Light-mediated release of dissolved organic carbon by phytoplankton

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ABSTRACT

Laboratory and field studies were carried out to examine the effects irradiance variability on dissolved organic carbon (DOC) extracellular release by phytoplankton (ER) and the response of natural bacteria assemblages. In axenic laboratory cultures, ER was 3x greater in cultures shifted to 330 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ compared to cultures kept at their cultured irradiance, 110 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Natural bacterial assemblages incubated in the dark for 24 h in algal-free culture filtrate generated from both light treatments consumed the DOC from the high-light treatment at a faster rate than that for the low-light treatment. Field measurements in the coastal waters of the northeastern Gulf of Mexico (GOM) and the Eastern North Pacific (ENP) mirrored the laboratory findings, with short-term increases in DOC concentrations occurring concurrently with short-term increases in irradiance, followed by rapid consumption by bacteria. Where no diurnal irradiance increase was observed (overcast skies), no increase in DOC concentration was observed. An experiment using ^{14}C as a tracer for plankton interactions (GOM) was consistent with data on bulk DOC concentrations. For all the field measurements, the rate of irradiance

change was correlated with the quantity of DOC released. Collectively these results indicated that release of DOC by phytoplankton populations as a function of incident irradiance can be significant and may have important implications for estimates of ocean carbon flux.

1. Introduction

DOC in the ocean is one of the largest reservoirs of carbon on the planet (Hansell and Carlson, 1998). Small changes in this inventory, therefore, can profoundly affect fluxes of carbon across the ocean surface and the export flux of carbon to depth (Carlson, 2002). Although sources and sinks for DOC have been identified (Hansell and Carlson, 2001), limited data exist regarding changes to the inventory. Marine DOC concentrations are generally found in a narrow range (40-80 $\mu\text{M C}$; see Carlson, 2002) within larger variations in net primary production (Behrenfeld and Falkowski, 1997). These relative variations may indicate tight coupling between production and consumption processes (Carlson and Ducklow, 1996). For example Lancelot and Billen (1984) demonstrate tightly coupled short-term oscillations between primary and bacterial production in the North Sea and the English Channel. Experiments conducted by Carlson and Ducklow (1996) in the northwestern Sargasso Sea show increased bacterial production when the ambient labile DOC concentration is higher than the average mixed layer concentration. Similarly rapid turnover of labile plankton-derived DOC has been demonstrated in northeastern Pacific surface waters (Cherrier et al., 1996; Cherrier and Bauer, 2004). On the other hand, Fouilland and Mostajir (2010) in their analysis of an extensive database going back to Cole et al. (1988), conclude that bacteria are independent of phytoplankton activities, a controversial conclusion (see Morán and Alonso-Sáez, 2011; Fouilland and Mostajir, 2011).

Current satellite based estimates put oceanic primary production at 41-77 Gt yr⁻¹ (del Giorgio and Duarte, 2002). As satellite sensors cannot ‘see’ dissolved primary production, estimates from satellite ocean color probably miss the effects of dissolved organic carbon (DOC) release on net community production. In fact, much of the organic carbon contributed by phytoplankton to the upper ocean (possibly more than 50%) is in the dissolved form (Williams, 2000; del Giorgio and Cole, 1998; Williams, 2004). For this reason, the oceanic flux of carbon may be underestimated (Marañón et al., 2004).

After decades of research, the consensus (Carlson, 2002) is that extracellular release (ER) is a normal function of phytoplankton cells. It averages about 13% of primary production, while varying between 0 and 80%, and appears to be related to the level of productivity (Lancelot, 1983; Lancelot and Billen, 1984; Zlotnik and Dubinsky, 1989; Baines and Pace, 1991; Morán and Estrada, 2001). Beyond this, Carlson (2002) notes little agreement on the environmental conditions influencing ER, that is, how it might be affected by high or low irradiance, or nutrients. Literature reports going back to the 1970’s suggest that phytoplankton release DOC under changing irradiance conditions (Thomas, 1971; Mague et al., 1980; Hu and Smith, 1998; Wood et al., 1992; Panzenbock, 2007).

Despite the recognition of the relationship between light and DOC release, this phenomenon has not been formally quantified with respect to its potential contribution to the global carbon budget nor has it been analyzed with respect to its impact on the microbial food web. Here we report experiments that examine the effects of short-term changes in irradiance on dissolved organic carbon release by phytoplankton and the response of heterotrophic bacteria. We first present results from laboratory experiments demonstrating ER in a diatom in response to a transition to high irradiance, and utilization of the released DOC by natural assemblages of

bacteria. In a second set of experiments, we show that the quantity of DOC in surface waters can vary with natural irradiance variability, and use ^{14}C to trace the flow of carbon into the phytoplankton, to released DOC, and into bacteria over the course of a photoperiod.

2. Methods

2.1 Culture experiments

Time series culture experiments were carried out first to assess DOC release by the centric marine diatom, *Thalassiosira weissflogii*, as a function of increased irradiance, and second, to evaluate the utilization of the light-mediated DOM exudate by indigenous bacterial populations. Axenic cultures of *T. weissflogii* were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). Cultures were grown and maintained with sterile f/10 media (Guillard, 1975) in a temperature-controlled circulating water tank kept at 20°C. The cultures were grown at $110 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (saturating for this species) supplied by Phillips 40 W cool-white fluorescents on a 12:12-h light:dark cycle. Cultures were periodically tested for the presence of marine bacteria (i.e. General Marine Test Medium, CCMP), and were monitored daily for growth via *in vivo* fluorescence (Turner Designs 10-AU). Cultures were considered acclimated when there was no significant difference between the slopes of four or more growth curves (F-test, Zar, 1996). Single-day experiments were performed when cultures were in early-exponential growth and timed so that they occurred while all flasks exhibited similar fluorescence readings.

For the first culture-based experiment, inocula from the axenic *T. weissflogii* cultures in early-exponential growth were placed in 4 L flasks with 3 L of sterile f/10 media, and returned to the tank. At the beginning of the photoperiod 4 d later (T0), flasks were either exposed to higher

irradiance ($330 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) or maintained at the same irradiance levels ($110 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Due to a limitation of temperature-controlled circulating water tanks for this experiment, incubations with light-shifted cultures and those maintained at lower light were carried out sequentially. The average chlorophyll-a (Chla) per cell at the beginning of the incubations was $1.3 \text{ pg Chla cell}^{-1}$. Both the low- and high-light cultures were sampled at intervals over the course of the 12 h incubation, for enumeration and bulk DOC concentration measurements. Phytoplankton samples (10 ml) were preserved with 2% acid Lugol's solution (Thronsdon, 1978). The remaining 100 ml was gently vacuum-filtered through precombusted GF/F filters (effective pore size, $0.7 \mu\text{m}$) and the filtrate was distributed into precombusted (525°C for 4 h) EPA VOA vials with HCl-washed Teflon-lined caps, and frozen for subsequent analysis of DOC. Incubations were run in triplicate.

There was not enough culture filtrate after the above experiment for subsequent time series incubations to evaluate bacterial utilization of the light-mediated DOC exudate. Thus, a separate experiment was conducted using the same protocol for both growing the *T. weissflogii* and exposing the cultures to varying irradiances, but no subsamples were removed over the 12-h incubation period. Instead, after 12 h, the diatoms were removed via gentle vacuum filtration through pre-combusted GF/F filters and the filtrate saved to serve as the inoculum media for the bacterial utilization experiment. The average DOC concentration of the filtrate produced by light-shifted cultures was $151 \mu\text{M}$ and that for cultures maintained at lower light was $68 \mu\text{M}$ (Fig. 1b).

Natural planktonic bacterial assemblages were concentrated from seawater collected before dawn at Shell Point, FL, a pristine estuary approximately 50 km south of Tallahassee, FL. Seawater samples were collected from just below the surface into HCl-leached polycarbonate

carboys and stored in the dark for transport back to the lab (within 2 h). In the laboratory, the seawater was first filtered through precombusted GF/F filters (as previously described) to remove larger organisms and detritus, and the bacteria were concentrated into 0.2 μm micro-culture capsules (Pall Corp.). This protocol ensured that no extraneous particulate carbon substrates were introduced into the incubation, but also possibly resulted in an underestimate of bacterial utilization of the light mediated DOM exudates because we were removing all bacteria $>0.7 \mu\text{m}$ as well as any attached to particles.

The concentrated planktonic bacteria were then resuspended into the appropriate light treatment filtrate within 1 h of the removal of the diatoms. Bacterial concentration at the initiation of the experiment was $10^8 \text{ cells L}^{-1}$. Each of the bacterial incubations were run in 3 L flasks, in triplicate, and incubated in the dark at 20°C for 24 h. Flasks were subsampled at 6-h intervals over the next 24 h for enumeration of bacterial cells (Daley and Hobbie, 1975) and determination of DOC. For DOC, 100 ml was filtered through a 0.2- μm acid-washed PTFE syringe filter (Millipore). The filtrate was then distributed into precombusted EPA VOA vials with acid washed Teflon-lined caps and stored at -70°C for subsequent DOC analysis.

3.2 Field Studies

Two kinds of time series incubations were carried out to evaluate the role of daily fluctuations in light intensity on natural phytoplankton community extracellular DOC release and subsequent uptake by bacteria: the first, an in situ incubation, and the second in incubation using ^{14}C -bicarbonate tracer. Diurnal, in situ incubations (light and dark bottles) were carried out from research vessels in both the coastal Northeastern Gulf of Mexico (GOM), $29^\circ37.98'\text{N}$, $85^\circ10.38'\text{W}$ (July 2006 and March 2007) and the coastal eastern North Pacific (ENP),

49°10.38'N, 25°41.56'W (August 2006). Light intensity was measured using a LI-COR LI-250 photometer (Li-Cor Biosciences, Lincoln, NE) (GOM, July 2006 and ENP August 2006), and a GUV511C solar radiometer with a PUV500 profiling radiometer (Biospherical Instr., San Diego CA) during the GOM March 2007 experiment.

In the evening prior to the incubation, seawater was collected using Niskin bottles from either 1 m (GOM) or 4 m (ENP) depth, pre-filtered, passed through a 335- μ m nylon mesh and dispensed into 12-L HCl-leached polycarbonate carboys. All carboys were suspended and incubated at the depth from which the water was collected and allowed to acclimate overnight prior to initial sampling. Light incubations were in clear carboys, while for the dark incubations, the carboys were covered with gray duct tape. All treatments were run in duplicate and samples were drawn from each carboy at selected time intervals.

At each time point, 150 ml aliquots were collected from the incubated containers and put into HCl-leached polycarbonate bottles. Phytoplankton samples (10 ml) were withdrawn and preserved with 2% acid Lugol's solution (Thronsdon, 1978). Samples for bacteria (10 ml) were preserved with 1% formalin (Daley and Hobbie, 1975). The remaining 130 ml were gently vacuum-filtered through pre-combusted GF/F filters, and handled as described above for subsequent DOC analysis.

The ^{14}C bicarbonate tracer experiment was conducted in July 2010. Water was collected approximately 2 km offshore of the Florida State University Coastal and Marine Lab (FSUCML; 29°54.9'N, 84°30.7'W) from 1 m depth at 1600 h using a peristaltic pump with silicon tubing. The water was passed through nylon netting (335 μ m) to remove grazers and debris, and brought back to the lab in an acid cleaned polycarbonate container. In the laboratory, the water was allowed to acclimate at *in situ* temperature for approximately 6 h, and then dispensed into HCl-

174 leached 40 ml Teflon bottles. The bottles were placed in flowing seawater tanks overnight. A
175 total of 20 bottles were used for ^{14}C additions in each experiment, eight of which were blackened
176 with electrical tape. Changes in bulk DOC concentrations were concurrently monitored
177 throughout the time series incubation in a parallel set of clear 40 ml Teflon bottles to which no
178 ^{14}C -bicarbonate was added.

179 Approximately 1 h before dawn, a set of bottles were spiked with ^{14}C -bicarbonate (49
180 mCi/mmol; Perkin Elmer, Shelton, CT) to a nominal final concentration of $2.5\ \mu\text{Ci ml}^{-1}$ and
181 returned to the flowing seawater tables. At each time point, a set of four sample bottles (3 light
182 and 1 dark) was removed from the flowing seawater tables for total and size-fractionated carbon
183 assimilation. An initial 4 ml was removed from each of the four bottles, placed in a scintillation
184 vial and acidified with HCl and shaken for 12 h before adding scintillation cocktail. This sample
185 represents the total fixed carbon (both particulate and dissolved). The remaining contents from
186 the replicates were filtered through 1- μm pore-size polycarbonate filters (Poretics) that were
187 rinsed with 5 ml of filtered seawater. These filters were then placed in a vial and treated as
188 described above. These filters represent the ^{14}C incorporated into phytoplankton biomass. The <1
189 μm filtrate was collected and filtered onto a 0.2- μm pore-size polycarbonate filter (Poretics), and
190 filters were subsequently rinsed with filtered seawater and shaken in a vial with 5 μl
191 concentrated HCl for 12 h. These filters represent the ^{14}C incorporated into bacterial biomass.
192 Four ml of the remaining $<0.2\ \mu\text{m}$ filtrate were acidified and shaken for 12 h. This filtrate
193 represents ^{14}C that was fixed by phytoplankton and released as DO^{14}C . The ^{14}C samples were
194 analyzed at University of West Florida using a Perkin-Elmer Tricarb 2200 CA Liquid
195 Scintillation Counter. All ^{14}C assimilation values were corrected for uptake in the dark. At each

time point, two to three bottles to which no tracer was added were sacrificed, treated as described above for DOC concentration measurements and stored for subsequent analysis.

3.3 Sample Analysis

Dissolved organic carbon. DOC was measured by high-temperature catalytic oxidation with a Shimadzu TOCN-V analyzer (modified from Suzuki, 1992). Samples are first acidified and sparged with CO₂-free air to remove all inorganic carbon, and then combusted to measure all organic carbon. All DOC results were referenced against certified reference materials (CRM, University of Miami, Rosenstiel School of Marine and Atmospheric Sciences). Analytical precision was 2 to 3 $\mu\text{M C}$.

Phytoplankton. Phytoplankton samples were settled in 10 ml chambers for 24 h, then analyzed using the $\ddot{\text{U}}$ termohl method (Hasle, 1978) with a Zeiss Axiovert 10 inverted microscope (at 200 and 320X). Phytoplankton cells were also counted using a Multisizer Coulter Counter (Beckman Coulter, Inc.), employing a Sedgwick-Rafter counting cell (APHA 1976). Chlorophyll concentration was determined by fluorescence in acetone extracts. For the laboratory cultures, diatom volumes were estimated using the regression equations derived by Menden-Deuer and Lessard (2000), and cell size data obtained from CCMP; for these centric diatom cultures, cell length = 12-22 μm , and cell width = 10-12 μm . The resulting cell volume ranges from 1130 to 4560 μm^3 . Cell carbon was estimated by employing the regression equations for carbon determined by Menden-Deuer and Lessard (2000) using the volumes calculated above. Using this regression, we estimated that the carbon content of diatom cells used in this study was between 87-195 pg C cell^{-1} , and the central value to be 141 pg C cell^{-1} .

Bacteria. Bacteria were counted by epifluorescence microscopy using a DAPI (4',6-diaminidino-2-phenylindole) method, adapted from Coleman (1980). Ten fields or a minimum of 400 cells

per sample were counted. Bacterial carbon production (BP) was estimated from the product of bacterial abundance and a constant biomass conversion factor of 30.2 ± 12.3 fg C bacterial cell⁻¹ (Fukuda et al., 1998). This represents net BP.

3. Results

3.1 Laboratory experiments

Axenic Diatom Culture. DOC release was significantly greater for cultures that were shifted to higher light than for cultures that were maintained under their lower, growth irradiance (Fig 1a). The bulk DOC concentration for the cultures shifted to high light increased by 171 ± 20 μ M C over the 12 h incubation period, with the greatest increase occurring between 2 and 6 h following the shift to higher light (Fig. 1a). The net increase in bulk DOC during this initial 4-h period (165 ± 15 μ M C) represents the bulk (87%) of that released over the full 12-h. On the other hand, DOC concentrations in the cultures kept at their growth irradiance increased only marginally (48 ± 21 μ M C) over the same period.

Phytoplankton cell abundance stayed constant for cultures shifted to higher light. Conversely, cell abundance in cultures maintained at moderate light doubled once during the 12-h sampling period. We estimate that the amount of carbon released per cell over the course of the 12-h incubation for cultures shifted to higher light was 330 ± 74 pg C cell⁻¹. If we assume the central value of carbon per cell to be 141 pg C cell⁻¹, then the amount of carbon released by the cells for this light treatment was more than twice as much as what we have estimated to be present in the cell based on volume. In a similar calculation for the cultures kept at their growth irradiance, extracellular release was 12 ± 8 pg C cell⁻¹ representing approximately 9% of their biomass. Chlorophyll remained stable in the high-light shifted cultures, while doubling in the cultures kept

at the growth irradiance. No appreciable change in the chlorophyll-a content per cell occurred over the course of the incubation in either light treatments.

Bacterial response to light-mediated ER. Bacteria incubated with filtrate from the *T. weissflogii* cultures shifted to higher light consumed approximately 97% of the DOC during the 24-h incubation period ($\Delta\text{DOC} = 147 \pm 18 \mu\text{M C}$; Fig. 1b). The average DOC uptake rate for the 24-h incubation was approximately $6.1 \mu\text{M C h}^{-1}$ with the highest rate 6-12 h into the incubation ($10.9 \mu\text{M C h}^{-1}$), and representing almost 45% of total DOC uptake over the entire 24-h period. Bacteria incubated with filtrate from the cultures of *T. weissflogii* maintained at their growth irradiance consumed 79% of the DOC ($\Delta\text{DOC} = 54 \pm 16 \mu\text{M C}$; Fig. 1b). The average DOC utilization rate measured for this treatment was $2.3 \mu\text{M C h}^{-1}$.

3.2 Field Studies

In situ incubations. In both the July 2006 and March 2007 GOM incubations, increases in DOC concentrations were observed during the first 4-5 h following sunrise, and appeared to be concurrent with increases in solar irradiance (Fig. 2). For the August 2006 ENP incubations, there was no increase in either DOC concentration or solar irradiance over the day (Table 1, Fig. 2). In the July 2006 GOM samples, the net DOC increase over the 5 h following sunrise was $16 \pm 1 \mu\text{M C}$, a 15% increase over its sunrise value, and declined rapidly thereafter. This net increase was concurrent with an increase in light intensity of $853 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. No significant change in DOC was observed in the dark treatments (data not shown). Similar trends were observed in March 2007 GOM samples when an increase in DOC concentration in the light bottles was observed in the first four hours following sunrise (Table 1, Fig. 2b). The net DOC

accumulation during this period was $13 \pm 6 \mu\text{M C}$, a 10% increase over the sunrise concentration. Again, the observed increase in DOC was concurrent with an increase in light intensity ($749 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, Fig. 2b). After this net increase, DOC concentrations fell back to their early morning values and remained relatively constant for the rest of the sampling period. As before, no changes in DOC were observed in the dark bottles.

In July 2006 GOM samples, a small increase in phytoplankton abundance was observed between 1 and 3 h after sunrise in the light bottles, while no significant changes were observed in the dark (data not shown). In the March 2007 GOM samples as well as in the August 2006 ENP samples, phytoplankton cell counts were highly variable and showed no significant changes during the hours following sunrise in either the light or dark treatments (data not shown). Phytoplankton species composition varied temporally and between sampling sites (Table 1). In the July 2006 GOM samples, significant increases in bacterial abundances were observed in the light treatments between 3 and 5 h after sunrise ($2.4 \times 10^9 \pm 0.38 \times 10^9 \text{ cells L}^{-1}$), and bacterial carbon production was high ($6.8 \mu\text{M C}$, Table 1). This pattern was not observed in the dark treatments (data not shown). In both the light and dark treatments in March 2007 GOM samples, bacterial abundance remained relatively stable throughout sampling with negligible net bacterial production (Table 1). In the August 2006 ENP light treatments, bacterial abundances increased sharply between 3 and 5 h after sunrise, and then remained relatively stable. Bacterial production was observed to be very high ($10.5 \mu\text{M C}$) in the light treatments between 5-9 h after sunrise (Table 1).

Tracer Experiment. During the course of the incubation, light intensity increased steadily, but with high variability between 2 and 7 hours after sunrise, rising to $\sim 2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$

(Fig. 3). Most of the total fixed ^{14}C was found in the phytoplankton biomass. The amount of ^{14}C appearing in the DOC fraction by the end of the day (% extracellular release, PER) was about 20%, while that appearing in the bacteria was 6-7% of the total (Fig. 3). The time courses of C assimilation into the phytoplankton, appearing in the DOC, and assimilated into the bacteria, however, were strongly influenced by irradiance (Fig. 3). As irradiance increased in the morning between 0900 and 1100, the percentage of ^{14}C fixed by phytoplankton actually decreased from 85 to 67% while the DOC fraction increased from 6 to 27% (Fig 3). During a cloudy period at mid-day, the opposite occurred. The phytoplankton fraction increased from 67 to 82%, and the DOC fraction declined from 27 to 12%. In response to the afternoon increase in light 1200-1300, the phytoplankton fraction declined and the DOC fraction increased in percentage, 82 to 73%, and 12 to 20%, respectively (Fig. 3). Bacterial incorporation increased steadily throughout the day, following the appearance of DO^{14}C , and remained a more or less constant percentage of total C assimilated. Bacterial uptake of DOC follows the trend of ER, but the hour-to hour variability is not always matched with the availability of substrate as represented by phytoplankton-derived DOC. Still, there is an overall correlation between the bacterial C assimilation and its availability as DO^{14}C . As observed in ^{14}C bicarbonate tracer studies, small but significant net increases ($\Delta\text{DOC} = 15 \pm 4 \mu\text{M C}$; data not shown) in bulk DOC concentrations were also seen during the course of the incubation and corresponded with dramatic shifts in light intensity. Similar to that seen both the GOM in situ incubations and the ^{14}C tracer studies, bulk DOC accumulation lagged rapid increases in light intensity by approximately 1 h. Collectively, these results show an increase in DOC extracellular release (ER) accompanying increases in irradiance.

4. Discussion

Our results can be summarized as follows. First, axenic cultures of *Thalassiosira weissflogii* release significant amounts of DOC in the first hours after exposure to a higher irradiance. DOC release by *T. weissflogii* in cultures shifted to higher light was 3.6 times greater than that observed over the same time period for cultures kept at their prior growth irradiance (Fig. 1a). Second, the DOC from ER in the *T. weissflogii* culture exposed to higher irradiance appears to be preferentially utilized by natural populations of bacteria, or at least utilized at a higher rate (Fig. 1b). Third, natural communities also exhibit short-term, diurnal, increases in DOC concentrations associated with rapid increases in solar irradiance. When no change in irradiance is observed, i.e., with overcast skies, there is no observed diurnal variability in DOC concentrations. Fourth, the results from the radiotracer experiment are consistent with the bulk DOC measurements from the in situ incubations. There is evidence of short-term increases in DO^{14}C in response to variability in irradiance, and subsequent utilization by bacteria. We now discuss these results in greater detail.

4.1 Light-mediated increases in DOC

The results from both the laboratory cultures and field observations agree well with what others have found in both laboratory (Mague et al., 1980) and field (Thomas, 1971; Panzenbock 2007) experiments in which increased amounts of DOC are released by phytoplankton following a change from lower to higher irradiance. Although the conditions and methodologies for our study and others are different, all three illustrate light-mediated ER of DOC by phytoplankton.

There are two competing models for ER. The ‘overflow’ model predicts active release of both low- and high-molecular weight (LMW and HMW, respectively) DOC when

photosynthesis outpaces cell growth. The ‘passive diffusion’ model (Bjornsen, 1988) predicts the release of LMW DOC as a result of steep concentration gradients created by heterotrophic uptake. Research exists to support both models (e.g., Wood and Van Valen, 1990; Mague et al., 1980) and it is likely that the two are not mutually exclusive. Rather, dynamic local conditions determine which model operates at any time (Carlson, 2002). Support for the overflow model came from studies under nutrient-limited conditions, that is, where there is an environmental stress. A light-mediated release of DOC under nutrient replete conditions could also act as an environmental driver, and in that sense supports the overflow model.

The rate of light intensity increase may also be a determining factor for DOC release. For the GOM incubations, the rate of light increase was greatest in the hour before the peak net DOC increase was observed (Figs. 2, 3). Furthermore, following the peak DOC release, concentrations returned to baseline levels. The decrease in DOC concentrations may be the result of the phytoplankton becoming physiologically acclimated to the light levels and releasing less DOC, as we demonstrated with axenic *T. weissflogii* cultures (Fig. 1a), or, additionally (see below) a balance between light mediated phytoplankton DOC production and bacterial uptake.

4.2 Bacterial utilization of DOC from ER

Studies have shown that phytoplankton-released dissolved compounds are labile, and quickly consumed by marine bacteria (Baines and Pace, 1991; Biddanda et al., 2001), although the results, regionally, can be variable (Morán et al., 2002). Our results from the laboratory experiments show that natural populations of bacteria can quickly consume recently released DOC (Fig. 1b). We conclude that the 15-20% transient increase followed by decline in in situ

DOC concentrations (Fig. 2b) results from light-mediated release by phytoplankton and consumption by bacteria.

The quality of excreted DOC helps determine its fate, and the response of the microbial community. For example, Judd et al. (2006) and Chauhan et al. (2009) have shown that bacterial production and community structure are affected by transient variations in DOC composition and lability and thus may have influenced the seasonal differences in bacterial response to light-mediated DOC variations that we observe. Two recent publications support these results. First, Teeling et al. (2013) describe the change in bacterial populations in response to phytoplankton-derived organic matter during and after a spring bloom. Second, Fouilland et al. (2013) find that the connection between bacteria and phytoplankton, via DOC, is enhanced under conditions of high nutrients, which was the case for all of our experiments.

The released DOC also affects bacterial production (BP). We found that BP for natural bacterioplankton incubated with DOC obtained from phytoplankton exposed to high light is significantly higher ($4.6 \mu\text{g C L}^{-1} \text{d}^{-1}$) than with DOC released by phytoplankton grown under lower light ($0.12 \mu\text{g C L}^{-1} \text{d}^{-1}$). The response found in the laboratory experiments (Fig. 1b) may explain shifts in BP over the course of the incubation observed in the July 2006 GOM study, where BP for the first 5 h after sunrise was two times higher than the afternoon (Table 1). Results from the present study suggest that whether or not light-enhanced DOC release is taken up into bacterial biomass or subsequently respired will be a function of environmental conditions, the taxonomic make up of the phytoplankton and bacterioplankton communities (Teeling et al., 2013), and the energetic status of the bacterial communities.

4.3 Tracer experiment of microbial interactions

Use of a radiocarbon tracer allowed us to track DOC production and bacterial uptake over time with greater sensitivity than net DOC concentration measurements alone. Significant DOC production was clearly observed in the ^{14}C tracer study, and appears to be strongly influenced by irradiance (Fig. 3). Similar to that observed in *in situ* GOM incubation studies, this release was followed by rapid bacterial uptake (Fig. 3). The fact that we did not see significant changes in phytoplankton abundances in any of the light treatments together with the anti-symmetrical behavior between the ^{14}C -labeled fractions (phytoplankton and DOC) (Fig. 3) implies that the cells are not lysing but rather responding to changes in light intensity. (Similarly, in the laboratory culture, cell lysis cannot explain the changes in DOC observed.)

Although the data are limited, the results from both the *in situ* and ^{14}C tracer incubations (i.e. Figs. 2, 3) can be combined by plotting the hourly change in irradiance against the hourly change in DOC. Fig. 4 suggests a dependence of DOC release on (positive) irradiance change demonstrating that similar behavior to the change in irradiance is observed whether the results are from isotope-based assays (Fig. 3) or the less sensitive chemical analysis of bulk DOC (Fig. 2).

5. Concluding remarks

Our data indicate that release of DOC by phytoplankton populations as a function of incident irradiance is variable but can be significant. We observe a close coupling between light-mediated DOC production and bacterial consumption that at times resulted in enhanced bacterial production. These observations illustrate how transient bursts in primary production and DOC release may be captured in bacterial rather than phytoplankton biomass and supports the

contention by Williams et al. (2004) that heterotrophic consumption of DOC produced by discontinuous primary production dampens the ability to observe variations in production. Collectively these results may have important implications for estimates of ocean carbon flux and might also help to explain, at least in part, the perceived imbalance between autotrophic production and heterotrophic consumption processes in marine systems. As such, the relative impact of light-mediated DOC released by phytoplankton and the subsequent transient changes in bacterial production should be considered, better to parameterize models of coastal carbon flux.

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Figure Captions

Fig. 1. a. Change in DOC concentration for axenic *Thalassiosira weissflogii* cultures shifted to 330 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (●) and those kept at their growth irradiance (110 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (○) over the course of a 12 h photoperiod. Error bars represent ± 1 standard deviation of triplicate samples. Experimental incubations were carried out sequentially. b. Change in DOC concentration over a 24 h period for bacteria incubated in filtrate derived from the light shifted culture (●) and those kept at their growth irradiance (○). Error bars represent ± 1 standard deviation of triplicate samples.

Fig. 2. a. Solar irradiance and b. changes in DOC concentration) for in situ incubations in the Gulf of Mexico in March 2007 (▲) and July 2006 (◆), and in the Eastern North Pacific in August 2006 (■). Error bars represent ± 1 standard deviation (n=4).

Fig. 3. a. Solar irradiance and ^{14}C tracer assimilation, and b. changes in DOC concentration for FSU Marine Lab ^{14}C bicarbonate tracer experiments July 2010. Phytoplankton ($>1 \mu\text{m}$) C fixation (○) and bacterial (0.2-1 μm) C assimilation (△), and the appearance of labeled DOC (◆) over a daylong time course. C fixation and ER are $\mu\text{mols l}^{-1}$; PAR is $\mu\text{mols m}^{-2} \text{s}^{-1}$. Closed circles are total assimilation, and crosses are the summed bacterial, DOC, and phytoplankton assimilation. Error bars represent ± 1 standard deviation (n= 3).

Fig. 4 Change in DOC (from ^{14}C) against the hourly change in irradiance for the data in Figs. 1 (●). Also plotted are data (■) from chemical analyses of DOC (average of 6 replicates) in Fig. 2. ^{14}C -derived ER and DOC are $\mu\text{mols l}^{-1}$; PAR is $\mu\text{mols photons m}^{-2} \text{s}^{-1}$.

Table 1. Dominant phytoplankton community members and net changes in DOC concentrations and bacterial production (BP) following sunrise, and throughout the sampling period, for in situ incubation. Error represents ± 1 standard deviation (sd) (n=2). ud: undetectable

Date & Time	Δ [DOC] ($\mu\text{M C} \pm \text{sd}$)	BP ($\mu\text{M C}$)
GOM July 2006		
Most abundant phytoplankton species: <i>Gyrodinium</i> sp, <i>Scrippsiella trochoidea</i> , <i>Thalassiosira</i> sp, <i>Asterionellopsis glacialis</i> and <i>Cylindrotheca closterium</i>		
Sunrise - 5 h post sunrise	16 ± 1	6.8
5 - 9 h post sunrise	-15 ± 4	3.2
Full sampling period	3 ± 4	10.7
GOM March 2007		
Most abundant phytoplankton species: <i>Chaetoceros curvisetus</i> , <i>Chaetoceros similis</i> , <i>Skeletonema costatum</i> and <i>Thalassionema nitszchioides</i>		
Sunrise - 6 h post sunrise	13 ± 6	ud
6 - 9 h post sunrise	-10.7 ± 6	ud
Full sampling period	0.5 ± 6	ud
ENP August 2006		
Most abundant phytoplankton species: <i>Skeletonema costatum</i> , <i>Guinardia striata</i> and <i>Thalassiosira punctigera</i>		
Sunrise - 5 h post sunrise	4 ± 3	ud
5 - 9 h post sunrise	3 ± 5	10.5
Full sampling period	0.6 ± 4	3.2

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