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PHYSIOLOGICAL RESPONSE OF 10 PHYTOPLANKTON SPECIES EXPOSED TO MACONDO OIL AND THE DISPERSANT, COREXIT[1]

Laura Bretherton[2]

Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas 77553, USA

Alicia Williams

Department of Marine Sciences, University of New England, Biddeford, Maine 04005, USA

Jennifer Genzer, Jessica Hillhouse, Manoj Kamalanathan

Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas 77553, USA

Zoe V. Finkel

Environmental Science, Mount Allison University, Sackville, New Brunswick, Canada E4L 1E4

and Antonietta Quigg

Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas 77553, USA
Department of Oceanography, Texas A&M University, College Station, Texas 77843, USA

Culture experiments were conducted on ten phytoplankton species to examine their biological and physiological responses during exposure to oil and a combination of oil and dispersant. The species tested included a range of taxa typically found in the Gulf of Mexico such as cyanobacteria, chlorophytes, and diatoms. Cultures were exposed to Macondo surrogate oil using the water accommodated fraction (WAF), and dispersed oil using a chemically enhanced WAF (CEWAF) and diluted CEWAF, to replicate conditions following the Deepwater Horizon spill in the Gulf of Mexico. A range of responses were observed, that could broadly class the algae as either "robust" or "sensitive" to oil and/or dispersant exposure. Robust algae were identified as *Synechococcus elongatus*, *Dunaliella tertiolecta*, two pennate diatoms *Phaeodactylum tricornutum* and *Navicula* sp., and *Skeletonema grethae* CCMP775, and were largely unaffected by any of the treatments (no changes to growth rate or time spent in lag phase relative to controls). The rest of the phytoplankton, all centric diatoms, exhibited at least some combination of reduced growth rates or increased lag time in response to oil and/or dispersant exposure. Photophysiology did not have a strong treatment effect, with significant inhibition of photosynthetic efficiency (F_v/F_m) only observed in the CEWAF, if at all. We found that the effects of oil and dispersants on phytoplankton physiology were species-dependent, and not always detrimental. This has significant implications on how oil spills might impact phytoplankton community structure and bloom dynamics in the Gulf of Mexico, which in turn impacts higher trophic levels.

Key index words: **chlorophyll; dispersant; Gulf of Mexico; oil spills; photosynthesis; phytoplankton; water accommodated fraction**

Abbreviations: **CEWAF, chemically enhanced water accommodated fraction; DCEWAF, dilute chemically enhanced water accommodated fraction; DwH, Deepwater Horizon; EOE, estimated oil equivalence; GOM, Gulf of Mexico; PAH, polycyclic aromatic hydrocarbon; WAF, water accommodated fraction**

On April 20, 2010 an explosion on the Deepwater Horizon (DwH) oil rig resulted in the continuous release of over 4 million barrels of crude oil into the Gulf of Mexico (GOM; Crone and Tolstoy 2010, McNutt et al. 2012). Remediation strategies not only involved the application of dispersants at both the surface and wellhead 1,500 m below the surface but also introduced more oil into the water column (Kujawinski et al. 2011), making components such as the highly toxic polycyclic aromatic hydrocarbons (PAHs) more bioavailable (e.g., Wolfe et al. 1998, 2001). Dispersants can facilitate the degradation of hydrocarbons by marine bacteria (Lu et al. 2012), and many studies have focused on the impact of the DwH spill on deep-sea bacterial communities in the GOM (Hazen et al. 2010, Kostka et al. 2011, Baelum et al. 2012, Mason et al. 2014). Less focus has been given to the ecotoxicological effects of oil and dispersants on phytoplankton, despite their importance to the marine environment.

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[2]Author for correspondence: e-mail lbretherton@mta.ca.
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Oil exposure has been shown to negatively impact the growth (Østgaard et al. 1984) and motility (Garr et al. 2014) of some microalgae, and some studies have demonstrated that they can accumulate high levels of crude oil even when exposed to low concentrations (Lee 1975, Wolfe et al. 2001). However, in some cases oil has no observable effect (Harrison et al. 1986), or even a stimulatory effect (Ozhan et al. 2014a) on phytoplankton growth. Rapid recovery from acute exposure has been observed, sometimes within 24 h (Lee 1975, Pérez et al. 2010), and some cyanobacteria can even store crude oil within the interthylakoid spaces (Al-Hasan et al. 2001). Community-level studies show that these responses are almost certainly taxa specific (González et al. 2009, Adekunle et al. 2010, Gilde and Pinckney 2012), and are further confounded by differences in type, concentration and application of oil across studies.

A commonly used standard for assessing the toxicity of compounds in phytoplankton research is the LC_{50}, the concentration of a toxicant which reduces the growth rate by 50% (e.g., Hook and Osborn 2012, Garr et al. 2014). However, growth rates in phytoplankton are known to be driven by many different environmental factors (Sett et al. 2014, Feng et al. 2017) and even small changes in growth rates could alter community structure and function. LC_{50} values are often much higher than concentrations found in the environment following an oil spill (Sammarco et al. 2013), and arguably only offer a measure of relative sensitivity which makes the data difficult to relate back to a real-life scenarios.

Additionally, oil toxicity studies have only developed standardized methods of exposure in the last 20 years (Singer et al. 2000). Many studies prior to the 21st century either used a single compound, or added oil directly to the culture vessels. Since oil has many fractions, many of which are not water soluble, this method does not ensure exposure to toxicants (Girling et al. 1992). Hence, the LC_{50} values from such studies may be overestimating the concentrations at which the oil has a negative effect. Furthermore, it is difficult to be certain that the observed response is due to toxicant exposure, rather than indirect effects of oil slicks such as shading or limited gas exchange.

In this experiment, we prepared a water accommodated fraction (WAF) of oil which has the advantage of exposing phytoplankton to oil water-soluble components (i.e., those that are directly available). These can also be some of the most toxic components of the oil; for example, PAHs are known to have extremely detrimental effects on marine biota at short-term exposure (Neff and Stubblefield 1995) and can bioaccumulate in marine food webs (Meador et al. 1995). Rather than using a range of concentrations to find the LC_{50}, we used concentrations that would have been present in the GOM during the DwH spill (0.5–2.5 mg · L^{-1}; Sammarco et al. 2013) and observed the effects of these environmentally relevant conditions on the growth kinetics and photophysiology of ten different phytoplankton species/strains originating from the GOM. These species covered a range of taxa, from cyanobacteria and chlorophytes, to centric and pennate diatoms, which are all ecologically significant to the GOM (Strom and Strom 1996, Mulholland et al. 2006, Macintyre et al. 2011).

We prepared four treatments: (i) a WAF of the MC252 Louisiana crude oil (Macando surrogate oil) obtained from BP in 2015 (WAF), (ii) a dispersed oil or chemically enhanced WAF (CEWAF) prepared with a 20:1 oil:dispersant mixture, (iii) a diluted CEWAF treatment (DCEWAF) prepared by diluting the CEWAF, and (iv) a control (seawater). This information was intended to help make insightful choices into which phytoplankton species would be ideal candidates for further research.

METHODS

Cultivation of phytoplankton. A total of 10 phytoplankton strains were used for this study. Cultures of *Thalassiosira pseudonana* CCMP 1335, *Synechococcus elongatus* CCMP 1334, *Skeletonema grethae* CCMP 775, *S. grethae* CCMP 776, *Odontella mobiliensis* CCMP 597, and *Lithodesmium undulatum* CCMP 472 were obtained from the National Center for Marine Algae (NCMA), while *Dunaliella tertiolecta* UTEX LB999, *Phaeodactylum tricornutum* UTEX 646, *Navicula* sp. UTEX BSP11, and *Skeletonema costatum* UTEX LB2308 were obtained from the Culture Collection of Algae at The University of Texas at Austin (UTEX). Species and genera common to the GOM or, ideally, isolated from the GOM were preferentially chosen for investigation (see Table 1).

Phytoplankton were grown in sterilized natural seawater enriched with f/2+ Si medium (Guillard and Ryther 1962, Guillard 1975). Natural seawater was acquired from the GOM (off Galveston Island, TX) and treated with activated charcoal to remove particulates. The seawater media was further sterilized by filtering through a 0.45 μm polycarbonate filter cartridge before adding f/2 nutrients, trace metals and vitamins. All cultures were grown in triplicate 1 L glass bottles at 19°C ± 1°C under a 12:12 h light:dark (L:D) cycle and light intensity of 100–130 μmol · m^{-2} · s^{-1}.

Preparation of treatments. The preparation of WAF was carried out as described by the CROSERF method (Singer et al. 2000) with some modifications to prepare CEWAF and DCEWAF. Preprepared f/2 media (see above) was transferred to 1 L glass aspirator bottles with bottom spigots and glass stoppers. To prepare WAF, 400 μL of MC252 Louisiana crude oil was added per 1 L aspirator, and CEWAF was prepared by premixing the dispersant Corexit with oil in a 1:20 ratio, and adding 400 μL of that mixture per 1 L aspirator. The aspirators were then stirred to create a vortex that occupied the upper ~25% of the bottle, covered and left to mix at room temperature for 24 h. This allows the water-soluble fraction of oil to dissolve into the seawater media, and breaks up the nonsoluble fractions into small particles. After 24 h, each 1 L aspirator was decanted into a larger 9 L aspirator to form the stock solution for each treatment. During this process, the media was sieved with a 20 μm nylon mesh to remove large particles, and the surface slick in each 1 L aspirator was not allowed to pass through the spigot.

The DCEWAF was prepared from the CEWAF stock solution. A 5 mL aliquot was taken from each stock and the

TABLE 1. Details of species and strains examined in the study.

Species	Strain	Described by	Origin of Isolation
Cyanobacteria			
Synechococcus elongatus	CCMP 1334	Nageli	33.74° N, 67.49° W N Atlantic
Green algae			
Dunaliella tertiolecta	UTEX 999	Butcher	Oslofjord, Norway
Pennate diatoms			
Phaeodactylum tricornutum	UTEX 646	Bohlin	Segelskär, Finland
Navicula sp.	UTEX SP11	Bory	36.44° N, 98.15° W Oklahoma, USA
Centric diatoms			
Thalassiosira pseudonana	CCMP 1335	Hasle et Heimdal	40.75° N, 72.82° W New York, USA
Skeletonema grethae	CCMP 776	Zingone et Sarno	28.95° N, 95.36° W Gulf of Mexico
S. grethae	CCMP 775	Zingone et Sarno	28.90° N, 89.48° W Gulf of Mexico
Odontella mobiliensis	CCMP 597	(Bailey) Grunow	28.62° N, 89.75° W Gulf of Mexico
Lithodesmium undulatum	CCMP 472	Ehrenberg	28.62° N, 89.75° W Gulf of Mexico
Skeletonema costatum	UTEX 2308	(Greville) Cleve	Galveston, TX Gulf of Mexico

estimated oil equivalent (EOE; see below) measured spectrofluorometrically. A volume of the CEWAF stock solution was then diluted with f/2 media until the EOE matched that of the WAF stock. This diluted media became the DCEWAF stock solution.

The WAF, CEWAF, and DCEWAF (700 mL) stock solutions were decanted into 1 L glass Duran bottles, and inoculated with 150 mL of phytoplankton culture. Control cultures were prepared by adding 700 mL of fresh f/2 media and 150 mL of phytoplankton culture to 1 L glass bottles. In addition, 500 mL each of WAF, CEWAF, and DCEWAF were decanted into a 1 L bottle and kept in the same conditions to form non-biological controls. All experimental bottles were sampled every 2 d for 14 d.

Estimated oil equivalents. To determine EOE ($mg \cdot L^{-1}$), 10 mL aliquots were taken from each experimental bottle, as well as initial stock solutions of WAF, CEWAF, and DCEWAF, and were extracted into 10 mL dichloromethane (DCM) in 20 mL scintillation vials. Use of DCM allows for detection of oil as low as 0.7 $\mu g \cdot L^{-1}$ (Wade et al. 2011). Approximately 3 mL of the DCM fraction was transferred into a quartz cuvette, and the maximum intensity was measured at an excitation wavelength of 322 nm and an emission wavelength of 376 nm in a Shimadzu spectrofluorophotometer (RF-5301PC; Shimadzu, Houston, TX, USA) corresponding to the MI from the MC252 Louisiana crude oil described in Wade et al. (2011). A calibration curve was made using dilutions of crude oil in DCM in order to calculate the EOE in each sample. This method is able to measure hydrophilic hydrocarbons, and does not capture fractions such as *n*-alkanes.

The EOE was calculated according to the following calibration curve equations:

$$\text{EOE} = 0.0104 \times (\text{Spectrofluorometer Fluorescence Intensity}) + 0.209 \quad (1)$$

$$\text{EOE} = 0.0179 \times (\text{Spectrofluorometer Fluorescence Intensity}) \quad (2)$$

Equation 1 was used for *Thalassiosira pseudonana*, *Synechococcus elongatus*, *Skeletonema grethae* CCMP775 and CCMP776, *Dunaliella tertiolecta*, *Odontella mobiliensis*, and *Phaeodactylum tricornutum*, while Equation 2 was used for *Navicula* sp., *Skeletonema costatum*, and *Lithodesmium undulatum*.

Chlorophyll a *measurements.* Algal growth was monitored using a benchtop fluorometer (10AU Fluorometer, Turner Designs, San Jose, CA, USA). A 4 mL sample was collected every 2 d for a period of 14 d from each of the treatments and immediately dark-acclimated for a period of 15 min. Chlorophyll fluorescence intensity was measured on each culture sample and was used to calculate the chlorophyll *a* concentration ($\mu g \cdot L^{-1}$). A fresh aliquot of f/2 medium, as well as from the WAF, CEWAF, and DCEWAF nonbiological controls were used to correct for interference from background fluorescence (Cullen and David 2003). A chlorophyll standard extracted from the alga *Anacystis nidulans* (Sigma-Aldrich, St Louis, MO, USA) and was used to prepare a standard curve according to the Environmental Protection Agency Method 445.0 (Arar and Collins 1997). Cell counts were taken alongside fluorescence measurements for *Navicula* sp. and *S. costatum* in order to establish the relationship between cell density and chlorophyll. When plotted against each other, the adjusted R^2 values were 0.84 and 0.75, respectively (data not shown).

Calculation of growth rates and lag periods. Chlorophyll-specific growth rates ($\mu \cdot d^{-1}$) were calculated using the following equation:

$$\mu = \frac{\ln C_t - \ln C_0}{t} \quad (3)$$

where μ is the average specific growth rate between time 0 and time t (d^{-1}), C_t is the chlorophyll concentration at time t, C_0 is the chlorophyll concentration at the start of the experiment, and t is the time considered (days).

Lag time (λ, d) was calculated by log transforming the chlorophyll concentrations and plotting them over time using the online application GeoGebra (https://www.geogebra.org/). Two lines were then plotted, one where $y = C_0$, and

one through the points in exponential phase. The intercept of these two points was used as an estimate of λ.

Fluorescence induction and relaxation parameters. The fluorescence induction and relaxation (FIRe) fluorometer system (Satlantic, Halifax NS, Canada) was used to measure the FIRe parameters from the single turnover (ST) component (Kolber et al. 1998, Kromkamp and Forster 2003). A 4 mL aliquot was taken from each experimental bottle every 2 d and dark-acclimated for 15 min before being placed in the FIRe fluorometer to measure minimum and maximum fluorescence values ($F_{o(ST)}$ and $F_{m(ST)}$, respectively). Variable fluorescence (F_v) was calculated as the difference between these two [$F_{o(ST)} - F_{m(ST)}$] after correction for the sample blank (prepared as described above for the chlorophyll fluorescence measurements). F_v/F_m (maximum quantum yield of photosystem II (PSII) photochemistry), σ_{PSII} (functional absorption cross-section of PSII, Å^2 per quanta), ρ (connectivity factor defining the excitation energy transfer between individual photosynthetic units), and T (time constant of the relaxation kinetics of the fluorescence yield following the ST flash, μs) were used from mid-exponential growth where applicable.

Comparison of growth and photophysiology. Because treatments like the CEWAF resulted in no detectable growth in several of the species tested, it was often difficult to compare treatment effects. Therefore, the area under the curve of both chlorophyll *a* and F_v/F_m plotted over time was calculated using the trapezoid method:

$$A = \Delta t\left[\left(\frac{N_0 + N_1}{2}\right) + \left(\frac{N_1 + N_2}{2}\right)\dots + \left(\frac{N_t + N_{t+1}}{2}\right)\right]$$

where A is the area under the curve, t is time in days, and N is either chlorophyll *a* concentration or F_v/F_m. This calculated area under the curve was considered "total biomass accumulation" for the chlorophyll *a* data, and "overall photosynthetic performance" for the F_v/F_m data. The percentage difference in total biomass accumulation and overall photosynthetic performance was calculated for each treatment relative to the control values, for each species.

Statistical analysis. Data represent means ± 1 SE as each experiment was performed in triplicate. An analysis of variance (ANOVA) test was used to determine statistical significance between treatments, within species, for growth rate, lag period and the photosynthetic parameters displayed in Table 4. $P < 0.05$ were used as standard for statistical significance.

RESULTS

EOE changes over time. The EOE declined over time in all treatments (see Table 2), with the biggest declines observed in the CEWAF cultures. The EOE values in this treatment decreased by 89%–99% after 14 d, while on average the WAF and DCEWAF values fell by 62% and 65%, respectively. CEWAF treatments always had the highest starting EOE values, while WAF and DCEWAF were typically similar to each other, but not always.

Chlorophyll a *and growth kinetics.* Chlorophyll *a* evolution over time varied between species (Fig. 1), with the highest chlorophyll concentrations observed in *Phaeodactylum tricornutum*, and the lowest in *Lithodesmium undulatum*. In several species, chlorophyll *a* concentrations fell below detection limits in the CEWAF treatment after several days, and in *L. undulatum* and *Skeletonema costatum*, there was no detectable chlorophyll *a* in this treatment for the entire experiment.

Lag phase duration (Table 3) was most affected in the CEWAF treatment (relative to Control treatment), with two species spending significantly longer in this phase (*Synechococcus elongatus* and *Navicula* sp.; one-way ANOVA, $P < 0.05$, $F_{3,8} = 6.303$ and $F_{3,8} = 25.93$, respectively), while in another five species (all centric diatoms except for *Skeletonema grethae* CCMP776), the lag period could not be calculated because they either did not achieve exponential growth during the experiment, or produced no detectable amounts of chlorophyll. The DCEWAF treatment also resulted in significantly longer lag periods in some species (one-way ANOVA, $P < 0.05$), doubling the lag period in *Thalassiosira pseudonana* ($F_{2,6} = 7.11$) and *Lithodesmium undulatum* ($F_{2,6} = 9.131$), and tripling that of *Skeletonema costatum* ($F_{2,5} = 5.882$) and *S. grethae* CCMP776 ($F_{2,6} = 21.86$). While WAF exposure did cause increased lag periods in several species, this difference was only significant in *S. grethae* CCMP776. *Dunaliella tertiolecta*, *Phaeodactylum tricornutum*, and *S. grethae* CCMP775 showed no significant changes in lag time in any treatment.

Chlorophyll-specific growth rates (Table 3) were undetectable in five species; in *Thalassiosira pseudonana* and *Odontella mobiliensis* growth rates were otherwise not significantly different from the Control in the other treatments, in *Skeletonema grethae* CCMP776, *Lithodesmium undulatum*, and *Skeletonema costatum* growth rates were significantly lower than the Controls in all treatments as well (one-way ANOVA, $P < 0.05$, $F_{2,6} = 139.1$, $F_{2,5} = 15$, and $F_{2,5} = 286.2$, respectively). *Synechococcus elongatus* also exhibited lower growth rates in all treatments compared to the control, but this difference was not significant in the DCEWAF treatment. The two pennate diatoms, *P. tricornutum* and *Navicula* sp., showed a significantly lower growth rate in the CEWAF treatment only (one-way ANOVA, $P < 0.05$, $F_{3,8} = 5.651$, and $F_{3,8} = 23.27$), while growth rates of *D. tertiolecta* and *S. grethae* CCMP775 were not affected in any treatment.

Photosynthetic efficiency and photophysiology. Photosynthetic efficiency (F_v/F_m) varied with species and treatment (Fig. 3). *Thalassiosira pseudonana*, *Skeletonema grethae* CCMP776, *Odontella mobiliensis*, *Lithodesmium undulatum*, and *Skeletonema costatum* all had F_v/F_m values below detection limit in the CEWAF treatment. In *Dunaliella tertiolecta*, *Phaeodactylum tricornutum*, and *Skeletonema grethae* CCMP775, photosynthetic efficiency was very stable over time in all treatments. In *Navicula* sp., F_v/F_m in the CEWAF treatment was initially severely inhibited (<0.1), but recovered to values similar to the other treatments by day 10. *Synechococcus elongatus* showed variable F_v/F_m over time, ranging between 0.1 and 0.4 in all treatments.

TABLE 2. Initial EOE concentrations ($mg \cdot L^{-1}$) for each culture and each treatment, its decline over the time course of the growth experiments (Δ_{EOE}; $mg \cdot L^{-1} \cdot d^{-1}$) and the corresponding decline of EOE in the controls (f/2 medium, no phytoplankton added). Water accommodated fraction of oil (WAF), chemically enhanced WAF (CEWAF), and a diluted CEWAF (DCEWAF) were tested daily. Values in brackets represent standard error (n = 3). N/D indicates values that could not be measured/calculated.

	WAF		CEWAF		DCEWAF	
Species	$EOE_{initial}$	Δ EOE	$EOE_{initial}$	Δ EOE	$EOE_{initial}$	Δ EOE
S. elongatus	0.99 (0.008)	−0.62 (0.04)	52.12 (2.94)	−50.19 (1.76)	1.17 (0.13)	−0.73 (0.07)
D. tertiolecta	0.99 (0.008)	−0.67 (0.03)	52.12 (2.94)	−48.81 (1.88)	1.17 (0.13)	−0.81 (0.07)
P. tricornutum	0.64 (0.05)	−0.34 (0.03)	212.66 (21.60)	−210.10 (12.72)	0.70 (0.05)	−0.44 (0.04)
Navicula sp.	0.11 (0.04)	−0.23 (0.05)	62.56 (4.61)	−60.14 (2.67)	4.46 (0.14)	−4.62 (0.11)
T. pseudonana	0.99 (0.008)	−0.66 (0.04)	52.12 (2.94)	−49.32 (2.64)	1.17 (0.13)	−0.80 (0.08)
S. grethae CCMP775	0.64 (0.05)	−0.35 (0.04)	212.66 (21.60)	−211.48 (12.68)	0.70 (0.05)	−0.47 (0.02)
S. grethae CCMP776	0.99 (0.008)	−0.61 (0.03)	52.12 (2.94)	−49.35 (1.71)	1.17 (0.13)	−0.77 (0.06)
O. mobiliensis	0.64 (0.05)	−0.31 (0.06)	212.66 (21.60)	−211.13 (12.66)	0.70 (0.05)	−0.45 (0.03)
L. undulatum	0.47 (0.05)	−0.41 (0.04)	16.00 (1.38)	−14.24 (2.04)	0.77 (0.09)	−0.72 (0.06)
S. costatum	0.11 (0.04)	−0.34 (0.03)	62.56 (4.61)	−58.29 (2.40)	4.46 (0.14)	−4.51 (0.04)

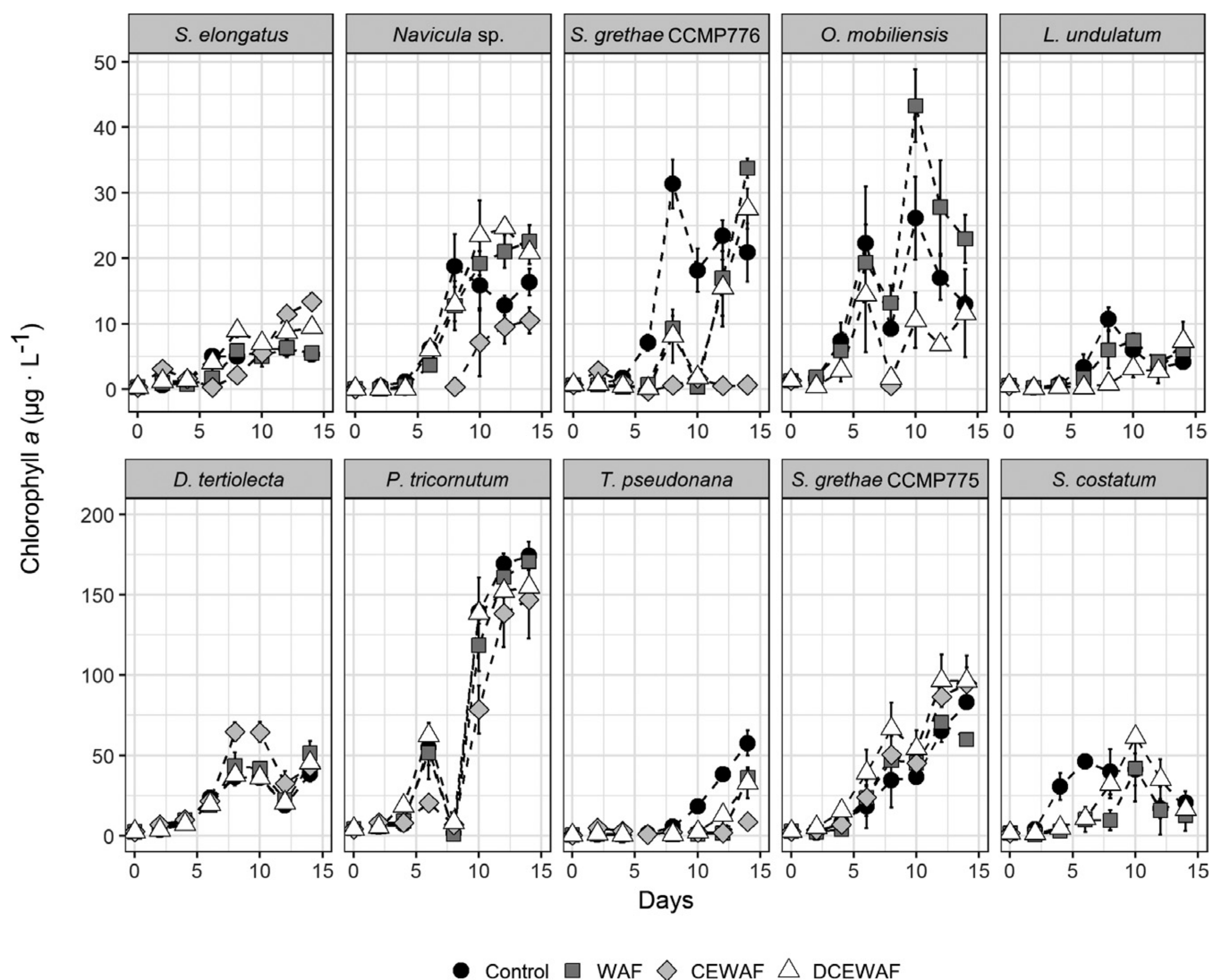

FIG. 1. Chlorophyll *a* concentrations ($\mu g \cdot L^{-1}$) over 14 d in 10 phytoplankton species grown in Control, WAF, CEWAF, and DCEWAF. Values below zero (i.e., below detection) have been omitted. Error bars represent 1 standard error (n = 3).

Other photophysiological parameters did not show any trends with treatment, and were more species dependent (see Table 4). The lowest σ_{PSII} values and highest τ values were observed in *Synechococcus elongatus* (82.33–89.67 $\AA^2$ per quanta and 457–665.33 μs, respectively). For all other species, σ_{PSII} typically ranged between 200 and 350, with τ ranging higher from 236 to 460. PSII connectivity factors (ρ) were also more dependent on species than treatment. The highest values (>0.30) were observed in *Synechococcus elongatus*, *Dunaliella tertiolecta,* and *Lithodesmium undulatum.* These parameters were not able to be calculated by the FIRe fluorometer in the CEWAF treatment for some species.

Comparison of area under the curve. A summary of the percent change in total biomass accumulation and overall photosynthetic performance (area under the curve) is displayed in Figure 2. The total biomass accumulation was inhibited in all treatments relative to the control in *Thalassiosira pseudonana, Skeletonema grethae* CCMP776, *Lithodesmium undulatum,* and *Skeletonema costatum.* In *Odontella mobiliensis,* total biomass accumulation was also inhibited in both CEWAF and DCEWAF treatments, but increased in the WAF treatment. The two pennate diatoms, *Phaeodactylum tricornutum* and *Navicula* sp., only showed an effect on biomass accumulation in the CEWAF treatment. *Synechococcus elongatus* biomass increased in both CEWAF and DCEWAF, while *D. tertiolecta* and *S. grethae* CCMP775 showed an increase in biomass in all treatments relative to Control.

Overall photosynthetic performance was largely unaffected by treatment in any species. Significant inhibition of photosynthetic performance was observed only in the CEWAF treatment in *Thalassiosira pseudonana, Skeletonema grethae* CCMP776, *Lithodesmium undulatum, Odontella mobiliensis* and *Skeletonema costatum.*

DISCUSSION

Overall, we found three distinct responses across the ten species tested, summarized in Figure 3:

1 *Synechococcus elongatus, Dunaliella tertiolecta, Phaeodactylum tricornutum, Navicula* sp., *Skeletonema grethae* CCMP775—are resistant to the oil and oil and dispersant treatments in this study and therefore we refer to them as “robust”; their time spent in lag phase and their chlorophyll-specific growth rates were either unaffected, or only negatively impacted in the CEWAF treatment (apart from *S. elongatus,* where a decrease in growth rate was also observed in the WAF treatment), and biomass accumulation either tended to increase or not change in any treatment (apart from *Navicula* sp., where a decrease in biomass was observed

Table 3. Time spent in lag phase (λ, d), chlorophyll-specific growth rates (μ, d^{-1}), and maximum chlorophyll *a* measured (Chl_{max}, $\mu g \cdot L^{-1}$) for 10 phytoplankton species grown in f/2 medium (control), water accommodated fraction of oil (WAF), chemically enhanced WAF (CEWAF), and a diluted CEWAF (DCEWAF). Values in brackets represent standard error (n = 3). N/A indicates values that could not be measured/calculated.

	Control			WAF			CEWAF			DCEWAF		
Species	λ	μ	Chl_{max}	λ	μ	Chl_{max}	λ	μ	Chl_{max}	λ	μ	Chl_{max}
S. elongatus	1.24 (0.27)	0.46 (0.012)	68.52 (7.35)	2.73 (0.46)	0.28 (0.011)	70.10 (15.73)	5.58 (0.77)	0.24 (0.007)	145.67 (13.86)	2.07 (1.17)	0.36 (0.020)	103.20 (5.48)
D. tertiolecta	1.29 (0.66)	0.33 (0.017)	414.90 (53.00)	0.61 (0.33)	0.35 (0.023)	558.67 (81.77)	0.14 (0.13)	0.40 (0.011)	703.33 (62.78)	1.02 (0.44)	0.33 (0.010)	494.00 (35.23)
P. tricornutum	3.72 (0.87)	0.33 (0.017)	1,874.76 (94.32)	2.40 (0.72)	0.29 (0.007)	1,837.64 (14.12)	3.08 (0.63)	0.24 (0.007)	1,582.47 (260.66)	2.03 (0.85)	0.33 (0.005)	1,666.51 (62.37)
Navicula sp.	1.33 (0.58)	0.52 (0.035)	203.88 (52.70)	3.49 (0.42)	0.43 (0.012)	244.76 (26.39)	8.91 (0.73)	0.27 (0.014)	115.03 (21.70)	2.97 (0.77)	0.45 (0.019)	267.43 (9.59)
T. pseudonana	4.24 2.11)	0.36 (0.007)	621.56 (82.67)	7.64 (1.38)	0.30 (0.011)	389.07 (56.95)	N/A (–)	N/A (–)	94.53 (12.54)	8.86 (0.70)	0.30 (0.034)	354.80 (103.36)
S. grethae CCMP775	4.45 (2.05)	0.24 (0.011)	896.56 (13.27)	3.97 (0.49)	0.28 (0.004)	760.68 (24.34)	2.55 (1.28)	0.24 (0.007)	1,020.13 (109.64)	1.37 (0.88)	0.28 (0.019)	1,038.80 (175.41)
S. grethae CCMP776	2.87 (0.47)	0.44 (0.015)	338.81 (54.93)	8.61 (0.82)	0.26 (0.006)	369.67 (15.76)	N/A (–)	N/A (–)	26.00 (1.43)	8.49 (0.80)	0.24 (0.002)	303.00 (32.23)
O. mobiliensis	2.11 (0.84)	0.27 (0.019)	282.64 (68.91)	2.2 (0.50)	0.29 (0.010)	466.89 (59.35)	N/A (–)	N/A (–)	7.82 (0.90)	5.04 (2.23)	0.27 (0.067)	156.88 (94.20)
L. undulatum	3.63 (0.25)	0.32 (0.012)	117.41 (18.69)	4.49 (0.32)	0.23 (0.025)	81.77 (11.42)	N/A (–)	N/A (–)	N/A (–)	8.61 (1.47)	0.18 (0.012)	81.04 (3.00)
S. costatum	1.10 (0.44)	0.54 (0.008)	497.90 (47.68)	3.53 (1.34)	0.34 (0.008)	451.95 (223.54)	N/A (–)	N/A (–)	N/A (–)	3.67 (0.20)	0.35 (0.004)	662.24 (11.50)

in the CEWAF treatment). Overall photosynthetic performance was either unaffected or decreased in CEWAF treatment only.

2 *Thalassiosira pseudonana*, *S. grethae* CCMP776, *Lithodesmium undulatum*, *Skeletonema costatum*—are termed "sensitive" species; their lag times, growth rates, and total biomass accumulation were all negatively impacted in every treatment, with no detectable growth in the CEWAF treatment. Overall photosynthetic performance was either unaffected or decreased in CEWAF treatment only.

3 *Odontella mobiliensis*—the WAF treatment did not cause any changes to growth rate or time spent in lag phase, and resulted in an increase in biomass accumulation. However, in the DCEWAF treatment this species had a much longer lag time, and reduced biomass accumulation, and there was no detectable growth in the CEWAF treatment.

Cyanobacteria have shown resistance to oil exposure in previous studies, consistent with our findings for *Synechococcus elongatus*. In soil samples, *S. elongatus* persisted within the bacterial community after 15 d of exposure to diesel fuel (Bastida et al., 2010). Gilde and Pinckney (2012) conducted microcosm experiments on estuarine phytoplankton communities, and found that cyanobacteria were generally resistant to exposure to MC252 crude oil and Texas crude oil (10–100 $\mu L \cdot L^{-1}$), and even increased in relative abundance in some cases. Following the DwH incident, cyanobacteria were identified in some samples taken from surface sheens (Redmond and Valentine 2012), but overall did not contribute much at all to surface bacterial communities immediately following the spill (Yang et al. 2016). The population size of cyanobacteria in the GOM after the DwH incident increased by 39% following the spill (Parsons et al. 2015), and they can locally almost entirely dominate GOM surface communities (93%–94% relative abundance), but this only occurred in samples from sites with no detectable surface slicks (Liu and Liu 2013). Therefore, despite being able to tolerate oil, cyanobacteria are likely not important components of post-spill bacterial communities.

Dunaliella tertiolecta has not been extensively studied with regard to crude oil toxicity. Culture work by Dunstan et al. (1975) found that low concentrations (<100 $\mu g \cdot L^{-1}$) of several types of oil stimulated *D. tertiolecta* growth, and even up to 10 $mg \cdot L^{-1}$ had little to no effect on its growth relative to controls. Short-term exposure to petroleum and diesel oil can rapidly suppress growth and inhibit photosynthetic performance in *D. tertiolecta*, however, it can recover from long-term exposure (Romero-Lopez et al. 2012). These results are consistent with our findings. Generally, chlorophytes are successful in oil toxicity mesocosm experiments

TABLE 4. Photosynthetic efficiency (Fv/Fm; dimensionless), PSII antenna size (σPSII; Å^2 per quanta), PSII connectivity factor (ρ; dimensionless) and PSII re-oxidation time (μsec) measured via fast repetition rate fluorescence on 10 phytoplankton species in controls (f/2 media), water-accommodated fraction of oil (WAF), chemically enhanced WAF (CEWAF), and a diluted CEWAF (DCEWAF). Values are from mid-exponential phase, N/A indicates cultures where exponential growth was not observed.

	Control				WAF				CEWAF				DCEWAF			
Species	F_v/F_m	σ_{PSII}	ρ	τ	F_v/F_m	σ_{PSII}	ρ	τ	F_v/F_m	σ_{PSII}	ρ	τ	F_v/F_m	σ_{PSII}	ρ	τ
S. elongatus	0.388	85.00	0.33	457.00	0.340	89.67	0.35	610.00	0.359	82.33	0.24	494.00	0.422	83.33	0.21	665.33
D. tertiolecta	0.555	206.00	0.31	386.67	0.558	188.67	0.28	459.33	0.555	183.00	0.29	424.33	0.558	208.67	0.32	416.00
P. tricornutum	0.516	352.67	0.06	380.33	0.500	351.33	0.06	400.00	0.568	307.33	0.08	418.33	0.502	344.00	0.06	361.33
Navicula sp.	0.568	202.33	0.13	371.67	0.571	202.00	0.19	369.00	0.586	189.67	0.28	379.67	0.571	199.67	0.14	299.67
T. pseudonana	0.478	304.00	0.10	383.00	0.590	271.67	0.11	345.33	N/A	N/A	N/A	N/A	0.578	261.33	0.12	347.00
S. grethae CCMP 775	0.579	255.00	0.20	368.33	0.592	238.33	0.18	390.00	0.628	213.00	0.21	372.00	0.563	253.00	0.11	354.33
S. grethae CCMP 776	0.594	243.00	0.21	320.33	0.596	257.00	0.17	357.67	N/A	N/A	N/A	N/A	0.585	233.00	0.21	348.00
O. mobiliensis	0.536	246.67	0.20	352.00	0.479	290.67	0.11	327.00	N/A	N/A	N/A	N/A	0.519	256.33	0.20	281.33
L. undulatum	0.593	222.00	0.36	257.67	0.557	206.33	0.33	405.67	N/A	N/A	N/A	N/A	0.586	203.33	0.40	335.00
S. costatum	0.594	223.33	0.21	344.67	0.435	520.67	0.14	236.67	N/A	N/A	N/A	N/A	0.553	218.00	0.17	407.33

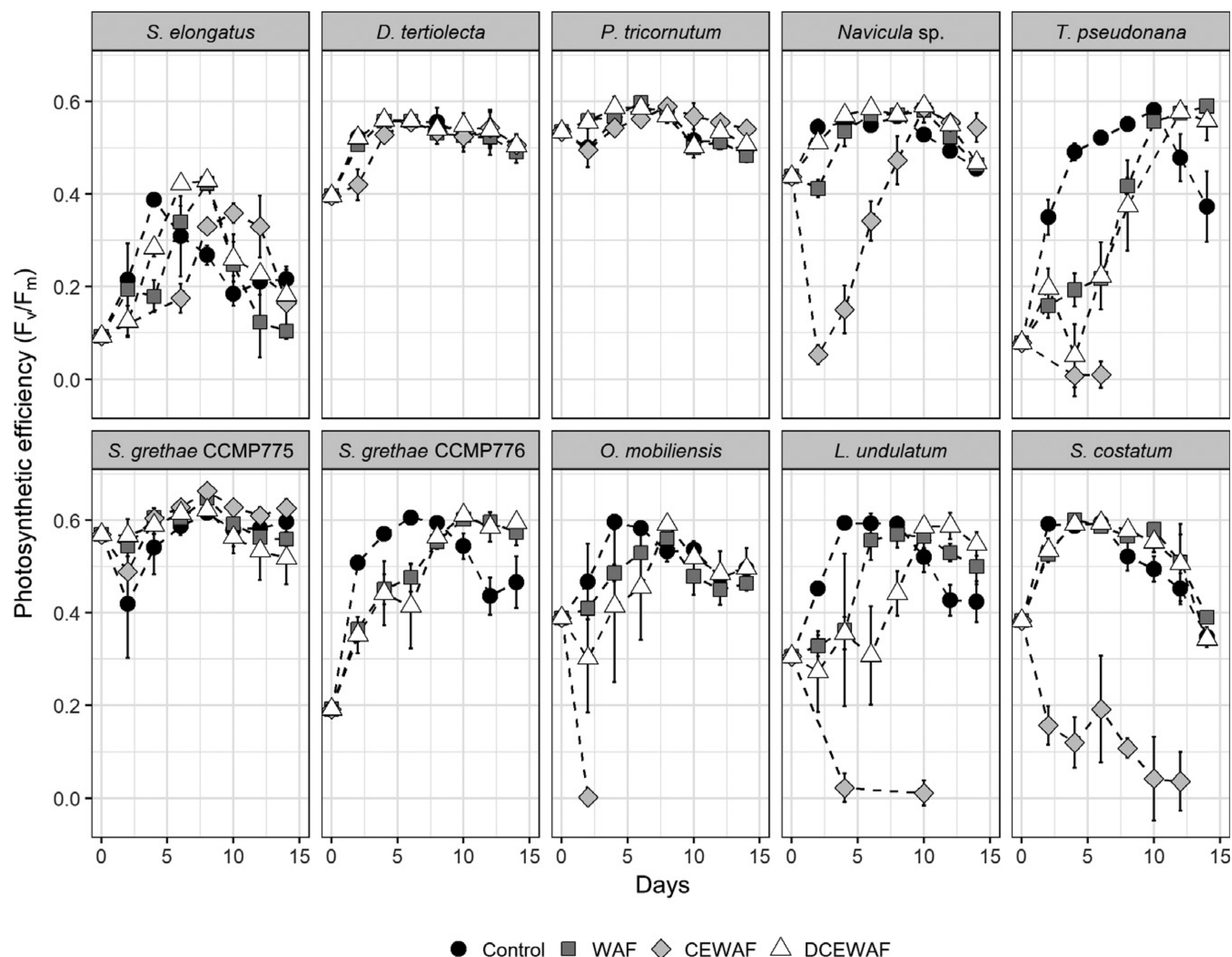

FIG. 2. Photosynthetic efficiency (F_v/F_m) over 14 d in 10 phytoplankton species grown in Control, WAF, CEWAF, and DCEWAF. Values below zero (i.e., no detectable photosynthesis) are omitted. Error bars represent 1 standard error ($n = 3$).

(Bott and Rogenmuser 1978, Gilde and Pinckney 2012), and have shown resilience to a variety of toxicants such as heavy metals (Baos et al. 2002, López-Rodas et al. 2008) and sulfur (Flores-Moya et al. 2005). However, it should be noted that not only was the baseline population of chlorophytes (pre-spill, 2010) in the Gulf of Mexico low (1.5%), they disappeared from the phytoplankton community entirely in 2010 following the DwH spill (Parsons et al. 2015). This implies that many chlorophytes are sensitive to oil and either do not survive to the end of studies on natural communities, or do not have a chance to recover within the time frame of these experiments. A wider range of species from this group needs to be studied.

Both pennate diatoms (*Phaeodactylum tricornutum* and *Navicula* sp.) exhibited little response to the WAF and DCEWAF treatments, only showing signs of growth inhibition in the CEWAF treatment, which was the most toxic treatment. This is consistent with previous studies. *Phaeodactylum tricornutum* has been found to be much more sensitive to dispersants than to WAF, and more sensitive still to CEWAF, with both dispersants and CEWAF causing membrane damage (Hook and Osborn 2012). The observed toxicity was not dependent on PAH concentrations or total petroleum hydrocarbon, suggesting that a specific but unknown component of the oil was causing toxicity to the diatom (Hook and Osborn 2012). *Phaeodactylum tricornutum* has shown sensitivity to a range of dispersants, but it is concentration dependent (Rial et al. 2013).

All centric diatoms studied showed high sensitivity to all treatments, except for *Skeletonema grethae* CCMP775. Centric diatoms in culture have shown a varied response to oil. *Chaetoceros teniussimus*, for example, could maintain active growth in WAF up to concentrations of $1.18\ mg \cdot L^{-1}$ (Deasi et al. 2010), and a *Chaetoceros* sp. did not show significant inhibition of growth in a WAF made of MC252 crude oil, but did in a CEWAF treatment (Garr et al. 2014). Work on *Skeletonema costatum* has suggested this species has a high sensitivity to oil

COLOR

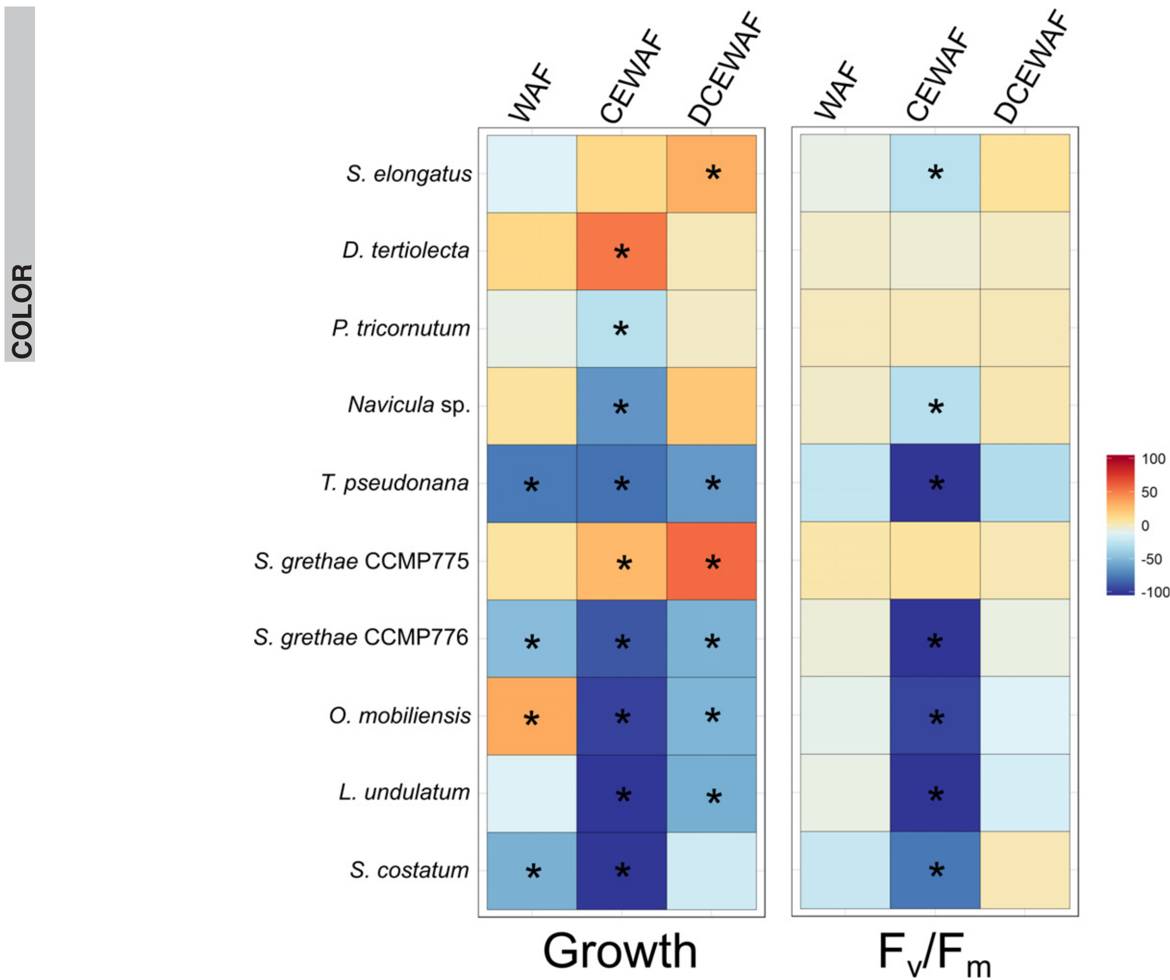

Fig. 3. Heatmap showing the percent change in growth (total biomass accumulation) and F_v/F_m (overall photosynthetic performance) over 14 d in 10 phytoplankton species exposed to WAF), CEWAF, and DCEWAF, relative to Control. Asterisks (*) indicate treatments where values were significantly different from control values (one-way ANOVA).

exposure (Østgaard et al. 1984, Deasi et al. 2010, Chao et al. 2012), although some studies have found it can tolerate certain types of oil (Dunstan et al. 1975, Morales-Loo and Goutx 1990). More generally, diatoms are relatively successful in mesocosm experiments treated with oil, with genera like *Chaetoceros* (González et al. 2009, Ozhan and Bargu 2014), *Skeletonema* (Jung et al. 2012), and pennate diatoms (Ozhan and Bargu 2014) often becoming the dominant algae by the end of the study. However, in this study we have shown that the response to oil and dispersants can vary within a single genus (*Skeletonema*), so the response of diatoms in mesocosm experiments is likely dependent on starting community.

Interestingly, the two strains of *Skeletonema grethae* (CCMP775 and CCMP776) had contrasting physiological responses to oil and oil and dispersant. While *S. grethae* CCMP775 was one of the most tolerant of the phytoplankton strains tested, *S. grethae* CCMP776 was by far one of the most sensitive. To our knowledge, this is the first study to demonstrate intraspecific differences in oil and dispersant tolerance. Different ecotypes of globally distributed phytoplankton have been shown to respond different to environmental changes, such as CO_2 availability (Langer et al. 2009, Müller et al. 2015), and can have significantly different physiologies (Saravanan and Godhe 2010), so this result is not surprising. These intraspecific differences demonstrate that different populations within the same species have the capacity to respond to oil in very different ways, which means that depending on where a spill happens, how an individual species fares can vary. For example, *S. grethae* CCMP775 was isolated from an offshore location, while *S. grethae* CCMP776 was isolated closer to the coast. While it is not possible to discern why these two populations might behave so differently from each other, it underscores the genetic plasticity that can be found within algae species.

Species identified as sensitive above all exhibited severe inhibition in their ability to accumulate

biomass over the 14 d period in every treatment (Fig. 3). However, it is important to note that decreases in growth rate across all treatments were only observed in *Thalassiosira pseudonana*, *Skeletonema grethae* CCMP776, and *Skeletonema costatum*. In some species, growth rates remained unchanged, and the increase in the duration of the lag phase was the cause of lower biomass. This suggests that growth rate is not necessarily always an effective tool at identifying sensitive species. There are already data to suggest that the phytoplankton community response is different depending on time of year (González et al. 2009, 2013), but growth phase (i.e., cells in lag phase vs. in exponential phase) could perhaps be an important factor as well. Delays in lag time could, for example, result in delays in bloom onset, which can have potential effects on the higher trophic levels (Townsend et al. 1994, Wiltshire and Manly 2004). Based on some of the species we tested, if an oil spill were to occur just before the onset of the annual spring bloom it could potentially delay the bloom by up to a week. The reason species have longer lag phases than others may be due to differences in how individual species allocate resources, but is beyond the scope of this work.

It is interesting to note that F_v/F_m did not vary in the WAF or even DCEWAF treatments; significant decreases in F_v/F_m were only observed in CEWAF treatments—if observed at all (Fig. 3). Hydrocarbons have been shown to cause short-term inhibition of F_v/F_m (Carrera-Martínez et al. 2010, Gorbunov and Falkowski 2011, Romero-Lopez et al. 2012), although in longer term mesocosm experiments, F_v/F_m either recovers after an initial decline (González et al. 2009) or is not affected by oil exposure (González et al. 2013). Since we only observed a significant decline in F_v/F_m in the CEWAF treatment, effects on photosynthesis are likely concentration dependent, and appear to have a high threshold. Even when we observed significant inhibition of growth, F_v/F_m did not always change, which means it may also not be an effective tool for assessing oil and dispersant sensitivity in phytoplankton. Ultimately, the way in which stress or sensitivity manifests appears to be species-specific, thus a more inclusive method of assessment needs to be adopted in phytoplankton studies.

The variety of physiological responses observed in this study support the idea that phytoplankton community composition is affected by oil and dispersant exposure (e.g., González et al. 2009, Adekunle et al. 2010, Jung et al. 2012, Ozhan and Bargu 2014). *Synechococcus elongatus*, *Dunaliella tertiolecta*, and the two pennate diatoms *Phaeodactylum tricornutum* and *Navicula* sp. were all very robust to WAF and DCEWAF treatments, consistent with other findings for cyanobacteria, chlorophytes, and pennate diatoms, while the majority of the centric diatoms were sensitive to WAF and DCEWAF treatments. However, as the intraspecific differences found within *Skeletonema grethae* demonstrate, making general statements about how a taxonomic group—or even species—responds to oil and dispersants should be avoided. Importantly, phytoplankton community composition during a bloom can have effects on higher trophic levels, and diatom composition in particular can cause reproductive failure in many species of copepod (Irigoien et al. 2002, Ianora et al. 2004), which, in turn, are very selective with their prey items (Leising et al. 2005). The structure and function of pelagic marine food webs is critically dependent on the energy and matter supplied by phytoplankton photosynthesis. Hence, understanding how hydrocarbons affect different phytoplankton species is an important issue.

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