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Has the importance of photoautotrophic picoplankton been overestimated?

Abstract—Postincubation differential filtration (PIDF), preincubation differential filtration (PreIDF), and track autoradiography (TA) were compared for estimating cell-specific and total photoautotrophic picoplankton production. Experiments were performed in Lakes Michigan and Huron and in the Gulf of Mexico. When *Synechococcus* dominated the photoautotrophic picoplankton community (>70% of total picoplankton abundance), PIDF estimates of cell-specific and total picoplankton production were ~3.0× (range, 2.0–3.8×) higher than TA estimates. PreIDF estimates of cell-specific and total picoplankton production, however, were only slightly higher than TA estimates (mean, 1.4×; range, 1.4–1.5×). The higher PIDF estimates were attributable to breakage and damage of larger photoautotrophs during postincubation filtration and to retention of this labeled material on the smaller (0.2 μm) pore-size filter. Results from PIDF experiments must be viewed with caution and previous estimates of picoplankton production, cell-specific or total, based solely on PIDF may need to be re-evaluated.

Research during the past decade in a wide variety of environments (*see* Stockner and Antia 1986) has demonstrated the importance of photoautotrophic picoplankton as significant contributors to primary production and photoautotrophic biomass. Photoautotrophic picoplankton can contribute as much as 90% to total primary production (Li et al. 1983; Iturriaga and Marra 1988). Many of these estimates of picoplankton production—particularly the very high estimates—have been made with postincubation differential filtration (PIDF) experiments (Glover et al. 1986; Iturriaga and Mitchell 1986; Hagstrom et al. 1988; Iturriaga and Marra 1988). Likewise, much of the rate process information for *Synechococcus* and other picoplankton-sized organisms has been made with PIDF experiments (Iturriaga and Mitchell 1986; Prézelin et al. 1986). Preincubation differential filtration (PreIDF) has been used to estimate picoplankton production (Waterbury et al. 1986), but because this technique may introduce possible artifacts it has been viewed with caution (Furnas 1987).

The accuracy of PIDF for estimating picoplankton production has been questioned. Waterbury et al. (1986) noted that the percent of primary production attributed to picoplankton often exceeded 100% and could be as much as 175% of total primary production if the PIDF protocol were used. They attributed these biased estimates of picoplankton production to disruption of eucaryotic phytoplankton during filtration and to retention of cellular fragments on the small pore-size filters. Also, Iturriaga and Marra (1988) noted that ^{14}C-based growth rates from PIDF experiments were about twofold higher than estimates from track autoradiography (TA).

Although these studies questioned the accuracy of PIDF procedures for estimating picoplankton production, they provided only a cursory evaluation of the PIDF technique. In this note, we build on the work of Waterbury et al. (1986) and Iturriaga and Marra (1988)

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to assess the usefulness of PIDF techniques for estimating cell-specific picoplankton rates and total picoplankton production. We evaluate the importance of photoautotrophic picoplankton only in terms of their contribution to primary productivity and do not consider the transfer of carbon within the food web or other ecologically important processes.

Samples were collected at two offshore stations in Lake Huron (southern, 43°56′N, 82°21′W; northern, 45°25′N, 82°55′W) and Lake Michigan (43°1′N, 86°37′W) and at two stations in the northern Gulf of Mexico (28°27′N, 91°57′W; 28°14′N, 98°49′W). In the Great Lakes, water samples were collected with clean 5- or 10-liter PVC Niskin bottles; Gulf of Mexico samples were collected with clean 30-liter Go-Flo bottles. All water samples were collected from the surface mixed layer, except those taken on 15 March from the deep chlorophyll layer (50 m) in the Gulf of Mexico. Water-column temperature profiles were measured with an electronic bathythermograph or Sea-Bird CTD. Underwater scalar irradiation was measured with a LiCor LI-193SB sensor and LI-188B integrating meter. Chlorophyll concentrations were determined fluorometrically from 90% acetone-extracted samples.

Water samples (raw and fractionated) for microscopic analysis were transferred into 250-ml amber bottles and preserved with glutaraldehyde (1% final concn) buffered with sodium cacodylate (0.1 M final concn). Picoplankton samples were then cooled (~5°C) until duplicate slides were prepared within 24 h (Fahnenstiel and Carrick 1992) and then frozen (−20°C). Slides were counted within 24 h to minimize error due to autofluorescent fading. Photoautotrophic picoplankton biomass and composition on each slide were estimated by enumerating a minimum of 500 units (<5% counting error assuming Poisson statistics). A Leitz Laborlux microscope (1,250×) was equipped to distinguish the dominant bright autofluorescent emission of an individual cell (Fahnenstiel and Carrick 1992). This protocol provides quantitative enumeration of the total photoautotrophic picoplankton community in the Great Lakes as demonstrated by comparisons between flow cytometry and epifluorescent microscopy (Fahnenstiel et al. 1991). If any photoautotrophic picoplankton were missed by our sample preparation and counting protocol, they were relatively rare and were not a dominant component (>20%) of the photoautotrophic picoplankton community.

Samples for PIDF experiments were placed in clean 1–2-liter polycarbonate bottles, inoculated with $H^{14}CO_3^-$ (100–250 μCi liter^{-1}), and incubated for 1–2 h. Trace-metal clean techniques (Fitzwater et al. 1982) were used for work in the Gulf of Mexico, while more traditional ("less clean") procedures (Fahnenstiel and Scavia 1987) were used in the Great Lakes. All incubations were performed in shipboard incubators that simulated conditions in the surface-mixed layer (12–25% of surface irradiance) or, on one occasion, conditions in the deep chlorophyll layer (6% of surface irradiance).

Following incubations in PIDF experiments, separate subsamples from the bottles were postfractionated through 1- and 3-μm Nuclepore membranes (47 mm). A portion of unfractionated water was retained to serve as an estimate of total primary production. Very low vacuum pressure was used for all filtrations (<30 mm of Hg). Samples from all pre- and postincubation filtrations also were preserved with glutaraldehyde for enumeration of picoplankton as outlined above. Three subsamples from the <1- and 3-μm filtrations were refiltered (0.22-μm Millipore), decontaminated with 0.5 ml of 0.5 N HCl for 4–6 h, placed in vials with 12 ml of scintillation cocktail, and assayed with a Packard Tri-Carb scintillation counter. Dark and time-zero controls were taken with each experiment; the activities associated with these controls were ~40–100 dpm, which represented <5% of the activity in the <1-μm fraction. Time-zero controls were subtracted from all samples; dark bottle activities were not subtracted. Counting efficiencies were determined by external standards.

Our PIDF protocol involved a combination of parallel filtrations (3 and 1 μm) and then a subsequent serial filtration (0.22 μm). Although this distinction is important for methodological considerations, it is a minor consideration for the general application of our results (*see below*). Total available C was determined from alkalinity, temperature, and pH measurements for both the fractionation and autoradiography estimates. On three occasions, PreIDF experiments were also performed. The experimental protocol was sim-

Table 1. Characteristics of photoautotrophic picoplankton communities from Lake Michigan, Lake Huron, and the Gulf of Mexico.

	Depth (m)	Total pico.*	Single cyano.†	Picoplankton <1 μm‡	Picoplankton <3 μm§
Lake Michigan					
11 May 1988	5	22,254	30	23	70
14 Jun 1988	5	24,076	41	14	45
30 Jul 1991	5	32,511	81	47	82
Lake Huron					
26 Jul 1988	5	19,295	74	36	64
13 Oct 1988	5	56,966	87	37	77
Gulf of Mexico					
9 Mar 1991	10	38,675	94	60	97
15 Mar 1991	50	62,425	95	61	92

* Total photoautotrophic picoplankton abundance (cells ml^{-1}).
† Percent of total picoplankton that were single chroococcoid cyanobacteria.
‡ Percent of total picoplankton that passed a 1-μm Nuclepore filter.
§ Percent of total picoplankton that passed a 3-μm Nuclepore filter.

ilar to that of PIDF except that fractionation (filtration through a 1-μm Nuclepore filter) was done before incubation.

Autoradiographs were prepared by preserving raw water and <1-μm fraction samples with 1% glutaraldehyde buffered with 0.1 M sodium cacodylate. Samples of 10–40 ml were concentrated onto 0.2-μm Nuclepore membranes and these organisms were transferred onto subbed coverslips. These coverslips were mounted onto slides and then dipped and developed for track autoradiography (Knoechel and Kalff 1976). Cells on coverslips were counted, noting the number of tracks, size, shape, and pigment composition of each cell. To determine the loss associated with preservation, we performed time-series filtration with the <1-μm fraction. In all cases, loss associated with preservation was <5%.

Picoplankton production estimates were made as follows. From PIDF and PreIDF experiments, production in the 0.22–1-μm fraction was assigned to cells enumerated in that fraction with epifluorescent microscopy for a cellular production estimate (fg $cell^{-1}$ h^{-1}). Total picoplankton production rate (μg $liter^{-1}$ h^{-1}) was determined by normalizing production in this fraction to total picoplankton abundance. In PIDF experiments, all phototrophs fix labeled C, and separation into the picoplankton fraction occurs at the end of the experiment. However, in the PreIDF protocol, picoplankton (<1 μm) are separated before inoculation with ^{14}C and labeled C is fixed only by cells in the 0.22–1.0-μm fraction. Both PIDF and PreIDF estimates are also estimates of differential filtration (DF). From PIDF-incubated samples, TA was performed on samples for a direct estimate of cellular production (fg $cell^{-1}$ h^{-1}). Because TA estimates of cell-specific production were similar for both the <1-μm and raw samples (t-test, $P > 0.1$), we used the TA estimate from the <1-μm fraction for all comparisons. This production estimate was then multiplied by picoplankton abundance to determine total picoplankton production. For the TA estimate, individual picoplankton cells were identified and the activity associated with each cell was counted.

Photoautotrophic picoplankton communities from Lakes Huron and Michigan and from the Gulf of Mexico have several similarities that facilitated comparisons between TA and DF. First, colonial or aggregated picoplankton as described by Fahnenstiel and Carrick (1992) were not abundant (<8% of total abundance) in any sample. Colonial or aggregate picoplankton can confound comparisons between DF and TA because aggregates are not fractionated precisely. Second, with two exceptions (11 May and 14 June 1988) single chroococcoid cyanobacteria dominated the photoautotrophic picoplankton communities (>70% of total abundance, Table 1). Single chroococcoid cyanobacteria are relatively robust and easily enumerated with epifluorescent microscopy-track autoradiography. TA procedures did not affect our ability to count chroococcoid cyanobacteria; epifluorescent counts before and after autoradiography were not significantly different ($P > 0.05$). Third, the <1-μm size fraction was almost exclusively picoplankton (>95%), as only a few larger cells (nanoplankton) passed intact through the 1-μm filter. Thus, the passage of fixed labeled C by larger intact phytoplankton will not confuse the interpretation of our experiments. We also performed 3-μm fractionations, but the <3-μm fraction usually had more contamination by larger nanoflagellates. Therefore, most of our discussion will be limited to comparisons with the <1-μm size class.

For cells adequately enumerated with epifluorescent microscopy, such as *Synechococcus*, TA must be considered the standard for determining cellular production rates. TA pro-

Table 2. Cellular picoplankton production rates (fg cell^{-1} h^{-1}) determined by TA, PIDF, and PreIDF. Error estimates are 95% confidence intervals. Production rates from TA were determined by counting the disintegrations associated with each cell; rates from PIDF and PreIDF were determined by assigning the production in the <1-μm size class to the number of intact cells in that size fraction.

	Production				
	TA	PIDF	PreIDF	PIDF:TA	PreIDF:TA
11 May 1988	9.6±2.0	84.6±9.0		8.8±2.1	
14 Jun 1988	5.3±1.3	21.4±1.5		4.0±1.0	
26 Jul 1991	3.1±0.6	14.5±1.4		3.5±0.8	
30 Jul 1988	5.5±1.3	21.2±2.2	7.6±0.8	3.8±1.0	1.4±0.4
13 Oct 1988	5.3±1.0	11.9±1.1		2.0±0.4	
9 Mar 1991	10.4±2.0	34.2±4.8	14.1±1.5	3.3±0.7	1.4±0.3
15 Mar 1991	7.1±1.4	19.8±1.3	10.7±0.8	2.8±0.6	1.5±0.3

vides a direct estimate of cell-specific production by counting the activity of each individual cell. These estimates have been theoretically and empirically demonstrated to be accurate (Knoechel and Kalff 1976; Carney and Fahnenstiel 1987). Moreover, we estimated cellular production rates of a cultured isolate of *Synechococcus* from Lake Huron in exponential-phase growth with liquid scintillation counting and TA and found no significant difference between techniques (*t*-test, $P > 0.05$; mean ±95% C.I.: liquid scintillation = $1.2 \pm 0.2 \times 10^{-3}$ dpm cell^{-1}; track autoradiography = $1.1 \pm 0.3 \times 10^{-3}$ dpm cell^{-1}).

For all experiments in the Gulf of Mexico and Great Lakes, DF (1-μm fractionation) cellular production rates were higher than TA estimates (Table 2). On average, PIDF estimates were 4.0× (range, 2–8.8) higher than TA estimates, whereas PreIDF estimates were 1.4× (range, 1.4–1.5) TA estimates. The differences between PIDF and TA were largest when red-fluorescing cells were abundant. Because red-fluorescing picoplankton can be difficult to count with epifluorescent microscopy (Chisholm et al. 1988), particularly when combined with TA (G. Fahnenstiel unpubl. data), the estimates of cellular production from these samples must be viewed with caution. If we exclude the 11 May and 14 June experiments from our comparisons, the PIDF estimates were on average 3× the TA estimates. It should be noted that if DF estimates of picoplankton production were calculated for the <3-μm fraction rather than the <1-μm fraction, differences between TA and PIDF estimates would be much greater, as estimates of <3-μm production were ~50% higher than <1-μm estimates (Table 3).

These differences between TA and PIDF estimates suggest that PIDF can provide systematically high estimates of cellular production for photoautotrophic picoplankton. For example, in the Gulf of Mexico where *Synechococcus* constituted ≥94% of total photoautotrophic picoplankton abundance, estimates of PIDF cellular production for *Synechococcus* were 2.8–3.3× those determined by TA (Table 2). Because most estimates of cellular production for *Synechococcus* were based on a similar PIDF experimental protocol with similar assumptions concerning the dominance of *Synechococcus,* we should view these previous estimates with caution. Regardless of the source of the differences between TA and DF estimates, it is clear that PIDF experiments can produce systematically high estimates of cell-specific production.

PreIDF estimates were only slightly higher (1.4–1.5×) than TA estimates and more similar to TA estimates than PIDF estimates (Table 2). These results are somewhat surprising

Table 3. Comparison of PIDF estimates of cell-specific production (fg cell^{-1} h^{-1}) for <1- and <3-μm size fractions. In both cases, PIDF estimates were determined by assigning production in the specific size class to the number of intact cells in that same size class. Error estimates are 95% confidence intervals.

	Picoplankton production	
	<1-μm fraction	<3-μm fraction
11 May 1988	84.6±9.0	73.0±5.5
14 Jun 1988	21.4±1.5	45.2±3.7
26 Jul 1991	14.5±1.4	21.1±2.1
30 Jul 1988	21.2±2.2	31.1±2.5
13 Oct 1988	11.9±1.1	21.4±1.8
9 Mar 1991	34.2±4.8	52.8±5.5
15 Mar 1991	19.8±1.3	21.8±1.7

Table 4. Picoplankton production (μg liter^{-1} h^{-1}) from TA, PIDF, and PreIDF experiments. Error estimates are 95% confidence intervals. The TA estimate was determined by multiplying estimates of cellular production and total picoplankton abundance. The PIDF and PreIDF estimates were determined by normalizing production in the <1-μm size fraction to total picoplankton abundance.

	Production		
	TA	PIDF	PreIDF
11 May 1988	0.21±0.05	1.88±0.24	
14 Jun 1988	0.13±0.03	0.51±0.05	
26 Jul 1991	0.08±0.02	0.28±0.03	
30 Jul 1988	0.18±0.04	0.69±0.09	0.25±0.03
13 Oct 1988	0.34±0.07	0.68±0.06	
9 Mar 1991	0.40±0.08	1.15±0.15	0.55±0.07
15 Mar 1991	0.44±0.09	1.08±0.08	0.67±0.05

given the results of previous work on preincubation filtration and the potential artifacts associated with these techniques (Furnas 1987). Our work and that of Waterbury et al. (1986) suggest that preincubation filtration may be less undesirable than previously thought, and in some cases, may be more desirable than postincubation filtration. Because our picoplankton communities were dominated by *Synechococcus,* estimates of total production are comparable to cell-specific estimates, i.e. PreIDF estimates are only slightly higher than TA estimates (1.4–1.5×), but PIDF estimates were much higher than TA estimates (2.0–9.0×, Table 4).

The most likely source(s) of the differences between DF and TA estimates is (are) production by cells not enumerated with epifluorescent microscopy (missed cells) and production by larger cells (nonpicoplankton) that are damaged or broken during filtration but are retained on the smallest filter (0.2 μm). Other sources, such as filter-retainable extracellular material (Carney and Fahnenstiel 1987), passage of larger intact cells, and uptake of labeled DOC released from photoautotrophs may contribute to the differences among estimates, but it is unlikely that these sources could explain the 2–3-fold differences.

If the differences between DF (PIDF and PreIDF) and TA are due to production by missed picoplankton, then DF estimates are better for estimating total picoplankton production. TA estimates would still be useful as cell-specific estimates of readily identified cells, but would underestimate total picoplankton production. If, however, the differences between estimates were due to retention of fixed material from larger (nonpicoplankton) cells that passed intact or were damaged or broken during the filtration process, then DF estimates may be biased.

Provided no artifacts or trauma are associated with preincubation filtration, comparisons of PIDF and PreIDF are particularly insightful, because both techniques measure production of cells in the same specific size fraction (0.22–1.0 μm). To evaluate the potential for artifacts with PreIDF experiments, we compared rates of *Synechococcus* cellular production from pre- and postfiltered samples (9 and 15 March) with TA. Prefiltration rates were slightly higher (13–17%) than postfiltration rates, but these differences were not significant (t-test, $P > 0.05$; Pre = 12.2 and 8.0 fg cell^{-1} h^{-1}, respectively; Post = 10.4 and 7.1 fg cell^{-1} h^{-1}, respectively).

Although these comparisons were limited to *Synechococcus,* they suggest that preincubation did not produce artifacts or trauma in our experiments and that comparisons of PIDF and PreIDF experiments are robust. Therefore, if the differences between DF and TA estimates were due to missed picoplankton or the passage of larger, intact phytoplankton, then PIDF and PreIDF estimates should be similar, but distinctly different from TA estimates. This was not the case however, as PreIDF estimates were more similar to TA estimates than to PIDF estimates. The relatively good agreement between PreIDF and TA estimates suggests that material from damaged or broken nonpicoplankton was contributing to higher PIDF estimates. In PreIDF experiments, uptake of labeled C is limited to cells that passed a 1-μm filter, whereas in PIDF experiments, all photoautotrophs fix labeled C. Fractionation at the end of the experiment (PIDF) separates picoplankton from larger phytoplankton and thus, a large pool of fixed labeled C is available to bias estimates of picoplankton production. If larger nonpicoplankton cells are damaged during postfiltration, and fragments and other cellular material pass through the 1-μm filter and are retained on the 0.22-μm filter, then PIDF estimates would be higher than both TA and PreIDF estimates. This appears to be the case.

Previous work in a variety of environments also supports our premise that the PIDF technique overestimates picoplankton production due to retention of cellular material from broken or damaged larger cells during the postincubation filtration procedure. During routine filtrations, several investigators have noted artifacts associated with the release of dissolved and particulate material from phytoplankton (Fuhrman and Bell 1985; Goldman and Dennett 1986). The integrity of many cells, particularly fragile nanoflagellates, is adversely affected during routine filtration, and some cells are completely destroyed (Bloem and Bär-Gilissen 1988; Lignell 1992). These problems seem to be most severe with the use of polycarbonate filters like the ones used in this and many other PIDF experiments (Fuhrman and Bell 1985; Goldman and Dennett 1986; Lignell 1992).

We cannot entirely dismiss the possibility that other factors (release and uptake of labeled DOC, filter-retainable extracellular activity, etc.) are responsible for some of the differences between DF and TA estimates, but information on the composition and contribution of picoplankton and phytoplankton communities in the Great Lakes strongly supports our premise of production bias due to damage and breakage of larger nonpicoplankton cells during filtration. For the Great Lakes experiments, the contribution of picoplankton to primary production was relatively small (range, 3–12%; mean, 7%), whereas the contribution of photoautotrophic nanoflagellates (PN) and photoautotrophic protozoans (PP) was relatively large (PN, 24–65%; PP, 40–90%; Carrick 1990). Thus, only a small percentage of photoautotrophic nanoflagellate and protozoan production would need to be released during filtration to significantly bias our estimates of picoplankton production. Moreover, the most probable alternative explanation (missed picoplankton) is not supported by available information. As noted earlier, it is unlikely that a large component of the picoplankton community in the Great Lakes would be missed with routine epifluorescent microscopy. Prochlorophytes do not appear to be abundant and epifluorescent microscopy is adequate for enumerating the entire photoautotrophic picoplankton community (Fahnenstiel et al. 1991). Although we do not have information on the abundance of prochlorophytes and other red-fluorescing cells in the Gulf of Mexico, it is unlikely that we would have completely missed a large population of red-fluorescing cells from the deep chlorophyll layer where pigment fluorescence is greater and where prochlorophytes have been detected with routine epifluorescent microscopy (Chisholm et al. 1988).

Our results and those of Waterbury et al. (1986) and Iturriaga and Marra (1988) clearly question the accuracy of PIDF techniques for estimating photoautotrophic picoplankton production and therefore our current paradigms on the importance of photoautotrophic picoplankton. The broad application of our results to other studies may be limited due to differences in experimental protocol and to the structure and composition of phytoplankton and picoplankton communities. Filter type and pore size may influence results as much as the abundance of delicate photoautotrophs. Although it is clear that differences in experimental protocol may limit the application of our results, one protocol difference that will have limited effect is the use of serial vs. parallel filtration. Our differential filtrations were done in series (first 1 μm and then 0.2 μm); other investigators have used parallel filtrations (Glover et al. 1986; Iturriaga and Mitchell 1986). Regardless of the type of filtration, all PIDF procedures will overestimate picoplankton production as long as the filter used to separate picoplankton (1 μm in our experiments) from larger photoautotrophs allows some production or activity from larger nonpicoplankton cells to pass, and the smallest filter (0.22 μm) retains all photoautotrophic production or activity.

Future work is needed in environments where other photoautotrophic picoplankton are abundant and where different experimental protocols have been used. In many respects, the conditions in our study (i.e. dominance of the picoplankton community by *Synechococcus*, large contribution of small photoautotrophic nanoflagellates, etc.) were ideal for comparisons of TA and DF procedures; however, in other environments these comparisons may be more difficult and interpretations more equivocal. Until these evaluations are com-

plete, investigators must take precautions to ensure the accuracy of PIDF experiments.

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