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Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting

Abstract—A partitioning of ultraphytoplankton primary production among prochlorophytes, cyanobacteria, and eucaryotic algae was made by shipboard flow cytometric sorting of ^{14}C-labeled cells. Aggregate primary production was derived from the sum, over all three ultraplankton groups, of the product of cell abundance and cell-specific rate of ^{14}C uptake which ranged from 0.03 to 4 fg C cell^{-1} h^{-1} for prochlorophytes and 0.2 to 10 fg C cell^{-1} h^{-1} for cyanobacteria. Results indicated that the dominant primary producer was not necessarily the numerical dominant nor necessarily the group with the highest cell-specific rate of ^{14}C uptake. Generally, eucaryotic ultraphytoplankton are dominant because of their high cell-specific rate of ^{14}C uptake and in spite of their relatively low abundance. Less often, it seems, procaryotic picoplankton may dominate in spite of their low cell-specific rate of ^{14}C uptake because of their high abundance.

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Primary production, meaning the rate at which carbon is converted from inorganic to organic form by photosynthesis, is often measured by the rate at which phytoplankton become radiolabeled when supplied with $NaH^{14}CO_3$. Various methods exist which allow this production to

be ascribed to particular cell groups (Li 1993). Most notable of these are microautoradiography, isolation by micropipette of single cells visible under a dissecting microscope, and separation of taxon-specific carotenoids by high-performance liquid chromatography. In general, these methods cannot be easily applied toward the *direct* measurement of ^{14}C uptake by cells as small as the ultraplankton (<5–10 μm), and especially the picoplankton (<2 μm). In contrast, the separation of ^{14}C-labeled phytoplankton by flow cytometric sorting appears to be well suited for this purpose because large numbers of small cells can be isolated with the assurance that there is a strict proportionality between the amount of ^{14}C assayed and the number of cells sorted (Li 1993).

In this note, I report measurements of ^{14}C uptake per cell for prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton at several sites in the North Atlantic. The results were obtained by shipboard flow cytometric sorting of live (unpreserved) cells labeled with ^{14}C during a prior period of incubation under simulated in situ conditions. Seawater was collected with Niskin bottles. Samples of <3 ml were analyzed fresh within minutes with a benchtop flow sorter (FACSort, Becton Dickinson). Fluorescence at wavelengths longer than 650 nm (FL3 instrument channel) was ascribed to chlorophyll *a*; fluorescence centered at 585 nm (FL2 instrument channel) was ascribed to phycoerythrin. The flow cytometer was operated to emphasize the smallest and most abundant phytoplankters (i.e. instrument set for high photomultiplier gains).

The cytogram signatures generally revealed the presence of four cell groups (Fig. 1). Phycoerythrin-containing cyanobacteria (CYA) were distinguished by their fluorescence in the orange waveband (Fig. 1A,C). Of the cells not containing phycoerythrin, a distinction into three groups could be made based on the intensity of light scatter (Fig. 1B,D) which increased from prochlorophytes (PRO) to small eucaryotes (SEUC) to large eucaryotes (LEUC). The taxonomic identities of the eucaryotes are not known. However, by size fractionation, SEUC was found to pass through 1-μm polycarbonate membranes (Nuclepore) and LEUC was retained on them. Most of the LEUC passed through 2-μm membranes (Fig. 1C,D) but some did not; this is evident in the cytograms of ultraphytoplankton (Fig. 1A,B) and picophytoplankton (Fig. 1C,D) which differed only with respect to the few cells larger than indicated by light scatter of ~200 units. The presence of diverse groups in 2-μm, and even 1-μm, filtrates indicates the difficulty of partitioning ^{14}C activity among such groups by conventional "size-fractionation" experiments. In the samples analyzed here, PRO, CYA, and LEUC were always present, but SEUC were sometimes absent.

Cell-specific rates of ^{14}C uptake were measured following the "presort incubation" protocol for which Rivkin et al. (1986) have shown that cellular radioactivity is unaffected by flow sorting. A 20-ml sample of seawater was placed in a polycarbonate centrifuge tube (Nalgene No. 3118-0030) and spiked with $NaH^{14}CO_3$ (ICN No. 17441H, 2 mCi ml^{-1}) to final activities of 50 or 100 μCi ml^{-1}. Radiolabeled samples were incubated for 8 h in simulated in situ deck incubators and then kept dark (unpreserved) in the laboratory as subsamples were successively removed for flow cytometric sorting over ~3 h. Sorted cells, which were diluted into large volumes of sheath fluid (filtered seawater), were collected by filtration onto membrane filters which were then fumed over concentrated HCl to remove inorganic ^{14}C. For each cell group, successive sorts were made, each attaining a different number of sorted cells; the amount of ^{14}C uptake per cell could thus be estimated from the regression slope of disintegrations per minute (dpm) on number of sorted cells (Fig. 2). Positive values of the *y*-intercept in these regressions were indicative of methodological blanks and background radioactivity.

Using high levels of radioisotope, I could easily detect sorted cells based on a signal of 50 dpm above background. For example, at station 20, this amount of radioactivity was found in ~10,000 CYA (Fig. 2B) and in ~50,000 PRO (Fig. 2C). Table 1 lists, for three different samples, the abundance of each cell group *i* (n_i, cells $liter^{-1}$), the rate of primary production per cell (p_i, fg C $cell^{-1}$ h^{-1}), and their product which is the group-specific rate of primary production (P_i, ng C $liter^{-1}$ h^{-1}). The results indicate that $n_{PRO} > n_{CYA} \approx n_{SEUC} > n_{LEUC}$, but $p_{PRO} < p_{CYA} \approx p_{SEUC} < p_{LEUC}$. In other words, small cells were more abundant than large cells, but cellular ^{14}C uptake was less for the smaller cells.

The reliability of the sorting procedure was

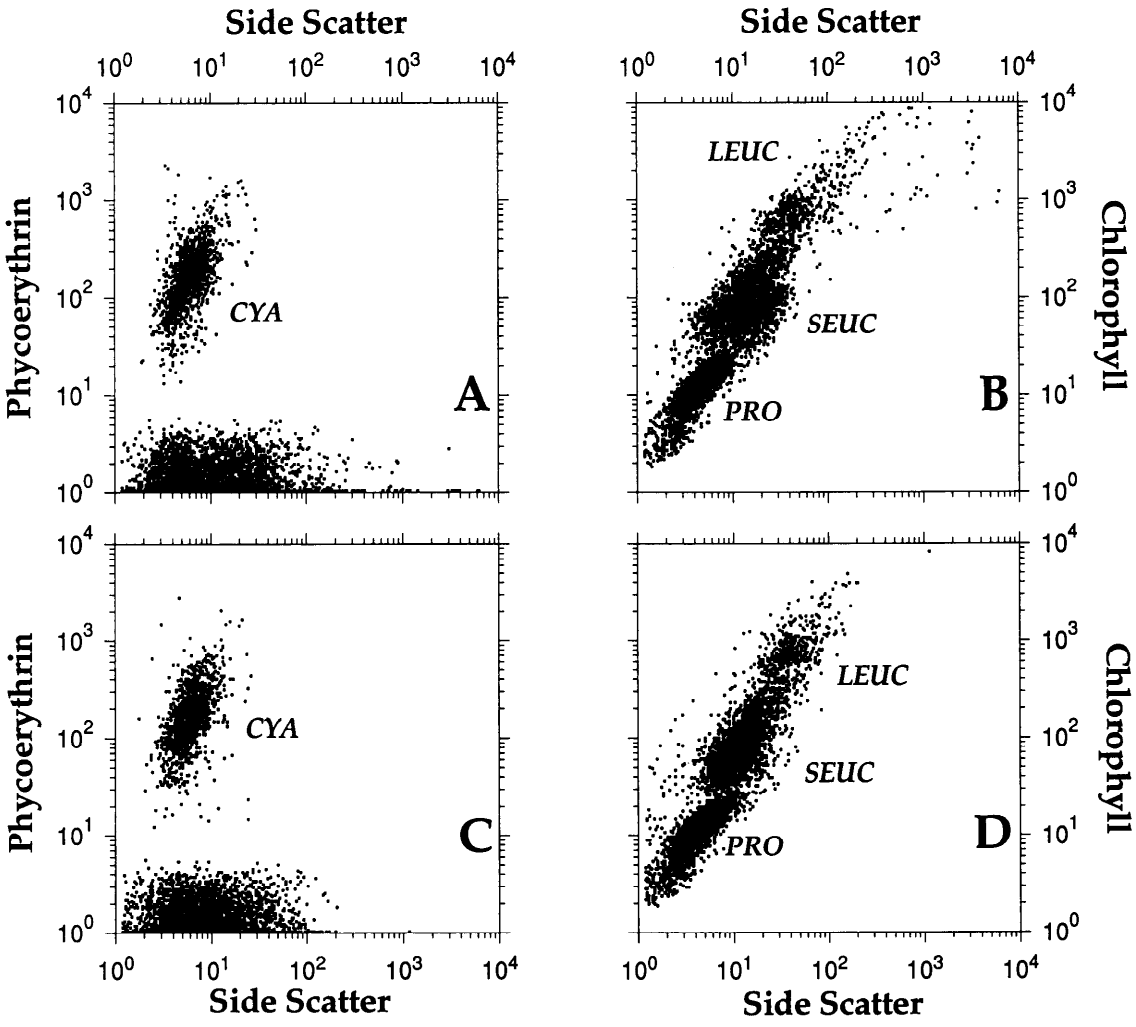

Fig. 1. Flow cytometric signature of 60-m ultraphytoplankton at station 45 (31°14.0′N, 10°47.4′W). A,B. Ultraphytoplankton counted from unfiltered sample. C,D. Picophytoplankton counted from the filtrate of 2.0-μm Nuclepore polycarbonate membrane. Cytograms of chlorophyll vs. side scatter (B,D) indicate only those cells whose FL2 intensity (phycoerythrin) was <10 units, namely noncyanobacterial cells.

checked by comparing the sum of group-specific ^{14}C uptake (ΣP_i) against the aggregate amount of ^{14}C uptake (cf. Yentsch and Campbell 1991). The latter was independently measured by filtering various volumes (≤ 3 ml) of unsorted radiolabeled subsamples onto membrane filters; the regression slope of dpm on filtered volume was an estimate of aggregate ^{14}C uptake per ml (Fig. 2A). The sorting procedure accounted for $>90\%$ of the aggregate ^{14}C uptake (Table 1), giving a good degree of confidence to values of n_i and p_i. It is important to note that the aggregate amount of ^{14}C uptake as measured need not necessarily equal the total amount of primary production expressed by all the phytoplankton. The reason is that the aggregate amount is based on only small volumes (≤ 3 ml) which would likely not include a proportional representation of the less abundant cells. For example, in a volume of 1 ml, a cell group for which the abundance is 1,000 individuals per liter would assuredly be undersampled. Since an inverse relationship exists between abundance and size of oceanic particles (e.g. Stramski and Kiefer 1991), it is evident that large cells would be underrepresented in samples of small volumes.

The significance of the present results (Table

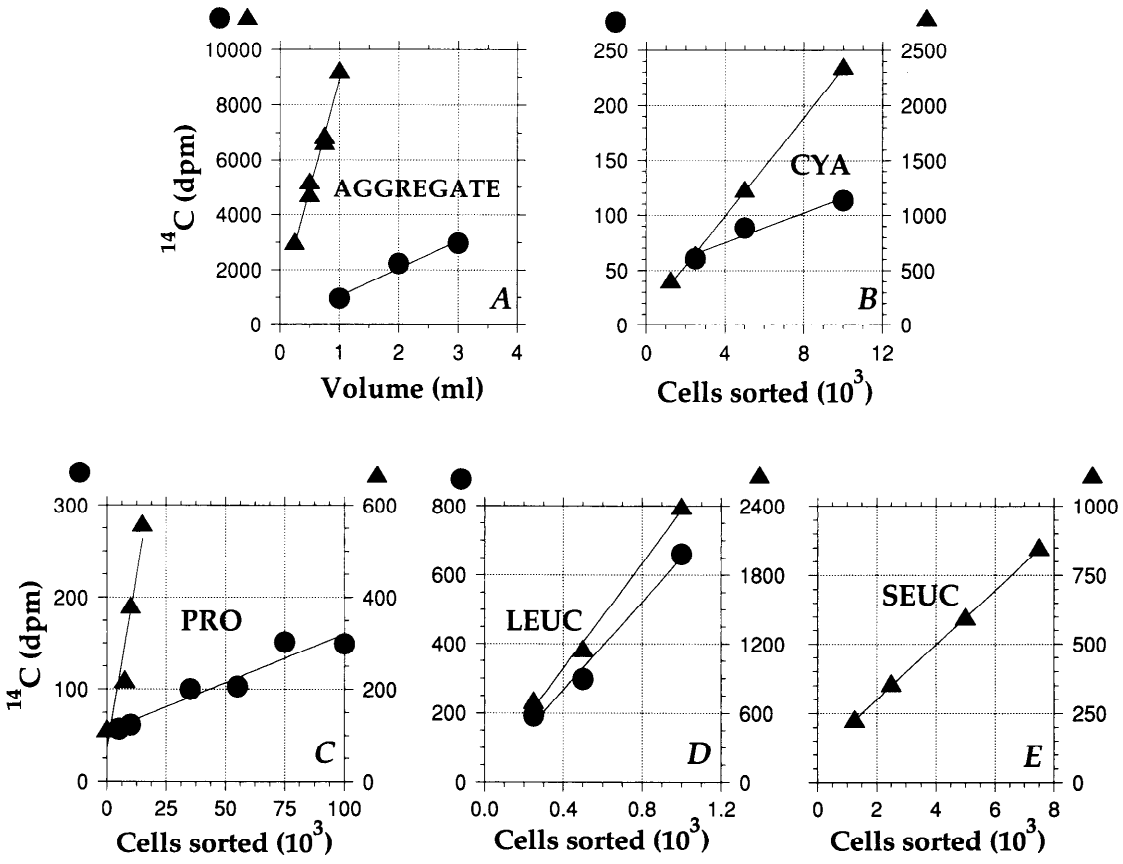

Fig. 2. Uptake of ^{14}C by (A) the aggregate plankton and by (B) cyanobacteria, (C) prochlorophytes, (D) large eucaryotic ultraphytoplankton, and (E) small eucaryotic ultraphytoplankton. The sample collected at station 45 (▲) received ^{14}C at 50 μCi ml^{-1}; the sample collected at station 20 (●) received ^{14}C at 100 μCi ml^{-1}.

Table 1. Primary production of three North Atlantic ultraphytoplankton samples.

Sta.	Location	z (m)	$\%I_0$*	Chl (μg liter^{-1})	Group†	n_i (cells liter^{-1})	p_i (fg C cell^{-1} h^{-1})	P_i‡	% agg
14	32°39.5′N, 26°39.2′W	60	4	0.21	PRO	1.62×10^8	0.27	44.2	57
					CYA	2.35×10^6	0.82	1.9	2
					LEUC	1.02×10^6	24.09	24.5	32
					sum			70.6	91
					agg			77.4	
20	29°00.1′N, 19°16.3′W	60	2	0.15	PRO	1.57×10^8	0.03	4.6	16
					CYA	3.34×10^6	0.19	0.6	2
					LEUC	1.24×10^6	17.93	22.3	79
					sum			27.5	97
					agg			28.3	
30	31°13.9′N, 10°48.0′W	1	91	0.37	PRO	6.63×10^7	0.81	53.6	11
					CYA	1.27×10^7	7.68	97.8	20
					SEUC	8.49×10^6	7.32	62.1	13
					LEUC	1.25×10^6	193.16	241.1	49
					sum			454.5	92
					agg			493.4	

* Irradiance as percent of intensity at sea surface.

† PRO—prochlorophytes; CYA—cyanobacteria; SEUC—small eucaryotic ultraphytoplankton; LEUC—large eucaryotic ultraphytoplankton; sum—PRO+CYA+SEUC+LEUC; agg—aggregate from filtration of bulk sample ≤3 ml.

‡ $P = (n \cdot p)10^{-6}$, ng C liter^{-1} h^{-1}.

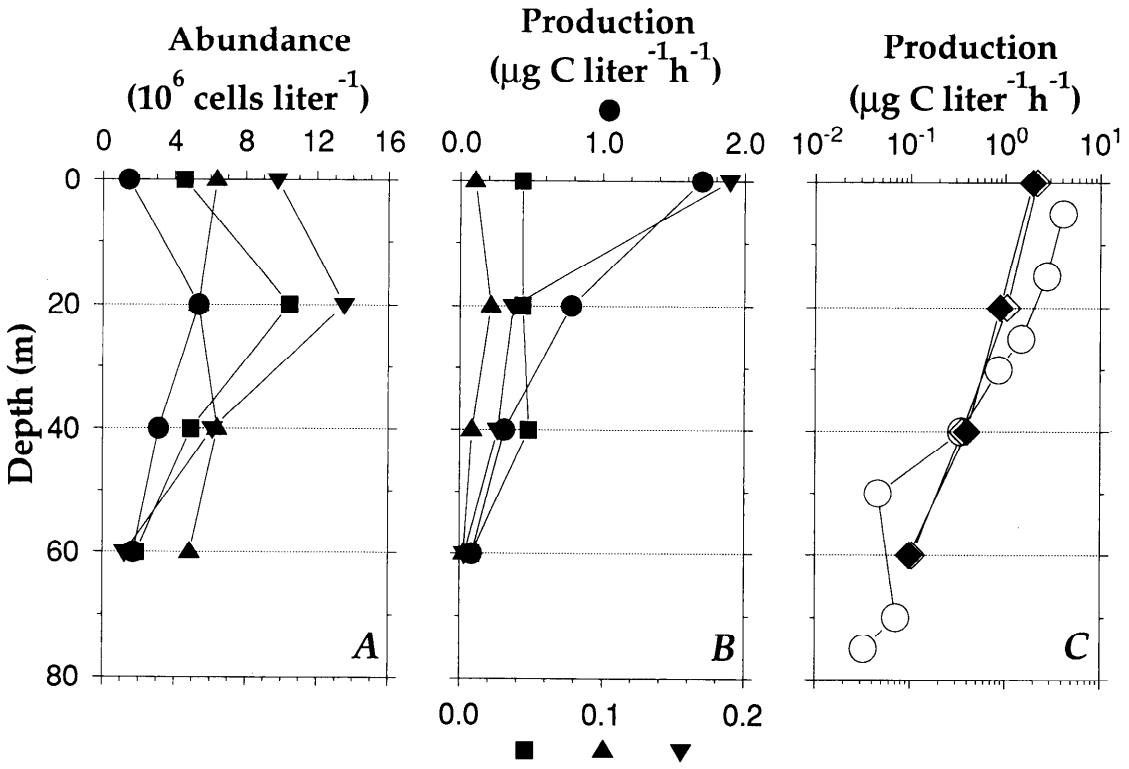

Fig. 3. Vertical distribution of (A) ultraphytoplankton abundance, (B) group-specific primary production, and (C) summed, aggregate, and total primary production near Morocco. The profiles represent composites constructed from measurements made on four successive days in 1992 at 31°14.0′N, 10°47.4′W (4 October, 40 m), 31°14.0′N, 10°51.1′W (5 October, 0 m), 31°13.8′N, 10°59.1′W (6 October, 60 m), and 31°8.8′N, 11°6.0′W (7 October, 20 m). In panel B, the top labels refer to LEUC (●) and the bottom labels to PRO (▲), CYA (■), and SEUC (▼). In panel C, sum (◆)—PRO + CYA + LEUC + SEUC; aggregate (◇)—filtration of bulk sample ≤3 ml (cf. Fig. 2A); total (○)—filtration of bulk 500-ml sample (data of W. G. Harrison). The average value of sum : aggregate over the four sampled depths was 95%.

1) is that the contribution to aggregate production from particular groups is not necessarily in proportion solely to numerical abundance nor solely to cell-specific rate of ^{14}C uptake. Thus, at stations 14 and 20, where samples were collected near the base of the euphotic zone, values of n_i were very similar between the stations; however, both the aggregate primary production and its partitioning among the cell groups were different. As a percentage of aggregate production, the major primary producers were PRO at station 14 (57%) and LEUC at station 20 (79%). In both these shade communities, the contributions to primary production from CYA was very small (2%). In contrast, at station 30 where the sample was collected near the sea surface and the aggregate primary production was much higher, the distribution among PRO (11%), CYA (20%), and SEUC (13%) was more equitable. In this sun community, LEUC were the major producers (49%).

Additional results were obtained at an upwelling station off the Moroccan coast where all four ultraphytoplankton groups were present (Fig. 3A). Again, measurements of aggregate production (Fig. 3C) were similar to values summed from the contributions of the four groups (Fig. 3B). As a percentage of aggregate primary production, the depth-averaged contribution from CYA was 7%, from PRO was 2%, from SEUC was 6%, and from LEUC was 80%. At this station, data are available (courtesy of W. G. Harrison) for primary production estimated from simulated in situ incubations and subsequent bulk filtrations of much larger volume seawater samples (500 ml). Results from these large-volume incubations (which are here called "total" primary production) agree with aggregate production only at depths

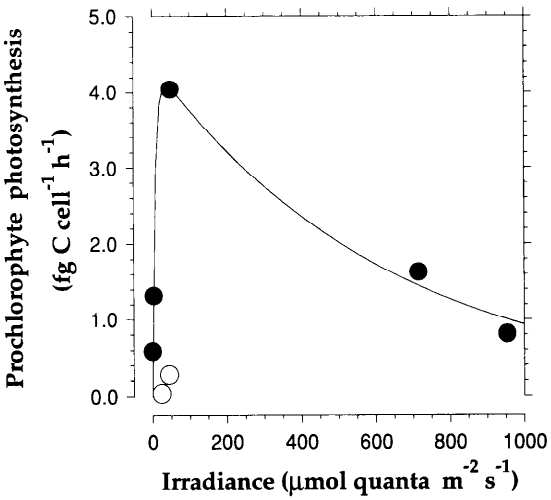

Fig. 4. Prochlorophyte cellular photosynthesis (fg C cell^{-1} h^{-1}) as a function of average irradiance experienced over 8 h in simulated in situ incubations. Moroccan upwelling station (●) and deep (60 m) open-ocean stations (○).

≥40 m (Fig. 3C). The discrepancy between total and aggregate production at shallow depths might indicate the role of primary producers (those less abundant and of larger size) not taken into account by flow cytometric analysis.

In recent years, the abundance and distribution of oceanic ultraphytoplankton have been studied in detail. However, much less is known about their cell-specific rates of ^{14}C uptake (p_i). It is certainly not surprising that the present measurements indicate $p_{PRO} < p_{CYA} \approx p_{SEUC} < p_{LEUC}$. However, the contribution of each group to aggregate production must be weighted by abundance (n_i) and it is then not so obvious how the groups rank in importance until calculations are made of $P_i = n_i \cdot p_i$. From the small number of experiments reported here, it appears that among the ultraphytoplankters, the eucaryotes are generally the dominant primary producers—but exceptions occur (e.g. prochlorophytes at Sta. 14, Table 1). In other words, eucaryotic ultraphytoplankton are important because of their high cell-specific rate of ^{14}C uptake and in spite of their relatively low abundance. A similar conclusion has also been stated regarding the general importance of eucaryotic ultraphytoplankton on the basis of their contribution to carbon biomass inferred from flow cytometric measurements of Coulter volume (Li et al. 1992, 1993*a*), light scatter (Li et al. 1993*b*), and image cytometric measurements of cell dimensions (Furuya 1990). However, exceptions are also to be expected. In the North Atlantic, Li et al. (1993*a*) recorded a change in ultraphytoplankton biomass dominance from eucaryotes to cyanobacteria coincident with a change in water mass. In the central Pacific, Campbell et al. (1994) reported that prochlorophytes appear to comprise two-thirds of the total photosynthetic biomass.

The present measurements of cell-specific rates of primary production compare favorably to results obtained by others. For cyanobacteria (*Synechococcus*), estimates by microautoradiography range from 3 to 12 fg C cell^{-1} h^{-1} in the northwest Atlantic (Iturriaga and Marra 1988) and from <0.5 (light-limited rate) to 6 fg C cell^{-1} h^{-1} (light-saturated rate) in Lakes Huron and Michigan (Fahnenstiel et al. 1991). The present estimates for cyanobacteria are 0.2–0.8 fg C cell^{-1} h^{-1} for cells at the base of the euphotic zone in the open Atlantic (Table 1) and 4–10 fg C cell^{-1} h^{-1} for cells in the Moroccan upwelling (Fig. 3, Table 1). For prochlorophytes in the Sargasso Sea, Chisholm et al. (1988) reported doubling times ranging from 2 to 10 d based on flow cytometric sorting of ^{14}C-labeled cells. These doubling times translate to values of 0.3–1.5 fg C cell^{-1} h^{-1}. The present estimates for prochlorophytes are low (0.03–0.3 fg C cell^{-1} h^{-1}) for cells at the base of the euphotic zone in the open Atlantic (Table 1) but higher (0.6–4.0 fg C cell^{-1} h^{-1}) for cells in the Moroccan upwelling (Fig. 4). With the average irradiance experienced by cells incubated over 8 h, a photosynthesis-irradiance curve was constructed for prochlorophytes at the upwelling station (Fig. 4). The number of data points is too few to justify a mathematical parameterization of the curve. Nevertheless, it is evident that the flow-sorting experiments yield results very close to those from laboratory experiments on cultured clones of *Prochlorococcus* spp. (Partensky et al. 1993) showing saturation at rather low irradiances and significant photoinhibition at modest to high irradiances. The maximum cellular rate of photosynthesis recorded here (4 fg C cell^{-1} h^{-1}) is very similar to those reported by Partensky et al. (1993) for *Prochlorococcus* clones cultured in the laboratory (3–6 fg C cell^{-1} h^{-1}).

In conclusion, the successful partitioning of ultraphytoplankton primary production among prochlorophytes, cyanobacteria, and eucaryotic algae demonstrates the utility of shipboard flow sorting in the ecological study of these cell groups. It is a significant step toward an important goal in biological oceanography: namely, the recovery of the bulk properties of phytoplankton from the details of the properties of the constituents.

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