Radioisotopic Method for Measuring Cell Division Rates of Individual Species of Diatoms from Natural Populations†

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Silicon is an essential element for diatom frustule synthesis and is usually taken up only by dividing cells. With 68Ge, a radioactive analog of Si, the cell cycle marker event of frustule formation was identified for individual species of diatom. The frequency of cells within a population undergoing this division event was estimated, and the cell division rate was calculated. In laboratory cultures, these rates of cell division and those calculated from changes in cell numbers were similar. By dual labeling with 68Ge(OH)₄ and NaH¹⁴CO₃, rates of cell division and photosynthesis were coincidently measured for diatoms both in laboratory cultures and when isolated from natural populations in estuarine, offshore, and polar environments. These techniques permit the coupling between photosynthesis and cell division to be examined in situ for individual species of diatom.

Growth and cell division are the most relevant measures of the success of an algal species in nature. The growth rate of naturally occurring phytoplankton is a critical yet difficult parameter to measure accurately (19, 29). Growth rates based on isotopic flux measurements are equivocal because of uncertainties in estimating phytoplankton biomass (18, 19, 48), and only relative growth rates can be derived from the measurement of chemical composition ratios or changes in nucleotide content of particulate matter (20, 21, 26–28).

Currently, only by microscopic counting of the frequency of cells undergoing mitosis can absolute rates of cell division be calculated for phytoplankton in natural waters (17, 50, 52–54). This method, which was first used over 75 years ago (1, 22), has in recent years been greatly improved (11, 54). It nevertheless remains limited to algal species with persistent and morphologically distinct life cycle stages and requires observing or preserving cells during the typically short interval of nuclear or cell division. Owing to the brevity of these cell cycle events in diatoms, it is particularly difficult to measure their in situ cell division rates (30, 50, 56).

Herein is described a new technique to measure the species-specific cell division rate of diatoms. This technique identifies those individuals of a species which have undergone the division event of silicon frustule formation. Diatoms have an absolute requirement for orthosilicic acid, Si(OH)₄, and its incorporation is coupled to the cell cycle (9, 12–14, 32, 41, 42). The cellular pool free (unpolymerized) silicon is usually small (7, 51, 55), and dividing cells rapidly take up and polycondense silicon into newly forming frustules during a typical 10- to 60-min uptake window (4, 5, 7, 51). Measurement of silicon uptake would be facilitated by using a radioactive tracer; however, radioisotopes of Si have a half-life (t₁/₂) of 157 min or less. However, germanium, the chemical analog of silicon, has a radioactive nuclide (⁶⁸Ge) with a t₁/₂ of 282 days. ⁶⁸Ge undergoes copolycondensation with Si(OH)₄ when it is present in a low (<10⁻⁴) molar ratio of Si to Ge (2, 55). Thus, the incorporation of ⁶⁸Ge(OH)₄ accurately models Si(OH)₄ uptake (2–6, 9). By incubating a sample with ⁶⁸Ge(OH)₄ and subsequently isolating a large number of single cells of the same species into separate liquid scintillation vials, the frequency of dividing cells (F) can be determined. From estimates of the frequency of dividing cells of a given species within the population, the species-specific cell division rate was calculated. In laboratory cultures, cell division rates determined by ⁶⁸Ge incorporation and from changes in cell numbers were very similar. By dual labeling samples with ⁶⁸Ge(OH)₄ and NaH¹⁴CO₃, both cell division and photosynthesis were coincidently measured for diatoms from estuarine, oceanic, and polar environments. These techniques permit the study of the coupling of photosynthesis and cell division of individual species of diatom isolated from natural assemblages.

MATERIALS AND METHODS

Culture conditions. Laboratory experiments were carried out with unialgal cultures of four common diatoms (see Table 1). Cultures were maintained in 30% salinity seawater with half-strength f enrichment (23) at 12 to 13°C on a 12-h light–12-h dark photoperiod of cool-white fluorescent illumination of about 200 microeinstein m⁻² s⁻¹. Cell counts were routinely done in a Palmer-Maloney counting chamber. Cell dimensions were measured, and volumes were calculated by the appropriate geometric formulae. Batch cultures of each alga were inoculated at a low initial cell density and grown at a range of irradiances. Cultures were preconditioned to each irradiance level for 1 week prior to measurement of cell division rates.

⁶⁸Ge labeling experiments. Carrier-free ⁶⁸Ge in 0.5 N HCl (New England Nuclear Corp.) was neutralized with a slight excess of 0.5 N NaOH to convert ⁶⁸GeCl₂ to ⁶⁸Ge(OH)₄. ⁶⁸Ge has a t₁/₂ of 282 days and decays by electron capture to ⁶⁸Ga, a nuclide with a t₁/₂ of 68 min. ⁶⁸Ga decays to ⁶⁸Zn with an 88% β⁻ emission of 1.90 MeV. This energetic emission can be counted by Cerenkov counting. In biological uptake experiments with ⁶⁸Ge, samples must be stored for about 12 h (at least 10 t₁/₂ for ⁶⁸Ga) after incorporation has stopped to permit ⁶⁸Ga to come into transient equilibrium with ⁶⁸Ge. Thus, all of the measured ⁶⁸Ga activity is derived from incorporated ⁶⁸Ge.

During early- to mid-exponential phase, as determined by daily cell counting, cultures were inoculated at the beginning of the light period with ⁶⁸Ge to a final activity of 0.5 to 0.7 µCi ml⁻¹ (about 1.1 to 1.5 pmol of Ge added in the form of...
68Ge). Silicon was 50 to 75 μM, and the molar ratio of Ge to Si was about 10−7. After 6, 12, and 24 h, 50-ml samples of the culture were removed, and cells were collected on an 8.0-μm (pore size) Nuclepore filter of a 10-μm nitex screen. The algae were washed three times with 100 ml of unlabeled seawater and backwashed off the filter or screening. Single cells (n = 50 to 75) were transferred through several serial washes before isolation into separate liquid scintillation vials (44, 45). The short chains of Thalassiosira rotula and T. nordenskioldii were disrupted by agitating samples on a vortex mixer; this facilitated the isolation of single cells. Killed controls (CN− or HgCl2) were used to evaluate abiotic frustule adsorption of 68Ge. This was a negligible component of total uptake.

Dual labeling. The rates of photosynthetic carbon uptake and cell division were coincidently measured by dual labeling the cultures with 14C and 68Ge. At the beginning of the light period, cultures were spiked with NaH14CO3 (ICN Pharmaceuticals, Inc.) and 68Ge(OH)4 to final activities of 0.1 and 1.0 μCi ml−1, respectively. In this experiment, photosynthesis was measured by 14C incorporation according to standard (filter retained) methodology (45). Rates of carbon uptake were calculated during 6- and 12-h incubation periods. The synergistic effects of coincident addition of 68Ge and 14C on cell division and photosynthesis were examined. Exponentially growing cultures were divided into five parts. 68Ge was added to part A. 14C was added to part B, and both 68Ge and 14C were added to parts C, D, and E. After 12 and 24 h, single cells were collected and isolated as described above and in reference 44. Rates of cell division and photosynthesis were measured and compared for cells incubated in the presence of single and dual additions of the radioisotopes.

Field experiments. Rates of cell division and photosynthetic carbon uptake were measured for individual species of diatom isolated from the Chesapeake Bay (36°55′ N, 73°55′ W) aboard the R/V Cape Hatteras cruise Corsair 4 (March 1984), from the continental shelf slope break (35°34′ N, 73°42′ W) aboard the R/V Ridgecrest, 1000 fathom cruise Cor108 (July 1984), and from planktonic communities under the annual sea ice and at the ice edge in McMurdo Sound, Antarctica (78°25′ S, 166°30′ W) (December 1984). Water density structures were determined with a conductivity temperature probe (InterOcean Systems Inc.) or a salinometer (Yellow Springs Instruments). Downwelling and incident irradiances were usually measured with a quantum photometer (Li Cor Lambda Instrument Co.). Plankton samples were collected from the surface mixed layer in either a polyvinyl chloride Niskin (General Oceanics Corp.) bottle or with 0.5-m-diameter, 20-μm-aperture, doubling-plankton nets. Within 10 min of collection, samples were passed through a 50-μm (aperture size) nitex screen to remove larger zooplankton, placed in borosilicate incubation bottles, and spiked with either 68Ge alone or 68Ge plus 14C. Final activities were 0.5 to 1.0 μCi ml−1. Bottles were incubated at ambient seawater temperatures with incident irradiances attenuated with neutral-density filters and subsampled periodically for cell isolations as described above.

Radioactivity counting. Radioactivity was counted in 7-ml plastic or glass vials on a Packard Instrument Co., Inc., Tri-Carb model 460C or Beckman Instruments, Inc., model 6800 liquid scintillation spectrometer. In single-label experiments with 68Ge, β− emissions were measured by Cerenkov counting. Counting efficiency, determined by addition of internal standards, was about 35 to 45%. In dual-label experiments, cells were oxidized with 0.3 ml of 0.2 N perchloric acid for 12 h prior to adding Biofluor (New England Nuclear) liquid scintillation cocktail. The efficiency of counting in Biofluor was about 85 to 95% for both 68Ge and 14C. All sample counts were corrected both for quench by the external standard method and for background radiation. Background radiation was typically less than 5 dpm for Cerenkov counting and 15 dpm for counting in Biofluor, and sample counts were typically 3 to 400 times that of the background counting rate and significantly different (see reference 44 for details).

Treatment of experimental results. Cell division rates (μN doublings per day) were determined from the slope of the linear regression between log of the cell number and time during exponential growth in laboratory cultures. The statistical error of the division rate was derived from the standard error of the slope of this regression. The maximum proportion of cells (F) undergoing the division event of silicon frustule formation was estimated by determining the fraction of the total number of cells (of that species) which contained 68Ge. This does not require quantitative determination of the 68Ge incorporated, only detection of a radiolabel in newly formed cells and discrimination between labeled and unlabeled cells.

The frequency distribution of disintegrations per minute per cell can be bimodal with a theoretical disintegrations per minute per cell ratio of 2 to 1. Two types of radiolabeled diatom can occur in a sample spiked with 68Ge(OH)4. There can be type A diatoms, which have formed new frustules, undergone cytokinesis, and been released from the parent cell wall. These daughter cells are independent individuals and, when isolated into separate scintillation vials, have x disintegrations per minute per cell. There can also be type B diatoms, which have formed new frustules but have not undergone cytokinesis. These daughter cells are still retained within the parental cell wall and, when isolated into separate scintillation vials, have 2x disintegrations per minute per cell. The third category, type C, are diatoms which have not formed new frustules and thus will not contain 68Ge. The frequency of dividing cells (F) was estimated from (54)

$$F = \frac{(B + 0.5A)/(0.5A + B + C)}$$

The mean cell division rate (μF, doublings per day) was calculated from

$$\mu F = \frac{\ln(1 + F)\ln2}{t}$$

where t is typically an incubation period of 1 day for phased or synchronous division; however, if division is asynchronous, t is the incubation interval (34).

RESULTS

Laboratory experiments. The irradiance-dependent cell division rates (μN) of the four diatoms are shown in Fig. 1. In all cases, cell division rates were light saturated at or below about 200 microeinsteins m−2 s−1. There was no clear relationship between cell size and division rate. The two smaller diatoms, T. rotula and T. nordenskioldii divided more rapidly than the larger species (Table 1; Fig. 1). However, Ditylum brightwellii, which was larger than Coscinodiscus escentricus, divided two to three times as fast (Fig. 1; Table 1).

The cell division rate estimated from changes in cell number (μN) and that calculated from equations 1 and 2 (μF) were compared at growth-limiting and saturating light irradiance (Table 1). For each alga, μF was estimated on three
FIG. 1. Cell division rate of *C. escentricus* (○), *D. brightwellii* (△), *T. rotula* (●), and *T. nordenskiöldii* (▲) as a function of growth irradiance. Each value is the mean (± 1 standard error) of three replicate culture flasks.

Table 1. Cell division rates calculated from changes in cell numbers (μN) and from the frequency of cells which incorporated 68Ge (μF)*

<table>
<thead>
<tr>
<th>Alga (clonal designation)</th>
<th>Culture irradiance (microeinstein m⁻² s⁻¹)</th>
<th>Cell vol (μm³)</th>
<th>Mean (± SE) cell division rate (doublings/day)</th>
<th>μN</th>
<th>μF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. escentricus</em> (C-38B)</td>
<td>200</td>
<td>50</td>
<td>0.31 ± 0.08</td>
<td>0.28 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>53</td>
<td>0.20 ± 0.06</td>
<td>0.21 ± 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>47</td>
<td>0.10 ± 0.04</td>
<td>0.14 ± 0.05</td>
<td></td>
</tr>
<tr>
<td><em>D. brightwellii</em> (L1332)</td>
<td>100</td>
<td>ND*</td>
<td>0.62 ± 0.12</td>
<td>0.71 ± 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>82</td>
<td>0.25 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td></td>
</tr>
<tr>
<td><em>T. rotula</em> (1492)</td>
<td>200</td>
<td>22</td>
<td>1.05 ± 0.05</td>
<td>1.10 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>26</td>
<td>0.60 ± 0.08</td>
<td>0.68 ± 0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>23</td>
<td>0.20 ± 0.05</td>
<td>0.27 ± 0.04</td>
<td></td>
</tr>
<tr>
<td><em>T. nordenskiöldii</em> (T. Nord)</td>
<td>200</td>
<td>8.0</td>
<td>1.20 ± 0.10</td>
<td>1.14 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7.0</td>
<td>0.72 ± 0.14</td>
<td>0.64 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8.1</td>
<td>0.40 ± 0.07</td>
<td>0.48 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

* The duration of incubation with 68Ge(OH)₄ was 12 and 24 h for *T. rotula* and *T. nordenskiöldii* and 24 h for *D. brightwellii* and *C. escentricus*. Diatoms were grown on a 12-h light–12-h dark cycle.

** Field experiments. Rates of photosynthesis and cell division were measured for phytoplankton from natural populations in the Chesapeake Bay, in temperate coastal and offshore regions, and in Antarctica. A detailed description of the physical, chemical, and biological conditions and the interactions between photosynthesis and cell division within each system will be published separately (manuscript in preparation). Photosynthesis was measured at five to seven irradiances during 2- to 3-h incubation periods, and cell division rates were measured at three irradiances during 24-h periods. The majority of cells assayed were type A cells (about 1x disintegrations per minute). This was probably the result of daughter cells being released almost immediately upon formation. Over the course of a 12- or 24-h incubation period, there was little difference between F calculated either according to equation 1 or by dividing the number of labeled cells by the total number of cells assayed. The frequency of dividing cells was determined during incubation periods subsampled several times during the photoperiod. When grown at saturating irradiances on a 12-h light–12-h dark cycle, *C. escentricus* and *D. brightwellii* incorporated 68Ge(OH)₄ into newly forming frustules primarily during the second half of the light portion of the photoperiod (i.e., between the 6- and 12-h incubation intervals; Table 2). When grown in continuous light, the diatoms incorporated 68Ge(OH)₄ at a relatively constant rate throughout the 24-h period (Table 2).

The synergistic effects of coincident additions of up to 5 μCi each of 14C and 68Ge ml⁻¹ (molar ratio of Ge to Si < 10⁻⁶) on measurements of rates of cell division and photosynthesis were determined. Cultures were inoculated at the beginning of the light period with 68Ge, 14C, or combinations of 68Ge plus 14C (Table 3). Carbon uptake was measured only during the light period, and 68Ge uptake was measured after 12- and 24-h incubation periods. There was no significant difference between the rates of cell division or carbon uptake when single radioisotope additions were compared with dual additions (P = 0.05 by Student t test; Table 3). In addition, the photosynthesis-irradiance relationships for *D. brightwellii* and *T. rotula* measured in the absence or presence of 1 μCi of 68Ge ml⁻¹ were identical (Fig. 2). Since there was no apparent synergistic effect of the radioisotopes, it was possible to measure the rates of photosynthesis and cell division coincidently and accurately, for algae isolated from natural populations, by dual labeling with NaH14CO₃ and 68Ge(OH)₄.
incubation periods at ambient temperatures (Table 4; Fig. 3). Several different photosynthesis-irradiance relationships were seen (Fig. 3). Carbon uptake by Corethron criophilum, isolated from the edge of the annual sea ice in McMurdo Sound, Antarctica, saturated at a very low irradiance and was photoinhibited above 75 microeinsteins m\(^{-2}\) s\(^{-1}\) (Fig. 3). The photosynthetic rate of Rhizosolenia styliformis, isolated at the shelf-slope break, saturated at about 400 microeinsteins m\(^{-2}\) s\(^{-1}\) and was not photoinhibited. Photosynthesis of D. brightwellii, isolated from a well-mixed region at the Chesapeake Bay mouth, saturated at 230 microeinsteins m\(^{-2}\) s\(^{-1}\) and was not photoinhibited. Cell division rates for the six species examined ranged from about 0.25 day\(^{-1}\) for Coscinodiscus sp. to 0.9 day\(^{-1}\) for D. brightwellii, and there was no apparent relationship between \(\mu F\) and irradiance during the 24-h incubation period (Table 4).

**DISCUSSION**

This study shows that cell division rates of diatoms isolated from natural phytoplankton populations can be estimated by pulse-labeling with \(^{68}\)Ge(OH)\(_4\). In laboratory cultures, there were no significant differences in cell division rates calculated from changes in cell number (\(\mu N\)) and from the frequency of cells containing \(^{68}\)Ge (\(\mu F\)) (Table 1). Dual labeling with \(^{68}\)Ge(OH)\(_4\) and NaH\(^{14}\)CO\(_3\) allows coincident measurement of rates of both cell division and photosynthesis by diatoms from natural populations. Cells labeled with \(^{68}\)Ge could be isolated from Formalin-preserved samples to estimate \(F\). During frustule formation, \(^{68}\)Ge is copolycondensed with Si into a crystalline structure relatively resistant to dissolution under natural conditions (25, 31, 55). Thus, samples incubated on board ship can be preserved in 1 to 2% (neutralized) Formalin and then isolated under more stable conditions in the laboratory. This potentially allows the cell division rates of nanoplanktonic diatoms (<10 \(\mu\)m) to be measured. This type of postpreservation isolation is not possible with radioactive carbon or phosphorus. For example, algae release variable proportions of their incorporated \(^{14}\)C and \(^{32}\)P upon preservation (24, 49).

For most eucaryotic algae, the cell division or life cycle is composed of four discrete, sequential intervals (8, 40).

**TABLE 3. Rates of photosynthesis and cell division measured by single- and dual-isotope labeling**

<table>
<thead>
<tr>
<th>Alga</th>
<th>Irradiance(^a)</th>
<th>Photosynthesis (pg of carbon cell(^{-1}) h(^{-1}))</th>
<th>Cell division (doublings/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without (^{6})Ge</td>
<td>With (^{6})Ge at:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (\mu)Ci ml(^{-1})</td>
<td>1 (\mu)Ci ml(^{-1})</td>
</tr>
<tr>
<td>(T.\ rotula)</td>
<td>200</td>
<td>53</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>(D. brightwellii)</td>
<td>200</td>
<td>180</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>(C. escentricus)</td>
<td>200</td>
<td>425</td>
<td>395</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>(T. nordenskioldii)</td>
<td>200</td>
<td>160</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>44</td>
<td>54</td>
</tr>
</tbody>
</table>

\(^a\) Photosynthesis was measured as \(^{14}\)C incorporation in the absence of \(^{68}\)Ge and in the presence of 5 or 1 \(\mu\)Ci of \(^{68}\)Ge(OH)\(_4\) ml\(^{-1}\). The cell division rate was calculated from the frequency of cells which took up \(^{68}\)Ge(OH)\(_4\) in the absence of \(^{14}\)C and in the presence of 5 or 1 \(\mu\)Ci of NaH\(^{14}\)CO\(_3\) ml\(^{-1}\). The cultures to which 1 \(\mu\)Ci each of \(^{68}\)Ge and \(^{14}\)C ml\(^{-1}\) was added were used for coincident measurements of cell division and photosynthesis.

\(^b\) This was both the growth and incubation irradiance.

**FIG. 2.** Photosynthesis-irradiance relationship of \(D. brightwellii\) (○, ●) and \(T. rotula\) (□, △) in the absence (open symbols) and presence (closed symbols) of 1 \(\mu\)Ci of \(^{68}\)Ge(OH)\(_4\) ml\(^{-1}\). Cultures were grown at 200 microeinsteins m\(^{-2}\) s\(^{-1}\). Each point is the mean (= the standard deviation) of three replicate samples.
FIG. 3. Photosynthesis-irradiance relationship of Corethron criophilum (○), Rhizosolenia styloformis (△), and D. brightwellii (□) isolated from natural phytoplankton populations from antarctic, oceanic, and coastal-estuarine environments, respectively. Photosynthesis was measured during dual-labeling experiments with NaH\(^{14}\)CO\(_3\) and \(^{68}\)Ge(OH)\(_4\). Each value is the mean (± 1 standard deviation) of 8 to 10 replicate determinations.

Typically, two temporal gaps (G-1 and G-2 stages) separate the period of DNA synthesis (S stage) from cell division (D stage; e.g., karyokinesis and cytokinesis). The time it takes to complete one cell division cycle is the cell generation or doubling time. The number of individual cells (N) can only increase when the cell cycle passes through the D stage, whereas cell mass (B) can increase throughout the G-1, G-2, and S stages (36). Thus, for a population, the specific division rate (μN) is

$$\mu'N = (dN/dt)(1/N)$$

which is distinct from the specific growth rate (μ'B)

$$\mu'B = (dB/dt)(1/B)$$

It is μ'B which is usually estimated for natural phytoplankton assemblages, whereas μN or μF can be calculated from changes in cell numbers or by enumerating the daily maximum proportion of cells in a population undergoing mitosis, respectively. Typically, F is estimated by identifying doublet cells or cells with paired nuclei. However, F can be calculated by identifying cells which have undergone any "cell cycle marker event", that is, an event which occurs only once during a cell cycle (35, 36). In this study, the cell cycle marker event of silicon frustule formation was used to identify F. Incorporation of \(^3\)H-thymidine has also been used to identify the cell cycle marker event of DNA replication and to calculate F in eucaryotic microalgae (R. B. Rivkin, J. Phycol., in press).

Various formulae have been used to calculate division rates from F; these have been reviewed by McDuff and Chisholm (34). They suggest that it is necessary to (i) estimate the duration of the paired division or mitotic stage and (ii) observe or identify all the dividing cells in a given day to calculate μF accurately. The \(^{68}\)Ge technique satisfies these criteria. All cells which have gone through the division event of silicon frustule formation incorporate \(^{68}\)Ge(OH)\(_4\) and can be identified on isolation and radioactivity counting. This technique, which identifies all the division events taking place during the incubation interval, does not require that a short mitotic event be observed while it is taking place. It is, therefore, unnecessary to estimate the duration of the paired division or mitotic stage.

When grown in a light-dark cycle, the diatoms examined in culture seemed to divide during the second half of the light

![Graph showing photosynthesis-irradiance relationship](http://aem.asm.org/)

**TABLE 4. Cell division rates and photosynthetic characteristics of diatoms isolated from McMurdo Sound (Antarctica) the shelf-slope break off the east coast of the United States, and the mouth of the Chesapeake Bay**

<table>
<thead>
<tr>
<th>Environment and alga</th>
<th>Photosynthesis(^a)</th>
<th>Cell division (doublings/day) at an incubation irradiance of(^b):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P_{max})</td>
<td>α</td>
</tr>
<tr>
<td>Antarctic(^c)</td>
<td>Corethron criophilum</td>
<td>295</td>
</tr>
<tr>
<td></td>
<td>Coscinodiscus furcatus(^d)</td>
<td>ND</td>
</tr>
<tr>
<td>Oceanic shelf(^f)</td>
<td>Rhizosolenia styloformis</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>Thalassiosira decepiens</td>
<td>ND</td>
</tr>
<tr>
<td>Estuarine coast(^g)</td>
<td>Ditylum brightwellii</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>Coscinodiscus sp.</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) The maximum photosynthetic rate (\(P_{max}\), pg of C cell\(^{-1}\) h\(^{-1}\)), the slope of the light-limited region of the photosynthesis-irradiance relationship (α; pg of C cell\(^{-1}\) h\(^{-1}\) [microeinsteins m\(^{-2}\) s\(^{-1}\)]), and the saturation irradiance, the intersection of \(P_{max}\) and α (\(I_i\); microeinsteins m\(^{-2}\) s\(^{-1}\)), were calculated from the photosynthesis-irradiance relationship.

\(^b\) Incubation irradiance as a percentage of incident insolation.

\(^c\) The incubation temperature was \(-1.8^\circ\)C, and the maximum incident insolation during incubation was about 1,100 microeinsteins m\(^{-2}\) s\(^{-1}\).

\(^d\) Tentative identification.

\(^e\) ND, Not determined.

\(^f\) The incubation temperature was 27°C, and the maximum incident insolation during incubation was about 1,800 microeinsteins m\(^{-2}\) s\(^{-1}\).

\(^g\) The incubation temperature was 6°C, and the maximum incident insolation during incubation was about 1,500 microeinsteins m\(^{-2}\) s\(^{-1}\).
phase of each photoperiod. Few cells took up $^{68}$Ge in the first half of the light phase or during the dark phase. For diatoms growing under continuous irradiance, approximately equal proportions of cells divided during all incubation intervals (Table 2). To determine whether the timing of division was influenced by the time of day at which $^{68}$Ge was added, cultures were spiked at the beginning of the dark cycle rather than the beginning of the light cycle. In those cultures, $^{68}$Ge(OH)$_4$ was still taken up primarily during the second half of the subsequent light cycle. Although division periodicity in diatoms has been extensively examined (9, 10, 37-39, 50), the time when division takes place is not generally predictable. Thus, the division of these four diatoms during the light period should not be considered a general pattern.

For diatoms isolated from natural populations, the rate of carbon uptake was clearly dependent on incubation irradiance (Fig. 3). Cell division rates, however, were apparently independent of incubation irradiance. At least one incubation irradiance was limiting and one was saturating for photosynthesis (Fig. 3; Table 4). This apparent independence of cell division rate from incubation irradiance suggests that (i) the division rates of these diatoms saturated at the lowest incubation irradiance, (ii) these diatoms used carbon storage compounds to maintain high cell division rates temporarily at suboptimal irradiances (46, 47), or (iii) the diatoms “attained a commitment to divide” prior to the beginning of the incubation period and completed the division event with reduced rates (or in the absence) of carbon uptake (15, 16, 33). At this time, the data are inadequate to allow speculation on the mechanism of this apparent independence of division rate on short-term incubation irradiances. However, possibilities i and ii above are currently being examined for both diatoms and dinoflagellates in situ with these single-species radioisotope techniques (43-46), and the results will be reported separately.

The pulse-labeling technique described here identifies the cell cycle marker event of silicon frustule formation in individual species of diatoms isolated from natural populations. The high sensitivity required to measure species-specific cell division rates is possible by using radiolabeled substrates and liquid scintillation counting of single, isolated cells. The absolute requirement for silicon by diatoms, its rapid uptake and polycondensation within the silicalemma, and the coupling of incorporation to the cell cycle allow $^{68}$Ge(OH)$_4$ to be used as a cell cycle marker event for estimation of $\mu F$. In addition, the rate of carbon uptake can be coincidently and conveniently determined. These high-resolution, single-species techniques reduce the physiological background noise associated with interspecific variability or changes in the floristic composition of the sample. Thus, the coupling of photosynthesis and cell division can be examined with almost the same sensitivity and precision as in unialgal cultures in the laboratory.

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LITERATURE CITED


