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Extracellular dissolved organic carbon released from phytoplankton as a source of carbon for heterotrophic bacteria in lakes of different humic content

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Key words: dissolved organic carbon, PDOC, bacterial production, humic lakes

Abstract

The quantitative importance of photosynthetically produced dissolved organic carbon (PDOC) released from phytoplankton as a source of carbon for pelagic, heterotrophic bacteria was investigated in four temperate Swedish lakes, of which two had low ($\sim 20\ mg\ Pt\ l^{-1}$), and two moderately high ($60–80\ mg\ Pt\ l^{-1}$) humic content. The bacterial assimilation of PDOC was estimated with the ^{14}C method, and the total production of the heterotrophic bacteria was estimated with the [^{3}H]thymidine incorporation method. The release of PDOC from natural communities of phytoplankton was not restricted to periods of photosynthesis, but often continued during periods of darkness. Heterotrophic bacteria often assimilated the labile components of the PDOC at high rates (up to 73% of the released PDOC was assimilated during the incubation in our experiments). The contribution of PDOC to bacterial production exhibited large within-lake seasonal variations, but PDOC was at certain times, both in humic and non-humic lakes, a quantitatively very important carbon source for the heterotrophic bacteria. Under periods of comparatively low primary production, heterotrophic bacteria in humic lakes appear to utilize allochthonous, humic substances as a substrate.

Introduction

The dissolved organic carbon (DOC) in the pelagic zone of lakes consists of both allochthonous substances produced in the watershed and transported to the lake in its inflows, and autochthonous substances produced by phytoplankton and macrophytes within the lake, and entering the DOC pool by direct release by living organisms or degradation of dead material. The DOC pool is normally dominated by the allochthonous compounds (Wetzel, 1983). In theory, the whole pool of DOC is available to the heterotrophic bacteria, but while the large pool of allochthonous substances are relatively refractory to bacterial utilization (Søndergaard & Schierup, 1982; Cole *et al.*, 1984), the photosynthetically produced dissolved organic carbon released from phytoplankton (PDOC) may be readily available to bacterial utilization and assimilated at high rates (Coveney, 1982; Chróst, 1983; Jensen, 1983; Feuillade *et al.*, 1988). Bacterial dependence on PDOC released from phytoplankton has been suggested to be a major reason for the positive relationship between phytoplankton biomass (often measured as concentration of chlorophyll *a*) and bacterial numbers in pelagic environments (reviews by Bird & Kalff, 1984; Cole *et al.*, 1988). This relationship is

not always clear-cut however, and Scavia & Laird (1987) found an almost inverse relationship between chlorophyll concentration and bacterial numbers in Lake Michigan. In their recent review of simultaneous measurements of phyto- and bacterioplankton production rates from both marine and freshwater environments, Cole *et al.* (1988) found a significant positive relationship between phytoplankton primary production and bacterial secondary production. The relationship contained considerable scatter, however, which could be due to fluctuations both in phytoplankton release rates and bacterial uptake rates of PDOC, as well as bacterial utilization of allochthonous DOC.

To assess the quantitative importance of PDOC as a carbon source for the bacteria, the rates of bacterial uptake of PDOC should be directly compared with the total bacterial carbon production. These kinds of comparisons have demonstrated that PDOC often contributes $> 50\%$ to the total bacterial carbon demand (Cole *et al.*, 1982; Bell *et al.*, 1983; Brock & Clyne, 1984; Søndergaard *et al.*, 1985; Chrzanowski & Hubbard, 1989).

These general trends have been established in non-humic systems, and may not hold for humic brown-water lakes, where allochthonous humic substances constitute an even more dominant portion of the total DOC than in clear-water lakes. For example, bacterial isolates from humic lakes are able to degrade dissolved humic substances (Sederholm *et al.*, 1973; De Haan, 1977), and Hessen (1985) reported that the bacterial biomass in brown-water lakes was not correlated with phytoplankton biomass but rather was positively correlated with water colour. Also, Jones & Salonen (1985) found that PDOC was not a significant source of substrate for total bacterial respiration in two polyhumic lakes. Additionally, Tranvik (1989) compared the primary and bacterial secondary production in two lakes of similar abiotic and biotic characteristics but different humic content, and found that the ratio of bacterial production to primary production was significantly higher in the humic lake. Taken together, these results strongly imply that in humic lakes, allochthonous dissolved organic carbon is a significant carbon source for bacterioplankton.

In this paper we present estimates of the epilimnetic bacterial assimilation of PDOC (measured with the ^{14}C-bicarbonate tracer technique involving fractionated filtration) and the total bacterial secondary production (measured as [^{3}H]thymidine incorporation) in two non-humic and two moderately humic temperate lakes in central Sweden. If the heterotrophic bacteria of the humic lakes utilize allochthonous, humic material as substrate, we expect the uptake of PDOC to make a smaller contribution to the total bacterial production in these lakes.

Materials and methods

Description of lakes

Some basic abiotic characteristics of the lakes are presented in Table 1. Of the two non-humic lakes, Lake Vallentunasjön is small, shallow and highly eutrophic whereas Lake Erken is larger, and gen-

Table 1. Some general characteristics of the investigation lakes. Values given for total phosphorus and water colour are approximate mean values for the ice-free season[1].

	Lake area km^2	Maximum depth m	Mean depth m	Total phosphorus μg P l^{-1}	Water colour mg Pt l^{-1}
Lake Vallentunasjön	6.2	5.0	2.7	100	20
Lake Erken	24	20	9.0	20	20
Lake Siggeforasjön	0.76	11	4.2	15	80
Lake Sörmogen	3.7	10	–	15	80

[1] For more details on the abiotic and biotic characteristics of the lakes consult Boström *et al.* (1989) (Lake Vallentunasjön), Petterson (1990) (Lake Erken), Heyman & Blomqvist (1984) (Lake Siggeforasjön) and Sundh (1989) (Lake Sörmogen).

erally of a mesotrophic character. Both the humic lakes (Lakes Sörmogen and Siggeforasjön) are comparatively small forest lakes and have mesotrophic to oligotrophic character.

Sampling

All details on the sampling, incubation and filtration procedures refer to the diel experiments in Lakes Vallentunasjön, Erken and Siggeforasjön. No diel incubations, and slightly different filtration methods (Sundh, 1989) were used in Lake Sörmogen.

Composite samples, from the euphotic zone during circulation and from the epilimnion during stratification, were taken with a 2 m long tube sampler. A subsample was preserved with formaldehyde (final concentration 4%) for enumeration and biomass determination of bacteria. In five experiments (Lake Vallentunasjön April 29, 1988 and September 27, 1989 and Lake Erken May 5, and August 30, 1988 and August 1, 1989) sampling was done in the afternoon of the day before start of the experiments and the water samples stored in darkness at *in situ* temperature until experiments were started the following morning. On the other occasions, sampling was performed in the morning (07.00–09.00) and incubations with ^{14}C were started within three hours of sample collection.

Bacterial abundance and biomass

Bacteria were enumerated and cell volumes estimated by acridine orange staining and epifluorescent microscopy according to Bell *et al.* (1983). For biomass determinations the dimensions of at least 125 randomly chosen cells were measured and biovolumes calculated using geometric formulas.

Heterotrophic bacterial production

Heterotrophic bacterial production was assessed via [*methyl*-^{3}H]thymidine (40–60 Ci mmol^{-1}; Amersham) incorporation into macromolecules (Bell *et al.*, 1983). Triplicate 10–20 ml samples (100-μm prefiltered) were incubated with [^{3}H]thymidine (final concentration 25–30 nM in Lakes Vallentunasjön and Erken and 40–50 nM in Lakes Sörmogen and Siggeforasjön) at *in situ* temperature for $\approx$1 hour. The incubations were stopped by adding formaldehyde to a final concentration of 2%. A formaldehyde-killed blank (2% final conc.) was always included. In these lakes, 70–85% of the [^{3}H]thymidine incorporation into macromolecules (material insoluble in cold 5% trichloroacetic acid) is in DNA (Bell & Kuparinen, 1984; Bell, 1986; Bell, 1990). Bacterial production in Lake Siggeforasjön on August 8, 1988 was assessed via [^{3}H]adenine incorporation into DNA (Bell & Riemann, 1989). Triplicate 15 ml subsamples were prefiltered (2 μm) to separate algae from bacteria, and incubated with 100 nM [^{3}H]adenine (25 Ci mmol^{-1}; Amersham) for $\approx$4 hours. The samples were subsequently extracted and filtered according to Bell & Riemann (1989). Thymidine and adenine incorporation were converted to carbon units using a conversion factor of 2×10^{18} cells mol^{-1} (Bell *et al.*, 1983; Smits & Riemann, 1988; Riemann & Bell, 1990) and assuming a bacterial carbon content of 0.22 pg μm^{-3} (Bratbak & Dundas, 1984). Bacterial production was assessed twice during each diel cycle.

The phytoplankton community during autumn in Lake Vallentunasjön was dominated by colonial cyanobacteria (primarily *Microcystis* spp.). These contain numerous bacteria in their gelatinous sheaths (Pearl, 1982; Bern, 1985). To assess the respective contribution of attached and non-attached bacteria to total bacterial production the [^{3}H]thymidine uptake during these periods was size-fractionated by post-filtration through a 2 μm filter prior to TCA extraction.

Primary production, percentage extracellular release and bacterial assimilation of PDO^{14}C

Before the start of the ^{14}C tracer incubations, the mixed water samples were gently sieved through a 100 μm net to remove large zooplankton. Three transparent glass flasks with plastic screw caps

were each filled with 500 ml of the 100 μm filtered water, and ^{14}C labelled bicarbonate (0.23–1.6 μCi ml^{-1} sample) was added to two of them. The unlabelled flask was subsequently used to take subsamples for measurements of the inorganic carbon uptake in the bacterial size fraction (0.2–2 μm) and for [^{3}H]thymidine incorporation assays. All three flasks were incubated *in situ* at ≈0.7 m depth (Lake Erken May 25, 1987 and July 20, 1988) or under *in situ* temperature in the laboratory at 110–250 μE m^{-2} s^{-1} during the light periods and in darkness during the night. The incubations lasted at least 24 hours, and always included a dark period between two light periods. At intervals, subsamples were taken for determination of ^{14}C in the phytoplankton (>2 μm), the bacterial (0.2–2 μm) and dissolved fractions (<0.2 μm). Aliquots of 5–15 ml were filtered sequentially through a 2 μm (Nuclepore polycarbonate) and 0.2 μm (Schleicher & Schüll membrane) filter. Aliquots for zero time radioactivity of the different size fractions were taken immediately after addition of the ^{14}C. To avoid breakage of phytoplankton cells retained by the 2 μm filter, the filtration was allowed to proceed under gravity, but a slight vacuum (<100 mm Hg) was necessary for the 0.2 μm filtration. The filters were placed in scintillation vials and a few drops of 0.5 M HCl were added to remove radioactive inorganic carbon. The filtrates were collected in glass scintillation vials. The pH was lowered (to ≈2) by addition of 0.5 M HCl and excess radioactive inorganic carbon was removed by air bubbling for 45 minutes. The radioactivity of the filters and filtrates was subsequently measured by liquid scintillation counting, with quench correction according to the external standard channels ratio method. In the calculations of the ^{14}C activity in the particulate and dissolved fractions, the zero time values were subtracted.

To correct for possible autotrophic uptake of ^{14}C labelled bicarbonate in the bacterial size fraction (0.2–2 μm), we prefiltered aliquots from the unlabelled flasks through a 2 μm (Nuclepore polycarbonate) filter. After addition of ^{14}C labelled bicarbonate to the filtrates (0.4–3.9 μCi ml^{-1}) and 3.3–12 hours incubation at *in situ* temperature, the incubation was terminated by filtration through a 0.2 μm filter (Schleicher & Schüll membrane). The filters and filtrates were subsequently treated as described above. The inorganic carbon uptake in the bacterial size fraction was determined during both the first day and the night.

The total concentration of inorganic carbon was calculated from pH (measured on a Radiometer pHM 62), alkalinity (titration with HCl under N_2 flow and with a mixed indicator) and conductivity (measured on a Radiometer CDM 2d). For transformation of ^{14}C in the different size fractions to units of carbon, the same specific ^{14}C activity was assumed in these fractions as the specific activity of inorganic carbon at the start of the experiment. The total carbon assimilation was calculated as the sum of radioactivity of the two filters and the filtrate, and the percentage extracellular release (PER) as the sum of the filtrate and the 0.2 μm filter (corrected for bicarbonate uptake in the 0.2–2 μm size fraction) in percent of the total assimilation. The bacterial incorporation of $PDO^{14}C$ was obtained from the radioactivity of the 0.2 μm filter (corrected for bicarbonate uptake in this size fraction), and transformed to total carbon uptake assuming 50% respiration of $PDO^{14}C$ (Cole *et al.*, 1982; Chrost & Faust, 1983). The total ^{14}C assimilation, PER and bacterial incorporation of $PDO^{14}C$ was calculated both for subsamples taken after the first light period and after the first light + the dark period.

Results

Bacterial production and abundance

Bacterial abundance and biomass was highest and the within-lake variation greatest in eutrophic Lake Vallentunasjön (Table 2). Bacterial abundance and biomass was comparable in Lake Erken and the humic Lake Siggeforasjön, whereas abundance and biomass was ≈3 times greater in the equally humic Lake Sörmogen.

Except for Lake Erken on August 1 1989, when the bacterial production was four times higher

Table 2. Bacterial abundance and biomass and total bacterial production. Columns denoted 'light' shows results from subsamples taken after the first light period of incubation, and 'light + dark' from subsamples taken after the dark period.

	Bacterial abundance $10^9\ l^{-1}$	Bacterial biomass $\mu g\ C\ l^{-1}$	Total bacterial production[1] $\mu g\ C\ l^{-1}\ h^{-1}$	Specific bacterial production day^{-1}	Bacterial production in % of prim. prod.	
					Light	Light + dark
Lake Vallentunasjön						
April 29 1988	4.3	140	0.44	0.07	1.0	1.7
Sep 5 1988	7.1	323	6.2	0.46	3.4	8.4
Sep 27 1989	15.6	717	6.1	0.20	1.6	4.0
Lake Erken						
May 25 1987	–	–	0.09	–	5.5	8.0
May 5 1988	1.1	25	0.12	0.12	0.6	1.2
July 20 1988	1.3	28	1.19	1.02	12	19
Aug 30 1988	1.6	30	0.30	0.24	5.4	12
August 1 1989	1.8	42	0.75	0.43	6.3	15
Lake Siggeforasjön						
Aug 8 1988	1.5	34	0.34[2]	0.24	5.0	8.3
Sep 19 1988	1.4	29	0.20	0.17	19	40
Aug 1 1989	1.2	26	0.15	0.14	1.5	3.0
Lake Sörmogen						
June 25 1986	2.6	85	0.95[3]	0.27	23	–
July 9 1986	3.5	117	0.95[3]	0.19	17	–
Sep 3 1986	4.5	149	0.45[3]	0.07	14	–

[1] Mean of the afternoon and morning determinations.
[2] Bacterial production measured as adenine incorporation into DNA.
[3] Mean of two determinations a few days before and after the dates of ^{14}C incorporation experiments.

after the night, and Lake Vallentunasjön on September 5 1988 (21% higher after the night), the bacterial production was somewhat lower (4–33%) at the end of the dark period than during the first light period. Because the differences between the two determinations were moderate, the mean was used for all calculations involving total bacterial production. Considering the non-humic lakes, the total bacterial production was higher in eutrophic Lake Vallentunasjön (0.44–6.2 $\mu g\ C\ l^{-1}\ h^{-1}$) than in mesotrophic Lake Erken (0.092–1.19 $\mu g\ C\ l^{-1}\ h^{-1}$, Table 2). The bacterial production rates in the humic lakes exhibited the same pattern as the bacterial biomass, i.e., a higher production rate in Lake Sörmogen (0.45–0.95 $\mu g\ C\ l^{-1}\ h^{-1}$) than in Lake Siggeforasjön (0.15–0.34 $\mu g\ C\ l^{-1}\ h^{-1}$). The specific bacterial production (day^{-1}) did not exhibit any significant difference between the lakes, and except for Lake Erken on July 20, 1988 (1.02 day^{-1}), it varied between 0.072 and 0.46 day^{-1} (Table 2).

Primary production, PER and bacterial uptake of PDO^{14}C

Three examples of the diel time course of ^{14}C labelling of the phytoplankton, bacterioplankton and dissolved fractions are shown in Fig. 1. The most common pattern was that the ^{14}C of the phytoplankton size fraction increased during the first day, decreased slightly during the night and increased again during the second light period, while the labelling of the bacterial size fraction, and particularly the filtrate, continued to increase during the night, implying that phytoplankton release of PDO^{14}C did not cease when photosynthesis stopped.

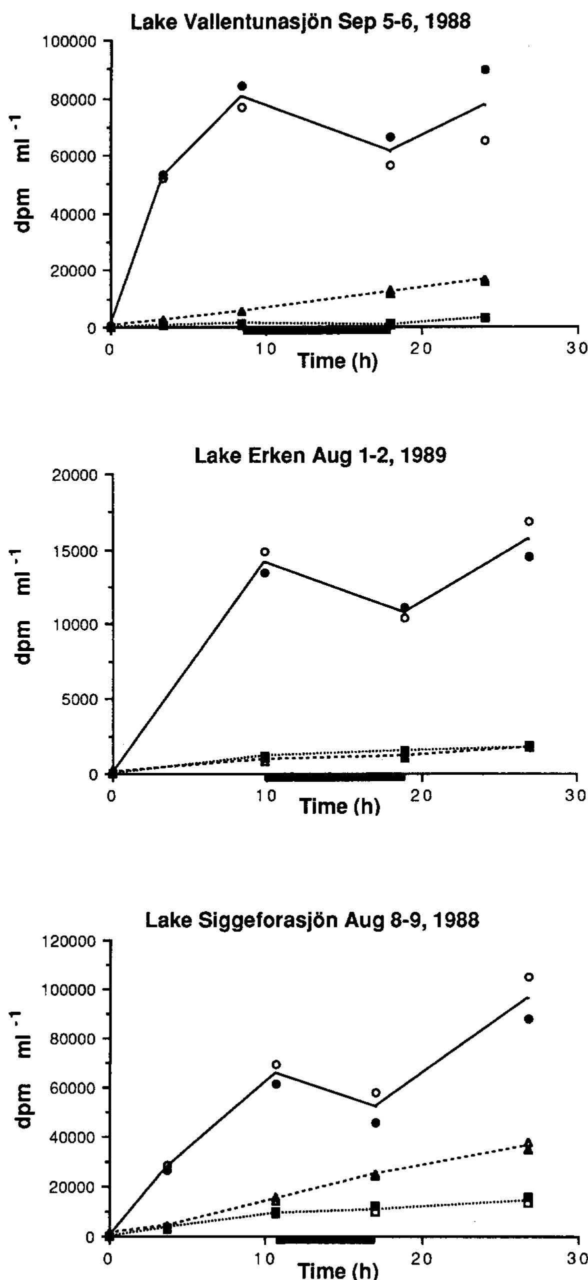

Fig. 1. Three examples of the diel time-course of ^{14}C labelling of phytoplankton (circles), bacterioplankton (squares) and dissolved (triangles) fractions. Light and dark symbols represent replicate flasks, and the lines connect the mean values. The thickened section of the x-axis shows the duration of the dark period.

The total ^{14}C assimilation in Lakes Erken, Siggeforasjön and Sörmogen was comparable (diel assimilation ranging between 1.15–10.4 μg $C l^{-1} h^{-1}$ in Lake Erken and 0.50–4.97 μg $C l^{-1} h^{-1}$ in Lake Siggeforasjön), but the total assimilation in eutrophic Lake Vallentunasjön was an order of magnitude higher (range of 25.6–151 μg $C l^{-1} h^{-1}$, Table 3). The short incubations in Lake Sörmogen yielded PER values between 36% and 55% of the total assimilation. In Lakes Vallentunasjön, Erken and Siggeforasjön, the continued release of PDO^{14}C during the night consistently resulted in significantly higher release rates (in several experiments about twice as high) if the whole period (light + dark) is considered, and the diel rates of release ranged between 6–29%, 9–35% and 21–40% in Lakes Vallentunasjön, Erken and Siggeforasjön, respectively (Table 3).

The percentage of the released PDO^{14}C recovered in the bacterial size-fraction after the incubation varied between 5% and 57% (Table 3). The PDO^{14}C appears to be more efficiently utilized in Lake Erken (23–57% of the total released PDO^{14}C incorporated on a diel basis) compared to the other lakes (5–17% in Lake Vallentunasjön and 6–31% in Lake Siggeforasjön). The same trend is apparent when the bacterial incorporation of PDO^{14}C is expressed as a percentage of the total primary production (Table 3).

Uptake of PDO^{14}C in relation to total bacterial carbon demand

To assess the total bacterial uptake of carbon and the contribution of PDOC, the bacterial incorporation of PDO^{14}C was transformed to gross carbon uptake assuming 50% respiration of PDOC, and the bacterial production measured as thymidine incorporation transformed to total bacterial carbon uptake by assuming a growth yield of 50% (Table 4). Frequently, the uptake of PDO^{14}C exceeded the estimated total uptake of carbon measured with the [^{3}H]thymidine technique, thus resulting in higher than 100% contribution of PDOC. Except for Lake Sörmogen, the contribution of PDOC exhibited very large variations within the lakes (Table 4), and consequently there is a large overlap between the lakes.

Table 3. Total ^{14}C assimilation, percentage extracellular release and bacterial assimilation of $PDO^{14}C$. Columns denoted 'light' shows results from subsamples taken after the first light period of incubation, and 'light + dark' from subsamples taken after the dark period.

	Total ^{14}C ass. $\mu g\ C\ l^{-1}\ h^{-1}$		PER %		Bacterial ass. of $PDO^{14}C$ % of release		Bacterial ass. of $PDO^{14}C$ % of prim. prod.	
	Light	Light + dark	Light	Light + dark	Light	Light + dark	Light	Light + dark
Lake Vallentunasjön								
April 29 1988	42.6	25.6	14	29	10	10	1.5	2.9
Sep 5 1988	183	74	7	17	13	5	0.9	0.9
Sep 27 1989	383	151	4[1]	6[1]	16[1]	17[1]	0.7[1]	0.9[1]
Lake Erken								
May 25 1987	1.68	1.15	34	35	49	42	16	14
May 5 1988	19.9	10.4	5[1]	9[1]	39[1]	28[1]	1.9[1]	2.7[1]
July 20 1988	10.3	6.12	21	33	16	23	3.2	7.4
Aug 30 1988	5.55	2.45	15	22	21	38	3.1	8.6
Aug 1 1989	12.0	5.11	11	17	57	57	6.3	9.6
Lake Siggeforasjön								
Aug 8 1988	6.78	4.11	26	40	40	31	10	9.6
Sep 19 1988	1.04	0.50	25	37	12	6	2.8	2.4
Aug 1 1989	10.1	4.97	10	21	28	13	2.7	2.6
Lake Sörmogen								
June 25 1986	4.13	–	55	–	31	–	17	–
July 9 1986	5.62	–	36	–	14	–	5.0	–
Sep 3 1986	3.25	–	45	–	13	–	5.8	–

[1] No correction for bicarbonate uptake in the bacterial (0.2–2 μm) size fraction

During September in Lake Vallentunasjön, when colonial cyanobacteria dominated the phytoplankton, more than half the total [^3H]thymidine incorporation was by the $> 2\ \mu m$ fraction (Table 4). Since the $PDO^{14}C$ uptake is measured for the 0.2–2 μm fraction, it is more accurate to compare the measured $PDO^{14}C$ uptake with the [^3H]thymidine uptake based estimate of carbon uptake for only the 0.2–2 μm size fraction. Based on this later comparison PDOC constitutes $\approx 120\%$ of total bacterial production at this time (Table 4). Thus, when considering only the $< 2\ \mu m$ bacterial size fraction, the contribution of PDOC (short light incubations) is higher in the non-humic (147%) than the humic (90%) lakes. This difference, however, is not significant at the 0.05 level (student's t-test). The contribution of PDOC to total bacterial production in Lake Erken was large during the spring experiments but smaller in summer.

Discussion

Phytoplankton PDOC release

In most experiments, the radioactivity of the phytoplankton size fraction decreased during the night while the activity in the bacterial size fraction and filtrate continued to increase (Fig. 1). A continued night-time release, although at lower rates, has been noted earlier (Saunders, 1972; Berman & Kaplan, 1984). Clearly, the decrease in phytoplankton radioactivity during darkness is not only due to respiration losses, but also to continued release of $PDO^{14}C$. In consequence,

Table 4. Bacterial uptake of $PDO^{14}C$ and its contribution to total bacterial carbon uptake, assuming growth yields of 50% both for $PDO^{14}C$ uptake and total carbon uptake. Columns denoted 'light' shows results from subsamples taken after the first light period of incubation, and 'light + dark' from subsamples taken after the dark period.

	Bacterial uptake of $PDO^{14}C$ $\mu g\ C\ l^{-1}\ h^{-1}$		Total bacterial carbon uptake $\mu g\ C\ l^{-1}\ h^{-1}$	Bacterial carbon uptake, 0.2–2 μm fraction $\mu g\ C\ l^{-1}\ h^{-1}$	Uptake $PDO^{14}C$, % of total bacterial carbon uptake %		Uptake $PDO^{14}C$, % of bacterial carbon uptake in 0.2–2 μm fraction %	
	Light	Light + dark			Light	Light + dark	Light	Light + dark
Lake Vallentunasjön								
April 29 1988	1.28	1.50	0.88	–	145	170	–	–
Sep 5 1988	3.24	1.28	12.4	2.96	26	10	109	43
Sep 27 1989	5.26	2.80	12.2	3.96	43	23	133	71
Lake Erken								
May 25 1987	0.54	0.32	0.18	–	293	174	–	–
May 5 1988	0.74	0.56	0.24	–	308	233	–	–
July 20 1988	0.66	0.90	2.4	–	28	38	–	–
Aug 30 1988	0.34	0.42	0.60	–	57	70	–	–
Aug 1 1989	1.52	0.98	1.51	–	101	65	–	–
Lake Siggeforasjön								
Aug 8 1988	1.36	0.98	0.68	–	200	144	–	–
Sep 19 1988	0.058	0.024	0.40	–	15	6	–	–
Aug 1 1989	0.54	0.26	0.30	–	180	87	–	–
Lake Sörmogen								
June 25 1986	1.16	–	1.90	–	74	–	–	–
July 9 1986	0.38	–	1.90	–	29	–	–	–
Sep 3 1986	0.40	–	0.90	–	42	–	–	–

the PER from the long incubations (light + dark period) are consistently higher than when only the first light period is considered (Table 3). This indicates, as suggested by Søndergaard *et al.* (1985) that diel incubations are necessary to obtain reliable PER estimates. A continued night-time release is not unexpected, when the possible mechanisms of release are considered. Death and subsequent autolysis may be an important loss factor for phytoplankton (Jassby & Goldmann, 1974; Pollinger & Serruya, 1976; Heyman & Blomqvist, 1984). The activity of zooplankton ('sloppy feeding', excretion or defecation) may contribute to the continued release of $PDO^{14}C$ at night (Lampert, 1978; Olsen *et al.*, 1986; Jumars *et al.*, 1989). In our experiments, large zooplankton were removed by filtering the sample through 100 μm, but small crustacean zooplankton, rotifers, ciliates and phagotrophic flagellates were not removed by this procedure. Another possible explanation for continued release in darkness has been suggested by Bjömsen (1988). He hypothesized that phytoplankton release of PDOC can be explained by diffusive leakage of organic molecules over the plasma membrane, driven by the steep concentration gradient, and that the release should be interpreted in terms of biomass as opposed to production of biomass. This release mechanism is only possible for low molecular weight metabolic intermediates, however, and it has been shown repeatedly (Sundh, 1989 and references therein) that high molecular weight substances often make large contributions to *in situ* PDOC. Clearly, the release of PDOC from *in situ* phytoplankton communities is a result of several mechanisms acting simultaneously, and

the PDOC is a mixture, containing compounds released by direct excretion from intact cells as well as autolysis products and substances released as a result of the activities of grazing zooplankton and protozoans. Diffusive leakage may be an important mechanism for the direct excretion of low molecular weight compounds from intact cells, while the grazing component of the release is obviously dependent on the prevailing grazing pressure. In addition, it is theoretically possible that the $PDO^{14}C$ contains $DO^{14}C$ released by bacteria. It has been suggested, however, that $DO^{14}C$ released by bacteria is not important when incubations are shorter than 24 hours (Dunstall & Nalewajko, 1975; Iturriaga & Zsolnay, 1981).

Overall, the large seasonal variations of PER within the lakes probably result from the taxonomic succession of phytoplankton. The release rates differ widely between different species in laboratory cultures (Hellebust, 1965). We observed that the PER was frequently higher when small forms of flagellates and diatoms (the humic lakes, and spring conditions in Lakes Vallentunasjön and Erken) dominated the phytoplankton biomass (Sundh, in prep.), perhaps partly due to microzooplankton grazing on those edible forms of phytoplankton, leading to higher contribution to $PDO^{14}C$ release from microzooplankton activities.

Bacterial assimilation of $PDO^{14}C$

Although the bacterial uptake rates of $PDO^{14}C$ (expressed as percent of released $PDO^{14}C$ processed during the incubation) did not vary significantly between the lakes, there was a tendency for higher utilization rates in Lake Erken. Assuming 50% respiration of PDOC, the bacteria removed 18–28% (10–29% on a diel basis) in Lake Vallentunasjön, 28–73% in Lake Erken (37–73% on a diel basis), 21–57% in Lake Siggeforasjön (11–47% on a diel basis) and 23–47% in Lake Sörmogen. Our results thus corroborate earlier findings of high uptake rates of the labile components of PDOC.

When large colonial cyanobacteria dominated the phytoplankton biomass (September experiments in Lake Vallentunasjön), more than half the [^3H]thymidine incorporation was by the $>2\ \mu m$ size fraction (Table 4). If the bacterial incorporation of $PDO^{14}C$ is likewise predominately by the large and attached bacteria not passing the 2 μm filter, it follows that the incorporation of $PDO^{14}C$ (and to some extent the PER) in these experiments are seriously underestimated. Thus, it is possible that the actual uptake rates in the September experiments in Lake Vallentunasjön were about twice as high as indicated in Table 3. We can not be sure whether attached bacteria made a significant contribution to the heterotrophic activity in the other experiments. We know, however, from the microscopic direct counts of bacteria, that aggregates of bacteria or bacteria attached to large particles made a negligible contribution to total bacterial biomass. Thus, in these experiments it is unlikely that the bacterial assimilation of $PDO^{14}C$ is underestimated due to uptake by attached bacteria.

$PDO^{14}C$ contribution to total carbon uptake

The goal of this study was to assess the quantitative importance of newly released PDOC to total bacterial carbon demand. This kind of comparison places demands on the methodology. Both the measurements of release and bacterial assimilation of PDOC, as well as thymidine incorporation, have uncertainties that are difficult to assess for every sample. We discuss these problems below, but are convinced that our results are internally consistent and the general trends are real. For example, accumulating evidence shows that thymidine uptake and other methods give equivalent estimates of bacterial production (Bell & Riemann, 1989; Simon & Azam, 1989; Chin-Leo & Kirchman, 1988; Riemann *et al.*, 1990).

The bacterial uptake of PDOC exceeded the estimated total bacterial carbon uptake in several of our experiments. Likewise, Søndergaard *et al.* (1985) and Chrzanowski & Hubbard (1989) (also

using the ^{14}C and [^{3}H]thymidine methods) frequently obtained PDOC contribution to total carbon production exceeding 100%. The contribution of PDOC to total bacterial production of biomass estimated from [^{3}H]thymidine incorporation, however, must not necessarily be lower than 100%. Large bacterial cells contain more carbon per unit of DNA than smaller cells (Simon & Azam, 1989; Riemann *et al.*, 1990). Thus, when cells are increasing in size, bacterial carbon uptake would exceed the carbon uptake estimated from DNA synthesis. Temporal rhythms of carbon uptake and DNA synthesis would also uncouple PDOC uptake and thymidine incorporation. Riemann & Bell (1990) found that the ratio of leucine incorporation (= estimate of protein synthesis) to thymidine incorporation varied by a factor of ≈ 5 over periods of weeks to months in enclosures in both an oligotrophic and a eutrophic lake. Diurnal rhythms in carbon incorporation and DNA synthesis could also result in actual carbon uptake exceeding the uptake calculated from the thymidine incorporation. Diurnal variations in DNA synthesis are comparatively small, however (Riemann & Söndergaard, 1984), compared to diurnal variation in leucine incorporation (Bell *et al.*, in prep).

We used a constant factor for converting moles of thymidine incorporated into bacterial cell production, and in the literature this factor varies by more than an order of magnitude (Bell, 1990). However, empirically determined conversion factors are very dependent on the concentration of thymidine used for calibration, and it is difficult to compare different studies. Bell (1990) argues that using a high (≥ 20 nM) concentration of [^{3}H]thymidine will minimize *de novo* synthesis, giving conversion factors of $\approx 2 \times 10^{18}$ cells mol^{-1}. This factor has been derived for several Swedish (Bell *et al.*, 1983; Bell & Kuparinen, 1984; Bell, 1990) and Danish lakes (Smits & Riemann, 1988) and approaches the factor expected on theoretical grounds (Bell, 1990; Riemann & Bell, 1990). The weakest links in converting rates of thymidine incorporation into carbon production are the estimation of bacterial cell volumes and the factor for carbon per unit bacterial biovolume. At present, most workers use the factor of 0.22 pg μm^{-3} of Bratbak & Dundas (1984) which lies in the middle of the range of literature values (Riemann & Bell, 1990).

To convert thymidine incorporation to gross bacterial carbon uptake, we assumed a bacterial growth yield of 50%. It has been suggested that 30% is a more realistic estimate for natural bacterial communities (Bjørnsen & Riemann, 1988; Schwaerter *et al.*, 1988). The most realistic estimate of bacterial respiration of PDOC is 50%, however (Cole *et al.*, 1982; Chrost & Faust, 1983; Coveney & Wetzel, 1989), and our calculations reveal that on several occasions, PDOC is the dominant (or the sole) bacterial substrate. Therefore, we do not think that use of different respiration quotas for PDOC uptake and total bacterial carbon uptake can be justified for our experiments.

Another reason for the fact that the PDO^{14}C uptake at times exceeds total bacterial production concerns the methodology of PDO^{14}C uptake measurements. Several studies have shown that inorganic carbon uptake in the 'bacterial' size fraction may lead to serious overestimations of the bacterial assimilation of PDO^{14}C measured with the fractionated filtration method (Larsson & Hagström, 1982; Sundh, 1989). With the exception of Lake Erken on May 5, 1988 (when the correction resulted in negative uptake rate) and Lake Vallentunasjön on September 27, 1989 (the prefiltered samples were lost), the diel assimilation of inorganic carbon in the bacterial size fraction was 5–10%, 14–39% and 7–58% of the total radioactivity of the bacterial filter after fractionated filtration, in Lakes Vallentunasjön, Erken and Siggeforasjön, respectively, stressing the importance of this type of correction. In addition, it is possible that fragile phytoplankton cells break when they are trapped on the 2 μm filter (Kuosa, 1988) and, if so, fragments of the cells may pass the filter and be trapped on the 0.2 μm filter instead, resulting in overestimations of the PDO^{14}C uptake. The same effect may result from postfiltration rinsing of filters (Goldman & Dennet, 1985). In the diel experiments in Lakes Vallentunasjön, Erken and Siggeforasjön the effect of such

errors was minimized by not using vacuum for the 2 μm filtration, and excluding the rinsing of filters, and should thus be moderate. The bacterial $PDO^{14}C$ uptake measurements, however, may be underestimated in two ways. Besides underestimation because isotopic equilibrium in the $PDO^{14}C$ released from the cells was not attained (Smith, 1982; Jensen *et al.*, 1985), presence in the water of unlabelled substances identical to the released PDOC, leads to dilution of the released substances and underestimation of uptake. Evidence presented elsewhere (Sundh, 1989) for experiments in Lake Sörmogen, however, suggests that isotopic equilibrium may not have been a major problem in our experiments.

The mean contribution of PDOC to total bacterial carbon uptake was lower in the humic than in the non-humic lakes (Table 4) and thus our results, in agreement with other studies (Hessen, 1985; Jones & Salonen, 1985; Tranvik, 1989), suggest that pelagic bacteria in humic lakes utilize allochthonous, humic substances as a significant, additional carbon source. The lower contribution of PDOC to bacterial production in the humic lakes was not statistically significant on the 0.05 level, however (student's t-test). 'Humic lakes' is an arbitrary distinction, however, and compared to the lakes studied by Jones & Salonen (1985, water colour up to 300 mg Pt l^{-1}) and Tranvik (1989, water colour up to 180 mg Pt l^{-1}), Lakes Sörmogen and Siggeforasjön must be considered moderately humic. It should be emphasized, therefore, that the small difference between non-humic and humic lakes in our data may be an effect of the comparatively low humic content of Lakes Siggeforasjön and Sörmogen. Another factor that would tend to render the difference in PDOC contributions to bacterial production in the humic and clear-water lakes an underestimate, is that we only used one light level (probably close to the level of maximum photosynthesis) for the ^{14}C incubation, and thus our data are volume- and not area-based. Assuming that in the epilimnion, bacterial production is less variable with depth than primary production (Bell *et al.*, in prep.), and that the depth of the euphotic zone relative to the depth of the epilimnion is smaller in the humic lakes, it is probable that on an areal basis, the bacterial production in relation to primary production and bacterial assimilation of PDOC, differs more between the humic and the non-humic lakes than is suggested from our data.

Seasonal variations in activity of heterotrophic bacteria

The contribution of PDOC to total bacterial production (Table 4), exhibited large within-lake variation. Especially in Lake Erken, the tendency is that PDOC contributes more to bacterial production during spring conditions than later in summer. A possible explanation for this situation is that since quite a large fraction ($\approx 50\%$ in Lake Erken) of the released PDOC is not immediately assimilated by bacteria, these substances will accumulate in the water. In spring, after the ice-covered period with very low primary production, the water may be almost depleted of DOC utilizable to the bacteria. When primary production (and thus production of PDOC) increases during the spring bloom and with the passing of summer, the PDOC not immediately assimilated by bacteria accumulates, and may subsequently serve as an additional bacterial substrate during summer stratification. Similarly, Scavia & Laird (1987) found that during summer stratification in Lake Michigan, not even the total primary production could meet the total bacterial carbon demand, and their explanation was that in summer the bacteria utilized PDOC that had accumulated in the water during the spring chlorophyll peak.

In Lake Siggeforasjön, the contribution of PDOC to bacterial production was much higher (87% and 144%, which is well within the range of the non-humic lakes) during summer stratification (August experiments) than in the September experiment (6%), indicating larger utilization of allochthonous, humic substances during autumn conditions. Interestingly, the low contribution of PDOC in the September experiment occurs simultaneously with the lowest rates of $PDO^{14}C$ assimilation (6% of released $PDO^{14}C$ recovered in the bacteria). Tranvik (1989) ob-

tained the highest rates of bacterial production to primary production in a humic lake in autumn, compared to earlier in the summer. Thus, the bacterial assemblage of humic lakes appears to be able to utilize other substrates than recently released PDOC when necessary to keep the production on a high level, but nevertheless seems to prefer PDOC under conditions of high primary production and release of PDOC.

Bacterial growth rates (production/biomass) also showed seasonal trends. The bacterial growth rates tended to be lowest during spring and autumn when the water temperature is lower than during summer stratification. Likewise, Hobbie & Cole (1984) found a close relationship between [^{3}H]thymidine incorporation and water temperature in marine enclosures. Also, Scavia & Laird (1987) found that when water temperatures were lower than 10 °C, bacterial production was positively correlated to the water temperature, but not to primary production. These studies strongly imply that water temperature exerts a major influence on the activity of pelagic communities of heterotrophic bacteria.

It is interesting that, overall, the bacterioplankton in Lake Erken utilized the highest percentage of released PDOC (Table 3). The role of bacteria in pelagic carbon and phosphorus cycling has attracted considerable attention (Vadstein *et al.*, 1988, 1989; Jürgens & Güde, 1990). The common paradigm has been that bacteria, while outcompeting algae for phosphorus, are nonetheless dependent on the algae for carbon, creating a type of mutualism (Bratbak & Thingstad, 1985). This view is being modified since recent evidence suggests that bacteria may at times also be limited by phosphorus (Vadstein *et al.*, 1988). Two lines of evidence suggest that bacterioplankton in Erken may be more limited by carbon than phosphorus. The molar C:P ratio of bacteria in Erken is ≈ 30 (Pettersson & Bell, unpubl.) which, based on chemostat studies, suggests C-limitation (Vadstein & Olsen, 1989). Likewise, from May through August 1989, $\approx 15\%$ of the alkaline phosphatase activity was by the bacterial size-fraction (Pettersson *et al.*, unpubl.) suggesting that it is the phytoplankton that are P-limited. In this context it seems logical that the bacteria in Erken would utilize a higher percentage of PDOC. Perhaps the percentage of released PDOC that is utilized by the bacteria can be an index of the extent to which bacteria are 'C-limited' and of the availability of other carbon sources.

In short, the most striking conclusion in terms of seasonal variations of bacterial dependence of PDOC and total bacterial activity is that the variations within the lakes appear to be at least as large as the variations between the non-humic and the humic lakes. The most important governing factors for these within lakes seasonal variations can probably be found in the seasonal succession of phytoplankton, both in terms of biomass (influencing the quantity of PDOC) and taxonomic composition (influencing both the quantity and quality of PDOC), as well as the seasonal fluctuations of water temperature.

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