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Dark fixation of ^{14}C: Variations related to biomass and productivity of phytoplankton and bacteria

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Abstract

In various North Atlantic sites, the rate of dark ^{14}C fixation by microbial plankton was significantly correlated with the rate of light ^{14}C fixation, with the rate of bacterial [^{3}H]thymidine incorporation, and with chlorophyll biomass. For plankton sampled near the sea surface (<5 m) and at the depth of the maximum light ^{14}C fixation, the median value for the ratio of dark to light fixation was only 1%. At the depth of the subsurface chlorophyll maximum where less light was available, the median value for this ratio was still quite low at 7%. Our results confirm early claims that generally in the oceans, including oligotrophic regions where plankton biomass is low, dark ^{14}C fixation is high in relation to light fixation only at depths where photosynthesis is severely limited by light. We infer that direct ^{14}C fixation by both bacteria and phytoplankton are significant processes that contribute to measured ^{14}C fixation in opaque bottles during 12 h dawn-to-dusk incubations.

In a bottle containing ocean plankton to which $H^{14}CO_3^-$ has been introduced, the amount of radiolabel fixed by the microbial assemblage depends, among other factors, on whether the bottle is incubated in the light or in the dark. If the incubation is short compared to microbial generation times, the amount of ^{14}C fixed by autotrophs indicates the rate at which carbon is converted from inorganic to organic form—a measure of primary productivity. Generally, we can discount the contribution to dark ^{14}C fixation arising from chemosynthesis by nitrifying bacteria, both because of the low abundance of these bacteria compared to the total bacterial population and because of low oxidation rates (*see* Banse 1993). Since autotrophic biomass in oceanic euphotic zones is predominantly phototrophic rather than chemotrophic, the incubation must be performed under illumination. In principle, corrections should be made to account for ^{14}C fixation that is not due to carboxylation carried out by photoautotrophic cells. The correction required for abiotic processes (e.g. adsorption) and biocalcification (coccolith production) is straightforward and based on the removal of inorganic ^{14}C by acidification. The correction required for biotic processes other than autotrophic carboxylation is much more difficult to obtain. The "dark bottle" aims to estimate this latter correction. However, even >40 yr after the paper of Steemann Nielsen (1951) was published, uncertainty remains concerning the validity of subtracting ^{14}C fixed in the dark from ^{14}C fixed in the light (Banse 1993).

The interpretation of dark ^{14}C values is difficult because several direct and indirect (tro-

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Table 1. List of field programs.

	Location
9–19 Apr 1984	Grand Banks, Newfoundland
1–18 Dec 1984	Caribbean Sea
6 Jan–6 Apr 1987	Bedford Basin, Nova Scotia
11 Jun–8 Jul 1987	New England seamounts, North Atlantic
7–17 May 1988	Labrador shelf, Newfoundland
7–21 Sep 1988	Northern Sargasso Sea
20 Apr–18 May 1989	Western North Atlantic (NABE)
6–28 Apr 1990	Western North Atlantic
4–20 Apr 1991	Scotian shelf and northern Sargasso Sea

phic transfer) means for fixation of inorganic C into cells exist. Most important of these are the β-carboxylation reactions due to heterotrophic organisms (bacteria and animals) and phytoplankton. In heterotrophs, these reactions represent anaplerotic sequences and obviously do not contribute toward primary production. The rate of anaplerotic C fixation depends on the rate of growth (Overbeck 1984): to correct for anaplerotic fixation of ^{14}C by heterotrophs in the light bottle, it must be assumed that rates of growth in the light and dark are the same. In phytoplankton, β-carboxylation can be a significant feature of light-dependent C assimilation which accompanies the primary carboxylation catalyzed by ribulose-1,5-bisphosphate carboxylase (Morris 1980; Kerby and Raven 1985; Descolas-Gros and Fontugne 1990). There is evidence that phytoplankton β-carboxylations occurring in the light are different from those in the dark (Mortain-Bertrand et al. 1987, 1988), implying that they do not simply constitute "background" dark metabolism. In brown macroalgae, considerations of the path of C in β-carboxylations led Kremer (1981) to suggest that nonphotosynthetic CO_2 fixation could justifiably be included in calculations of gross productivity. The different paths by which C can be assimilated led Morris et al. (1971) to question the general validity of the dark bottle.

In this paper, we report data from the North Atlantic that allow us to make inferences concerning dark ^{14}C fixation based on correlation of variables. We investigated whether the rate of dark ^{14}C fixation was significantly correlated with phytoplankton stock (chlorophyll biomass), with the rate of light ^{14}C fixation, with bacterial stock (numerical abundance), and with bacterial productivity (thymidine incorporation). The nature of the measurements did not allow any conclusions concerning the mechanism of dark ^{14}C fixation; however, it was clear that both bacteria and phytoplankton could be implicated in the process. Of particular significance is that dark ^{14}C fixation was strongly correlated with both chlorophyll biomass and the rate of light ^{14}C fixation; these findings re-emphasize the above concern of Morris et al. (1971).

Methods

Measurements were made during the course of nine field programs from 1984 to 1991 (Table 1). Seawater was collected with 12-liter Niskin bottles that were custom designed (Crooks Mechanical Systems, Dartmouth) to circumvent possible toxicity from rubber by using an external closing mechanism and Teflon closure seals. For measurements of light and dark ^{14}C fixation, the general procedure was as follows. A water sample from each of 11 depths was collected before sunrise, placed into six clear and three opaque bottles, traced with $NaH^{14}CO_3$ (20–50 μCi per bottle), and incubated in situ attached to a free-floating drifter (clear bottles) or in dark, temperature-controlled laboratory chambers (opaque bottles). Prior to 1987, Pyrex bottles were used to contain the samples; from 1987 onward, polycarbonate bottles were used. Incubations were stopped at sunset by filtering the radiolabeled samples onto glass-fiber filters (Whatman GF/F). Inorganic ^{14}C was removed by fuming the filters over HCl. Measurements of ^{14}C fixation are reported in units of dpm (total added dpm)$^{-1}$ (12 h)$^{-1}$ and represent means of the replicate bottles. In the 1987 time-series study of Bedford Basin and the 1987 cruise to the New England seamounts, ^{14}C incubations were carried out for 24 h instead of the usual 12. As will be shown later, results from 12- and 24-h incubations cannot be considered in the same way; for this reason, the 1987 data were omitted from all correlation analyses that were based on a 12-h incubation period.

Procedures for the sampling and measurement of chlorophyll, bacterial abundance, and [^{3}H]thymidine (Tdr) incorporation appear

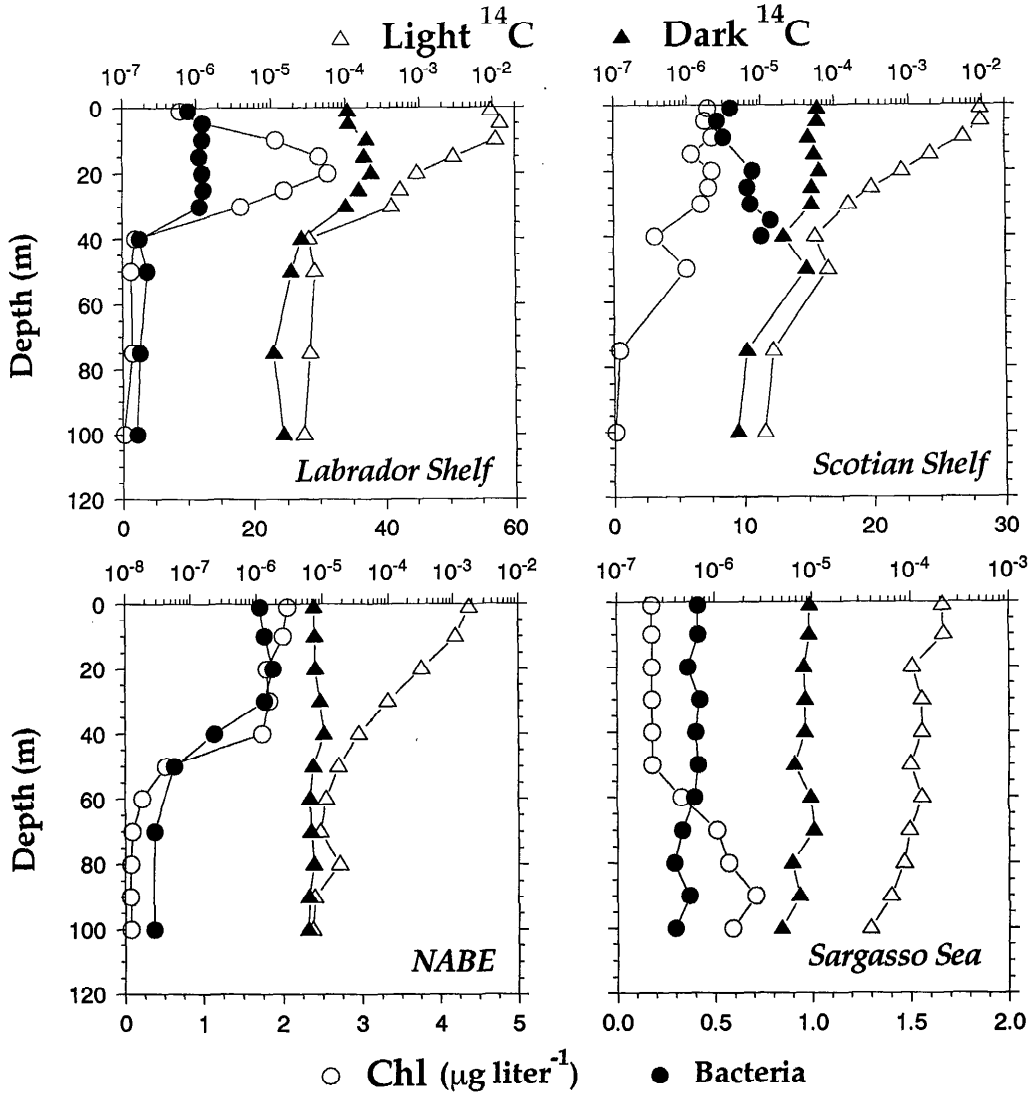

Fig. 1. Depth profiles of Chl *a*, bacteria, light and dark fixation of ^{14}C. Units for bacteria—10^5 cells ml^{-1} for Labrador shelf and Scotian shelf, 10^6 cells ml^{-1} for NABE and Sargasso Sea; fixation of ^{14}C—particulate dpm (total added dpm)$^{-1}$ (12 h)$^{-1}$.

elsewhere (Li et al. 1992, 1993). Briefly, chlorophyll was measured by fluorometric analysis of acetone extracts; bacteria were counted in DAPI-stained preparations under epifluorescence microscope; rates of [^{3}H]Tdr incorporation were estimated from simulated in situ deck-incubations lasting ≤3 h.

In this paper, all statistical tests were performed on the logarithm of variables. Linear regression of log-transformed variables was based on model 2 theory (Laws and Archie 1981) which is appropriate when both X and Y variables are subject to natural variability.

Results

Depth profiles of dark ^{14}C fixation—Figure 1 presents a contrast of four situations that differ in the abundance and vertical distribution of chlorophyll and bacteria. On the Labrador and Scotian shelves, dark ^{14}C fixation decreased with depth; but in the oceanic North Atlantic and the Sargasso Sea, dark ^{14}C fixation

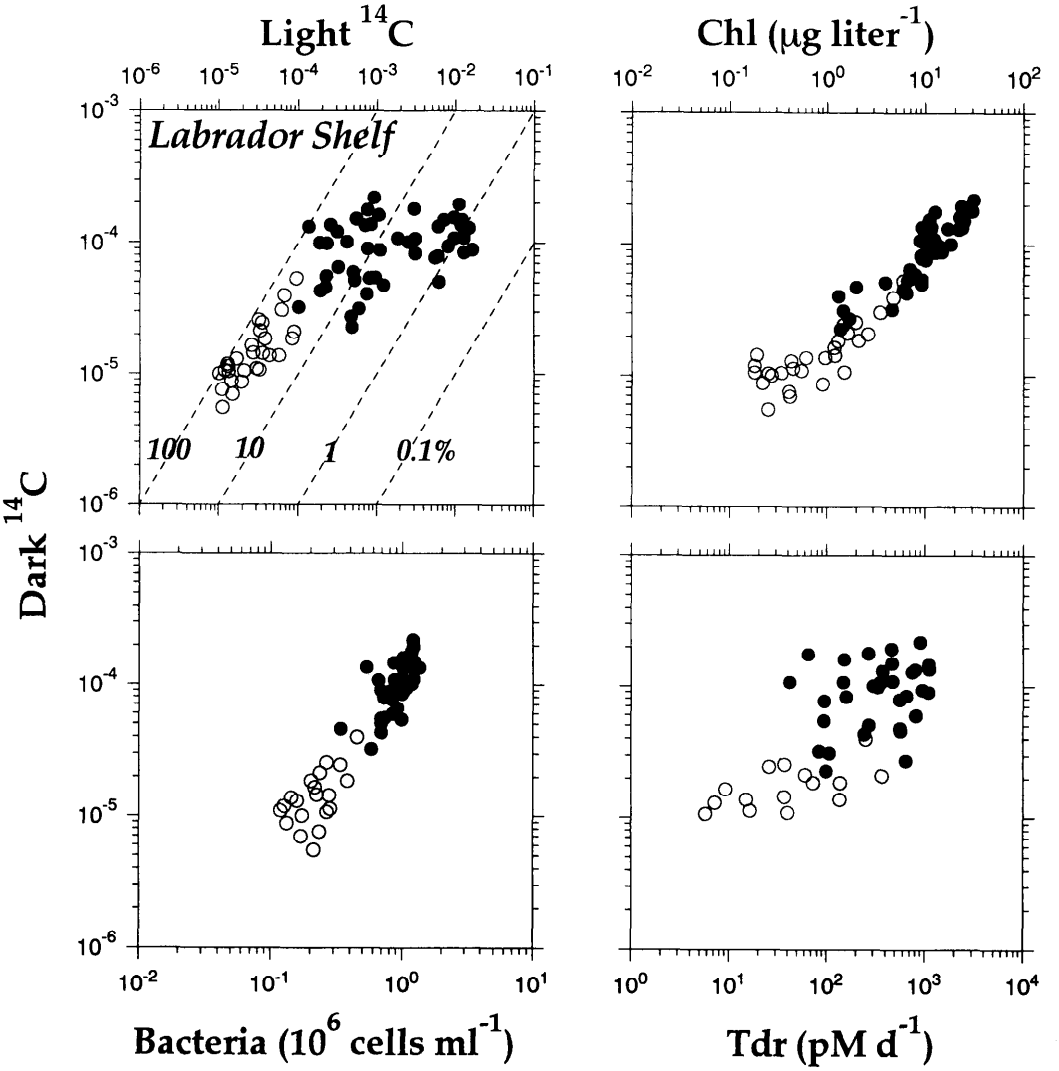

Fig. 2. Depth-related variation on the Labrador shelf: scatterplots of dark ^{14}C fixation vs. light ^{14}C fixation, Chl, bacterial abundance, and rate of [^{3}H]Tdr incorporation. Depths <40 m—●; depths ≥40 m—○. Dashed lines indicate dark : light ratio of 100, 10, 1, and 0.1%. Units for fixation of ^{14}C—particulate dpm (total added dpm)$^{-1}$ (12 h)$^{-1}$.

remained invariant with depth. The Labrador shelf data present the clearest example in which depth-related variations in dark ^{14}C fixation were correlated with light ^{14}C fixation, chlorophyll, bacteria, and [^{3}H]Tdr incorporation (Fig. 2). Conversely, the North Atlantic bloom experiment (NABE) data present an example of depth-invariant dark ^{14}C fixation uncorrelated with any other measured variable (Fig. 3).

Dark ^{14}C fixation at selected depth regimes—To analyze patterns of dark ^{14}C fixation unrelated to depth-dependent variations, we took subsets of the data from all cruises (except 1987) pertaining to three particular depth regimes: depths <5 m ($z < 5$ m), the depth at which light ^{14}C fixation was maximal [$z(P_{max})$], and the depth at which chlorophyll was maximal [$z(\text{Chl}_{max})$]. At each of these three depth regimes, dark ^{14}C fixation was significantly correlated ($P < 0.01$) with chlorophyll and [^{3}H]Tdr incorporation but not ($P > 0.05$) with bacterial abundance (Table 2, Fig. 4).

Ratio of dark to light ^{14}C fixation—At each of the three selected depth regimes, the rate of dark ^{14}C fixation (D) was significantly corre-

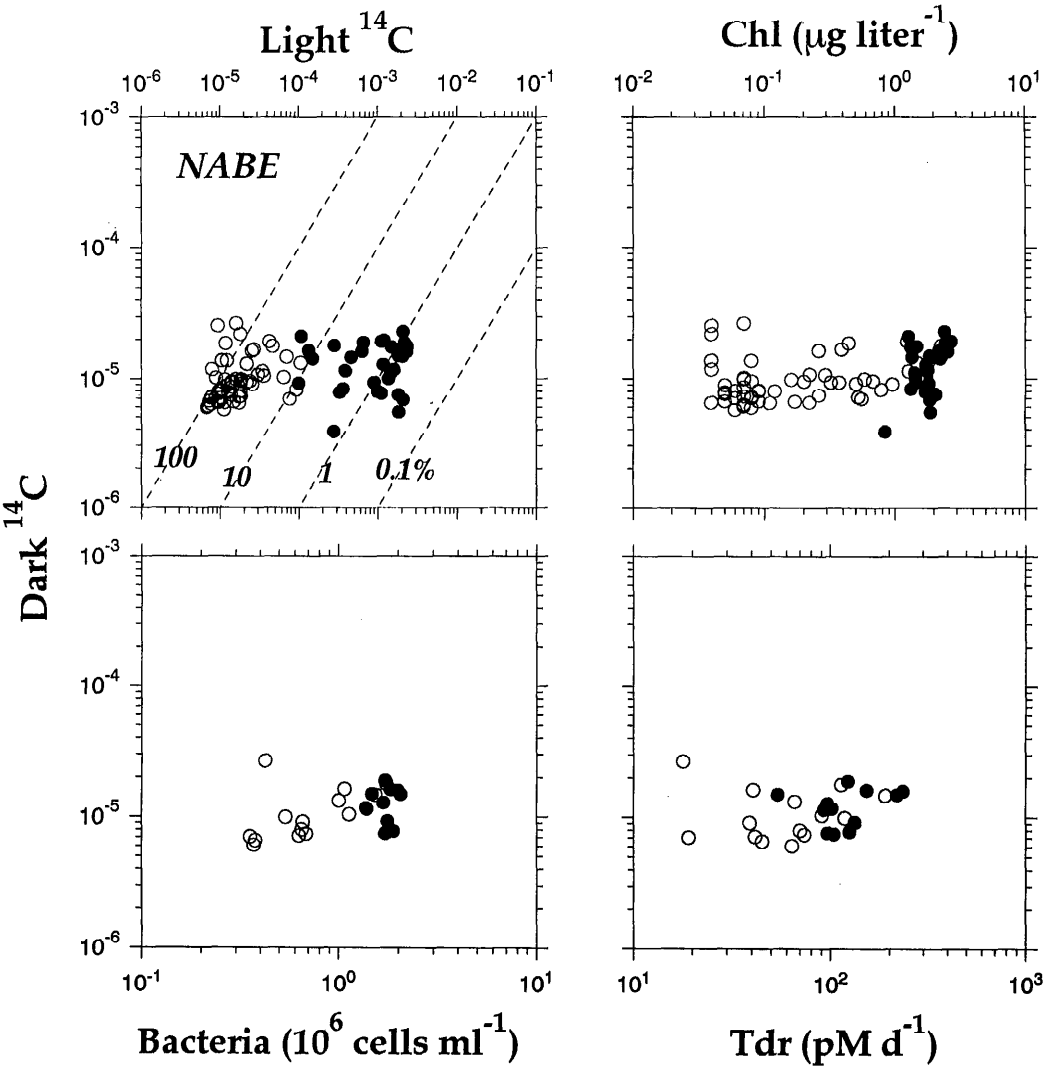

Fig. 3. As Fig. 2, but measurements refer to the North Atlantic bloom experiment (NABE, cruise 89-003).

lated ($P < 0.01$) with the rate of light ^{14}C fixation (L) (Fig. 5):

$$\log D = a + b(\log L). \quad (1)$$

The ratio of $D:L$ was therefore given by

$$D/L = 10^a \times L^{(b-1)}. \quad (2)$$

At each of the three selected depth regimes, the value of b was significantly ($P < 0.01$) < 1. This result implies that the ratio $D:L$ increased as L decreased. The median and mean values of $(D:L) \times 100\%$ were respectively 1.2 and 2.4% at $z < 5$ m, 1.1 and 2.2% at $z(P_{max})$, 6.6 and 10.3% at $z(Chl_{max})$.

Time series in Bedford Basin—The 1987 spring phytoplankton bloom in Bedford Basin was clearly evident in the coincident increase and decrease of Chl and light ^{14}C fixation (Fig. 6). In a generally parallel manner, spring values of dark ^{14}C fixation were also higher than winter values. It is notable that the exponential increase in dark ^{14}C fixation started several days later than did the increase in Chl and light ^{14}C fixation (Fig. 6). Nevertheless, dark ^{14}C fixation was correlated with both the latter variables.

12-h vs. 24-h incubations—In opaque bottles, the amount of ^{14}C fixed after a dawn-to-dawn 24-h incubation was between 1.2 (shallow) and 2.8 (deep) times the amount fixed

Table 2. Model 2 regressions (log-log) of light and dark ^{14}C fixation [particulate dpm (total added dpm)$^{-1}$ (12 h)$^{-1}$] vs. Chl (μg liter^{-1}) bacterial abundance (10^6 cells ml^{-1}), and Tdr incorporation rate (pM d^{-1}) for plankton at the near-surface ($\leq$5 m), at the depth of maximum photosynthesis, and at the depth of maximum chlorophyll. Data from all cruises except 87-022 and 87-BB. r—Correlation coefficient; df—degrees of freedom; ν—model 2 slope; ν_L, ν_U = 95% C.L. on model 2 slope. All correlations are significant ($P < 0.01$) except those of dark ^{14}C fixation vs. bacteria (ns—not significant, $P > 0.05$).

	Chlorophyll					Bacteria					Thymidine				
Depth	r	df	ν	ν_L	ν_U	r	df	ν	ν_L	ν_U	r	df	ν	ν_L	ν_U
$Z \leq 5$ m															
Light ^{14}C	0.84	69	0.87	0.76	0.99	0.57	30	2.89	2.14	3.91	0.86	24	0.93	0.75	1.16
Dark ^{14}C	0.76	69	0.61	0.52	0.72	ns	—	--	—	—	0.75	24	0.64	0.49	0.85
Z at P_{max}															
Light ^{14}C	0.84	49	0.87	0.74	1.01	0.65	15	2.54	1.69	3.82	0.85	16	0.99	0.75	1.31
Dark ^{14}C	0.80	49	0.58	0.49	0.69	ns	—	--	—	—	0.77	16	0.66	0.47	0.91
Z at Chl_{max}															
Light ^{14}C	0.46	49	1.18	0.92	1.52	0.77	12	2.35	1.58	3.47	0.73	13	1.01	0.67	1.50
Dark ^{14}C	0.87	49	0.84	0.73	0.97	ns	—	--	—	—	0.71	13	0.82	0.55	1.24

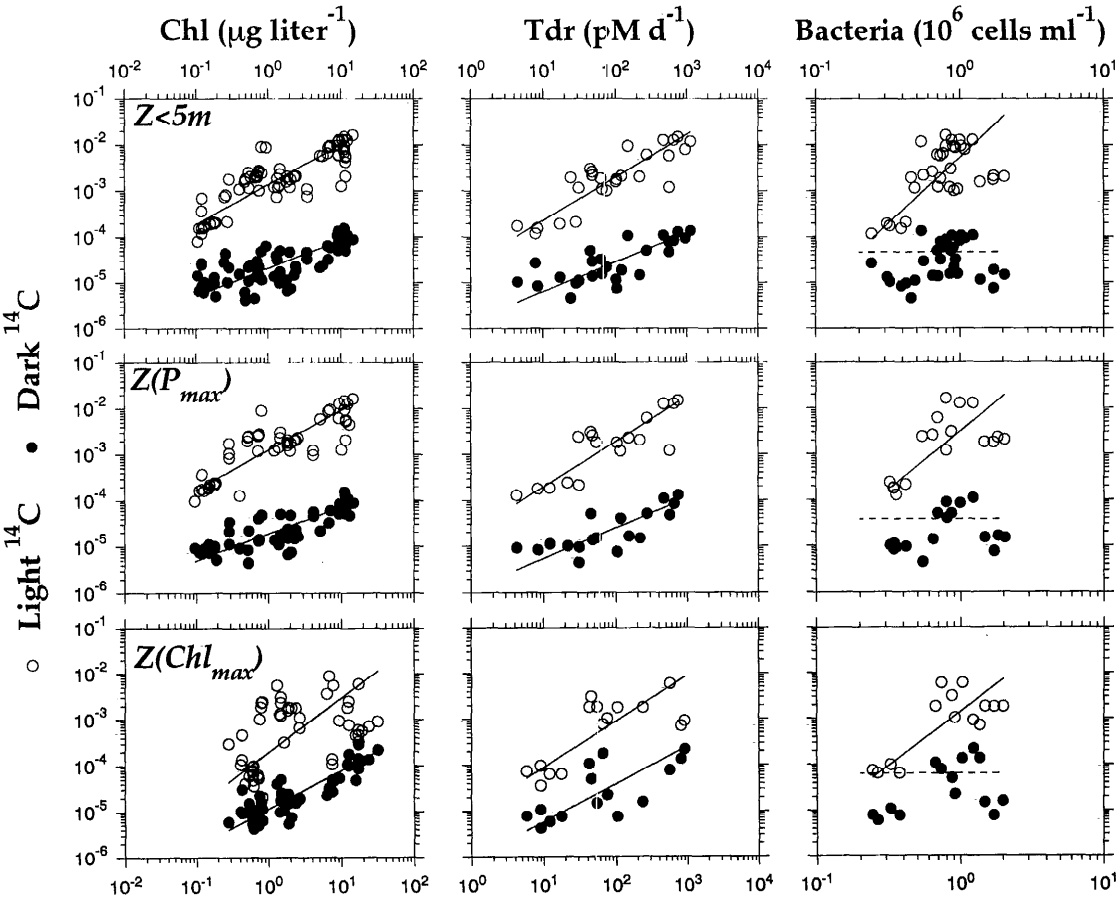

Fig. 4. Correlation between rates of light and dark ^{14}C fixation vs. Chl concentration, Tdr incorporation rate, and bacterial abundance for plankton sampled at depths <5 m, at the depth of maximum photosynthesis, and at the depth of maximum chlorophyll. Solid lines are model 2 regression fits; dashed horizontal line indicates that no significant correlation exists between dark ^{14}C fixation and bacterial abundance at any depth ($P > 0.05$). Units for fixation of ^{14}C—particulate dpm (total added dpm)$^{-1}$ (12 h)$^{-1}$.

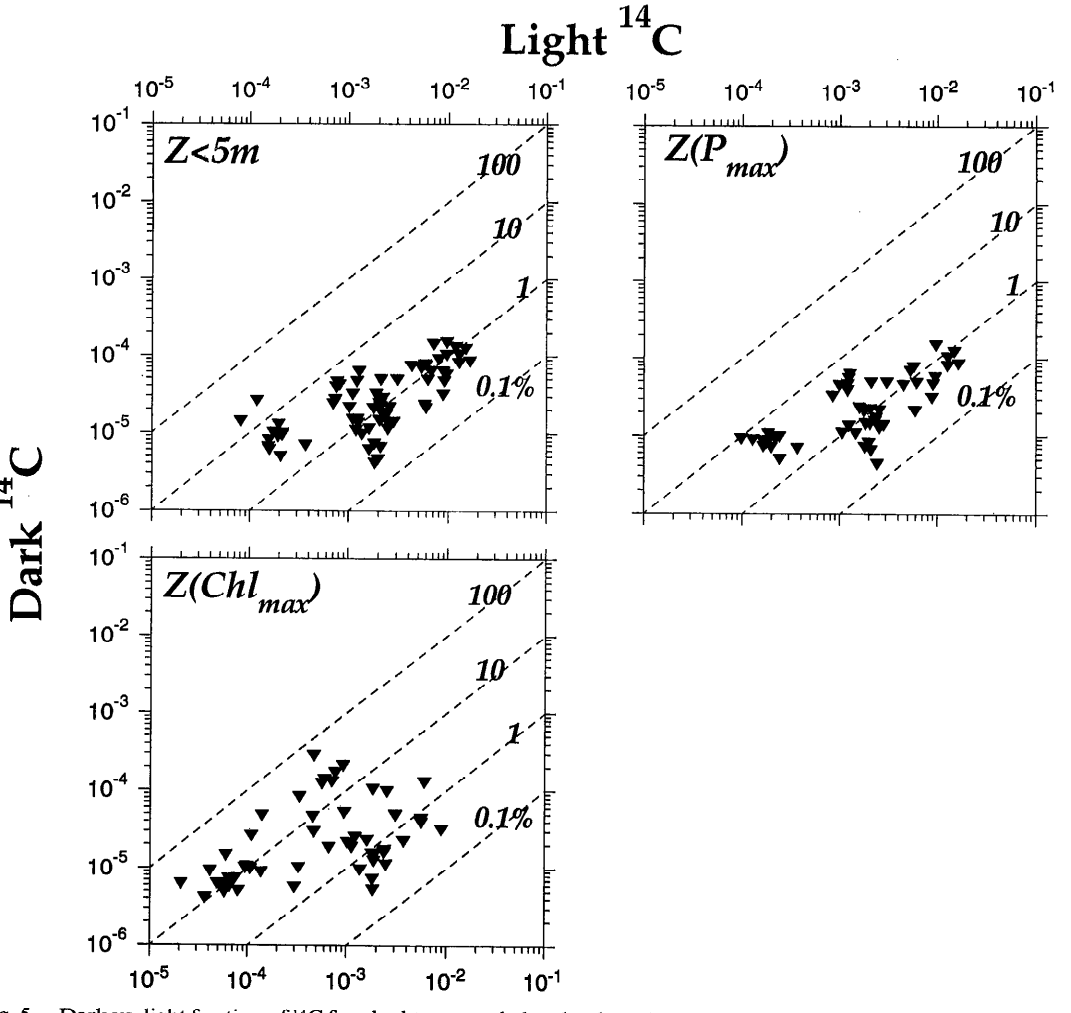

Fig. 5. Dark vs. light fixation of ^{14}C for plankton sampled at depths <5 m, at the depth of maximum photosynthesis, and at the depth of maximum chlorophyll. Dashed lines indicate dark : light ratio of 100, 10, 1, and 0.1%. Units for fixation of ^{14}C—particulate dpm (total added dpm)$^{-1}$ (12 h)$^{-1}$.

after a dawn-to-dusk 12-h incubation (Fig. 7). In other words, dark fixation of ^{14}C occurred throughout the 24-h period, although not necessarily at a linear rate, which would have been indicated by 24-h values being twice as large as 12-h values.

On the other hand, in clear bottles, there was a loss of fixed ^{14}C from dusk-to-dawn in ≤40-m plankton (Fig. 7). This was the expected nighttime loss of ^{14}C (respiration and grazing) fixed during the initial 12-h light period. In unexpected contrast, clear bottles at ≥50 m showed a net gain of fixed ^{14}C at night (Fig. 7).

As a result of these changes, the $D : L$ ratio at any depth was higher after 24 h than after 12 (Fig. 7). This ratio ranged from 0.006 (shallow) to 0.9 (deep) after 12 h, but from 0.008 (shallow) to 1.6 (deep) after 24 h (Fig. 7). In other words, dark ^{14}C never exceeded light ^{14}C after 12 h; however, after 24 h, deep plankton (≥60 m) kept in opaque bottles contained more ^{14}C than those kept in clear bottles.

Discussion

In general, should dark ^{14}C fixation be subtracted from light ^{14}C fixation? Although this problem has many facets (Banse 1993), our discussion focuses on only two issues: dark ^{14}C

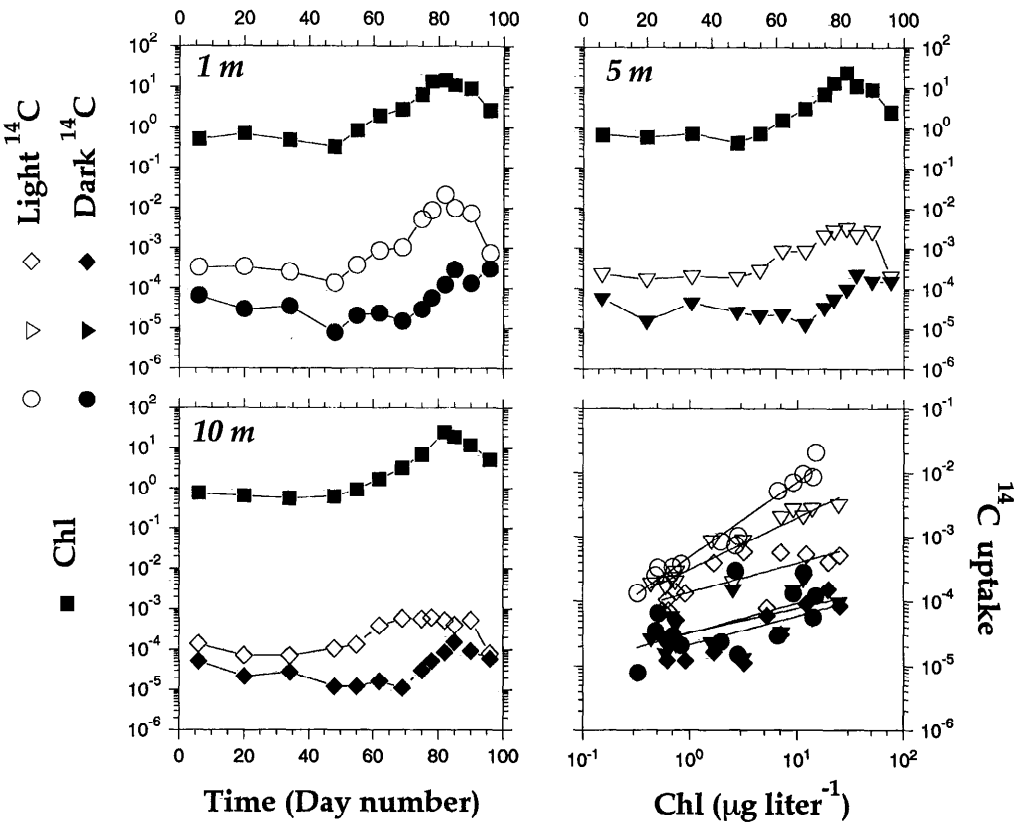

Fig. 6. Time series (1987) of Chl and light and dark fixation of ^{14}C in Bedford Basin at 1, 5, and 10 m. Lower right—scatterplots of light and dark fixation vs. Chl for data from the three depths. Units for fixation of ^{14}C—particulate dpm (total added dpm) $^{-1}$ (24 h) $^{-1}$.

fixation by heterotrophic bacteria and by autotrophic phytoplankton.

If the measured dark fixation of ^{14}C is due solely to bacterial processes that proceed similarly in clear and opaque bottles, then the answer should be affirmative. This assertion brings out three points to consider. First, can bacteria be implicated in contributing to dark ^{14}C fixation? Second, does ^{14}C fixation by bacteria differ in clear and opaque bottles? Third, are bacteria the sole contributors to measurable dark ^{14}C; if there is also demonstrable dark ^{14}C fixation by phytoplankton, then the question of whether to exclude such fixation becomes complicated by uncertainties in the biochemical pathways of carbon.

It seems clear that bacteria can indeed be implicated in measurable dark ^{14}C fixation. The significant correlation of dark ^{14}C fixation with [^{3}H]Tdr incorporation demonstrated in our results (Fig. 4, Table 2) is consistent with an important role for bacteria. In separate experiments reported earlier, we also showed that the specific rates of increase for ^{3}H-amino acid uptake and [^{3}H]Tdr incorporation were similar to that for dark ^{14}C fixation (Li and Dickie 1991). Steemann Nielsen and Aabye Jensen (1957) were first to point out the probable involvement of bacteria in dark fixation. Later, a UNESCO (1973) guide to the measurement of marine primary production illustrated the potential involvement of bacteria in dark ^{14}C fixation by an experiment in which different concentrations of bacteria were added to an algal culture. Elsewhere, Jones et al. (1958) measured bacterial abundance (pour-plate method) and ^{14}C fixation in dark bottles containing natural plankton; they calculated that after 24 h, 79% of the C fixed could be attributable to bacterial growth. A similar calculation indicated that growth of bacteria (epifluorescence microscopy) might reasonably

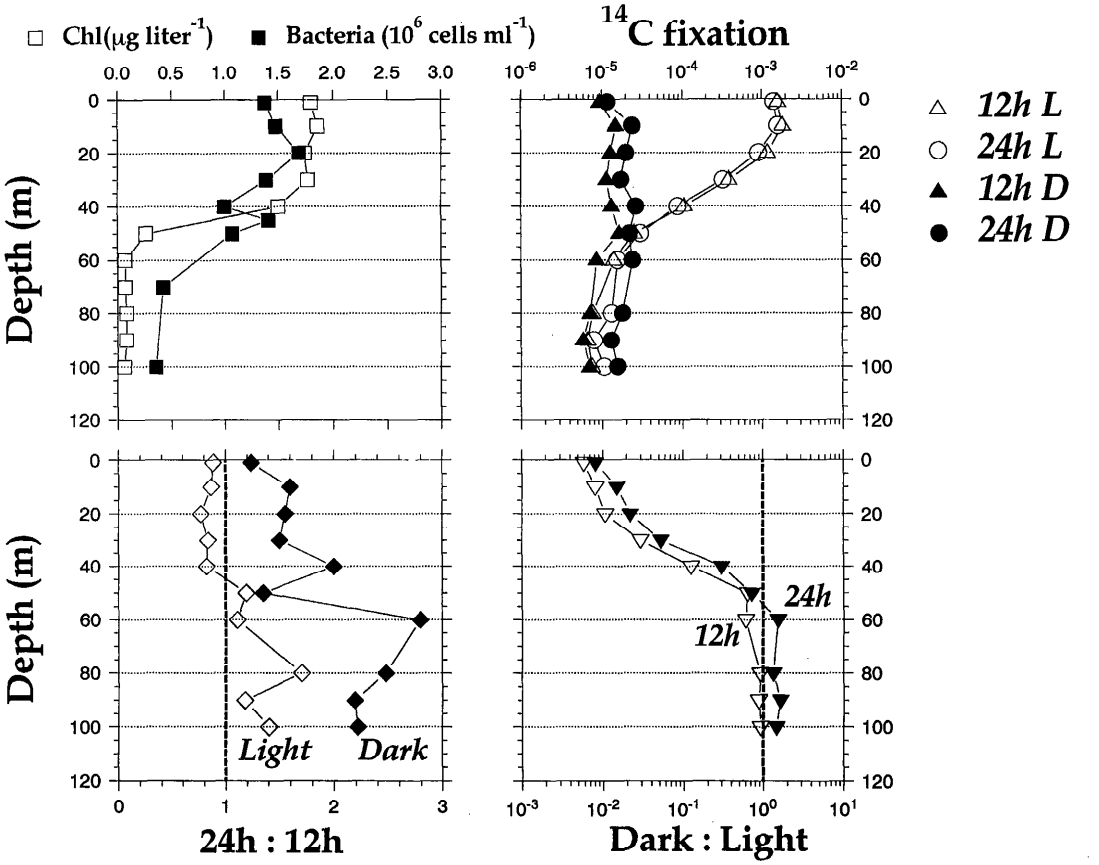

Fig. 7. Difference between 12-h (dawn-to-dusk) and 24-h (dawn-to-dawn) incubations in clear and opaque bottles. Upper left—profiles of Chl and bacterial abundance at the time of sampling (40°38′N, 47°10′W, 28 April 1989). Upper right—profiles of 12-h and 24-h ^{14}C fixation in clear and opaque bottles. Units for fixation of ^{14}C—particulate dpm (total added dpm) $^{-1}$ (incubation period) $^{-1}$. Lower left—ratios of 24-h : 12-h fixation in clear and opaque bottles. Lower right—ratios of opaque : clear bottles at 12 and 24 h.

account for much of the dark ^{14}C fixation measured in deep (i.e. dimly lit) plankton of the central North Atlantic (Li and Dickie 1991). In fact, some workers view the relationship between dark ^{14}C fixation and bacterial production to be an effective basis on which to derive an estimate of the latter from a measurement of the former (Romanenko 1964; Overbeck 1984; Sorokin 1990).

Recently, Harris et al. (1989) suggested that high values of dark ^{14}C fixation were associated with rapid bacterial growth, but that such rapid growth was an experimental artifact associated with the sampling and containment of fragile plankton. This artifact was prevalent in warm, oligotrophic subtropical and tropical waters, but inconsequential in cool, temperate waters. Our previous experiments in North Atlantic subtropical waters (32° and 37°N, 26°C) also indicated that high dark ^{14}C fixation was accompanied by significant growth and metabolic activity of bacteria (Li and Dickie 1991); however, we had no basis to judge whether the events were outcomes of experimental artifact. Our results here show convincingly that dark ^{14}C fixation is not high (in relation to light ^{14}C fixation) in surface waters (Fig. 5): the median and mean $D:L$ ratios were only 1 and 2% respectively. At the depth of the subsurface chlorophyll maximum which is dimly lit, these ratios were still quite low at 7 and 10% respectively (Fig. 5). We analyzed the data of Steemann Nielsen (1960) and found them similar to our own in two respects (Fig. 8): there

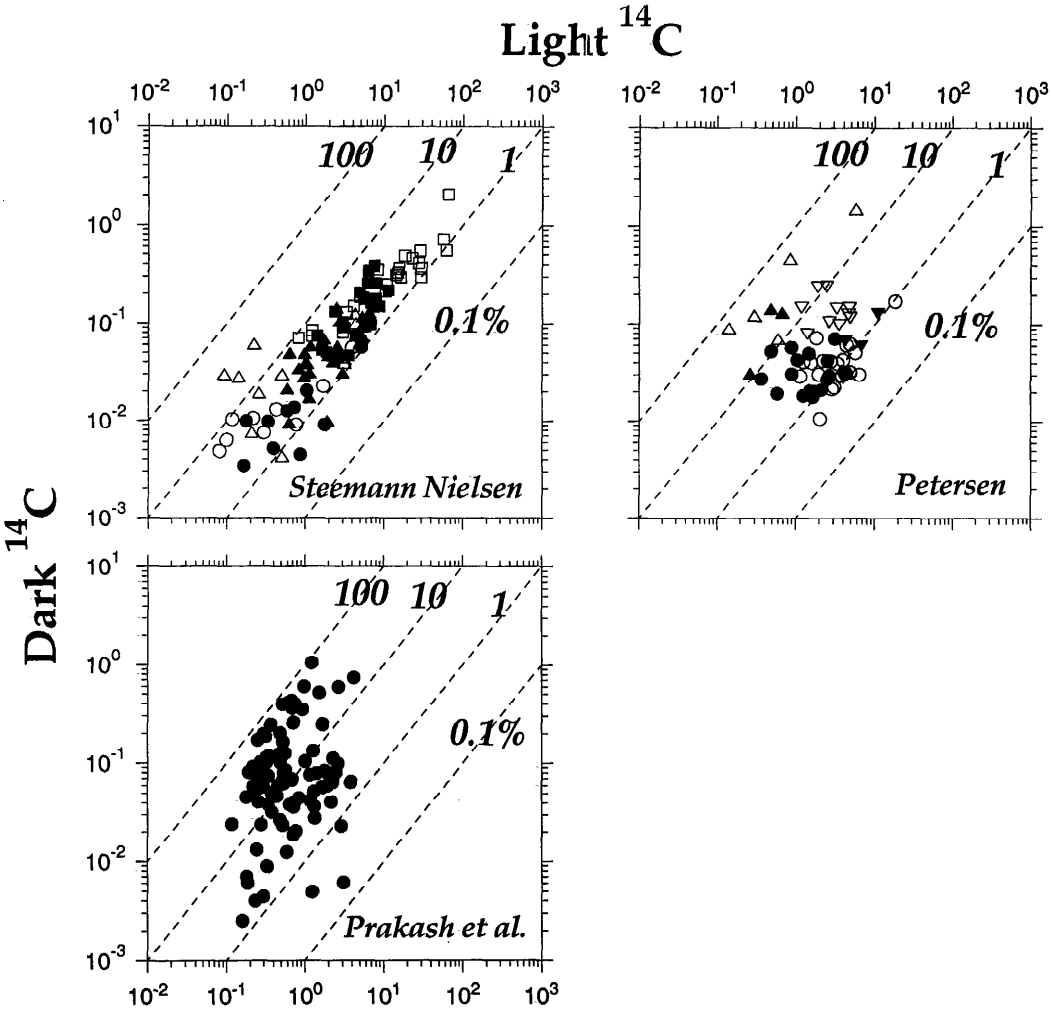

Fig. 8. Dark vs. light fixation of ^{14}C in published studies. Steemann Nielsen 1960—plankton sampled from the Øresund off Helsingør (●—surface; ○—depth of 15–20 m), from the North Atlantic and Greenland waters (▲—near surface; △—depth of 1% surface green light), and from the Grand Canal in Venice (■—high tide; □—low tide). Petersen 1979—surface-water plankton sampled from Godhavn (△), from the East Greenland current (▲), from the subarctic eddy (▽), from the 51° (●) and 62° (○) sections, and from off Frederikshåb (▼). Prakash et al. 1991—plankton sampled from <5-m depth at midocean stations from high northern to high southern latitudes in both the Pacific and Atlantic Oceans. Dashed lines indicate dark : light ratio of 100, 10, 1, and 0.1%. Units for fixation of ^{14}C—mg C m^{-3} h^{-1}.

was a significant correlation ($P < 0.01$) between dark and light ^{14}C fixation, and the median and mean $D:L$ ratios were low at 2 and 4%. In contrast, the data of Petersen (1979) and those of Prakash et al. (1991) showed no significant correlation ($P > 0.01$) between dark and light ^{14}C fixation (Fig. 8). The median and mean $D:L$ ratios were low (2 and 7%) for the data of Petersen, but higher (13 and 20%) for the data of Prakash et al.

In the waters for which we have data (Table 1), it is evident that dark ^{14}C fixation is low in 12-h dawn-to-dusk incubations. There are at least three circumstances under which the $D:L$ ratio assumes higher values. Two of these are rather obvious. First, the $D:L$ ratio is higher in deep samples (Figs. 2, 3, 5, 7) mainly because of low ^{14}C fixation in the clear bottles which receive only a little light. Second, the $D:L$ ratio is higher when the incubation is prolonged, especially if the clear bottles experience nighttime darkness (Fig. 7), because

^{14}C fixation in opaque bottles is continuous, because there is nighttime loss of fixed ^{14}C from plankton in the clear bottles, and also because there is a possibility that artifacts arising from containment of plankton become significant with time. The third circumstance under which the $D:L$ ratio assumes higher values is more intriguing. On the basis that the parameter b in Eq. 2 was <1, our results indicated that the $D:L$ ratio was higher at lower L. Since the rate of light ^{14}C fixation was also correlated with chlorophyll, bacteria, and Tdr incorporation (Fig. 4), it is not possible to say which, if any of these variables, was the factor of primary importance. Our results are consistent with the notion that the $D:L$ ratio is higher at lower levels of phytoplankton biomass (Morris et al. 1971; Prakash et al. 1991). The explanation for this empirical observation remains obscure.

While it seems convincing that bacteria can contribute to measurable ^{14}C fixation, it is less certain that this process is the same in clear and opaque bottles. Although it may be reasonable to suppose that β-carboxylation reactions in heterotrophic bacteria are light-independent, there does not appear to be a general consensus as to whether the rates of growth and metabolism (upon which the rate of β-carboxylation depends) in these organisms are the same in clear and opaque bottles (Li 1986; Banse 1993). Particular experiments may (e.g. Li and Dickie 1985) or may not (e.g. Li and Dickie 1991) indicate any difference. The bacterial measurements described here (abundance and Tdr incorporation in clear bottles) are not relevant to the issue of light–dark differences in bacterial processes. Specific experiments clearly aimed at this problem are needed.

We now question whether phytoplankton can also be implicated in the measurable fixation of ^{14}C in opaque bottles. The significant correlations we found between dark ^{14}C fixation and Chl (Figs. 4, 6) and between dark and light ^{14}C fixation (Fig. 5) do not allow us to dismiss a role for phytoplankton in dark fixation. However, as pointed out by Banse (1993), correlation analysis is not amenable to strong inference because the underlying basis for the relationship may not be a direct one. For example, the positive correlation between dark fixation and the phytoplankton attributes (i.e. light fixation, chlorophyll) may reflect an underlying positive relation between heterotrophic and phytoplankton attributes. Thus, a system in which phytoplankton are abundant and photosynthesizing at high rates is also a system in which the microbial loop may be more active. Be that as it may, and without independent direct evidence to the contrary, we can weakly infer that phytoplankton are responsible for some of the ^{14}C fixed in opaque bottles; there is no question that phytoplankton have the biochemical capability for ^{14}C fixation in the dark.

In algae, light-independent ^{14}C fixation is accomplished by β-carboxylating enzymes such as phosphoenolpyruvate (PEP) carboxylase, PEP carboxykinase, and pyruvate carboxylase (Glover 1989). Although these β-carboxylations occur in both the light and the dark, the reactions may perhaps be different in some respects: e.g. the origin of the enzyme substrate and the possibility of distinct pools of enzymes differentially activated by light or dark (Mortain-Bertrand et al. 1987, 1988). In the light, the substrates for these enzymes, PEP and pyruvate, are undoubtedly supplied by the Calvin cycle. However, in the dark, these substrates originate from an intracellular carbohydrate pool in the Phaeophyceae (Kremer 1981) and possibly also in the Bacillariophyceae (Mortain-Bertrand et al. 1987, 1988). Kremer (1981) suggested that nonphotosynthetic CO_2 fixation could justifiably be included in calculations of productivity on the theoretical basis that dissimilation of 1 mol of mannitol yields 2 mol of PEP which results in refixation of 2 mol of CO_2. However, Kerby and Raven (1985) cautioned that more information is needed on the kinetics of in vivo light-independent CO_2 fixation and on the ultimate fate of the C_4 products of β-carboxylation.

Our results (Fig. 5) confirm that in general, including oligotrophic regions where plankton biomass is low, dark ^{14}C fixation is high in relation to light fixation only at depths where photosynthesis is severely limited by light (Steemann Nielsen 1960). This confirmation is reassuring in view of apparently conflicting data sometimes recorded in the past 40 yr. Thus, the question of whether to subtract dark values from light values is significant only in the deep parts of the euphotic zone. As Banse (1993) demonstrated, this subtraction be-

comes important in calculations of primary production integrated through the water column. We have inferred that both bacteria and phytoplankton are directly responsible for the fixation of ^{14}C in opaque bottles. In the future, it may be possible to test this idea by flow cytometric sorting of bacteria and phytoplankton labeled in the dark with ^{14}C. Perhaps of more importance will be a clearer appreciation of the light–dark differences in both biochemical and trophic processes.

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