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Trait-dependent variability of the response of marine phytoplankton to oil and dispersant exposure

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ABSTRACT

The Deepwater Horizon oil spill released millions of barrels of crude oil into the Gulf of Mexico, and saw widespread use of the chemical dispersant Corexit. We assessed the role of traits, such as cell size, cell wall, motility, and mixotrophy on the growth and photosynthetic response of 15 phytoplankton taxa to oil and Corexit. We collected growth and photosynthetic data on five algal cultures. These responses could be separated into resistant (*Tetraselmis astigmatica*, *Ochromonas* sp., *Heterocapsa pygmaea*) and sensitive (*Micromonas pusilla*, *Prorocentrum minimum*). We combined this data with 10 species previously studied and found that cell size is most important in determining the biomass response to oil, whereas motility/mixotrophy is more important in the dispersed oil. Our analysis accounted for a third of the variance observed, so further work is needed to identify other factors that contribute to oil resistance.

1. Introduction

The 2010 Deepwater Horizon oil spill resulted in the release of over 4 million barrels of oil into the Gulf of Mexico (McNutt et al., 2012), making it the largest oil spill to date. As a means of remediation, large quantities of the chemical dispersant Corexit were applied to vast areas of the Gulf both on the surface and directly at the burst wellhead at 1500 m depth (Kujawinski et al., 2011). Chemical dispersants lower surface tension at the oil/water interface and allow for the formation of smaller oil droplets that can become accommodated into the water column (Quigg et al., 2016; Schwehr et al., 2018). While application of dispersants can stimulate bacterial degradation of hydrocarbons (Bacosa et al., 2018; Baelum et al., 2012; Campo et al., 2013; Doyle et al., 2018; Kamalanathan et al., 2018b), it can also produce conditions that are toxic to other microbes such as microalgae (Bretherton et al., 2018; Kamalanathan et al., 2018a; Özhan et al., 2014) and cause substantial changes to phytoplankton community structure (Bretherton et al., 2019; Gilde and Pinckney, 2012; Özhan and Bargu, 2014a).

Some phytoplankton are particularly resilient to exposure to either oil or dispersed oil (Bretherton et al., 2018; Harrison et al., 1986; Özhan et al., 2014). While the exact mechanisms of oil toxicity are not known, there are several characteristics that could make organisms more resistant to its effects, based on the literature. For example, different taxonomic groups often respond differently, with groups such as the diatoms (González et al., 2009; Koshikawa et al., 2007; Özhan and Bargu, 2014b) and green algae (Gilde and Pinckney, 2012; Sargian et al., 2007) often being more robust. In some studies, dinoflagellates end up dominating phytoplankton communities exposed to oil and/or dispersants (Gemmell et al., 2018; Jung et al., 2010; Taş et al., 2011).

Physiological traits could contribute to a species' ability to survive exposure to oil and/or dispersants. The presence, and structure, of cell walls could provide protection from the toxic effects of oil spills, as exposure to Corexit (Hook and Osborn, 2012) and some components of oil such as polycyclic aromatic hydrocarbons (Carvalho et al., 2011) can cause membrane damage to phytoplankton cells. Cell size can have profound impacts on physiology (see Finkel et al., 2010 for a review), and smaller phytoplankton species are typically more sensitive to oil toxicity due to their higher surface-area-to-volume ratio (Echeveste et al., 2011, 2010). Motile species may be able to avoid or move out of polluted regions. However, motility also comes at a metabolic cost (Raven and Richardson, 1984) and could potentially make these species more susceptible to stress from oil toxicity. Mixotrophic algae often thrive in wastewater conditions by utilising organic pollutants, and can be an effective form of bioremediation (Pittman et al., 2011).

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Table 1
Details of algal cultures used in this study.

Species	Strain	Described By	Origin of Isolation	Data From	Motility	Mixotrophy	Cell size	Cell wall
Ochromonas sp.	CCMP 1393	Vysotskii	38.70° N, 72.37° W N Atlantic	This study	Yes – flagellar	Yes	4–6 μm	Chitin fibrils
Tetraselmis astigmatica	CCMP 880	R.E. Norris et Hori	48.23° N, 122.64° W Washington, USA	This study	Yes – flagellar	Yes	5–15 μm	Cellulose theca
Micromonas pusilla	RCC 1614	(Butcher) Manton et Parke	56.98° N, 3.98° E North Sea, Norway	This study	Yes – flagellar	Yes	1–3 μm	Naked
Prorocentrum minimum	CCMP 2233	(Pavillard) J. Schiller	38.59° N, 75.10° W Delaware, USA	This study	Yes – flagellar	Yes	16–20 μm	Cellulose plates
Heterocapsa pygmaea	UTEX 2421	Lobelich III, R. J. Schmidt et Sherley	44.06° N, 9.92° E La Spezia, Italy	This study	Yes – flagellar	Yes	10–18 μm	Cellulose plates
Synechococcus elongatus	CCMP 1334	Nageli	33.74° N, 67.49° W N Atlantic	Bretherton et al. (2018)	Yes – flagellar	No	1–3 μm	Naked
Dunaliella tertiolecta	UTEX 999	Butcher	Oslofjord, Norway	Bretherton et al. (2018)	Yes – flagellar	Yes	8–12 μm	Naked
Phaeodactylum tricornutum	UTEX 646	Bohlin	Segelskär, Finland	Bretherton et al. (2018)	Yes – gliding	No	10–14 μm	Silica frustule
Navicula sp.	UTEX SP11	Bory	36.44° N, 98.15° W Oklahoma, USA	Bretherton et al. (2018)	Yes – gliding	No	10–12 μm	Silica frustule
Skeletonema grethae	CCMP 775	Zingone et Sarno	28.90° N, 89.48° W Gulf of Mexico	Bretherton et al. (2018)	No	No	4–8 μm	Silica frustule
Skeletonema grethae	CCMP 776	Zingone et Sarno	28.95° N, 95.36° W Gulf of Mexico	Bretherton et al. (2018)	No	No	4–8 μm	Silica frustule
Skeletonema costatum	UTEX 2308	(Greville) Cleve	Galveston, TX Gulf of Mexico	Bretherton et al. (2018)	No	No	4–6 μm	Silica frustule
Thalassiosira pseudonana	CCMP 1335	Hasle et Heimdal	40.75° N, 72.82° W New York, USA	Bretherton et al. (2018)	No	No	4–6 μm	Silica frustule
Lithodesmium undulatum	CCMP 472	Ehrenberg	28.62° N, 89.75° W Gulf of Mexico	Bretherton et al. (2018)	No	No	31–63 μm	Silica frustule
Odontella mobiliensis	CCMP 597	(Bailey) Gunrow	28.62° N, 89.75° W Gulf of Mexico	Bretherton et al. (2018)	No	No	24–90 μm	Silica frustule

Degradation of compounds found in crude oil has been described in several algal taxa, such as the chrysophytes (Semple and Cain, 1996), the chlorophytes (Kneifel et al., 1997; Todd et al., 2002), and a variety of cyanobacteria (see Subashchandrabose et al., 2013 for a summary). The presence of oil and dispersed oil can also cause an increase in bacterial abundance (e.g. Doyle et al., 2018), which in turn could benefit mixotrophs, particularly those that rely on these mutually beneficial symbiotic relationships.

A previous study (Bretherton et al., 2018) looked at the responses of ten different microalgae to mixtures of oil and dispersed oil. Most of these species were found to be sensitive to oil and were mostly small centric diatoms. We grew a further five species of algae to cover taxonomic groups not examined in the previous study (dinoflagellates and chrysophytes) and include phytoplankton that exhibited some of the traits mentioned above such as mixotrophy or the presence of cell walls (Table 1). We hypothesize that some of this variability is due to biochemical and biophysical differences across species tested, but not taxonomy alone. The algae were exposed to Macondo surrogate oil using the water accommodated fraction (WAF), and a chemically enhanced water accommodated fraction (CEWAF) made of a mixture (20:1) of crude oil and the dispersant Corexit. A third treatment of a diluted CEWAF (DCEWAF) was used to account for the high oil concentration present in the CEWAF. Cell growth and photosynthetic performance were monitored over a 6-day period to examine their response. These data were combined with the previous study to test statistically the importance of five traits (taxon, motility, mixotrophy, cell size, cell wall) in dictating sensitivity to oil using a linear regression model.

2. Methods

2.1. Algal culturing methods

Cultures of *Micromonas pusilla* (RCC1614), *Tetraselmis astigmatica* (CCMP880), *Ochromonas* sp. (CCMP1393), *Heterocapsa pygmaea* (UTEX 2421), and *Prorocentrum minimum* (CCMP2233) were obtained from the Roscoff Culture Collection (RCC) and the National Center for Marine Algae and Microbiota (NCMA). A summary of the details of each culture is presented in Table 1. The microalgal cultures were maintained in sterilised natural seawater collected from the Gulf of Mexico off Galveston, TX and enriched with f/2 nutrients, metals and vitamins (Guillard, 1975). The cultures were kept in a climate controlled room at a temperature of 19 °C with a light:dark cycle of 12:12 h and an irradiance of 100 μmol $m^{-2} s^{-1}$.

2.2. Preparation of treatments

WAF, CEWAF, and DCEWAF treatments were prepared using the CROSERF method (Singer et al., 2001) with some modifications as described in Bretherton et al. (2018). Stock solutions of WAF and CEWAF were prepared in 1 L glass aspirators with bottom spigots by adding 400 μL of either Macondo crude oil (to make WAF) or a mixture of the dispersant Corexit and oil in a 20:1 ratio (to make CEWAF) to fresh f/2 seawater media. Each aspirator was then stirred at such a speed that a vortex occupied the upper ~25% of the volume. Stirring was done in the dark for 24 h at room temperature. WAF is a non-homogenous mixture that is difficult to reproduce between batches (Wade et al., 2017). As a result, the starting oil concentrations for each treatment differed slightly between species (see Fig. 1).

After stirring, the WAF and CEWAF mixtures were each pooled into a 9 L glass aspirator bottle with a bottom spigot, forming the stock solutions for these two treatments. During transfer, the mixtures were passed through a 20 μm nylon mesh sieve to remove larger oil droplets, and any surface slicks were not allowed to pass through the spigots of the 1 L aspirator bottles. To make the DCEWAF stock solution, a volume of the CEWAF was transferred to a third 9 L aspirator bottle and diluted with fresh f/2 media by a factor of 10.

Stock solutions (850 mL) were transferred into sterile 1 L glass bottles and inoculated with 150 mL of exponentially growing algal culture. Control cultures were prepared by inoculating 850 mL of fresh

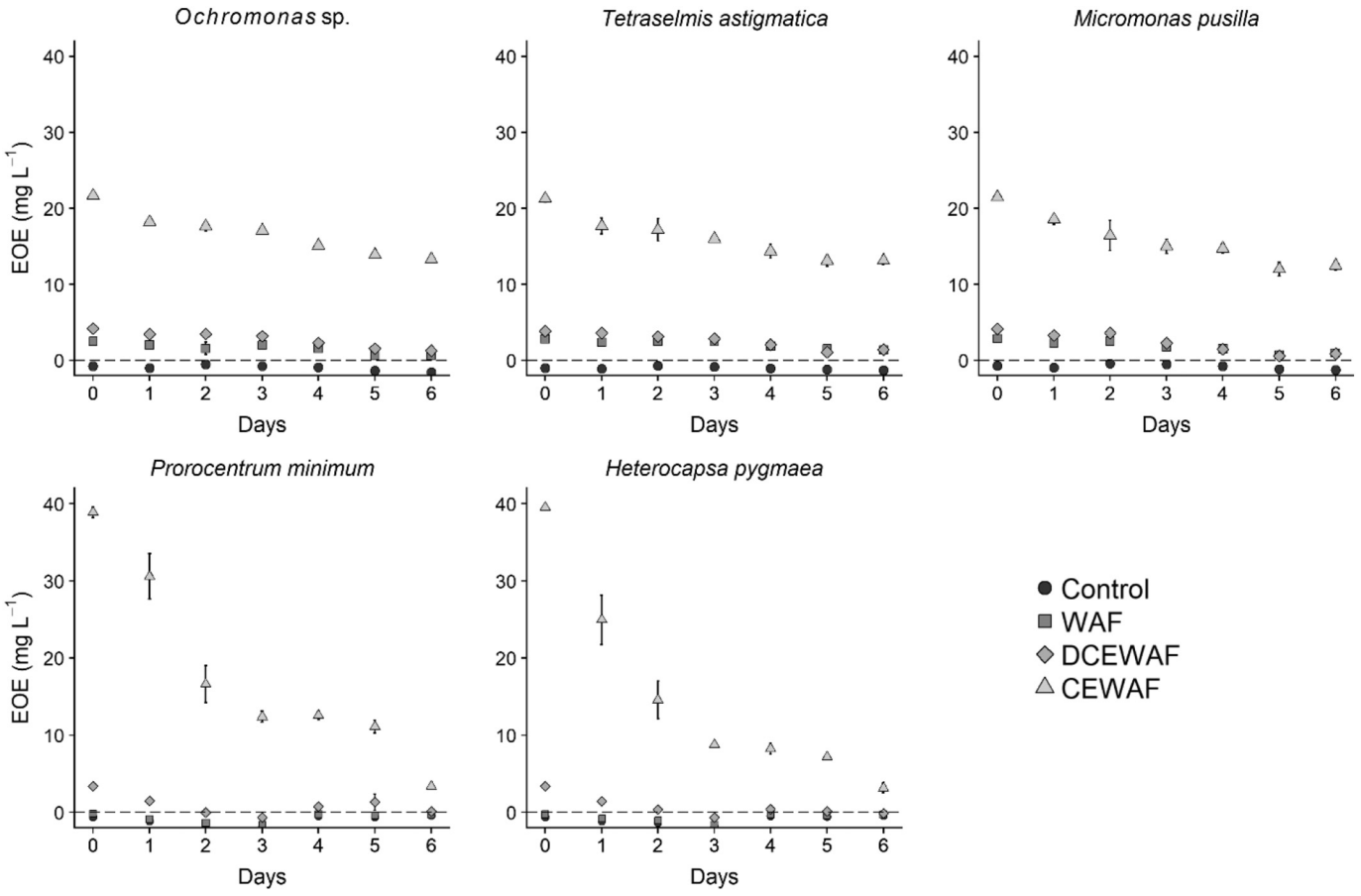

Fig. 1. Changes in estimated oil equivalents (EOE) over time in cultures of *Ochromonas* sp., *T. astigmatica*, *M. pusilla*, *P. minimum* and *H. pygmaea* in four different treatments. Values in Control cultures were always below reliable detection limits (0.02 μg L^{-1}), error bars represent standard error ($n = 3$).

f/2 media with 150 mL of algal culture. All treatments were prepared in triplicate. Additionally, 500 mL of WAF, CEWAF and DCEWAF stock solutions were each transferred to 1 L sterile glass bottles with no algal culture added to form non-biological controls. These served to correct for background fluorescence from both the oil and Corexit for many of the measurements made on the experimental cultures (see below). All experimental bottles were kept in a climate controlled room at 19 °C with a light:dark cycle of 12:12 h and an irradiance of 100 μmol m^{-2} s^{-1} for a period of 6 days.

2.3. Quantification of oil

The concentration of oil in each experimental bottle was monitored every 24 h by measuring the estimated oil equivalents (EOE). Briefly, a 10 mL sample from each experimental bottle was extracted into 5 mL of the solvent dichloromethane (DCM) in a 20 mL scintillation vial. Extraction with DCM allows for the detection of oil as low as 0.7 μg L^{-1} (Wade et al., 2011). The DCM fraction (3 mL) was transferred to a quartz cuvette, and the fluorescence was measured at an excitation wavelength of 322 nm and an emission wavelength of 376 nm using a spectrofluorophotometer (RF-5301PC, Shimadzu, Houston, TX, USA). A calibration curve made using a serial dilution of crude oil in DCM was used to calculate the EOE in each sample in mg L^{-1}. The initial EOE of the WAF, CEWAF and CEWAF stock solutions was also measured in the same way.

2.4. Cell density

Cell growth was monitored using a benchtop Turner fluorometer (10 AU, Turner, San Jose, CA). Every 24 h, a 4 mL sample was transferred from every experimental bottle to a glass cuvette. Samples were dark acclimated for 20 min prior to analysis with the Turner fluorometer. Fluorescence resulting from the oil and/or dispersant was accounted for using the non-biological controls. The Turner fluorometer was calibrated using a serial dilution of a chlorophyll standard, which was prepared using powdered chlorophyll extracted from the alga *Anacystis nodulans* (Sigma-Aldrich) in 90% acetone. A repeated-measures ANOVA (rmANOVA) was used to test for significant differences between treatments.

2.5. Photophysiological measurements

Photosynthetic performance was monitored using a Fluorescence Induction and Relaxation (FIRe) fluorometer (Satlantic, Halifax, Canada). A 4 mL sample from each experimental bottle was dark acclimated for 20 min prior to analysis with the FIRe in order to ensure all photosystem II (PSII) reaction centers are open, yielding a baseline fluorescence (F_o). Once in the fluorometer, samples are exposed to saturating pulses of blue light that close all PSII reaction centers and yield the maximum fluorescence (F_m). The difference between F_o and F_m is the variable fluorescence (F_v). F_v/F_m is termed the maximum PSII quantum yield, and is used as an indicator of photosynthetic efficiency (Kolber et al., 1998). Other photophysiological parameters examined from the FIRe included the PSII absorption cross-section (σ_{PSII}), the PSII connectivity factor (ρ) and PSII re-oxidation time ($\tau 1$). Fresh aliquots of f/2 medium, as well as from the WAF, CEWAF, and DCEWAF non-biological controls were used to correct for interference from background fluorescence (Cullen and David, 2003). A rmANOVA was used to test for significant effects of treatment on all parameters.

2.6. Analysis of adaptive traits in phytoplankton

The magnitude, direction, and timing of biomass changes varied across species, so an integrated biomass was calculated as the integral of chlorophyll concentration over time. The percent change in integrated chlorophyll concentration relative to the Control values was calculated for each treatment in each species, as in Bretherton et al. (2018). Values for this experiment were combined with the values previously calculated for 10 other phytoplankton species (see Table 1 for a summary), and the similarities in the responses were measured using the heatmap.2 function in *R* (v 3.5.2) to plot a dendrogram based on Euclidean distance. A summary of the traits (motility, mixotrophy, cell wall, cell size) associated with all 15 phytoplankton species, along with the percent change in biomass accumulate on, was compiled. Motility, mixotrophy and cell size were binary traits (either yes/no, or smaller than 10 μm/larger than 10 μm), cell wall had three potential options (theca/frustule/naked) and taxon used the phylum for each species (with the exception of *Synechococcus elongatus*, which was simply designated Cyanobacteria). A linear regression model was used to model the percent change in integrated biomass relative to the control for each treatment as a function of the traits and taxonomy to assess the importance of each of these factors. The estimated coefficients were used to assess the relative effects of each trait (or taxon) in determining the response to the treatments.

3. Results

3.1. Quantification of oil concentrations in WAF, DCEWAF and CEWAF cultures

The EOE values in the cultures of *M. pusilla*, *P. minimum*, *H. pygmaea*, *T. astigmatica* and *Ochromonas* sp. all declined over time following first order decay kinetics (Fig. 1). In all cases, the highest initial EOE was measured in the CEWAF cultures; ~21 mg L^{-1} in the *M. pusilla*, *T. astigmatica* and *Ochromonas* sp. cultures; ~39 mg L^{-1} in the *H. pygmaea* and *P. minimum* cultures (Fig. 1). The initial EOE in the DCEWAF ranged between 2 and 4 mg L^{-1}, while the WAF ranged between 0.2 and 2 mg L^{-1}. In both the WAF and DCEWAF treatments, the EOE fell below detection limit by the end of the experiment (see Fig. 1). Samples from Control bottles were always below reliable detection limits (0.02 μg L^{-1}). These EOE concentrations are similar in magnitude to those used in previous studies in which phytoplankton were exposed to WAF, CEWAF or DCEWAF (e.g., Bretherton et al., 2018; Kamalanathan et al., 2018a) and represent concentrations which were observed either immediately after the DwH oil spill or in the days and weeks following (Wade et al., 2016).

3.2. Changes in phytoplankton biomass over time

The five species presented here did not show any significant changes in biomass when exposed to the oil treatments except for the CEWAF (Fig. 2). The chlorophyll concentrations in the *Ochromonas* sp. and *T. astigmatica* cultures were higher in all the treatments than those measured in the Control cultures throughout the experiment. In fact, for both species, the highest chlorophyll values were observed in the highest EOE concentrations (CEWAF cultures). On Day 6, chlorophyll in the controls for *Ochromonas* sp. and *T. astigmatica* cultures were 16.71 and 7.13 μg L^{-1} respectively, but ranged from 23.27 to 42.43 μg L^{-1} and 18.28 to 24.37 μg L^{-1} in the treated cultures (Fig. 2). *H. pygmaea* did not show any significant change in growth with any treatment, and overall grew slowly compared to the other species (Fig. 2). Both *M. pusilla* and *P. minimum* were very negatively impacted in the CEWAF, but no significant differences between the Control, WAF or DCEWAF were observed (Fig. 2).

3.3. Changes in photosynthetic performance over time

F_v/F_m ranged between 0.4 and 0.6 in Control cultures across all species, and showed sensitivity to different treatments in all but *T. astigmatica* (Fig. 3). CEWAF caused the biggest perturbations in F_v/F_m, especially in *M. pusilla*, *H. pygmaea* and *P. minimum*. After 3 days of exposure, F_v/F_m values started declining in the WAF cultures of *M. pusilla*, falling below Control and DCEWAF values. In *P. minimum*, F_v/F_m values in the WAF cultures increased and were higher than both Control and DCEWAF values by the end of the experiment. In both species, the WAF F_v/F_m values were significantly different from the other treatments (rmANOVA, $p < 0.05$).

The σ_{PSII}, ρ, and τ1 values for each species are summarised in Table 2. *Ochromonas* sp. and *T. astigmatica* were both very similar to each other, and showed little variation in their physiology between the treatments. The σ_{PSII} values of *M. pusilla* and *H. pygmaea* declined significantly in CEWAF compared to Control values, while they increased significantly in *P. minimum*. In both *M. pusilla* and *P. minimum*, the ρ values increased while τ1 values decreased significantly in CEWAF compared to Control values, while ρ declined and τ1 remained unchanged *H. pygmaea*.

3.4. Trait-based analysis of phytoplankton responses

The dendrogram analysis (Fig. 4) of the integrated biomass response divided the 15 species into two groups of "resistant" species (*Dunaliella tertiolecta*, *Ochromonas* sp., *T. astigmatica*, *Skeletonema grethae* CCMP775, *Synechococcus elongatus*, *Navicula* sp., *H. pygmaea*, *Phaeodactylum tricornutum*) and "sensitive" species (*Odontella mobiliensis*, *Lithodesmium undulatum*, *Skeletonema costatum*, *M. pusilla*, *P. minimum*, *S. grethae* CCMP776, *Thalassiosira pseudonana*). The growth of species in the "resistant" group was either unaffected or stimulated by the presence of oil and/or Corexit, while those in the "sensitive" group had their growth inhibited by at least the CEWAF treatment.

A linear regression model was used to weight several traits in their importance of determining the response of integrated biomass to WAF, DCEWAF and CEWAF relative to the Control in all 15 phytoplankton species (Table 3). A different trait was identified as the most important in each treatment; cell size was identified as the most important trait in predicting the response in WAF, while motility/mixotrophy was important in the growth response to DCEWAF/CEWAF. Performing a type I ANOVA on the data shows that both motility and mixotrophy are significant variables in predicting the response observed in both DCEWAF and CEWAF, depending on the order the variables are entered into the model (not shown). This suggests that these traits co-vary to the point that it is not possible to confidently distinguish the effect between the two (i.e. all the mixotrophic organisms in the analysis are also motile).

4. Discussion

The changes in growth for *Ochromonas* sp., *T. astigmatica*, *M. pusilla*, *P. minimum* and *H. pygmaea* are consistent with other published studies for these genera. For example, *P. minimum* has previously been observed to be tolerant of low oil concentrations (Morales-Loo and Goutx, 1990; Özhan and Bargu, 2014b). Several *Prorocentrum* species, such as *Prorocentrum balticans* and *Prorocentrum micans*, have also been observed to increase in abundance following oil spills off the coasts of Spain (Varela et al. 2006) and Turkey (Taş et al., 2011). In the Gulf of Mexico, *Prorocentrum texanum* dominated phytoplankton communities following the Texas City "Y" spill off the coast of Galveston (Gemmell et al., 2018). These data corroborate our findings, where *P. minimum* was tolerant of WAF and DCEWAF (low oil concentrations) but died off in the CEWAF treatments. Data on phytoplankton community changes following the Deepwater Horizon oil spill show that three *Prorocentrum* species, including *P. minimum*, all decreased in relative abundance off

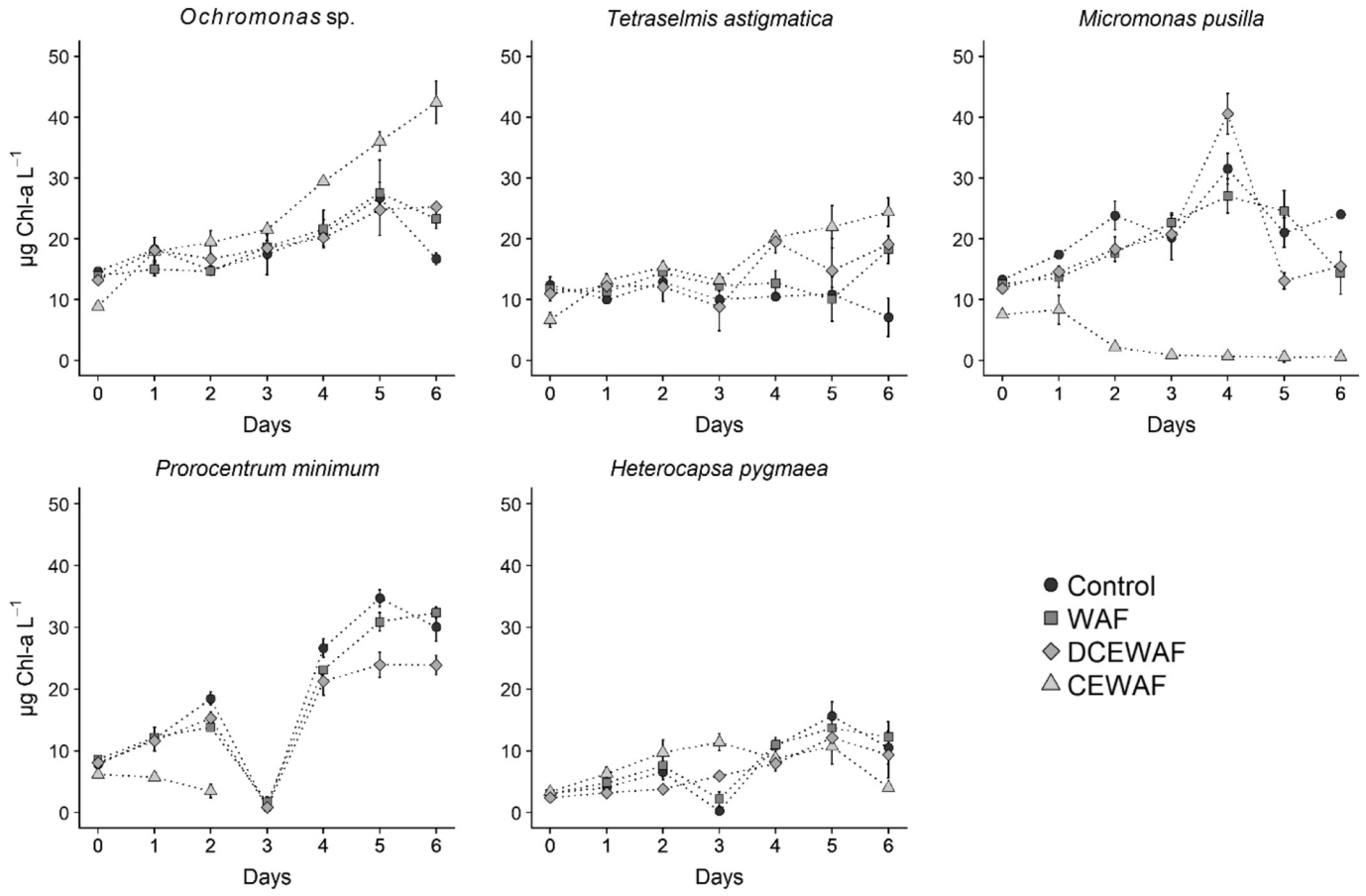

Fig. 2. Evolution of chlorophyll *a* (Chl-a) over time in cultures of *Ochromonas* sp., *T. astigmatica*, *M. pusilla*, *P. minimum* and *H. pygmaea* exposed to oil (WAF) and dispersed oil (DCEWAF and CEWAF). Error bars represent standard error ($n = 3$), missing values indicate days where measurements were below detection limits.

the Louisiana coast (Parsons et al., 2015). Further, it was found when grown as a monoculture, *P. texanum* grown in the presence of either oil or dispersed oil grew slower than control cultures (Gemmell et al., 2018). This is likely because large ciliates such as *Strombidium* sp., which feed on *P. texanum*, were very sensitive to oil (Gemmell et al., 2018), and the response of *P. texanum* (and perhaps *Prorocentrum* spp. in general) to oil may be driven by relieving grazing pressure. Low concentrations of oil did not impact the growth of *Tetraselmis suecica*, and much like *T. astigmatica* in this study, its growth was stimulated by dispersed oil (Fabregas et al., 1984).

Consistent across all 15 species is the insensitivity of F_v/F_m to oil exposure except in the most toxic conditions (CEWAF), even if growth was highly sensitive (Supplementary Fig. 1). This suggests that phytoplankton are either capable of protecting the photosynthetic apparatus from hydrocarbon exposure, or that oil compounds target pathways associated with growth and cell cycle. Transcriptomic analyses suggest that PAHs can arrest the cell cycle in *T. pseudonana* by inhibiting silica uptake (Carvalho et al., 2011; Carvalho and Lettieri, 2011). This could explain why most of the diatoms tested in this and the previous study are in the sensitive group, but since several diatoms were not sensitive, and some of the sensitive species are not diatoms, this alone cannot explain the consistent trend of growth being more sensitive than photosynthesis.

Species-specific F_v/F_m responses have been reported previously to these pollutants (e.g., Bretherton et al., 2018) as well as others (e.g., engineered nanomaterials, persistent organic pollutants); in all cases, phytoplankton exposed to toxic materials may maintain their F_v/F_m even when the growth rate was lowered (Gorbunov and Falkowski, 2011; Miao et al., 2009; Zhao et al., 2019). Our observations reveal a dose dependent response with CEWAF > DCEWAF > WAF in general. This also suggests that Corexit may play a larger role in eliciting a toxic response, but further studies are required to determine if this is related to the overall higher concentrations of oil when Corexit is present or if the Corexit itself is the more harmful component. Passow et al. (2019) recently showed that more oil becomes associated with individual phytoplankton cells in the presence of Corexit than when it is absent, even at the same oil concentrations in seawater. Further, if F_v/F_m responses (as well as σ_{PSII}, ρ, and τ1) indeed suggest the cells are protecting their photosynthetic apparatus, then future studies should examine potential mechanisms and pathways.

4.1. Assessing factors that predict oil resistance

From the linear regression model, different traits influence the growth rate response to WAF and DCEWAF/CEWAF. This suggests that the dispersant generates an environment that requires a different adaptive strategy than exposure to oil by itself. Cell size has a well-documented impact on algal physiology, from photosynthesis (Suggett et al., 2009) to nutrient uptake (Aksnes and Egge, 1991; Edwards et al., 2011). Due to their larger surface area:volume ratio, smaller cells can be more sensitive to toxins including polycyclic aromatic hydrocarbons and oil (Echeveste et al., 2011, 2010). Of the five species grown in this study, two out of the three robust species were larger than 10 µm in length, while the smallest species tested, *M. pusilla*, was one of the most sensitive (Fig. 4). However, *Ochromonas* sp. is smaller than 10 µm and was one of the most resistant species, while *P. minimum*, which is ~20 µm in length, was in the sensitive group. In the context of the ten previously studied algae species, size was identified as a significant variable when predicting the response in the WAF treatment (Table 3). While there are some notable exceptions to this (very small cells like *S.*

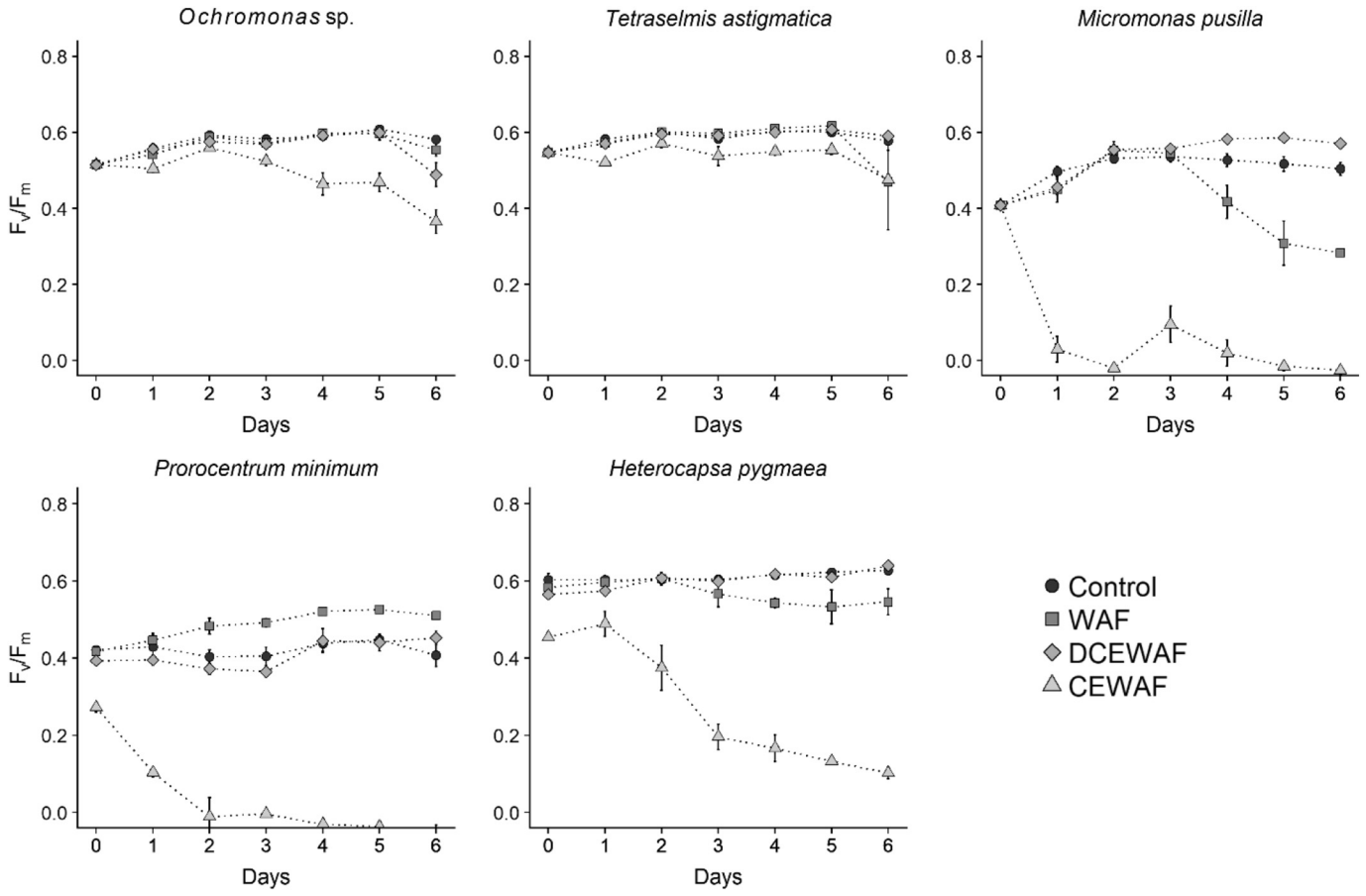

Fig. 3. The PSII quantum yield (F_v/F_m) over time in cultures of *Ochromonas* sp., *T. astigmatica*, *M. pusilla*, *P. minimum* and *H. pygmaea* exposed to oil (WAF) and dispersed oil (DCEWAF and CEWAF). Error bars represent standard error ($n = 3$), missing values indicate days where measurements were below detection limits.

Table 2
The photosystem II (PSII) absorption cross section (σ_{PSII}; $Å^2$ $quanta^{-1}$), PSII connectivity factor (ρ; dimensionless), and PSII reoxidation time (τ1; μs) of five phytoplankton grown in each treatment. Values shown are for final day measurements, standard error is in brackets ($n = 3$). ND = not detected (i.e. below detection limits).

	Control			WAF			DCEWAF			CEWAF		
	σ_{PSII}	ρ	τ1	σ_{PSII}	ρ	τ1	σ_{PSII}	ρ	τ1	σ_{PSII}	ρ	τ1
Ochromonas sp.	205.0 (3.6)	0.36 (0.01)	297.3 (39.6)	185.0 (2.7)	0.34 (0.02)	463.7 (27.2)	178.0 (7.0)	0.33 (0.03)	409.7 (54.1)	193.0 (2.1)	0.24 (0.03)	537.0 (46.7)
T. astigmatica	200.0 (4.04)	0.37 (0.01)	332.3 (28.6)	181.3 (1.2)	0.31 (0.11)	414.7 (37.7)	180.7 (3.0)	0.40 (0.01)	372.3 (19.5)	178.7 (3.2)	0.29 (0.03)	455.0 (8.02)
M. pusilla	450.3 (17.7)	0.07 (0.003)	323.0 (19.8)	386.0 (10.7)	0.05 (0.00)	465.0 (21.5)	433.0 (13.01)	0.08 (0.00)	437.7 (16.6)	227.3 (78.2)	0.16 (0.03)	18.3 (1.9)
P. minimum	220.0 (5.5)	0.07 (0.01)	356.3 (68.3)	190.0 (4.4)	0.08 (0.00)	261.0 (6.8)	210.3 (2.4)	0.07 (0.01)	320.0 (29.7)	414.7 (208.6)	0.42 (0.2)	12.0 (1.0)
H. pygmaea	192.0 (6.9)	0.36 (0.003)	374.0 (26.8)	208.7 (1.2)	0.36 (0.00)	382.7 (48.4)	200.0 (2.0)	0.35 (0.01)	484.7 (45.6)	76.7 (3.7)	0.07 (0.02)	295.3 (28.4)

elongatus and *S. grethae* both being highly resistant, while larger centric diatoms like L. *undulatum* was very sensitive), the data suggest that for exposure to oil only, cell size is an important factor in predicting sensitivity. However, in the dispersed oil treatments, cell size is a less important factor, which suggests that there are other traits that confer an advantage in these situations.

None of the linear regression models identify cell wall as an important variable (Table 3). There is evidence to suggest that dispersants impact algal physiology by disrupting cellular membranes (Carvalho et al., 2011; Hook and Osborn, 2012). Algae with thick cellulose cell walls, and in particular those with sporopollenin structures on them, are far less sensitive to exposure to surfactants such as linear alkylbenzene sulfate, a major component in laundry detergents (Biedlingmaier et al., 1987). In the present study, the two more sensitive species are either naked (*M. pusilla*) or have a thin cell wall (*P. minimum*). Two of the more resistant species have thicker cell walls made up of either several layers of cellulose in the case of *T. astigmatica*, or plates of cellulose as in *H. pygmaea*. However, *Ochromonas* sp. was also very resistant to the treatments, and does not possess a cell wall. Instead, cells from the genus *Ochromonas* have a network of microtubules that give the cell a distinct tapered shape (Bouck and Brown, 1973). These microtubules are made of chitinous fibrils (Herth et al., 1977), perhaps making the cell membrane of *Ochromonas* sp. more robust than other naked cells and explaining its resistance to oil. Even

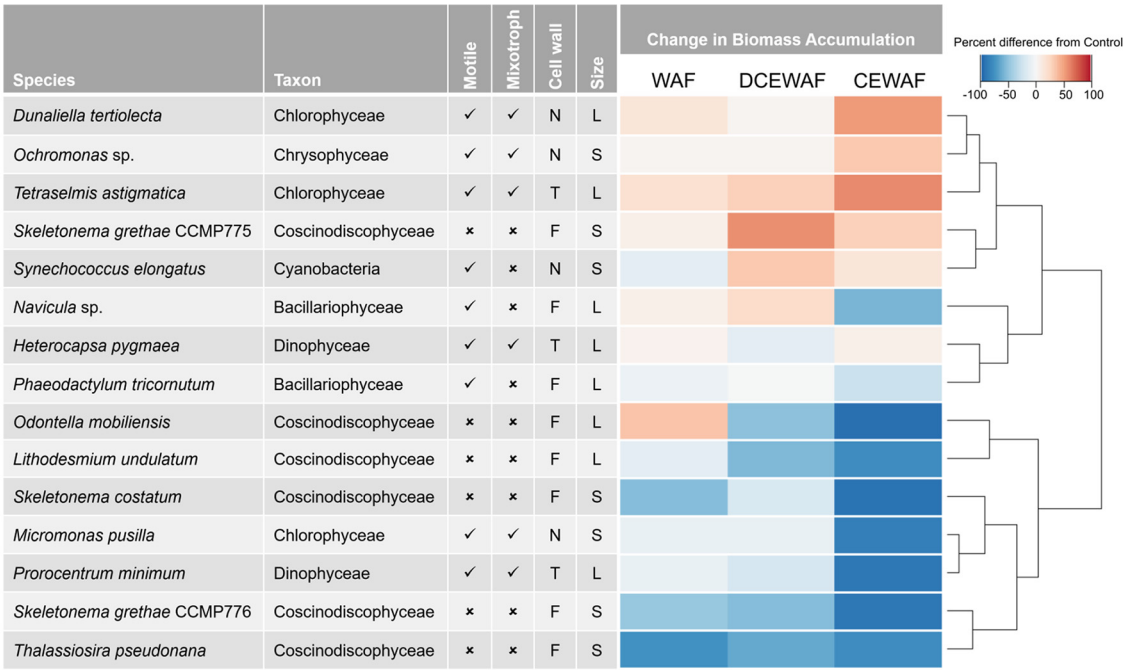

Fig. 4. Species traits, heatmap of percent change in integrated biomass in each treatment relative to Control, and dendrogram clustering species with similar responses for 15 microalgae species. The upper cluster of species (top 8 rows) benefitted or was relatively insensitive to the treatments compared to the lower cluster (bottom 7 rows).

Table 3
Traits listed in order of importance for determining response (integrated biomass) in each treatment. The coefficients were calculated by fitting the data to a linear regression model.

Treatment	Model *P*-value	Adjusted R^2	Variable	Coefficient
WAF	0.0016	0.30	Size	38.875*
			Mixotrophy	21.816
			Cell wall	−10.982
			Taxon	2.453
			Motility	2.374
DCEWAF	0.104	0.10	Motility	42.444*
			Size	−21.161
			Mixotrophy	6.076
			Taxon	4.643
			Cell wall	4.163
CEWAF	0.0012	0.31	Mixotrophy	119.443*
			Taxon	20.777
			Motility	9.333
			Size	1.951
			Cell wall	−0.567

* Significant predictor variables ($p < 0.05$).

so, there are other species that are not sensitive to oil and dispersant exposure, such as *D. tertiolecta* and *S. elongatus*, that do not possess cell walls. Thus, the presence of a cell wall does not necessarily always predict resistance to crude oil or dispersant exposure.

Both mixotrophy and motility were identified as important factors for determining sensitivity to CEWAF and DCEWAF respectively (Table 3). Mixotrophy has been observed in all five of the species grown in the present study (Fig. 4). The ability to rely on sources of organic carbon to grow may help to deal with oil toxicity. Some species in the *Ochromonas* genus have been reported to utilise organic compounds that are sometimes found in oil, such as phenols (Semple and Cain, 1996), which could explain the success of *Ochromonas* sp. in all the treatments. While there is no evidence that members of the *Tetraselmis* genus are able to utilise such compounds, it has been described in other genera of the green algae, such as *Chlorella* and *Scenedesmus* (Kneifel et al., 1997; Todd et al., 2002). Of the 15 total species that have been tested, six are capable of mixotrophy (either in the form of bacteriophagy or direct uptake of organic carbon), and four of them fall into the robust group (Fig. 4). It is important to note that motility and mixotrophy are closely related traits, in that all species identified as mixotrophic are also motile, and there are only three motile species that are not mixotrophic. The advantage that motility might confer is less readily obvious. Some studies have found that exposure to crude oil can in fact limit motility, though much of this data comes from the haptophyte *Isochrysis galbana* (Garr et al., 2014; Pérez et al., 2010). In the natural environment, motile cells may be able to move away higher concentrations of oil and thus avoid toxic effects, but it is less likely that this is the case in a culture environment.

Importantly, the five traits tested could only account for, at most, around a third of the variance observed (see Table 3), which suggests that there may be other traits and trait interactions that are more important in predicting sensitivity to oil in microalgae. Exposure to crude oil can cause damage to genes in some diatoms (Deasi et al., 2010), and thus the ability to quickly repair DNA might be a trait of particularly resistant species. Cell morphology can also impact responses to environmental changes (Alves-de-Souza et al., 2008), and the dominant morphology of entire phytoplankton communities can change in response to seasonal variability (Stanca et al., 2013). It is possible that cells that are more tolerant are better able to adjust their surface area-to-volume ratio, but this data was not collected during these experiments. The production of polysaccharides and other organic materials might also be related to oil tolerance. It is well established that phytoplankton release extracellular polymeric substances (EPS) into the surrounding seawater during times of stress (Kahl et al., 2008). Suppression of chrysolaminarin synthesis, a polysaccharide produced by many phytoplankton groups, resulted in increased sensitivity to WAF exposure in *T. pseudonana* (Kamalanathan et al., 2019). The role that polysaccharide and EPS production play in the oil response of marine algae is uncertain, though several studies have observed an increase in

EPS and aggregate formation following oil exposure (Passow et al., 2017; Yan et al., 2016). EPS production is also influenced by algae-associated bacteria (Gutierrez et al., 2013), and algae in turn can alter these bacterial communities through the production of organic compounds (Kamalanathan et al., 2019). As such, bacterial associations may also be an important factor for predicting oil resistance.

The phytoplankton response to oil and dispersants is highly variable and can be explained by the fact that different taxa possess different traits that contribute differently to the way they deal with hydrocarbons. However, this is confounded by the fact that phytoplanktons are highly variable between species, and even intra-specific variability in the oil response has been observed. As such, the traits we analyzed here (cell wall, motility, mixotrophy, cell size) can only explain a portion of the variability, and it is likely that more data from a broader range of species is required to better predict what traits are ideal for resistance to oil and dispersant exposure.

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CRediT authorship contribution statement

Laura Bretherton:Data curation, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.**Jessica Hillhouse**:Investigation, Writing - original draft, Writing - review & editing.**Manoj Kamalanathan**:Investigation, Formal analysis, Writing - original draft, Writing - review & editing.**Zoe V. Finkel**:Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.**Andrew J. Irwin**:Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.**Antonietta Quigg**:Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aksnes, D., Egge, J., 1991. A theoretical model for nutrient uptake in phytoplankton. Mar. Ecol. Prog. Ser. 70, 65–72. https://doi.org/10.3354/meps070065.

Alves-de-Souza, C., González, M.T., Iriarte, J.L., 2008. Functional groups in marine phytoplankton assemblages dominated by diatoms in fjords of southern Chile. J. Plankton Res. 30, 1233–1243. https://doi.org/10.1093/plankt/fbn079.

Bacosa, H.P., Kamalanathan, M., Chiu, M.-H., Tsai, S.-M., Sun, L., Labonté, J.M., Schwehr, K.A., Hala, D., Santschi, P.H., Chin, W.-C., Quigg, A., 2018. Extracellular polymeric substances (EPS) producing and oil degrading bacteria isolated from the northern Gulf of Mexico. PLoS One 13, e0208406. https://doi.org/10.1371/journal.pone.0208406.

Baelum, J., Borglin, S., Chakraborty, R., Fortney, J.L., Lamendella, R., Mason, O.U., Auer, M., Zemla, M., Bill, M., Conrad, M.E., Malfatti, S.A., Tringe, S.G., Holman, H.-Y., Hazen, T.C., Jansson, J.K., 2012. Deep-sea bacteria enriched by oil and dispersant from the Deepwater horizon spill. Environ. Microbiol. 14, 2405–2416. https://doi.org/10.1111/j.1462-2920.2012.02780.x.

Biedlingmaier, S., Wanner, G., Schmidt, A., 1987. A correlation between detergent tolerance and Cell Wall structure in green algae. Zeitschrift für Naturforschung C 42, 245–250. https://doi.org/10.1515/znc-1987-0313.

Bouck, G.B., Brown, D.L., 1973. Microtubule biogenesis and cell shape in *Ochromonas*: I. the distribution of cytoplasmic and mitotic microtubules. J. Cell Biol. 56, 340–359. https://doi.org/10.1083/jcb.56.2.340.

Bretherton, L., Williams, A., Genzer, J., Hillhouse, J., Kamalanathan, M., Finkel, Z.V., Quigg, A., 2018. Physiological response of 10 phytoplankton species exposed to macondo oil and the dispersant, Corexit. J. Phycol. 54, 317–328. https://doi.org/10.1111/jpy.12625.

Bretherton, L., Kamalanathan, M., Genzer, J., Hillhouse, J., Setta, S., Liang, Y., Brown, C.M., Xu, C., Sweet, J., Passow, U., Finkel, Z.V., Irwin, A.J., Santschi, P.H., Quigg, A., 2019. Response of natural phytoplankton communities exposed to crude oil and chemical dispersants during a mesocosm experiment. Aquat. Toxicol. 206, 43–53. https://doi.org/10.1016/j.aquatox.2018.11.004.

Campo, P., Venosa, A.D., Suidan, M.T., 2013. Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 °C. Environ. Sci. Technol. 47, 1960–1967. https://doi.org/10.1021/es303881h.

Carvalho, R.N., Lettieri, T., 2011. Proteomic analysis of the marine diatom *Thalassiosira pseudonana* upon exposure to benzo(a)pyrene. BMC Genomics 12, 159. https://doi.org/10.1186/1471-2164-12-159.

Carvalho, R.N., Bopp, S.K., Lettieri, T., 2011. Transcriptomics responses in marine diatom *Thalassiosira pseudonana* exposed to the polycyclic aromatic hydrocarbon benzo[a]pyrene. PLoS One 6, e26985. https://doi.org/10.1371/journal.pone.0026985.

Cullen, J.T., David, R.F., 2003. The blank can make a big difference in oceanographic measurements. Limnology and Oceanography Bulletin 12, 29–34. https://doi.org/10.1002/lob.200413229.

Deasi, S.R., Verlecar, X.N., Ansari, Z.A., Jagtap, T.G., Sarkar, A., Vashistha, D., Dalal, S.G., 2010. Evaluation of genotoxic responses of *Chaetoceros tenuissimus* and *Skeletonema costatum* to water accommodated fraction of petroleum hydrocarbons as biomarker of exposure. Water Res. 44, 2235–2244. https://doi.org/10.1016/j.watres.2009.12.048.

Doyle, S.M., Whitaker, E.A., De Pascuale, V., Wade, T.L., Knap, A.H., Santschi, P.H., Quigg, A., Sylvan, J.B., 2018. Rapid formation of microbe-oil aggregates and changes in community composition in coastal surface water following exposure to oil and the dispersant Corexit. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00689.

Echeveste, P., Agustí, S., Dachs, J., 2010. Cell size dependent toxicity thresholds of polycyclic aromatic hydrocarbons to natural and cultured phytoplankton populations. Environ. Pollut. 158, 299–307. https://doi.org/10.1016/j.envpol.2009.07.006.

Echeveste, P., Agustí, S., Dachs, J., 2011. Cell size dependence of additive versus synergetic effects of UV radiation and PAHs on oceanic phytoplankton. In: Environmental Pollution, Adaptation of Forest Ecosystems to Air Pollution and Climate Change. vol. 159. pp. 1307–1316. https://doi.org/10.1016/j.envpol.2011.01.023.

Edwards, K.F., Klausmeier, C.A., Litchman, E., 2011. Evidence for a three-way trade-off between nitrogen and phosphorus competitive abilities and cell size in phytoplankton. Ecology 92, 2085–2095. https://doi.org/10.1890/11-0395.1.

Fabregas, J., Herrero, C., Veiga, M., 1984. Effect of oil and dispersant on growth and chlorophyll a content of the marine microalga *Tetraselmis suecica*. Appl. Environ. Microbiol. 47, 445–447.

Finkel, Z.V., Beardall, J., Flynn, K.J., Quigg, A., Rees, T.A.V., Raven, J.A., 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. J. Plankton Res. 32, 119–137. https://doi.org/10.1093/plankt/fbp098.

Garr, A.L., Laramore, S., Krebs, W., 2014. Toxic effects of oil and dispersant on marine microalgae. Bull. Environ. Contam. Toxicol. 93, 654–659. https://doi.org/10.1007/s00128-014-1395-2.

Gemmell, B.J., Bacosa, H.P., Dickey, B.O., Gemmell, C.G., Alqasemi, L.R., Buskey, E.J., 2018. Rapid alterations to marine microbiota communities following an oil spill. Ecotoxicology 27, 505–516. https://doi.org/10.1007/s10646-018-1923-7.

Gilde, K., Pinckney, J.L., 2012. Sublethal effects of crude oil on the community structure of estuarine phytoplankton. Estuar. Coasts 35, 853–861. https://doi.org/10.1007/s12237-011-9473-8.

González, J., Figueiras, F.G., Aranguren-Gassis, M., Crespo, B.G., Fernández, E., Morán, X.A.G., Nieto-Cid, M., 2009. Effect of a simulated oil spill on natural assemblages of marine phytoplankton enclosed in microcosms. Estuar. Coast. Shelf Sci. 83, 265–276. https://doi.org/10.1016/j.ecss.2009.04.001.

Gorbunov, M.Y., Falkowski, P.G., 2011. Analysis of Biophysical, Optical and Genetic Diversity of DoD Coral Reef Communities Using Advanced Fluorescence and Molecular Biology Techniques.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp. 29–60. https://doi.org/10.1007/978-1-4615-8714-9.

Gutierrez, T., Berry, D., Yang, T., Mishamandani, S., McKay, L., Teske, A., Aitken, M.D., 2013. Role of bacterial exopolysaccharides (EPS) in the fate of the oil released during the deepwater horizon oil spill. PLoS One 8, e67717. https://doi.org/10.1371/journal.pone.0067717.

Harrison, P.J., Cochlan, W.P., Acreman, J.C., Parsons, T.R., Thompson, P.A., Dovey, H.M., Xiaolin, C., 1986. The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure. Mar. Environ. Res. 18, 93–109. https://doi.org/10.1016/0141-1136(86)90002-4.

Herth, W., Kuppel, A., Schnepf, E., 1977. Chitinous fibrils in the lorica of the flagellate chrysophyte Poteriochromonas stipitata (syn. Ochromonas malhamensis). J. Cell Biol. 73, 311–321. https://doi.org/10.1083/jcb.73.2.311.

Hook, S.E., Osborn, H.L., 2012. Comparison of toxicity and transcriptomic profiles in a diatom exposed to oil, dispersants, dispersed oil. Aquat. Toxicol. 124–125, 139–151. https://doi.org/10.1016/j.aquatox.2012.08.005.

Jung, S.W., Park, J.S., Kown, O.Y., Kang, J.-H., Shim, W.J., Kim, Y.-O., 2010. Effects of crude oil on marine microbial communities in short term outdoor microcosms. J. Microbiol. 48, 594–600. https://doi.org/10.1007/s12275-010-0199-2.

Kahl, L., Vardi, A., Schofield, O., 2008. Effects of phytoplankton physiology on export flux. Mar. Ecol. Prog. Ser. 354, 3–19. https://doi.org/10.3354/meps07333.

Kamalanathan, M., Schwehr, K.A., Bretherton, L., Genzer, J., Hillhouse, J., Xu, C., Williams, A., Santschi, P., Quigg, A., 2018a. Diagnostic tool to ascertain marine phytoplankton exposure to chemically enhanced water accommodated fraction of oil using Fourier transform infrared spectroscopy. Mar. Pollut. Bull. 130, 170–178. https://doi.org/10.1016/j.marpolbul.2018.03.027.

Kamalanathan, M., Xu, C., Schwehr, K., Bretherton, L., Beaver, M., Doyle, S.M., Genzer, J., Hillhouse, J., Sylvan, J.B., Santschi, P., Quigg, A., 2018b. Extracellular enzyme activity profile in a chemically enhanced water accommodated fraction of surrogate oil: toward understanding microbial activities after the Deepwater horizon oil spill. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00798.

Kamalanathan, M., Chiu, M.-H., Bacosa, H., Schwehr, K., Tsai, S.-M., Doyle, S., Yard, A., Mapes, S., Vasequez, C., Bretherton, L., Sylvan, J., Santschi, P., Chin, W.-C., Quigg, A., 2019. Role of polysaccharides in diatom *Thalassiosira pseudonana* and its associated bacteria in hydrocarbon presence. Plant Physiol. https://doi.org/10.1104/pp.19.00301. pp.00301.2019.

Kneifel, H., Elmendorff, K., Hegewald, E., Soeder, C.J., 1997. Biotransformation of 1-naphthalenesulfonic acid by the green alga *Scenedesmus obliquus*. Arch. Microbiol. 167, 32–37. https://doi.org/10.1007/s002030050413.

Kolber, Z.S., Prasil, O., Falkowski, P.G., 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochim. Biophys. Acta Bioenerg. 1367, 88–106. https://doi.org/10.1016/S0005-2728(98)00135-2.

Koshikawa, H., Xu, K.Q., Liu, Z.L., Kohata, K., Kawachi, M., Maki, H., Zhu, M.Y., Watanabe, M., 2007. Effect of the water-soluble fraction of diesel oil on bacterial and primary production and the trophic transfer to mesozooplankton through a microbial food web in Yangtze estuary, China. Estuar. Coast. Shelf Sci. 71, 68–80.

Kujawinski, E.B., Kido Soule, M.C., Valentine, D.L., Boysen, A.K., Longnecker, K., Redmond, M.C., 2011. Fate of dispersants associated with the Deepwater horizon oil spill. Environ. Sci. Technol. 45, 1298–1306. https://doi.org/10.1021/es103838p.

McNutt, M.K., Camilli, R., Crone, T.J., Guthrie, G.D., Hsieh, P.A., Ryerson, T.B., Savas, O., Shaffer, F., 2012. Review of flow rate estimates of the Deepwater horizon oil spill. Proc. Natl. Acad. Sci. 109, 20260–20267. https://doi.org/10.1073/pnas.1112139108.

Miao, A.-J., Schwehr, K.A., Xu, C., Zhang, S.-J., Luo, Z., Quigg, A., Santschi, P.H., 2009. The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. Environ. Pollut. 157, 3034–3041. https://doi.org/10.1016/j.envpol.2009.05.047.

Morales-Loo, M.R., Goutx, M., 1990. Effects of water-soluble fraction of the Mexican crude oil “isthmus Cactus” on growth, cellular content of chlorophyll a, and lipid composition of planktonic microalgae. Mar. Biol. 104, 503–509. https://doi.org/10.1007/BF01314357.

Özhan, K., Bargu, S., 2014a. Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant Corexit® EC9500A under different nutrient regimes. Ecotoxicology 23, 370–384. https://doi.org/10.1007/s10646-014-1195-9.

Özhan, K., Bargu, S., 2014b. Responses of sympatric *Karenia brevis*, *Prorocentrum minimum*, and *Heterosigma akashiwo* to the exposure of crude oil. Ecotoxicology 23, 1387–1398. https://doi.org/10.1007/s10646-014-1281-z.

Özhan, K., Miles, S.M., Gao, H., Bargu, S., 2014. Relative phytoplankton growth responses to physically and chemically dispersed South Louisiana sweet crude oil. Environ. Monit. Assess. 186, 3941–3956. https://doi.org/10.1007/s10661-014-3670-4.

Parsons, M.L., Morrison, W., Rabalais, N.N., Turner, R.E., Tyre, K.N., 2015. Phytoplankton and the Macondo oil spill: a comparison of the 2010 phytoplankton assemblage to baseline conditions on the Louisiana shelf. Environ. Pollut. 207, 152–160. https://doi.org/10.1016/j.envpol.2015.09.019.

Passow, U., Sweet, J., Quigg, A., 2017. How the dispersant Corexit impacts the formation of sinking marine oil snow. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2017.08.015.

Passow, U., Sweet, J., Francis, S., Xu, C., Dissanayake, A.L., Lin, Y.-Y., Santschi, P.H., Quigg, A., 2019. Incorporation of oil into diatom aggregates. Mar. Ecol. Prog. Ser. 612, 65–86. https://doi.org/10.3354/meps12881.

Pérez, P., Fernández, E., Beiras, R., 2010. Fuel toxicity on *Isochrysis galbana* and a coastal phytoplankton assemblage: growth rate vs. variable fluorescence. Ecotoxicol. Environ. Saf. 73, 254–261. https://doi.org/10.1016/j.ecoenv.2009.11.010.

Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology, Special Issue: Biofuels - II: Algal Biofuels and Microbial Fuel Cells 102, 17–25. https://doi.org/10.1016/j.biortech.2010.06.035.

Quigg, A., Passow, U., Chin, W.-C., Xu, C., Doyle, S., Bretherton, L., Kamalanathan, M., Williams, A.K., Sylvan, J.B., Finkel, Z.V., Knap, A.H., Schwehr, K.A., Zhang, S., Sun, L., Wade, T.L., Obeid, W., Hatcher, P.G., Santschi, P.H., 2016. The role of microbial exopolymers in determining the fate of oil and chemical dispersants in the ocean. Limnology and Oceanography Letters 3–26. https://doi.org/10.1002/lol2.10030.

Raven, J.A., Richardson, K., 1984. Dinophyte flagella: a cost-benefit analysis. New Phytol. 98, 259–276. https://doi.org/10.1111/j.1469-8137.1984.tb02736.x.

Sargian, P., Mas, S., Pelletier, É., Demers, S., 2007. Multiple stressors on an Antarctic microplankton assemblage: water soluble crude oil and enhanced UVBR level at Ushuaia (Argentina). Polar Biol. 30, 829–841. https://doi.org/10.1007/s00300-006-0243-1.

Schwehr, K.A., Xu, C., Chiu, M.-H., Zhang, S., Sun, L., Lin, P., Beaver, M., Jackson, C., Agueda, O., Bergen, C., Chin, W.-C., Quigg, A., Santschi, P.H., 2018. Protein: polysaccharide ratio in exopolymeric substances controlling the surface tension of seawater in the presence or absence of surrogate Macondo oil with and without Corexit. Mar. Chem. 206, 84–92. https://doi.org/10.1016/j.marchem.2018.09.003.

Semple, K.T., Cain, R.B., 1996. Biodegradation of phenols by the alga *Ochromonas danica*. Appl. Environ. Microbiol. 62, 1265–1273.

Singer, M.M., Aurand, D.V., Coelho, G.M., Bragin, G.E., Clark, J.R., Sowby, M., Tjeerdema, R., 2001. Making, measuring, and using water-accomodated fractions of petroleum for toxicity testing. International Oil Spill Conference 2001, 1269–1274. https://doi.org/10.7901/2169-3358-2001-2-1269.

Stanca, E., Cellamare, M., Basset, A., 2013. Geometric shape as a trait to study phytoplankton distributions in aquatic ecosystems. Hydrobiologia 701, 99–116. https://doi.org/10.1007/s10750-012-1262-2.

Subashchandrabose, S.R., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K., Naidu, R., 2013. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. Environ. Int. 51, 59–72. https://doi.org/10.1016/j.envint.2012.10.007.

Suggett, D.J., Moore, C.M., Hickman, A.E., Geider, R.J., 2009. Interpretation of fast repetition rate (FRR) fluorescence: signatures of phytoplankton community structure versus physiological state. Mar. Ecol. Prog. Ser. 376, 1–19. https://doi.org/10.3354/meps07830.

Taş, S., Okuş, E., Ünlü, S., Altiok, H., 2011. A study on phytoplankton following ‘Volgoneft-248’ oil spill on the north-eastern coast of the sea of Marmara. J. Mar. Biol. Assoc. U. K. 91, 715–725. https://doi.org/10.1017/S0025315410000330.

Todd, S.J., Cain, R.B., Schmidt, S., 2002. Biotransformation of naphthalene and diaryl ethers by green microalgae. Biodegradation 13, 229–238. https://doi.org/10.1023/A:1021245404675.

Wade, T.L., Sweet, S.T., Sericano, J.L., Guinasso, N.L., Diercks, A.R., Highsmith, R.C., Asper, V.L., Joung, D., Shiller, A.M., Lohrenz, S.E., Joye, S.B., 2011. Analyses of water samples from the Deepwater horizon oil spill: Documentation of the subsurface plume. In: Monitoring and Modeling the Deepwater Horizon Oil Spill: A Record Breaking Enterprise. American Geophysical Union, pp. 77–82. https://doi.org/10.1029/2011GM001103.

Wade, T.L., Sericano, J.L., Sweet, S.T., Knap, A.H., Guinasso, N.L., 2016. Spatial and temporal distribution of water column total polycyclic aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (TPH) from the Deepwater horizon (Macondo) incident. Mar. Pollut. Bull. 103, 286–293. https://doi.org/10.1016/j.marpolbul.2015.12.002.

Wade, T.L., Morales-McDevitt, M., Bera, G., Shi, D., Sweet, S., Wang, B., Gold-Bouchot, G., Quigg, A., Knap, A.H., 2017. A method for the production of large volumes of WAF and CEWAF for dosing mesocosms to understand marine oil snow formation. Heliyon 3, e00419. https://doi.org/10.1016/j.heliyon.2017.e00419.

Yan, B., Passow, U., Chanton, J.P., Nöthig, E.-M., Asper, V., Sweet, J., Pitiranggon, M., Diercks, A., Pak, D., 2016. Sustained deposition of contaminants from the Deepwater horizon spill. Proc. Natl. Acad. Sci. U. S. A. 113, E3332–E3340. https://doi.org/10.1073/pnas.1513156113.

Zhao, Yirong, Tang, X., Quigg, A., Lv, M., Zhao, Yan, 2019. The toxic mechanisms of BDE-47 to the marine diatom *Thalassiosira pseudonana* - a study based on multiple physiological processes. Aquat. Toxicol. 212, 20–27. https://doi.org/10.1016/j.aquatox.2019.04.010.