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Exposure to oil from the 2015 Refugio spill alters the physiology of a common harmful algal bloom species, *Pseudo-nitzschia australis*, and the ubiquitous coccolithophore, *Emiliania huxleyi*

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ABSTRACT: Short-term oil exposure impacts on the harmful algal bloom (HAB) species *Pseudo-nitzschia australis* and the coccolithophore *Emiliania huxleyi* were studied by investigating physiological responses in growth, production of transparent exopolymer particles (TEP), *P. australis* domoic acid (DA) content, and *E. huxleyi* calcification, with the goal of better understanding the potential effects of the 2015 oil spill near Refugio State Beach in the Santa Barbara Channel, California (USA). While oil exposure negatively impacted growth of both species, *P. australis* appeared to be more sensitive to oil exposure compared to *E. huxleyi*. Increased cellular TEP (both species) and DA (*P. australis*), and abnormal calcification (*E. huxleyi*) were observed in the presence of oil. The physiological changes detected in these locally important phytoplankton species have implications for other ecosystem processes, human health, and the fate of the spilled oil. The results from this study enhance our understanding of the repercussions of oil pollution events, but further research is necessary to explore ecosystem-wide impacts and chronic or long-term effects of oil in marine environments.

KEY WORDS: Oil · Phytoplankton · Refugio oil spill · Domoic acid · *Pseudo-nitzschia australis* · *Emiliania huxleyi*

INTRODUCTION

The annual release of oil from all sources worldwide into the marine environment has been estimated to exceed 1 Mt (NRC 2003). Major oil spills are not the most significant source of oil into the ocean; natural seeps and other chronic sources (e.g. land runoff) are more significant due to the long timescales of input, but large spills can have more dramatic impacts due to highly localized and concentrated pollution (NRC 2003). After oil spill events, much of the ecosystem assessment focuses on marine mammals, birds, and other locally important macrofauna because of the observable impacts such as strandings and deaths of oil-coated organisms. However, although phytoplankton are at the base of the marine food web and key to ocean biogeochemical processes, they are relatively understudied in the context of oil pollution. In the limited but growing number of studies on the effects of oil exposure on individual phytoplankton species or natural phytoplankton assemblages, enormous variability in physiological responses has been demonstrated. Generally, growth and primary production appear to decrease or be inhibited during oil exposure (e.g. Østgaard et al. 1984, González et al. 2009, Brussaard et al. 2016), but some studies have found that at low oil concentrations, growth or primary production can remain un-

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changed (e.g. Batten et al. 1998, González et al. 2013, Li et al. 2017) or even increase (e.g. Parsons et al. 1976, Vargo et al. 1982, Hu et al. 2011). Both individual and community-level studies have shown that species-specific phytoplankton sensitivity to crude oil and oil exposure can result in a change in the community composition (González et al. 2009, Özhan et al. 2014a, Ozhan et al. 2014b). However, there is no apparent general trend in how oil exposure affects the structure of phytoplankton communities, as many factors operate simultaneously, including the initial community structure (González et al. 2009, Huang et al. 2011), oil concentration and type (Hsiao et al. 1978), nutrient concentrations (Ozhan & Bargu 2014a), and water temperature (Huang et al. 2011).

On 19 May 2015, an underground pipeline near Refugio State Beach, about 32 km west of Santa Barbara, California, USA, leaked between 101 000 and 140 000 gallons (~382 327 to 529 958 l) of oil with approximately 21 000 gallons (79 494 l) entering the ocean (www.refugioresponse.com/go/doc/7258/2588430/). Although not a large spill compared to others in the marine environment, this event was the largest accidental release of crude oil into the Santa Barbara Channel (SBC) since the historic 1969 oil well blowout. The release, and subsequent flow of oil from the land to the ocean, and the timing of the spill, also make the 2015 event unique in the context of marine spills. Notably, the spill occurred at a time of high phytoplankton primary productivity in the SBC and the dominance of the toxic coastal pennate diatoms *Pseudo-nitzschia* spp. (www.sccoos.org/data/habs/history.php?location=Stearns%20Wharf; McCabe et al. 2016). This diatom genus is known for its production of domoic acid (DA), a neurotoxin transferred through the marine food web that causes sickness or death in marine mammals, birds, and humans (Lefebvre et al. 2002). The species *P. australis*, one of the most prolific DA producers and the main perpetrator of the extensive 2015 blooms along the entire North American west coast (McCabe et al. 2016), typically blooms in warm water off the central and southern California coast (e.g. Fritz et al. 1992, Schnetzer et al. 2007, Sekula-Wood et al. 2011) and has been documented to form massive blooms with high DA production in the SBC (Anderson et al. 2006). The production of DA by *Pseudo-nitzschia* spp. varies dramatically, and even strains of the same species can produce different amounts of DA depending on environmental conditions (e.g. Maldonado et al. 2002, Kudela et al. 2008, Thorel et al. 2014). This makes predictions of toxic events difficult, therefore necessitating DA monitoring and the shutting down of local fisheries when required. Weeks after the Refugio oil spill, in early June, an unprecedented coccolithophore bloom, mainly composed of *Emiliania huxleyi*, was observed in the SBC (P. G. Matson unpubl. data). This species is the most abundant and widespread calcareous phytoplanktonic organism in the world's oceans, where it commonly forms extensive blooms at high latitudes (Westbroek et al. 1989, Tyrell & Merico 2004). In the SBC, *E. huxleyi* is present year-round and is considered ubiquitous (Grelaud et al. 2009), but with the exception of the 2015 event (P. G. Matson unpubl. data), populations in the SBC do not appear to form blooms.

The aim of this study was to explore how a large and abrupt input of oil into a productive coastal marine ecosystem can impact phytoplankton physiology and morphology, including growth, DA cellular content, and calcium carbonate ($CaCO_3$) production. A limited number of studies have explored the effect of oil pollution on phytoplankton communities that include *Pseudo-nitzschia* or coccolithophores (e.g. Ignatiades & Mimicos 1977, Ozhan & Bargu 2014a) as well as individual toxic phytoplankton taxa (not including any DA-producers) (e.g. Özhan & Bargu 2014b), but this is the first study to test the effect of oil exposure on the physiology of either *E. huxleyi* or *P. australis*, or any representative of either coccolithophores or *Pseudo-nitzschia* spp. individually. Additionally, this is the first study to explore oil impacts on phytoplankton in regards to the SBC, a location with significant offshore oil production, natural hydrocarbon seeps, and a highly productive coastal ecosystem. We used 2 species representing the functionally distinct phytoplankton groups dominating the SBC phytoplankton community at the time of the spill. We discuss the implications of this study on phytoplankton succession, HAB dynamics, and the fate of the spilled oil.

MATERIALS AND METHODS

Water accommodated fraction preparation and analysis

The oil used for the experiments included crude oil from offshore Platform Holly (34.389° N, 119.906° W) collected in 2012 (0.915 g ml^{-1}, calculated American Petroleum Institute [API] gravity = 23°) and crude oil from the Plains All American Line 901 collected during the oil spill in May 2015 (0.946 g ml^{-1}, calculated API gravity = 18°, Passow et al. 2017). Although the focus of this study was to test oil originating from the

Refugio oil spill, the Refugio spill oil was not available until more than 6 mo after the spill occurred. Therefore, we used Platform Holly oil to conduct initial tests and to provide comparisons to other oil present in SBC waters. The Platform Holly oil used was similar to the spilled oil (a fraction of the spilled oil originated from Platform Holly), and the use of this crude oil gives this study a broader context and insight into whether the Refugio oil had any unique impacts on physiology that may not have been measured directly (such as additives in the oil to improve transportation performance within the pipelines).

The water accommodated fraction (WAF) of crude oil was prepared for each experiment separately according to the protocol outlined by Aurand & Coelho (2005). Briefly, 6–7 l of prepared culture medium were poured into ~8 l borosilicate glass bottles, leaving approx. 20–25% headspace. Crude oil (~2–5 g l^{-1}) was added using a syringe, the bottle was covered to prevent light exposure, and the mixture was stirred at 200 rpm for 24 h at 16 ± 1°C. Immediately after removal from the stir plate, the WAF was drained from a spigot at the bottom of the bottle and aliquoted into 1 l culture vessels either undiluted or diluted with fresh uncontaminated medium (50% WAF, 50% medium) to a final volume of 800 ml. Samples for initial oil chemistry were taken directly from the WAF bottle before aliquoting into the culture vessels and stored in 1 l amber glass bottles at 4°C for analysis of total petroleum hydrocarbon (TPH) (C9–C44) (US EPA 2003), polycyclic aromatic hydrocarbon (PAH) (US EPA 2014), and saturated hydrocarbon (SH) (US EPA 2003) analyses by gas chromatography with flame ionization detection (GC-FID) and gas chromatography mass spectrometry (GC/MS) (Alpha Analytical).

Culture conditions

To explore the effects of oil exposure on the dominant phytoplankton present around the time of the Refugio oil spill, 4 separate batch culture experiments were conducted with 2 strains of *Pseudo-nitzschia australis*, 1 strain of *Emiliania huxleyi*, and 2 different oil sources. The 2 strains of *P. australis* were isolated in March 2015 from Monterey wharf (HAB 207) and Santa Cruz wharf (HAB 197), while the *E. huxleyi* strain (150604 A9) was isolated in June 2015 from the SBC. Multiple *P. australis* strains were used due to the loss of strain HAB 207 in culture after Experiment (Expt) 3 (see below) was conducted, which allowed for comparisons between strains with inherently different cellular DA content.

Experiments are described as follows: (1) *E. huxleyi* only, exposed to 1.105 and 2.21 mg l^{-1} TPH from Platform Holly oil; (2) *E. huxleyi* only, exposed to 0.72 and 1.44 mg l^{-1} TPH from the Refugio pipeline spill; (3) *P. australis* (HAB 207) only, exposed to 1.39 and 2.78 mg l^{-1} TPH from the Refugio pipeline spill; and (4) *E. huxleyi* and *P. australis* (HAB 197) using the same WAF, exposed to 1.185 mg l^{-1} TPH from the Refugio pipeline spill (Table 1). The goal of Expt 4 was to compare the response of the 2 species tested

Table 1. Descriptions of 4 independent oil exposure experiments conducted to test physiological responses of *Emiliania huxleyi* and *Pseudo-nitzschia australis*. Concentrations of total petroleum hydrocarbons (TPHs), polycyclic aromatic hydrocarbons (PAHs), and saturated hydrocarbons (SHs) shown represent the initial measured concentrations in the concentrated (100%) water accommodated fraction (WAF) before any dilution or addition of phytoplankton cells. Growth rates (μ) are displayed as the mean ± SD of triplicate cultures for each treatment. Asterisks represent a significant difference ($\alpha = 0.05$) in oil treatment growth rates compared to the control for each experiment. Data that was not measured is represented by a dash

Expt	Oil source	Species (strain)	Growth media	Oil added for 100% WAF (g oil l^{-1})	[TPH] 100% WAF (mg l^{-1})	[PAH] 100% WAF (mg l^{-1})	[SH] 100% WAF (mg l^{-1})	μ (d^{-1}) 100% WAF treatment	μ (d^{-1}) 50% WAF treatment	μ (d^{-1}) control treatment
1	Platform Holly 2012	*E. huxleyi* (150604 A9)	mod f	3.80	2.21	0.0531	0.017	−0.1 ± 0.1*	0.70 ± 0.04*	0.99 ± 0.09
2	Refugio pipeline spill	*E. huxleyi* (150604 A9)	mod f	4.25	1.44	0.0351	0.019	0.63 ± 0.07*	0.71 ± 0.07*	0.92 ± 0.04
3	Refugio pipeline spill	*P. australis* (HAB 207)	f/2	3.94	2.78	0.0385	0.027	−0.5 ± 0.2*	−0.3 ± 0.1*	1.0 ± 0.2
4	Refugio pipeline spill	*E. huxleyi* (150604 A9) *P. australis* (HAB 197)	f/2	2.59	2.37	0.0513	0.043	–	0.5 ± 0.3* −0.3 ± 0.1*	1.02 ± 0.01 0.8 ± 0.1

under the same WAF (and nutrient) concentrations, but due to sampling time, culture volume, and space limitation, intensive sampling was staggered by 1 d between *E. huxleyi* and *P. australis* (*P. australis* cultures were monitored for 1 d longer than *E. huxleyi*) and only 1 concentration of WAF was tested. Clonal batch cultures were inoculated from exponentially growing cultures and maintained in modified f medium (supplemented with 100 µM nitrate, 6.24 µM phosphate, and f/2 concentrations of trace metals and vitamins; e.g. Langer et al. 2006, Iglesias-Rodriguez et al. 2008) for *E. huxleyi* only experiments (Expts 1 and 2) or f/2 supplemented medium (Guillard & Ryther 1962) for the *P. australis* only experiment (Expt 3) and the *P. australis* and *E. huxleyi* experiment using the same WAF (Expt 4) prepared with filter-sterilized (0.22 µm Steritop filtration units, EMD Millipore) SBC seawater (Andersen 2005). The modified f medium was used because *E. huxleyi* has a relatively low nutrient requirement, and high phosphate concentrations have been shown to impact calcification (e.g. Paasche 1998). Silicate was not added or measured in Expts 1 or 2. Cultures were maintained at 16 ± 1°C under a 12:12 h light:dark cycle at ~120 µmol photons m^{-2} s^{-1} (EnviroGro T5 Hydrofarm). Experimental parameters were monitored in triplicate cultures of each treatment for 4 or 5 d to test short-term exposure in early exponential phase growth (to avoid additional stressors due to nutrient, light, or carbon limitation).

Cell density, growth rates, and nutrient utilization

Cell densities of each culture were determined every day of the experiments (except on Day 1 for *P. australis* in Expt 4) by light microscopy using a Sedgewick-Rafter counting chamber. Growth rates (µ, d^{-1}) for each replicate culture were determined by calculation of the slope of a linear fit of the natural log of cell density (cells ml^{-1}) versus day of the experiment. Time points that were not linear (suggesting a lag phase or approaching stationary phase) were removed from the fit and not used to calculate experiment-wide growth rates. Nutrient concentrations (nitrate + nitrite, phosphate, ammonium, and silicate [not measured in Expts 1 or 2]) were monitored throughout the experiments by sampling ~20 ml from each culture, filtering through 0.45 µm syringe filters, and storing at −20°C until flow injection analysis (QuikChem 8000, Zellweger Analytics). This work was conducted at the UCSB Marine Science Institute Analytical Laboratory.

Dissolved and cellular DA content

Dissolved DA samples were collected daily by filtering 2 ml aliquots through 0.45 µm syringe filters into cryovials, immediately flash frozen in liquid nitrogen, and stored at −80°C until analysis. Particulate DA samples were taken at 3 time points (initial, middle, and final) throughout the experiment by filtering 50–100 ml aliquots onto 25 mm glass fiber filters (EMD-Millipore), collecting the filters in 2 ml cryovials, flash freezing in liquid nitrogen, and storing at −80°C until analysis. Both dissolved and particulate DA samples were analyzed in the Kudela lab at the University of California, Santa Cruz, using well established methods. Briefly, DA analysis was conducted using an Agilent 6130 LC-MS system with an Agilent Zorbax Rapid Resolution column using an 8-point dilution series of CRM DA-f DA standards (National Research Council Canada) for calibration. Sample preparation followed Wang et al. (2007). The LC-MS was operated with a gradient elution of acidified water (0.1% formic acid) and acidified acetonitrile (0.1% formic acid). DA was identified by the presence of a 312 amu peak in positive scanning ion mode (SIM) with concentration determined by signal integration of the peak area and back-calculations based on the standard curve. Cellular DA, expressed in pg DA $cell^{-1}$, was determined by normalizing particulate DA to *P. australis* cell abundance.

Transparent exopolymer particle analysis

Duplicate or triplicate 20 ml aliquots were sampled for transparent exopolymer particle (TEP) analysis. Samples were filtered through 0.4 µm polycarbonate filters (Isopore, EMD Millipore) under gentle vacuum, stained with 0.5 ml of pre-calibrated Alcian Blue dye, and stored in 15 ml tubes at −20°C until analysis. Samples were analyzed colorimetrically as described by Passow & Alldredge (1995). Briefly, 5 ml of 80% sulfuric acid were added to the tubes containing the filter and allowed to sit for longer than 2 h with occasional gentle mixing. Samples were then transferred into cuvettes, and the absorbance at 787 nm was measured on a Genesys 10S Vis spectrophotometer. Absorbance values were converted to gum xanthan equivalents (determined by calibration with the Alcian Blue dye) and expressed as gum xanthan equivalents $cell^{-1}$ (GXeq $cell^{-1}$) after normalization by *E. huxleyi* or *P. australis* cell abundance.

Coccolith morphology and cellular $CaCO_3$

Scanning electron microscopy (SEM) was used to observe coccolith morphology. SEM samples were collected at the beginning and the end of each experiment by filtering 3–7 ml of culture onto a 13 mm 0.4 µm polycarbonate filter (Isopore, EMD-Millipore) and allowing filters to dry overnight at room temperature. Each filter was mounted on an aluminum stub and sputter-coated with gold prior to being observed with a Zeiss EVO 40 XVP scanning electron microscope at the Santa Barbara Museum of Natural History (CA, USA). For each replicate filter, a minimum of 50 coccospheres (cells with surrounding coccoliths) were imaged for analysis of coccolith morphology. Coccospheres were counted and recorded as either normal (containing ≤1 incomplete or malformed coccolith, e.g. Langer et al. 2011) or abnormal (containing >1 incomplete or malformed coccoliths, e.g. Langer et al. 2011).

Samples for cellular $CaCO_3$ were collected and processed following Iglesias-Rodriguez et al. (2008). Briefly, 100 ml aliquots were filtered through 47 mm, 0.2 µm polycarbonate filters (Isopore, EMD-Millipore), rinsed with a dilute ammonium hydroxide solution (pH ~9), and stored at −20°C before further processing. Approximately 50 ml of 0.1 M HNO_3 were added to the frozen filters and left for 24 h at room temperature to dissolve the $CaCO_3$ on the filter. The solution was then filtered through 0.45 µm syringe filters and analyzed for Ca^{2+} and Na^{2+} concentrations on a Perkin-Elmer Optima 7300DV inductively coupled plasma–optical emission spectrometer (ICP-OES) at the University of California, Riverside Environmental Sciences Research Laboratory. Sodium concentrations were used to correct for seawater contamination. Cellular $CaCO_3$ was determined by normalizing $CaCO_3$ concentration to *E. huxleyi* cell abundance.

Flow cytometry analysis

Aliquots of 9 ml were preserved with a buffered formaldehyde/glutaraldehyde solution (1% formaldehyde, 0.05% glutaraldehyde final concentration) and stored at −20°C until processing on a BD Influx Flow Cytometer (BD Biosciences) in the Iglesias-Rodriguez lab at the University of California, Santa Barbara. Identification of *E. huxleyi* cells and detached coccoliths (non-living calcified particles) were identified using chlorophyll fluorescence (cells only), polarization-sensitive detectors, and forward scatter signals (as in von Dassow et al. 2012). The ratio of detached coccoliths to cells was calculated for each culture replicate. Coccosphere and cell sizes were estimated by concurrently running size standard beads (Sphero™ particle size standard kit; Spherotech) and quality control beads (Ultra Rainbow Beads; Spherotech) with acidified (addition of dilute HCl) and unacidified samples. Cell volume estimates assumed cells were spherical in shape, and biovolumes were calculated by multiplying *E. huxleyi* cell volume by the culture cell density.

Bacterial abundance

Bacterial cell densities were measured through epifluorescence microscopy each day throughout the 4 experiments. Briefly, aliquots of 9 ml were preserved with formalin and stored at 4°C for <48 h, then filtered onto Irgalan Black stained 47 mm 0.2 µm polycarbonate filters (Isopore, EMD-Millipore). Addition of 0.5 ml of the nucleic acid stain DAPI (5 µg ml^{-1} solution) to each filter followed and was allowed to sit for 3 min before filtering the remaining volume. Filters were subsequently mounted onto microscope slides with immersion oil and stored in the dark at −20°C before counting cells with epifluorescence microscopy.

Statistical analyses

Several statistical tests were used to examine both within-experiment responses and across-experiment responses to oil exposure. Analyses were performed using R software (version 3.4.2) and JMP Pro 12 software. Data were initially examined for homogeneity of variances and normality of residuals to determine if parametric or non-parametric tests were necessary. If the assumptions of a parametric test were met, 1-way ANOVAs, repeated measures ANOVAs, linear regression analyses, or logistic regression analyses were implemented to examine differences between treatments at each time point measured. As necessary, the false discovery rate (FDR) post hoc test (best compromise between type I and type II errors) was used to identify significant differences (reported as q-values [FDR-adjusted p-values]) between each treatment. To compare trends across experiments, measurements of physiological parameters were normalized through division by the mean of the control replicates at each time point in each experiment and then averaged across all time points sampled. Nor-

malized values were grouped by control or oil treatments and compared using nonparametric confidence intervals for the median. Overall differences between control and oil groups were considered significant if the confidence intervals did not overlap at the 95% confidence level. Significant differences are reported as p-values <0.05.

RESULTS

Phytoplankton growth and oil chemistry

Across all experiments, growth rates (μ, d^{-1}) of *Pseudo-nitzschia australis* and *Emiliania huxleyi* were negatively affected by exposure to crude oil in the form of WAF (Figs. 1 & 2, Table 1). In all experiments, oil exposure completely inhibited the growth of *P. australis* (strains HAB 207 and HAB 197) ($\mu \leq 0$ d^{-1}). Growth rates of exponentially growing *E. huxleyi* cultures significantly decreased as TPH ($p < 0.0001$, $R^2 = 0.660$), PAH ($p < 0.0001$, $R^2 = 0.633$), and SH ($p < 0.0001$, $R^2 = 0.672$) concentrations increased. Growth of *E. huxleyi* was completely inhibited during exposure to Platform Holly crude oil WAF at high concentrations ([TPH] = 2.21 mg l^{-1}, [PAH] = 0.0531 mg l^{-1}, [SH] = 0.017 mg l^{-1}). Under the same initial WAF concentrations using Refugio spill oil in Expt 4, *E. huxleyi* cultures grew exponentially while *P. australis* (HAB 197) growth was inhibited. Nutrients were replete throughout the course of each experiment, so growth was not limited by availability of inorganic nutrients (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m603p061_supp.pdf).

Cell volumes of *E. huxleyi* were measured and culture biovolumes were calculated on Days 2 and 4 of Expts 1, 2, and 4 and compared across treatment (Figs. S1 & S2, Table S2). In Expt 1, cell volumes of the growth-inhibited high oil treatment were on average more than 16 μm^3 (Day 2) ($q = 0.001$) and 33 μm^3 (Day 4) ($q = 0.003$) larger than cells in the control treatment while the low oil treatment had cells that were more than 10 μm^3 larger than the control on Day 2 ($q = 0.0002$), but similar cell sizes to those in the control treatment on Day 4 ($q = 0.85$). For Expt 2, *E. huxleyi* cells in both oil treatments were consistently larger than those in the control treatment with cells in the high oil treatment and low oil treatment being more than 3 μm^3 ($q = 0.003$) and 2 μm^3 ($q = 0.005$) larger than control cells on Day 2 and on average 4.5 μm^3 ($q = 0.002$) and 3.9 μm^3 ($q = 0.002$) larger

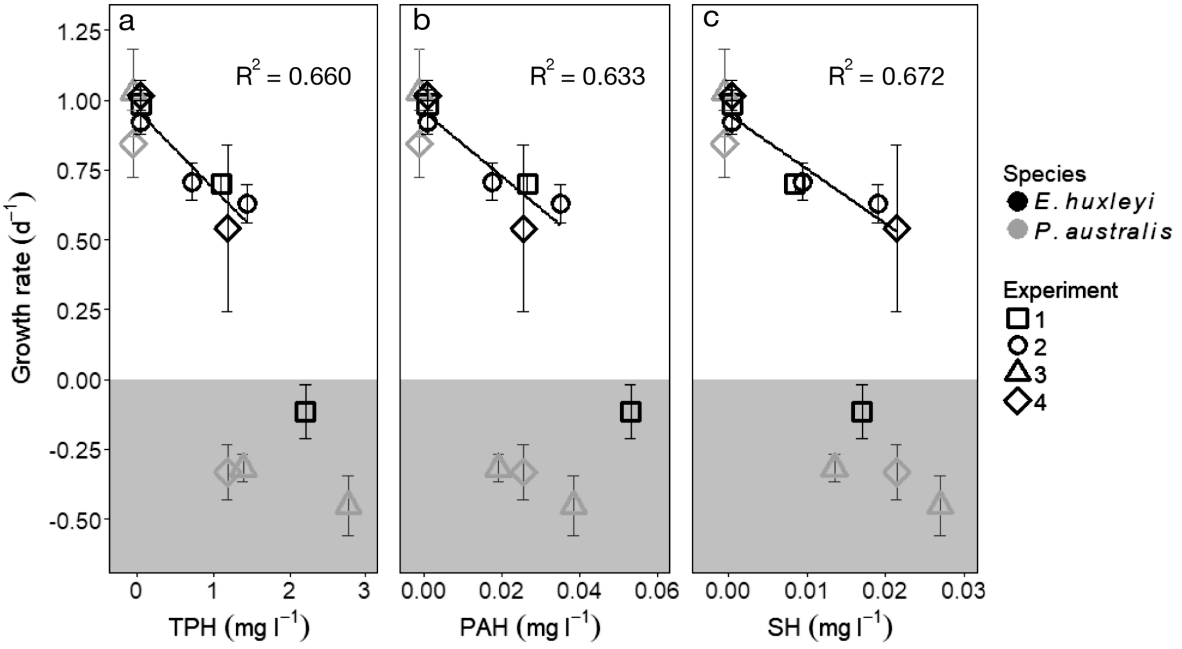

Fig. 1. Mean growth rates as a function of initial (a) total petroleum hydrocarbon (TPH), (b) polycyclic aromatic hydrocarbon (PAH), and (c) saturated hydrocarbon (SH) concentrations of triplicate cultures of *Emiliania huxleyi* (black) and *Pseudo-nitzschia australis* (grey) from 4 independent experiments; see Table 1 and the Materials and Methods for details of the experiments. Control treatments (no oil added) were assumed to have negligible TPH, PAH, and SH concentrations, but points are slightly offset to avoid overlapping data points. The grey shaded area represents treatments that had complete inhibition of growth. Linear regression fits (and R^2) of *E. huxleyi* triplicates (individual data points not presented) displaying exponential growth are shown. Error bars are ±1 SD (n = 3)

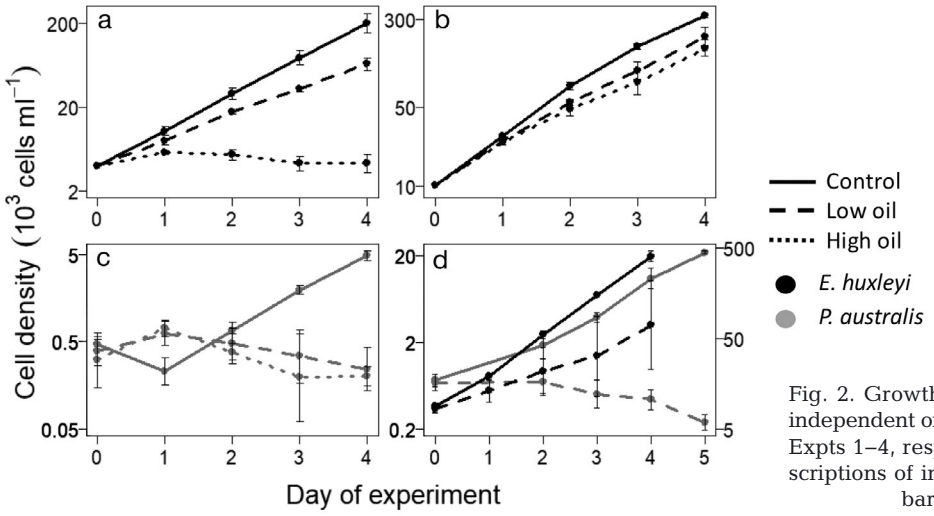

Fig. 2. Growth curves (log-scale) for the 4 independent oil exposure experiments. (a–d) Expts 1–4, respectively (see Table 1 for descriptions of individual experiments). Error bars are ±1 SD (n = 3)

than control cells by Day 4. On the other hand, Expt 4 did not show significant differences between the oil and control cell volumes on either Day 2 ($p = 0.12$) or Day 4 ($p = 0.080$). Although significant differences were not detected during all time points in all experiments, across all 3 *E. huxleyi* experiments, cell volumes in oil-exposed treatments were on average (median) 16% greater than control treatments ($p < 0.004$).

Biovolume calculations showed that although cell size was larger in oil-exposed treatments, the culture biovolume was still generally lower in oil-exposed treatments compared to control treatments (Fig. S2, Table S2). Specifically, control *E. huxleyi* cultures in Expt 1 had on average more than 1.4 ($q < 0.020$) and 3 ($q < 0.0029$) times more biovolume than the low and high oil treatments on Days 2 and 4, respectively. In Expt 2, the control treatment had more than 1.3 times more biovolume than the high oil and low oil treatments on Day 2 ($q = 0.010$) and 1.6 times more than the high oil treatment on Day 4 ($q = 0.015$). The control treatment in Expt 4 had consistently more biovolume than the oil treatment, with 1.8 times and 3.4 times more on Days 2 ($p = 0.024$) and 4 ($p = 0.015$), respectively. Normalized culture biovolumes calculated throughout all experiments confirmed that on average (median), culture biovolumes of oil-exposed treatments were 49% lower than control treatments ($p < 0.004$).

The initial WAF chemistry for the 4 experiments is shown in Table 1 and Figs. S3 & S4. The TPH ($p = 0.619$), PAH ($p = 0.326$), and SH ($p = 0.100$) concentrations did not appear to be correlated with the initial mass of oil added to make the WAF. The relative concentrations of measured compounds also varied between experiments even when using the same source oil, but naphthalene (parent compound + alkylated homologs) was always the dominant PAH, making up approximately 94–96% of the total measured PAH concentration.

Dissolved and cellular DA content in *P. australis* cultures

Dissolved DA normalized to cell abundance and cellular DA were compared across treatments at each sampled time point for both *P. australis* strains (Figs. 3 & 4, Table S2). For *P. australis* strain HAB 207 (Expt 3), dissolved DA was below the limit of detection (0.52 ng ml^{-1}) at the start of the experiment (T_0) but had measurable dissolved DA ranging from 1.60 to 2.04 ng ml^{-1} in all treatments for Days 1–4. One replicate in the control treatment on Day 2 as well as 1 replicate in the low oil treatment on Day 3 had dissolved DA concentrations below the detection limit and were not included in the analyses (substitution of values below the detection limit was avoided to limit bias; Helsel 2006). On Day 1, the control treatment had approximately 3 times higher dissolved DA $cell^{-1}$ than the oil treatments ($q = 0.028$) while by Days 3 and 4, the oil treatments had on average more than 4 ($q < 0.0012$) and 20 ($q = 0.010$) times higher dissolved DA concentrations per cell than the control, respectively. In *P. australis* strain HAB 197 (Expt 4), dissolved DA concentrations were not detected until Day 4 in both the control and oil treatments, and when normalized to cell abundance, dissolved DA $cell^{-1}$ was approximately 11 and 72 times higher in the oil treatment than the control on Days 4 ($p = 0.0019$) and 5 ($p = 0.0018$), respectively. Across both experiments, using 2 different strains of *P. australis*,

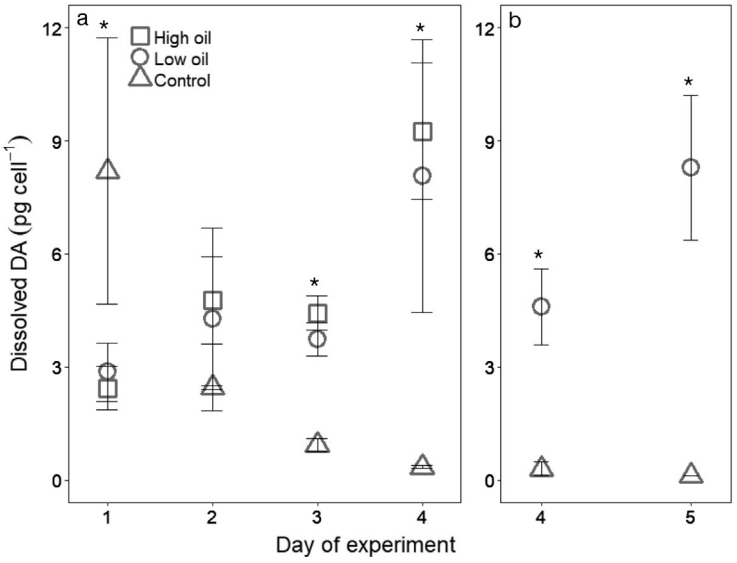

Fig. 3. Dissolved domoic acid (DA) normalized to cell abundance measured in the growth media over time in (a) Expt 3 and (b) (see Table 1 for descriptions of individual experiments). The days for which the data are not shown (Day 0 in Expt 3 and Days 0–3 in Expt 4) had dissolved DA concentrations below the limit of detection (0.52 ng ml^{-1}). Asterisks represent significant differences among treatments at each time point (α = 0.05). Error bars are ±1 SD (n = 3 or 2 [below detection limit values removed])

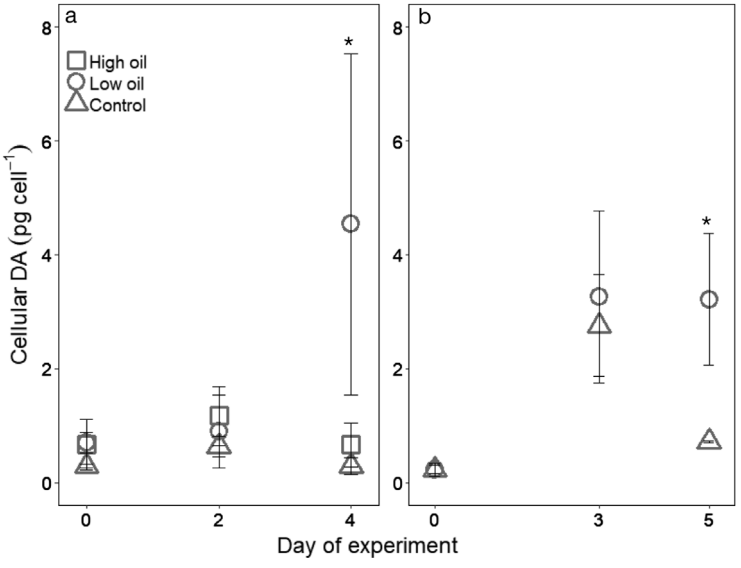

Fig. 4. Cellular domoic acid (DA) measured over time in (a) Expt 3 and (b) Expt 4 (see Table 1 for descriptions of individual experiments). Asterisks represent significant differences among treatments at each time point (α = 0.05). Error bars are ±1 SD (n = 3)

nonparametric confidence intervals for normalized dissolved DA cell^{-1} suggest that oil-exposed treatments of *P. australis* had on average (median) 10 times more dissolved DA cell^{-1} compared to control treatments ($p < 0.03$).

Cellular DA in *P. australis* HAB 207 (Expt 3) did not differ significantly between treatments except on Day 4 where the low oil treatment was on average more than 6 times higher than the control or high oil treatments ($p = 0.045$), although pairwise comparisons did not show significant differences between any 2 individual treatments. In *P. australis* HAB 197 (Expt 4), cellular DA in the oil treatment was 4.5 times higher than in the control treatment on Day 5 ($p = 0.020$). Across both experiments, nonparametric confidence intervals constructed with normalized cellular DA show that on average (median) cellular DA in oil-exposed treatments was 2.6 times higher than in control treatments ($p < 0.03$). The consistent effect of treatment across experiments using multiple strains shows that oil exposure increases cellular DA in *P. australis* under growth-inhibiting conditions.

TEP content in *P. australis* and *E. huxleyi* cultures

The concentration of cellular TEP in both *P. australis* and *E. huxleyi* was compared across treatments at multiple time points throughout the 4 experiments (Figs. 5 & 6, Table S2). Cellular TEP in *P. australis*

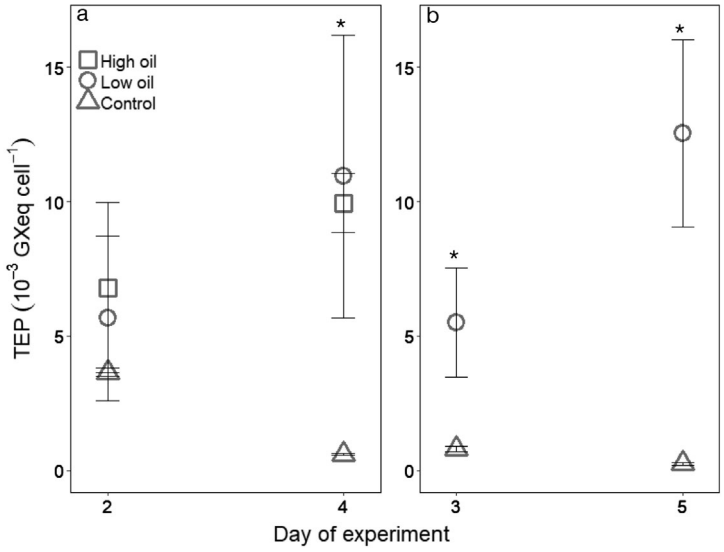

Fig. 5. Transparent exopolymer particle (TEP) concentration normalized to *Pseudo-nitzschia australis* cell abundance (gum xanthan equivalents cell^{-1}) for (a) Expt 3 and (b) Expt 4 (see Table 1 for descriptions of individual experiments). Asterisks represent significant differences among treatments at each time point ($\alpha = 0.05$). Error bars are ±1 SD (n = 3)

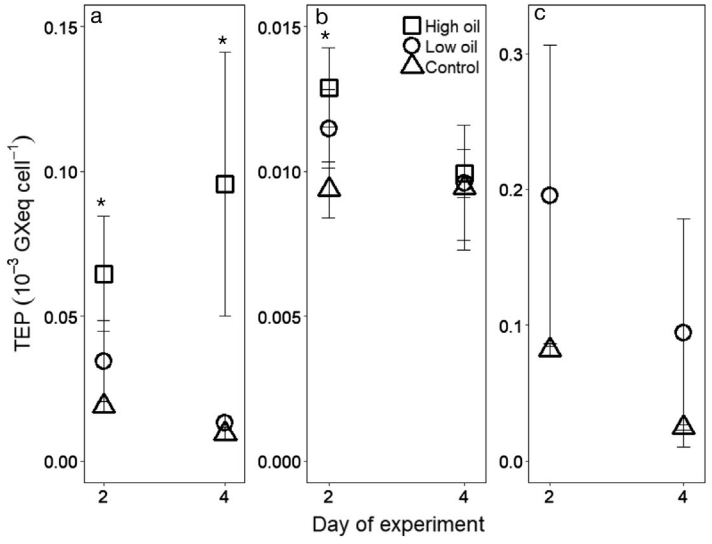

Fig. 6. Transparent exopolymer particle (TEP) concentration normalized to *Emiliania huxleyi* cell abundance (gum xanthan equivalents cell^{-1}) for (a) Expt 1, (b) Expt 2, and (c) Expt 4 (see Table 1 for descriptions of individual experiments). Asterisks represent significant differences among treatments at each time point ($\alpha = 0.05$). Error bars are ±1 SD (n = 3)

HAB 207 (Expt 3) in oil-exposed treatments was more than 16 times higher than the control treatment on Day 4 ($q = 0.015$) in contrast with Day 2, when no significant differences were observed ($p = 0.37$). For *P. australis* HAB 197 (Expt 4), cellular TEP in the oil-exposed treatment was approximately 7 times and 50 times higher on Days 3 ($p = 0.016$) and 5 ($p = 0.0036$), respectively, compared to the control treatment. Across both *P. australis* experiments, normalized cellular TEP in oil-exposed treatments was overall on average (median) 11 times higher than control treatments ($p < 0.03$).

For *E. huxleyi* in Expt 1, cellular TEP in the growth-inhibiting high oil treatment was more than 1.8 times higher compared to the control treatment on Day 2 ($q = 0.022$) and more than 7 times higher than the control ($q = 0.013$) and low oil treatment on Day 4 ($q = 0.013$) of the experiment, respectively. Cellular TEP in exponentially growing *E. huxleyi* cultures did not significantly differ between the oil and control treatments at most time points during the 3 experiments. The only significant difference between exponentially growing *E. huxleyi* cultures was on Day 2 during Expt 2 when the high oil treatment was 1.4 times higher than the control treatment ($q = 0.041$). Although statistical differences in *E. huxleyi* cellular TEP were not observed at every time point, nonparametric confidence intervals for normalized cellular TEP across all *E. huxleyi* experiments showed that on average (median), oil treatments had 35% higher cellular TEP than control treatments ($p < 0.004$) and even when excluding the growth-inhibited high oil treatment from Expt 1, significant differences are apparent at the 95% confidence level.

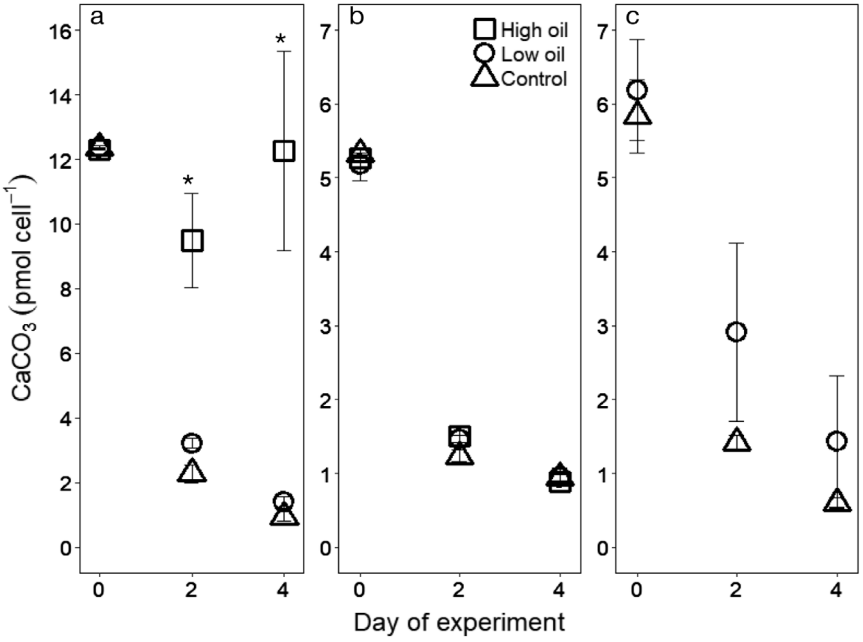

Fig. 7. Cellular calcium carbonate ($CaCO_3$) in *Emiliania huxleyi* cultures for (a) Expt 1, (b) Expt 2, and (c) Expt 4 (see Table 1 for descriptions of individual experiments). Asterisks represent significant differences among treatments at each time point ($\alpha = 0.05$). Error bars are ±1 SD (n = 3)

Cellular $CaCO_3$, coccolith morphology, and shedding of coccoliths by *E. huxleyi*

The amount of $CaCO_3$ cell^{-1} in *E. huxleyi* was measured in the 3 *E. huxleyi* experiments and compared across treatments (Fig. 7, Table S2). The growth-inhibited high oil treatment in Expt 1 had more than 3 and 8 times the concentration of $CaCO_3$ cell^{-1} than the low oil and control treatments on Days 2 ($q = 0.0002$) and 4 ($q = 0.0005$) of the experiment, respectively. The other experiments did not show significant differences in $CaCO_3$ cell^{-1} except for Day 2 of Expt 2, when the oil treatments appeared to have more $CaCO_3$ cell^{-1} than the control ($p = 0.038$), but pairwise comparisons did not show significant differences between any treatments. Across all experiments, $CaCO_3$ cell^{-1} was found to be on average (median) 44% greater in oil-exposed treatments compared to control ($p < 0.04$), but when the growth-inhibited treatment was excluded from the analysis, a difference between oil and control treatments was not apparent at the 95% confidence level. Quantification of abnormal coccosphere morphology (determined as coccospheres that contained 2 or more incomplete or malformed coccoliths) using SEM revealed that at the end of each *E. huxleyi* experiment, the proportion of abnormal coccospheres increased with increasing TPH concentration ($p < 0.001$) (Fig. 8c). The number

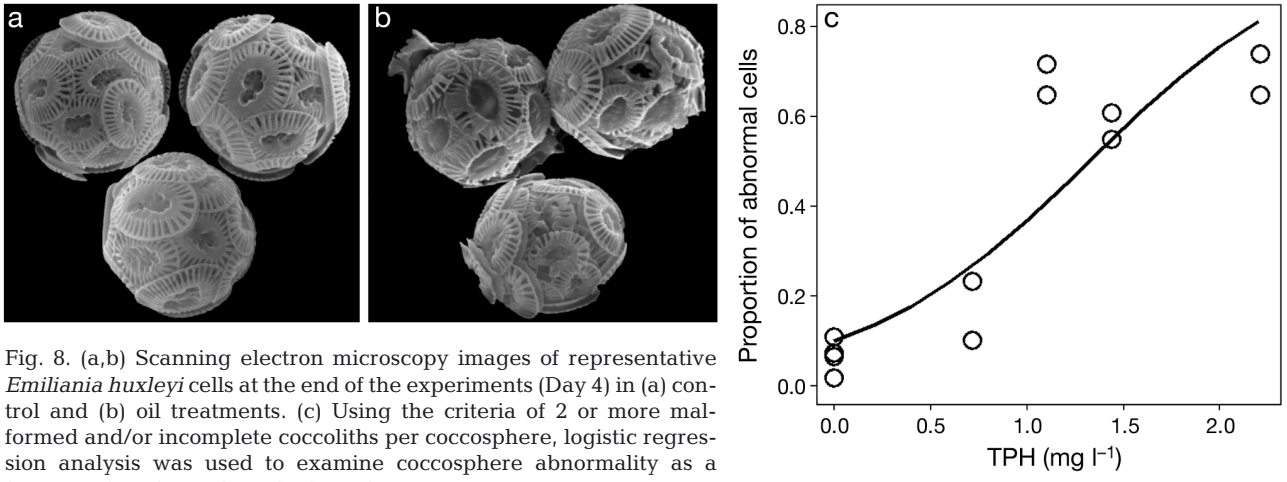

Fig. 8. (a,b) Scanning electron microscopy images of representative *Emiliania huxleyi* cells at the end of the experiments (Day 4) in (a) control and (b) oil treatments. (c) Using the criteria of 2 or more malformed and/or incomplete coccoliths per coccosphere, logistic regression analysis was used to examine coccosphere abnormality as a function of total petroleum hydrocarbon (TPH) concentration in Expts 1 and 2 (see Table 1 for descriptions of individual experiments)

of detached coccoliths cell^{-1} also appeared to be different between control and oil treatments at some time points throughout the *E. huxleyi* experiments (Fig. S5, Table S2). Specifically, on Day 2 of Expt 1 using Platform Holly oil, the low oil treatment had the highest number of detached coccoliths cell^{-1}, with 1.1 more detached coccoliths cell^{-1} than the control ($q = 0.020$) and 1.4 more detached coccoliths cell^{-1} than the high oil treatment ($q = 0.0015$). In Expt 4 using Refugio spill oil, the *E. huxleyi* treatment exposed to oil had 2.4 and 1.7 more detached coccoliths cell^{-1} than the control treatment on Days 2 ($p = 0.026$) and 4 ($p = 0.018$), respectively. Normalized coccolith:cell ratio measurements across all experiments showed that on average (median), oil-exposed treatments had 10% higher coccolith:cell ratios than control treatments ($p < 0.04$).

Bacterial abundance

Across all 4 experiments, bacterial abundances did not differ significantly between treatments (Fig. S6, Table S2). For the first experiment (*E. huxleyi* with Platform Holly oil), a repeated measures ANOVA failed to detect differences in bacterial abundances between treatments ($p = 0.58$), but there was a significant change over time ($p = 0.019$). Independent analysis of each time point showed that bacterial abundances were comparable between treatments at any time point (Table S2). In Expt 2 (*E. huxleyi* with Refugio spill oil), repeated measures ANOVA revealed no differences in bacterial abundances between treatments throughout the experiment ($p = 0.99$), but in agreement with the first experiment (with Platform Holly oil), bacterial abundance changed over time ($p = 0.0002$). For each day, this trend was consistent as there were no significant differences between treatments at any time point (Table S2). A repeated measures ANOVA for Expt 3 with *P. australis* and Refugio spill oil showed that bacterial abundances did not significantly differ either across treatments ($p = 0.82$) or through time ($p = 0.074$). Analysis at each time point was consistent with the results of the repeated measures ANOVA (Table S2). For Expt 4 (conducted with both *E. huxleyi* and *P. australis*), bacterial abundances were not significantly different between the oil and control treatments for either phytoplankton tested (*E. huxleyi*: $p = 0.18$, *P. australis*: $p = 0.80$) but both appeared to have a significant change in bacterial abundance over time (*E. huxleyi*: $p = 0.0021$, *P. australis*: $p = 0.0015$). Analysis of each day separately showed that for *P. australis* there were no differences in bacterial abundance at any time during the experiment, but for *E. huxleyi*, at the initial time point, the oil treatment appeared to have on average 2.4 times more bacteria than the control while bacterial abundance did not differ at any other time point (Table S2).

DISCUSSION

The results of the 4 independent experiments conducted in this study indicate short-term impacts of oil exposure on the physiology and morphology (*Emiliania huxleyi*) of the 2 dominant phytoplankton species blooming in the SBC around the time of the Refugio oil spill. Although extrapolating laboratory experiments to the natural environment is challenging due to the many interacting and complex factors governing physiology in natural phytoplankton populations, this study provides insight into the physiological responses of 2 ecologically and biogeochemically important species that were highly abundant during the Refugio oil spill.

Short-term responses of phytoplankton growth to oil exposure

Overall, we observed a decrease and/or inhibition of growth in *E. huxleyi* and *Pseudo-nitzschia australis* during exposure to oil in the form of WAF. Although increases in cellular size have the potential to offset decreases in growth rates in terms of biomass, biovolume measurements in *E. huxleyi* cultures suggest that even though cell sizes increased in the presence of oil, the overall biovolume of the population was still significantly lower in oil-exposed cultures compared to control cultures. Observations that bacterial densities in the cultures were not significantly different in oil-exposed treatments suggests that indirect effects on phytoplankton physiology due to growth of bacterial populations were unlikely to have significantly impacted our results. In addition, a follow-up study on *E. huxleyi* culture bacterial communities during oil exposure revealed that bacterial community composition did not appear to differ significantly between oiled and control treatments within the first 5 d of oil exposure (J. A. Bullington unpubl. data). Our results agree with many others that have shown a decrease in phytoplankton growth in the presence of oil (e.g. Østgaard et al. 1984, González et al. 2009, Brussaard et al. 2016), which may suggest that phytoplankton growth

in spill-impacted areas of the SBC was depressed with repercussions for food web dynamics and upper trophic levels. However, due to the fact that in some cases, especially at low oil concentrations, growth can increase (e.g. Parsons et al. 1976, Siron et al. 1991, Huang et al. 2011) or remain unaffected (e.g. Batten et al. 1998, González et al. 2013, Li et al. 2017), it is possible that on longer timescales, using different types of oil, and at different oil concentrations, the responses to oil exposure could differ. Also, due to the abundance of natural hydrocarbon seeps in the SBC, locally adapted phytoplankton may respond differently to oil compared to strains from other locations.

It is common to observe a shift in phytoplankton community structure in mesocosm and microcosm experiments exposed to oil pollution (González et al. 2013, Brussaard et al. 2016, Li et al. 2017) as well as in the few field studies that have available information on phytoplankton communities before and after oil pollution events (Parsons et al. 2015, Brussaard et al. 2016). Differential sensitivities of phytoplankton groups or species have also been demonstrated in experiments on individual phytoplankton populations (e.g. Echeveste et al. 2010, Hook & Osborn 2012, Özhan & Bargu 2014b). Here, we found that at the same oil concentrations (the same WAF), *E. huxleyi* cultures showed growth while *P. australis* cultures did not. Our results are consistent with other studies that have found that diatoms tend to be more sensitive to oil exposure than other groups (e.g. Harrison et al. 1986, Siron et al. 1991, Nomura et al. 2007), while smaller phytoplankton taxa are more resistant (Huang et al. 2011, González et al. 2009). On the other hand, some studies have revealed contradictory results to these general trends. For example, Sargian et al. (2007) found that smaller phytoplankton (picophytoplankton) were more sensitive to oil exposure than larger nanophytoplankton, and Echeveste et al. (2010) showed that phytoplankton with larger cell sizes had higher toxicity thresholds to PAH exposure than smaller cells. Additionally, some studies have suggested that diatoms can be more resistant to oil exposure than other groups (e.g. González et al. 2009, Ozhan & Bargu 2014a), and Ozhan & Bargu (2014a) showed that under nutrient-limited conditions in microcosm experiments, *Pseudo-nitzschia* spp. were more tolerant to crude oil than other pennate diatoms. Although many other factors are likely to influence shifts in the phytoplankton community structure, our results suggest that in the presence of oil, *E. huxleyi* could have a competitive advantage over *P. australis*, and we cannot discard the possibility that the oil spill may have created a competitive niche for *E. huxleyi* in the SBC, where it formed the first ever reported SBC *E. huxleyi* bloom just 2 wk after the spill (P. G. Matson unpubl. data). However, 2015 proved to be an anomalous year for oceanic conditions in the California Current system with the influence of the 'warm blob' (Bond et al. 2015) and El Niño, and therefore other factors also likely influenced *E. huxleyi* success (Cavole et al. 2016, McCabe et al. 2016, Zaba & Rudnick 2016). A shift in the phytoplankton community from toxic diatoms to smaller calcified coccolithophores has implications for the local ecosystem including decreasing the productivity of an important HAB species in the California Current (while simultaneously increasing cellular DA content) and the alteration of food web dynamics.

Variability of phytoplankton responses to oil exposure

The chemistry and concentrations of WAF as well as the methods of oil addition are important factors for phytoplankton responses to oil exposure. The decrease in *E. huxleyi* growth with increasing TPH concentrations observed in our study is in agreement with results for other phytoplankton taxa (e.g. Adekunle et al. 2010, Özhan et al. 2014a, Li et al. 2017). Overall concentrations of PAHs and SHs showed a similar trend, with lower *E. huxleyi* growth at higher concentrations, but for the *E. huxleyi* experiment using Platform Holly oil (Expt 1), the SH concentration in the growth-inhibiting high oil treatment was lower than in the other experiments that supported exponential growth in *E. huxleyi*. This suggests that SHs are unlikely to be solely responsible for the overall toxicity of the WAF, and other components of the oil may have greater impacts on the decrease or inhibition of phytoplankton growth.

As a group of compounds, PAHs have been suggested to be particularly toxic due to their strong adsorption affinity for particulate material, DNA-damaging properties (Gelboin 1980), and interaction with cellular membranes (Neff 1979). The WAF made with Platform Holly oil used in the first experiment on *E. huxleyi* had 5 additional high molecular weight PAH compounds that were not detected in the experiments conducted with Refugio spill oil (Fig. S3). It is therefore possible that these compounds may have caused the observed inhibition of growth by *E. huxleyi* in the high oil treatment. However, the overall PAH concentration and naphthalene (parent compound + alkylated homologs), as the dominant PAH

measured in all WAFs, were also higher in the Platform Holly WAF, while the *E. huxleyi* growth trends were otherwise comparable across experiments using different oil. This suggests that there may have also been a PAH (or naphthalene) concentration threshold beyond which growth was inhibited. There was no correlation between the amount of oil added to make the WAF and the WAF chemistry (e.g. TPH, PAH, or SH concentrations) (Table 1). This is consistent with findings by Hook & Osborn (2012), who suggested that although the individual hydrocarbon compounds do not seem to be saturated, the total hydrocarbon capacity of the seawater may be saturated. Our values of TPH differed across experiments even with the same oil type, and therefore unmeasured hydrocarbon compounds (e.g. volatile, low molecular weight compounds) or other factors (potentially small differences in stirring speed and time, WAF bottle headspace, and temperature across experiments) may have influenced the solubility of the hydrocarbons measured in our study. Interestingly, Hook & Osborn (2012) found that phytoplankton growth did not correlate with TPH or PAH concentrations but rather with the oil loading concentration. They suggested that the observed toxicity in their study must have been due to unidentified compounds within the WAF rather than the PAHs or overall TPHs. This is in contrast to the results of our study, where it appears that the measured groups of hydrocarbon compounds, especially the TPHs and PAHs, controlled the toxicity of the WAF.

Unfortunately, seawater oil chemistry measurements could not be made in the field during the time of the Refugio oil spill, and so direct comparisons to the magnitude, extent, and overall time of oil exposure in the SBC are not possible. However, information on *in situ* TPH and PAH concentrations is available for other oil spill scenarios, which can help provide guidance for our study. In this study, concentrations of TPHs and PAHs ranged from 0.72–2.78 mg l^{-1} and 18–53 µg l^{-1}, respectively, which is higher than field observations for some spills such as the 'Prestige' spill (max PAH = 5.8 µg l^{-1}; González et al. 2006), the 'Baltic Carrier' spill (max PAH = 1.45 µg l^{-1}; Pécseli et al. 2003), and the 'Exxon Valdez' spill (max PAH <1.0 µg l^{-1}; Neff & Stubblefield 1995), but within the observed concentrations for other spills such as the 'Volgoneft-248' spill (max TPH = 2.17 mg l^{-1}; Taş et al. 2011), the 'North Cape' spill (max TPH = 3.94 mg l^{-1}, max PAH = 115 µg l^{-1}; Reddy & Quinn 1999), and the Deepwater Horizon (i.e. Macondo) oil spill (max TPH = 11400 mg l^{-1}, mean TPH = 202 mg l^{-1}, max PAH = 1231 µg l^{-1}, avg PAH = 47 µg l^{-1}; Sammarco et al. 2013). Additionally, dissolution and solubility of oil in seawater varies due to many factors such as water temperature, pH, salinity, oil type (e.g. viscosity), and physical processes (e.g. mixing and wave action) (Rice et al. 1976), making it difficult to estimate or compare seawater concentrations across oil spill scenarios. Both the Refugio and Platform Holly oil originating from the Monterey Formation are relatively heavy (calculated API gravity = 23° [Refugio] or 18° [Platform Holly]) and are therefore less soluble in seawater than lighter oils such as the Macondo oil (API gravity = 37°; SL Ross Environmental Research 2010). However, the oil from the 'Prestige' and 'Volgoneft-248' spills were both heavy oils with API gravity around 11° (Otay & Yenigun 2000, Castanedo et al. 2006), and given that the concentrations used in our study are comparable to some measured in the field, it is possible that in some areas near the spill, phytoplankton in the SBC were exposed to concentrations relevant to this study during the Refugio oil spill.

Dissolved and cellular DA content in *P. australis* cultures

This is the first study to explore the effect of oil exposure on DA content in *P. australis* cultures, and here we showed that 2 *P. australis* strains with inherently different cellular DA content generally appeared to produce more DA $cell^{-1}$ in growth-inhibited cultures exposed to oil than when growing in the absence of oil (Figs. 3 & 4). In a study by Özhan & Bargu (2014b), the effects of oil exposure on toxin production in dinoflagellates and a raphidophyte revealed that low oil levels enhanced intracellular toxin concentrations, although when growth was inhibited by high oil concentrations, intracellular toxin concentrations were lower or not significantly different than the control. DA production in *Pseudo-nitzschia* spp. has generally been shown to increase with decreasing growth rates (Pan et al. 1998, Thorel et al. 2014) associated with nutrient limitation (specifically phosphate, silicate, and iron) (e.g. Pan et al. 1998, Maldonado et al. 2002, Santiago-Morales & García-Mendoza 2011) and increasing pH (Lundholm et al. 2004). Different sources of organic and inorganic nitrogen have also been shown to affect both growth rate and DA cellular content but growth rates were not correlated with cellular DA (Martin-Jézéquel et al. 2015). Here, nutrients were not limited in any culture throughout the experiments, but growth was inhibited by oil exposure, and, as others

have suggested, this may have contributed to increased DA cellular content due to excess photosynthetic energy from arresting cell division (e.g. Pan et al. 1998, Mos 2001). It is also possible that oil addition altered the seawater chemistry directly (e.g. adding organic sources of nitrogen) or indirectly (e.g. bacteria remineralization and respiration) so that DA content was indirectly affected. Whether cellular and dissolved DA content might differ in exponentially growing *P. australis* cultures is unclear, but our results revealed that even though growth was inhibited by oil exposure, *P. australis* cells appear to continue to produce DA. Although inhibition of growth and competition by other phytoplankton species may have reduced the SBC *Pseudo-nitzschia* populations at the time of the Refugio spill, oil exposure could have resulted in a short-term increase in DA concentrations in the field, particularly if populations with high cell densities were present immediately preceding or at the time of the spill. A closure in commercial and recreational fisheries was enacted immediately following the Refugio spill, and the fisheries remained closed for 6 wk due to potential toxicity from the oil directly (www.noozhawk.com/article/santa_barbara_county_fishery_closures_lifted_after_refugio_oil_spill). However, shortly after the Refugio spill, high levels of DA also caused the closure of bivalve shellfish, anchovy, sardine, and crab fisheries in Santa Barbara County from 3 July to 13 November (bivalves and small finfish) or 31 December 2015 (Dungeness and rock crab) (California Ocean Science Trust 2016). Elevated levels of DA have historically been an issue in the SBC (Anderson et al. 2006, 2008, Sekula-Wood et al. 2011), and 2015 also proved to be an anomalous year for the entire US West Coast with unprecedented *Pseudo-nitzschia* blooms and DA production from California to Alaska (McCabe et al. 2016). However, from our study, it is not possible to determine the extent to which the oil impacted overall DA production in the SBC and the resulting fishery closures.

TEP content in *E. huxleyi* and *P. australis* cultures

TEPs are important components of carbon pools in marine and freshwater environments, where they can play a large role in biogeochemical cycling and the structuring of food webs (Passow 2002). Due to the sticky nature of TEPs, they are efficient facilitators of particle aggregation. Production by phytoplankton was suggested as one of the possible mechanisms for the formation of large amounts of oil-incorporated marine snow observed after the Deepwater Horizon oil spill (Passow et al. 2012). Here we showed that overall, cellular TEP for both *P. australis* and *E. huxleyi* across all experiments appeared to increase in oil-exposed treatments. This is consistent with other findings showing that cellular TEP content, as a carbon overflow mechanism, increases as growth rates decrease (Engel et al. 2002) while it has been suggested to be a response to physiological stress (e.g. Passow & Alldredge 1995, Moriceau et al. 2007, Kahl et al. 2008). Given that TEPs are known to enhance sedimentation and export of matter to the sea floor (Passow et al. 2001, Seebah et al. 2014), an increase in the amount of phytoplankton-derived TEP during the Refugio oil spill may have influenced the fate of the spilled oil. Specifically, high abundances of phytoplankton cells combined with sticky TEP could have been aggregated with oil droplets (as in Passow et al. 2012), allowing oil to sink out of the surface ocean where it could eventually be deposited onto the ocean floor.

Production, morphology, and shedding of $CaCO_3$ by *E. huxleyi*

Calcification in coccolithophores is of great significance for the carbon cycle through both the formation of coccoliths and the role of the high density $CaCO_3$ for transporting organic matter to depth. The formation of 'white waters' as a result of the production and shedding of coccoliths has also been suggested to impact higher marine trophic levels. For example, *E. huxleyi* blooms in the Eastern Bering Sea have coincided with widespread seabird mortality (Baduini et al. 2001) and poor salmon runs (Vance et al. 1998), while visual predators and zooplankton grazers seem to avoid bloom areas (Eisner et al. 2005). Here we showed that although the amount of $CaCO_3$ per *E. huxleyi* cell did not vary significantly between exponentially growing cultures, exposure to oil impacted the appearance and shedding of *E. huxleyi* coccoliths. Specifically, oil exposure increased the relative abundance of cells that had abnormal (containing malformed and/or incomplete coccoliths) coccospheres and the relative number of detached coccoliths $cell^{-1}$. This suggests that oil exposure is interfering with the cellular process of calcification while not inhibiting coccolith formation. Malformed coccoliths have been proposed to be detrimental to the overall fitness of the cell (Rosas-Navarro et al. 2016), but because it is unclear whether the process of calcification, the product of calcification, or both

are important for coccolithophore fitness, it is difficult to determine how malformation of coccoliths may impact the cell overall. Coccolith shedding or detachment also varies across *E. huxleyi* strains and environmental conditions, where it is dependent on growth rates and growth phase (e.g. Balch et al. 1993, Chow et al. 2015, Matson et al. 2016). The detachment of coccoliths in the field can have a large influence on particle aggregation and flux as well the optical properties of the water column. Due to the small size of individual coccoliths, detachment allows the free-floating coccoliths to remain suspended in the surface ocean for longer than if they were attached to cells. This leads to the accumulation of these tiny calcite particles in the surface ocean and an increase in turbidity with implications for visual predators. The accumulation of coccoliths in the surface ocean also increases the probability of particle aggregation. Any aggregates that incorporate enough of the dense $CaCO_3$ particles will be efficiently exported to depth with implications for the fate of the oil and the impacts on other organisms throughout the water column.

CONCLUSIONS

It is difficult to determine the effects of an unexpected oil pollution event in the marine environment due to the clear dependence on, among other factors, the physical conditions at the time of the spill, timing of the spill, initial microbial communities present, and the amount and method of oil addition. However, it is important to gain a better understanding of biological responses to these events so that management, clean-up, and future prevention can be optimized. This study focused on the 2 dominant phytoplankton species present around the time of the Refugio oil spill to elucidate their physiological responses to oil exposure. This is the first study to test a representative DA producer, *P. australis*, or a calcifying, bloom-forming coccolithophore, *E. huxleyi*, in response to oil exposure, and our findings provide insight into potential implications of oil spills on phytoplankton communities like those in the SBC. Food web structure and function in the SBC was likely impacted, as oil appeared to disproportionately decrease growth of the tested phytoplankton species, potentially leading to a decline in productivity and shifts in the phytoplankton community composition. Other physiological changes observed in DA content, calcification, and cellular TEP could also have implications for food web structure and function, ecosystem and human health, and the fate of the spilled oil in the environment. This study highlights the need for more comprehensive research into oil spill effects across ecosystem components and trophic levels because the direct and indirect effects, as well as the interactions between individual phytoplankton populations and other parts of the ecosystem, are currently unclear. This study also brings to light further questions regarding the possibility for oil spills to select for oil-adapted groups in areas with natural hydrocarbon seeps, and how seep oil may have a chronic impact on phytoplankton communities and their physiology.

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