Phytoplankton exudates
Size fractionation
Exsudats planctoniques
Fractionnement en taille

Photosynthesis and bacterial utilization of phytoplankton exudates: results from pre- and post-incubation size fractionation

B. B. Ward
Institute of Marine Resources, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA.

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ABSTRACT

Size fractionation $^{14}CO_2$ experiments were used to investigate the pathways of carbon assimilation in the small (<1 μm) fraction of natural plankton populations. Label distribution among the fractions provided evidence for the transfer of recently incorporated labeled carbon from "phytoplankton" to "bacterial" fractions. In the <1 μm fraction, both photosynthetic CO_2 assimilation and heterotrophic assimilation of labeled exudates contributed significantly to carbon assimilation, comprising up to 18 and 33% of the whole sample assimilation, respectively.

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RÉSUMÉ

Photosynthèse et utilisation bactérienne des exsudates planctoniques : résultats des fractionnements en taille avant et après l'incubation

L'assimilation du carbone par le plancton de taille inférieure à 1 μm a été étudiée par fractionnement en taille et incubation $^{14}CO_2$. La répartition obtenue a mis en évidence le transfert du carbone récemment incorporé du « phytoplancton » aux fractions « bactériennes ». Au-dessous de 1 μm, l'assimilation de CO_2 photosynthétique et l'assimilation hétérotrophique, des exsudats contribuent de manière significative à l'assimilation du carbone, jusqu'à 18 et 33% respectivement.

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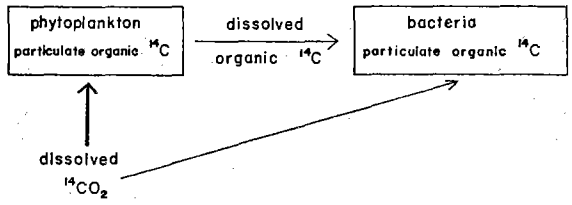
INTRODUCTION

The radiocarbon tracer method is sensitive and versatile for the study of carbon assimilation pathways in aquatic communities (Carpenter, Lively, 1980; Peterson, 1980). Size fractionation in conjunction with $^{14}CO_2$ incubations has been used to determine the distribution of label incorporation among various size (and perhaps trophic) components of the natural plankton assemblages (Derenbach, Williams, 1974; Chrétiennot-Dinet, Vacelet, 1978; Larsson, Hagström, 1979; 1982; Jordan, Likens, 1980; Platt *et al.*, 1983; Li *et al.*, 1983). While some ^{14}C-labeled constituents appear in all fractions after incubation with $^{14}CO_2$, the labeled compound actually assimilated may not always be $^{14}CO_2$. The autotrophic or heterotrophic nature of carbon assimilation pathways in the "picoplankton" fraction has been debated (Platt *et al.*, 1983; Li *et al.*, 1983). Although debate surrounds its nature and significance, release of newly synthesized organic compounds by actively photosynthesizing phytoplankton is well documented at least under some conditions (Hellebust, 1965; Sharp, 1977; Sharp *et al.*, 1980; Mague *et al.*, 1980). Immediate assimilation of this labeled exudate by heterotrophic bacteria results in indirect labeling of the "bacterial" fraction in size fractionation experiments utilizing post-incubation fractionation (Larsson, Hagström, 1979). Results of such experiments imply that substantial but variable fractions of photosynthetically-fixed $^{14}CO_2$ are released as dissolved organic compounds and are rapidly assimilated by bacteria (Derenbach, Williams, 1974: 1 to 30% of primary production; Larsson, Hagström, 1979: 32% of primary production on an annual basis; Jordan, Likens, 1980: 16 to 34% of net primary production). Bell and Sakshaug (1980) demonstrated differences in use of phytoplankton exudates by bacte-



ASSIMILATORY PATHWAY	FRACTION W_L	W_D	$<1\mu m_{L\,post}$	$<1\mu m_{L\,pre}$	$<1\mu m_{D\,post}$	$<1\mu m_{D\,pre}$
> 1µm photosynthesis	+					
< 1µm photosynthesis	+		+	+		
> 1µm dark CO_2 assimilation	+	+				
< 1µm dark CO_2 assimilation	+	+	+	+	+	+
assimilation of exudate from > 1µm photosynthate	+		+			
assimilation of exudate from < 1µm photosynthate	+		+	+		

Figure

Top: possible routes of ^{14}C incorporation into "bacterial" and "phytoplankton" fractions of natural populations.
Bottom: CO_2 assimilation pathways and their occurrence in fractions from parallel pre- and post-incubation fractionation experiments.

ria depending on what algal species produced the exudates. Sharp (1977) concluded that many of the higher values and much of the scatter in algal exudate-based heterotrophic production result from experimental techniques and handling problems. Smith *et al.* (1977) and Mague *et al.* (1980) however, concluded from careful field and laboratory experiments that extracellular release is a normal physiological aspect of photosynthesis.

Two main pathways for incorporation and transfer of label among fractions are possible (Fig.). In a routine productivity bottle, $^{14}CO_2$ incorporation could occur *via*: 1) photosynthesis by a wide size spectrum of organisms; 2) assimilation by heterotrophic microorganisms of soluble organic materials released into the medium by actively photosynthesizing phytoplankton; 3) "dark" fixation reactions including chemoautotrophic and anapleuratic CO_2 incorporation. Only the "dark" fixation reactions would occur in both light and dark bottles. In the light, the main route of label incorporation is $^{14}CO_2$ uptake by phytoplankton and then organic ^{14}C-carbon exudate uptake by bacteria. In the dark, phytoplankton production of labeled exudates is greatly reduced or zero and assimilation of $^{14}CO_2$ is the only mode of label incorporation by all fractions. The experiments described here were designed to distinguish among these pathways and to sort out the relative contribution of carbon assimilation by autotrophic and heterotrophic groups in the smallest size fraction. Size fractionation in light and dark bottle $^{14}CO_2$ experiments was used to: 1) compare incorporation of label by the <1.0 µm ("bacterial") fraction directly from added $^{14}CO_2$ and indirectly from *in situ* synthesized organic carbon; and 2) to compare the contribution of photosynthesis in the >1.0 µm fraction to photosynthesis and exudate assimilation in the <1.0 µm fraction.

Interpretation of the following size fraction data requires the assumption that fractionation by filtering does not alter the size composition of the incubated population nor change its viability. The assumption that size fractionation is complete and can be accomplished along trophic divisions is unnecessary in the parallel pre- and post-incubation fractionations because photosynthetic and heterotrophic carbon metabolism in the smallest size fraction can be independently distinguished (*see* "Methods"). In addition, the experimental design employed here assumes that heterotrophic bacterial metabolism is not influenced by light.

Experimental results from several marine environments presented here show that both photosynthetic CO_2 assimilation and heterotrophic assimilation of exudates by the <1.0 µm fraction contributed significantly (up to 20 and 30%, respectively) to whole sample carbon assimilation in routine $^{14}CO_2$ uptake experiments.

METHODS

Cruise and station location information is provided in the list of experiments (Tab. 1).

Radiocarbon tracer technique

Radioisotope stocks ($NaH^{14}CO_3$) were prepared from liquid (New England Nuclear) by the methods of Anderson and Zeutschel (1970). Incubation conditions are listed in Table 1. At the termination of incubations, samples were collected on 0.22 µm 25 mm diameter filters (Millipore) (after fractionation as described below) in blackened Pyrex filter holders in semi-darkness. Filters were rinsed twice (10 ml total) with filtered seawater and held over fuming HCl for 10 sec. and placed in glass scintillation vials containing 10 ml scintillation fluor. Samples were counted on an ISOCAP/300 (Nuclear Chicago, experiments 1 and 2), a Packard Tri-Carb (experiments 3 through 11) or a Beckman LS-100C (experiments 13, 14 and 15) scintillation counter. Counting efficiency was determined by the channels ratio method. Carbon assimilation ($\mu g\,C\,l^{-1}$) was calculated using the equation of Strickland and Parsons 1972).

Pre-incubation size fractionation

Twenty liters of seawater were collected using a surface bucket or a large volume plastic sampling bottle and transferred to a 25-l plastic carboy. With mixing between subsampling, replicate aliquots (about 2 l) were removed from the carboy by siphon and filtered by vacuum (less than 100 mm Hg) through 1.0 µm 47 mm diameter Nuclepore filters (several parallel fractions ranging from 0.6 µm to 202 µm were prepared in some experiments, but 1.0 µm was the cutoff used for most experiments to distinguish "phytoplankton" and "bacterial" fractions). Fractioned and unfractioned samples

Table 1

List of experiments: a) percent of surface radiation except experiment 3 which was incubated in front of artificial lights; b) samples collected off the dock at Friday Harbor Laboratories, San Juan Island, Washington; c) samples collected in the central basin of Puget Sound, Washington; d) cruise W8009C of the Oregon State University RV Wecoma, several stations on the Washington shelf; e) samples collected in the northern end of Dabob Bay, Puget Sound, Washington; f) experiments from cruise SCBS-22 in the Southern California Bight.

Experiment	Station		Date	Depth (m)	Incubation Light Intensity[a]	Length of Incubation (h)	Specific Activity (μCi/ml added)
	Lat. (N)	Long. (W)					
Pre-incubation Fractionation							
1[b]	48°32.7	123°00.5	29 JUN 80	0	100	8	0.0656
2[b]	48°32.7	123°00.5	5 JUL 80	0	100	8	0.0656
3[c]	48°6	122°22	19 AUG 80	2		6	0.2626
4[d]	47°21.0	125°05.0	26 SEP 80	0	100	24	0.0486
5[d]	47°32.0	124°32.0	2 OCT 80	2	100	24	0.0486
6[e]	47°45	122°49	7 APR 81	2	100	24	0.1313
Post-incubation Fractionation							
7[d]	47°07.3	124°57.4	6 OCT 80	2	100	24	0.0304
8[d]	47°07.3	124°57.4	6 OCT 80	50	3	24	0.0304
9[d]	47°13.2	124°30.0	9 OCT 80	2	100	24	0.0304
10[d]	47°13.2	124°30.0	9 OCT 80	20	3	24	0.0304
11[e]	47°45	122°49	7 APR 81	2	100	24	0.0328
Simultaneous pre- and post-incubation Fractionation							
12[e]	47°45	122°49	3 APR 82	2	100	24	0.0262
13[f]	33°39.0	118°35.0	19 MAY 83	2	100	8	0.0196
14[f]	33°30.0	118°37.0	24 MAY 83	2	100	8	0.0196
15[f]	33°30.0	118°37.0	24 MAY 83	35	8	8	0.0196

were distributed into 125 ml Pyrex bottles, 3 light and 3 dark for each fraction. Incubation bottles had been washed with soap, rinsed with tap water, then washed with 10% HCl and rinsed with tap and then distilled water. ^{14}C-bicarbonate was added to the bottles, which were then incubated under simulated *in situ* light conditions in running seawater incubators for 6 to 24 h (Tab. 1). One exception is experiment 3 which was incubated in front of artificial lights in a 20°C constant temperature room.

Post-incubation size fractionation

Twenty or more liters of seawater were collected using large volume plastic sampling bottles and transferred to plastic carboys. After mixing, 4-1 replicate subsamples were measured out and placed in 4.6-1 pyrex bottles, cleaned as described above. ^{14}C-bicarbonate solution was added to the bottles and the bottles placed in running seawater deck incubators for 24 h simulated *in situ* incubations. When samples from subsurface depths were used, light attenuation was achieved with neutral density screening (Perforated Products, Inc., Brookline, MA). Parallel filtration of aliquots from the 4-1 bottles through Nuclepore filters as described above was performed at the end of 24 h incubations.

Parallel pre- and post-incubation size fractionation

At 4 stations (experiments 12-15) the above procedures were combined for parallel experiments on replicate samples. For these samples, the following rates can be determined:

1) photosynthetic CO_2 assimilation by the whole sample = $W_L - 1\,\mu m_{D_{pre}}$;

2) photosynthetic CO_2 assimilation by the <1.0 μm fraction = $1\,\mu m_{L_{pre}} - 1\,\mu m_{D_{pre}}$;

3) nonphotosynthetic CO_2 assimilation by the >1.0 μm fraction = $W_D - 1\,\mu m_{D_{pre}}$;

4) nonphotosynthetic CO_2 assimilation by the <1.0 μm fraction = $1\,\mu m_{D_{post}} = 1\,\mu m_{D_{pre}}$;

5) exudate assimilation by the <1.0 μm fraction = $1\,\mu m_{L_{post}} - 1\,\mu m_{L_{pre}}$;

6) photosynthetic CO_2 assimilation by the >1.0 μm fraction (by difference) = $W_L - 1\,\mu m_{L_{pre}}$;

where W_L = incorporation by the unfractioned sample in the light;

W_D = incorporation by the unfractioned sample in the dark;

$1\,\mu m_{D_{pre}}$ = incorporation by the <1.0 μm fraction (fractionated before incubation) in the dark;

$1\,\mu m_{L_{pre}}$ = incorporation by the <1.0 μm fraction (fractionated before incubation) in the light;

$1\,\mu m_{D_{post}}$ = incorporation by the <1.0 μm fraction (fractionated after incubation) in the dark;

$1\,\mu m_{L_{post}}$ = incorporation by the <1.0 μm fraction (fractionated after incubation) in the light.

Fractionation and filtration of these samples were as described above for pre- and post-incubation fractionation experiments.

Chlorophyll analysis

Chlorophyll analyses by the fluorometric technique (Lorenzen, 1966) were performed on replicate subsamples of all fractions involved in these experiments. In most cases, analyses were performed within an hour of sample collection. For experiments 3 and 12 through 15, samples were filtered, the filters frozen and analyzed upon return to the laboratory.

RESULTS

Chlorophyll distribution

Although the distribution of chlorophyll among several size fractions was measured in some experiments, 1.0 μm was chosen as the most consistently meaningful cutoff. The total chlorophyll *a* concentration and the percent of chlorophyll found in the >1.0 μm and <1.0 μm fractions for all experiments is shown in Table 2.

Table 2
Percent of chlorophyll a found in ""phytoplankton" fraction of size fractionation experiments.

Expetiment	Cut-off (μm)	Total chlorophyll *a* ($\mu g\ l^{-1}$)	Percent of chlorophyll *a* in"phytoplankton" fraction
1	1.0	1.05	97.1
2	1.0	3.61	94.5
3	3.0	2.11	91.7
4	1.0	.38	86.8
5	0.6	.95	100.0
6	1.0	4.71	98.3
7	1.0	.38	83.3
8	1.0	.36	94.5
9	1.0	6.60	97.3
10	1.0	.49	89.8
11	1.0	4.71	98.3
12	1.0	2.59	95.7
13	1.0	.12	88.4
14	1.0	.11	68.1
15	1.0	.41	76.5

Size fractionation hypotheses

The following hypotheses concerning phytoplankton production of labeled exudates and their assimilation by heterotrophic bacteria were tested in the size fraction experiments reported here. The components of carbon assimilation found in the various fractions are listed in the Figure, and the direct test of each hypothesis is listed in terms of these components following its verbal description. 1) Bacterial assimilation of CO_2 is unaffected by light: *i.e.*, $1\ \mu m_{D_{pre}} = 1\ \mu m_{L_{pre}}$. 2) Bacteria are capable of assimilating organic material released by phytoplankton during photosynthesis, and this pathway of transfer is light dependent because of the light dependence of photosynthesis: *i.e.*, $1\ \mu m_{L_{post}} > 1\ \mu m_{D_{post}}$. 3) In natural assemblages, bacteria assimilate both phytoplankton exudates and CO_2: *i.e.*, $1\ \mu m_{L_{post}} > 1\ \mu m_{L_{pre}}$. Photosynthesis may also occur in the "bacterial" fraction if microphytoplankton forms are present (Waterbury *et al.*, 1979; Li *et al.*, 1983; Platt *et al.*, 1983). 4) Bacteria are responsible for most of the dark CO_2 assimilation observed in natural assemblages: *i.e.*, $1\ \mu m_{D_{pre}} > W_D - 1\ \mu m_{D_{pre}}$.

Pre-incubation size fractionation

The Wilcoxon Rank Sum was used to test the null hypothesis that the $^{14}CO_2$ assimilation by the "bacterial" fraction in the dark equals that in the light. In 4 of the 6 cases (Tab. 3) the null hypothesis cannot be rejected, *i.e.*, CO_2 assimilation by the small size fraction is due to a light insensitive process ($1\ \mu m_{D_{pre}} = 1\ \mu m_{L_{pre}}$). In the two cases where light uptake by the "bacterial" fraction exceeded the dark uptake, the presence of significant chlorophyll in the <1.0 μm fraction (Tab. 2) suggests that active photosynthesis by small phytoplanktonic forms occurred in this fraction. Since post-incubation fractionation was not done on replicate samples, photosynthetic and heterotrophic contributions in the <1.0 μm fraction cannot be distinguished (*see* below for parallel pre- and post-incubation fractionation results).

Table 3
*Results (mean of three replicates) from pre-incubation fractionation experiments: * = significant difference ($p < .05$) between light ($1\ \mu m_{L_{pre}}$) and dark ($1\ \mu m_{D_{pre}}$) values.*

Experiment	^{14}C assimilation by "bacterial" fraction ($\mu g\ C\ l^{-1}$)	
	$1\ \mu m_{L_{pre}}$	$1\ \mu m_{D_{pre}}$
1	2.14	2.67
2	2.32	2.46
3	0.27*	0.22
4	2.96*	0.45
5	4.79	3.59
6	0.58	1.47

Post-incubation size fractionation

The null hypothesis that there is no difference between dark and light incorporation of label in the "bacterial" fraction is contradicted in 3 of 5 cases (Tab. 4); *i.e.*, label assimilation by the "bacterial" fraction is enhanced by uptake of labeled exudates in the light ($1\ \mu m_{L_{post}} > 1\ \mu m_{D_{post}}$). In the other two cases where light assimilation of label by the "bacterial" fraction was less than dark assimilation, the difference was quite small and very likely due to experimental error at such low measurement levels.

Parallel pre- and post-incubation size fractionation

Carbon assimilation rates via photosynthetic and non-photosynthetic pathways in the whole sample and 2 fractions for the parallel experiments are shown Table 5. In all cases, most or all of the photosynthetically fixed carbon was associated with the >1.0 μm fraction. At the Southern California Bight stations, significant amounts of chlorophyll were measured in the <1.0 μm fractions and significant rates of photosynthetic CO_2 assimilation also occurred in this fraction. Assimilation of labeled exudates by the <1.0 μm fraction was found in all 4 parallel experiments (*i.e.*, $1\ \mu m_{L_{post}} > 1\ \mu m_{L_{pre}}$). This assimilation equaled up to 33% of the whole sample photosynthetic CO_2 assimilation. Photosynthetic CO_2 assimilation in the <1.0 μm fraction was significant only at the Southern California Bight stations and equaled less than 20% of the whole

sample photosynthesis. Nonphotosynthetic CO_2 assimilation (Tab. 5) was generally negligible in the >1.0 μm fraction but equaled almost 20% of whole sample photosynthesis in the <1.0 μm fraction in some experiments, *i.e.*, $1\,\mu m_{D_{pre}} > W_D - 1\,\mu m_{D_{pre}}$.

DISCUSSION

The enhancement of light over dark label incorporation into the <1.0 μm fraction of post-incubation fractionation experiments in 3 cases provides support for the hypothesis that the "bacterial" fraction indirectly assimilated $^{14}CO_2$ *via* exudates released during routine bottle incubations. The portion of label incorporation in the light that can be attributed to photosynthesis plus uptake of secondarily labeled substrates in these 3 examples equaled 3.5 to 36% of the whole sample light incorporation (Tab. 4). In pre-incubation fractionation experiments, the 4 cases where label assimilation by the <1.0 μm fraction was equal in light and dark incubations show that this enhancement did not occur when the source of the exudates was removed prior to incubation. The cases (experiments 3, 4, 7, 8) where results were not in agreement with the critical hypotheses had in common that the samples all had very low productivity levels and low chlorophyll concentrations. In the case of pre-incubation fractionation, enhanced label uptake by the <1.0 μm fraction in the light is probably indicative of active phytoplankton photosynthesis in this fraction. There is no evidence for a direct effect of light on bacterial heterotrophic metabolism (Williams, Yentsch, 1976). In the post-incubation fractionation experiments 7 and 8, incorporation by the <1.0 μm fraction in the light was less than in the dark. This result might imply inhibition by light of some CO_2 fixing reaction (Eisenstark, 1971; Horrigan *et al.*, 1981); however, these data alone are not sufficient to explain this observation. Primary productivities in these 2 experiments were among the lowest encountered in this study; negative values here probably reflected combined errors in the component measurements, rather than real phenomena.

Parallel pre- and post-incubation fractionations allowed for direct comparison of label incorporation by the <1.0 μm fraction in the presence and absence of >1.0 μm photosynthetic organisms and in the light and dark. Label incorporation which was attributed to assimilation of photosynthetically produced exudates (secondarily labeled substrates) was detected in all experiments at a level equivalent to 5 to 33% of the whole sample photosynthetic rate. Photosynthetic CO_2 assimilation in the <1.0 μm fraction was also detected in most cases, in rough agreement with the increasing proportion of chlorophyll found in the <1.0 μm fraction [in the pre-fractionated 1 μm samples, the quantity $(1\,\mu m_{L_{pre}} - 1\,\mu m_{D_{pre}})$ included not only photosynthesis by the <1 μm fraction, but also assimilation of photosynthate produced by the <1 μm phytoplankton during the incubation. These two processes cannot be distinguished in this experimental design, but the latter is probably quite small (a few percent of photosynthesis by the <1 μm fraction, in analogy to the relative importance of assimilation of exudates produced by the >1 μm fraction). Thus the quantity $(1\,\mu m_{L_{pre}} - 1\,\mu m_{D_{pre}})$ is comprised mainly of incorporation due to photosynthesis, but includes a small but unknown portion of exudate assimilation].

However, the contribution of heterotrophic assimilation via phytoplankton exudates in also important and can be larger than the photosynthetic contribution in the <1.0 μm fraction. Factors contributing to the variation in the contributions of photosynthesis and exudate assimilation in the <1.0 μm fraction probably include biomass and productivity level (eutrophic status) and plankton community composition. Length of the incubation period might influence the relative contribution of exudate assimilation because respiratory loss during

Table 4
Results from post-incubation fractionation experiments. Assimilation (mean of 2 replicates) in μg C l^{-1} over the incubation period.

Experiment	$1\,\mu m_{L_{post}}$	$1\,\mu m_{D_{post}}$	$1\,\mu m_{L_{post}} - 1\,\mu m_{D_{post}}$	W_L	$\left(\frac{1\,\mu m_{L_{post}} - 1\,\mu m_{D_{post}}}{W_L}\right)100$
-7	-1.58	1.86	—0.28	3.93	—
-8	-1.00	1.62	—0.62	5.31	—
-9	23.06	2.30	20.76	343.70	-6.0
10	-7.02	1.75	-5.27	-14.53	36.3
11	17.89	5.99	11.90	340.08	-3.5

Table 5
Results from parallel pre- and post-incubation fractionation experiments. Assimilation in μg C l^{-1} over the incubation period, and as percent of W_L.

		$W_L - 1\,\mu m_{L_{pre}}$		$1\,\mu m_{L_{pre}} - 1\,\mu m_{D_{pre}}$		$W_D - 1\,\mu m_{D_{pre}}$		$1\,\mu m_{D_{pre}}$		$1\,\mu m_{L_{post}} - 1\,\mu m_{L_{pre}}$	
Experiment	W_L	μg C l^{-1}	(%)	μg C l^{-1}	(%)	μg C l^{-1}	(%)	μg C l^{-1}	(%)	μg C l^{-1}	(%)
12	21.20	21.02	(99.13)	.18	(.87)	.70	(3.31)	1.23	(5.81)	6.99	(32.99)
13	28.73	27.16	(94.52)	1.57	(5.48)	.09	(0.30)	5.67	(19.75)	5.33	(18.55)
14	38.81	31.91	(82.21)	6.90	(17.79)	0	(0)	3.65	(9.42)	4.45	(11.46)
15	27.06	22.41	(82.83)	4.65	(17.17)	0	(0)	5.15	(19.05)	1.38	(5.09)

the night of photosynthetically fixed carbon would lower the net photosynthetic rate measured in a 24 h incubation compared to a daylight only incubation. Having provided evidence for the importance of both carbon pathways in the <1.0 μm fraction, further experiments will be necessary to determine the controlling environmental factors.

It is evident that either pre- or post-incubation fractionation alone is not sufficient to make trophic distinctions among size fractions. Pre-fractionation alone separates the main source of exudates from the bacterial consumers, thus preventing the measurements of this component of the food web. Post-fractionation alone does not distinguish photosynthetic and exudate assimilation pathways (*e.g.* Platt *et al.*, 1983). Both procedures together provide much more information on the pathways of carbon in the incubated samples even when size fractionation does not result in separation of trophic groups.

Uptake of $^{14}CO_2$ directly by the <1.0 μm fraction (measured as $1\,\mu m_{D_{pre}}$ or $1\,\mu m_{D_{post}}$) was as large as or larger than label incorporation attributed to exudate assimilation, in some cases equaling 20% of the whole sample photosynthetic carbon assimilation (Tab. 5). Relatively high dark bottle values are often observed in samples with very low productivity (Peterson, pers. comm.; Lorenzen, pers. comm.; Ward, unpublished observations). Data from the pre-incubation fractionation experiments showing the distribution of light and dark uptake between the >1.0 μm and <1.0 μm fractions (Tab. 6) are consistent with the above results. Most of the dark CO_2 assimilation occurred in the <1.0 μm fraction, although most of the productivity, chlorophyll (and biomass) was found in larger fractions. Autotrophic bacteria may be responsible for some of this dark CO_2 assimilation but their contribution is probably negligible in all except a few rare cases (Horrigan, 1981; Karl *et al.*, 1984). Several interpretations of dark $^{14}CO_2$ assimilation have been proposed (Sorokin, 1961; Romanenko, 1964; Jordan, Likens, 1980; Billen, 1976; Somville, 1978; Horrigan, 1981). The proper interpretation of high levels of dark label incorporation in $^{14}CO_2$ experiments remains unresolved.

Although the experimental design employed here allowed differentiation among the main carbon pathways in the various fractions, microscopic observation of the efficiency of fractionation and effects of fractionation upon the population would verify the presence/absence and abundance of photosynthetic and nonphotosynthetic populations in the fractions. The concentration of exudates in the incubated samples was not monitored and no direct measurements of exudate production or consumption were made. However, the transfer of this material between trophic groups was demonstrated by the distribution of ^{14}C label. Direct evidence of the reported transfer of recently photosynthesized organic exudates from "phytoplankton" to "bacterial" fractions could be obtained by autoradiographic examination of labeled populations from size fractionation experiments like those described here. Size fractionation over time course experiments are underway (Ward, unpublished data) to study the kinetics of carbon assimilation via the various photosynthetic and heterotrophic pathways.

The experiments reported here present evidence for the existence of a step in the oceanic food web linking heterotrophic carbon metabolism directly to primary production. This pathway has implications for the efficiency and rapidity of carbon cycling within the photic zone. Both photosynthetic and heterotrophic carbon metabolism are responsible for carbon assimilation in the <1 μm fraction in at least some environments. The contribution of the <1 μm fraction to carbon assimilation by natural populations is significant and variable. Further study is necessary to determine what environmental and hydrographic variables control its relative importance in marine productivity and carbon cycling.

Table 6

Distribution of dark CO_2 assimilation in μg C l^{-1} over the incubation period (and as per cent of total dark assimilation) in pre-incubation fractionation experiments.

Experiment	$W_D - 1\,\mu m_{D_{pre}}$	$1\,\mu m_{D_{pre}}$	$W_L - 1\,\mu m_{D_{pre}}$	$1\,\mu m_{L_{pre}}$
1	.08	2.67 (97.1)	31.39	2.14 (6.4)
2	.20	2.46 (92.5)	57.65	2.32 (3.9)
3	.77	.22 (22.2)	35.22	.27 (0.8)
4	0	.45 (100)	21.29	2.96 (12.2)
5	.44	3.59 (97.3)	89.66	4.79 (5.1)
6	.10	1.47 (93.6)	148.14	.58 (0.4)

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