

Release of dissolved organic matter by coastal diatoms

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Abstract

Dissolved organic matter (DOM) production was examined in axenic batch cultures of five coastal diatom species. For *Chaetoceros decipiens*, dissolved organic carbon (DOC) accumulated beginning in late exponential growth as a result of increased cell density. For *Cylindrotheca closterium*, DOC actually decreased in late exponential growth and reached zero by the end of the experiment. This coincided with continued particulate organic carbon (POC) production and a threefold increase in the per-cell concentration of transparent exopolymer particles after nutrients were depleted. DOC release rates varied between species but were significantly higher for all five species in exponential or transition growth than during stationary growth. On average, 5% of the total fixed C was released as DOC for four of the diatoms, whereas *C. decipiens* released ~21% of its fixed C as DOC. The percentage of fixed C released as DOC varied little with nutrient availability or diatom growth stage. The DOM produced by some diatom species adheres to filters and is measured in the particulate organic matter (POM) fraction when cells are separated from the medium by filtration. This may be an important problem when diatom species with known benthic life histories are prevalent. In contrast, for species like *Chaetoceros* that have no benthic life history, DOM release rates estimated by bulk measurements or ¹⁴C appear to be accurate. Overall, these results indicate that the species composition of phytoplankton blooms has the potential to influence the relative importance of POM and DOM production and can complicate interpretation of those measurements.

Diatoms are ubiquitous in surface water of the world's oceans. They are capable of fast growth relative to other phytoplankton taxa and are well adapted to form large blooms in high-nutrient coastal systems and in oceanic systems that are subjected to pulses of nutrients (Margalef 1978). Indeed, diatoms are the largest contributor to oceanic primary production and new production (e.g., Dugdale and Wilkerson 1992; Sarthou et al. 2005). Given the magnitude of diatom production relative to other phytoplankton taxa in the ocean, their fate has important implications for global carbon (C) and nitrogen (N) cycles. For instance, diatoms are capable of relatively rapid sinking rates, both as individual cells and in aggregates, and are responsible for a significant portion of the sinking C flux out of the euphotic zone (Smetacek 1985). Diatoms are also an important component of marine food webs, fueling both pelagic (Ryther 1969) and benthic secondary production (Graf 1992).

Several recent studies have shown that the dissolved organic matter (DOM) pool may be an important, but overlooked, fate for fixed C and N. For instance, in high-productivity upwelling systems where diatoms dominate

production, autochthonous-produced DOM may accumulate on several timescales, ranging from that of bloom duration (days to weeks) to seasonal (months) (Williams 1995; Alvarez-Salgado et al. 2001; Hill and Wheeler 2002). Given that phytoplankton cells are permeable to a variety of organic compounds, release by the blooming phytoplankton is likely an important source of the accumulating DOM. That this DOM accumulates in situ indicates that a fraction of the C fixed by phytoplankton, including diatoms, will not be capable of sinking and less may be available to higher trophic levels because of the greater number of trophic steps in the microbial food web (Ducklow et al. 1986).

Early research on phytoplankton DOM production was hampered by methodological problems that prevented unambiguous determination of whether healthy phytoplankton released DOM (Sharp 1977). The careful study by Mague et al. (1980) showed that healthy phytoplankton do release DOM and that under certain conditions, this released DOM could be a significant component of total primary production. Diffusion is a possible mechanism leading to the release of small organic compounds from phytoplankton cells as a result of the permeability of their cell membranes (Bjørnsen 1988). However, Baines and Pace (1991) argued that DOM release is tied to the pool of recently fixed organic matter and not to the total cellular biomass on which the diffusion model is based. Other studies have shown that high C:N DOM, composed mainly of carbohydrates, may also accumulate in blooms that have become nutrient starved (e.g., Ittekkot et al. 1981; Biddanda and Benner 1997). This led to the suggestion that DOM production may be enhanced under nutrient-depleted conditions as a mechanism for dissipating cellular energy, particularly under relatively high-light conditions (e.g., Fogg 1983; Wood and van Valen 1990). Support for

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Table 1. Description of diatom species used in this study.

Species	Source	Morphology	Biovolume (μm^3)	Surface area to biovolume
<i>Bellerochea</i> sp.	CCMP (#143)	Prism on triangle	75 to 150	1.5 to 2.0
<i>Cylindrotheca closterium</i>	CCMP (#1855)	Prolated spheroid + two cylinders	110 to 340	1.1 to 1.9
<i>Skeletonema</i> sp.	Inner shelf, Oregon coast	Cylinder + two half spheres	500 to 2,000	0.5 to 0.9
<i>Chaetoceros decipiens</i>	CCMP (#173)	Elliptical prism	1,500 to 9,000	0.3 to 0.7
<i>Odontella longicruris</i>	Inner shelf, Oregon coast	Elliptical prism	18,000 to 24,000	0.2

(e.g., Smith and Underwood 2000) and against this idea (e.g., Marañón et al. 2004) point to the fact that this issue has been difficult to resolve.

In addition to complicating C export estimates and estimates of C available for trophic transfer, DOM production is generally not accounted for in field-based primary production studies (i.e., from satellites, ^{14}C uptake studies, etc.), adding a degree of uncertainty to C budgets that rely on those types of measurements. Potential variability in DOM release rates with phytoplankton growth stage makes use of a single “correction factor” to account for DOM release unadvisable. Further complications arise when one considers that there may be species-specific differences in DOM release rates. If diffusion is important, then theoretically DOM release rates should be positively correlated with the surface area to volume relationship of cells. There is little information in this regard, although Malinsky-Rushansky and Legrand (1996) provide some evidence for enhanced release by small phytoplankton compared to larger phytoplankton. However, that study, like many other studies on this subject, involved use of nonaxenic cultures, making interpretation of C fluxes tenuous at best. Furthermore, the assumption that size/morphology alone determines DOM release rates may be an oversimplification, given that it does not take into account the various life histories of phytoplankton or ambient environmental conditions. In this study, DOM and particulate organic matter (POM) production by axenic cultures of five diatom species was examined. The fluxes of fresh phytoplankton C and N to these pools were determined under controlled environmental conditions, with specific goals of addressing whether there are growth stage and species-specific differences in these fluxes.

Methods

Diatom cultures—Axenic cultures of three diatom species, *Chaetoceros decipiens*, *Cylindrotheca closterium*, and *Bellerochea* sp., were obtained from the Center for the Culture of Marine Phytoplankton (Table 1). Cultures of two other species, *Odontella longicruris* and *Skeletonema* sp., were isolated from inner-shelf surface waters collected off Oregon during the summer (Table 1). Axenic strains of these cultures were established through single cell isolations and transfer to sterile media containing antibiotics. Cultures were maintained on a 14:10 light:dark cycle at $\sim 205 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ by eight 40-W cool white fluorescent bulbs and two full-spectrum aquarium lights. The fluorescent bulbs were distributed at regular intervals

along the side and top of the incubation chamber such that light levels were constant throughout the entire volume of the experimental carboys or bottles. Cultures were grown in *f/40* media at 12°C . Release of DOM was studied by two methods; measurement of bulk DOM accumulation and tracking ^{14}C -bicarbonate uptake and release as DOC. Because of the cost and tedious nature of the bulk measurements, only three species were studied by that technique. DOM release from all five species was studied by the ^{14}C tracer technique.

Bulk DOM accumulation experiments—Three of the five diatoms—*C. decipiens*, *C. closterium*, and *Bellerochea* sp.—were grown as batch cultures, analogous to coastal bloom development, for determination of how their bulk organic matter is partitioned. For each species, triplicate acid-washed 20-liter polycarbonate Clearboy carboys were filled with sterile filtered ($0.2 \mu\text{m}$) seawater collected from Yaquina Bay (Newport, Oregon) on the incoming tide. Salinities for all experiments were 31–32. After the carboys were filled with seawater, they were autoclaved and allowed to cool to 12°C . Nutrients were then added by means of sterile techniques to achieve $\sim f/40$ concentrations. Finally, a small ($\sim 5 \text{ mL}$) inoculum of exponentially growing culture was added to each carboy 1 d before the start of experiments, and the carboys were placed under the lights described above. The carboys were manually mixed twice daily. Samples were collected daily at 2.5 h before the beginning of the dark period. To ensure that there was no bacterial contamination, 5-mL samples were collected at selected time points (beginning, middle, and end) during each experiment, fixed with 1% formalin, stained with DAPI, and filtered onto $0.2\text{-}\mu\text{m}$ polycarbonate filters. The filters were examined by epifluorescence microscopy.

Cell counts and biomass measurements were made by the Utermöhl technique from samples that had been fixed with 3% Lugol's solution and settled for 18–24 h immediately after collection. Size measurements were made on approximately 25–50 cells from each sample with a calibrated ocular micrometer.

Samples for nutrient analyses were gently prefiltered through combusted GF/F filters into acid-washed 30-mL HDPE bottles and immediately frozen at -30°C . Samples were analyzed on a Technicon AA-II according to standard wet chemical methods of Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made before each run by diluting a primary standard with low nutrient surface seawater. Deionized water (DIW) was

used as a blank, and triplicate DIW blanks were run at the beginning and end of each run to correct for baseline shifts. Nitrate was determined by subtracting nitrite from nitrate plus nitrite ($\text{NO}_3^- + \text{NO}_2^-$). Detection limits for $\text{NO}_3^- + \text{NO}_2^-$ and phosphate were $0.21 \mu\text{mol L}^{-1}$ and $0.03 \mu\text{mol L}^{-1}$, respectively.

Total nitrogen (TN) samples were collected in acid-washed 30-mL Teflon bottles and immediately frozen at -30°C until analysis. Organic N was converted to nitrate by a persulfate wet oxidation method (Libby and Wheeler 1997), and then analyzed with a Technicon AA-II. Instrument calibration was performed daily by a standard curve prepared from triplicate digested leucine standards at three concentrations. Fresh standards were made before each run by diluting a primary standard with artificial seawater. Digested artificial seawater was used as a blank, and the standard curve was corrected for N content of the blank by determining the concentration of N in the persulfate solution and then calculating the amount of N in the artificial seawater. Artificial seawater N content was estimated as the difference between the blank and persulfate signals.

Total organic carbon (TOC) samples were collected in triplicate in acid-washed borosilicate vials with Teflon cap liners. Each vial contained approximately 5 mL of seawater that was preserved with 50 μL of 90% phosphoric acid. Samples were stored at room temperature until processing using the high-temperature catalytic combustion method on a Shimadzu TOC-5000A analyzer. Standard curves were run twice daily using a DIW blank and four concentrations of an acid potassium phthalate solution. Three to five subsamples were taken from each standard and water sample and injected in sequence. Variance between subsamples was $\leq 6.8\%$ (mean = $1.2\% \pm 0.9\%$). Certified Reference Material Program (CRMP) deep-water standards of known TOC concentration were injected in triplicate at the beginning, middle, and end of every run to check for baseline shifts. Baseline drift was calculated from changes in the deep-water concentrations during a run, and a drift correction was applied to the raw data. For the *C. decipiens* and *C. closterium* experiments, average daily CRMP TOC concentrations (12–00 batch) were $45.3 \pm 2.2 \mu\text{mol L}^{-1}$. For the *Belleriochea* sp. experiment, the average daily CRMP TOC concentration (05–04 batch) was $43.2 \pm 2.9 \mu\text{mol L}^{-1}$. The data from each individual run were normalized to the average daily CRMP TOC concentration.

Particulate organic carbon (POC) and particulate nitrogen (PN) were determined from material collected on precombusted GF/F filters. Depending on the expected amount of material in a sample, between 50 to 1000 mL of experimental water was vacuum filtered ($<27 \text{ kPa}$) onto precombusted GF/F filters. After filtration, samples were stored in glass Vacutainers and immediately frozen at -30°C until laboratory analysis. Samples were processed within 3–4 months of collection. Filters were fumed with concentrated HCl to remove inorganic C and dried, followed by analysis by a Control Equipment Corp. 440HA CHN elemental analyzer calibrated with acetanilide. During analysis, filter blanks were run after every 12

samples. Filter blanks averaged $18.1 \pm 2.6 \mu\text{g C}$ and $0.6 \pm 0.8 \mu\text{g N}$, and these values were subtracted from each measured value as a blank correction.

Dissolved organic nitrogen (DON) was determined by subtracting PN and dissolved inorganic nitrogen (DIN) (NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$) from TN (Eq. 1).

$$\text{DON} = \text{TN} - \text{PN} - \text{DIN} \quad (1)$$

The standard deviation for DON was calculated by propagation of error by using standard deviations for TN, PN, and DIN (Bevington 1969). DOC was determined by subtracting POC values from TOC values (Eq. 2).

$$\text{DOC} = \text{TOC} - \text{POC} \quad (2)$$

The standard deviation for DOC was calculated by propagation of error by using standard deviations for TOC and POC (Bevington 1969).

Transparent exopolymer particle (TEP) production was determined for *C. closterium*. TEP was not quantified for the other two species because it was not included in our original sampling regime. However, after DOC dynamics were found to be dramatically different for *C. closterium* than *C. decipiens*, the experiments were rerun for *C. closterium* to include TEP (reported here with other concomitant measurements). TEP were determined from 5–10 mL samples that had been filtered ($<27 \text{ kPa}$) onto 0.4- μm polycarbonate filters. The filtered samples were stained with 500 μL of prefiltered (through 0.2- μm filter) 0.02% Alcian blue (pH 2.5) for $<5 \text{ s}$. Finally, the filters were rinsed twice with DIW and frozen until analysis. Sample filters, blank filters, and standard filters were soaked in 80% H_2SO_4 for 2 h, during which they were gently agitated three to five times. Standards consisted of six different concentrations of xanthan gum filtered onto 0.4- μm filters and extracted as above. Absorption of each extracted sample, blank, or standard was measured spectrophotometrically at 787 nm.

¹⁴C tracer experiments—¹⁴C was used to assess POC and DOC production rates for the five diatom species described above. For each species, rates were measured over the light period (14 h) or over the entire light/dark period (24 h) for cells in the exponential, transition (defined here as the time period when the diatoms just began to enter stationary growth; i.e., $<1 \text{ d}$ after first entering stationary growth), or stationary growth phases. Dark period rates were determined by subtracting the cumulative light period production from the cumulative daily (light/dark) production and dividing that by the duration of the dark period (10 h). Under subdued lighting and within 30 min before the start of the light period, $\sim 5.92 \times 10^5 \text{ Bq}$ of $\text{NaH}^{14}\text{CO}_3$, previously determined not to contain organic ¹⁴C, was added to each of 12–50-mL acid-washed and DIW-rinsed transparent polyethylene media bottles containing the diatom cultures. Coincident with addition of ¹⁴C label, an additional 300–350 mL of culture was collected for determination of dissolved inorganic C concentrations. One of the ¹⁴C-labeled bottles was processed immediately for

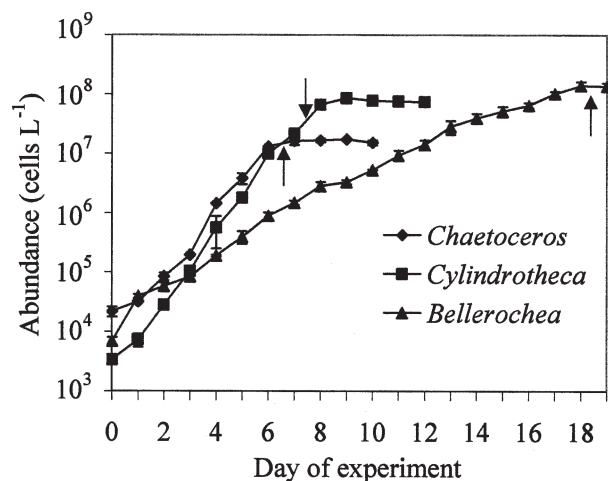


Fig. 1. Growth curves (cells L^{-1}) for three diatom species used in bulk measurement experiments. Arrows indicate where nitrate and phosphate were depleted.

determination of specific activity, which involved collection of a 50- μL subsample and addition of it to 300 μL of β -phenylethylamine in a glass scintillation vial, and finally addition of 10 mL of scintillation cocktail. Another bottle was used for a $t = 0$ blank and was processed immediately upon addition of the isotope. The additional 10 bottles were incubated in the light (previously described) or in darkness and processed after 14 h (light period) or 24 h (light/dark period).

For processing of the $t = 0$ and time course samples, a 5-mL subsample was withdrawn from each bottle under subdued lighting and gently vacuum filtered through a 0.2- μm polycarbonate filter. The filtrate and filter from each were placed in glass scintillation vials, and 100 μL of 50% HCl or 1 mL of 10% HCl was added to the filtrate or filter, respectively, for removal of dissolved inorganic C. The samples were exposed to acid for 24 h, and then 15 mL or 10 mL of scintillation cocktail was added to the filtrate or filter, respectively. Finally, samples were analyzed with a Wallac 1409 liquid scintillation counter with an internal quenching curve. Results from the acidified $t = 0$ samples showed that inorganic ^{14}C was completely removed through the acidification process. POC/DOC production rates were determined by subtracting dark bottle POC/DOC values ($\times 2$ per incubation period) from light-exposed bottle values ($\times 3$ per incubation period).

Results

Bulk DOM accumulation experiments—All three species in these experiments grew exponentially until depletion of N and P, after which growth ceased within 1 d (Fig. 1). *C. closterium* exhibited the highest growth rate, averaging $1.16 \pm 0.46 d^{-1}$, followed by *C. decipiens*, which averaged $0.95 \pm 0.58 d^{-1}$, and *Bellerochea* sp., which averaged $0.55 \pm 0.35 d^{-1}$. Empty frustules, probably resulting from cell death (lysis), only amounted to 0.5–3.0% of live cell abundances throughout each of the experiments. No bacterial contamination was found during each of the bulk

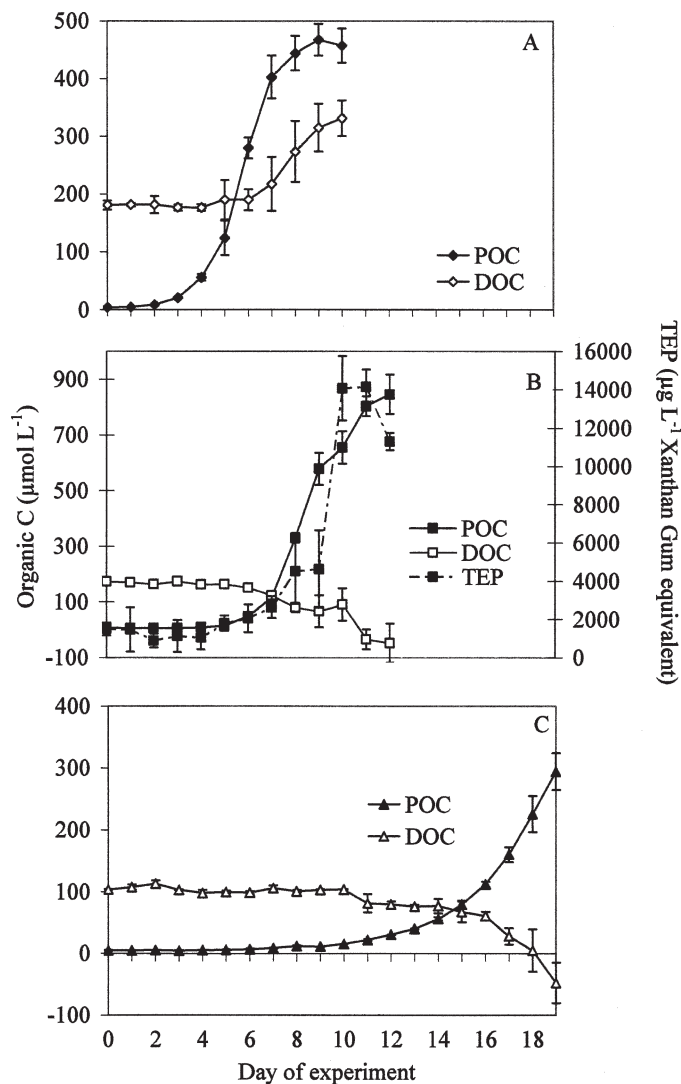


Fig. 2. Concentrations of POC and DOC ($\mu mol L^{-1}$) in bulk measurement experiments for (A) *Chaetoceros decipiens*, (B) *Cylindrotheca closterium*, and (C) *Bellerochea* sp. Also indicated in (B) is TEP ($\mu g L^{-1}$ xanthan gum equivalent).

measurement experiments for these species (data not shown).

POC increased exponentially for *C. decipiens* until ~ 1 d after the transition to the stationary growth phase, when accumulation ceased (Fig. 2A). Likewise, PN increased exponentially until nutrient depletion, then decreased by $\sim 6.8 \mu mol L^{-1}$ by the end of the experiment (Fig. 3A). During exponential growth, the C:N (mol:mol) of each day's newly produced POM averaged 7.2 ± 1.3 , similar to the C:N of the total accumulated POM pool (Fig. 4A). However, the C:N of the newly produced material that accumulated during the transition and early stationary growth phases approached infinitely high values as expected for carbohydrate accumulation (Fig. 4A). Likewise, the C:N of the total accumulated POM pool increased to 12.1 ± 1.7 in the stationary growth phase (Fig. 4A). Noticeable accumulation of both DOC and DON began in the late exponential growth phase and

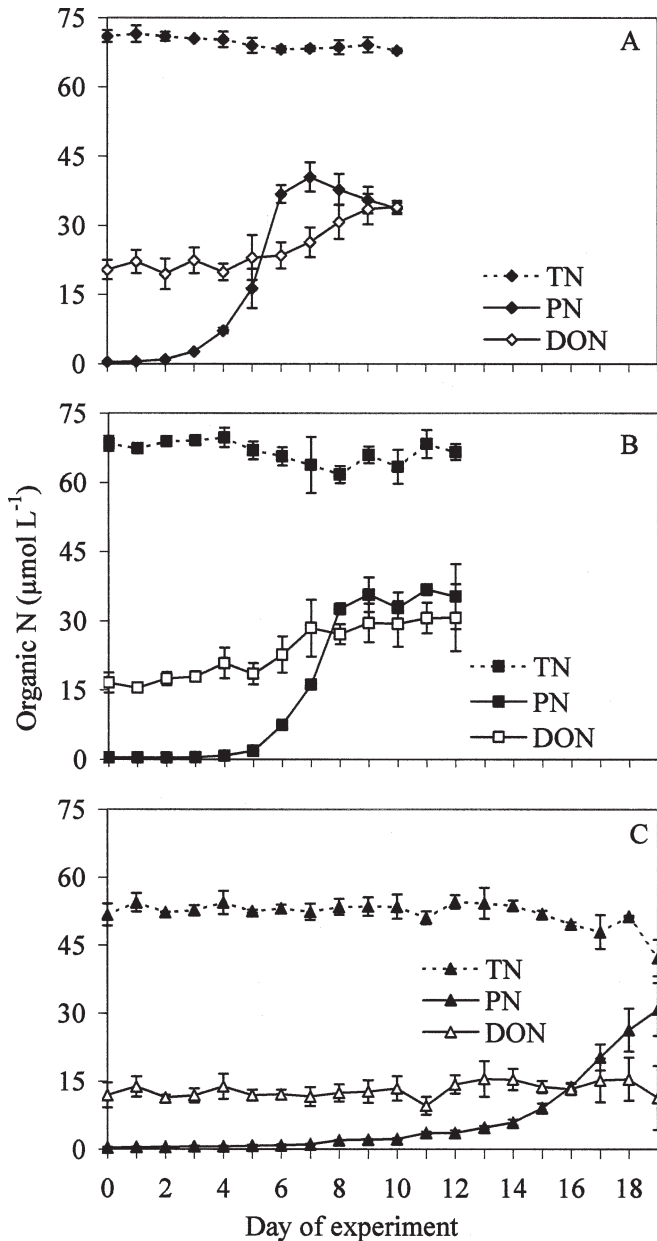


Fig. 3. Concentrations of TN, PN, and DON ($\mu\text{mol L}^{-1}$) in bulk measurement experiments for (A) *Chaetoceros decipiens*, (B) *Cylindrotheca closterium*, and (C) *Bellerochea* sp.

continued until termination of the experiment (Figs. 2A, 3A). The average C:N of each day's newly produced DOM from late exponential growth through the end of the experiment averaged 17.2 ± 9.8 , indicative of mainly carbohydrate accumulation, but also some N-containing organic compounds (Fig. 4A).

POC increased exponentially for *C. closterium*, but unlike for *C. decipiens*, production continued for more than 3 d after nutrient depletion and the accumulation rate only decreased slightly by the end of the experiment (Fig. 2B). PN increased exponentially until nutrient depletion, after which production stopped (Fig. 3B). The C:N of the newly produced POM varied considerably with

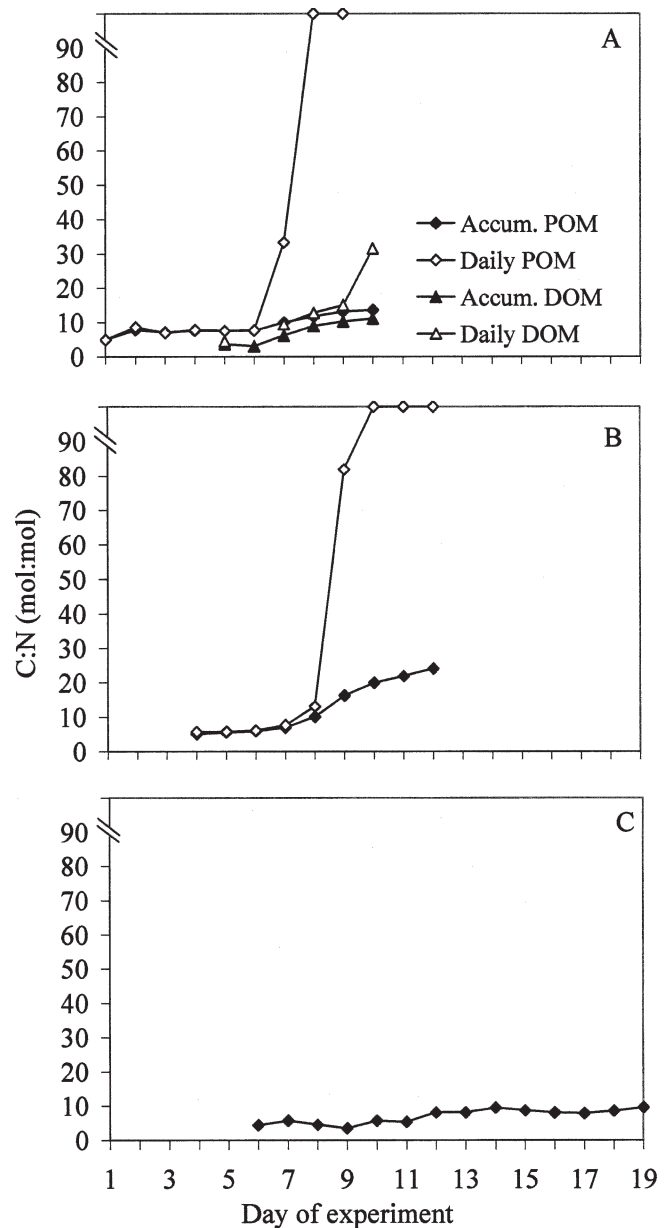


Fig. 4. Molar C:N of the total accumulated POM, daily accumulated POM, total accumulated DOM, and daily accumulated DOM for (A) *Chaetoceros decipiens*, (B) *Cylindrotheca closterium*, and (C) *Bellerochea* sp. C:N values >90 could not be estimated accurately because no cellular N accumulated.

growth stage, averaging 6.3 ± 1.0 during exponential growth, $13.1\text{--}82.8$ during the transition to stationary growth, and finally reaching an infinitely high value in stationary growth (Fig. 4B). Likewise, the C:N of the total accumulated POM pool averaged 6.7 ± 2.0 during exponential growth, but increased to 22.0 by the end of the experiment (Fig. 4B). DOC began to decrease during late exponential growth and reached zero by the end of the experiment (Fig. 2B). Negative DOC values were not statistically different from zero (two-tailed *t*-test; $p > 0.05$). In contrast, DON increased dramatically for two days during late exponential growth (by $9.9 \mu\text{mol L}^{-1}$),

then subsequently increased by only $\sim 2.2 \mu\text{mol L}^{-1}$ during the rest of the experiment (Fig. 3B). There is some degree of uncertainty associated with the PN and DON measurements, particularly during the mid- to late exponential phase, because recovery of TN was incomplete. The error associated with this missing TN ranged from $\sim 6.7 \mu\text{mol L}^{-1}$ during late exponential growth to $\sim 1.8 \mu\text{mol L}^{-1}$ at the end of the experiment (Fig. 3B). TEP production strongly followed the dynamics of the TOC pool (Fig. 2B). Under nutrient replete conditions, the proportion of TEP to TOC (μg xanthan gum equivalent : μg TOC) was fairly constant, averaging 0.7 ± 0.2 (Fig. 2B). However, within 1 d of nutrient depletion, the proportion of TEP to TOC increased nearly twofold and averaged 1.2 ± 0.5 for 4 d of stationary growth.

Bellerochea sp. POC increased exponentially until nutrient depletion, as did PN (Figs. 2C, 3C). Unfortunately, the experiments were terminated within 1 d of the cells reaching stationary growth, and coincident with complete nutrient depletion. Therefore, we cannot say anything about POM dynamics during the later stages of stationary growth. The daily C:N of the accumulating POM was much more variable than the other species, averaging >11 while the cells were in exponential growth. This variability is likely due to the slower growth and the smaller daily accumulation rates of POC/PN of *Bellerochea* sp., which adds uncertainty to estimates of the POM C:N. DOC began to decrease in midexponential growth and reached zero by the end of the experiment (Fig. 2C). Again, the negative DOC was not statistically different from zero (two-tailed *t*-test; $p > 0.05$). DON did not significantly increase, although it may have been underestimated near the end of the experiment as a result of incomplete recovery of the TN (by $2\text{--}7 \mu\text{mol L}^{-1}$; Fig. 3C).

¹⁴C tracer experiments—The three species used in the bulk measurement experiments (*C. decipiens*, *C. closterium*, *Bellerochea* sp.) grew at similar rates in the ¹⁴C tracer experiments as in the bulk measurement experiments. *O. longicruris* grew at $0.86 \pm 0.52 \text{ d}^{-1}$, whereas *Skeletonema* sp. grew at $0.90 \pm 0.58 \text{ d}^{-1}$ in the ¹⁴C tracer experiments. Cell-C normalized DOC release rates during the entire light/dark cycle (i.e., 24-h period) varied between species, ranging $0.4\text{--}5 \times 10^{-3} \text{ pmol DOC pmol cell C}^{-1} \text{ h}^{-1}$ for cells in exponential growth or at the transition to stationary growth (Fig. 5A). Stationary phase release rates were much lower, ranging $<0.1\text{--}0.7 \times 10^{-3} \text{ pmol DOC pmol cell C}^{-1} \text{ h}^{-1}$. Nighttime DOC release rates were not significantly different than light/dark rates for *Bellerochea* sp. but were reduced on average by 21–32% for *C. closterium*, *O. longicruris*, and *Skeletonema* sp. (Fig. 5B). For *C. decipiens*, nighttime rates were reduced to near zero in the exponential growth phase, although there was considerable variability associated with that sample. In the other two growth phases, nighttime release rates were reduced by 43–45% relative to day/night rates. There was no statistically significant POC production at night for any of the species except *C. closterium* (data not shown). *C. closterium* continued to produce POC at night, but only in the transition and stationary growth phases and at reduced

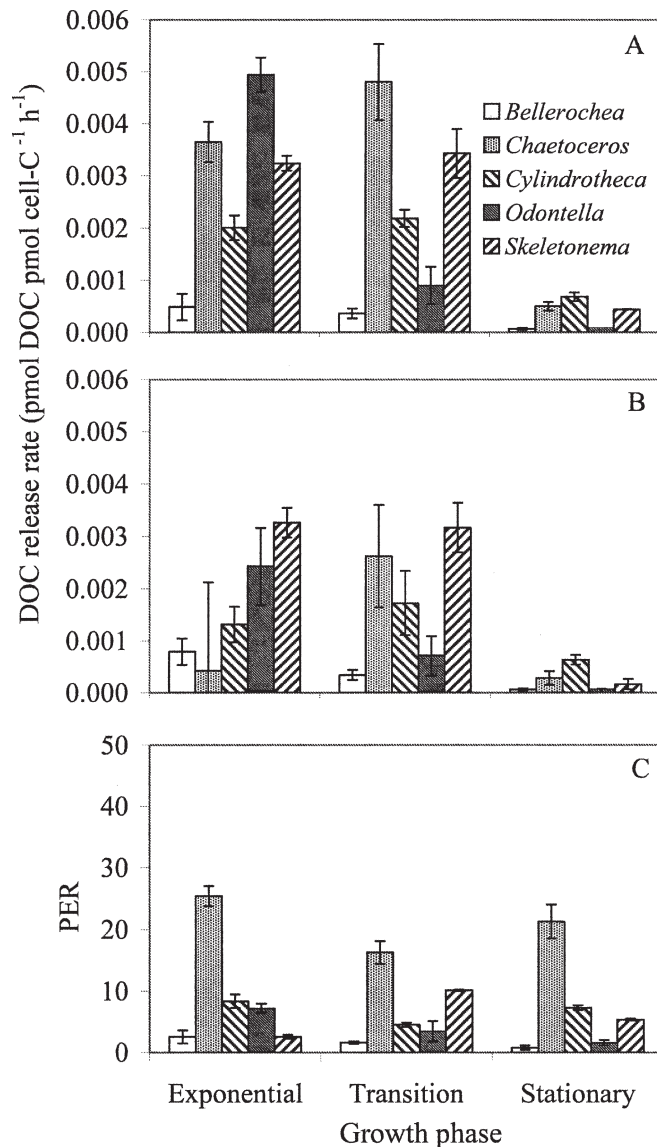


Fig. 5. DOC release rates ($\text{pmol DOC pmol cell C}^{-1} \text{ h}^{-1}$) for cells in exponential, transition, or stationary growth phases and (A) over a 24-h light/dark cycle, or (B) at night. (C) PER for cells in exponential, transition, or stationary growth phases.

rates relative to daily (light/dark) rates. Nighttime POC production rates for *C. closterium* were reduced by $\sim 33\%$ over daily rates in the transition phase, and by $\sim 75\%$ over daily rates in the stationary phase. The percentage of DOC released to total C fixed (hereafter “PER”) ranged from 0.8 to 10.1% (mean = $4.6\% \pm 3.1\%$) for the diatoms, excluding *C. decipiens* (Fig. 5C). There were no discernible trends in PER related to growth stage. *C. decipiens* PER was consistently higher than the other species, averaging $21.0\% \pm 4.6\%$ and varying little with growth stage.

Discussion

Studies of DOM production have often relied on nonaxenic field samples or cultures and/or short term (less

than a few hours) ^{14}C tracer studies. When designed properly, valuable information has been obtained from these types of studies. However, in many cases, bacteria acting on and influencing the fresh phytoplankton DOM pool creates uncertainties in the magnitude of C and N fluxes to that pool. In the case of the short-term ^{14}C tracer studies, Underwood et al. (2004) found that there was an ~3-h time lag between addition of ^{14}C label to diatom cultures and the subsequent production of labeled extracellular polysaccharides, a potentially large component of phytoplankton-derived DOM. Thus, short (less than a few hours) ^{14}C studies may completely miss C dynamics associated with the extracellular polysaccharide pool, a point noted in the earlier studies of Mague et al. (1980) and Smith (1982). Our study shows that even with careful accounting for possible sources of error associated with POM and DOM measurements (e.g., through use of axenic cultures and careful analytical techniques), the dynamics of those pools are still quite complex and their interpretation in the context of phytoplankton physiology/bloom dynamics remains a daunting task.

In many coastal systems, phytoplankton bloom development is analogous to batch culture growth. Hence, DOM production studies from coastal systems are often interpreted in the context of well-defined phytoplankton growth stages. In this study, cellular C-based ^{14}C DOC production rates were much higher for all species in exponential and transition growth phases than during stationary growth. Higher DOC release rates in the exponential/transition growth phases compared to the stationary growth phase have been noted previously in batch cultures of other diatom species (Granum et al. 2002; Underwood et al. 2004). Even if some of the DOC was actually measured as POC, which we suspect for *C. closterium* and *Belleriochea* sp., the stationary growth phase rate of ^{14}C TOC production (i.e., true DOC + attached or TEP DOC + true POC) was lower on average by $59\% \pm 3\%$ for *C. closterium* and *Belleriochea* sp., and by $90\% \pm 5\%$ for the other three diatoms.

In the bulk measurement experiments, *C. decipiens* POC production ceased within 1 d of N depletion, and DOC production slowed by late stationary growth. As N became limiting, the C:N of newly produced POM approached infinity as a result of C accumulation in the absence of N accumulation, pointing to carbohydrate or lipid accumulation (Mykkestad 1974; Biddanda and Benner 1997). The C:N of the newly produced DOM was also increased over Redfield stoichiometry, averaging ~17 and indicative of the presence of mainly carbohydrates or lipids, but also some N-containing compounds and probably TEP-like material (e.g., Corzo et al. 2000). However, even if TEP production occurred in stationary growth, the rapid slowdown of C production after N depletion would imply that the cumulative effect on both POC and DOC and the potential for conversion of DOC to POC through TEP dynamics was minimal. Thus, it appears that for *C. decipiens*, bulk POM and DOM measurements may actually provide accurate measures of true POM and DOM. It should be noted that PER was much higher for *C. decipiens* than the other diatom species. Although not well

understood, several studies have found that various *Chaetoceros* sp. tend to exhibit higher PER than other diatoms (e.g., Mykkestad 1974, 1977). The fact that PER was not increased for *C. decipiens* under nutrient limitation, that the bulk DOC accumulation could be accounted for in the absence of increased release rates under N limitation, and that some of the DOC release continued at night in two of three growth phases argues that the overflow mechanism may not be as pronounced in *C. decipiens* compared to other phytoplankton.

In contrast to results for *C. decipiens*, the dynamics of the POM and DOM pools were more complex and the distinction between the two pools less evident for *C. closterium* and *Belleriochea* sp. During exponential growth, the newly accumulated C:N of POM for *C. closterium* was near Redfield stoichiometry. After nutrient depletion, however, POM with a C:N approaching infinity continued to accumulate coincident with DOC decreasing to zero. Some of the POM may have been intracellular carbohydrates, which Underwood et al. (2004) showed to increase twofold per cell for *C. closterium* during stationary growth. However, a significant portion of the continued POC increase, especially that which occurred in the last few days of the experiment, was likely due to release of TEP precursors (polysaccharides) and their subsequent binding or aggregation with other DOC in the media. In fact, TEP per cell increased dramatically just after nutrient depletion and was threefold higher in stationary growth, consistent with previous work on this species (e.g., Smith and Underwood 2000; Underwood et al. 2004). The C content of TEP has been measured and appears to be species-specific with slopes of the TEP-C versus xanthan gum equivalents ranging from 0.51 to 0.88 (Engel and Passow 2001). A mean slope of 0.75 and an intercept of 110 applied to our TEP results for *C. closterium* yield TEP-C values that exceed TOC by approximately 20%. To achieve a quantitative estimate of TEP-C, we calculated cell C from the biovolume measurements and a diatom carbon to volume relationship of $\text{pg C cell}^{-1} = 0.288 \times \text{volume}^{0.811}$ (Menden-Deuer and Lessard 2000) and assumed that the TOC during stationary growth of *C. closterium* was present exclusively as cell C and TEP-C. This inverse approach gave a slope of 0.55 for the TEP-C versus xanthan equivalents for *C. closterium* and is close to the values reported for cultures of *Chaetoceros gracilis* and *Thalassiosira weissflogii* (Engel and Passow 2001). Our estimates of TEP-C produced by *C. closterium* during the stationary phase averages $613 \pm 75 \mu\text{mol L}^{-1}$ C and is $80\% \pm 12\%$ of the C produced.

Similar results have been reported for *C. closterium* isolated from the Adriatic Sea (Alcoverro et al. 2000). Overall, these results suggest that because TEP is empirically measured as POC in most protocols, determination of DOC released and estimates of PER without inclusion of TEP are inadequate measures for species and ecosystems where "gel organic material" is a significant part of organic C production (Verdugo et al. 2004). TEP aggregation and binding with other DOC is now widely recognized as an important process for creating POC from DOC (Passow 2002; Verdugo et al. 2004) and was likely an important factor in the DOC decrease, especially given the large

amount of TEP that accumulated. Although TEP can “plug” filters (e.g., Verdugo et al. 2004) and may have caused a slight overestimation of POC in this study, possibly leading to the slightly negative DOC values, the reduction in filtration volumes for the POC measurements as the cultures became dense suggests should have minimized the overall contribution of this process to the DOC decrease. The DOC in the media of *Bellerochea* sp. also disappeared as POC increased. By the time nutrients became depleted in *Bellerochea* sp. cultures, the C:N of newly produced POM approached 15, indicating the accumulation of a mixture of carbohydrates or lipids and N-containing compounds. The disappearance of the DOC coincident with the accumulation of POC suggests that this species may also be producing TEP-like material that binds to the background DOC.

In this study, PER averaged ~5% for four of five species tested, and was ~21% for *C. decipiens*. Several recent reviews of PER from the literature indicate that PER generally averages 5–15% (e.g., Baines and Pace 1991; Nagata 2000). Despite the relatively low PER, large amounts of DOC may still accumulate as phytoplankton biomass reaches bloom proportions, as demonstrated in the *C. decipiens* experiment. However, these low PER values and the concomitant DOC fluxes may not be sufficient to support the C demand of bacteria in many marine ecosystems (reviewed by Nagata 2000). Hence, although phytoplankton DOC release is important, other mechanisms such as grazer excretion and viral lysis are also important, and further complicate our understanding of DOC fluxes in situ.

With respect to nutrient availability, PER remained relatively constant in nutrient-replete versus nutrient-deplete conditions. Recent work by Marañón et al. (2004) showed that PER is relatively invariant across eutrophic-oligotrophic environmental gradients, arguing against the overflow mechanism as being important in marine systems. However, as demonstrated in our study, this interpretation may be complicated by the fact that for some species, DOC may actually be measured as POC. PER values for *C. closterium* and *Bellerochea* sp. were within the average ranges previously reported for other diatom species, but it appears that a significant portion of the DOC that they produce is measured as POC. As discussed earlier, inclusion of TEP-C to estimate PER for *C. closterium* suggests that PER may approach 80% for this and other similar diatom species in nutrient depleted conditions. This suggests that the carbohydrate-based “overflow” mechanism may be more relevant to some species, including *C. closterium* (Alcoverro et al. 2000; Smith and Underwood 2000; Underwood et al. 2004), and presumably *Bellerochea* sp. *C. closterium* is commonly found in the benthos and is known to retain an extracellular polysaccharidic capsule and also produces copious amounts of extracellular polysaccharides (Hoagland et al. 1993; Smith and Underwood 2000). These traits are well known to many other species of diatoms that have some form of benthic life history (e.g., Smith and Underwood 2000).

Hence, we speculate that in field and culture situations where these types of diatoms are prevalent, routine POM

and DOM measurements using GF/F filters may give misleading results in terms of the dynamics of those pools. *C. closterium* is known to form blooms in coastal surface waters and thus its distribution is not limited to the benthos (e.g., Gilabert 2001). This is probably the case for most other benthic diatom species as well, especially in shallow coastal systems subjected to wind-driven resuspension (e.g., Shaffer and Sullivan 1988). In contrast to results for *C. closterium* and *Bellerochea* sp., it appears that the DOC release rates and PER estimated for *C. decipiens* in this study may be accurately used to predict bulk DOC accumulation, despite the fact that this genus of diatoms is known to produce extracellular polysaccharides (Mykkestad 1974, 1977; Corzo et al. 2000). The PER estimated for *Skeletonema* sp., 2.5–10%, is also consistent with previous studies of this species (Mykkestad 1974; Granum et al. 2002) and its DOC appears to be measured as DOC with separation using GF/F filters (e.g., Biddanda and Benner 1997). Therefore, we speculate that for some diatom species, organic matter dynamics studied using either bulk measurements (with GF/F filters) or ¹⁴C to measure POC and/or DOC accumulation rates may be accurate.

It is important to note that our studies were done under carefully controlled environmental conditions (nutrients, temperature and light). How do the DOM release processes described in this study compare to field situations where different nutrients may limit bloom growth or where temperature and light conditions fluctuate? Laboratory and field studies have shown that as with the N and P limiting conditions here, the release of a variety of organic compounds (including extracellular polysaccharides) also occurs under strictly N or P limiting conditions (e.g., Alcoverro et al. 2000). DOM release relative to total C fixation has been shown to be relatively invariant under normal growth temperatures ranging from 5°C to 20°C (Verity 1981). Less clear is the effect of light conditions on DOM release. The DOM release rate relative to total C fixed may be increased at extremely high or low light levels, but under those conditions, the absolute rate of DOM release will probably be reduced as a result of reductions in cell growth and photosynthesis (Zlotnik and Dubinsky 1989). In nature though, phytoplankton are exposed to fluctuating, not static, light conditions. To the best of our knowledge, only one study has looked at how sudden changes in light intensity may affect DOM release. Mague et al. (1980) shifted a water sample collected at 12 m depth in the Gulf of Maine and incubated at the corresponding light intensity (~10% of surface light intensity) to either higher or lower light intensities (i.e., from 1–100% of surface light intensity). Their results were variable and DOC release was only measured several hours after the shift in light intensity. Nonetheless, when the phytoplankton were shifted to 100% surface light intensities, extracellular DOM release was enhanced. Clearly, more work is needed to characterize the impact that fluctuating light (and nutrient) conditions may have on phytoplankton photosynthesis and DOM release, particularly over shorter timescales (i.e., seconds to minutes) than used in the Mague et al. (1980) study.

This study highlights the necessity for future work to determine whether field measurements of POM and DOM are biased by species-specific differences. The bound or aggregated polysaccharides may have a much different fate than “true” DOC, both in terms of the potentials for export through sinking (reviewed by Passow 2002) and in differences in the potential rates of microbial turnover of the polysaccharides versus smaller monomeric compounds (e.g., Hama et al. 2004). Hence, the relative amount of these different types of organic matter that are produced during blooms may significantly affect the C cycle dynamics. Determining whether there are species-specific biases in POM/DOM production may be difficult given the likely need for time-course measurements during bloom development and because some blooms are dominated by mixtures of phytoplankton. Nonetheless, it is an important issue that needs to be addressed because of reliance by many large field programs (e.g., JGOFS, GLOBEC) and local studies on separation of DOM from POM using GF/F filters. Controversy has already arisen over these types of measurements because of the frequent neglect of the DOM adsorption blank (Moran et al. 1999), and our work adds further complexity to interpretation of POM/DOM dynamics.

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