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# A ribosomal sequence-based oil sensitivity index for phytoplankton groups

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ABSTRACT

Species-level variability has made it difficult to determine the relative sensitivity of phytoplankton to oil and mixtures of oil and dispersant. Here we develop a phytoplankton group sensitivity index using ribosome sequence data that we apply to a mesocosm experiment in which a natural microbial community was exposed to oil and two oil-dispersant mixtures. The relative sensitivity of four phytoplankton taxonomic groups, diatoms, dinoflagellates, green algae, and Chrysophytes, was computed using the log of the ratio of the number of species that increase to the number that decrease in relative abundance in the treatment relative to the control. The index indicates that dinoflagellates are the most sensitive group to oil and oil-dispersant treatments while the Chrysophytes benefit under oil exposure compared to the other groups examined. The phytoplankton group sensitivity index can be generally applied to quantify and rank the relative sensitivity of diverse microbial groups to environmental conditions and pollutants.

## 1. Introduction

Millions of tonnes of petroleum enter the marine environment each year, both from natural seeps and as a result of anthropogenic events (Farrington, 2013; Transportation Research Board, National Research Council, 2003). During oil spill events, additional petrochemicals in the form of dispersants may be added to break up large masses of oil and help remove it from the surface and coastal ocean. During the 2010 Deepwater Horizon oil-spill event in the Gulf of Mexico, an estimated 4–6 million barrels of sweet crude oil was released into the marine environment (Crone and Tolstoy, 2010; McNutt et al., 2012) and ~7.9 million liters of the dispersant Corexit 9527 and 9500A was added to mitigate some effects of the oil spill (Kujawinski et al., 2011). The impact of this oil spill and the application of the dispersant on the marine ecosystem are still being evaluated.

Phytoplankton are a crucial component of marine ecosystems, forming the base of the food web and performing about half of global primary production (Behrenfeld et al., 2009). Evidence is accumulating to show that oil has a wide range of effects on phytoplankton, ranging from highly destructive (Østgaard et al., 1984) to possibly enhancing growth (Özhan et al., 2014) and photosynthesis (Hu et al., 2011; Tang et al., 2019) in some species. These effects are known to vary from species to species, although little is known about the biological basis for these differences (Bretherton et al., 2018). Chemical dispersants facilitate the formation of smaller oil droplets and can accelerate oil degradation by bacteria (Bælum et al., 2012; Chakraborty et al., 2012; Lindstrom and Braddock, 2002) but can also increase the bioavailability of the toxic components of oil to phytoplankton and other marine organisms (Wolfe et al., 1998, 2000). Phytoplankton can play a major role in exporting oil from the surface to the deep sea. Phytoplankton, other microbes, and mineral particles can form aggregates with oil, termed marine oil snow (MOS), that can sink rapidly to the sea floor. Evidence suggests that 15–30% of the oil from the Deepwater Horizon event was exported from the surface to the ocean floor and that phytoplankton MOS played a role in this sedimentation (Chanton et al., 2015; Passow and Ziervogel, 2016; Romero et al., 2017; Yan et al., 2016). More knowledge of the effects of oil and dispersant on phytoplankton community structure will help inform our understanding of the consequences for the base of the food web and processes that degrade and remove oil from the surface ocean.

After the Deepwater Horizon spill, the relative abundance of all photoautotrophic groups (apart from cyanobacteria) declined in the coastal waters of Louisiana (Parsons et al., 2015). In contrast, the presence of oil from the Deepwater Horizon spill and hydrocarbon seeps

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has been shown to be correlated with higher levels of phytoplankton biomass (chlorophyll) in surface waters of the Gulf of Mexico (D'souza et al., 2016; Li et al., 2019). Community-level and single species experiments indicate that phytoplankton responses to oil and dispersant exposure are highly varied (Bretherton et al., 2018, 2019; González et al., 2009; Harrison et al., 1986; Hook and Osborn, 2012; Özhan et al., 2014). Monoculture studies have demonstrated that while some phytoplankton species are sensitive to oil (Deasi et al., 2010), some are relatively insensitive (Garr et al., 2014), or even grow better in the presence of hydrocarbons (Bretherton et al., 2018; Özhan et al., 2014). The addition of dispersant and ecological interactions can further complicate phytoplankton responses to oil: altering toxicological responses, grazing pressure (Gemmell et al., 2018; Ortmann et al., 2012) and competition with bacteria for mineral resources (Fleeger et al., 2003; Severin and Erdner, 2019).

Marine eukaryotic plankton are extremely diverse, consisting of approximately 150,000 operational taxonomic units (de Vargas et al., 2015), making it impossible to assess the toxicological and ecological responses of the majority of individual species to oil. Typically, predictive models of phytoplankton cluster species into groupings based on taxonomy and similarities in physiology and ecological or biogeochemical role in the ecosystem (Allen and Polimene, 2011; Flynn et al., 2015; Irwin and Finkel, 2018). Characteristics of phytoplankton groups are frequently defined from experimental work on a relatively small number of species. It has been suggested that smaller-sized phytoplankton groups may be more sensitive to oil exposure due to their higher surface-area to volume ratios (Echeveste et al., 2010, 2011). Other studies indicate that diatoms are relatively unaffected by hydrocarbon exposure compared to other taxonomic groups, and therefore often dominate phytoplankton communities treated with oil (Bretherton et al., 2019; Gilde and Pinckney, 2012; González et al., 2009; Koshikawa et al., 2007). However, this is not always the case, and some studies have found that diatoms are more susceptible to oil exposure than phytoflagellates (Adekunle et al., 2010; Jung et al., 2010; Taş et al., 2011). There is also some evidence that some chlorophytes and euglenophytes are resistant to oil (Gilde and Pinckney, 2012; Sargian et al., 2007) and dispersed oil (Bretherton et al., 2019).

Clearly more work and a new approach is required to obtain a synoptic view of the relative sensitivity of phytoplankton taxonomic or functional groups to oil and oil-dispersant exposure. Molecular methods are revolutionizing our ability to assess the response of microbial communities to pollutants such as oil. Assessments of changes in microbial diversity rely upon constantly-changing and incomplete databases, meaning that it is difficult to know how well we can assess diversity, especially across taxonomic groups because of uneven representation of microbial taxonomic groups within databases relative to the environment. Here we describe a ribosome sequence-based sensitivity index for higher-level taxonomic groups that is less sensitive to these biases. We apply this approach to a mesocosm experiment in which a natural Gulf of Mexico plankton community is exposed to oil and oil-dispersant mixtures for three days. We used RNAseq data collected for a separate metatranscriptome study, but widely-available amplicon data could be used. First we quantify the relative abundance of species within the mesocosms based on 18S rRNA reads. We then quantify the relative sensitivity of phytoplankton species within taxonomic groups to oil treatments relative to the control using the change in relative abundance. Finally, we quantify the sensitivity of phytoplankton groups to oil treatments by the log ratio of the number of species that increase in relative abundance to the number that decrease in relative abundance within each taxonomic group in each treatment relative to the control. Our goal is to develop a molecular approach to quantify how large numbers of species within taxonomic groups respond to oil and oil-dispersant mixtures in conjunction with metatranscriptomic and metagenomic analyses.

## 2. Methods

### 2.1. Mesocosm experiment

Natural seawater was collected from a pipeline situated ~1 km off the coast of Galveston, Texas on 17 October 2015 and transferred to a holding tank at Texas A&M University at Galveston (TAMUG). The seawater was charcoal filtered to remove larger particles, but not sterilized, and then used to prepare the three different treatments: a water accommodated fraction of oil (WAF), a chemically enhanced water accommodated fraction of dispersant and oil (CEWAF), a 10-fold diluted CEWAF (DCEWAF), and a control (seawater only). WAF is a water-oil mixture. CEWAF is a water-oil mixture with an added chemical dispersant (Corexit) that acts to decrease the size of oil droplets and increase the equivalent oil concentration. DCEWAF is a diluted version of CEWAF. The WAF and CEWAF were prepared as described in Wade et al. (2017). Briefly, the WAF was prepared by mixing 5 mL of Macondo surrogate oil (a sweet crude oil that is representative of the oil spilled during the Deepwater Horizon incident) every 30 min for 2.5 h into 130 L of natural seawater in a baffled recirculating tank. The mixture was gently mixed for 24 h from the initial addition of oil and then transferred via an osmotic pump into the mesocosm tanks. To prepare the CEWAF, Corexit (the primary dispersant used during the Deepwater Horizon incident) was mixed with the Macondo surrogate oil in a ratio of 1:20 before being added to 130 L of seawater in the same manner described for WAF preparation. After 24 h of mixing, a volume of the CEWAF was diluted with the original natural seawater by a factor of 10 to produce the DCEWAF. Both the CEWAF and DCEWAF were then transferred to treatment tanks via an osmotic pump. All work was conducted in dim light at room temperature. Each treatment was prepared in triplicate 100 L mesocosm tanks to a final volume of 87 L. The estimated oil equivalents (EOE) were determined by fluorescence, after Wade et al. (2011). At the start of the experiment, the EOE was 0.26 mg $L^{-1}$ ( ± 0.01 mg $L^{-1}$) in the WAF tanks, 41.5 mg $L^{-1}$ ( ± 3.4 mg $L^{-1}$) in the CEWAF tanks, and 2.8 mg $L^{-1}$ ( ± 0.5 mg $L^{-1}$) in the DCEWAF tanks. Full details are available in Doyle et al. (2018).

Plankton were collected from a dock at TAMUG using tow nets (≥63 μm nylon mesh) and transferred to polycarbonate bottles after being pre-filtered with a 115 μm mesh to exclude larger grazers including shrimp and cnidaria. Immediately prior to beginning the experiment, 2 L of the plankton "soup" was added to each mesocosm tank. This community was added to the microbial community left in the filtered seawater that was used to fill the tanks and make the WAF, CEWAF, and DCEWAF. The tanks were illuminated with full spectrum fluorescent lamps (Sylvania GRO-LUX) at an intensity of ~50 μmol $m^{-2}$ $s^{-1}$ under a 12:12 h light:dark cycle. Tanks were open to the atmosphere and maintained at ambient room temperature (21 °C).

### 2.2. RNA sampling

RNA was harvested from each of the 12 mesocosm tanks (3 replicate tanks for each treatment, control, WAF, CEWAF, DCEWAF) 72 h after the initiation of the experiment. Several hundred mL (250 to 4000 mL) were rapidly and gently filtered onto two 47 mm, 0.8 μm polycarbonate filters. Total filtration time was limited to 20 min. It took more time to filter water from the CEWAF tanks, and therefore less volume was filtered from this treatment than the WAF and DCEWAF treatments. The filters and denaturating solution (Ambion Simply RNA) was added to Y-matrix bead beater tubes (MoBio). The samples were lysed using a SuperFastprep2 bead beater (30 s at the maximum setting) and immediately stored in a −80 °C freezer. RNA was extracted by exposing samples immediately after thawing to two additional 30 s rounds in the SuperFastprep2 bead beater. The Ambion Total RNA kit (ThermoFisher AM1910) was used to extract RNA, followed by DNA removal with the Ambion Turbo DNAfree kit (ThermoFisher AM1907) as per the

**Table 1**
Phytoplankton group responses to oil and oil-dispersant treatments using the full-length 18S sequences. For each group and treatment, the table reports the total number of species detected, the number of species increasing or decreasing in relative abundance in the treatment relative to the control, the number increasing or decreasing statistically significantly in relative abundance (*t*-test, $p < 0.05$) in treatment relative to the control, and the sensitivity index. The sensitivity index is equal to the log of the ratio of the number of increasing to decreasing species. Confidence intervals (95%) are reported in parentheses below each number.

| Phytoplankton group | Treatment | Total species | Increase | Decrease | Significant increase | Significant decrease | Sensitivity index |
|---|---|---|---|---|---|---|---|
| Diatoms | WAF | 402 | 167 | 230 | 10 | 26 | −0.32 |
| | | | (156, 171) | (223, 238) | (9, 15) | (18, 27) | (−0.41, −0.27) |
| | DCEWAF | | 271 | 130 | 34 | 16 | 0.73 |
| | | | (262, 275) | (124, 136) | (30, 40) | (11, 18) | (0.66, 0.8) |
| | CEWAF | | 135 | 259 | 6 | 30 | −0.65 |
| | | | (130, 145) | (244, 260) | (4, 9) | (24, 34) | (−0.69, −0.52) |
| Green algae | WAF | 199 | 105 | 87 | 10 | 8 | 0.19 |
| | | | (100, 109) | (81, 91) | (8, 14) | (4, 9) | (0.095, 0.28) |
| | DCEWAF | | 129 | 65 | 17 | 6 | 0.69 |
| | | | (125, 133) | (60, 68) | (16, 21) | (4, 8) | (0.61, 0.8) |
| | CEWAF | | 100 | 95 | 4 | 6 | 0.051 |
| | | | (92, 102) | (92, 101) | (3, 6) | (4, 9) | (−0.083, 0.1) |
| Chrysophytes | WAF | 48 | 40 | 8 | 16 | 3 | 1.6 |
| | | | (39, 42) | (6, 9) | (13, 18) | (2, 3) | (1.5, 1.9) |
| | DCEWAF | | 18 | 30 | 3 | 7 | −0.51 |
| | | | (16, 21) | (27, 31) | (2, 4) | (6, 8) | (−0.63, −0.25) |
| | CEWAF | | 32 | 16 | 1 | 4 | 0.69 |
| | | | (29, 32) | (16, 19) | (1, 3) | (4, 4) | (0.42, 0.69) |
| Dinoflagellates | WAF | 225 | 74 | 150 | 5 | 13 | −0.71 |
| | | | (68, 78) | (144, 155) | (4, 7) | (9, 17) | (−0.82, −0.61) |
| | DCEWAF | | 100 | 125 | 6 | 15 | −0.22 |
| | | | (95, 103) | (121, 129) | (5, 9) | (11, 19) | (−0.31, −0.17) |
| | CEWAF | | 63 | 162 | 3 | 23 | −0.94 |
| | | | (57, 66) | (157, 167) | (1, 5) | (19, 28) | (−1.1, −0.87) |

manufacturer's instructions. RNA quality was assessed by spectral absorption (260/280 and 260/230 ratios > 1.8). Samples that contained residual salt (260/230 < 1.5) were re-precipitated by adding 1/10 volume of 3 M sodium acetate (pH 4.5), followed by 2–2.5 volumes of 100% EtOH.

### 2.3. RNA sequencing

RNA was sequenced as 125 bp paired-end reads using Illumina HiSeq 2000 RNAseq and the TruSeq mRNA stranded library preparation protocols for paired-end reads by the Genome Quebec Innovation Centre. PolyA selection was used to remove the majority of the rRNA using the NEBNext Poly(A) mRNA magnetic isolation module kit from New England Biolabs. Approximately 2–10% of the original rRNA sequences pass through this step and are sequenced (Abernathy and Overturf, 2016).

### 2.4. Bioinformatic analyses

Trimmomatic was used to remove Illumina adapters and low quality bases were identified using Phred scores (Bolger et al., 2014). KrakenUniq (Breitwieser et al., 2018) was used to classify the taxonomy and count reads at the rank of species against all the sequences for Bacillariophyta, Dinoflagellata, Chlorophyta, and Chrysophyceae in the PR2 database (release 4.11.1) (Guillou et al., 2012). For simplicity, in our results we code these groups as diatoms, dinoflagellates, green algae, and Chrysophytes, respectively. Counts were normalized to the same total across all replicates and treatments and reported as transcripts per million (TPM). In addition to using the full-length 18S rRNA sequences in PR2, we trimmed the PR2 sequences to the V4 region and repeated the read counting and taxonomic identification. The two sets of counts are identified here as “full-length” and “V4 only”. The V4 analysis is included to illustrate the potential application of this method to amplicon data.

Species not detected in a replicate or treatment were counted as having zero abundance. Principal component analysis (PCA) was performed in R using the normalized count data (TPM) to visually assess differences among replicates relative to treatments. Species were classified as increasing or decreasing in relative abundance according to changes in the mean TPM. (A small number of species did not change and were not included in these counts.) Additionally, species were classified as having a statistically significant increase or decrease in relative abundance through *t*-tests with $p < 0.05$.

### 2.5. Ribosome sequence based oil pollution index

A treatment sensitivity index was computed for each phytoplankton group (diatoms, dinoflagellates, green algae, and Chrysophytes) in each treatment relative to the control. The index was defined as the log of the ratio of the number of species that increased in relative abundance (mean TPM) to the number that decreased in relative abundance within each group. Species not detected in any replicate (zero mean abundance) of the control and treatment had no change in abundance and were not counted. An increase in average abundance in the treatment results in a positive index value, while a decrease results in a negative index. The log transform ensures that proportional changes up or down in abundance are reported as symmetric deviations from 0. Rare species contributed a stochastic element to this index since small counts mean that a change in counts between treatment and control may often be attributed to chance fluctuations. An exclusive focus on dominant species, or species for which mean abundance changes could be detected with t-tests, resulted in only a small number of species being assessed. We opted to include all species in our analysis and used resampling to incorporate this stochastic variation in confidence intervals for the index statistic. Error bars representing 95% confidence intervals were computed by bootstrap resampling 1000 times. Simulated relative transcripts per million (TPM) were computed for each replicate from $10^6$ species occurrences sampled with replacement according to the relative probabilities reflected in the TPM scores in the data.

## 3. Results

We quantified the relative abundance of 874 phytoplankton species using the full length 18S rRNA sequence match and a smaller set of 357

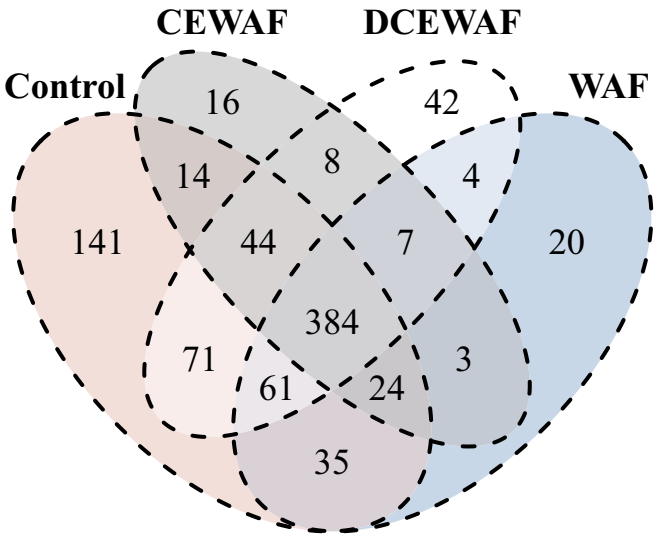


**Fig. 1.** The number of species identified (abundance > 0 in two or three replicates) for each treatment and combination of treatments using the full-length 18S sequence. Colours correspond to treatments: pink (Control), blue (WAF), gray (CEWAF), white (DCEWAF). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

species using the more restrictive matches to the V4 region (Table 1, Supplementary Table 1). A visualization of the species composition in the mesocosms using the PR2 taxonomic hierarchy is provided in Supplementary Fig. 1. The results below primarily focus on the findings using the full length 18S rRNA sequence; the V4 analyses is provided in the supplementary materials (Supplementary Tables 1 and 2, Figs. 3 and 4).

The oil and oil-dispersant mixtures altered the relative taxonomic composition of the mesocosms with clear differences across treatments relative to the differences across replicates within treatments (Supplementary Fig. 2). The control mesocosms had the highest number of detected species (Table 1) and the plurality of all species (384) were detected in all treatments (Fig. 1, Supplementary Fig. 3). 141 species were detected in the control but in none of the treatments, while 100 species were detected in one or more treatments, but not in the control. Statistically significant changes in relative abundance with a treatment were detected in a minority of species (Table 1, 9–12% of the diatoms, 5–12% of the green algae, 8–12% of the dinoflagellates, and 10–40% of the Chrysophytes; *t*-test, $p < 0.05$). The Chrysophytes exhibited the highest percentage of species that change significantly in relative abundance, especially under the WAF treatment.

Since so few species demonstrated statistically significant changes in relative abundance, we computed the total number of species that increased or decreased in relative abundance in treatments relative to the control. The log of the ratio of the number of species within the higher taxonomic groupings (diatoms, green algae, Chrysophytes, and dinoflagellates) that increased or decreased in relative abundance in the treatments relative to the controls was tabulated to determine the relative sensitivity of the groups to the treatments (Table 1). These counts might be noisy estimates of the number of species affected by the treatment, due to sampling variation in species with low relative abundance or very similar relative abundance in the control and another treatment. To guard against over interpreting these potentially noisy counts, we performed bootstrap resampling of the underlying relative abundance data and computed 95% confidence intervals on the resulting statistics. The 95% confidence intervals on the log ratio clearly distinguish distinct differences in this ratio among most of the groups within treatments.

Our oil sensitivity index ranged from roughly −2 to +2 corresponding to a ratio of about 7 in the number of species positively or negatively affected by each treatment (Fig. 2). All the treatments (except for green algae in CEWAF) resulted in a sensitivity index significantly different from 0, indicating there is a change in relative abundance aggregated over species within our taxonomic groups in

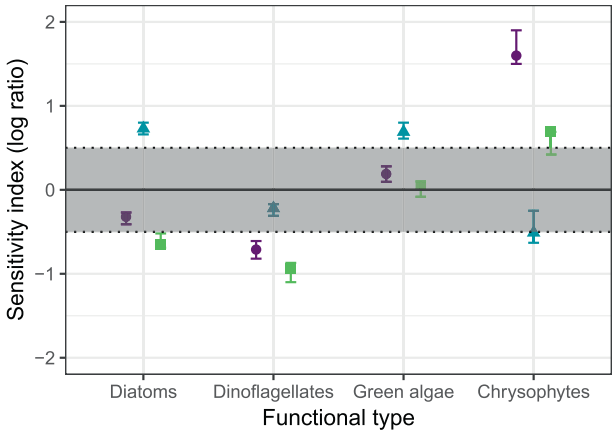


**Fig. 2.** The sensitivity of phytoplankton groups to three different oil and dispersant treatments, relative to a control treatment using the full-length 18S sequences. The sensitivity index is the log of the ratio of the number of species increasing in relative abundance to the number of species decreasing in relative abundance in the treatment relative to the control. Colours and symbols indicate treatments (purple circle = WAF, blue triangle = DCEWAF, green square = CEWAF). Error bars represent the 95% confidence interval on the mean determined from 1000 bootstrap resamplings of the data. The gray region masks out small effects, covering the range of index values from −0.5 to 0.5, corresponding to changes in relative abundance of < 65% relative to the control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

most treatments, but with the magnitude and sign varying across the taxonomic groups. The largest positive index occurs in WAF and the most negative is associated with CEWAF. The most negative values are associated with the dinoflagellates under WAF and CEWAF suggesting this group is the most sensitive to these treatments. The most positive values are found for Chrysophytes under WAF suggesting they get by far the largest relative benefit from this treatment. Small positive and negative values < 0.5 in magnitude are found for the green algae under WAF and CEWAF, suggesting this group is relatively insensitive to these treatments. Similar patterns of sensitivity are obtained whether the full length 18S rRNA or V4 region are used to identify species (Supplementary Table 2 and Supplementary Fig. 4).

Although fewer unique phytoplankton species are identified using the V4 region versus the full length of the 18S rRNA, the relative changes in relative abundance across the treatments are similar (Supplementary Table 2 and Supplementary Fig. 4). The number of

species detected is sensitive to the molecular target sequence and content of the database used. While gaps in the PR2 ribosomal sequence database, the choice of molecular sequence to identify species, or other methodological choices can cause biases in the quantification of relative abundance of species within a community, the quantified changes in relative abundance within groups across treatments should be mostly immune to these effects.

## 4. Discussion

Marine ecosystem models often group phytoplankton species into categories based on higher taxonomic identity and function (Allen and Polimene, 2011; Flynn et al., 2015; Irwin and Finkel, 2018). Trait values for phytoplankton groups are often determined from observations that are made on a small number of model species under laboratory conditions. These species often grow well under laboratory conditions, but may not be representative of the whole group, or their behavior in nature. Furthermore, phytoplankton groups represent a large number of species with potentially very different trait values, so the representation of a group by an average trait value of a single species may not be appropriate. Alternatively, there is evidence that species within large taxonomic groups may often have similar responses to environmental forcing (Mutshinda et al., 2016; Vergnon et al., 2009).

The physiological responses of phytoplankton to oil and dispersant vary widely across the species and communities examined (Bretherton et al., 2018, 2019; Gilde and Pinckney, 2012; González et al., 2009; Ozhan and Bargu, 2014). This has made it difficult to assign trait values for how various phytoplankton groups respond to oil and oil-dispersant mixtures. For example, community-level studies have suggested that diatoms can be both resistant (Bretherton et al., 2019; Gilde and Pinckney, 2012; Ozhan and Bargu, 2014) and sensitive (Adekunle et al., 2010) to oil. Phytoflagellates, a taxonomically diverse group that typically includes green algae and the Chrysophytes, are often resistant to oil exposure (Gilde and Pinckney, 2012), and can even out-compete diatoms as the dominant group in some oil experiments (Adekunle et al., 2010). Dinoflagellates have also demonstrated mixed responses, appearing sensitive in some studies (Özhan et al., 2014), while able to tolerate oil in others (González et al., 2009, 2013; Ozhan and Bargu, 2014). The sensitivity of grazers is possibly an important factor in dictating their oil response (Gemmell et al., 2018). As a result we lack critical information to create the next-generation of marine ecosystem models to predict phytoplankton group responses to oil spills and dispersant application. Using a molecular approach, we describe a sensitivity index for phytoplankton groups based on the proportion of species within the taxonomic group that increase or decrease in relative abundance in response to oil and oil and dispersant in a natural community. This approach should provide a more robust indication of the relative impact of oil on different phytoplankton groups under a range of natural conditions. Our index weights all detected species equally within a taxonomic group, since we are interested in the treatment effects on all species and not just the dominant species in the particular community studied here.

Taxonomic identification of microbes is challenging. Microscope identification and counts are time consuming and expensive. Microscopic identification requires highly trained experts, identification can vary across experts, and results in chronic under-sampling of rare species (Culverhouse et al., 2003; Dromph et al., 2013; Zingone et al., 2015). Cryptic diversity is also high; many species are genetically and physiologically distinct, while being exceedingly difficult or impossible to distinguish morphologically (Degerlund et al., 2012; Lundholm et al., 2012; Montresor et al., 2003; Šlapeta et al., 2006). Although there are known biases, molecular methods that sequence RNA or DNA marker sequences provide higher estimates of diversity, and have the potential to provide more consistent estimates of microbial community composition (de Vargas et al., 2015; Medlin et al., 2006; Zingone et al., 2015). Furthermore, environmental sequencing of RNA and DNA can quantify metabolic capacity and physiological responses to environmental conditions of large numbers of species.

Our sensitivity index indicates that the four taxonomic groups examined are significantly affected by all the oil and oil-dispersant treatments and each have distinct responses to the treatments (Fig. 2). The dinoflagellates have the largest negative index values under CEWAF and WAF, indicating they are most sensitive to oil and the concentrated oil-dispersant mixture. The Chrysophytes, in contrast, have the largest positive sensitivity index under WAF, indicating they are the group most likely to relatively benefit under oil exposure. It is plausible that bacteria and bacteriovorous Chrysophytes may have proliferated in the WAF treatment. The green algae are relatively insensitive to both CEWAF and WAF, with an index value close to zero, and have a relatively high positive index under DCEWAF exposure, suggesting they may have mechanisms to resist oil and dispersant penetration and toxicity. For example, many green algae have thick cell walls of cellulose that can protect membranes from being damaged by surfactants (Biedlingmaier et al., 1987). There is some evidence that a number of Chrysophytes and green algae are phagotrophic (Andersen, 2011; McKie-Krisberg and Sanders, 2014). As bacterial cell counts increased in all treatments (see Doyle et al., 2018), it is possible that phagotrophy allowed these two groups to continue growing in the presence of oil and Corexit. The diatoms exhibit a mixed response to the treatments; a high positive sensitivity index in response to DCEWAF and relatively high negative sensitivities to WAF and CEWAF. These results indicate that the effects of oil-dispersant mixtures on phytoplankton communities can be expected to differ significantly from the effects of oil alone on phytoplankton communities, corroborating previous studies that compare these two conditions (Bretherton et al., 2019; Hsiao et al., 1978; Özhan et al., 2014).

## 5. Conclusions

We have introduced a phytoplankton taxonomic group sensitivity index based on ribosome sequences to quantify the effects of treatments on complex microbial communities. Assessments based on richness will be biased by database quality, which often varies widely across taxonomic groups. This index allows us to assess the overall sensitivity of a taxonomic group to a treatment by pooling data from many species within taxonomic groups, based on changes in relative abundance in detected species. As an application we use RNAseq reads counted against an 18S sequence database to identify changes in the relative abundance of species in a natural phytoplankton community following exposure to oil and oil-dispersant mixtures. Our index weights all species equally, but includes a bootstrapping stage to guard against undue influence of minor constituents of the community and sampling variation, in an effort to obtain a robust estimate of the effect of treatments on phytoplankton groups that are not influenced by a few dominant species in a particular natural community. Further work will be needed to test the practical robustness of this index and to explore applications to other treatments.

Private link to Figshare data for use during review: https://figshare.com/s/8d3e35c2641453681c27. Public doi for data will be: https://doi.org/10.6084/m9.figshare.8766389. Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.110798.

## CRediT authorship contribution statement

**Zoe V. Finkel:**Conceptualization, Methodology, Writing - original draft, Writing - review & editing.**Yue Liang:**Investigation, Writing - original draft, Writing - review & editing.**Deepak Nanjappa:**Investigation, Writing - original draft, Writing - review & editing.**Laura Bretherton:**Writing - original draft, Writing - review & editing.**Chris M. Brown:**Investigation.**Antonietta Quigg:**Conceptualization, Writing - original draft, Writing - review & editing.**Andrew J.**

**Irwin:**Conceptualization, Methodology, Investigation, 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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