Diffusion chamber studies of carbon flux from living algae to heterotrophic bacteria

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Abstract

The flux of recently photosynthetically fixed, dissolved organic carbon (PDOC) from phototrophs to heterotrophic microorganisms was measured directly in microplankton samples from Lake Kinneret using a diffusion chamber technique. The minimum amounts of PDOC incorporated into small <3 μm heterotrophs during a 12 h light period ranged from 0.8 to 38.4 μg(C) l^{-1}, or from 0.2% to 6% of net primary productivity. Although quantitatively small in comparison with total photosynthetic fixation, this PDOC flux could yield about 2 to 3 $\times 10^6$ bacterial cells.ml^{-1} daily. This experimental result is consistent with previous estimates of algal PDOC release in this lake.

Introduction

Much attention has focussed on the release of newly photosynthetically fixed, dissolved organic carbon (PDOC) from living algae (Blaauboer *et al.*, 1982; Bell & Sakshaug, 1980; Fogg, 1958, Sharp, 1977; Meffert & Overbeck, 1977, 1981) and the extent to which this pathway represents a significant source of available substrates for heterotrophic microorganisms in the aquatic environment (Cole *et al.*, 1982; Chrost, 1978; Derenbach & Williams, 1974; Ituriaga & Hoppe, 1977; Mague *et al.*, 1980; Nalewajko *et al.*, 1980). In some situations, especially under environmental stress such as caused by high irradiation or low nutrient levels, the amounts of PDOC released by algae, relative to total photosynthetic fixation, are considerable (Allen, 1973; Berman & Holm-Hansen, 1974; Larsson & Hagstrom, 1979; Wiebe & Smith, 1977). However, in many cases the extent of PDOC release appears to be small, either in terms of absolute amounts of carbon, or compared to total photosynthetic fixation (Coveney *et al.*, 1977; Sheldon & Sutcliffe, 1978; Smith *et al.*, 1977; Thomas, 1971; Waterbury *et al.*, 1979).

In previous studies, levels of PDOC release averaging 3.7% of the photosynthetic fixation in the euphotic zone of Lake Kinneret were reported (Berman, 1976). Subsequently we used a differential filtration technique to check whether these results had been seriously underestimated because of potentially rapid PDOC uptake by small (<3 μm) heterotrophic bacteria and concluded that this was probably not the case (Berman & Gerber, 1980). We now report the results of a series of experiments with diffusion chambers which have enabled us to detect the direct incorporation of PDOC by heterotrophic microorganisms in the near surface waters of this lake.

This method has permitted us to measure the PDOC flux in these waters without separating in time the microorganisms responsible for production and uptake, and without selecting for any particular component either of the phototrophic or

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heterotrophic populations. We have been able to compare estimates of the minimal potential yields of bacteria growing on PDOC derived by this method with those calculated independently from PDOC excretion rates measured directly in Lake Kinneret (Berman 1976). We suggest that this approach could prove useful not only for studies of carbon flux between algae and bacteria, as in the present instance, but also for further investigations of interactions between different size components of planktonic populations.

Methods

The outline of our experiments is shown schematically in Fig. 1. We used glass diffusion chambers (kindly supplied by Dr Z. Dubinsky, Bar Ilan University) which have a total volume capacity of about 600 ml. Each half chamber has separate input ports and a flat, round-glass rim, permitting the two halves to be firmly clamped over a filter or nylon net.

Lake water was taken with a bottle sampler from depths of 1 to 3 m at a representative pelagic station and brought to the laboratory. Part of the sample was filtered under gentle vacuum (~100 mm Hg) through a 3 μm Nuclepore filter and distributed into previously sterilized chamber compartments B, C and D (200 ml) and flasks F and H (20 ml) (Fig. 1). Unfiltered water was added to compartment A (200 ml) and to flasks E and G (20 ml). Compartments A and B were separated by a 0.4 μm Nuclepore membrane while compartments C and D were separate and not in contact with any other chamber. To start the experiment, equal portions of radioactive ^{14}C-bicarbonate (about 0.2 μCi ml^{-1}) were added to chambers A, B, C and D. Flasks E and F, and G and H received 0.1 μCi ml^{-1} of uniformly labelled D-^{14}C-glucose (0.04 nM ml^{-1}) or 0.1 μCi ml^{-1} of ^{3}H-amino acid mixture (approximately 0.4 pM ml^{-1} total amino acids) respectively. Compartments A, B and C were incubated for an initial period (4–10 h) in the light (at 80 to 100 μEin m^{-2}s^{-1}) then in the dark for 10–12 h, and subsequently for about 4 hours in the light. Compartments D, E, F, G and H were incubated in the dark only. All incubations were carried out at room temperature (20–22 ° C) with agitation on a rotary shaker (~80 rpm).

Immediately after isotopic addition and at various time intervals thereafter, duplicate 20 ml samples were withdrawn from chambers A, B, C and D and filtered on presoaked 0.45 μm Millipore filters. After brief fuming in HCl fumes and drying, the radioactivity retained on these filters was counted by liquid scintillation. Duplicate 10 ml portions were taken from flasks E, F, G and H after 3 hours' incubation in order to determine the relative size distribution of heterotrophic (glucose, or amino acid) uptake activity. Also, these experiments gave

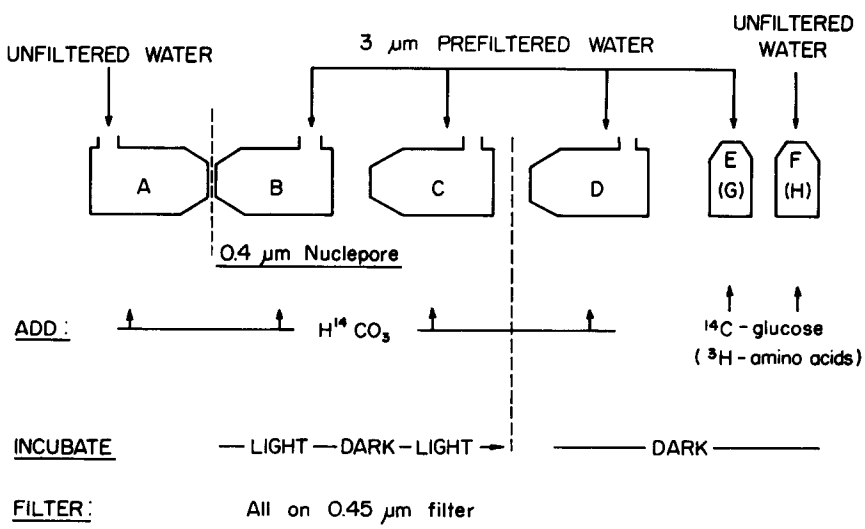

Fig. 1. Schematic outline of experimental design, see text for explanation.

the turnover, or residence, times for these substrates which were calculated by dividing the total dpm of added radioactive material by the dpm of incorporated radioactivity in the microplankton retained on 0.45 μm filters after 3 hours' incubation in the dark. In parallel, the concentrations of chlorophyll in unfiltered and in 3 μm filtered water samples were measured by fluorometry (Holm-Hansen *et al.*, 1965).

By comparing the incorporation of ^{14}C into the total particulate fractions of chambers B and C we could determine if there had been any direct uptake of PDOC ^{14}C released from photoautotrophs in chamber A, by the microorganisms in chamber B. Any dissolved compound released by organisms in chamber A would be diluted by half as it diffused across the membrane and became evenly distributed between A and B at equilibrium. Because of this, at equilibrium, in comparison to heterotrophic microorganisms in control chamber C, those in chamber B would have *at most*, only one half of the concentration of released PDOC available to them, assuming that PDOC uptake was much less rapid than diffusion across the membrane (see below). We therefore corrected for this effect by doubling the difference in counts observed between chamber B and chamber C i.e. $(cmp_B - cmp_C) \times 2$. Chamber D served as dark control and gave a measure of dark fixation of ^{14}C-bicarbonate by organisms < 3 μm.

Results and discussion

Our results are based on an experimental series which covers a complete limnological cycle in Lake Kinneret. In Table 1 we show the percentage of chlorophyll, photosynthetic and heterotrophic incorporation associated with organisms which passed through 3 μm Nuclepore filters, relative to the total microplankton. As previously noted (Berman & Gerber, 1980; Berman & Stiller, 1977) the majority of the chlorophyll and photosynthetic activity in the trophogenic zone of this lake was attributed to phytoplankton larger than 3 μm, in contrast to many oceanic situations (Berman, 1975; Waterbury *et al.*, 1979). The highest proportions of chlorophyll and primary production associated with <3 μm microorganisms occurred towards the end of the annual dinoflagellate bloom of *Peridinium cinctum* (early June) and immediately after destratification (in December), when netplankton metabolism was minimal and nanoplankton were most active (Pollingher & Berman, 1978).

In contrast to the photosynthetic organisms, the majority of heterotrophs active in assimilating amino acids and glucose passed through the 3 μm filters. Thus heterotrophic microorganisms present in chamber B should have been capable of taking up any suitable substrates released as PDOC from the photosynthesizing algae in chamber A provided that no extreme depletion of the substrates oc-

Table 1. Percentage of chlorophyll, photosynthetic and heterotrophic incorporation associated with microorganisms <3 μm, in near-surface waters of Lake Kinneret. Also shown (in parentheses) are turnover times for amino acids and glucose in non-fractionated samples.

Date	Chlorophyll %	Photosynthesis[a] %	Heterotrophy[b]			
			Amino acids		Glucose	
			%	(h)	%	(h)
9 Apr. 80	3.3	4.0	–	–	53	(27)
4 May 80	1.3	1.3	44	(81)	–	–
2 Jun. 80	13.3	19.4	85	(60)	–	–
15 Jun. 80	22.7	14.7	88	(21)	–	–
17 Jun. 80	7.9	5.2	86	(30)	61	(38)
29 Jun. 80	2.7	1.6	90	(18)	74	(16)
12 Oct. 80	4.6	6.8	85	(16)	80	(20)
27 Oct. 80	2.2	3.4	–	–	–	–
29 Oct. 80	16.8	26.5	93	(78)	93	(115)
8 Jan. 81	13.6	5.3	87	(116)	91	(130)
12 Feb. 81	4.0	2.8	54	(72)	66	(79)

[a] Measured after light exposure of 3 to 5 hours.

[b] Uptake measured in the dark. Turnover times (hours) given in parentheses.

curred in chamber A prior to diffusion across the membrane (see below). Similarly to previous observations in Lake Kinneret (Berman & Gerber, 1980; Berman *et al.*, 1979) the turnover times for amino acids and glucose were relatively long, averaging 55 hours and 61 hours respectively (Table 1). Generally turnover times for amino acids were more rapid than those for glucose.

In most, but not all, experiments, we found some stimulation of ^{14}C incorporation in chamber B which could be attributed to heterotrophic uptake of the PDOC released by algae in chamber A (i.e. c.p.m. in chamber B > c.p.m. in chamber C). The time courses of two experiments are shown in Fig. 2.

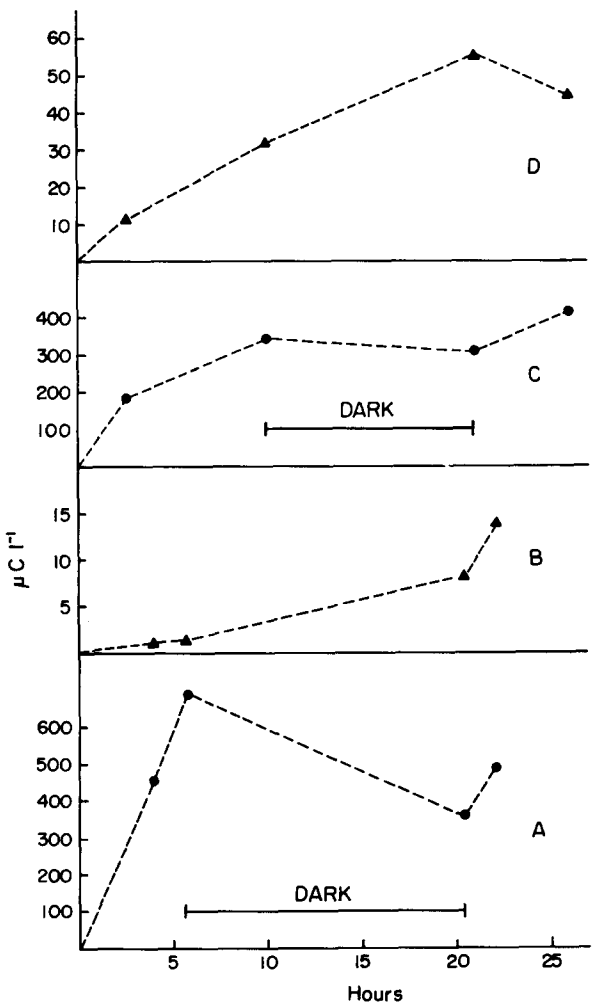

Fig. 2. Time course of net incorporation of carbon into the particulate fraction in Chamber A (●--●) and uptake of PDOC by <3 μm microorganisms (▲--▲). A and B: - experiment of 9 April 1980. C and D: - experiment of 15 June 1980.

Table 2. Total algal net photosynthesis and minimal estimates of PDOC incorporated by small (<3 μm) heterotrophs compared to total algal net photosynthesis.

Date	12 hour light*		Subsequent 12 hour dark
	Phytoplankton photosynthesis μg (C).l^{-1}	PDOC uptake* μg (C).l^{-1}	PDOC uptake μg (C).l^{-1}
9 Apr. 80	1300	2.6 (0.2)	5.4 (0.4)
4 May 80	480	1.0 (0.2)	not measured
2 Jun. 80	198	no effect	(c.p.m. in B=c.p.m. in C)
15 Jun. 80	640	38.4 (6.0)	23.6 (3.6)
17 Jun. 80	383	4.6 (1.2)	not measured
29 Jun. 80	591	7.1 (1.2)	3.3 (0.5)
12 Oct. 80	182	10.9 (6.0)	15.9 (8.0)
27 Oct. 80	284	7.4 (2.6)	4.1 (1.4)
29 Dec. 80	210	1.3 (0.6)	0.9 (0.4)
8 Jan. 81	130	0.8 (0.6)	not measured
12 Feb. 81	240	no effect	(c.p.m. in B=c.p.m. in C)

* In parentheses, the PDOC uptake is given as a percentage of the net photosynthesis during 12 hours light. All values were recalculated for 12 hours light and 12 hours dark from actual experimental exposure times.

We summarize in Table 2 the results of eleven experiments in this series, nine of which indicated a definite flux of PDOC to <3 μm heterotrophs. In this table, we list the daily rates of phytoplankton photosynthesis and compare these to minimum estimates of PDOC (as μg (C) l^{-1}) assimilated by <3 μm heterotrophs during 12 hours of light and in a subsequent 12 hours dark period. Both as a percentage of the total primary production and as total carbon the amounts of PDOC flux were usually low (e.g. experiment of 9 April 1980, Figs. 2A and B). An exception to this was provided by the experiment of 15 Jun 1980 (Figs. 2C and D) which was made with a water sample taken towards the end of the *Peridinium* bloom when senescent dinoflagellate cells release relatively greater amounts of PDOC (Berman, 1975) and maximum heterotrophic uptake rates are observed (Berman *et al.*, 1979; Cavari & Hadas, 1979). At this time, a combination of physical and chemical factors causes the rapid decline of *Peridinium* in the lake (Pollingher & Berman, 1978). Most of the dinoflagellate cells are degraded in the water column (Hertzig *et al.*, 1981) with a concomitant release of dissolved organic matter and particulate detritus.

With increasing time, we usually found a continuous rise in the amount of PDO^{14}C taken up by the <3 μm fraction (Fig. 2). In our experience, we have

often found that release of PDOC by algae continues in the dark, although this does not always appear to be the case (Mague *et al.*, 1980).

The diffusion chamber technique which we used has enabled us to demonstrate clearly that a flux of PDOC does occur from photosynthetic organisms to heterotrophs of dimensions smaller than 3 μm. For several reasons, the quantitative measures of PDOC uptake presented in Table 2 must be regarded as minimal estimates. A major difficulty in achieving an absolute measurement is due to uncertainty about the rate at which the PDOC pool in the system (bicarbonate → algal intracellular carbon → PDOC → heterotroph cellular carbon) reaches, or approaches, isotopic equilibrium. This problem has bedevilled PDOC studies with radioactive ^{14}C tracers since their inception (see, for example, Berman, 1975). If the specific activities of the carbon pools were very different then considerable underestimation of PDOC flux could have occurred. However, as the PDOC pool in Lake Kinneret is generally a small fraction (<5%) of the photosynthetically incorporated carbon, and as the turnover of many algal carbon pools appears to be relatively rapid (less than 3 to 4 hours; Berman & Gerber, 1980; Mague *et al.*, 1980) this underestimate may not be excessive.

Another potential problem is the delay time for PDOC molecules passing from chamber A to chamber B across the 0.4 μm Nuclepore membranes. We found experimentally that, when solutions of ammonia, nitrate, glucose or amino acids were added to one side of the membrane, full equilibrium in both chambers was obtained only after 3 to 3.5 hours, even with shaking. The multiplication factor of 2 which was used to correct for the concentration of PDOC in the combined volumes of chambers A and B relative to chamber C, would be valid only if equilibrium was established across the membrane instantaneously. Since this is not the case, heterotrophic microorganisms in A would tend to deplete the released PDOC. We note, however, that in Lake Kinneret the measured turnover times (Table 1) for amino acids and glucose (which were presumably among the most readily available substrates) were always considerably longer (on average, ten- to twenty-fold) than the time required for substrate equilibrium to be established between chambers A and B. This would indicate that the error due to the non-instantaneous diffusion time might not be greater than about 10%. Clearly our present techniques would need to be modified for environments where the residence times for organic substrates are much shorter (Allen, 1978).

Table 3. Minimal estimated yields of bacteria (cells × 10^5) ml^{-1} from D PDOC flux[a].

Date	12 h light		Subsequent 12 h dark	
	minimum	corrected[b]	minimum	corrected
9 Apr. 80	4.1	7.7	8.9	16.8
4 May 80	1.2	2.7	not measured	
15 Jun. 80	64.0	72.7	39.3	44.6
17 Jun. 80	7.7	9.0	not measured	
29 Jun. 80	11.8	13.1	5.5	6.1
12 Oct. 80	18.1	21.2	6.5	7.6
27 Oct. 80	12.3	–	6.8	–
29 Dec. 80	2.2	2.4	1.5	1.6
8 Jan. 81	1.3	1.5	not measured	

[a] Calculated on the basis of a carbon content per bacterium of 6×10^{-15} μg (C).

[b] Correction based on % distribution of amino acid heterotrophic uptake in >3 μm abd <3 μm particulate fractions. (When amino acid data was unavailable, glucose uptake results were used.)

Heterotrophic uptake by clumped bacteria or microorganisms larger than 3 μm in chamber A could also lead to underestimation of the PDOC flux in these experiments. As previously shown (Table 1) the >3 μm heterotrophic fraction was usually responsible for only 20% or less of the total glucose or amino acid uptake. If the proportional uptake of PDOC by <3 μm and >3 μm heterotrophic was similar to that for amino acids or glucose (Table 1), then a correction for the incorporation due to the >3 μm fraction can be made (Table 3). No growth attachment of bacteria on the Nuclepore membrane surfaces was observed when we examined these microscopically at the end of several experiments. In view of the above considerations we suggest that neither the delayed diffusion of compounds across the Nuclepore membrane nor the uptake of organics by heterotrophs in chamber A, caused a severe underestimation of the PDOC fluxes as given in Table 2.

Our results were derived from measurements made under optimum light intensities (i.e. at, or close to, maximum photosynthetic levels) and thus probably reflect the maximum amounts of PDOC flux in the water column. With decreasing photosynthesis, the quantities of released PDOC usually

decline, although there may be an increase in the percentage extracellular release (PER) of PDOC relative to net photosynthetic fixation (Berman, 1976). Also PER tends to increase when algae are inhibited by high light intensities but this situation is generally limited to a very small portion (0–1 m) of the euphotic water column which ranged from 10 to 15 m.

From the values of PDOC uptake measured in these experiments, and considering the limitations noted above, some estimations could be made of the minimum numbers of bacterial cells which might be derived from this source of organic carbon in near surface waters of the lake (Table 3). If we assume an average carbon content of 6×10^{-15} g(C) per bacterium (Hobbie & Wright, 1979), the uptake of PDOC (Table 2) would account for a total heterotrophic bacterial production averaging 2×10^6 cells $ml^{-1}.12\ h^{-1}$, with a 50% to 100% increase in the subsequent 12 hour dark period. (The incorporation of PDOC only into <3 μm microorganisms would average 1.4×10^6 cells.$ml^{-1}.12\ h^{-1}$.) Note that we have estimated net PDOC incorporation and have no measure of PDOC respired or recycled by heterotrophs. (Unfortunately we did not have an epifluorescent microscope available which would have enabled us to follow changes of bacterial cell numbers directly in chambers B and C.)

These numbers may be compared to potential bacterial yields from PDOC in Lake Kinneret calculated from previous data for primary productivity (Pollingher & Berman, 1978) and algal extracellular release (Berman, 1976). At optimum light depth, the average daytime net photosynthetic carbon fixation was approximately 300 μg (C).$l^{-1}.d^{-1}$ of which about 4% was released as PDOC. Depending on the respiration of this PDOC (reported to be ~5% (Schleyer, 1980; Wiebe & Smith, 1977) or about 30% (Bell & Sakshaug, 1980), this would imply a potential incorporation into heterotrophic biomass of between 8.4 to 11.4 μg (C)$l^{-1}d^{-1}$ or approximately 1 to 2×10^6 cells.$ml^{-1}.d^{-1}$ which is close to our experimentally derived results.

Clearly the present data are insufficient to quantify all parameters of the PDOC flux in Lake Kinneret. In common with most studies which have examined the release of newly photosynthesized organic carbon from living algae and its subsequent uptake, our results were based on a few, relatively short-term observations following an isotope pulse of dissolved inorganic ^{14}C. For most natural situations the more or less continuous release of PDOC is accompanied by a concomitant heterotrophic uptake (Smith & Higgins, 1978) although there may not always be short-term synchrony between the two processes (Mague *et al.*, 1980). The kinetic coefficients of release and uptake will then determine the ambient concentration of PDOC at any time. For non-steady state systems, such as in our single isotope pulse experiments, it is impossible to determine statistically satisfactory coefficients without increased sampling times and information on labelling patterns of the major carbon pools (Volohonsky, pers. comm.). Some of these difficulties may be overcome with the use of continuous flow diffusion chambers which are currently being developed (Olie, pers. comm.).

The diffusion chamber technique as described here has given us a direct approximation of the minimal quantities of PDOC taken up by heterotrophs in Lake Kinneret. The bacterial carbon biomass or cell yields which might be derived from these amounts of PDOC appear to be consistent with those calculated independently from measurements of PDOC release *in situ*. Our studies for this eutrophic lake environment give a picture similar to that proposed by Mague *et al.* (1980) for the role of PDOC in the marine waters of the Gulf of Maine, and Cole *et al.* (1982) for oligotrophic Mirror Lake. Quantitatively, the amounts of carbon released as PDOC are generally small compared to photosynthetic fixation. However, in the euphotic zone of Lake Kinneret this carbon source can at times support, perhaps in a selective manner (Bell *et al.*, 1975), significant populations of heterotrophic bacteria.

Summary

1. A diffusion chamber technique was developed to study the flux of PDOC from natural populations of living algae to heterotrophic bacteria.
2. In nine out of eleven experiments, covering a complete limnological cycle in Lake Kinneret, a significant uptake of PDOC by small (<3 μm) bacteria was detected. Minimal values for PDOC incorporated by bacteria ranged from 0.2 to 6.0% of the photosynthetically fixed carbon over a 12 hour light period. The release and uptake of

PDOC also continued during a subsequent dark period. In terms of carbon a minimum estimate for net PDOC uptake ranged from 0.8 to 38.4 μg (C).l^{-1}.12 h^{-1}.

3. These amounts of PDOC flux might account for a total heterotrophic bacterial production averaging about 2×10^6 cells.ml^{-1}.12 h^{-1} with a 50% to 100% increase in the subsequent dark period.
4. Calculations of PDOC flux based on previous direct measurements of algal extracellular release (Berman, 1976) gave values (8.4 to 11.4 μg (C).l^{-1} d^{-1}) compatible to those obtained with the diffusion chamber technique.
5. In Lake Kinneret, the majority of chlorophyll and photosynthetic activity in the euphotic zone was usually associated with organisms >3 μm. Small (<3 μm) organisms (mainly bacteria) were generally responsible for >80% of heterotrophic uptake.
6. Turnover times for model organic substrates (glucose and amino acids) were on average about ten to twenty fold longer than the time required for equilibrium to be reached in the diffusion chambers (3 to 2.5 hours). This characteristic minimised the problems associated with non-instantaneous concentration equilibrium in the experimental system. Some underestimation of PDOC flux could be due to non achievement of isotopic equilibrium.
7. In comparison to total photosynthetic carbon fixation in Lake Kinneret the amount of PDOC passing to heterotrophs is relatively low. Nevertheless, significant populations of heterotrophic bacteria could be supported on these organic substrates, which may act in a selective manner.

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