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Direct Measurement of Dissolved Organic Carbon Release by Phytoplankton and Incorporation by Microheterotrophs

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Abstract

It is now possible to divide particulate primary production into algal and heterotrophic components without physical separation. This depends on two innovations, the introduction of isotope in the form of labelled dissolved product(s) of primary production and the employment of a data analysis specifically designed for tracer kinetic incorporation experiments. The ^{14}C technique described by Steemann Nielsen (1952) is inapplicable in the analyses of certain classes of systems and kinetic tracer incorporation experiments must be employed instead. We show that measurement of PDOC production rate requires such kinetic tracer analyses. Measurements made in the laboratory on water taken from 2 m depth in South West Arm of the Port Hacking estuary showed that: (1) the steady-state rate of PDOC production was 0.10 to 0.13 mg $C.m^{-3}.h^{-1}$; (2) the rate of PDOC incorporation into microheterotroph particulate organic carbon was 0.10 to 0.12 mg $C.m^{-3}.h^{-1}$; (3) the rate at which PDOC was respired to CO_2 was 0.001 to 0.003 mg $C.m^{-3}.h^{-1}$. (4) the PDOC makes up only about 0.1% of the total dissolved organic carbon. The size class of particles associated with PDOC production differed from the size class responsible for uptake of PDOC. More than 50% of the PDOC production was associated with particles having a nominal diameter range of 20 to 63 µm, while this fraction was responsible for <10% of the incorporation.

Introduction

The $^{14}CO_2$ method of Steemann Nielsen (1952) has been used extensively to estimate primary production by phytoplankton. From the beginning it was recognized that, during photosynthesis, algae release some of their photosynthate into the medium as PDOC[1], and that this material can be metabolized by microheterotrophs. Thus, some of the ^{14}C-labelled POC actually represents PDOC incorporation by microheterotrophs rather than only $^{14}CO_2$ fixed by algae. While this distribution of label does not alter the quantity of particulate primary production measured by the $^{14}CO_2$ method, it may determine which size classes of organisms can make use of that primary production. From trophic food web and energetic viewpoints, it is essential to know the pathways of production utilization (Pomeroy, 1974).

The release of PDOC by algae has been investigated by several workers (e.g. Fogg *et al.*, 1965; Watt, 1966; Thomas, 1971; Choi, 1972; Berman and Holm-Hansen, 1974; Hellebust, 1974). The amount of ^{14}C-labelled PDOC remaining in the medium after a few hours incubation has been reported to vary from <1% to about 50% of the total amount fixed. In general, the more nutrient-depleted the waters, the greater the percentage of photosynthate released as PDOC, although this matter is by no means settled. It is clear, however, that the amount of photosynthate released sometimes constitutes a significant percentage of the total carbon fixed. The total quantity of PDOC released cannot be determined from the types of studies referred to above, since only the amount of PDOC remaining after an incubation period has been measured; some of the PDOC may have been incorporated into heterotrophs or respired.

[1]Abbreviations used in this paper: DOC, dissolved organic carbon; PDOC, photosynthetically produced DOC; DIC, dissolved inorganic carbon; POC, particulate organic carbon; MFSW, membrane-filtered seawater (pore size, 0.22 µm).

The ability of microheterotrophs in planktonic communities to utilize organic compounds has been investigated by three methods. (1) Parsons and Strickland (1962), Wright and Hobbie (1965, 1966) and Crawford *et al.* (1974) added specific ^{14}C-labelled organic substrates to plankton samples and examined incorporation and dissimilation. (2) Azam and Holm-Hansen (1973) and Derenbach and Williams (1974) modified this "heterotrophic potential" technique; they separated microheterotrophs from autotrophs after $^{14}CO_2$ incubation in light by differential membrane filtration. (3) Derenbach and Williams (1974) also measured PDOC metabolism by monitoring the loss of PDOC from the medium when plankton samples were incubated in the dark subsequent to their being incubated in the light.

These initial studies on PDOC formation and metabolism have provided us with an insight into the roles of microheterotrophs but have left several important questions unanswered. Among these are: at what rate is PDOC produced? The required tracer kinetic experiments have not been performed to obtain an answer to this question. How much of the PDOC is evolved as CO_2? This information is necessary to assess the fraction of the PDOC that is incorporated into microheterotrophs or respired. Are the organisms responsible for PDOC production also responsible for its utilization?

In this paper we describe techniques for measuring PDOC production, and subsequent incorporation and CO_2 evolution; the use of these techniques is examined in an Australian estuary.

Materials and Methods

Sampling Locality

Water-column samples were collected from 2 m depth in the South West Arm of the marine-dominated Port Hacking estuary, (34°05'S; 151°06'E) N.S.W. Australia.

Sample Preparation

Water samples were taken with a Jitts' Sampler (Jitts, 1964), passed gently through a 124 µm nylon mesh into a 5 l Erlenmeyer flask and transported to the laboratory within 20 min. The 5 l water sample was transferred to a glass beaker and continuously stirred to ensure homogeneity during subsampling. One hundred ml aliquots of seawater were dispensed into 125 ml De Long culture flasks which were placed in an Aquatherm water bath (Model G86, New Brunswick Co.) set at the ambient estuarine water temperature (17.6° to 18.1°C), shaken at 180 rpm, and illuminated by a bank of seven 40 W fluorescent lamps (daylight) yielding an average illumination of 180 µE m^{-2} sec^{-1}. Dark controls and killed cell controls, obtained by adding 1 ml of 18% (w/v) formaldehyde to each flask, were run for each experiment.

Sample Concentration and Particle Size Fractionation

A 4 l beaker and a Perspex cylinder (14.6 cm inner diameter), closed at the bottom with nylon gauze, were employed to fractionate the particulate matter (>10 µm diameter) in seawater on the basis of nominal particle diameter (i.e., on the basis of the mesh size of the gauze). A 2 l sample was added to the beaker; the Perspex cylinder was inserted and allowed to sink to the bottom of the beaker. The liquid inside the Perspex cylinder contained those particles having diameters less than the mesh size of the screen; the liquid surrounding the cylinder (300 ml) contained all the particles with nominal diameters greater than the mesh size. This latter size class was essentially freed of particles having smaller diameters than the mesh size of the gauze by removing 1700 ml from the inside of the cylinder and replacing it with an equal volume of MFSW. A specific particle size fraction was obtained by removing the liquid inside the Perspex cylinder and repeating the process described above using a Perspex cylinder with a finer mesh size. In this manner a series of discrete particle size fractions were obtained.

Particles which passed through the 10 µm nylon gauze were fractionated with membrane filters (Nuclepore Corp. Pleasanton, California) having pore sizes of 5.0, 3.0 and 0.22 µm. A small volume (50 ml) and a 2.5 cm diameter filter held in a SWIN-LOK filter holder (Nuclepore Corp.) were employed to obtain particle size fractions less than 5.0 µm diameter. Aliquots of samples were examined using a Celloscope Model No. 112TS particle counter; all nylon gauzes and membrane filters were found to be free of by-pass leakage.

Tracer Bolus Preparation

A ^{14}C-labelled PDOC preparation (1.56×10^5 dpm ml^{-1}, specific activity = 4.075×10^7 dpm/µ mole C) was obtained by incubating in seawater containing only radioactive DIC (18.0 mg $^{14}C.l^{-1}$) phyto-

plankton (10 to 124 µm nominal diameter) concentrated to 33 times the ambient particle concentration. After 63 min the light incubation was terminated, the flask contents poured through 10 µm mesh nylon gauze and then filtered under vacuum (<100 mm Hg) onto a 0.22 µm pore-size membrane filter. The filtrate was acidified (pH ≃ 2) with 1.0 N HCl and bubbled for 5 min with N_2 to collect the major fraction of the $^{14}CO_2$ in a NaOH trap. The filtrate then was bubbled vigorously for 1 h with carrier $^{12}CO_2$ to remove any residual $^{14}CO_2$ left in the PDOC preparation. This procedure ensures quantitative removal of the $^{14}CO_2$ if the tracer employed is obtained by collecting $^{14}CO_2$ liberated from commercially available $Ba^{14}CO_3$ (Smith and Wiebe, 1976). The labelled PDOC preparation was adjusted to pH = 8.3 with 1.0 N NaOH, filter sterilized, sealed in glass ampoules, and stored at -70°C (see "Discussion" for comments on the chemical composition of the PDOC preparation).

For use in an experiment, the contents of a thawed PDOC ampoule (ca. 30 ml) were transferred to a 100 ml volumetric flask, and the solution brought to volume with MFSW. Three replicate 1.0 ml samples of the tracer bolus were transferred to counting vials containing 9.0 ml MFSW and 10.0 ml of Instagel (Packard Instruments). The tracer bolus was counted in a liquid scintillation spectrometer (Packard Instruments Model 3390) and the dpm calculated from previously established quench correction curves by the method of external standardization.

In kinetic experiments, each flask received 1.0 ml of the PDOC tracer bolus containing from 3.8×10^4 to 4.75×10^4 dpm. In addition, a set of flasks was incubated with ^{14}C-labelled DIC instead of PDOC. The ^{14}C DIC, previously distilled to eliminate any radioactivity which might be nonvolatile in acid solution, was added to flasks in volumes of 1.0 ml containing from 3.591×10^7 dpm to 5.143×10^7 dpm, depending upon the experiment.

In an experiment designed to determine which particle size fractions were responsible for the production and utilization of PDOC, an additional set of flasks was included wherein tracer (5.8×10^6 dpm) was introduced as D-glucose-uniformly labelled ^{14}C (Cal-Atomic) at a final concentration of 0.5 $\mu g.l^{-1}$.

Sample Incubation and Analysis

Three sets of flasks were incubated with the labelled PDOC. One set, consisting of from 12 to 16 flasks, was incubated in the light; an identical second set was incubated in the dark. The third set of 4 to 8 flasks contained formaldehyde-killed cells. Three sets of flasks, labelled with ^{14}C DIC, were also prepared; 4 flasks were incubated in the light, 3 in the dark, and 3 were formaldehyde-killed controls.

The times of tracer addition and filtration were recorded to the nearest second for each flask. At intervals, one flask from each set was removed and the contents filtered under vacuum (<100 mm Hg) using a 25 mm diameter 0.45 µm Millipore membrane filter. Before filtration, a 2 ml portion of carrier PDOC solution was passed through each filter. The carrier PDOC was produced in the same manner as the labelled PDOC except that no $^{14}CO_2$ was employed.

The filters, containing the POC fraction of the sample, were exposed to concentrated HCl fumes for 5 min to remove any $^{14}CO_2$ produced by respiration during the incubation with PDOC or added as DIC, then dried overnight *in vacuo* over KOH. The filters were transferred to counting vials containing 15 ml of Permablend I (Packard Instruments Co.), counted to 2×10^4 counts, and the cpm converted to dpm by employing previously constructed quench correction curves and the method of external standardization.

A 10 ml portion of each sample filtrate was counted to 2×10^4 counts and the dpm calculated in the manner described for measuring the tracer bolus activity. The remainder of the filtrate and all the filtrate from flasks incubated with ^{14}C-labelled DIC were acidified by the addition of 1.0 ml of 37.5% (w/v) H_3PO_4 and bubbled vigorously with carrier $^{12}CO_2$ for 20 min to remove $^{14}CO_2$. A 10 ml portion of these filtrates was counted in the manner described for the other filtrate and the dpm of the PDOC calculated using appropriate quench correction curves.

Data Analysis

A complete kinetic tracer analysis permits one to measure not only a net production rate but each of the associated rate constants (Berman *et al.*, 1962; Bergner, 1964, 1965). In addition, the pathways of mass transfer (e.g. foodweb structure) can be defined by conducting kinetic tracer experiments and applying a multicompartmental analysis to the experimental curves of time-varying radioactivities. Although non-linear interactions between the components (e.g. competition, symbiosis, etc.) cannot be

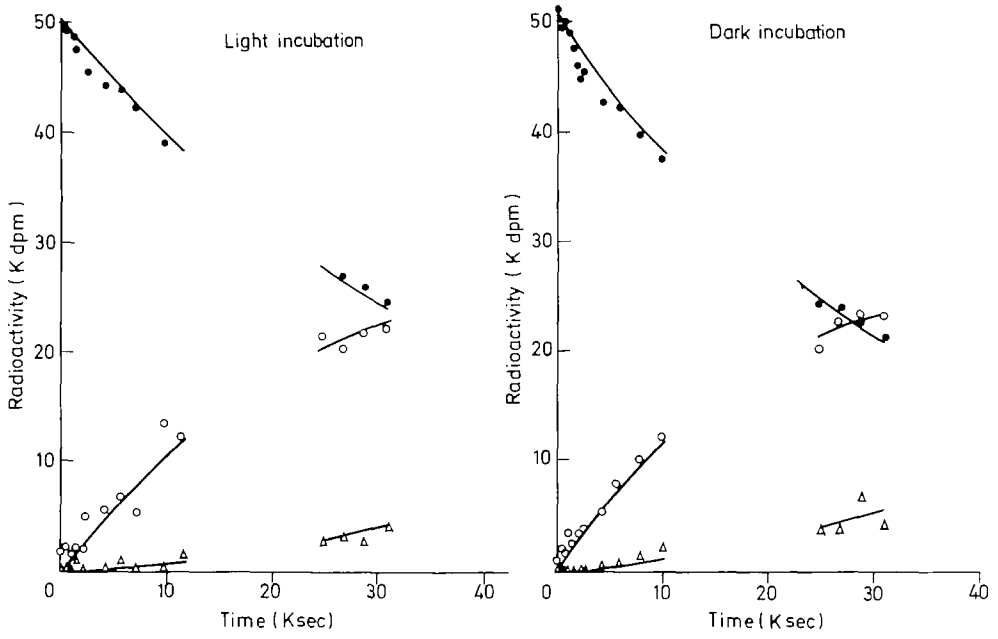

Fig. 1. Secondary production in water sample collected on 18 July 1975. A tracer bolus consisting of the ^{14}C-labelled dissolved organic carbon released by phytoplankton, PDOC (filled circles), was added to sample and the time-varying radioactivities in the particulate organic carbon, POC (open circles), and in the respired dissolved inorganic carbon, DIC (triangles), were recorded for subsequent multicompartmental analysis. Breaks in these curves arise from fact that flasks were incubated for both short and long terms in order to ensure that neither large nor small exponents would be omitted during subsequent data analysis

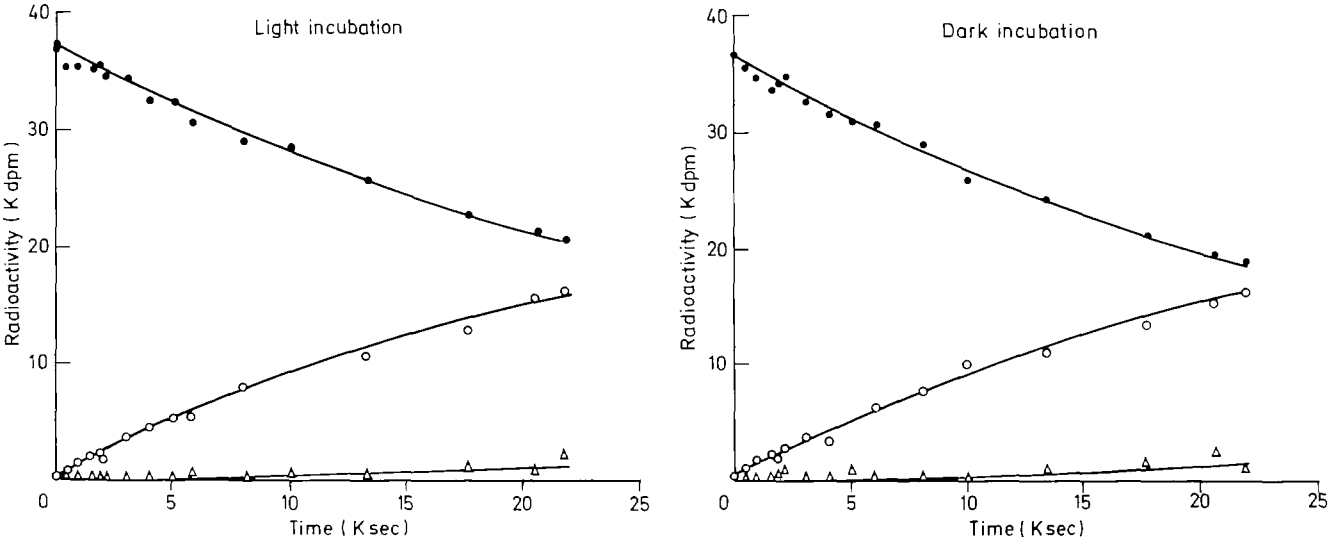

Fig. 2. Secondary production in water sample collected on 8 August, 1975. Experimental details as in Fig. 1 and in text. Radioactivities of PDOC are given by filled circles, of POC by open circles, and of DIC, by triangles

observed in the course of a single kinetic tracer experiment, they can be discovered and quantitatively expressed by applying a non-linear analysis to data obtained from a set of tracer experiments (Smith, 1974b).

All time-varying radioactivity curves such as those obtained for PDOC, POC and CO_2 in this study can be resolved into the sums of exponentials or sums of exponentials plus a term proportional to time (Berman and Schoenfeld, 1956; Hart, 1957; Rescigno and Segre, 1964; Mann and Gurpide, 1966, 1969; Smith, 1974). The data points of the time-varying radioactivities of POC, PDOC and CO_2 were simultaneously fitted to a single exponential sum using SAAM 25 (Mathematical Research Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. 20013, USA) a program designed for analysis of tracer kinetic data which permits a global fitting strategy. A global fitting permits one to obtain estimates of the exponents which simultaneously minimise the sums of squares for all the compartmental radioactivities. However, every term of this single sum of exponentials does not typically appear in the function describing the time-varying radioactivity of each compartment; zero coefficients make some terms disappear because some compartments do not connect with each other (Smith, 1974a). Thus, one can obtain the equations and parameter values which, when plotted, reproduce the time-varying radioactivities of PDOC, POC, and CO_2 and, from these, rates of production and loss of PDOC, POC, and CO_2 can be obtained (Smith, 1974a).

Results

The kinetic tracer data are represented as graphs of the time-varying distribution of radioactivity between the compartments of the experimental system (Figs. 1, 2). The tracer was introduced as labelled PDOC and the radioactivity subsequently appeared in the POC and DIC compartments. In separate experiments the tracer was introduced as DIC and the time-varying radioactivity of the PDOC measured. The curve obtained from such an experiment (Fig. 3) results from an increasing specific activity of a PDOC pool of constant size (Smith, 1974a). The plateau region of the curve occurred because the PDOC compartment size was constant for the duration of the experiment and because the specific activity of the DOC pool became equal to that of the DIC. The PDOC pool sizes (Table 1) in the experiments shown in Figs. 1 and 2 were calculated from the specific activities of the administered DIC and measurements of the total radioactivity of the PDOC at isotopic equilibrium.

To obtain the rates of PDOC uptake and production it was first necessary to fit the curves shown in Figs. 1 and 2 to the following sets of equations:

$$\varrho_1(t) = \varrho_1(0)\, e^{-\lambda_{21} t} \tag{1}$$

$$\varrho_2(t) = [\lambda_{21}\varrho_1(0)/(\lambda_{32}-\lambda_{21})] \cdot (e^{-\lambda_{21} t} - e^{-\lambda_{32} t}) \tag{2}$$

$$\varrho_3(t) = [\lambda_{32}\lambda_{21}\varrho_1(0)/(\lambda_{32}-\lambda_{21})] \cdot [(\lambda_{32}-\lambda_{21})/(\lambda_{32}.\lambda_{21}) - (\tfrac{1}{\lambda_{21}})\, e^{-\lambda_{21} t} + (\tfrac{1}{\lambda_{32}})\, e^{-\lambda_{32} t})] \tag{3}$$

where $\varrho_1(0)$ = radioactivity added as PDOC, at time zero, and $\varrho_1(t)$, $\varrho_2(t)$, and $\varrho_3(t)$, are the time-varying radioactivities of the PDOC, POC, and DIC, respectively. The notation employed is that of Berman and Schoenfeld (1956), λ_{ij} is the fractional turnover rate from the jth compartment to the ith (k sec^{-1}) and $\varrho i(t)$ is the total radioisotope content of the ith compartment at time, t.

These equations are the integrated form of the differential equations defined by the model of PDOC flux shown in Fig. 4. The rationale for selecting this model is given under "Discussion". The data points were fitted to Eqs. (1)-(3) by multiple non-linear regression using SAAM 25; the fitting resulted in the estimates of the equation parameters, λ_{21}, λ_{32} and $\varrho_1(0)$, given in Table 2. The product of the PDOC fractional turnover rates, λ_{21} and λ_{32}, and the PDOC pool size yield the rates of PDOC disappearance and production. These rates, given in Table 3, have been expressed in units of mg carbon.m^{-3}.h^{-1}.

The rates of labelled PDOC disappearance and of labelled PDOC production from ^{14}C-labelled DIC for various particle size fractions found in a seawater sample are given in Table 4. The size fraction responsible for producing most of the PDOC (20 to 63 µm) was clearly not the size fraction responsible for the disappearance of the PDOC.

Table 1. Steady-state PDOC pool sizes

Date of experiment (1975)	Incubation period (ksec)	Radioactivity in PDOC/100 ml (kdpm)	DIC/100 ml (mg carbon)	Radioactivity in DIC/100 ml (kdpm)	PDOC/100 ml (µg carbon)
18 July					
	17.9	2.72			0.119
	18.0	2.84	2.37 ± 0.01[a]	5.399×10^4	0.125
	23.4	2.82			0.124
				Mean =	0.123
8 August					
	16.98	1.48			0.105
	19.00	1.79	2.55 ± 0.3[a]	3.592×10^4	0.127
	31.81	1.81			0.129
				Mean =	0.120

[a] Average of 3 determinations.

Table 2. Parameter estimates defining rates of carbon flux due to secondary productivity in water-column samples

Secondary productivity parameters	Date of experiment (1975) 18 July Light	18 July Dark	8 August Light	8 August Dark
Minimum sums of squares calculated	58.7	65.1	4.35	4.99
$Q_1(t=0)$ (kdpm)	50.68 ± 2.4%	50.46 ± 2.6%	37.26 ± 1.3%	36.59 ± 1.5%
λ_{21} ($ksec^{-1}$)	0.0243 ± 6.5%	0.0285 ± 6.2%	0.0276 ± 2.7%	0.0308 ± 3.9%
λ_{32} ($ksec^{-1}$)	0.00097 ± 19%	0.00124 ± 16.1%	0.00063 ± 16.5%	0.00078 ± 15.8%

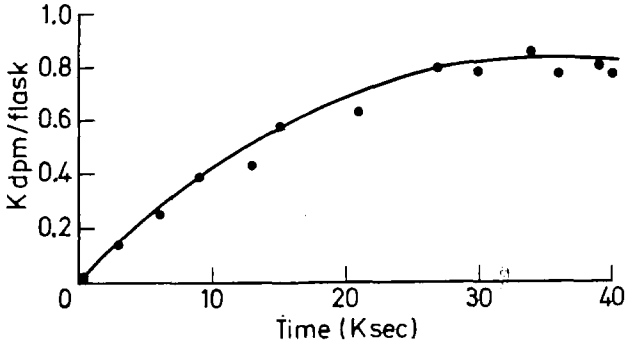

Fig. 3. Kinetics of radioisotope incorporation into PDOC during light incubation of water samples. ^{14}C-labelled DIC was added. Monotonically decreasing slope and plateau are characteristic features of isotope distribution curves which would arise from introducing ^{14}C-labelled DIC to system shown in Fig. 4 at steady state

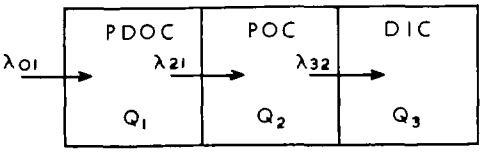

Fig. 4. Compartmental model of radiocarbon flux in water sample incubated with ^{14}C-labelled dissolved organic carbon produced by phytoplankton (PDOC). Q_1, a function of time, is total radioactivity in the PDOC compartment, Q_2 is that of POC, and Q_3 is that of DIC. The λ's are fractional turnover rates of the compartments, where λ_{ij} denotes a flow from the jth to the ith compartment. λ_{01} refers to the production of unlabelled PDOC during incubation

Table 3. Dissolved organic carbon release and incorporation

PDOC pool size and rates of production and loss	Experiment of 18 July, 1975		Experiment of 8 August, 1975	
	Light	Dark	Light	Dark
PDOC pool size (mg C.m^{-3})	1.23[a]	1.23	1.20[a]	1.20
Production of POC from PDOC (mgC.m^{-3}.h^{-1})	0.104	0.126	0.116	0.133
Respiration of PDOC (mgC.m^{-3}.h^{-1})	0.0043	0.0055	0.0027	0.0034
Production of PDOC (mgC.m^{-3}.h^{-1})	0.104	Unobserved[b]	0.116	Unobserved[b]

[a] Portions of each water sample were taken for PDOC estimations just prior to removing aliquots for the subsequent light and dark incubations.

[b] Although no dark fixation of DIC was found, techniques employed in this study would not measure any PDOC production in the dark that might occur by release of cellular polymers, preformed in the light.

Table 4. Rates of production and loss of PDOC by different particle size fractions in seawater

Particle size range[a] (μm)	Rate of production of labelled PDOC from ^{14}C-labelled DIC[b] (dpm/flask/ksec)		Rate of loss of added PDOC[c] (dpm/flask/ksec)	
	Light	Dark	Light	Dark
Unfractionated	651.3	22.6	629.0	234.0
(124.106)	113.8	35.9	78.0	585.0
(106-63)	636.7	39.2	598.0	219.0
(63-20)	2,093.9	60.9	2,033.0	109.0
(20-10)	216.8	30.0	187.0	202.0
(10-5)	325.7	85.5	240.0	78.0
(5-3)	260.1	43.7	216.0	81.0
(3-0.45)	294.0	92.3	202.0	183.0

[a] Before dispensing samples, each size fraction was suspensed in a volume of filtered seawater equal to that of original sample.

[b] Each flask received 2.419 x 10^8 dpm and contained 100 ml of sample at a DIC concentration of 24 mg C.l^{-1}.

[c] Each flask received 2.1250 x 10^4 dpm of PDOC and was incubated in the dark.

Discussion

The radioisotopic procedures and kinetic analyses used in this paper have been examined in detail by Smith (1974a, b; 1975). The time-varying curves of radioactivity of PDOC, POC and CO_2 were fitted to the sum of exponentials arising from the integrated differential equations defined by the compartmental model shown in Fig. 4. The rationale of fitting the experimental data points to the specific model shown in Fig. 4 arises from the following considerations.

First, note that the model possesses no λ_{i3}'s, indicating that there was no relabelling of PDOC or POC by labelled DIC arising from the respiration of labelled PDOC. This assumption, concerning the absence of λ_{i3}'s, is based on the fact that the amount of ^{14}C-labelling of DIC by respiration of PDOC was always small (Figs. 1 and 2) while the quantity of ^{12}C DIC was large (90 mg CO_2.l^{-1}). Hence, the specific activity of any respired $^{14}CO_2$ would be so diluted that any recycling of label could not be detected.

Second, from the analysis of the experimental curves shown in Figs. 1-3, the PDOC compartment was considered to be in steady state during the course of the light incubations. If this were not the case, a successful minimization of the sums of squares could not be accomplished by fitting the data points from the light incubations to the model shown in Fig. 4, since this assumes a variation only in compartmental specific activity. For example this model is one defined by the simple differential equation,

$$\frac{dQ_i}{dt} = \frac{dq_i}{dt} x_i, \qquad (4)$$

where Q_i = total radioactivity of the ith compartment, x_i = total mass of the ith compartment, a constant, $q_i \equiv Q_i / x_i$.

If both the specific activity and the pool size of PDOC changed simultaneously during the kinetic tracer experiment, a successful fitting of the data points would require increasingly complicated expressions of the form

$$\frac{dQ_i}{dt} = \frac{dq_i}{dt} x_i + \frac{dx_i}{dt} q_i. \qquad (5)$$

The kinetics of tracer incorporation into PDOC were obtained by adding ^{14}C-labelled DIC to flasks incubated in the light. The results, shown in Fig. 3,

confirmed that the PDOC compartment was in steady state during the course of the incubation experiment [for example, the total radioactivity in the PDOC compartment approaches a limiting value (Smith, 1974a)]. Furthermore, the shape of the radioactivity curve seen in Fig. 3 provides some insight into the intracellular origin of the PDOC. If the labelled DIC had been photosynthetically fixed in the form of endogenous precursors to PDOC, and the turnover times of the precursor pools were large compared to that of the PDOC, then the curve shown in Fig. 3 would have possessed an inflection point. The absence of an inflection point demonstrates either that any endogenous pools are minute in size compared to the PDOC compartment or that their turnover times are much more rapid than that of the PDOC compartment. In either case, the specific activity of the material leading to the PDOC production must be that of the administered DIC; hence, the PDOC compartment size can be calculated using any set of points from a curve such as that shown in Fig. 3. That is, the PDOC pool size can be calculated from any single point in the plateau region, given the specific activity of the DIC, or from a set of points obtained from the time-varying region of such a curve (Smith, 1974a).

Precisely because the pool size of PDOC can be calculated in the manner just described, it follows that the method proposed by Steemann Nielsen (1952) cannot be employed to estimate the rate of PDOC production. One of the constrictions that this author explicitly placed upon the system to be studied with his technique is that there should be negligible loss of incorporated label. The reason for this is obvious if one examines the curve shown in Fig. 3. From approximately 20 to 40 ksec there is virtually no change in radioactivity. Thus, any attempt to estimate production rate by dividing a sample activity by the incubation period results in an experimental artifact. The estimate obtained is solely a measure of a constant pool size divided by an arbitrarily chosen incubation period. An incubation period of 20 ksec would yield an estimate of production rate twice that of a 40 ksec incubation period. It should be noted that even when measuring POC production, one may be dealing with exponential curves. Although the long incubation of samples was precluded in Steemann Nielsen's paper of 1952, the results of 24 h tracer incubation experiments have been described by Eppley and Sharp (1975). The radioactivity of phytoplankton POC is essentially constant over 6, 15, and 24 h incubation periods and the authors report photosynthetic rates greatest in the 6 h incubation and least in the 24 h incubation. Unfortunately, no time series of samples incubated for less than 6 h were reported.

The results of the light and dark incubation experiments are particularly interesting, since mathematically one cannot separate the possibility that PDOC was released in the dark at the same rate as in the light from the possibility that no PDOC release took place in the dark. Both situations yield the same equations.

In the dark-incubated flasks we observed essentially no fixation of labelled DIC and no production of ^{14}C-labelled PDOC. Assuming that no PDOC was released in the dark, then the specific activity of the added PDOC would have remained unchanged but the quantity of PDOC would have decreased as it was incorporated into POC. If this were the case, the differential equation defining the rate of loss of radioactivity in the PDOC pool would be:

$$\frac{dQ_1}{dt} = q_1 \frac{dX_1}{dt} \qquad (6)$$

but, from our model in Fig. 4,

$$\frac{dX_1}{dt} = -\lambda_{21} X_1. \qquad (7)$$

Therefore, the equation defining the rate of loss of labelled PDOC in the dark is

$$\frac{dQ_1}{dt} = -\lambda_{21} X_1 q_1 = -\lambda_{21} Q_1, \qquad (8)$$

which is precisely the same equation defining the rate of change of label in PDOC in the light, i.e.,

$$\frac{dQ_1}{dt} = -\lambda_{21} Q_1. \qquad (9)$$

As this equation defines the following two equations of the model, i.e.,

$$\frac{dQ_2}{dt} = \lambda_{21} Q_1 - \lambda_{32} Q_2, \qquad (10)$$

$$\frac{dQ_3}{dt} = \lambda_{32} Q_2, \qquad (11)$$

the single set of integrated equations given in the text must describe the time-varying radioactivities in both the light and dark incubations.

The route by which ^{14}C-label in PDOC is transferred to $^{14}CO_2$ is implicitly

defined by the model shown in Fig. 4. It might have been expected that a fraction of the PDOC was respired directly to CO_2, i.e., a λ_{31} existed, while simultaneously, a fraction was being assimilated into POC. Such a model would describe a mammillary system, with the PDOC occupying the central compartment. The model shown in Fig. 4 describes a catenary system and requires that the labelled PDOC first must be assimilated into POC before CO_2 can be respired. The experimental data were fitted to both models; the fitting of both the light and dark incubation data to the catenary model decreased the final sums of squares by 18 and 12% for data collected on 18 July and 8 August, 1975, respectively.

A further and independent examination was made of the validity of accepting the catenary model by examining the differences between the observed data points and those calculated by SAAM 25 for the theoretical curves. These residual differences were examined for both the behaviour of the time-sequence plot of residuals and for changing amplitude of the residuals with time (Draper and Smith, 1966), but no evidence was obtained for rejecting the catenary model; i.e., such analyses cannot validate a model, but can furnish evidence that the model should be discarded.

The mathematical procedures that we employed have given us additional insight into the processes involved in PDOC uptake and respiration. We have demonstrated that the experiments took place under steady-state conditions; these are often assumed in these types of experiments but seldom examined. The CO_2 release from the PDOC was via POC, not a direct PDOC → CO_2 pathway. This implies that PDOC was not used primarily for energy metabolism but was first assimilated. This finding is somewhat surprising, since many authors (see review by Hellebust, 1974) have suggested that the PDOC consists primarily of small molecular weight carbohydrates, which would be expected to be respired rather than incorporated.

Pomeroy (1974) noted that the mode by which microheterotrophs utilize PDOC has two possible consequences: either the material is assimilated and thus made available to animals or it is respired, in which case the heterotrophs act as an energy sink. Derenbach and Williams (1974) subsequently demonstrated that bacterial incorporation of PDOC could account for 1 to 50% of the total primary production. They could not measure $^{14}CO_2$ release, however, since their incubation systems contained $^{14}CO_2$. In our study, the $^{14}CO_2$ released amounted to 2-4% of the total amount of PDOC taken up. While it is not possible to calculate the real efficiency of substrate utilization in short-term experiments, it is apparent that virtually all the PDOC was incorporated into POC. Microheterotrophs, at least in the short-term, did not act as energy sinks. Derenbach and Williams (1974) suggested that their data should be corrected for bacterial respiration by about 25%. This would be a large overestimate if our data are representative.

Derenbach and Williams (1974) measured bacterial assimilation by placing light-incubated samples subsequently in the dark and measuring bacterial POC by size separation. Because we could add highly radioactive PDOC in our experiments, it was possible to examine the POC production in both the light and the dark. The two sets of curves (Figs. 1, 2) are virtually identical; there is no light-facilitated uptake of PDOC. As explained above, PDOC release in the dark cannot be estimated directly. However, we do know that there is either no PDOC release in the dark or the release is at the s a m e rate as in the light; any deviation from these conditions would be detected. Laws and Caperon (1976) have shown that some algae release DOC in both light and dark; our results suggest that if this is so, then the release should be the same.

PDOC consumption appears tightly coupled to production. Thus, little or no PDOC can accumulate, therefore it cannot be responsible, directly, for the major amount of DOC in marine waters. Our calculations (Table 3) show that there was a pool size of PDOC of about 1.2 µg $C.l^{-1}$, while the estimate of total DOC in this region is 1 to 2 $mg.l^{-1}$. The turnover times (T_t) were calculated at 8.6 to 9.6 h for two complete data sets. Crawford *et al.* (1974) examined the T_t of 16 amino acids and glucose in the Pamlico Sound estuary for an entire year. Values range from 0.12 h for methionine on June 3 to 215 h for isoleucine on December 13. In general, their T_t values were tens-of-hours in winter and 1 to 10 hours in spring, summer and autumn. Our experiments were conducted in mid-winter (July and August); the PDOC T_t fall below most of the winter values of Crawford *et al.* (1974).

Results of preliminary studies on PDOC chemical composition show it to be complex in nature; at pH 8.3, 59% is negatively charged, 24% is positively charged, and 17% is neutral. More than

95% of it is <3,500 molecular weight, the ether-soluble fraction accounts for <2%, and on thin-layer chromatography there do not appear to be any nucleotides or nucleosides present. The absence of high molecular weight nucleic acid and low molecular weight precursors provides supporting evidence that the PDOC was not derived simply from damaged cells. Further investigations on the nature of the material are under way.

The size fractionation which investigated PDOC production and utilization provides some insight into the organisms responsible for these processes. In the open ocean, Derenbach and Williams (1974) and Hansen (1976) have shown that the microorganisms responsible for ^{14}C-glucose uptake are <3 µm in size. Hansen (1976) showed that ^{14}C-glucose uptake in a saltmarsh estuary was associated with particles >14 µm in size. In our study (Table 4), the highest rate of uptake of PDOC was in the fraction 106 to 124 µm, while the greatest production of PDOC was in the fraction 20 to 63 µm. Therefore, the organisms responsible for most of the PDOC uptake are not the same ones responsible for its production. The current theory is that bacteria are responsible for most of the uptake; in the open ocean this appears to be confirmed by the size-fractionation experiments. However, in estuaries, heterotrophic activity resides on large particles, indicating that either the bacteria are in the main attached to the particulate matter or that large organisms are themselves responsible for most of the PDOC uptake. As there is no evidence for this latter hypothesis, we interpret our data according to the former hypothesis, Hansen (1976) worked in a turbid, low-saline, Georgia saltmarsh estuary, while we worked in a clear, high-saline system. It is puzzling why there should be such different distribution patterns of bacteria.

Finally, the unfractionated water (Table 4) gives rates of PDOC production which are much less than the sum of the size fractions. This phenomenon results from physically separating the producer and consumer populations and is reversed when the fractions are mixed and concentrated to form a reconstituted unfractionated seawater sample (Table 4). We suggest that the uncoupling of production and utilization by separating different particle size classes may afford evidence of an inter-specific regulatory control loop, a view which is consistent with our finding that the PDOC pool is in steady state.

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Literature Cited

Azam, F. and O. Holm-Hansen: Use of tritiated substrates in the study of heterotrophy in seawater. Mar. Biol. *23*, 191-196 (1973)

Bergner, P.E.: Tracer dynamics and the determination of pool-sizes and turnover factors in metabolic systems. J. theor. Biol. *6*, 137-158 (1964)

- Exchangeable mass: determination without assumption of isotopic equilibrium. Science, N.Y. *150*, 1048-1050 (1965)

Berman, M. and R. Schoenfeld: Invariants in experimental data on linear kinetics and the formulation of models. J. appl. Phys. *27*, 1361-1370 (1956)

- , M.F. Weiss and E. Shahn: Some formal approaches to the analysis of kinetic data in terms of linear compartmental systems. Biophys. J. *2*, 289-315 (1962)

Berman, T. and O. Holm-Hansen: Release of photoassimilated carbon as dissolved organic matter by marine phytoplankton. Mar. Biol. *28*, 305-310 (1974)

Choi, C.I.: Primary production and release of dissolved organic carbon from phytoplankton in the western North Atlantic Ocean. Deep-Sea Res. *19*, 731-735 (1972)

Crawford, C.C., J.E. Hobbie and K.L. Webb: The utilization of dissolved free amino acids by estuarine microorganisms. Ecology *55*, 551-563 (1974)

Derenbach, J.B. and P.J. Le B. Williams: Autotrophic and bacterial production: fractionation of plankton populations by differential filtration of samples from the English Channel. Mar. Biol. *25*, 263-269 (1974)

Draper, N.R. and H. Smith: Applied regression analysis, 407 pp. New York: J. Wiley 1966

Eppley, R.W. and J.H. Sharp: Photosynthetic measurements in the central North Pacific: the dark loss of carbon in 24-h incubations. Limnol. Oceanogr. *20*, 981-987 (1975)

Fogg, G.E., C. Nalewajko and W.D. Watt: Extracellular products of phytoplankton photosynthesis. Proc. R. Soc. (Ser. B) *162*, 517-534 (1965)

Hansen, R.B.: Heterotrophic utilization and mineralization of dissolved organic carbon in a salt-marsh estuarine ecosystem. *In*: Annual Meeting Abstracts of the American Society for Microbiology, p. 178. Washington, D.C.: American Society for Microbiology 1976

Hart, H.E.: Analysis of tracer experiments: II. Non-conservative non-steady-state systems. Bull. math. Biophys. *19*, 61-72 (1957)

Hellebust, J.A.: Extracellular products. Bot. Monogr. *10*, 838-863 (1974)

Jitts, H.R.: A twin six litre sampler. Limnol. Oceanogr. *9*, p. 452 (1964)
Laws, E. and J. Caperon: Carbon and nitrogen metabolism by *Monochrysis lutheri*: measurement of growth-rate-dependent respiration rates. Mar. Biol. *36*, 85-97 (1976)
Mann, J. and E. Gurpide: Generalised rates of transfer in open systems of pools in the steady state. J. clin. Endocr. Metab. *26*, 1346-1354 (1966)
-- Interpretation of tracer data: some factors which reduce the number of terms in the specific activity functions in *n*-pool systems. Bull. math. Biophy . *31*, 473-486 (1969)
Parsons, T.R. and D.H. Strickland: On the production of particulate organic carbon by heterotrophic processes in sea water. Deep-Sea Res. *8*, 211-222 (1962)
Pomeroy, L.R.: The oceans foodweb, a changing paradigm. Bioscience *24*, 499-504 (1974)
Rescigno, A. and G. Segre: On some topological properties of the systems of compartments. Bull. math. Biophys. *26*, 31-38 (1964)
Smith, D.F.: Quantitative analysis of the functional relationships existing between ecosystem components. I. Analysis of the linear intercomponent mass transfers. Oecologia (Berl.) *16*, 97-106 (1974a)
- Quantitative analysis of the functional relationships existing between ecosystem components. II. Analysis of the non-linear relationships. Oecologia (Berl.) *16*, 107-117 (1974b)
- Quantitative analysis of the functional relationships existing between ecosystem components. III. Analysis of ecosystem stability. Oecologia (Berl.) *21*, 17-29 (1975)
- and W.J. Wiebe: Constant release of photosynthate from marine phytoplankton. Appl. envirl Microbiol. *32*, 75-79 (1976)
Steemann Nielsen, E.: The use of radioactive carbon (^{14}C) for measuring organic production in the sea. J. Cons. perm. int. Explor. Mer *18*, 117-140 (1952)
Thomas, J.P. Release of dissolved organic matter from natural populations of marine phytoplankton. Mar. Biol. *11*, 311-323 (1971)
Watt, W.D.: Release of dissolved organic material from the cells of phytoplankton populations. Proc. R. Soc. (Ser. B) *164*, 521-551 (1966)
Wright, R.T. and J.R. Hobbie: Uptake of organic dilutes in lake water. Limnol. Oceanogr. *10*, 220-228 (1965)
-- Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology *47*, 447-454 (1966)

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