Dark uptake of inorganic $^{14}$C in oligotrophic oceanic waters

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Abstract. Dark uptake of inorganic $^{14}$C by offshore plankton was measured at two depths at 36 stations in the Atlantic Ocean from 52°S to 26°N, mainly along 30°W. The samples were incubated for 2 h with and without inhibition of biological activity with HgCl$_2$. In addition, six time course experiments were performed. The mean dark uptake rate varied from 0.68 to 4.82 μmol C m$^{-3}$ h$^{-1}$ over the transect and showed a significant positive relationship with chlorophyll a. The dark uptake was usually <5% of the maximum photosynthetic capacity ($P_m$), and higher values relative to $P_m$ were associated with low values of $P_m$ and not with high absolute dark values. A linear relationship between dark uptake and $P_m$ was found with a background value ($y$-axis intercept) of 0.51 μmol C m$^{-3}$ h$^{-1}$ and a slope of 0.77% of $P_m$. A major fraction of the dark signal, 66-80% of the total signal, persisted in bottles treated with HgCl$_2$, indicating that most of the dark signal was independent of biological activity. Time course experiments showed a linear dark uptake with time for the first hours, whereafter the uptake ceased. At stations with low concentrations of inorganic nitrogen [<1 μmol (NH$_4^+$ + NO$_3^-$)], a second stage was observed after 3-8 h, probably due to an increase in bacterial activity. The results suggest three mechanisms for the dark value in short-term incubations in oligotrophic waters. A background value independent of biomass and incubation time which was the dominant part of the dark signal in samples with very low phytoplankton biomass (<0.3 μg Chl a l$^{-1}$). Another important part was residuals of $^{14}$C associated with plankton, probably adsorbed to compounds inside the cells. This fraction was dominant in short-term incubations at chlorophyll concentrations >0.3 μg Chl a l$^{-1}$. Active uptake by living cells (total minus 'HgCl$_2$ uptake') was only a minor part of the dark signal in short-term incubations, but dominated at longer incubation time (>3-9 h), probably driven by an increase in bacterial activity. A significant enhancement of the non-photosynthetic uptake of $^{14}$C was observed in light, probably associated with a carbon-concentrating mechanism in phytoplankton or light stimulation of β-carboxylation activity. The results strongly suggest that dark values should be subtracted from the light uptake. This correction is particularly important when photosynthetic rates are low, e.g. at low light or in short-term incubations where a time-zero background becomes a significant part of the total uptake in light.

Introduction

Since Steemann Nielsen (1952) introduced the $^{14}$C-method for measurement of primary production of phytoplankton, it has become a standard method for aquatic ecologists, due to its high sensitivity, reproducibility and the relative ease by which many samples can be handled. In oligotrophic plankton systems, the $^{14}$C-method is the only approach with sufficient sensitivity to measure primary production. It has, therefore, become the main source of data for our understanding of primary production in these ecosystems. The principle of the method is simple, but an extensive literature during the last 40 years proves that interpretation of the results is far from simple (e.g. Peterson, 1980; Dring and Jewson, 1982; Jespersen, 1994). The problems can be divided into two categories: (i) relating the measured uptake of isotope to photosynthetic fixation of inorganic carbon by phytoplankton; (ii) to what extent does containment of the plankton
community alter the photosynthetic rate compared with the \textit{in situ} rate. In this paper, I will consider an aspect of the first type of problem that relates to the blank correction in the method.

When Steemann Nielsen introduced the method in 1952, he was well aware that a prerequisite was that the correction for uptake in the dark was small compared with the light uptake. In a simple laboratory experiment, he found that dark values were only \(-1\%\) of the uptake in light and therefore not a problem (Steemann Nielsen, 1952). His concern about the dark correction, and about the effect of respiration on the measured uptake rate in light, however, led him to the statement that 'the \(^{14}\)C-method was not suitable for the measurement of the intensity of photosynthesis at very low light intensities' (Steemann Nielsen, 1952). Many users have later overlooked Steemann Nielsen's precaution and the method has been used under all circumstances possible. Later, dark samples were introduced as a standard part of the protocol with the purpose of obtaining a correction for the activity in the final counted samples that were unrelated to the photosynthetic uptake. During the last 35 years, several papers have reported that this correction can be considerably higher than \(1\%\) of the light-saturated uptake, and occasionally equal to the uptake in light (Morris \textit{et al.}, 1971; Petersen, 1979; Harris \textit{et al.}, 1989; Prakash \textit{et al.}, 1991; Li \textit{et al.}, 1993). These results were mainly from oligotrophic marine waters, and have caused the paradoxical situation that common practice in marine systems is to omit dark samples, whereas most freshwater ecologists do subtract the dark value from the light uptake.

Several mechanisms have been proposed to account for the radioactivity in dark samples. Some are physical phenomena, including labeled organic impurities in the isotope and adsorption of inorganic carbon to particles or to the filter, whereas others are associated with non-photosynthetic carboxylation processes in the dark by phytoplankton and bacteria. The focus for possible explanations of high dark values has been on the biological mechanisms (Legendre \textit{et al.}, 1983; Prakash \textit{et al.}, 1991; Li \textit{et al.}, 1993) where the physical phenomena are considered less important. Yet, a number of the earlier data sets indicate that a background signal (unrelated to biomass or incubation time) could play an important role, and probably account for some of the high values for relative dark uptake observed in oligotrophic waters (Banse, 1993).

Residuals of inorganic carbon are probably the most important part of a dark value. In 1952, Steemann Nielsen prescribed that filters should be treated with fuming HCl to remove residuals of inorganic carbon, but for various reasons some authors have later omitted that step. Steemann Nielsen (1960) suggested that this could be the cause for some of the first reports of high dark values. Lean and Burnison (1979) and Hitchcock (1986) have also pointed out the importance of the acidification step, in connection with a negative relationship between activity/volume and volume of water filtered. They recommend applying HCl directly on the filters after they are placed in vials, as also used in the present experiments. Thus, residuals of inorganic carbon must be considered a possible part of the dark value in experiments where the filters were not acidified, e.g. Morris \textit{et al.} (1971) and Ignatiades \textit{et al.} (1987), but probably also in experiments where acidification was carried out (see below). The removal of labeled inorganic
Dark uptake of $^{14}$C carbon is particularly important in low productive systems where high $^{14}$C activities are used and small residuals may constitute a large fraction of the total uptake, i.e. under the same conditions where problems with high dark values relative to the photosynthetic uptake are most frequently reported.

The objective of the present study was to obtain estimates of dark uptake of $^{14}$C over a range of oceanic conditions, and to divide the uptake into a physical uptake and a biological driven uptake. Part of the dark signal was due to adsorption of inorganic $^{14}$C to filters and the surface of particles. This is not an uptake in the sense that inorganic carbon has crossed the cell membrane; however, for the sake of simplicity, uptake is used as synonym for the activity measured on a filter after incubation.

Method

The experiments were carried out on the Spanish research vessel ‘BIO Hesperides’ in March and April 1995 as part of the project LATITUDE-I. Samples were taken on two transects in the South Atlantic Ocean (Figure 1). The first transect ran parallel to the coast of South America on the continental shelf. Chlorophyll concentrations were between 1 and 2 $\mu$g l$^{-1}$ at the surface and in the deep chlorophyll maximum (DCM). Transect II went through the central parts.

Fig. 1. Map showing the two transects.
of the Atlantic Ocean mainly along 30°W and chlorophyll concentrations varied from 0.03 to 0.9 μg l⁻¹, lowest at the surface between 10°S and 20°S, and highest in the DCM.

Samples were collected from 5 m and from the DCM with a rosette of opaque Niskin bottles. The DCM was determined from the peak in the fluorescence signal. Duplicate samples were transferred into rinsed 5 l bottles and kept in dim light before incubations were initiated within 30–60 min of sampling. A sample volume of 2 l was transferred to a darkened bottle placed on a magnetic stirrer and [¹⁴C]HCO₃⁻ was added. The isotope was obtained from ¹⁴C-Central, Hørsholm, Denmark. The activity added varied between 120 and 1500 μCi l⁻¹, depending on the chlorophyll concentration—highest at low concentrations. The sample was allowed to mix for 2 min before subsamples for specific activity were taken. Then, aliquots of 61.8 ml were dispensed into two sets of 14 flat tissue-culture flasks (Nunclon). Each set consisted of 10 light bottles (LB), two dark bottles (DB) and two dark bottles with HgCl₂ (final concentration 20 mg l⁻¹) added before dispensing into flasks (HgB). On eight stations at the end of transect II, DCMU was added to a final concentration of 32 μmol l⁻¹ to two dark bottles in each set, making each set 16 bottles. Duplicate sets were made at each station and depth.

The bottles were incubated in a linear incubator at in situ surface and DCM temperature, respectively, for 90–120 min. Irradiances ranged from 11 to 1190 μmol m⁻² s⁻¹ with a spectrum that resembled daylight. After incubation, the samples were filtered through Whatman GF/F filters and placed in scintillation vials. Then, 40 μl of 0.5 N HCl were placed on each filter and the vials were allowed to stand for 24 h before the scintillation cocktail (Ecoscint H, National Diagnostics, Atlanta, GA) was added. This technique gave a lower variability between samples compared to fuming with HCl (S.Markager, unpublished data), probably because the efficiency of HCl fumes depends on the amount of moisture on the filter that can take up the acid.

After a minimum of 24 h, the vials were counted in a Beckman Liquid Scintillation Counter, until the counting precision was better than 1.5% (95% CL) or for a maximum of 30 min. The latter were usually the case for the dark samples, and the counting precision was typically 4–5%. D.p.m. values were calculated from the c.p.m. values according to Beckman Instruments (1993) using a background quench curve made from vials with wet filters and a quench curve made from ¹⁴C standards.

The light uptake was calculated from the equation in Steemann Nielsen (1952) and the light-saturated uptake rate \( P_m \) was estimated from P–I curves using the P–I function described in Webb et al. (1974). The dark signal was not subtracted from the light uptake in this paper in order to avoid the dark signal appearing on both sides of the equation in the analysis of the relationship between dark uptake and \( P_m \).

Chlorophyll concentrations were obtained from S.Agustí and P.Murra (unpublished data), bacteria numbers and leucine uptake rates from J.Gasol and D.Vaqué (unpublished data), and data for inorganic carbon concentrations from Hein (1997).
Results

Dark uptake

Dark uptake rates at the coastal shelf were similar in surface and DCM samples, and ranged from 1.06 to 11.2 μmol C m⁻³ h⁻¹ with a mean value of 4.82 ± 1.14 (mean ± 95% CL; Figure 2). In the oligotrophic parts of the ocean (transect II), dark uptake rates decreased 5- to 7-fold to mean values of 0.68 ± 0.08 and 0.94 ± 0.12 μmol C m⁻³ h⁻¹ for surface and DCM samples, respectively. The range was from 0.35 to 2.35 μmol C m⁻³ h⁻¹. No latitudinal pattern could be detected, except the overall relationship to chlorophyll concentration (Figure 2). Chlorophyll-specific values at the surface ranged from 4 to 13 μmol C mg⁻¹ Chl h⁻¹ and were somewhat higher than DCM values (1-6 μmol C mg⁻¹ Chl h⁻¹) (Figure 3). Overall, there was a significant positive relationship between chlorophyll concentration and dark uptake of ¹⁴C (Spearman rank correlation coefficient = 0.69, P < 0.0001; Figure 4). This relationship could be described by the equation:

\[
\text{Dark signal (μmol C m}^{-3}\text{ h}^{-1}) = 0.65 \pm 0.14 \times \exp(\text{Chl} \times 1.46 \pm 0.14) \quad (1)
\]

\((r^2 = 0.92, \pm 95\% \text{ CL, Chl in μg l}^{-1})\) calculated with linear regression on log-transformed values of the dark signal. No significant relationships were detected when the four groups of samples were tested separately (transect I and II and

![Fig. 2. Dark uptake of inorganic carbon (●) and chlorophyll concentrations (○) in the Atlantic Ocean in samples from the surface and the deep chlorophyll maximum (DCM). Error bars for dark uptake (only shown when outside the symbol) indicate the range between two replicates. The transition from transect I (on the coastal shelf of South America) to transect II (Central Atlantic Ocean) is shown by ↓.](https://academic.oup.com/plankt/article-abstract/20/9/1813/1564406/fig-2)
Fig. 3. Chlorophyll-specific dark uptake rates. See also the legend to Figure 2.

Fig. 4. Relationship between dark uptake and chlorophyll concentration. The data are divided into surface values from transect 1 (■) and transect 2 (○), and DCM values from transect 1 (●) and transect 2 (□). The line is the equation: dark uptake = 0.65 ± 0.14 + exp(Chl × 1.46 ± 0.14), ± 95% CL. The point in parentheses was omitted from the estimation of the line.

surface and DCM samples, respectively). However, when samples from the surface and the DCM from transect II were merged, a significant linear relationship was found:

\[
\text{Dark signal (μmol C m}^{-3}\text{ h}^{-1}) = 0.61 \pm 0.14 + \text{Chl} \times 0.91 \pm 0.43 \quad (2)
\]

(± 95% CL). The positive y-axis intercept was significant in this model and in the
Dark uptake of $^{14}$C

previous one, which suggests a background value of $-0.63 \mu$mol C m$^{-3}$ h$^{-1}$ unrelated to phytoplankton biomass. The background explains the elevated chlorophyll-specific dark values at the surface for transect II where the lowest chlorophyll concentrations were found (Figure 3).

Dark values were positively correlated with $P_{m}$ (Spearman rank correlation coefficient $= 0.74, P < 0.0001$; Figure 5). The distribution of both variables was biased towards low values and the variance increased with the independent variable ($P_{m}$). Thus, linear regression could not be applied. Nonetheless, a visual inspection suggested an approximately linear relationship with a positive intercept (Figure 5) so the usual transformations that force the line through the origin were not appropriate. The parameters in a linear relationship were therefore estimated from the equation:

$$\log(D) = \log(D_{b} + P_{m} A/100)$$

(3)

Fig. 5. Relationship between dark uptake of $^{14}$C and maximum photosynthetic rate for all data (A) and for data where $P_{m} < 200 \mu$mol C m$^{-3}$ h$^{-1}$ (B). Estimated linear relationships are shown for all stations (---), for transect II (--------) and for stations where $P_{m} < 40 \mu$mol C m$^{-3}$ h$^{-1}$ (. . . . .). Parameters for lines are given in Table I. Legends for points are as for Figure 4.
by an iterative fitting procedure (DUD; Statistical Analysis Systems, 1990). This method gave an equal distribution of the two variables and normally distributed residuals. The parameters in equation (3) were: $D$, total dark signal; $D_b$, background dark value; $k$, dark signal as a percent of $P_m$.

A significant positive intercept ($D_b$) of $0.51 \pm 0.07 \mu\text{mol C m}^{-3} \text{h}^{-1} (\pm 95\% \text{ CL})$ for the entire data set shows that part of the dark value was unrelated to phytoplankton activity. The value for $k$ was $0.77 \pm 0.15\%$, showing that the dark uptake increased with $<1\%$ of $P_m$. When the relationship was tested for subsets of data, the values for $D_b$ increased with increasing mean Chl $a$ concentration and the slope decreased, but the overall pattern remained the same (Table I, Figure 5). However, a substantial part of the variation in the dark signal could not be related to $P_m$.

Dark uptake of $^{14}$C also showed significant positive relationships to bacterial number and leucine uptake. However, these variables were strongly correlated with chlorophyll concentration and $P_m$, and their capability to predict dark uptake rates was lower than for the Chl $a$ concentration and $P_m$. Residuals from the relationships between dark uptake and $P_m$ were tested for correlations to bacterial number, leucine uptake, latitude, temperature and activity added per bottle. In all cases, the relationship was less than significant ($P > 0.42$, tested with Spearman rank correlation coefficients) and the residuals showed no pattern when plotted against the above-mentioned independent variables.

Dark values were also expressed relative to $P_m$ at each station (Figure 6). The values range from 0.34 to 22.1% with mean values ($\pm 95\% \text{ CL}$) of $1.18 \pm 0.49$ and $4.08 \pm 0.73\%$ for transect 1 and 2, respectively. Median values were 1.03 and

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Fig. 6. Dark uptake rates as a percent of the light-saturated photosynthetic uptake ($P_m$). See also the legend to Figure 2.
Dark uptake of $^{14}$C

3.26%, respectively, and 82% of the values were <5% of $P_m$ and 94% were <10% of $P_m$. The few high values observed were all associated with low values of $P_m$ and not with high dark values. When plotted against $P_m$, a clear reciprocal pattern was observed (Figure 7A), as expected from the positive intercept in the linear relationship.

Table I. Statistics for a linear relationship between dark values and photosynthetic capacity ($P_m$).

<table>
<thead>
<tr>
<th>Data set</th>
<th>$D_0 \pm 95% CL$</th>
<th>$k \pm 95% CL$</th>
<th>$r^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>All data</td>
<td>0.51 (0.44–0.58)</td>
<td>0.77 (0.62–0.92)</td>
<td>0.58</td>
<td>131</td>
</tr>
<tr>
<td>Transect I</td>
<td>2.72 (2.15–4.75)</td>
<td>0.31 (0.14 to 0.76)</td>
<td>0.06</td>
<td>23</td>
</tr>
<tr>
<td>Transect II, surface</td>
<td>0.49 (0.40–0.57)</td>
<td>0.57 (0.28–0.86)</td>
<td>0.10</td>
<td>57</td>
</tr>
<tr>
<td>Transect II, DCM</td>
<td>0.71 (0.57–0.85)</td>
<td>0.48 (0.15–0.80)</td>
<td>0.15</td>
<td>51</td>
</tr>
<tr>
<td>Transect II, all samples</td>
<td>0.55 (0.46–0.63)</td>
<td>0.59 (0.36–0.82)</td>
<td>0.16</td>
<td>108</td>
</tr>
<tr>
<td>$P_m &lt;$ 40 μmol C m$^{-3}$ h$^{-1}$</td>
<td>0.38 (0.27–0.47)</td>
<td>1.72 (1.16–2.28)</td>
<td>0.27</td>
<td>81</td>
</tr>
</tbody>
</table>

Fig. 7. Relationships between relative dark uptake and light-saturated photosynthetic uptake (A) and absolute dark uptake rate (B). Lines are calculated from the linear relationship between dark uptake and $P_m$ for all stations (Table I, Figure 5).
relationship on Figure 5. When plotted against the absolute dark value, no positive relationship could be found, despite the absolute dark value appearing on both axes (Figure 7B). In fact, the relationship was also reciprocal, due to the overriding effect of the positive relationship between the absolute dark value and $P_m$ (Figure 5). This confirms that high dark signals relative to $P_m$ were associated with low $P_m$ values rather than with high dark values.

Dark values in bottles with HgCl$_2$ added constituted 66 ± 6.5% (mean ± 95% CL) and 80 ± 4.8% of the signal in the untreated dark bottles for surface and DCM samples, respectively. There was no clear pattern in the variation around the mean values with latitude or between the two transects, except for a tendency toward lower values in DCM samples south of 17°S (Figure 8). The 'HgCl$_2$ uptake' showed the same pattern in relation to Chl as the total signal, not surprising since it was such a large fraction of the total uptake. The relationship was described by the equation:

$$\text{HgCl}_2 \text{ signal (} \mu \text{mol C m}^{-3} \text{ h}^{-1} \text{)} = 0.37 \pm 0.04 \times \exp(\text{Chl} \times 1.50 \pm 0.19) \quad (4)$$

($r^2 = 0.92$, ± 95% CL, Chl in µg l$^{-1}$). The parameters were estimated as for the total uptake [equation (1)]. The efficiency of the inhibition was checked on several occasions by exposing bottles treated with HgCl$_2$ to light. The signal was always equal to the uptake in dark bottles treated with HgCl$_2$.

Addition of DCMU had no significant effect on the dark value. The mean value

![Figure 8](https://academic.oup.com/plankt/article-abstract/20/9/1813/1564406)

**Fig. 8.** Dark values in bottles with 20 mg HgCl$_2$ l$^{-1}$ added as a percent of the value in untreated dark bottles. Broken lines indicate the mean value for surface and DCM samples, respectively. Dotted lines indicate 100%, i.e. an uptake equal to the uptake in the untreated dark bottles.
Dark uptake of $^{14}$C

in the DCMU-treated bottles as a percentage of the dark value in untreated bottles was $95.7 \pm 7.3\%$ (95% CL) for the 32 samples (eight stations, two depths and two sets) with a variation from 68 to 110%.

Time course experiments

The uptake in the untreated dark bottles increased with time (Figure 9). The general pattern was a linear increase with time (equal to a constant rate) for 1–5 h. Then, the uptake slowed or ceased for a period, whereafter it started to increase again. However, in experiment 4 (3.2°N), the uptake rate was high throughout the experiment. The increase in the second stage was significant in experiments 1–4 (Figure 9A and B) where inorganic nitrogen concentrations

![Fig. 9. Time courses of $^{14}$C uptake in untreated dark bottles (filled symbols) and in bottles with 20 mg HgCl$_2$ l$^{-1}$ added. Additional information about the six experiments is given in Table II.](https://academic.oup.com/plankt/article-abstract/20/9/1813/1564406)
were low, but weak or absent in experiments 5 and 6 where the concentrations of inorganic nitrogen were higher (Table II). The uptake rate in the initial linear phase was estimated with linear regression (Table II) and varied between 0.29 and 2.05 \( \mu \text{mol C m}^{-3} \text{h}^{-1} \). In all experiments except no. 2, there was a significant positive time-zero intercept. A consequence of the non-linear pattern in the dark uptake with time was that the calculated mean rate in an endpoint incubation depended on the incubation time. The mean rate was higher in short-term incubations due to the positive time-zero intercept, whereafter it decreased with increasing incubation time. However, if the dark uptake increased after some time, the mean uptake rate did so as well, and the lowest mean rate over the incubation was observed at an intermediate (2-5 h) incubation time.

Bottles poisoned with \( \text{HgCl}_2 \) were included in four of the six time course experiments (Figure 9A and B). In these bottles, the signal was constant with time when tested by linear regression of signal versus time (\( P > 0.42 \)). The mean value in each experiment was between 0.93 and 1.30 \( \mu \text{mol C m}^{-3} \), or equal to the value in the standard 2 h experiments at the same stations. Thus, the signal in bottles treated with \( \text{HgCl}_2 \) represents an initial value which did not increase with time, at least not after 30 min, which was the shortest incubation time applied in the first four time course experiments. This also confirms that the addition of \( \text{HgCl}_2 \) was effective in inhibiting biological activity.

Five time course experiments with \( ^{14}\text{C} \) uptake at different irradiances showed a linear uptake in light with time for a certain period, after which the rate tended to decrease. Experiments 5 and 6 (Table III) had a higher time resolution during the first hours than the first three experiments, which made it possible to quantify the initial uptake pattern. The results for the first 50 min of experiment 6 are shown in Figure 10. Significant positive intercepts at time zero were found in both experiments 5 and 6 when calculated in the same manner as performed above for the dark uptake. Moreover, in all but one case, the intercepts were significantly higher that the corresponding intercepts for the dark value (Table III). In experiments 1–3, the time resolution was lower and the uptake rate in light started to

### Table II. Dark uptake of \( ^{14}\text{C} \) in six time course experiments. The time-zero intercept and the slope (± 95% CL) in untreated dark bottles were calculated by linear regression on data points (n) up to the time when the uptake rate started to decrease. The signal in bottles with \( \text{HgCl}_2 \) added did not change with incubation time and the value is given as the mean ± 95% CL for all samples in each experiments. Units are: Chl (\( \mu \text{g m}^{-3} \)), ammonium plus nitrate (\( \mu \text{mol l}^{-1} \)), intercept and \( \text{HgCl}_2 \) signal (\( \mu \text{mol C m}^{-3} \)), slope (\( \mu \text{mol C m}^{-3} \text{h}^{-1} \)) and time (minutes).

<table>
<thead>
<tr>
<th>Latitude</th>
<th>Depth</th>
<th>Chl ( a ) (NH(_4)(^+) + NO(_3)-)</th>
<th>Untreated dark bottles</th>
<th>( \text{HgCl}_2 ) bottles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intercept ± S.D.</td>
<td>Slope ± S.D.</td>
<td>Intercept ± S.D.</td>
</tr>
<tr>
<td>35.4°S</td>
<td>Surface</td>
<td>0.16 ± 0.09</td>
<td>1.34 ± 0.56</td>
<td>0.29 ± 0.24</td>
</tr>
<tr>
<td>22.2°S</td>
<td>DCM</td>
<td>0.16 ± 0.25</td>
<td>0.59 ± 0.99</td>
<td>0.45 ± 0.48</td>
</tr>
<tr>
<td>19.5°S</td>
<td>DCM</td>
<td>0.18 ± 0.16</td>
<td>0.62 ± 0.60</td>
<td>0.33 ± 0.21</td>
</tr>
<tr>
<td>3.2°N</td>
<td>Surface</td>
<td>0.22 ± 0.38</td>
<td>0.77 ± 0.44</td>
<td>0.80 ± 0.21</td>
</tr>
<tr>
<td>13.1°N</td>
<td>Surface</td>
<td>0.18 ± 1.56</td>
<td>0.63 ± 0.07</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td>24.5°N</td>
<td>DCM</td>
<td>0.37 ± 1.42</td>
<td>0.81 ± 0.24</td>
<td>2.05 ± 0.62</td>
</tr>
</tbody>
</table>

n.d., no data. 1824
Dark uptake of 14C
decrease after 70 min, providing only two points to calculate the initial uptake rate. However, when the slope between these points was extrapolated back to time zero, positive intercepts were found in all but one case (Table III). There was a tendency toward higher intercept values with increasing irradiance.

On three occasions, a 10 l sample was taken from the rosette of Niskin bottles and subsamples were transferred to 1 l glass bottles and incubated in a deck tank

Table III. Time-zero intercepts for linear relationships between carbon uptake in light and time. In experiments 1–3 (numbers correspond to numbers in Table II), intercepts were estimated from the slope between the uptake after 30 and 60 min of incubation. In experiments 5 and 6, the intercepts were calculated by linear regression on all data points before the photosynthetic rate started to decrease (see Figure 10). For these experiments, the intercept is given with 95% CL (t-test) and it is indicated whether the value was significantly higher than the corresponding value for the dark uptake (Table II). Units are: intercepts (μmol C m\(^{-2}\))

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Irradiance (μmol m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1186(^a)</td>
</tr>
<tr>
<td>1</td>
<td>-0.94</td>
</tr>
<tr>
<td>2</td>
<td>7.89</td>
</tr>
<tr>
<td>3</td>
<td>8.48</td>
</tr>
<tr>
<td>5</td>
<td>1.12 ± 0.07*</td>
</tr>
<tr>
<td>6</td>
<td>2.39 ± 0.22**</td>
</tr>
</tbody>
</table>

n.d., no data; -, not calculated.
Significance: *P < 0.05; **P < 0.01.

*In experiment 6, the irradiances were reduced to 760, 264, 91 and 15 μmol m\(^{-2}\) s\(^{-1}\), respectively.

Fig. 10. Time courses of 14C uptake in light (irradiances are given in μmol m\(^{-2}\) s\(^{-1}\)) and in the dark for experiment 6 (see Table II for details). Filled symbols are used for estimation of initial uptake rates by linear regression (Table III). The differences between the dotted lines indicate the magnitude of an uptake by a pool of inorganic carbon in the cells with an intracellular DIC concentration of 2-fold the concentration of DIC in sea water. See Discussion for assumptions and implications.
at *in situ* irradiance and temperature. At hourly intervals, a subsample was collected and incubated with $^{14}$C for 90–120 min, as for the standard samples. The dark uptake in the bottles with HgCl$_2$ remained constant regardless of the pre-incubation period, but the dark value in the untreated bottles increased with the pre-incubation time, although at different rates (Figure 11). The increase over 5 h of pre-incubation varied from ~2-fold in a surface sample from latitude 9.3°N and a DCM sample from 26.0°N to 6-fold in a surface sample from 18.3°S.

**Discussion**

*Mechanisms for dark uptake*

The activity measured on a filter after a dark incubation can arise from inorganic $^{14}$C, $^{14}$C in organic compounds and from contamination with other isotopes. Both forms of $^{14}$C activity can originate from several mechanisms that will all contribute to the final activity, but the quantitative role of each mechanism will vary with environmental and physiological conditions. The results presented here point at residuals of inorganic carbon as the single most important mechanism for dark uptake in short-term incubations in oligotrophic waters.

Residuals of inorganic $^{14}$C can be present in the bulk water on the filter, absorbed to the filter or to cell surfaces, and inside cells either in solution or bound to membranes or compounds; for example, Beardall and Raven (1981) have suggested that bicarbonate can be bound to proteins. Residual of inorganic carbon should be removed during the acidification step, but the efficiency of this process may not be as high as often assumed. In particular, inorganic carbon within the cell may not be exposed to the acid unless actions are taken to break up the cell membranes, e.g. with methanol (Li and Goldman, 1981). This is

![Fig. 11. Dark uptake of $^{14}$C during 2 h incubations in untreated dark bottles for samples that were pre-incubated in a deck tank at *in situ* light and temperature for 1–5 h. Time zero is the dark uptake in the routine incubations started <30 min after the subsampling (mean and range of two replicates [only visible when outside symbols]). • 18.3°S. ■ 9.3°N. ▲ 26.0°N.](https://academic.oup.com/plankt/article-abstract/20/9/1813/1564406)
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common in physiologically oriented studies (e.g. Mortain-Bertrand et al., 1988), but is seldom used in field experiments.

We can therefore divide the dark signal into three fractions. (i) A background fraction associated with inorganic carbon adsorbed to the filter \((D_b)\). This fraction will also include labeled particulate impurities in the isotope solution and is equivalent to a time-zero blank, i.e. a sample that is filtered immediately after the isotope is added to the sample. (ii) A second fraction is associated with inorganic carbon adsorbed to the cell surfaces or to structures inside the cells or in solution inside the cells \((D_i)\). This fraction will essentially depend on the total biomass of the plankton community. It does not require enzymatic activity, since CO\textsubscript{2} can enter the cells by passive diffusion, but an active CO\textsubscript{2}-concentrating mechanism might shorten the period before equilibrium is reached. (iii) The last fraction, where labeled carbon is incorporated into organic compounds \((D_o)\), is mediated by enzymatic processes, i.e. carboxylation. In darkness, this is likely to be primarily \(\beta\)-carboxylation where C-3 compounds such as pyruvate and phosphoenolpyruvate are carboxylated to oxaloacetic acid (C-4). These processes occur in all types of organisms and the rate is therefore likely to be related to the biomass and/or activity of all planktonic organisms. This division of the dark signal corresponds to the division in Legendre et al. (1983), except that the passive uptake is subdivided into a background value and a biomass-related uptake.

The background fraction \((D_b)\) in the present experiment was estimated from the \(y\)-axis intercepts unrelated to biomass or photosynthetic activity. The best estimates are probably from the relationships between chlorophyll concentration and the HgCl\textsubscript{2} signal \((0.37 \, \mu\text{mol} \, \text{m}^{-3} \, \text{h}^{-1})\) and total dark signal at low levels of \(P_m\) \((0.38 \, \mu\text{mol} \, \text{C} \, \text{m}^{-3} \, \text{h}^{-1})\). This value is equivalent to 0.75 \(\mu\text{mol} \, \text{C} \, \text{m}^{-3}\) over 2 h, which is in good agreement with the time-zero intercepts calculated in Table II \((0.59-1.34 \, \mu\text{mol} \, \text{C} \, \text{m}^{-3})\). This fraction is \(<5 \times 10^{-2}\) of the added activity and therefore only important in samples with low biomass (Figure 12). The size of the \(D_i\) fraction will vary with biomass, and can be estimated from the difference between \(D_b\) and the signal in bottles poisoned with HgCl\textsubscript{2}. In the present experiments, \(D_i\) was the major fraction in the standard 2 h experiments when the chlorophyll concentration exceeded 0.3 \(\mu\text{g} \, \text{l}^{-1}\), but less important in long-term incubations (Figures 9A and B and 12). The enzymatically driven uptake was only \(-20-34\%\) of the total dark value (total minus ‘HgCl\textsubscript{2} signal’) in the standard 2 h experiments (Figure 8). However, the time course experiments showed clearly that with longer incubation times, this fraction increased and eventually became the largest fraction of the dark signal (Figures 9A and B and 12).

\textit{Time course of dark uptake}

The time course of dark uptake has been investigated by several authors (Jones et al., 1958; Legendre et al., 1983; Ignatiades et al., 1987; Harris et al., 1989; Li and Dickie, 1991). The pattern most often observed agrees with the pattern observed here. A time-zero signal and/or a brief period with a fast uptake (Jones et al., 1958; Legendre et al., 1983; Ignatiades et al., 1987; Harris et al., 1989; Li and Dickie, 1991) followed by a period of one to several hours with no or a very slow uptake.
Later, usually after 3–9 h, a substantial increase in the uptake rate occurs (Jones et al., 1958; Harris et al., 1989; Li and Dickie, 1991). However, as also observed here, this pattern is variable, so the dark uptake can proceed at a constant rate (Harris et al., 1989, their Figure 5; Figure 9B in this study) or be virtually absent for up to 24 h (Harris et al., 1989, their Figure 4C; Figure 9C in this study). The three stages in the uptake correspond to the three fractions outlined above. First, a time-zero uptake related to the filter, then a passive uptake when CO₂ diffuses into the cells, and later, depending on the conditions, an active uptake mainly driven by increased bacterial activity. The enhancement of the initial uptake by light (Figure 10) is consistent with this model if we assume that CO₂, in addition to diffusion, is taken up by an active mechanism facilitated by light. The term active is used here for any process catalyzed by enzymes and therefore sensitive to inhibition by HgCl₂.

The increase in dark uptake after some hours of incubation is unlikely to be related to phytoplankton. Physiological studies show that the dark uptake associated with phytoplankton growth will decrease with time in the dark (Mortain-Bertrand et al., 1988), corresponding to a decrease in metabolic activity in algae with time in the dark (Markager and Sand-Jensen, 1989, 1994; Markager et al., 1992). Growth of bacteria has previously been shown to involve uptake of ¹⁴C.
Dark uptake of $^{14}$C due to $\beta$-carboxylation (Overbeck, 1984), and this mechanism has been used to estimate the growth rate of bacteria (e.g. Romanenko et al., 1972). Several authors have shown an increase in bacterial activity and/or number during bottle incubations (Jones et al., 1958; Ferguson et al., 1984; Li and Dickie, 1991). When this information is combined with observations of damage to plankton organisms during bottle incubations (Venrick et al., 1977) and decreases in chlorophyll concentration during incubations (Gieskes et al., 1979; Li and Dickie, 1991; Taguchi et al., 1993), it is likely that the increase in dark uptake over time is associated with bacterial activity fueled by compounds released from breaking cells (Harris et al., 1989). The possibility of a bottle effect on the dark uptake is also supported by the data by Gieskes et al. (1979) who showed that the dark uptake as a percent of light uptake increased 5- to 50-fold when the bottle volume was lowered from 3800 to 30 ml. A stimulation of bacterial growth can be due to release of dissolved organic carbon (DOC) from broken cells that is easily metabolized, but release of nitrogen-rich compounds might also be important. In Harris et al. (1989) and in this study, the increase in the dark uptake was most severe in nitrogen-deficient waters. If bacterial growth is nitrogen limited, a release of small amounts of nitrogen-rich compounds from lysing cells could cause a substantial increase in bacterial metabolism. The implication of this hypothesis is that high dark uptake rates might be a sign of severe damage to some of the organisms in the plankton community under the incubation conditions applied (time, bottle size, temperature, pre-handling, etc.). The consequences are major changes in the metabolic activity of the different components in the community, as shown by Li and Dickie (1991). Any rate estimates of photosynthesis or bacterial production which show high dark values relative to $P_m$ should therefore be treated with caution, as already noted by Petersen (1979) and Harris et al. (1989).

Patterns in dark uptake with latitude and biomass

The absolute rates of dark uptake presented here are comparable with the lowest values found in previous studies, but lower than rates reported in some frequently cited papers. Petersen (1979) found dark values between 0.8 and 13 $\mu$mol C m$^{-3}$ h$^{-1}$ in the North Atlantic Ocean (two outliers and values from a fjord affected by allochthonous material not included). Values in Li et al. (1993) were in the range 0.5–14 $\mu$mol C m$^{-3}$ h$^{-1}$ for samples with <2 $\mu$g Chl a l$^{-1}$ and Morris et al. (1971) found values from 2.6 to 13 $\mu$mol C m$^{-3}$ h$^{-1}$ (assuming 2.1 x 10$^6$ d.p.m. $\mu$Cr$^{-1}$) at chlorophyll concentrations between 0.13 and 2.2 $\mu$g Chl a l$^{-1}$. Both groups of authors worked in the Atlantic Ocean, mainly off the coast of North America. Dark values between 8 and 33 $\mu$mol C m$^{-3}$ h$^{-1}$ were found by Ignatiades et al. (1987) in the Aegean Sea for samples with chlorophyll concentrations between 0.26 and 1.74 $\mu$g l$^{-1}$. Prakash et al. (1991) reported data for dark uptake at the same transect as in this study, but from November–December instead of the period March–April. Their mean value for 23°N to 27°S (equivalent to transect II in this study) was 17-fold higher than the values presented here, and ranged from 0.5 to 80 $\mu$mol C m$^{-3}$ h$^{-1}$. This compilation of data for chlorophyll concentration <2 $\mu$g l$^{-1}$ shows that the normal range for dark uptake is between 0.5 and
10 \mu\text{mol C m}^{-2} \text{h}^{-1}. Higher values might be due to the use of longer incubation time or ineffective removal of inorganic carbon. The latter are probably the case for the data in Morris \textit{et al.} (1971) and Ignatiades \textit{et al.} (1987) where the filters were not acidified. The high values found by Prakash \textit{et al.} (1991) are peculiar, particularly since it is a large data set and from the same area as the data presented here. It seems unlikely that the biological conditions were sufficiently different in their study compared to this study to explain a 17-fold difference. Prakash \textit{et al.} (1991) used membrane filters and removed inorganic carbon by fuming HCl for 10 min after the filters were desiccated. Perhaps fuming HCl is not particularly effective if the filters are completely dry, since only crystallization water will be present to dissolve the acid.

The relationship between chlorophyll concentration and dark uptake in this study was similar to the relationship found by Li \textit{et al.} (1993). Their values for the slope between log(dark uptake) and log(Chl) were between 0.58 and 1.18, while the value for the present data set was 0.78 when calculated in the same way [model II regression to log(D) versus log (Chl)]. However, when a log(D)-log(Chl) model was applied to the present data set, there was a clear relationship between residuals and the chlorophyll concentration, so this model was less appropriate than the model used in Figure 4.

A relationship between dark uptake and chlorophyll concentration does not imply that phytoplankton are responsible for the dark uptake since a close correlation was found between chlorophyll concentration and bacterial activity in both investigations. Neither does the relationship imply that the dark uptake is an active process, since essentially the same relationship was found for uptake in the poisoned samples. We cannot evaluate the relative contribution from phytoplankton and bacteria in the present study, but given that the biomass of bacteria often equals or exceeds the biomass of phytoplankton (e.g. Cho and Farooq, 1990), it is likely that bacteria are responsible for a significant part of the dark uptake. However, the fact that chlorophyll is a better predictor for the dark signal than bacterial biomass and activity, and the lack of correlation between the residuals from equation (3) and bacterial abundance and activity, suggests that phytoplankton dominate the dark signal during the first hours of incubation.

The reciprocal relationship between $P_m$ and the dark signal as a percent of $P_m$ (Figure 7A) is the same pattern as found by Steemann Nielsen (1960) and Prakash \textit{et al.} (1991). In the present data set, the reciprocal relationship in Figure 7A was due to a background uptake in samples with low $P_m$ values and not related to high dark values. This pattern will likely be more pronounced with a higher level of dark uptake as found by Morris \textit{et al.} (1971), Ignatiades \textit{et al.} (1987) and Prakash \textit{et al.} (1991). Thus, geographical trends in relative dark values are probably an artifact created by low values of $P_m$ in some areas relative to a constant background. Nonetheless, the time series experiments presented here suggest that enhancement of dark uptake by bacteria during long incubations is most pronounced when nitrogen concentrations are low, so high relative uptake rates might also be an artifact of long incubation times in nutrient-depleted waters.

The high dark uptake rates found in the time series experiments after 24 h will add to other problems in the interpretation of the results from incubations lasting
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24 h or more (Smith et al., 1984; Collos et al., 1993; Taguchi et al., 1993; Mingelbier et al., 1995). The use of 24 h incubations, as recommended by Joint Global Ocean Flux Study (1988), should probably be reconsidered, and we should maybe follow the original recommendation of Steemann Nielsen (1952, 1960) and keep the incubation time below 4–6 h.

Finally, it should be noted that high dark uptake rates relative to $P_m$ can simply be an indication of a system where the autotrophic activity is low or absent and heterotrophic or chemoautotrophic processes dominate. High relative dark values in the winter (Taguchi and Platt, 1977) or from the monimolimnion of meromictic lakes (S. Markager and W. Vincent, unpublished data) are, therefore, to be expected. High dark values have also been observed in systems where heterotrophic activity was stimulated by allochthonous organic compounds (Petersen, 1979). The sensitivity of the $^{14}$C-method is ultimately determined by the dark signal. The signal for the total uptake can be increased by using more isotope and longer counting times. However, reliable estimates for the light-driven uptake can only be obtained if it is well separated from the dark signal.

**Light-stimulated non-photosynthetic uptake**

The dark values reported so far have been from the second replicate of the two dark bottles in each set (DB-2). The first replicate in each set (DB-1) received a small amount of light during the filling procedure and showed consistently higher uptake rates than DB-2. The enhancement was on average 93% for the surface sample and 43% for the DCM sample relative to the uptake in DB-2, and was highly significant ($P < 0.0001$, t-test). It is unlikely that the enhancement was due to photosynthesis. A high estimate of the light received by DB-1 would be 5 $\mu$mol m$^{-2}$ s$^{-1}$ in 30 s. Multiplied by the light utilization coefficient calculated from the P-I curves, this was equivalent to <1% of the observed enhancement. The enhancement agrees with the observation of positive time-zero intercepts in light during the time course experiments (Figure 10 and Table III) and both observations suggest an initial burst uptake of $^{14}$C in light that is largely completed before the first measurement after 5 min (time series experiments 5 and 6). The mechanism for such an uptake cannot be revealed from the present data, but information from the literature can provide some suggestions. It is known from the literature that many phytoplankton species possess mechanisms for concentration of inorganic carbon in light (Badger et al., 1980; Beardall, 1985, 1991; Raven, 1985; Raven and Lucas, 1985; Burns and Beardall, 1987), in some cases up to 2-fold the dissolved inorganic carbon (DIC) concentration in sea water (Raven, 1985). However, most of the information is from freshwater species and at low external DIC concentrations. Little is known about internal DIC concentrations in marine phytoplankton species at seawater levels of DIC. The possible contribution of a pool of inorganic carbon to the dark uptake is indicated in Figure 10 assuming an internal DIC concentration of 2-fold the DIC concentration in sea water. The total cell volume of phytoplankton for this estimate was calculated from the Chl a concentration, assuming a C:Chl ratio of 50 and using the cell volume:C ratio for 100 $\mu$m$^3$ cells provided in Verity et al. (1992). The
purpose of this calculation is only to show that an internal pool of inorganic carbon has the same magnitude as the difference between the initial values in light and darkness. Such a pool is, therefore, a possible explanation for the enhanced dark uptake in DB-1 and for the enhancement of the initial uptake by light.

Another possible mechanism responsible for the enhancement could be a stimulation of β-carboxylation by light. β-Carboxylation is known to play an important role in the uptake of inorganic carbon for many algae (Kerby and Raven, 1985) and in the growth metabolism (Mortain-Bertrand et al., 1987, 1988). As a whole, growth processes are stimulated by light so it is possible that β-carboxylation reacts in the same manner. Against the hypothesis that the stimulation of 'the dark uptake' by light is caused by β-carboxylation are the data by Mortain-Bertrand et al. (1987) which show that β-carboxylation starts at a slower rate than C-3 fixation in light incubations with Skeletonema costatum.

**Should the dark uptake be subtracted from the uptake in light?**

With the background provided here, the answer to this question is yes. First, a background from incomplete removal of inorganic $^{14}$C should be subtracted. This component forms the major fraction of the dark signal in short-term incubations (Figure 12; $D_0 + D_2$). Second, the uptake by bacteria should be subtracted unless the activity by bacteria in the dark is higher than in the light bottles. The latter seem unlikely, since excretion of organic compounds from phytoplankton occurs in light as a byproduct of photosynthesis, and these compounds are known to stimulate bacterial growth (Cole et al., 1982; Jensen, 1983). Li et al. (1993) showed that bacterial activity was equal in light and in the dark for three stations, and Li and Dickie (1985) found a higher uptake of organic compounds in light than darkness in two of three stations. Both results support the conclusion that, if anything, bacterial activity tends to be higher in light than in the dark.

The only component of the dark signal for which subtraction may not be valid is the dark uptake by phytoplankton associated with β-carboxylation (Legendre et al., 1983). To the extent that β-carboxylation serves the purpose of supporting anabolic processes in phytoplankton cells with C4-precursors, and the process is not balanced by decarboxylating processes, it should be regarded as carbon uptake by phytoplankton and should therefore not be subtracted (Kremer, 1981). β-Carboxylating processes could be responsible for part of the active uptake observed in this study, but since this fraction was $<0.76\%$ of $P_m$, probably much less, since part of the uptake is a passive process ($D_2$) or carried out by other types of organisms than algae, this correction is very small. Thus, the dark uptake associated with β-carboxylation is likely to be small and the positive error in primary production estimates introduced by not subtracting the dark uptake is, therefore, likely to be much more severe than the risk of a slightly negative bias.

The quantitative importance of the handling of dark values in primary production estimates depends on the photosynthetic rate and on the incubation time. With decreasing photosynthetic rates, e.g. due to low light, the relative importance of the dark signal will clearly increase (Steemann Nielsen, 1952). Deep in
the water column, the dark signal can be comparable to the uptake in light (Li et al., 1993), and it then becomes vital for a proper estimate of primary production that the dark uptake is measured and handled correctly (Banse, 1993). It is less well recognized that since part of the dark signal is a time-zero value, it becomes very important in short-term incubations, e.g. less than an hour, which have become increasingly popular during the last 15 years (Lewis and Smith, 1983). Figure 13 shows the effect of the handling of the dark signal at different incubation times with data from time series experiment 6. The uncorrected rates decreased with incubation time up to 1 h, thereafter the rates became stable \( I = 90 \, \mu\text{mol m}^{-2} \text{s}^{-1} \) or continued to fall slightly \( I = 264 \, \mu\text{mol m}^{-2} \text{s}^{-1} \), the latter probably because of photoinhibition. Rates corrected for the dark signal become stable after only 15 min. The decrease with incubation time before 15 min arises from the fact that the time-zero intercept is higher in the light than in the dark. Figure 13 also shows that the correction for dark uptake is large, up to 30% of the light uptake, for incubations <30 min. Against this background, very short incubation times should probably be avoided in primary production measurements in order to reduce the effect of an incorrect estimate of the dark signal and the uncertainty about the nature of the non-photosynthetic light-stimulated uptake of \(^{14}\text{C}\). An incubation time between 1 and 3 h may be the best compromise between this problem and the problems associated with high dark uptakes in long-term incubations. If very short incubation times are desirable for other reasons, e.g. to detect rapid changes in photosynthetic parameters (Lewis and Smith, 1983), dark incubations should be performed and not just time-zero blanks.

No matter how the dark values are handled in the calculation of primary production, they serve an important function as a quality check for the protocol.

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Fig. 13. The effects of subtracting the dark uptake on estimates of the mean photosynthetic rate in incubations with different duration. Data are from two irradiances from experiment 6. Closed symbols are the rates for the total light uptake and the open symbols are the rates after subtracting the dark uptake. Irradiances: 263 \( \mu\text{mol m}^{-2} \text{s}^{-1} \) (circles) and 91 \( \mu\text{mol m}^{-2} \text{s}^{-1} \) (squares).
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in all primary production estimates. Low dark uptake values are essential to prove that removal of inorganic carbon is efficient and that the plankton community examined can withstand the effects of containment without major shifts in the metabolism. A time-zero blank cannot serve the same purpose since it will not detect the latter problem. Thus, data for dark uptake should always be provided (Banse, 1993) and high values, e.g., >5% of $P_m$, suggest that there might be a problem with the protocol for the plankton community examined.

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