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A comparison of four methods for determining planktonic community production[1]

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Abstract

Samples from two coastal experimental ecosystems were incubated in vitro and sampled over 24 h. Production rates were measured by the ^{14}C method, the O_2 and CO_2 light-dark bottle methods, and the ^{18}O method. O_2 production in the experimental enclosures (volume $\sim 1.3 \times 10^4$ liters) was also measured directly.

Photosynthetic and respiratory quotients were close to 1.0. Gross production values determined by O_2 light-dark experiments, CO_2 light-dark experiments, and ^{18}O were similar. ^{14}C production ranged from 60 to 100% of gross production measured in CO_2 light-dark experiments, indicating that ^{14}C uptake is not precisely fixed with respect to other measures of community metabolism. There was no evidence that ^{14}C or any other method underestimated the rate of primary production in vitro by more than 40%. Productivities in vitro ranged from 35 to 100% of those in the mesocosm at similar light intensities.

In samples from one of the ecosystems, the rate of respiration in the light (calculated from ^{18}O data) was an order of magnitude greater than the rate in the dark. This difference may be ascribed to either photorespiration or light enhancement of mitochondrial respiration.

Turnover of microplankton populations in the ocean occurs on time scales of hours to days. Measurements of community turnover rates must be carried out with in vitro incubations, presenting two problems. First, it can never be claimed that processes occurring during bottle incubations exactly match those of populations in situ. Second, each technique for measuring production and respiration during in vitro incubations gives results which are often ambiguous and which fail to give a complete description of community metabolic rates.

One of the strategies adopted recently for resolving questions about the meaning of in vitro community production and respiration rate measurements involves comparing rates determined by different methods (e.g. Tjissen 1979; Postma and Rommets 1979; Gieskes et al. 1979; Davies and Williams 1984; Raine 1985). This approach has become more attractive with the development of high-precision analytical techniques for measuring seawater concentrations of O_2

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(Williams and Jenkinson 1982) and TCO_2 (total CO_2) (Johnson et al. 1985). These methods can be used to measure net community production in vitro in the light from the increase in $[O_2]$ or the decrease in $[TCO_2]$ and community respiration in the dark from the decrease in $[O_2]$ or increase in $[TCO_2]$.

In addition, an ^{18}O tracer method has been developed for measuring gross O_2 production in vitro (Grande et al. 1982). ^{18}O is an oxygen isotope with a natural abundance of 0.204 atom%; the major isotope is ^{16}O, with an abundance of 99.758%. The ^{18}O method involves spiking a water sample with $H_2{}^{18}O$, incubating in the light, and measuring the amount of ^{18}O-tagged O_2 produced during photosynthesis. All labeled O_2 is contained in a single well-defined phase (dissolved gas), and the ambient O_2 pool is so large ($\sim$250 μM) that only a negligible amount of O_2 will be recycled by respiration during an incubation. In any case, consumption has a very small effect on the $^{18}O : {}^{16}O$ of the remaining O_2. Therefore, the ^{18}O method measures gross O_2 production in vitro. The only exception is that intracellularly recycled O_2 cannot be discriminated: If a labeled O_2 molecule produced within an autotrophic cell is also consumed within the same cell, it will never enter the ambient seawater O_2 pool to be measured.

The method for determining gross O_2 production with an $H_2{}^{18}O$ spike, described above, differs from the method Brown and colleagues used in their visionary work (e.g. Mehler and Brown 1952; Brown 1953). Those investigators replaced dissolved natural O_2 (mostly $^{16}O_2$) in culture solutions with ^{18}O-labeled O_2 ($^{18}O : {}^{16}O$). They then determined the rate of photosynthesis from the rate of increase in the concentration of $^{16}O_2$, and the rate of respiration from the rate of decrease in the concentration of $^{18}O : {}^{16}O$. This method is very well suited to culture studies. Our method, which is more sensitive and simpler to implement, is better suited to studies of gross primary production in natural waters.

In this paper, we present results of a comparison of the four in vitro methods for determining rates of community production and respiration: ^{14}C assimilation, O_2 light-dark bottle production and consumption, CO_2 light-dark bottle consumption and production, and ^{18}O. Although our results can apply only to the systems studied, they provide examples which give considerable insight into the significance of community rates measured by the individual methods, as well as the relationships between physiological rates (e.g. photoassimilation) and community rates.

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Definitions and assumptions

The fundamental term in community energy and mass flows is the rate of gross primary production (GPP). This term has been defined frequently (e.g. Riley 1940; Platt et al. 1984; Steemann Nielsen 1963; Odum 1971). The general view is that GPP is the input of bond energy and organic material into the ecosystem and is normally defined as the rate of photosynthesis. We regard it as the rate of formation of organic carbon (C_{org}) or O_2 as a result of photosynthesis, regardless of the subsequent fate of O_2 or C_{org}. This definition places no constraint on the trophic location of the photosynthetic products at the time of measurement, which is consistent with the guidelines set forth by Worthington (1975). In this paper we adopt the simplifying assumption that there is no intracellular recycling of O_2 or CO_2 and make the drastic assumption that photorespiration results in complete oxidation of the C_{org} produced by this pathway. Violation of the first assumption would cause us to have underestimated all rates determined with light bottle measurements. Violation of the second (as in the case of C assimilation or DOC release associated with photorespiration) means that photorespiration to some extent contributes to net production as determined in light bottle CO_2 and O_2 experiments. In Table 1, we summarize the rate terms we have measured, indicate how they are determined, and outline their relation to other terms. All terms refer to community production or respiration rates unless we specify otherwise.

Because of the great importance of the ^{14}C method in biological oceanography, several

Table 1. Summary of measured rate terms, how they were determined, and their relationship to other variables of interest.

Term	Variable measured	Relative to other terms
^{18}O gross O_2 production ($[^{18}O]GP$)	Production of ^{18}O-labeled O_2 from ^{18}O	=GPP
O_2 net production (O_2NP)	$[O_2]$ increase in the light	=GPP + respiratory O_2 consumption in the light
O_2 dark respiration (O_2DR)	$[O_2]$ decrease in the dark	
O_2 gross production (O_2GP)		$=O_2NP + O_2DR$
CO_2 net production (CO_2NP)	$[CO_2]$ decrease in the light	=GPP − C_{org} respiration in light
CO_2 dark respiration (CO_2DR)	$[CO_2]$ increase in the dark	
CO_2 gross production (CO_2GP)		$=CO_2NP + CO_2DR$
$^{14}C_{org}$ production ($^{14}C_{org}P$)	($[^{14}C]$POC + DOC increase in light) − ($[^{14}C]$POC + DOC increase in dark)	$CO_2NP < {}^{14}C_{org}P < GPP$

recent papers have considered the relationship of ^{14}C production rates to other terms describing community metabolism (e.g. Peterson 1980; Carpenter and Lively 1980; Eppley 1980). $^{14}C_{org}$ production will be less than GPP if part of the $^{14}C_{org}$ pool is not collected and measured (e.g. if $[^{14}C]$DOC or a fraction of $[^{14}C]$POC is not analyzed) or if some $^{14}C_{org}$ is remineralized during the incubation. $^{14}C_{org}$ production will generally underestimate GPP by the rate of production of volatile C_{org} (e.g. CH_4), which is very rarely measured. Where the entire $^{14}C_{org}$ pool is analyzed, $^{14}C_{org}$ production will fall between CO_2 net production and GPP. If dark C_{org} respiration is slower than the rate of mitochondrial C_{org} respiration of "old" carbon (i.e. that existing before the incubation) in the light, $^{14}C_{org}$ production can be greater than CO_2 gross production.

The variables defined in Table 1 are related to two parameters of physiological interest—photoassimilation and net primary production. Photoassimilation (sensu Worthington 1975) is defined as GPP minus the rate of C_{org} or O_2 production associated with photorespiration. It is not precisely constrained by parameters we measure, but for O_2 it must lie between O_2 gross production and ^{18}O gross production, assuming that mitochondrial respiration is at least as rapid in the light as in the dark. For organic carbon, photoassimilation must be greater than CO_2 gross production or ^{14}C production. Net primary production (NPP) of O_2 and C_{org} is defined as the rate of photoassimilation minus the rate of dark respiration by autotrophs. For O_2, NPP must lie between O_2 net production and ^{18}O gross production. For C_{org}, NPP must be greater than CO_2 net production. It can be less than $^{14}C_{org}$ production, since algal cells present at the start of the incubation will respire.

Methods

Experimental setup—Our experiments were conducted with samples drawn from the tower tanks operated by the Marine Ecosystem Research Laboratory (MERL) of the Graduate School of Oceanography, University of Rhode Island. The tanks are closely monitored on a long-term basis for biomass, nutrient fluxes, physical parameters, and growth of contained plankton (Table 2). A description of the MERL facility can be found elsewhere (Pilson et al. 1980; Pilson 1985).

We performed two experiments, each lasting 24 h, on 6–7 April and 8–9 April 1983. MERL tank 9 was sampled for the first experiment, and tank 5 for the second. The basic procedure was to fill about 200 bottles (nominal volume, 125 ml) and subsample them throughout the next 24 h for analysis by the various methods. The incubation bottles were made of borosilicate

Table 2. Characteristics of the MERL tanks around the time of our experimennts.

	Tank 9			Tank 5		
	28 Mar	4 Apr	11 Apr	28 Mar	4 Apr	11 Apr
Salinity (‰)	28.65	28.46	27.67	28.75	28.48	27.58
Temp. (°C)	7.2	8.5	5.5	6.4	8.5	
NH_3 (μM)	9.32	6.04	10.75	3.57	3.06	0.21
NO_2^- + NO_3^- (μM)	3.21	4.28	5.19	2.76	3.70	0.15
PO_4^{3-} (μM)	1.44	1.49	1.89	0.45	0.47	0.02
SiO_2 (μM)	5.05	7.90	9.01	11.91	12.92	0.43
Chl *a* (μg liter^{-1})	4.8	13.2	4.5	0.8	2.9	16.7
	29 Mar	5 Apr	13 Apr	29 Mar	5 Apr	13 Apr
$[O_2]$ at dusk (μM)	368	378	333	310	309	428
pH at dusk	8.54	8.53	8.44	8.15	8.10	8.49
	22 Mar	5 Apr	21 Apr	22 Mar	5 Apr	21 Apr
Zooplankton (No. m^{-3})	31,000	92,000	69,000	7,100	7,100	18,000
Zooplankton (dry biomass, mg m^{-3})	113	167	125	31	35	38
	21 Mar	4 Apr	19 Apr	21 Mar	4 Apr	19 Apr
Phytoplankton live counts (No. ml^{-1})						
Microflagellates	4,800	2,100	3,900	2,200	300	800
Monads	4,800	13,000	15,500	4,100	6,900	5,500
Total phytoplankton	9,500	15,100	19,000	6,300	7,400	6,300

glass and had been soaked in dilute HCl (2% vol/vol) for several days before the experiments. About 12 h before use, the bottles were emptied and filled with deionized water. One hour before dawn of the day beginning an experiment, water was siphoned from the tanks into the incubation bottles. The bottles were flushed with at least three bottle volumes of sample. Samples were also taken for nutrient analyses and microscopic enumeration. Samples were not filtered to remove zooplankton.

All the bottles were placed in incubation chambers by dawn, ~0500 hours. Incubators were cooled with running seawater. The "light" bottles were incubated in a Plexiglas incubator; the "dark" bottles were incubated in double-thick black polyethylene bags inside a black plastic waste can. Samples were removed at 3- or 4-h intervals during the next 24 h.

During the 6 April experiment, samples were screened with two layers of neutral density screening, diminishing light intensity by 75%. During the 8 April experiment, samples were similarly screened after noontime, when conditions changed from cloudy to sunny.

Analytical methods—Light intensity was measured in the incubation chamber and in the MERL tanks. Nutrient analyses were done by autoanalyzer with standard techniques. Dissolved $[O_2]$ in all bottle incubations was measured by the automated Winkler technique of Williams and Jenkinson (1982). Dissolved O_2 in the tanks was measured with a pulsed O_2 electrode (Langdon 1984). Samples were analyzed in triplicate, and the standard deviation for each set was ±1.2 μM. $[TCO_2]$ was determined coulometrically as described by Johnson et al. (1985). Samples were analyzed in triplicate; the standard deviation was ±0.8 μM.

In both experiments gas bubbles were present in the bottles removed from the incubators late in the day. From their size, we judge that the bubbles in tank 9 probably caused inaccuracies in the O_2 analysis; the data are omitted from Fig. 1a. Although some small bubbles were also encountered in the tank 5 experiment, they are not believed to have caused serious error. Since most TCO_2 is present in HCO_3^-, gas bubbles do not produce a significant error in metabolic rates calculated from TCO_2 concentrations.

Cu, Fe, Cd, and Pb concentrations were measured on 0.4-μm-filtered samples by

graphite furnace atomic absorption spectrophotometry following ammonium pyrolidine dithiocarbamate (APDC) coprecipitation (Boyle 1976). Mn was measured by a direct injection, graphite furnace technique with a L'vov platform (Hunt 1983).

For $^{14}CO_2$ fixation measurements on the glass-bottle, time series samples, subsamples of 50 ml were withdrawn at each sampling point from each of four replicate bottles. These subsamples were filtered through a 0.45-μm Millipore filter. The filtrate was collected in a flask beneath the filter holder. The filter was placed in a glass scintillation vial with 0.10 ml of HCl solution (10% vol/vol), following the protocol of Lean and Burnison (1979). Meanwhile, the filtrate was acidified to pH 2 and bubbled with air for 40 min. An aliquot (9 ml) of the filtrate was then placed in a scintillation vial for subsequent counting. To the scintillation vial containing the filter, fluor (Aquasol) was added after a period of several hours. All samples were counted in a Beckman liquid scintillation counter. Counts per minute were converted to disintegrations per minute with data from quench curves. Eighty-five-milliliter samples in polycarbonate bottles were incubated in both the incubator and the tanks themselves for [^{14}C]POC productivity measurements according to the procedure of Hitchcock et al. (1985).

$\partial^{18}O$ of O_2 was measured with a method modified from that of Kroopnick (1971). Gases were extracted by siphoning about 50 ml from incubation bottles into flasks pre-evacuated to a pressure of $<10^{-5}$ atm. Extensive degassing was induced during the siphoning by introducing gases to the vacuum through a small orifice. The gases were allowed to equilibate for 1 h with the space in a second pre-evacuated 50-ml gas sample flask. The gas sample was then taken by sealing off a constriction in the latter flask. The extraction efficiency was 97±2% (similar to that expected from the known solubility of O_2), and the blank was <2%. The next step involved conversion of O_2 to CO_2. H_2O and CO_2 were removed from the gas sample with a liquid nitrogen trap and O_2 was combusted to CO_2 by circulation over a graphite tube, with platinum catalyst, heated to 900°C by an external furnace. At least 99.9% of the O_2 was combusted. In our system the isotope effect associated with the combustion was much less than that found by Kroopnick (1971), and incomplete combustion at the 0.1% level does not introduce a significant uncertainty in $\partial^{18}O$. CO_2 produced by the combustion was collected in a liquid N_2 trap. Residual gases (N_2 and Ar) were pumped away, water was removed with frozen isopropyl alcohol, and the $\partial^{18}O$ of CO_2 was measured with a mass spectrometer. Because of the high productivity, the incubated samples analyzed in this study had $\partial^{18}O$ values ranging up to +400‰, and analytical uncertainties were generally neg-

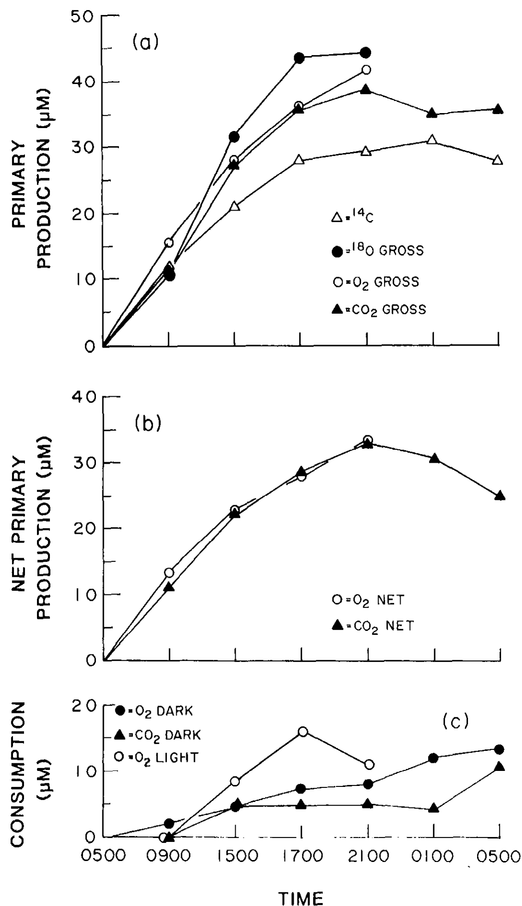


Fig. 1. Integrated values of (a) primary production vs. time of day for the tank 9 experiment, (b) net primary production vs. time of day, and (c) consumption (O_2 dark, CO_2 dark, and light O_2 respiration, taken as the difference between ^{18}O gross production and O_2 net production) vs. time of day.

Table 3. Comparison of trace metal concentrations in MERL tanks with those in water drawn from tanks and incubated. All analyses refer to dissolved component, except for Mn where the total was analyzed. Uncertainties are expressed as 68% C.L. Samples were analyzed in duplicate.

	Metal concentration (nM)				
	Cu	Fe	Cd	Pb	Mn
Tank 9					
Water from tank	30±2	35	1.8±0.2		
Water incubated in polycarbonate	28±1	34±1	2.0±0.6		
Tank 5					
Water from tank	23	21	1.7	0.35	282
Water incubated in glass	25±0	24±4	1.5±0.1	0.56±0.12	293±2

ligible. There is the possibility, however, that despite our precautions a few of our analyses were affected by contamination with water from the incubated sample, which was spiked up to a $\partial^{18}O$ of 4,000‰. In particular, it may be the cause of the anomalously high ^{18}O gross production value measured for one 1700-hour sample from the 8 April incubation. ^{18}O gross production values were calculated iteratively with the equations given by Bender and Grande (1987).

Conventions for expressing uncertainties—All analytical uncertainties and reproducibilities are expressed as 1 SD. Where terms are calculated from differences in measured values (e.g. O_2 and CO_2 net and gross production: Tables 3 and 4), uncertainties are expressed in terms of 68% C.L. These uncertainties are calculated following the guidelines of McCarthy (1957).

Results and discussion of trace metal analyses—Trace metals were analyzed in samples incubated in glass and polycarbonate bottles because contamination can reduce production rates (Fitzwater et al. 1982). Results are shown in Table 3. Except for slightly elevated Pb levels found in samples incubated in glass bottles, the samples were evidently free of contamination. Analyses of the ^{14}C spike showed negligible input of trace metals from this source. Hence contamination by trace metals does not appear to be a problem. This situation is not at all surprising, given the high concentrations of trace metals (with respect to open-ocean values) found in the tanks.

Tank 9

Table 2 lists the general characteristics of both tank 5 and tank 9. Tank 9 received a nutrient loading above that found in Narragansett Bay. NO_3^-, NH_4^+, and PO_4^{3-} concentrations as a function of time in glass incubation bottles are given in Table 4. [SiO_2] varied between 8 and 12 μM in light and dark incubation bottles and in the tank.

The flora in tank 9 was dominated by the flagellates *Gymnodinium* and *Pedinella*. As measured with glass incubation bottles, photosynthetic rates over the initial 12 h are in the range 2.3–3.6 μmol liter^{-1} h^{-1}. The Chl *a* content was 13.9 μg liter^{-1}. Integrated production rates for in vitro samples are given in Table 5. Dark respiration rates are in the range 0.4–0.6 μM C h^{-1}, and the ratio of photosynthesis to dark respiration (P : R) is about 6.9 (calculated as the ratio of CO_2 gross production divided by CO_2 dark respiration at 1700 hours). The midday assimilation number (photosynthetic rate normalized against Chl *a*) in the top meter of the tank is 2.6 μg C (μg Chl *a*)$^{-1}$ h^{-1}. This value was calculated with ^{14}C production values for samples incubated in polycarbonate bottles hung in the top meter of the tank. According to Humphrey (1975), a value of 2.6 is reasonable for a population

Table 4. Concentrations of nutrients as a function of time for in vitro samples drawn from tank 9. Values are given in units of μM.

	Light bottle nutrients			Dark bottle nutrients		
Time	[NO_3^-]	[NH_4^+]	[PO_4^{3-}]	[NO_3^-]	[NH_4^+]	[PO_4^{3-}]
0500	3.85	7.11	1.62	3.80	6.85	1.62
0900	3.79	6.50	1.55	3.84	6.99	1.57
1300	3.62	5.75	1.52	3.84	7.00	
1700	3.53	5.34	1.47	3.77	7.15	1.53
2100	3.56	5.16	1.44	3.77	7.26	1.50
0100	3.54	4.83	1.42	3.78	7.54	1.54
0500	3.57	4.79	1.42	3.79	7.71	1.51

dominated by flagellates. [^{14}C]DOC production accounts for 30–40% of total $^{14}CO_2$ fixation (Table 5).

[TCO_2], [O_2], and ^{18}O and ^{14}C production data are plotted vs. time in Fig. 1. Nutrient and TCO_2 concentrations are plotted vs. [O_2] in Fig. 2. All of our measurements reflect community activity, since we have no way to decouple effects of autotrophs and heterotrophs. However, because P : R ratios were so high in tank 9, we infer that primary production is the dominant cause of most chemical changes observed in the tanks. We neglect possible effects of heterotrophy in the ensuing discussion of bottle effects and nutrient uptake. Although heterotrophy is undoubtedly occurring in our samples, the rate is too slow to cause any of our conclusions to be seriously in error.

Results from two ancillary experiments shed light on the extent to which community production of incubated samples from tank 9 is enhanced or diminished relative to values in situ. In the first experiment, tank 9 samples in polycarbonate bottles were incubated along with glass bottles (Table 5). At 0900 and 1300 hours, productivities for samples incubated in glass and polycarbonate were nearly identical. This result shows that, in the first 8 h of the experiment, the material of which the bottles were made had no effect on production. This finding is surprising, because we have previously observed, microscopically, that *Gymnodinium* and *Pedinella* can rapidly lose their flagellae by colliding with walls of glass containers. No incubations in polycarbonate bottles were done at later times.

In the second ancillary experiment, tank 9 was mixed for a period ending at 1015 hours, and its (uniform) O_2 concentration was measured at this time. It was then left unmixed for 3 h 45 min. At 1400 hours, the O_2 concentrations were measured at various depths within the tank. The difference between initial and final [O_2] gives O_2 net production as a function of water depth and light intensity. Results are given in Table 6, along with values for Chl *a* concentration and average instantaneous irradiance. Rates of O_2 net production for these samples are calculated as $([O_2]_{t2} - [O_2]_{t1})/\Delta t$, where the subscripts $t2$ and $t1$ refer to the end and

Table 5. ^{14}C, O_2, and CO_2 gross production in samples incubated in glass bottles during the incubation for tank 9 samples. Values are also given for particulate $^{14}C_{org}$ production for samples incubated in polycarbonate bottles. Units are μM. 0100-hour and 0500-hour values of O_2 gross O_2 production are given as lower limits because bubbles in light bottles may have caused low [O_2] in these samples. Number of replicates given in parentheses; uncertainties expressed as 68% C.L. All ^{14}C samples were analyzed in triplicate. Irradiance is expressed as average instantaneous irradiance between two times, in units of Einst m^{-2} h^{-1}.

Time	Irradiance	$^{14}C_{org}$ production (glass)			^{14}POC prod. (polycarb)	^{18}O gross	O_2 net	O_2 gross	CO_2 net	CO_2 gross
		Part.	Diss.	Total						
0500										
	0.32									
0900		9.1±0.8	2.6±0.9	11.7	9.0±0.6	10.9(1)	13.5±0.3	15.5±0.5	11.2±1.1	11.0±3.7
	1.45									
1300		14.3±0.7	5.8±0.6	20.1	13.8±0.4	32.0(1)	23.0±0.3	27.6±0.4	22.7±1.3	27.5±1.0
	0.99									
1700		20.7±2.1	7.1±3.5	27.8		43.5±1.2(3)	28.0±2.3	35.4±2.4	28.4±1.6	33.2±2.6
	0.03									
2100		22.6±1.5	6.6±0.4	29.0		44.4±2.5(2)	33.6±1.5	41.8±1.5	33.5±1.9	38.7±2.1
0100		22.9±1.2	7.8±1.9	30.7			>25.6	>37.7	30.7±2.3	35.2±3.6
0500		22.7±1.5	5.0±1.2	27.7			>22.2	>35.8	25.0±3.0	35.7±3.3

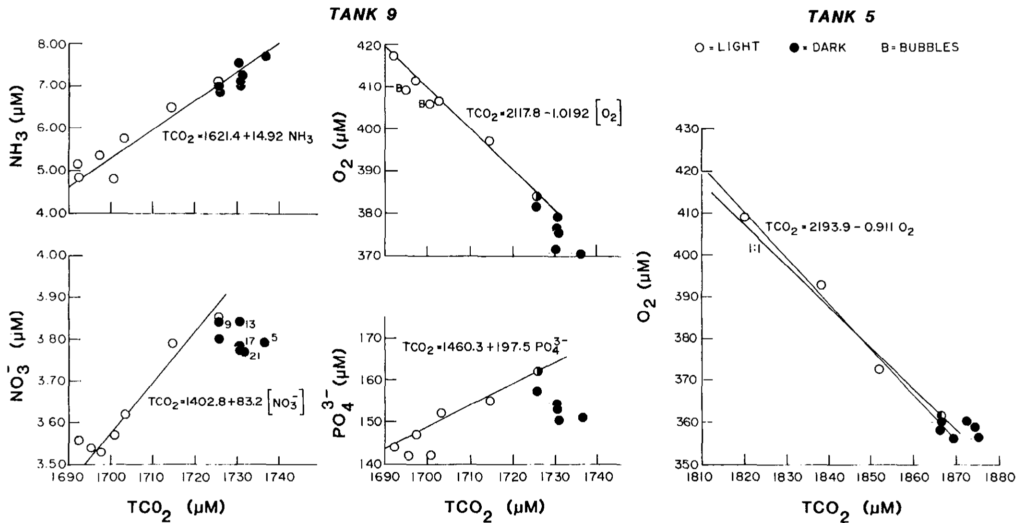


Fig. 2. Concentrations of nutrients and O_2 plotted vs. TCO_2 for the tank 9 (left) and tank 5 samples (right). The lines are least-squares linear regressions for points selected as described in the text.

beginning of the unmixed period, and Δt is the time interval between samplings. O_2 net production in the tank increases from 3.5 to 4.5 $\mu M\ h^{-1}$ as average instantaneous irradiance rises from 0.32 to 4.2 $\mu Einst\ m^{-2}\ h^{-1}$ (Table 6). For samples incubated in glass bottles, O_2 net production is 3.4 $\mu M\ h^{-1}$ between 0500 and 0900, 2.4 between 0900 and 1300, and 1.3 between 1300 and 1700 hours. We must compare bottle and tank production rates only for samples of similar average instantaneous irradiance. The irradiances of the 0500–0900 time-series samples are similar to that of the sample incubated at 2.5-m depth in the tank; rates of production for the two sets of samples are the same. The average irradiances of the time series samples between 0900 and 1300 and 1300 and 1700 hours are intermediate between values in the tank at 1 and 2.5 m, but O_2 net productivities of the in vitro samples are only 35–60% of the values in the tank. Thus by 0900 hours, production rates in glass bottles were being significantly lowered by bottle effects, showing that bottles may have had an adverse effect on community production. At the same time, the excellent reproducibility of our measures of production shows that the effects of containment in bottles are uniform.

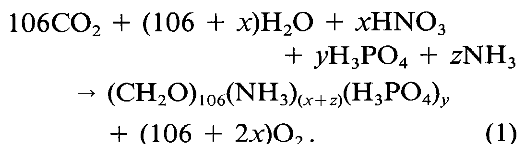
The photosynthetic efficiency (O_2 yield per unit of Chl *a* at low light intensities) measured from the increase in the O_2 concentration within the tank was about 2×10^{-3} $\mu mol\ O_2\ (\mu g\ Chl\ a)^{-1}\ h^{-1}\ \mu Einst^{-1}\ m^{-2}\ s^{-1}$. This value is within the range of photosynthetic efficiencies observed for culture experiments. For example, Langdon (1987) calculated a value of 2.5 ± 0.6, from his own data as well as that of Gallagher et al. (1984), Falkowski and Owens (1980), Dunstan (1973), and Takamura et al. (1985). The

Table 6. O_2 net oxygen production in situ, Chl *a*, and average instantaneous irradiance in tanks 9 and 5. The incubation period for tank 9 was 1015–1400 hours; for tank 5 it was 1000–1400 hours. Uncertainties in O_2 net oxygen production measurements are ± 0.4 μM (68% C.L.).

Depth (m)	O_2 net oxygen production ($\mu M\ h^{-1}$)	Chl *a* at end of incubation ($\mu g\ liter^{-1}$)	Average instantaneous irradiance ($Einst\ m^{-2}\ h^{-1}$)
	Tank 9		
0		13.0	5.35
0.5	4.5	12.1	4.23
1.0	4.3	18.6	1.91
2.5	3.5	15.8	0.32
4.5	0.4	10.0	0.022
	Tank 5		
0	6.5	8.9	2.19
0.5	7.2	14.8	1.62
1.0	6.9	15.7	0.99
2.5		18.4	0.22
4.5	2.1	16.4	0.021

good agreement between photosynthetic efficiencies measured in tank 9 with values previously measured in monospecific cultures shows that the phytoplankton in tank 9 are producing O_2 at about the expected rate. This result also strengthens the argument that heterotrophy played a small role in community metabolism.

Concentrations of TCO_2, O_2, and inorganic nutrients are related according to the stoichiometry of photosynthesis and respiration. The relationship can be represented by the following equation, which assumes a molar C : H : O ratio of 1 : 2 : 1 in organic matter (as in carbohydrate). The N : C ratio of the organic matter is equal to $(x + z)/106$, and the P : C ratio is $y/106$:



$$106CO_2 + (106 + x)H_2O + xHNO_3 + yH_3PO_4 + zNH_3 \rightarrow (CH_2O)_{106}(NH_3)_{(x+z)}(H_3PO_4)_y + (106 + 2x)O_2. \quad (1)$$

In Fig. 2, nutrients and O_2 are plotted vs. TCO_2 for the tank 9 experiment. The parameter x is equal to 106 times the slope in the NO_3^--TCO_2 plot, z is equal to 106 times the slope in the NH_3-TCO_2 plot, and y = 106 times the slope in the PO_4^{3-}-TCO_2 plot. $\Delta O_2 : \Delta TCO_2$ is then predicted to be $(106 + 2x) : 106$. This relationship would be satisfied only if the organic matter can in fact be represented as having C : H : O = 1 : 2 : 1. In general, PQ (the ratio of O_2 production to CO_2 consumption) is expected to be >1.0 during algal growth due to production of fat and proteins (Ketchum and Redfield 1949; Spoehr and Milner 1949). For our studies, the values of x, y, and z relect the stoichiometry of photosynthesis and respiration by the community rather than of any particular autotroph or heterotroph.

Consider first the NH_3 plot. The points are fit with the following line: TCO_2 = 1,621.4 + 14.92(NH_3), giving a value of 7.1 for z. For NO_3^-, not all points fall on a single straight line. The dark bottle samples have high TCO_2 but constant NO_3^- relative to the initial sample, reflecting respiration but the absence of nitrification. A best-fit line for light bottle samples taken before or at 1700 hours (Fig. 2) gives a value of x of 1.27. NO_2^- concentrations were very low, so that the C : N ratio of the newly formed organic matter is inferred to be 106 : (7.1 + 1.3) = 106 : 8.4, or about twice the Redfield value of 106 : 16. The uptake ratio of NH_3 to NO_3^-, calculated from the light bottle data for tank 9, is 5.6, whereas the concentration ratio of NH_3 to NO_3^- is about 1.6. Thus NH_3 uptake is favored over NO_3^- uptake, as would be expected from other studies (e.g. Glibert et al. 1982; Eppley et al. 1969).

The plot of PO_4^{3-} vs. TCO_2 for tank 9 shows that $[PO_4^{3-}]$ drops by about 0.1 μM in dark bottles. This decrease might reflect dark PO_4^{3-} uptake or adsorption on bottle walls. If the PO_4^{3-} decrease is due to adsorption, the C : P ratio inferred from the light bottle decrease is an upper limit. The C : P ($1/y$) value estimated from the best-fit of the data earlier than 1700 hours is then $>106 : 0.54$—clearly high with respect to the Redfield value (106 : 1).

The $\Delta O_2 : \Delta TCO_2$ ratio calculated for the above equation, and the observed value of x (reflecting NO_3^- uptake), is 1.02. It is in reasonable agreement with the value of 0.98 estimated from the fit of the light bottle points for the period up to 1700 hours. The low PQ value and the high C : N ratio of organic matter being synthesized suggest that the organic matter produced during the photoperiod was high in carbohydrate and poor in protein.

The rate of respiration in the light can be calculated from the difference between ^{18}O gross production and O_2 net production (given by the light bottle $[O_2]$ increase). We call this term the "light O_2 respiration rate." In the tank 9 experiment (Fig. 1c), light and dark respiration rates are the indistinguishable given the noise in the light O_2 respiration curve.

Integrated values of in vitro [^{14}C]POC and [^{14}C]DOC production as a function of time in the tank 9 experiment are given in Table 5. About 25% of $^{14}C_{org}$ production was due to [^{14}C]DOC. $^{14}C_{org}$ production is indistinguishable from CO_2 net production and clearly lower than CO_2 gross production. This relationship is within the limits expected from our earlier discussion in the definition of terms. It requires that light and dark respiration rates are similar (confirmed

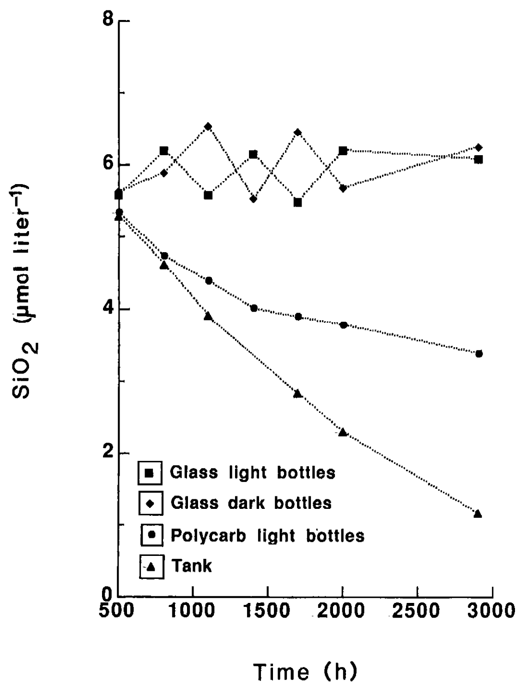


Fig. 3. $[SiO_2]$ vs. time in glass bottles incubated in the light and in the dark, in polycarbonate bottles incubated in the light, and in the tank during the tank 5 experiment.

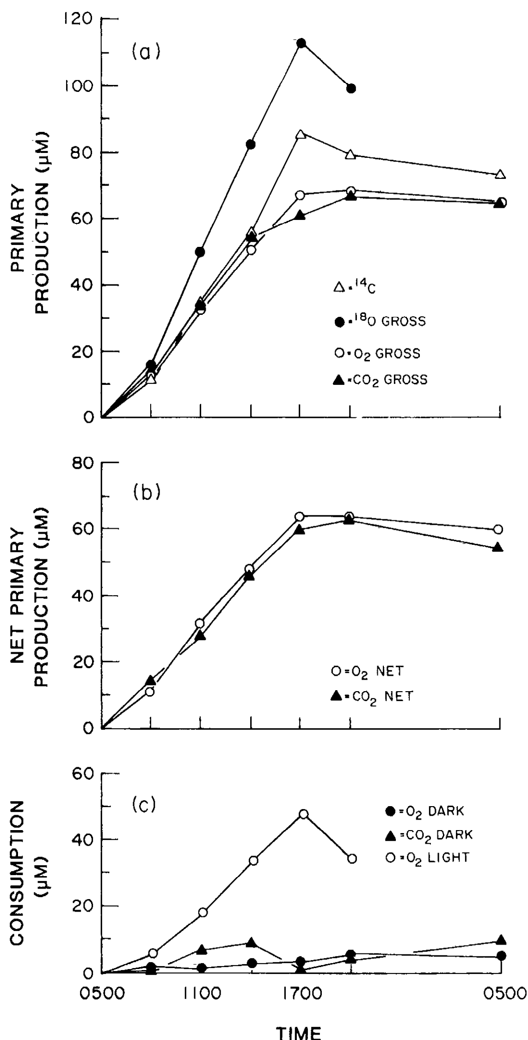


Fig. 4. As Fig. 1, but for tank 5 experiment.

by the O_2 and ^{18}O data) and implies that ^{14}C-tagged CO_2 produced during incubation is the major source of respired carbon.

Tank 5

Tank 5 was a control tank to which no nutrient additions were made. Its general characteristics around the time of our experiment are summarized in Table 2. The flora was dominated by the diatom *Thalassiosira pseudonana*. Photosynthetic rates in this tank were very high, ranging up to 10 $\mu M\ h^{-1}$. The community in this tank had a very high P : R, equal to 20, and an assimilation number of 5. P : R and assimilation number are calculated as for tank 9. There was little $[^{14}C]$DOC excretion in tank 5: the accumulation ratio of $[^{14}C]$DOC : $[^{14}C]$POC was only about 2%.

SiO_2 concentrations in bottles from the tank 5 experiment are plotted vs. time of day in Fig. 3, along with similar data for samples incubated in polycarbonate bottles and water in the tank itself. During the period of the experiment, $[SiO_2]$ in situ in the tank decreases from 5.3 to 1.2 μM. $[SiO_2]$ in the polycarbonate bottles also falls, although by a small amount. The decreases reflect uptake by diatoms. Integrated production rates in glass and polycarbonate bottles are given in Table 7 and plotted in Fig. 4. In both light and dark glass bottles, $[SiO_2]$ rises by about 1 μM during the experiment. Integrated $^{14}C_{org}$ production in glass bottles was higher than in polycarbonate bottles by 25% at 1100 hours and 68% at 2100 hours. We attribute this difference to enhancement of production by release of SiO_2 from the walls of glass bottles. Again,

reproducibility in glass bottles was excellent, and containment effects were uniform.

O_2 net production was measured directly in tank 5, as previously described for tank 9. Tank 5 was mixed for a period ending at 1000 hours and left unmixed until 1400. Productivities for four depths were determined from the rate of change of $[O_2]$ (Table 6). During the unmixed interval, the photosynthetic efficiency in tank 5 is 3 μmol O_2 (μg Chl *a*)$^{-1}$ h^{-1} μEinst m^{-2} s^{-1}, again within the range observed for culture studies (Langdon 1987). Time series samples from 0800–1100 and 1100–1400 (Table 4) both have average instantaneous irradiance values intermediate between those of the 0.5- and 1.0-m samples of the in situ experiment (Table 6). In vitro O_2 net productivities during the above two time intervals are 5.5 and 5.2 μM h^{-1}, on average 24% less than the values of 6.9 and 7.2 found for the two in situ samples. Thus even though there appears to be an enhancement of production within glass bottles due to release of SiO_2, production in glass is slightly slower than the rate in situ.

NO_3^-, NH_4^+, and PO_4^{3-} concentrations measured in all samples of the tank 5 experiment are extremely low. For all light and dark glass bottle samples, $[NO_2^- + NO_3^-]$ was <0.1 μM, $[NH_4^+]$ falls in the range 0.12 ± 0.05 μM, and $[PO_4^{3-}]$ falls in the range 0.05 ± 0.03 μM. During the experiment, TCO_2 in the light decreased by 63 μM, total fixed inorganic N ($NO_3^- + NO_2^- + NH_4^+$) fell by <0.15 μM, and $[PO_4^{3-}]$ dropped by <0.07 μM. The C : N ratio of organic matter being formed was $>106:0.25$, and the C : P was $>106:0.12$. The stoichiometric relationships thus show that, as in the tank 9 experiment, the organic matter produced was poor in N and P, but in tank 5 the depletions were much more extreme. NO_3^- assimilation obviously had no effect on the photosynthetic quotient, so that we expect $\Delta O_2 : \Delta TCO_2 = 1.00$ assuming C : H : N = 1 : 2 : 1. The calculated best-fit value of $\Delta O_2 : \Delta TCO_2$ is 0.9 (Fig. 2), again close to the value of 1.0 which would be expected for carbohydrate synthesis. The low PQ values, along with the high inferred C :N and C : P ratios of organic matter, suggest that the organic matter being synthe-

Table 7. As Table 5, but for tank 5, with uncertainties expressed as the standard deviation from the mean.

Time	Irradiance	$^{14}C_{org}$ production (glass) Part.	Diss.	Total	^{14}POC prod. (polycarb)	^{18}O gross	O_2 net	O_2 gross	CO_2 net	CO_2 gross
0500										
	0.17									
0800		11.6±0.9	0.17±0.02	11.8	8.3±1.7	16.2(1)	11.2±0.5	12.5±0.2	14.1±2.7	14.5±3.3
	1.01									
1100		34.1±2.4	0.36±0.05	34.5	27.5±1.1	49.5±4.9(4)	31.5±0.7	32.8±0.6	27.8±1.8	34.1±1.6
	1.29									
1400		54.1±2.0	1.57±0.66	55.7	39.4±0.9	81.7±2.9(3)	48.1±0.5	50.5±0.5	45.9±2.6	54.2±2.4
	0.165									
1700		83.5±2.7	1.65±0.21	85.2	49.3±0.3	112.2±12.1(3)	63.7±1.6	66.7±1.6	60.4±1.6	60.8±2.0
	0.01									
2000		76.3±1.4	1.83±0.38	78.1	46.3±0.3	98.4±6.1(3)	63.9±1.3	68.1±1.5	63.1±3.8	66.4±3.7
0500		70.3±1.0	2.39±0.65	72.7	35.6±6.6		60.2±1.2	64.9±1.1	55.0±2.8	64.0±2.7

sized by the algae is predominantly carbohydrate and poor in protein.

Rates of O_2 light respiration, calculated from ^{18}O data, are about an order of magnitude greater than those of O_2 dark respiration—a striking difference (Table 7, Fig. 4). The light respiration rate is about 40% of ^{18}O gross O_2 production, while the dark O_2 respiration rate is 3–4% of ^{18}O gross O_2 production.

We recognize three processes which may account for high rates of light respiration. The first is oxidation of photosynthate produced during the light incubation. A comparison of nighttime CO_2 respiration rates between bottles incubated in the light and in the dark gives some idea of the magnitude of the effect. Between 2000 hours on 8 April and 0500 on 9 April, 3.7 ± 1.8 μM O_2 was consumed in bottles incubated in the light and 3.2 ± 1.9 μM in bottles which had been incubated in the dark. This result suggests that, barring photoenhancement, an increase in rates of mitochondrial respiration associated with the presence of new photosynthate is completely inadequate to explain the ~1,000% increase in the rate of light O_2 respiration compared with that in the dark.

A second possible mechanism for explaining high rates of light respiration is photoenhancement of mitochondrial respiration. As noted by Lancelot and Mathot (1985), most workers have found that rates of dark respiration were unaffected or depressed by light (e.g. *see* Hoch et al. 1963). Falkowski et al. (1985) showed, for *T. pseudonana,* that rates of dark respiration rose by about 50% when measured immediately after samples were exposed to high intensities of light. Such a change could not account for the order of magnitude effect we have observed. Lancelot and Mathot (1985) found, however, that dark respiration rates for a late-summer coastal population were about 200% higher in the light than in the dark; respiration rates in the light were about 40% of carbon GPP. An effect of this magnitude would account for our results.

A third effect, photorespiration, also seems to be a reasonable explanation for our observation. There is considerable evidence that the process occurs in marine autotrophs (Burris 1977, 1980; Glover and Morris 1980; Smith 1974), and the rate in our experiments is not implausible. Explaining our findings as being due to photorespiration requires invoking photorespiratory C fixation at about 50% of the rate of C fixation along the normal pathway.

In the tank 5 experiment (Fig. 4), $^{14}C_{org}$ production agrees closely with CO_2 gross production, except for late in the afternoon, where it is greater than CO_2 gross production. Both these terms are less than ^{18}O gross O_2 production. We have seen that the comparison between rates of light and dark O_2 respiration indicates a light-driven respiration such that O_2 gross production and CO_2 gross production will underestimate true gross production. During the middle part of the photoperiod, underestimates of gross production (true gross production in the light) from carbon-based measurements can be explained by enhanced respiration in the light. ^{14}C would be respiring from the same carbon pool or pools as indicated by TCO_2. However, late in the photoperiod, $^{14}C_{org}$ production overestimates CO_2 gross C_{org} production. This relationship requires that the carbon source for respiration at this time is old, unlabeled carbon which existed at the start of the experiment. Otherwise, enhanced remineralization of C_{org} produced during the incubation would cause $^{14}C_{org}$ production, CO_2 net production, and CO_2 gross production to all be lowered in concert. To explain this within-photoperiod behavior, we must postulate a switch in the respiratory substrate, from new to pre-existing C_{org}, perhaps suggesting a changeover from photorespiration to mitochondrial respiration. We note that the rate of light respiration declines during the afternoon, which supports this idea. Bidwell (1977) has found a light dependence in dark respiration such that, at light intensities below saturation for photosynthesis, dark respiration is unaffected and old unlabeled C_{org} is the substrate. At irradiances above light saturation, both unlabeled CO_2 and ^{14}C-labeled CO_2 are produced, implying a substrate which includes recently fixed carbon. Bidwell's (1977) results would explain the close agreement between CO_2 gross production and ^{18}O gross O_2 production during the initial

time period, the differences between these two measures of production during the middle part of the day (when irradiances are highest), and the differences between $^{14}C_{org}$ production and CO_2 gross production late in the day. Still unexplained, however, is why ^{14}C production is not greater than CO_2 gross production during the early hours of the experiment when the specific activity of ^{14}C in the organic pool is expected to be low. (It may be because our techniques cannot detect differences at these levels.) The existence of at least two exchanging pools is consistent with evidence from laboratory (Smith and Platt 1984) and field (Bower 1981) studies. Resolution of these issues may come with a better understanding of how respiratory systems respond to the light.

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