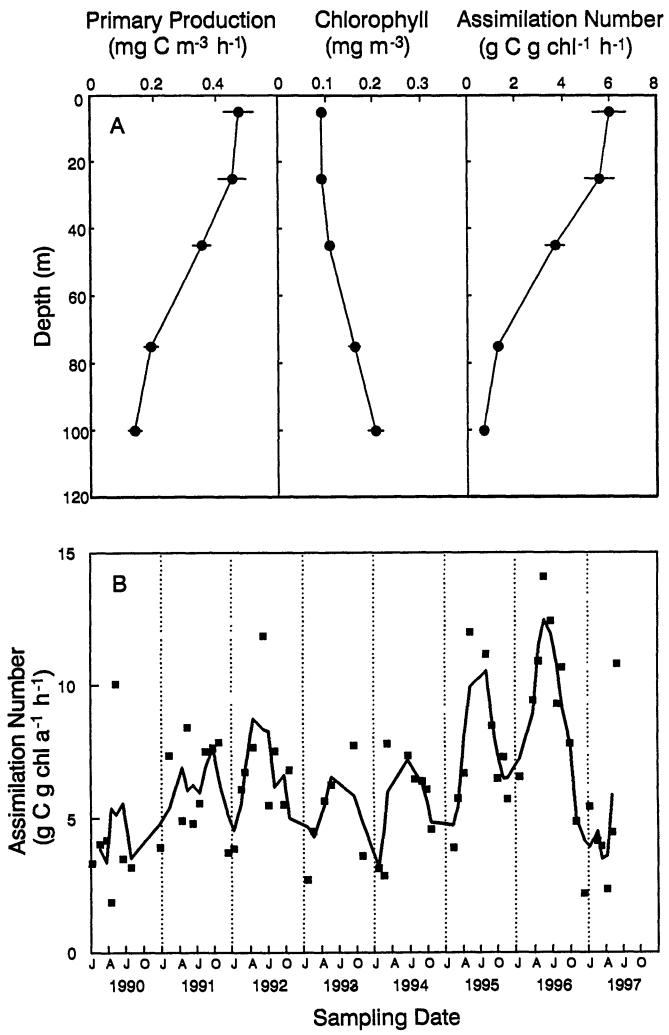


Fig. 10. (A) Mean and 95% confidence interval for primary production, Chl *a* concentration, and assimilation number for 0–100 m measured during 84 HOT program cruises to Station ALOHA between October 1988 and July 1997. (B) Temporal variability in the light-saturated mean assimilation number measured in the upper 25 m of the water column at Station ALOHA, showing an increasing trend during the past several years.

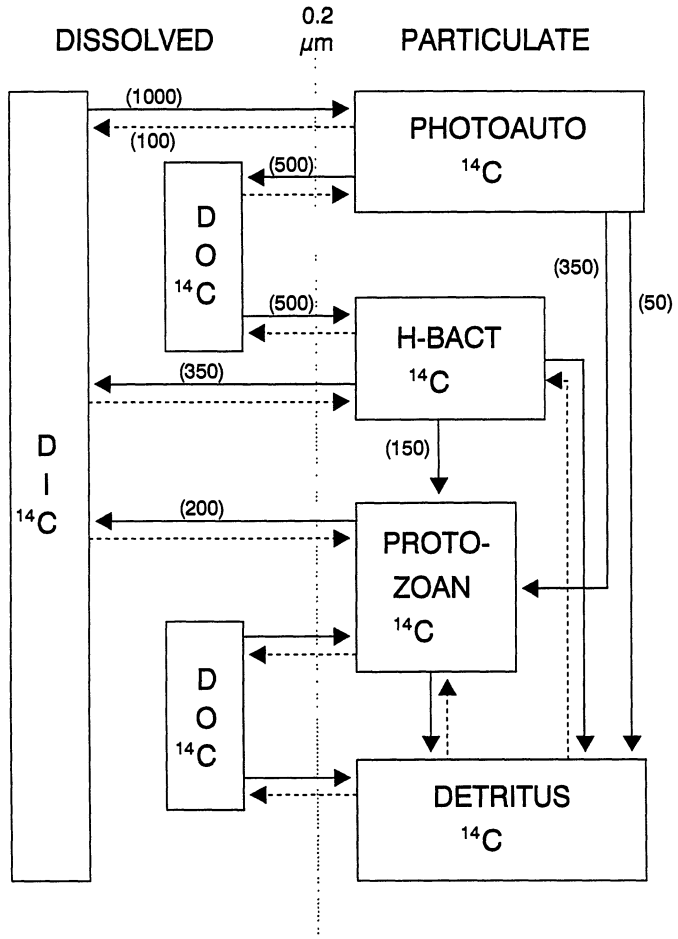


Fig. 11. Conceptual view of processes in a ^{14}C-based primary productivity incubation experiment. Depicted are some of the potential C pools, ^{14}C labeling pathways, and recycling mechanisms that occur following the addition of ^{14}C-bicarbonate to a sample of seawater contained in a clear polycarbonate incubation bottle. Although the assumed major pathway, uptake of DIC ^{14}C-DIC into photoautrophic C (PHOTOAUTO-^{14}C), is probably the dominant flux of ^{14}C, other pathways, especially the production of ^{14}C-DOC by several pathways and the transfer of PHOTOAUTO-^{14}C to other particulate pools (by both direct and indirect mechanisms), are probably important during a 12-h in situ primary production experiment. These coupled C production and C regeneration reactions are neither well understood nor well separated by current methodologies. The numbers assigned to selected fluxes (mg C m^{-2} d^{-1}) are the best guess mean values for the subtropical North Pacific Ocean, based on research conducted at Station ALOHA.

chlorococcus (i.e., ≥74% of the total gross carbon production; Liu et al. 1997), we speculate that *Prochlorococcus* may be responsible for a significant portion of the DOC flux reported here. If the DOC produced during photosynthesis is bioavailable, then coupled or subsequent uptake by microorganisms, including those organisms initially producing the DOC, is possible. From previous research at Station ALOHA, we know that there has been an accumulation of DOM in the upper ocean that greatly exceeds the accumulation of particulate matter (Karl et al. 1995, 1997; Karl and Tien 1997). The average chemical composition of the accumulated organic matter indicates that it is C rich and N and P poor, with an average C : N ratio of nearly 20. This net accumulation of organic matter implies a decoupling of production and utilization processes that, in the case of Station ALOHA, has been sustained for a period of several years. Also, it has been suggested recently that this accumulation of photosynthetically derived DOM in surface waters may be an indication of a malfunctioning microbial loop (Thingstad et al. 1997). Although this might be the case, we believe that it is an indication of nutrient limitation and, in the case of Station ALOHA, P limitation (Karl et al. 1995, 1997). Without further information on the chemical composition of the photosynthetically derived DOC pool, it may be premature to predict a coupled heterotrophic bacterial response, although it seems likely.

Based on ^{14}C-DOC flux measurements conducted in 1997,

we estimate that the rate of heterotrophic bacterial production could approach 150 mg C m^{-2} d^{-1} in the euphotic zone at Station ALOHA (Fig. 11). Furthermore, because the heterotrophic growth substrates are produced only during the light period, heterotrophic activities may be accelerated within the euphotic zone and may be stimulated in the light. The former prediction is supported by a recent study that showed that most of the heterotrophic bacterial production in the subtropical North Pacific occurred in the upper 40 m and was fueled by DOC production by phytoplankton (Jones et al. 1996). The reported value of heterotrophic bacterial production was about 150–200 mg C m^{-2} d^{-1} for the euphotic zone (Jones et al. 1996), a value that is consistent with our mass flux prediction (Fig. 11).

If a significant amount of C-rich DOM is accumulated during a primary production incubation experiment, then the realized photosynthetic quotient (PQ = $\Delta O_2/-\Delta CO_2$) may be different from that assumed to occur during balanced growth (Laws 1991). The PQ can range from 1.0 for carbohydrate synthesis to 3.0 for lipid biosynthesis. Under ecological conditions where large amounts of DOM are produced in response to inorganic nutrient limitation, such as those described here for Station ALOHA, the traditional particulate matter biological pump may be supplemented with a diffusion pump (Carlson et al. 1994; Ducklow et al. 1995), with numerous implications for export production and C sequestration (Toggweiler 1989). Consequently, knowledge of both the rates of DOC production and the chemical composition of the accumulated pools will be necessary before accurate biogeochemical and metabolic models can be formulated.

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