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Photosynthetically produced dissolved organic carbon: An important carbon source for planktonic bacteria[1]

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Abstract

A serial filtration technique was used to estimate microbial assimilation of photosynthetically produced dissolved organic carbon (PDOC) in Mirror Lake, New Hampshire. A single filtration at one pore size did not separate all heterotrophic microorganisms from photosynthetic algae. It was possible, however, to calculate microbial assimilation of PDOC if filters of several pore sizes were used simultaneously in combination with an independent estimate of heterotrophic activity in each size class.

Microbial net assimilation of algal PDOC in the epilimnion in summer (about 2 μg C·liter^{-1}·d^{-1}) was of a magnitude comparable to that of total bacterial production (4–8 μg C·liter^{-1}·d^{-1}) as estimated by the $^{35}SO_4$ technique. This comparison suggests that PDOC is an important source of carbon for planktonic microbes.

The amount of PDOC measured in the water is smaller than the amount actually released by phytoplankton because microbes metabolize PDOC rapidly. Failure to account for microbial utilization could lead to a serious underestimate of gross PDOC during measurements of primary production.

When algal cells grow and die some portion of their photosynthate is released as dissolved or colloidal material. This release may be due to autolysis (Golterman 1964), lysis by intra- or extracellular parasites (Mankaeva 1966; Daft and Stewart 1973; Gunnison and Alexander 1975; Burnham et al. 1976), mechanical breakage by zooplankton (Pourriot 1963) or to active excretion of metabolites. Fogg (1966) used the term extracellular production to denote only the release from healthy, growing cells. However, in natural waters all of the above processes occur simultaneously and an operational distinction between production by healthy cells and other modes of loss is not feasible. We use the term "photosynthetically produced dissolved organic carbon" (PDOC) to include all releases from algae (Wiebe and Smith 1977). Whatever its origin, PDOC supports some of the metabolism of planktonic bacteria (Waite and Duthie 1975; Wright and Shah 1975; Nalewajko and Schindler 1976; Smith et al. 1977). In fact, Bell and Mitchell (1972) proposed the term "phycosphere" for the area surrounding a lysed or leaky algal cell, in analogy to the rhizosphere (the area of the soil surrounding plant roots) where microbial activity is especially high. Because PDOC is metabolized by bacteria at the same time that it is being produced by algae, rates of both production and utilization have been difficult to measure (Nalewajko et al. 1976).

One way to evaluate the importance of PDOC would be to measure the total production of planktonic bacteria and compare this to bacterial net assimilation of PDOC. This measurement of total bacterial production has been made by several independent techniques in Mirror Lake, a small oligotrophic lake in New Hampshire (Jordan and Likens 1980). We report here our estimate of bacterial utilization of PDOC in the same lake. Our method is an extension of the differential filtration technique of Derenbach and Williams (1974); we followed the flux of added $H^{14}CO_3$ into algae, into a [^{14}C]PDOC pool, and then into bacteria.

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Methods

We added $H^{14}CO_3$ to lake water in clear and opaque glass bottles, incubated these in situ and then measured the distribution of ^{14}C in particles of different sizes. Heterotrophs were separated from autotrophs by serial filtration through several filters of different pore sizes and heterotrophic activity in each size class was calculated.

Heterotrophic activity—Two organic substrates, ([^{14}C]glucose and a soluble extract from ^{14}C-labeled populations of natural phytoplankton) were used to determine the activity distribution of planktonic heterotrophs in different size classes. These substrates were added to lake water in bottles which were incubated in situ. We then measured the incorporation of ^{14}C into particles of different size and the conversion of organic ^{14}C to $^{14}CO_2$ (*see below*).

Glucose, uniformly labeled with ^{14}C (Amersham; 230 $\mu Ci \cdot \mu mol^{-1}$) was diluted with sterile, deionized water. The diluted solution was sealed in glass ampoules and autoclaved (90°C, 10 min, twice). The ampoules were stored in the dark at 2°C.

Soluble plankton material (SPM) was prepared by extracting the water-soluble components from Mirror Lake phytoplankton. Lake water (plus about 100 μCi of $NaH^{14}CO_3$) was incubated in situ in 2.5-liter clear, glass bottles for 60 h. Growth then continued for an additional 12 h under artificial light. The labeled particulate material from 7.5 liters of this water was harvested by filtration through precombusted Whatman GF/C filters. The filters were fumed with dilute HCl to remove adherent inorganic C, then dried at 50°C, ground with a mortar and pestle, and extracted three times in warm (50°C) sterile water. The extract was centrifuged and the supernatant filtered, first through precleaned 0.45-μm pore-size Millipore filters and then through washed 0.1-μm pore-size Nuclepore filters. The filtered extract was sealed in glass ampoules, autoclaved (90°C, 10 min, twice) and then stored in the dark at 2°C.

Aliquots of SPM were added to lake water in clear 300-ml BOD bottles and incubated in situ (30,400 $dpm \cdot 300\ ml^{-1}$, equivalent to a final concentration of 0.5 $mg\ C \cdot liter^{-1}$). Glucose was added to 300-ml bottles at 75,000 $dpm \cdot 300\ ml^{-1}$, equivalent to a final concentration of about 1 $\mu g \cdot liter^{-1}$ and also incubated in situ. All incubations were in triplicate. After incubation, samples were immediately passed through a series of filters to determine the size distribution of the material into which radioactivity was incorporated (*see below*).

Radiorespirometry—Respiration ($^{14}CO_2$ production) and the incorporation of ^{14}C into particles was measured in replicate incubated 300-ml BOD bottles filled with 300 ml of lake water. Carbon dioxide was collected directly from the BOD bottle by the gas-sparging system of Cole and Likens (1979) and the $^{14}CO_2$ measured by liquid scintillation.

Bicarbonate additions—When $H^{14}CO_3$ is added to lake water, some portion is incorporated by autotrophs. A fraction of this incorporated C is subsequently released as [^{14}C]DOC which in turn may be incorporated and respired by heterotrophs, so that both auto- and heterotrophic organisms may become labeled with radioactivity. Sodium bicarbonate labeled with ^{14}C (Amersham; 40 $mCi \cdot mM^{-1}$) was diluted with sterilized deionized water adjusted to pH 9 with NaOH, sealed in glass ampoules, and then autoclaved (121°C, 1 h). The final activity of the diluted solution was about 10 $\mu Ci \cdot ml^{-1}$. A single batch of diluted bicarbonate solution was used throughout the experiments. More than 99.999% of added $H^{14}CO_3$ could be volatilized under acid conditions, indicating that contamination of the stock with labeled organic mate-

rials was quite low. Furthermore the results from the dark controls (*see below*) clearly demonstrated that such contamination was not a problem in our experiments.

Labeled bicarbonate was added to lake water in clear and opaque 300-ml BOD bottles at concentrations of 5–10 μCi·300 ml^{-1} in different experiments. The bottles were incubated in situ for various periods (5–84 h). At the end of an incubation, bottles were transported to the lab in a cold, dark, insulated chest and the plankton serially filtered immediately. The transit time was about 15 min.

Differential filtration—In all cases, samples were filtered sequentially through a series of Nitex nets and Nuclepore filters of decreasing pore size. Serial filtration has several advantages over parallel (direct) filtration. Serial filtration is faster, enables more sizes to be investigated simultaneously, and more important, does not require a difference estimate to determine the amount of radioactivity in each size class. Furthermore, the filtration scheme we used allowed most of the algal cells to be removed at very low vacuum, and thus may be a milder treatment than direct filtration through small pore-sized filters.

For sizes of $\geq$20 μm, Nitex screening was glued to the bottoms of plastic cups; the cups were stacked to form a serial tower. The entire contents (300 ml) of an incubation bottle were poured through the tower, followed by a rinse of 100 ml of filtered, sterile lake water. The particles caught by each net were rinsed from the net onto 0.8-μm Nuclepore filters. Nets were free of particles after 200–300 ml of rinsing. A vacuum of <5 cm of Hg was applied to the 0.8-μm filter to remove the water and the filter was then placed in a scintillation vial and fumed with dilute HCl for 3 h.

For pore sizes of $\leq$8 μm, 47-mm-diameter Nuclepore filters were used. A serial filtration tower was made by coupling Swin-lok filter holders with male-male Luer syringe couplers. Because filters with small pore sizes had slower filtration rates, the most convenient arrangement was to have two towers, a three-filter tower for sizes 8, 3, and 1 μm and a two-filter tower for sizes 0.4 and 0.2 μm. As a result, very low vacuum (<5 cm of Hg) was required to pass water through the first tower (i.e. through the 1-μm filter). As most algal cells in Mirror Lake are >3 μm, low vacuum filtration through the first tower should have minimized rupture of algal cells. A vacuum of 15–20 cm of Hg was required to pass water through the second (0.4–0.2 μm) tower.

Nuclepore filters were removed from the filter holders and then acid-fumed in scintillation vials. The vials were allowed to stand for several hours before adding 2 ml of 2-methoxyethanol and 0.2 ml of ethanolamine which dissolved the filters. As scintillation fluid we used 13 ml of 2:1 toluene : 2-methoxyethanol plus 6 g of PPO per liter.

Triplicate incubations were performed for each differential filtration experiment. Triplicate filtrations were done simultaneously.

Measurement of [^{14}C]DOC—Ten-milliliter samples of the filtrate from the second tower (<0.2-μm pore size) were added to 10-ml glass ampoules, acidified to pH <2, and bubbled vigorously to remove inorganic carbon. The sparged liquid was then combusted in sealed ampoules by persulfate oxidation (Menzel and Vaccaro 1964). The combusted liquid from one or more ampoules (usually three) was then transferred by wide cannula syringe to an alkaline solution in a 300-ml BOD bottle and $^{14}CO_2$ determined (Cole and Likens 1979). Because our definition of "dissolved" material is that which passes a 0.2-μm pore-size Nuclepore filter, PDOC may contain both colloidal and dissolved compounds.

This [^{14}C]DOC procedure provided enough ^{14}C in each scintillation vial for counting without serious contamination from [^{14}C]DIC. The method allows the addition of [^{14}C]DOC from 30 ml (or more) of incubation water to a single scintillation vial without loss of counting efficiency. Tests with [^{14}C]glucose showed that

about 90% of the added glucose was recovered as $^{14}CO_2$; the 10% loss is due to an inefficiency in trapping $^{14}CO_2$ and not to incomplete combustion of the glucose. Replicates usually agreed within 1% of the mean.

Size fractionation of biota—To determine what fraction of the plankton was caught by each filter, chlorophyll concentrations and the numbers of bacteria, algae, and zooplankton were determined in each of the size fractions described above. Bacteria were counted directly with the acridine orange–epifluorescence technique (Hobbie et al. 1977) in the filtrate from each filter. Algae in the filtrates were counted by the inverted microscope method (Utermöhl 1958). Zooplankton, none of which passes the 20-μm net, were counted in total.

Results

When soluble plankton material (SPM) or glucose was added to lake water most of the assimilated C was incorporated into the smaller particles (Fig. 1). When lake water samples were autoclaved before the substrate was added only trivial amounts were incorporated into particles or mineralized to CO_2 (Fig. 1). Similarly, antibiotics inhibited both uptake and mineralization. It is likely, then, that the uptake and mineralization of both substrates was due to biological processes, probably to bacteria (Cole and Likens 1979). Small algae may have been responsible for some of the metabolism of these substrates.

When $H^{14}CO_3$ was added to lake water its fate was quite different. The results of two incubations of different duration are shown in Fig. 2. Particulate ^{14}C was found in all fractions, including the dissolved (<0.2 μm). The results from the dark controls show that a negligible amount of [^{14}C]bicarbonate adhered to the filters and also that [^{14}C]DOC was produced only in the light. Two types of technical errors may cause overestimates of PDOC (Sharp 1977): preparations of $H^{14}CO_3$ sometimes contain small amounts of ^{14}C-labeled organic contaminants; inorganic labeled C may not be completely removed during sparging of samples for [^{14}C]DOC measurements. Our controls show clearly that neither of these errors was significant in our experiments.

As a test of the filtration system we filtered replicate samples that had been incubated with bicarbonate, glucose, or SPM directly through a 0.2-μm filter or through the entire filtration tower. The sum of radioactivity in the sizes separated by the tower was not statistically different from the amount caught directly by a 0.2-μm filter, indicating that the tower operated properly (Table 1).

Size fractionation of the biota—A large fraction of the total zooplankton biomass was caught by the 253-μm net, but many small forms, mostly rotifers, were caught by the 20- and 63-μm nets. Few algae were >253 μm, but a large fraction of the algal biomass was caught by the 20- and 63-μm nets. Although not much of the chlorophyll and little of the primary production is associated with large particles in Mirror Lake, a large fraction of algal biomass is in the 63-μm size class.

Most of the bacteria in Mirror Lake are between 1 and 0.4 μm, although a significant fraction passed the 0.4-μm filter (Table 2). We previously noted that bacteria <0.4 μm are at times active mineralizers of seston in Mirror Lake (Cole and Likens 1979). Bacteria also were caught by the 1- and 3-μm filters and even the 8-μm filter caught some bacterial aggregates and actinomycete filaments (Table 2).

Most of the chlorophyll was in particles <253 μm, but >1 μm (Table 2). On some dates, however, a significant amount of chlorophyll passed the 1-μm filter. No chlorophyll was detected by any procedure in the filtrate of a 0.4-μm filter. The chlorophyll passing the 1-μm filter appeared to be in the form of colonial cyanobacteria which became disaggregated as a result of filtration. We also noted very small, coccoid, unicellular cyanobacteria which could pass the 1-μm filter. Azam and Hodson (1977) found that while no chlorophyll from coastal samples (Pacific

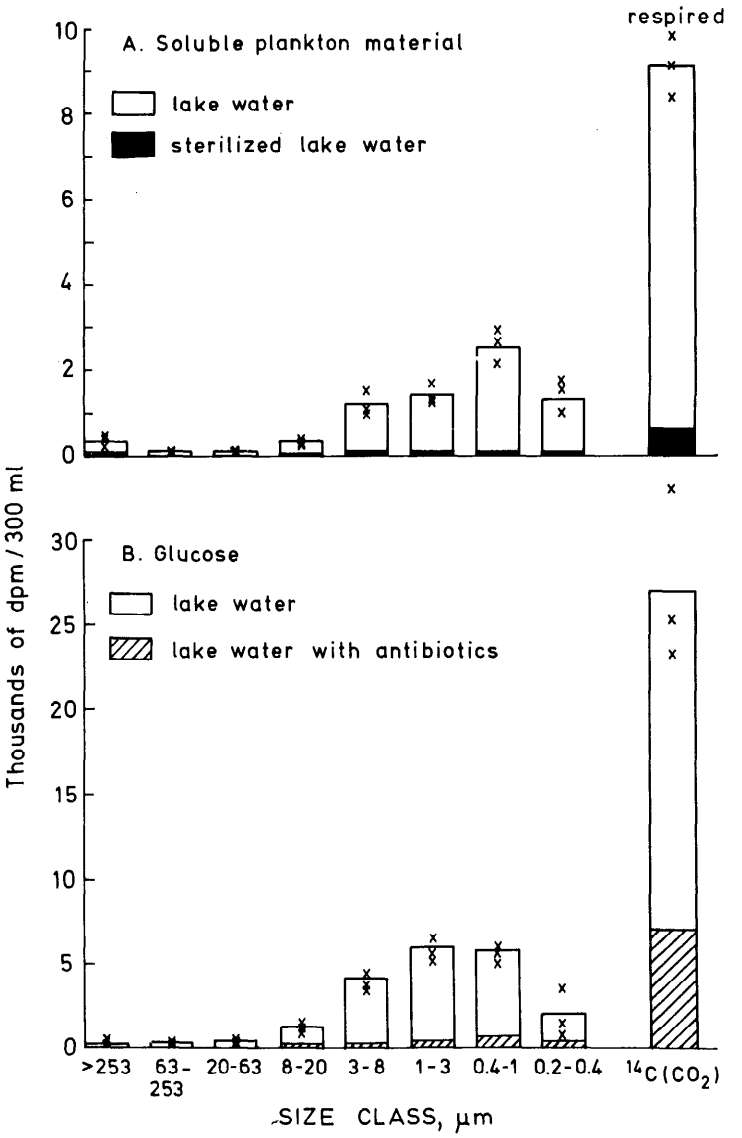

Fig. 1. Metabolism of SPM and glucose in lake water during 24-h incubations in situ in the epilimnion, August 1979. Shaded—sterile controls; hatching—results from addition of antibiotics (penicillin plus chloramphenicol plus streptomycin) at a final concentration of 9 mg C·liter^{-1}.

Ocean) passed a 0.5-µm filter, 16% of total chlorophyll did pass a 1-µm filter. It is quite clear from Table 2 that heterotrophic and autotrophic populations overlap in size in Mirror Lake.

Primary production—The relationship between measured particulate and measured dissolved "primary" production for 24-h experiments is shown in Fig. 3. On

Table 1. Serial differential filtration (Sum) and direct filtration (0.2 µm) for several types of incubations with ^{14}C-labeled substrates. Values are mean ± SD for triplicate incubations.

Substrate	Sum ± SD (dpm)	0.2 µm ± SD (dpm)
SPM	10,233±1,947	10,969±2,022
HCO_3	201,241±37,697	199,665±6,819
Glucose	18,003±817	18,400±3,566

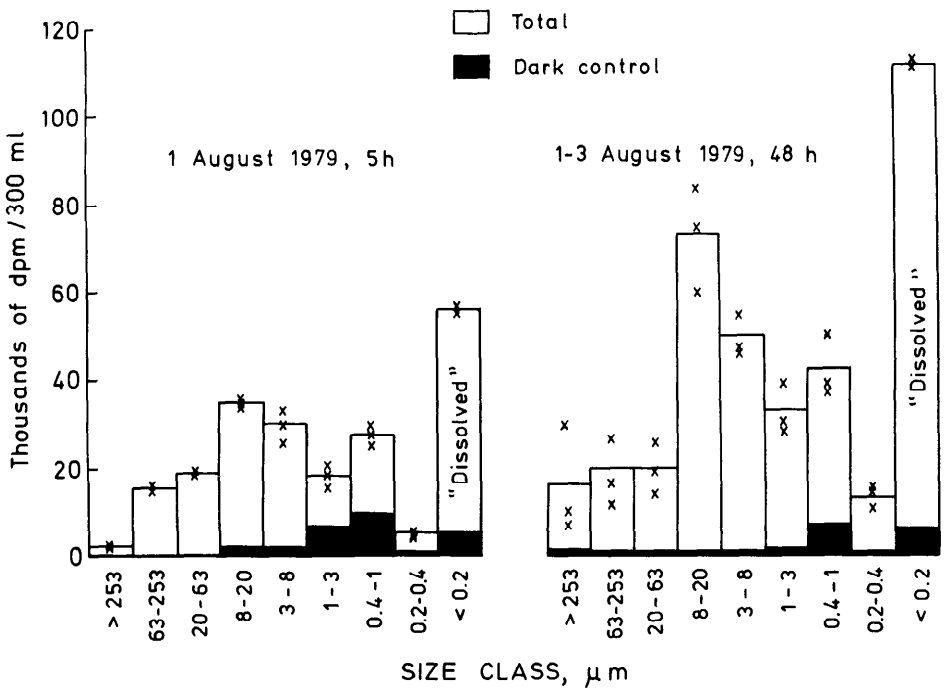

Fig. 2. Assimilation of $NaH^{14}CO_3$ added to water from 3-m depth in Mirror Lake, August 1979. Incubations were in situ for 5 (left) and 48 h (right). Two bar graphs are presented as typical results. Shaded—dark controls (incubations in opaque bottles). Mean and actual points shown for triplicate incubations.

the average during the ice-free season about 17% of primary production (corrected for dark controls) was in the dissolved fraction (also corrected for dark controls) when 0.45-µm Millipore filters were used to separate the two fractions; with 0.2-µm Nuclepore filters about 20% of production was in the dissolved phase. While some have found fractions comparable to this in the dissolved phase (e.g. Nalewajko and Schindler 1976) others have found lower fractions (e.g. Larsson and Hagström 1979). The discrepancy we observed in results from Millipore and

Table 2. Size fractionation of chlorophyll and bacterial numbers on several dates for epilimnetic water. Numbers of bacteria and amount of chlorophyll in each size class are expressed as percent of total amount in all size classes.

Size class (µm)	Chl (1 Aug (3 m))	Bacteria (1 Aug (3 m))	Chl 30 Aug (3 m)	Chl (17 Sep (3 m))	Bacteria (17 Sep (3 m))	Size class (µm)	Chl 9 Jun (3 m)
>253	0.0	0.0	0.0	3.4	0.0	>537	0.0
63–253	36.0	0.0	24.7	21.8	0.0	63–537	30.2
20–63	9.3	0.0	14.1	11.7	0.0	48–63	9.5
8–20	38.7	3.0	7.1	11.1	0.0	20–48	32.5
3–8	5.3	10.1	16.5	18.5	20.7	8–20	16.7
1–3	1.3	18.4	23.5	10.1	3.5	5–8	1.6
0.4–1	9.3	42.4	14.1	23.5	24.5	1–5	9.5
0.2–0.4	0.0	26.3	0.0	0.0	51.3	0.4–1	0.0
						0.2–0.4	0.0

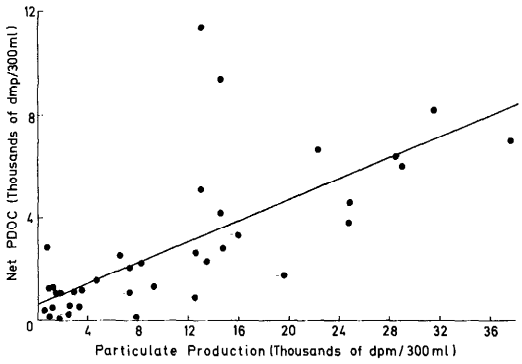

Fig. 3. Relationship between measured dissolved primary production and measured particulate primary production, both corrected for dark controls. "Particulate" is material caught by a 0.45-μm Millipore filter. Regression line: $Y - 0.2X + 673$ dpm significant at the 90% confidence level; $r^2 = 0.5$. Each point represents mean of duplicate incubations. Data from 11 dates.

Nuclepore filters may be related to the larger surface area of Millipore filters (Lean and Burnison 1979).

Uptake and mineralization of organic substrates—We found a strong linear relationship (significant at the 90% confidence level; $r^2 = 0.74$) between measured respiration and net incorporation of SPM at all times of the year (Cole 1981). During summer, respiration averaged 1.25 times net incorporation. For glucose the same ratio averaged 1.1. The relationship between mineralization and incorporation will be used in the calculations presented below.

Calculation of heterotrophic metabolism of PDOC—We seek an estimate of heterotrophic utilization of algal PDOC. Thus, we need to distinguish between algal uptake of inorganic C and heterotrophic uptake of algal PDOC in experiments in which $H^{14}CO_3$ is added to lake water. The data demonstrate, however, that heterotrophic and autotrophic populations overlap in size, making separation at any single pore-size cutoff impossible.

To distinguish between labeled carbon in bacterial and algal cells we made several assumptions. 1—No particulate algal carbon passes the 0.4-μm filter. We assume this because chlorophyll is never seen in the filtrate from a 0.4-μm filter and this filtrate has no detectable photosynthesis when incubated with $H^{14}CO_3$. 2—Heterotrophic organisms do not take up more than about 10% of their cellular carbon as CO_2. 3—SPM is metabolized by the same microorganisms that metabolize PDOC. That is, the size and activity distribution of microorganisms which assimilate SPM is identical to that of microbes which assimilate PDOC. We do not assume that SPM and PDOC are metabolized at the same rate. 4—Further, we assume that the ratio of respiration to net incorporation for PDOC is equal to that for SPM (1.1–1.3). We have measured the uptake and mineralization for a variety of ^{14}C-labeled organic compounds in Mirror Lake and found these ratios to vary between about 0.8 and 1.8. 5—We assume that distribution of heterotrophs remains relatively constant for periods up to 1 week in Mirror Lake. 6—Finally, we assume that changes in the unlabeled DOC pool do not affect the rate at which PDOC is metabolized. As the DOC in the epilimnion is quite constant in Mirror Lake (Likens 1982) we feel justified in making this assumption.

On the basis of the foregoing assumptions, simple ratios can be used to calculate heterotrophic incorporation of PDOC. All ^{14}C in the 0.2–0.4-μm fraction (corrected for dark controls) is assumed to be in bacterial cells. To determine the amount of ^{14}C in heterotrophs in the other size classes, we multiply the amount in the 0.2–0.4-μm fraction by the ratio of peak heights from the size and activity distribution for heterotrophic uptake of SPM (e.g. Fig. 1). For example in August, bacterial uptake in the 1–3-μm fraction would be 0.88 times the amount of ^{14}C in the 0.2–0.4-μm fraction; total bacterial uptake would be 4.97 times the amount in the 0.2–0.4-μm fraction. Respiration of PDOC then can be esitmated as 1.25 times net incorporation based on our experiments with SPM. The respiration of zooplankton, which may be large (Cole 1981), is not considered here. These results are shown in Fig. 4.

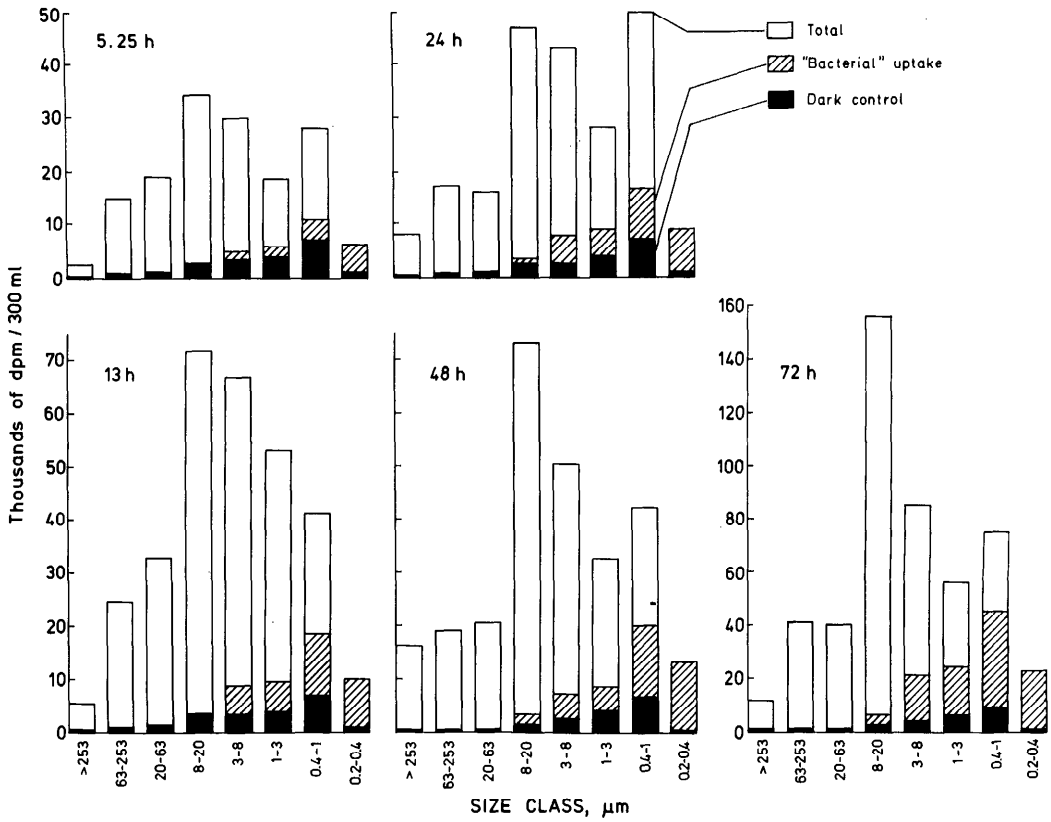

Fig. 4. Estimation of bacterial assimilation of algal PDOC from a serial differential filtration experiment, August 1979. Samples were incubated in situ for indicated duration in presence of $NaH^{14}CO_3$. Shaded—dark control; hatched—bacterial uptake of PDOC (*see text*).

Discussion

Heterotrophic microorganisms in the epilimnion rapidly assimilate a substantial portion of the PDOC released during algal growth. Clearly the amount of PDOC measured is smaller than the actual production because of this heterotrophic metabolism. We need to know, then, the fraction of total primary production that is solubilized and the fraction of this soluble material that is actually metabolized.

The gross production of PDOC, A, is the carbon potentially available to heterotrophs and includes D (measured net PDOC), the carbon assimilated by bacteria, B, plus the carbon respired by bacteria, B_r. Thus $A = D + B + B_r$. The fraction, F, of this dissolved carbon which is metabolized is $F = (B + B_r)/A$. For 24-h experiments F averages 0.43; for shorter incubations F averages 0.38 (Table 3). Roughly 40% of the gross production of PDOC is metabolized by heterotrophs during the incubation.

Measured net primary production (particulate plus dissolved) includes the ^{14}C in bacterial cells but excludes the ^{14}C respired by bacteria. If T is total net primary production then measured net primary production is $T - B_r$. Expressed as a fraction of T, A averages 45%. This result implies that particulate and dissolved primary production are of comparable magnitude although measured (net) PDOC is only 23% of measured net primary production (Table 3). Heterotro-

Table 3. Results of selected differential filtration experiments of various durations. T is total amount of primary production (particulate plus dissolved) and is always 100%; other quantities are expressed relative to T. Absolute amount of inorganic C assimilated during incubation is shown in first column ($T - B_r$). F is defined as $(B + B_r)/A$ and is the fraction of gross PDOC that is metabolized.

Incubation time (h)	Measured primary production (particulate + dissolved) $T - B_r$		Measured dissolved production (net PDOC) D	Bacterial net assimilation B	Gross dissolved production A	Metabolized C : Available C F	$(B + B_r)/T$ (%)
	(μg C·liter^{-1})	(% of T)		(% of T)			
				Aug 1979			
5	22	91.9	25.4	6.46	40.0	0.36	14.5
13	50	91.6	29.3	6.72	44.4	0.34	15.2
24	32	90.9	23.1	7.64	39.9	0.42	16.8
48	42	84.6	25.4	12.27	53.0	0.52	27.6
72	67	82.4	14.5	14.10	46.0	0.69	31.6
				Sep 1979			
13	34	93.3	20.2	5.50	32.6	0.38	12.4
48	34	82.4	9.4	14.10	41.1	0.77	31.6
				Jun 1979			
84	45	71.5	18.3	22.8	69.6	0.74	51.3

phic metabolism of PDOC then may greatly affect estimates of gross dissolved production in traditional primary production measurements.

What is the absolute magnitude of heterotrophic metabolism of PDOC? Measured primary production (particulate plus dissolved) in the epilimnion of Mirror Lake during summer ranged from 20 to 40 μg C·liter^{-1}·d^{-1}. Let 30 μg C·liter^{-1}·d^{-1} be taken as a representative value for $T - B_r$. Assuming that D is 23% of T and that $F = 0.42$ (Table 3: 24-h experiment), we calculate $B + B_r$ as follows.

$$B + B_r = FA = A - D,$$
$$A = D/(1 - F),$$
$$B + B_r = 0.42(D/0.58).$$

Then since

$$D = 6.9\ \mu\text{g C·liter}^{-1}\text{·d}^{-1},$$
$$B + B_r = 5.0\ \mu\text{g C·liter}^{-1}\text{·d}^{-1},$$

and

$$B = 2.2\ \mu\text{g C·liter}^{-1}\text{·d}^{-1}$$
(because $B_r = 1.25B$).

Through use of the $^{35}SO_4$ uptake technique, Jordan and Likens (1980) estimated that bacterial production in the water column of Mirror Lake averaged 4.7 μg C·liter^{-1}·d^{-1} during summer 1974 and 7.8 during summer 1975 (June through September volume-weighted average for entire water column). The ^{35}S technique measures, presumably, the production of all bacteria regardless of the carbon source used. A comparison of our results with those of Jordan and Likens (1980) suggests that during summer PDOC could support about 24–45% of epilimnetic bacterial production. Since most (80%) of the annual input of organic C to Mirror Lake is from phytoplankton production (Jordan and Likens 1975), it is reasonable that a substantial portion of bacterial production is supported by PDOC. The remainder of bacterial production might be supported by allochthonous sources or, more likely, by the decomposition of particulate plankton (Cole 1981).

Our results and the ^{35}S results must be compared cautiously. First, the ^{35}S technique has not been fully tested as a measure of bacterial production and problems remain, especially in estimating bacterial C:S ratios. Second, the data were from different years. However, as primary production, DOC, and bacterial abundance have not changed since the Jordan and Likens (1980) study we have no reason to expect large changes in bac-

terial production. Furthermore, the Jordan and Likens values are averages for the entire water column, and on most dates bacterial production increased with increasing depth. Bacterial production in the epilimnion is lower than the value for the whole water column and, correspondingly, the fraction supported by PDOC would be higher.

Finally, the differential filtration technique we used involves various untested assumptions. We assumed that no particulate algal material passed the 0.4-μm filter, but if algal cells were fragmented during filtration, pieces <0.4 μm could be formed and we would overestimate bacterial uptake of PDOC. If algal cells were ruptured during filtration we may also overestimate the production of PDOC.

We also assumed that the size and activity distribution of microorganisms using SPM (prepared from phytoplankton in early summer) was identical to that of microorganisms using PDOC in early, mid-, and late summer. In separate tests in which SPM prepared from early and late summer phytoplankton was added to lake water at one time, we found no difference in the size or activity distribution for either substrate. Further, the general similarity of the size and activity distribution for the uptake of glucose and SPM indicates that the pattern is not greatly dependent on the substrate.

From 12 to 51% of the PDOC is metabolized by bacteria ($B + B_r$); this percentage depends strongly on the duration of the incubation (Table 3). The dependence could be the result of time lag in the labeling of some pools or of a bottle effect. Nalewajko et al. (1976) suggested that there may be a delay in bacterial utilization of PDOC until some accumulation occurs. Confinement of lake water in a bottle could enhance such accumulation. Alternatively, senescent cells could be the major source of the soluble carbon. In a long incubation, cells senescent at the end could have been active at the start and therefore labeled. In a brief incubation only actively growing cells would be labeled. It is known that bacteria and fungi respond with more rapid growth to the filtrates of older than younger cultures (Waksman et al. 1937; Bell and Mitchell 1972) and are most abundant during the decline of an algal bloom (Canter and Lund 1953; Jones 1976). Fuhrman et al. (1980) found that bacterial abundance and activity were correlated with the standing stock of phytoplankton and pheopigments but not with primary production, suggesting that old or dead algae also sustain the bacteria.

Conclusion

In incubations of <24 h, bacterial uptake of algal PDOC is <10% of measured primary production in Mirror Lake. The measurement of algal production is not greatly affected by microbial uptake of algal products. The gross amount of PDOC, however, is about twice as large as the measured (net) amount because microorganisms rapidly metabolize this carbon, so that the production of algal PDOC will be underestimated if microbial metabolism is not taken into account. Perhaps the tremendous range in reported values for extracellular release is due, in part, to differences in heterotrophic metabolism of PDOC.

In Mirror Lake microorganisms metabolize as much as 40% of the gross PDOC each day and PDOC could support about a third of the total microbial production in the epilimnion. Soluble organic carbon from phytoplankton may, therefore, be important in regulating microbial growth in oligotrophic aquatic systems.

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