



## Contrasting transcriptomic responses of a microbial eukaryotic community to oil and dispersant<sup>☆</sup>

Deepak Nanjappa<sup>a,\*,1,4</sup>, Yue Liang<sup>a,2,3,4</sup>, Laura Bretherton<sup>a</sup>, Chris Brown<sup>b</sup>, Antonietta Quigg<sup>c</sup>, Andrew J. Irwin<sup>a,d</sup>, Zoe V. Finkel<sup>a</sup>

<sup>a</sup> Department of Oceanography, Dalhousie University, Halifax, NS, Canada

<sup>b</sup> Environmental Science Program, Mount Allison University, Sackville, NB, Canada

<sup>c</sup> Department of Marine Biology, Texas A&M University at Galveston, Galveston, TX, USA

<sup>d</sup> Department of Mathematics & Statistics, Dalhousie University, Halifax, NS, Canada

### ARTICLE INFO

#### JEL classification:

Biological sciences  
Environmental sciences

#### Keywords:

Metatranscriptomes  
Oil spill  
Chemical dispersant  
Phytoplankton  
Protists  
Ketogenesis  
Metabolic stress

### ABSTRACT

Dispersants can aid dispersion and biodegradation of oil in seawater, but the wider ecotoxicological effects of oil and dispersant to the base of marine food webs is unclear. Here we apply a metatranscriptomic approach to identify molecular responses of a natural marine microbial eukaryotic community to oil and chemically dispersed oil. Oil exposure stimulated the upregulation of ketogenesis in the eukaryotic community, which may alleviate carbon- and energy-limitation and reduce oxidative stress. In contrast, a chemically dispersed oil treatment stimulated eukaryotic genes and pathways consistent with nitrogen and oxygen depletion. These results suggest that the addition of dispersant may elevate bacterial biodegradation of crude oil, indirectly increasing competition for nitrogen between prokaryotic and eukaryotic communities as oxygen consumption induces bacterial anaerobic respiration and denitrification. Eukaryotic microbial communities may mitigate some of the negative effects of oil exposure such as reduced photosynthesis and elevated oxidative stress, through ketosis, but the addition of dispersant to the oil fundamentally alters the environmental and ecological conditions and therefore the biochemical response of the eukaryotic community.

### 1. Introduction

Oil spills damage the marine environment (Transportation Research Board and National Research Council, 2002). Dispersants can reduce beach pollution and elevate biodegradation, but may also increase the toxicity of oil by solubilizing oil components, including polycyclic aromatic hydrocarbons (PAHs), making them more bioavailable to marine organisms (Wolfe et al., 2000, 1998). The 2010 Deepwater Horizon (DwH) incident is one of the largest accidental oil spills in recent history, releasing an estimated 4–6 million barrels of Macondo oil into the Gulf of Mexico (Crone and Tolstoy, 2010; McNutt et al., 2012). The application of chemical dispersants following the DwH spill was unprecedented in both its scale (nearly 3 million liters) and its use directly at the

burst wellhead on the seafloor (Kujawinski et al., 2011). Considerable effort has been devoted to developing our understanding of how oil and dispersants influence the metabolic processes of prokaryotic microbes (bacteria and archaea) in the Gulf (Handley et al., 2017; Kimes et al., 2013; Mason et al., 2014; Rivers et al., 2013) but relatively little work has been done to quantify and understand the effects of oil and dispersants on marine eukaryotic microbes.

Marine eukaryotic communities are extraordinarily diverse, with very few species well-studied in culture. Data to date indicate that oil and chemical dispersants can coat and damage the cell wall and cellular membranes (Carvalho et al., 2011a; Hook and Osborn, 2012), damage DNA (Deasi et al., 2010), inhibit motility (Garr et al., 2014), and affect photosynthesis and growth rate (Bretherton et al., 2018; Hsiao et al.,

<sup>☆</sup> This paper has been recommended for acceptance by Dr. Sarah Harmon.

\* Corresponding author.

E-mail address: [deepak.nanjappa@stonybrook.edu](mailto:deepak.nanjappa@stonybrook.edu) (D. Nanjappa).

<sup>1</sup> Current address: Stony Brook University, School of Marine and Atmospheric Sciences, Southampton, New York, USA.

<sup>2</sup> Current address: Center of Deep Sea Research, Institute of Oceanology, Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China.

<sup>3</sup> Current address: Laboratory for Marine Mineral Resources, Pilot National Laboratory for Marine Science and Technology, Qingdao 266237, China.

<sup>4</sup> These authors contributed equally.

1978). Considerable variability in physiological responses to oil and oil-dispersant mixtures across eukaryotic species and communities has been reported (Finkel et al., 2020; Gemmell et al., 2018; Gilde and Pinckney, 2012). This variability impedes efforts to model the effects of oil spills and dispersant application on marine ecosystems. Some of the variability in the responses of eukaryotic microbes to oil versus chemically-dispersed oil is likely due to chemical interactions between eukaryotes, and between eukaryotes and prokaryotes in the community (Gutierrez et al., 2013; Thompson et al., 2017). For example, chemical dispersants can facilitate the biodegradation of hydrocarbons by bacteria, and this could facilitate changes in the microenvironment around the microbial eukaryotes by increasing the bioavailability of toxic oil compounds and biodegradation products and by increasing competition for nutrients between eukaryotic and bacterial members of the community.

Traditionally, research on the impact of oil and dispersants on marine eukaryotic communities has focused on quantifying taxonomic structure and bulk physiology (Bik et al., 2012; Bretherton et al., 2019; Campeão et al., 2017). Transcriptomic analyses provide unprecedented levels of detail into the physiological and biochemical responses of eukaryotes to stressors such as oil and chemical dispersants. Pioneering gene expression work has focused on model eukaryotic phytoplankton species such as the diatom *Thalassiosira pseudonana* (Bopp and Lettieri, 2007; Carvalho et al., 2011a, 2011b) and the diatom *Phaeodactylum tricornutum* (Hook and Osborn, 2012) with the goal of identifying cellular responses to polyaromatic hydrocarbons (components of crude oil) or single highly differentially expressed genes to be used as biomarkers for hydrocarbon exposure. Here we use a metatranscriptomic approach to identify the dominant gene- and pathway-level responses of the eukaryotic component of a natural Gulf of Mexico planktonic microbial community to a 72 h exposure to oil and diluted chemically dispersed oil treatments. This approach has the potential to identify changes in eukaryotic metabolism induced by direct responses to oil and dispersant plus indirect responses resulting from bacterial degradation of hydrocarbons. Using this approach, we find that the addition of dispersant fundamentally alters the biochemical response of the microbial eukaryotic community to oil.

## 2. Materials and methods

### 2.1. Experimental set-up and sampling

Twelve 100 L tanks were filled with Gulf of Mexico seawater collected from the Texas coastline on October 17, 2015, from a pipeline located ~100 m offshore from Galveston (Texas, USA) at 29.2726°N 94.8126°W and transferred to a holding tank at the Texas A&M University at Galveston (TAMUG) campus. Four treatments were prepared in triplicate, after the method described in Wade et al. (2017). Briefly, the water accommodated fraction (WAF) of oil was prepared by mixing 25 mL (5 mL every 30 min for 2.5 h) of Macondo surrogate oil (collected from the Marlin platform of the Dorado field 23 miles NE of Macondo) into 130 L of seawater in a baffled recirculating tank. Mixing ended 24 h after the initial oil addition. The WAF was then introduced into the WAF tanks and filled to 87 L and mixed. In order to make a chemically enhanced water accommodated fraction (CEWAF), Corexit was mixed with Macondo surrogate oil in a ratio of 1:20 and 25 mL of this mixture (5 mL every 30 min for 2.5 h) was added to 130 L of seawater. Mixing ended 24 h after the initial oil addition. The CEWAF was then introduced into the CEWAF tanks and filled to 87 L and mixed. Diluted CEWAF (DCEWAF) was prepared by mixing 9 L of CEWAF with 78 L of the original seawater for a total volume of 87 L to achieve a 9.7-fold dilution of CEWAF. Control tanks were filled with untreated seawater.

A coastal plankton slurry was used to seed the tanks and was collected at the TAMUG docks using tow nets (63 µm) and pre-filtered with a 115 µm mesh to exclude larger grazers. At the start of the experiment (time 0), 2 L of slurry was stirred into each tank. Banks of

fluorescent lights (Sylvania GRO-LUX) were placed behind each of the glass mesocosm tanks to achieve about 50–65 µmol photons m<sup>-2</sup> s<sup>-1</sup> at the illuminated face of the tank and a 12:12 light:dark cycle employed. Mesocosms were maintained at 19 °C. The estimated oil equivalents (EOE) were determined using Macondo surrogate oil as the calibration standard (Wade et al., 2011) for the fluorescence analyses (Horiba Scientific Aqualog Fluorometer). The EOE mean concentration of the three mesocosms for the control, WAF, DCEWAF and CEWAF at the start of the experiments were 0, 0.26, 2.74 and 41.5 mg/L, respectively and after 72 h were 0, 0.06, 1.03 and 17.3 mg/L, respectively. Oil droplet size distributions in the baffled recirculating tank used to make WAF, degradation rates over the three days of this experiment (M2), and GC/MS analysis of aromatic components of Macondo surrogate and chemically dispersed oil are provided by Wade et al. (2017). A nutrient auto-analyzer was used to measure macronutrient concentrations. Nitrate concentration at time 0 averaged 17.8 ± 1.6 µmol/L (n = 10, reporting 1 standard error as the uncertainty, after removing 2 outlier observations > 50 µmol/L) across all treatments. After 72 h, nitrate concentration had decreased to 4.5 ± 0.7 µmol/L (n = 3) in the control, 1.3 ± 0.8 µmol/L (n = 3) in DCEWAF, 1.0 ± 0.5 µmol/L in CEWAF and was below the detection limit (<0.1 µmol/L) for all n = 3 observations in WAF. Only minor changes were observed in phosphate, silicate, nitrite, ammonium, and urea concentrations. We chose 72 h after the addition of the plankton slurry to be the end point of the experiment as aggregates had formed, the oil concentration had changed (see Wade et al., 2017; Doyle et al., 2018), and we anticipated there would be a transcriptomic signal in the eukaryotic community.

### 2.2. RNA extraction and sequencing

At the end of the experiment (t = 72 h), plankton from the tanks were filtered on 0.8 µm polycarbonate filters to concentrate the eukaryotic component of the community. Filters were transferred quickly from -80 °C storage to Y-matrix bead tubes (MPBio) containing 900 µL denaturing buffer (Ambion Totally RNA kit, AM1910) and immediately disrupted by 2 cycles of 30 s (separated by 1 min on ice) at maximum speed with an MPBio SuperFastprep2, followed by centrifugation for 10 min at 13 000 rpm (Bretherton et al., 2019). RNA purification was performed using the Ambion Totally RNA kit, AM1910, followed by a DNase treatment with (Turbo DNase, Ambion AM1907) according to the manufacturer instructions. RNA quality was determined through gel electrophoresis and spectrophotometric measurements. Three replicate samples per treatment yielded good quality RNA and were used for library construction. mRNA was sequenced (Illumina HiSeq, 2000) by the Genome Quebec Innovation Centre McGill University using the TruSeq mRNA stranded library preparation protocols (Illumina) for 100 bp paired-end reads.

### 2.3. Sequence processing and taxonomic classification

The quality of the raw reads was assessed using FASTQC (version 0.11.6) (Andrews, 2016). Trimmomatic (version 0.32, Bolger et al., 2014) was used to remove bases with quality scores below 20 and reads shorter than 32 bp. Kraken (v1, standard parameters) was used to separate reads into three groups: (1) reads similar to bacterial genes or genes of prokaryotic origin, (2) reads similar to rRNA of prokaryotes or eukaryotes, and (3) reads which were presumed to be of eukaryotic origin using the standard Kraken library of bacterial, archaeal and viral genomes from RefSeq, the SILVA rRNA database (release 128), and the NCBI nucleotide database (Quast et al., 2012; Wood and Salzberg, 2014). Sequences that were categorized as eukaryotic in origin were used for assembly and gene expression analysis. The left and right paired reads were assembled into transcripts using Trinity (version 2.6.6, Grabherr et al., 2011; Haas et al., 2013) separately for each replicate and treatment. The transcripts were then combined into a single set and clustered to reduce redundancy using CD-HIT-EST module in CD-HIT

suite at 100% similarity threshold (Li and Godzik, 2006).

We determined the taxonomic identity of the RNA reads obtained from the mesocosms using a database of transcripts from the Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP) (Keeling et al., 2014). The MMETSP provides 678 transcriptomes of about 400 taxa and is the best compilation of marine eukaryotic transcriptomes, but due to the tremendous diversity of marine eukaryotes in the ocean we anticipated that many of our reads will not be identified using these data. The taxonomic origin of the assembled transcripts was identified using the Diamond annotation tool (Buchfink et al., 2015) through homology (blastx) searches against MMETSP peptide data, selecting the best match with a e-value cutoff of  $10^{-3}$ . Taxonomic matches are likely to be approximate, so we aggregated counts into the ten largest categories of transcriptomes: 178 Bacillariophyta, 119 Dinophyta, 74 Ochrophyta, 62 Chlorophyta, 62 Haptophyta, 27 Ciliophora, 22 Cryptophyta, 8 Lobosa, 3 Bicosoecida, 2 Choanozoa transcriptomes and 106 transcriptomes we aggregated into an “others” category, following the taxonomic identification provided with the MMETSP data.

#### 2.4. Gene expression and pathway enrichment

Transcript abundances (transcripts per million, TPM) were determined using Kallisto (version 0.43.1, Bray et al., 2016a). Gene annotations were obtained using the Diamond annotation tool (Buchfink et al., 2015) through best homology (blastx) searches against the Uniprot protein database at an e-value cutoff of  $10^{-3}$ . KEGG annotations were obtained through both the Uniprot database and the GhostKOALA (KEGG Orthology And Links Annotation) (Suzuki et al., 2014). Differential gene expression analysis was performed with sleuth (version v0.28.1, Pimentel et al., 2017) utilizing Kallisto counts aggregated according to KO gene annotation. KOs with q-values < 0.05 were considered significantly differentially expressed. KEGG pathways and Brite categories from the 4 groups: 1) Cellular Processes, 2) Environmental Information Processing, 3) Genetic Information Processing, and 4) Metabolism were selected for KEGG enrichment analyses. Pathways with detected KO ID counts less than 5 were removed from further analyses and pathway enrichment analysis was performed following Liang et al. (2019).

### 3. Results

A total of approximately 2.9 million (M), 2.3M, 1.5M and 1.7M transcripts belonging to control, WAF, DCEWAF and CEWAF treatments, respectively were assembled (Table S1). About 20–50% of these were annotated to taxa using the MMETSP data (53%, 54%, 22%, 41%, in each treatment, respectively). Of these, transcripts classified as Bacillariophyta (diatoms) dominated all samples, averaging 52.1, 43.6, 64.1 and 63.7% (Table S2) in the control and the WAF, DCEWAF and CEWAF treatments, respectively. In the control and DCEWAF treatment, Dinophyta (dinoflagellates) transcripts were the second dominant group, while in WAF and CEWAF, transcripts belonging to Ochrophyta (30.7 and 18.1%, respectively) were more abundant than Dinophyta (8.3 and 8.5%, respectively). Together these formed the three most abundant groups in all the three treatments. Bicosoecida, Ciliophora, and various other phyla formed the remainder of the taxonomic composition (Table S2).

A total of 11 895, 11 606, 10 840 and 10 744 distinct genes were counted in the control, WAF, DCEWAF and CEWAF treatments respectively, using Kallisto (version 0.43.1) with KEGG database identifications (KO IDs) belonging to 319 KEGG pathways (Bray et al., 2016b). Of these, 6960, 7219 and 6318 genes in WAF, DCEWAF and CEWAF, respectively, were filtered using sleuth (using default settings, Pimentel et al., 2017) and analyzed for differential gene expression (DE). Among the DE genes, DCEWAF had highest number of upregulated (1547 genes, 45% of the total 3437 DE genes), while in CEWAF most of the genes were

downregulated (2480 genes, 97%; total 2553 genes) (Fig. S1, Table S1). The WAF results were intermediate between the other treatments, with 63% (951) upregulated and 37% (556) downregulated (total 1507 genes). The differentially expressed genes vary across the treatments; there are only 12 commonly upregulated and 372 downregulated genes across the three treatments (Fig. S1). Between the treatments, WAF and DCEWAF had the most common upregulated genes (141) while DCEWAF and CEWAF had the most common downregulated genes (854). Individually, DCEWAF had the highest numbers of upregulated genes that were unique to the treatment (1295 genes) while CEWAF had the highest number of unique down-regulated genes (949). Due to the large proportion of downregulated genes in the CEWAF treatment, our pathway enrichment calculation cannot be applied, thus, we focus on the comparison of the WAF and DCEWAF treatments (Table 1).

Pathway enrichment analysis indicated 7, 22 and 0 pathways were enriched for upregulated genes and 5, 21, 20 pathways were enriched for downregulated genes in the oil (WAF), diluted chemically dispersed oil (DCEWAF) and concentrated chemically dispersed oil (CEWAF) treatments, respectively (Data S1). A total of 27 pathways were significantly enriched in upregulated genes, of which 5 were unique to WAF, 20 were unique to DCEWAF, and 0 were unique to CEWAF. A total of 30 pathways were significantly enriched in downregulated genes, of which 2 were unique to WAF, 10 were unique to DCEWAF, and 9 were unique to CEWAF. The lack of pathways enriched in upregulated genes under the CEWAF treatment reflects significant physiological stress and cell death, making it difficult to compare to the other treatments. The large proportion of differentially expressed genes in the CEWAF treatment

**Table 1**

KEGG metabolic pathways significantly enriched in up (yellow arrow) or down (purple arrow) regulated genes in oil (water accommodated fraction, WAF) and diluted chemically dispersed oil (DCEWAF) treatments (hypergeometric test, FDR-corrected  $p < 0.05$ ).

KEGG ID	Metabolic pathways	WAF	DCEWAF
Amino acid metabolism			
ko00250	Alanine, aspartate and glutamate metabolism		↑
ko00220	Arginine biosynthesis		↑
ko00380	Tryptophan metabolism	↑	
ko00280	Valine, leucine and isoleucine degradation	↑	
Carbohydrate metabolism			
ko00650	Butanoate metabolism	↑	↑
ko00660	C5-Branched dibasic acid metabolism		↑
ko00020	Citrate cycle (TCA cycle)	↑	
ko00640	Propanoate metabolism	↑	↑
ko00620	Pyruvate metabolism		↑
Energy metabolism			
ko00910	Nitrogen metabolism	↓	↑
ko00190	Oxidative phosphorylation	↓	↓
ko00194	Photosynthesis proteins		↑
ko00195	Photosynthesis		↑
Lipid metabolism			
ko00061	Fatty acid biosynthesis		↑
ko00071	Fatty acid degradation	↑	
ko00062	Fatty acid elongation	↑	
ko00600	Sphingolipid metabolism		↓
Metabolism of cofactors and vitamins			
ko00790	Folate biosynthesis		↑
ko00750	Vitamin B6 metabolism		↑
Metabolism of terpenoids and polyketides			
ko00906	Carotenoid biosynthesis		↓
Nucleotide metabolism			
ko00230	Purine metabolism		↑
ko00240	Pyrimidine metabolism		↑

reduced the utility of our pathway enrichment analysis. We focus our analysis on a comparison of the WAF and DCEWAF treatments and their impact on pathways associated with core metabolism (Table 1).

The enrichment of metabolic pathways and differential expression of genes differed substantially across the metatranscriptome in the oil and chemically dispersed oil treatments (Tables 1, S1, Fig. 1, S1). The only similarities in pathway enrichment were an enrichment in down-regulated genes associated with oxidative phosphorylation and an enrichment of upregulated genes associated with butanoate and propanoate metabolism, although the specific upregulation of genes within the butanoate and propanoate pathways differed across the treatments (Fig. S2). Nitrogen metabolism was the only pathway enriched in upregulated genes in DCEWAF and enriched in downregulated genes in WAF (Fig. S2). The raw sequence data and assembled transcriptome were deposited at NCBI. Gene annotations, counts, and differential expression and pathway enrichment analyses (Data S1–S4) were deposited at GRIIDC (see Data Availability).

#### 4. Discussion

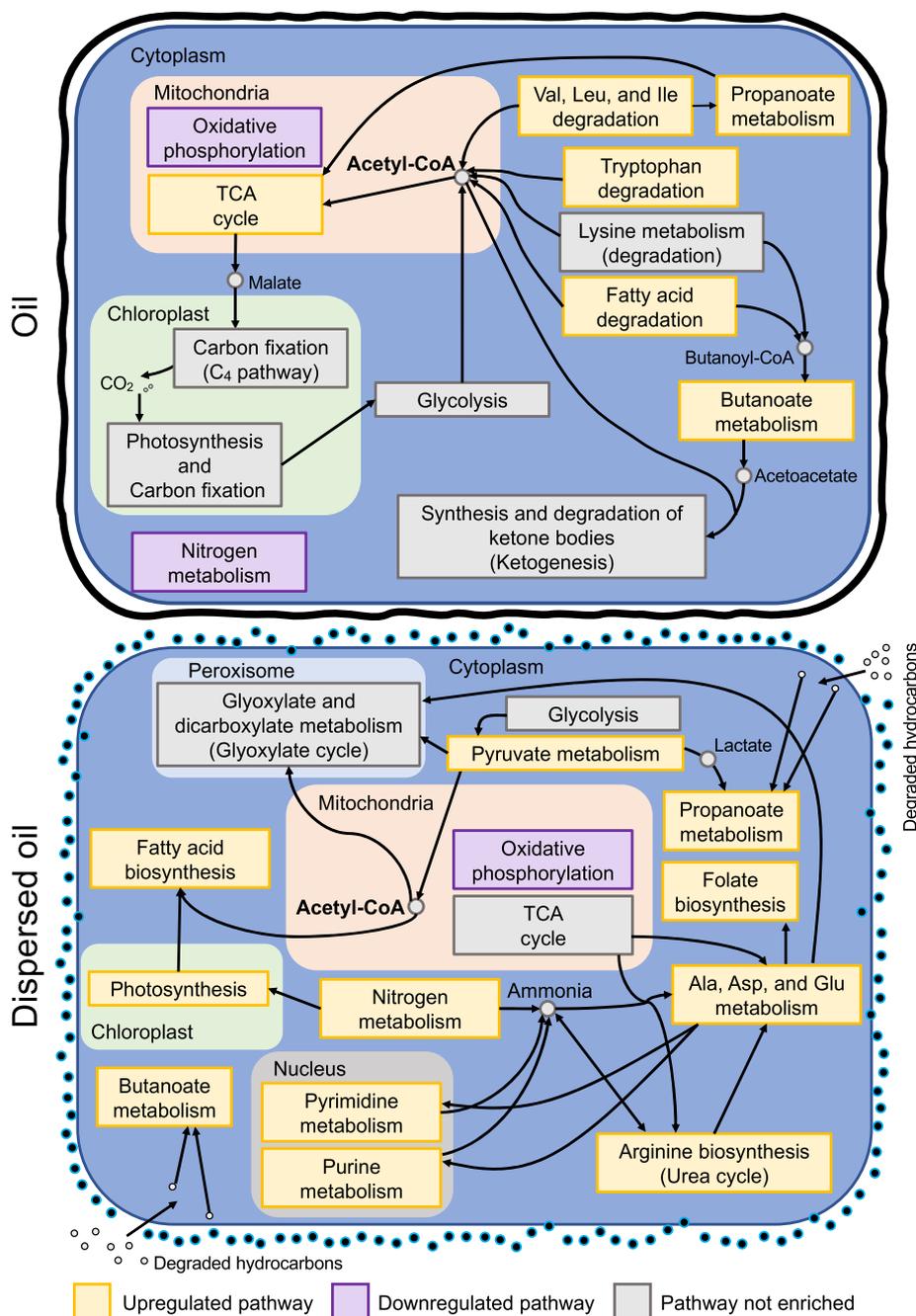
Oil can penetrate eukaryotic cells, damage membranes, disrupt cellular processes, and cause increased reactive oxygen stress (Carvalho et al., 2011b). Although the addition of dispersant facilitates biodegradation of oil by bacteria, it may also increase membrane and cellular damage and the bioavailability of toxic oil compounds to eukaryotes (Cerniglia and Sutherland, 2010; Hook and Osborn, 2012; Rughöft et al., 2020, 2021). Here we quantify the transcriptomic response of a natural community of eukaryotes, together with its associated prokaryotic community (but with the prokaryotic signal subtracted in the bioinformatic pipeline). We identify the genes and pathways that eukaryotes differentially regulate in response to exposure to oil and chemically dispersed oil to better understand the biochemical impact of dispersant on the eukaryotic base of the marine food web. We find that chemically dispersed oil stimulates a different set of differentially expressed genes and metabolic pathways than oil exposure alone (Table 1), indicating the addition of dispersant to oil dramatically alters the response of eukaryotes in marine microbial communities.

We observed an upregulation of several pathways in the marine eukaryotic metatranscriptome showing that crude oil exposure stimulates ketogenesis or a switch from metabolizing sugars to metabolizing lipids and producing ketone bodies. Specifically, the upregulation of tryptophan degradation (ko00380), valine, leucine and isoleucine degradation, and fatty acid degradation is consistent with a relative increase in the production of acetyl-CoA and the upregulation of the TCA cycle (Table 1, Fig. S2) (Hildebrandt, 2018) while oxidative phosphorylation, typically the primary sink for electrons from the TCA cycle, is downregulated (Fig. 1, S2, Table 1, specifically the electron transport chain (ko00195, Fig. S2)). In combination, this indicates that acetyl-CoA and reductant produced from the catabolism of fatty acids, tryptophan, leucine, and isoleucine is being shuttled to ketogenesis (ko00072, Fig. S3) (Aneja et al., 2002; Cahill, 2006; Krishnakumar et al., 2008; Robinson and Williamson, 1980). Furthermore, there is an enrichment in upregulated genes in the butanoate and propanoate pathways (Fig. S2). Fatty acid and lysine degradation (ko0031, Fig. S3) produce butanoyl-CoA while isoleucine degradation leads to propanoyl-CoA. These molecules can in turn be broken down into metabolites that can be used in the TCA cycle as well as for the biosynthesis of ketone bodies. Indeed, genes related to the ketone body biosynthesis pathway (ko00072, Fig. 1, S3) are significantly upregulated (Data S2). The final product of ketogenesis appears to be 3-hydroxybutanoate which is a substrate for the synthesis of poly-beta-hydroxybutyrate (PHB) (ko00072, Fig. S3) (Dedkova and Blatter, 2014). Although the genes for the conversion of 3-hydroxybutanoate to PHB have not been upregulated, many genes involved in the synthesis of PHB from butanoyl-CoA are upregulated. PHB synthesis is known to act as an electron sink under suboptimal conditions (Ackermann et al., 1995; Batista et al.,

2018). In summary, these results indicate that ketosis may be an important mechanism used by marine microbial eukaryotes to mitigate oil-related stress. Both the reduced gas and nutrient acquisition induced by oil exposure may be the cause of the elevated ketosis. Previous work suggests that oil exposure can negatively impact eukaryotic cell membranes through both physical and chemical disruption (Baker, 1970; O'Brien and Dixon, 1976). In addition, genes related to the C<sub>4</sub> dicarboxylic acid cycle (malate, pyruvate, and phosphoenol-pyruvate, ko00710, Fig. S3) are upregulated, providing additional support for the hypothesis that the oil treatment resulted in reduced CO<sub>2</sub> availability, and perhaps a relative increase in C<sub>4</sub> photosynthesis (Fig. 1) (Hesketh, 1963; Moss, 1962). The nitrogen metabolism pathway is also enriched in downregulated genes (Fig. S2), indicating the oil exposure treatment may have inhibited nutrient uptake by the microbial eukaryotes, although this signal may be due primarily to a relatively larger decline in nitrate concentration in the oil treatment relative to the control. Overall, our results suggest oil exposure compromises eukaryotic membranes, reducing the ability of both the autotrophic and heterotrophic members of the eukaryotic community to access inorganic and organic nutrition, leading to a breakdown of amino acids and fatty acids to generate energy and reduce oxidative stress.

The metatranscriptomic response of the eukaryotic microbial community to dilute chemically dispersed oil is significantly different from the response to oil alone (Table 1, Fig. 1). This is likely in part because the dilute chemically dispersed oil treatment is associated with higher levels of bacterial activity and the degradation of oil (see Doyle et al., 2018; Wade et al., 2017). Consequently, the eukaryotic components of the community are exposed to higher concentrations of oil biodegradation products, which have been shown to have toxic effects on cell membranes and membrane-dependent processes (Hook and Osborn, 2012). The dispersant could independently be responsible for some of the differences in the metatranscriptomic response, as it can also cause membrane damage, metabolic stress, and cellular damage (Rughöft et al., 2020, 2021). The upregulation of several genes within the butanoate pathway and propanoate pathways in this treatment strongly indicates it is the dispersed oil that is responsible for some of the unique responses in the eukaryotic metatranscriptome. In particular, the upregulation of acetaldehyde dehydrogenase (acetylating) [EC:1.2.1.10] (ko00650, Fig. S2) within the butanoate pathway is consistent with an increase in the absorption of 1-butanol, a product of bacterial degradation of aliphatic hydrocarbons (Güngörmedi et al., 2014; Preusting et al., 1990). The upregulation of genes within the propanoate pathway strongly indicate the activation of the 'methylcitrate cycle' (Fig. S2) (Brämer et al., 2002; Brämer and Steinbüchel, 2001; Brock and Buckel, 2004), a fungal pathway known to metabolize propanoate, a product of bacterial degradation of aromatic hydrocarbons (Chakka et al., 2015). In contrast, in the oil treatment, the propanoate pathway is enriched due to isoleucine degradation, while butanoate metabolism is upregulated due to fatty acid and lysine degradation (Fig. 1).

In the dilute dispersed oil treatment, we also see indications that the eukaryotic community is experiencing nitrogen limitation (Fig. 1); we hypothesize this is due to low oxygen conditions and denitrification. It is likely that the dispersed oil treatments stimulated elevated bacterial (and perhaps fungal) degradation of oil resulting in reduced oxygen within these experimental tanks (although oxygen was not measured in this experiment). Furthermore microbe-oil aggregates formed within the tanks (Doyle et al., 2018), and this likely would have resulted in localized hotspots of low oxygen concentration that could have enhanced denitrification (FdZ-Polanco et al., 2001). Evidence for this hypothesis includes an upregulation of genes involved in the conversion of pyruvate to acetate and ethanol in the glycolysis/gluconeogenesis pathway (ko00010, Fig. S3), indicating incomplete oxidation of glucose (Niimura et al., 1989), and the differential expression of genes involved in the conversion of lactate to acryloyl-CoA in the propanoate metabolism pathway (ko00640, Table 1, Fig. S2) (Buckel, 1992). Nitrogen metabolism is significantly enriched in upregulated genes (Table 1), and



**Fig. 1.** Summary of the effect of oil (top) and diluted chemically dispersed oil exposure (bottom) on selected metabolic pathways of a Gulf of Mexico marine eukaryotic community. Enriched pathways are highlighted in yellow (upregulated) and purple (downregulated). KEGG pathways that are not enriched are gray and key upregulated subpathways are in parentheses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

more specifically there is an upregulation of both dissimilatory and assimilatory nitrate reduction (ko00910, Fig. S2), suggesting the chemically dispersed oil treatment stimulated increased nitrate limitation due to increased bacterial denitrification under oxygen limitation (Fdz-Polanco et al., 2001). We observed reduced nitrate concentrations in all three treatments relative to control after 72 h, so we speculate that the microenvironments in aggregates and around cells likely influenced the metabolic response in the chemically dispersed oil treatments.

Alternatively or in addition, N-limitation could stem from the increased damage to cell membrane lipids and proteins due to increased availability of the oil compounds or the dispersant itself (Hook and Osborn, 2012). Photosynthetic eukaryotes are known to activate assimilatory nitrate reduction and/or catabolism of cellular nitrogen-containing compounds to obtain nitrogen under these conditions. Consistent with this, we observe an upregulation of the glutamate-glutamine cycle in the oil-dispersant treatment (ko00910,

Table 1, Fig. S2), which may reflect increased re-assimilation of ammonia from the catabolism of amino acids and nucleic acids. Gene expression further indicates that L-glutamine and L-glutamate are converted into folate (ko00790) and purine and pyrimidine (Table 1, Fig. S2). The upregulated genes in the pyrimidine metabolism pathway (Table 1, Fig. S2) suggest that *de novo* synthesized UMP and CMP were degraded to thymine whose later metabolites were further degraded to acetyl-CoA through the valine, leucine and isoleucine degradation pathway and the propanoate metabolism pathway (Green et al., 2016; Zrenner et al., 2006) while the upregulated genes in the purine metabolism pathway (Table 1, Fig. S2) indicates *de novo* synthesized IMP was converted to dGMP, dAMP, deoxyguanosine, deoxyinosine, and urea which were finally broken down to produce NH<sub>3</sub> and CO<sub>2</sub>. Of note the pathways for the synthesis of GTP, ATP, dGTP, dATP were all downregulated, indicating a net decrease of nucleotides. The urea cycle and arginine biosynthesis are also enriched in upregulated genes (Fig. S2).

The upregulated genes in arginine biosynthesis (Table 1, Data S2; Fig. S2) suggest the catabolism of arginine is followed by conversion to 2-oxoglutarate to fumarate through the urea cycle rather than the TCA cycle (Allen et al., 2011; Quintero et al., 2000). Taken together, these results reveal that chemically dispersed oil exposure can alter microbial eukaryotic community dynamics, speeding up oil degradation, and decreasing the bioavailability of oxygen and nitrate in our relatively closed system. In response the eukaryotic microbes upregulate pathways and genes associated with the catabolism of cellular nitrogen and biosynthesis of fatty acids, which is a typical response to N-limitation and reactive oxygen stress (ROS).

There are several lines of evidence suggesting a higher level of ROS in the dilute chemically dispersed oil versus oil treatment including the differential regulation of genes in the TCA cycle, from succinyl-CoA to isocitrate (Fig. S2), along with the upregulation of isocitrate lyase [EC: 4.1.3.1] and malate synthase [EC: 2.3.3.9] in the glyoxylate and dicarboxylate metabolism pathway (ko00630, Fig. S3, Data S1). The glyoxylate cycle is involved in the metabolism of H<sub>2</sub>O<sub>2</sub> in the peroxisome, regulating ROS (Ahn et al., 2016; Schnarrenberger and Martin, 2002). In addition, there is an enrichment of upregulated genes associated with fatty acid biosynthesis (Table 1, Fig. S2) and photosystem I, along with downregulation of the photosynthetic electron transport chain (ko00195, Table 1, Fig. S2). Fatty acid biosynthesis acts as an electron sink under a range of environmental stresses and has been associated with a ROS signal (Sekiya et al., 2008). Interestingly, in both the oil and the dilute chemically dispersed oil treatments, a potential accumulation of poly-beta-hydroxybutyrate (ko00072, Fig. S3) was observed which suggests this polymer may be involved in stress-compatible electron storage in the marine eukaryotes (Kadouri et al., 2003; Kulakovskaya et al., 2016; Obruca et al., 2018).

In conclusion, we find dramatic differences in the metatranscriptomic response of a Gulf of Mexico marine eukaryotic microbial community exposed to oil versus chemically dispersed oil. In response to oil exposure, the eukaryotes switch from oxidative phosphorylation to ketogenesis to mitigate nutrient limitation likely induced by the interaction of oil with cell membranes. Ketosis may reduce cellular reactive oxygen stress under moderate oil stress. The switch from metabolizing sugar to fatty acids and branched-chain amino acids may be a survival strategy that many eukaryotic microbes can employ when exposed to oil. In contrast, in response to chemically dispersed oil exposure, there is no evidence of ketogenesis and the eukaryotic metatranscriptomic response suggests there is an increase in the bioavailability of hydrocarbons, ROS, and reduced bioavailability of oxygen and nitrogen to the eukaryotes, especially those associated with oil-microbe aggregates, due to elevated biodegradation of oil by bacteria. The impact of oil and oil-dispersant mixtures on the microbial eukaryotic community is affected by community composition, environmental variables, the bacterial community and the concentration and composition of oil and dispersant. Complementary experiments in larger mesocosms or natural experiments in the open ocean are required to test these preliminary conclusions from our closed, minimally mixed experiment. More work is required to determine the reproducibility of these results across different eukaryotic communities, environmental conditions, and oil and dispersant concentrations before making recommendations for management.

#### 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.

#### Acknowledgements

The authors acknowledge the contribution of scientists and staff of the McGill University and Génome Québec Innovation Centre, Montréal,

Canada, for RNA library preparation and sequencing.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.117774>.

#### Author contributions

Z.V.F. and A.Q. designed the study, Z.V.F. and A.J.I. coordinated the project, C.B. and A.Q. conducted the experiments, D.N., Y.L., L.B., A.J.I., and Z.V.F. analyzed the data, D.N., Y.L., L.B., A.J.I., and Z.V.F. wrote the manuscript; all authors reviewed the manuscript.

#### Author statement

Deepak Nanjappa: Methodology, Investigation, Data curation, Visualization, Writing. Yue Liang: Investigation, Data curation, Visualization, Writing. Laura Bretherton: Investigation, Visualization, Writing. Chris Brown: Methodology, Investigation, Writing. Antonietta Quigg: Conceptualization, Investigation, Writing, Supervision, Funding acquisition. Andrew J, Irwin: Conceptualization, Investigation, Writing, Supervision, Funding acquisition. Zoe V. Finkel: Conceptualization, Investigation, Writing, Supervision, Funding acquisition.

#### Data and materials availability

Data were deposited at NCBI ([www.ncbi.nlm.nih.gov/bioproject/](http://www.ncbi.nlm.nih.gov/bioproject/)) under BioProject PRJNA489497. Raw RNA read files in the NCBI short read archive have accession codes SRR7796773-784. Metadata have BioSample accession codes SAMN09981211-14. The assembled transcriptome was deposited at the NCBI TSA ([www.ncbi.nlm.nih.gov/nuccore](http://www.ncbi.nlm.nih.gov/nuccore)) under the accession GICR01. Metatranscriptome counts obtained from the raw reads and the assembled transcriptome using Kallisto are reported in Data S3, with annotations in Data S4. NCBI accession codes, Data S1–S4 and Table S1 are available through the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) at [data.gulfresearchinitiative.org](http://data.gulfresearchinitiative.org), doi 10.7266/n7-43q9-xq06 (UDI R4.x263.189:0011) and doi 10.7266/2JWNDY5F (UDI R6.x807.000:0072).

#### Funding

This research was made possible by a grant from The Gulf of Mexico Research Initiative (GoMRI) to support the ADDOMEx (Aggregation and Degradation of Dispersants and Oil by Microbial Exopolymers) and ADDOMEx-2 Consortia.

#### References

- Ackermann, J., Müller, S., Lösche, A., Bley, T., Babel, W., 1995. *Methylobacterium rhodesianum* cells tend to double the DNA content under growth limitations and accumulate PHB. *J. Biotechnol.* 39, 9–20. [https://doi.org/10.1016/0168-1656\(94\)00138-3](https://doi.org/10.1016/0168-1656(94)00138-3).
- Ahn, S., Jung, J., Jang, I.-A., Madsen, E.L., Park, W., 2016. Role of glyoxylate shunt in oxidative stress response. *J. Biol. Chem.* 291, 11928–11938. <https://doi.org/10.1074/jbc.M115.708149>.
- Allen, A.E., Dupont, C.L., Obornik, M., Horák, A., Nunes-Nesi, A., McCrow, J.P., Zheng, H., Johnson, D.A., Hu, H., Fernie, A.R., Bowler, C., 2011. Evolution and metabolic significance of the urea cycle in photosynthetic diatoms. *Nature* 473, 203–207. <https://doi.org/10.1038/nature10074>.
- Andrews, S., 2016. FastQC: a quality control tool for high throughput sequence data [WWW Document]. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- Aneja, P., Dziak, R., Cai, G.-Q., Charles, T.C., 2002. Identification of an acetoacetyl coenzyme A synthetase-dependent pathway for utilization of l-(+)-3-hydroxybutyrate in *Sinorhizobium meliloti*. *J. Bacteriol.* 184, 1571–1577. <https://doi.org/10.1128/JB.184.6.1571-1577.2002>.
- Baker, J.M., 1970. The effects of oils on plants. *Environ. Pollut.* 1, 27–44. [https://doi.org/10.1016/0013-9327\(70\)90004-2](https://doi.org/10.1016/0013-9327(70)90004-2), 1970.
- Batista, M.B., Teixeira, C.S., Sfeir, M.Z.T., Alves, L.P.S., Valdameri, G., Pedrosa de, F.O., Sasaki, G.L., Steffens, M.B.R., de Souza, E.M., Dixon, R., Müller-Santos, M., 2018.

- PHB biosynthesis counteracts redox stress in *Herbaspirillum seropedicae*. *Front. Microbiol.* 9 <https://doi.org/10.3389/fmicb.2018.00472>.
- Bik, H.M., Halanuch, K.M., Sharma, J., Thomas, W.K., 2012. Dramatic shifts in benthic microbial eukaryote communities following the deepwater Horizon oil spill. *PLoS One* 7, e38550. <https://doi.org/10.1371/journal.pone.0038550>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Brämer, C.O., Silva, L.F., Gomez, J.G.C., Priefert, H., Steinbüchel, A., 2002. Identification of the 2-methylcitrate pathway involved in the catabolism of propionate in the polyhydroxyalkanoate-producing strain *Burkholderia sacchari* IPT101T and analysis of a mutant accumulating a copolyester with higher 3-hydroxyvalerate content. *Appl. Environ. Microbiol.* 68, 271–279. <https://doi.org/10.1128/AEM.68.1.271-279.2002>.
- Brämer, C.O., Steinbüchel, A., 2001. The methylcitric acid pathway in *Ralstonia eutropha*: new genes identified involved in propionate metabolism. *Microbiology* 147, 2203–2214. <https://doi.org/10.1099/00221287-147-8-2203>.
- Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016a. Near-optimal probabilistic RNA-seq quantification. *Nat. Biotechnol.* 34, 525–527. <https://doi.org/10.1038/nbt.3519>.
- Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016b. Near-optimal probabilistic RNA-seq quantification. *Nat. Biotechnol.* 34, 525–527. <https://doi.org/10.1038/nbt.3519>.
- Bretherton, L., Kamalanathan, M., Genzer, J., Hillhouse, J., Setta, S., Liang, Y., Brown, C.M., Xu, C., Sweet, J., Passow, U., Finkel, Z.V., Irwin, A.J., Santschi, P.H., Quigg, A., 2019. Response of natural phytoplankton communities exposed to crude oil and chemical dispersants during a mesocosm experiment. *Aquat. Toxicol.* 206, 43–53. <https://doi.org/10.1016/j.aquatox.2018.11.004>.
- Bretherton, L., Williams, A., Genzer, J., Hillhouse, J., Kamalanathan, M., Finkel, Z.V., Quigg, A., 2018. Physiological response of 10 phytoplankton species exposed to macondo oil and the dispersant. *Corexit*. *J. Phycol.* 54, 317–328. <https://doi.org/10.1111/jpy.12625>.
- Brock, M., Buckel, W., 2004. On the mechanism of action of the antifungal agent propionate. *Eur. J. Biochem.* 271, 3227–3241. <https://doi.org/10.1111/j.1432-1033.2004.04255.x>.
- Buchfink, B., Xie, C., Huson, D.H., 2015. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* 12, 59–60. <https://doi.org/10.1038/nmeth.3176>.
- Buckel, W., 1992. Unusual dehydrations in anaerobic bacteria. *FEMS Microbiol. Rev.* 8, 211–231. <https://doi.org/10.1111/j.1574-6968.1992.tb04989.x>.
- Cahill, G.F., 2006. Fuel metabolism in starvation. *Annu. Rev. Nutr.* 26, 1–22. <https://doi.org/10.1146/annurev.nutr.26.061505.111258>.
- Campeão, M.E., Reis, L., Leomil, L., de Oliveira, L., Otsuki, K., Gardinali, P., Pelz, O., Valle, R., Thompson, F.L., Thompson, C.C., 2017. The deep-sea microbial community from the amazonian basin associated with oil degradation. *Front. Microbiol.* 8, 1019. <https://doi.org/10.3389/fmicb.2017.01019>.
- Carvalho, R.N., Bopp, S.K., Lettieri, T., 2011a. Transcriptomics responses in marine diatom *Thalassiosira pseudonana* exposed to the polycyclic aromatic hydrocarbon benzo[a]pyrene. *PLoS One* 6, e26985. <https://doi.org/10.1371/journal.pone.0026985>.
- Carvalho, R.N., Burchardt, A.D., Sena, F., Mariani, G., Mueller, A., Bopp, S.K., Umlauf, G., Lettieri, T., 2011b. Gene biomarkers in diatom *Thalassiosira pseudonana* exposed to polycyclic aromatic hydrocarbons from contaminated marine surface sediments. *Aquat. Toxicol.* 101, 244–253. <https://doi.org/10.1016/j.aquatox.2010.10.004>.
- Cerniglia, C.E., Sutherland, J.B., 2010. Degradation of polycyclic aromatic hydrocarbons by Fungi. In: Timmins, K.N. (Ed.), *Handbook of Hydrocarbon and Lipid Microbiology*. Springer Berlin Heidelberg, Berlin, pp. 2079–2110. [https://doi.org/10.1007/978-3-540-77587-4\\_151](https://doi.org/10.1007/978-3-540-77587-4_151).
- Chakka, D., Gudla, R., Madikonda, A.K., Pandeeti, E.V.P., Parthasarathy, S., Nandavaram, A., Siddavattam, D., 2015. The organophosphate degradation (*opd*) Island-borne Esterase-induced metabolic diversion in *Escherichia coli* and its influence on p-Nitrophenol degradation. *J. Biol. Chem.* 290, 29920–29930. <https://doi.org/10.1074/jbc.M115.661249>.
- Crone, T.J., Tolstoy, M., 2010. Magnitude of the 2010 Gulf of Mexico oil leak. *Science* 330. <https://doi.org/10.1126/science.1195840>, 634–634.
- Deasi, S.R., Verlecar, X.N., Ansari, Z.A., Jagtap, T.G., Sarkar, A., Vashistha, D., Dalal, S.G., 2010. Evaluation of genotoxic responses of *Chaetoceros tenuissimus* and *Skeletonema costatum* to water accommodated fraction of petroleum hydrocarbons as biomarker of exposure. *Water Res.* 44, 2235–2244. <https://doi.org/10.1016/j.watres.2009.12.048>.
- Dedkova, E.N., Blatter, L.A., 2014. Role of  $\beta$ -hydroxybutyrate, its polymer poly- $\beta$ -hydroxybutyrate and inorganic polyphosphate in mammalian health and disease. *Front. Physiol.* 5 <https://doi.org/10.3389/fphys.2014.00260>.
- Doyle, S.M., Whitaker, E.A., De Pascuale, V., Wade, T.L., Knap, A.H., Santschi, P.H., Quigg, A., Sylvan, J.B., 2018. Rapid formation of microbe-oil aggregates and changes in community composition in coastal surface water following exposure to oil and the dispersant corexit. *Front. Microbiol.* 9 <https://doi.org/10.3389/fmicb.2018.00689>.
- Fdz-Polanco, F., Fdz-Polanco, M., Fernandez, N., Uruña, M.A., Garcia, P.A., Villaverde, S., 2001. New process for simultaneous removal of nitrogen and sulphur under anaerobic conditions. *Water Res.* 35, 1111–1114. [https://doi.org/10.1016/S0043-1354\(00\)00474-7](https://doi.org/10.1016/S0043-1354(00)00474-7).
- Finkel, Z.V., Liang, Y., Nanjappa, D., Bretherton, L., Brown, C.M., Quigg, A., Irwin, A.J., 2020. A ribosomal sequence-based oil sensitivity index for phytoplankton groups. *Mar. Pollut. Bull.* 151, 110798 <https://doi.org/10.1016/j.marpolbul.2019.110798>.
- Garr, A.L., Laramore, S., Krebs, W., 2014. Toxic effects of oil and dispersant on marine microalgae. *Bull. Environ. Contam. Toxicol.* 93, 654–659. <https://doi.org/10.1007/s00128-014-1395-2>.
- Gemmell, B.J., Bacosa, H.P., Dickey, B.O., Gemmell, C.G., Alqasemi, L.R., Buskey, E.J., 2018. Rapid alterations to marine microbiota communities following an oil spill. *Ecotoxicology* 27, 505–516. <https://doi.org/10.1007/s10646-018-1923-7>.
- Gilde, K., Pinckney, J.L., 2012. Sublethal effects of crude oil on the community structure of Estuarine phytoplankton. *Estuar. Coast* 35, 853–861. <https://doi.org/10.1007/s12237-011-9473-8>.
- Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 29, 644–652. <https://doi.org/10.1038/nbt.1883>.
- Green, C.R., Wallace, M., Divakaruni, A.S., Phillips, S.A., Murphy, A.N., Ciaraldi, T.P., Metallo, C.M., 2016. Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nat. Chem. Biol.* 12, 15–21. <https://doi.org/10.1038/nchembio.1961>.
- Güngörmedi, G., Demirebilek, M., Mutlu, M.B., Denkbaş, E.B., Çabuk, A., 2014. Polyhydroxybutyrate and hydroxyvalerate production by *Bacillus megaterium* strain A1 isolated from hydrocarbon-contaminated soil. *J. Appl. Polym. Sci.* 131 <https://doi.org/10.1002/app.40530>.
- Gutierrez, T., Berry, D., Yang, T., Mishamandani, S., McKay, L., Teske, A., Aitken, M.D., 2013. Role of bacterial Exopolysaccharides (EPS) in the fate of the oil released during the deepwater Horizon oil spill. *PLoS One* 8, e67717. <https://doi.org/10.1371/journal.pone.0067717>.
- Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N., Regev, A., 2013. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* 8, 1494–1512. <https://doi.org/10.1038/nprot.2013.084>.
- Handley, K.M., Piceno, Y.M., Hu, P., Tom, L.M., Mason, O.U., Andersen, G.L., Jansson, J.K., Gilbert, J.A., 2017. Metabolic and spatio-taxonomic response of uncultivated seafloor bacteria following the Deepwater Horizon oil spill. *ISME J.* 11, 2569–2583. <https://doi.org/10.1038/ismej.2017.110>.
- Hesketh, J.D., 1963. Limitations to photosynthesis responsible for differences among Species 1. *Crop Sci.* 3, 493–496. <https://doi.org/10.2135/cropsci1963.0011183X000300060011x>.
- Hildebrandt, T.M., 2018. Synthesis versus degradation: directions of amino acid metabolism during *Arabidopsis* abiotic stress response. *Plant Mol. Biol.* 98, 121–135. <https://doi.org/10.1007/s11103-018-0767-0>.
- Hook, S.E., Osborn, H.L., 2012. Comparison of toxicity and transcriptomic profiles in a diatom exposed to oil, dispersants, dispersed oil. *Aquat. Toxicol.* 124–125, 139–151. <https://doi.org/10.1016/j.aquatox.2012.08.005>.
- Hsiao, S.I.C., Kittle, D.W., Foy, M.G., 1978. Effects of crude oils and the oil dispersant corexit on primary production of arctic marine phytoplankton and seaweed. *Environ. Pollut.* 15, 209–221. [https://doi.org/10.1016/0013-9327\(78\)90066-6](https://doi.org/10.1016/0013-9327(78)90066-6), 1970.
- Kadouri, D., Jurkevitch, E., Okon, Y., 2003. Involvement of the reserve material poly- $\beta$ -hydroxybutyrate in *Azospirillum brasilense* stress endurance and root colonization. *Appl. Environ. Microbiol.* 69, 3244–3250. <https://doi.org/10.1128/AEM.69.6.3244-3250.2003>.
- Kimes, N.E., Callaghan, A.V., Aktas, D.F., Smith, W.L., Sunner, J., Golding, B.T., Drozdowska, M., Hazen, T.C., Sulflita, J.M., Morris, P.J., 2013. Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. *Front. Microbiol.* 4 <https://doi.org/10.3389/fmicb.2013.00050>.
- Krishnakumar, A.M., Sliwa, D., Endrizzi, J.A., Boyd, E.S., Ensign, S.A., Peters, J.W., 2008. Getting a handle on the role of coenzyme M in alkene metabolism. *Microbiol. Mol. Biol. Rev.* 72, 445–456. <https://doi.org/10.1128/MMBR.00005-08>.
- Kujawinski, E.B., Kido Soule, M.C., Valentine, D.L., Boysen, A.K., Longnecker, K., Redmond, M.C., 2011. Fate of dispersants associated with the deepwater Horizon oil spill. *Environ. Sci. Technol.* 45, 1298–1306. <https://doi.org/10.1021/es103838p>.
- Kulakovskaya, T., Rysanova, L., Dmitriev, V., Zvonarev, A., 2016. The role of inorganic polyphosphates in stress response and regulation of enzyme activities in yeast. In: Kulakovskaya, T., Pavlov, E., Dedkova, E.N. (Eds.), *Inorganic Polyphosphates in Eukaryotic Cells*. Springer International Publishing, Cham, pp. 3–14. [https://doi.org/10.1007/978-3-319-41073-9\\_1](https://doi.org/10.1007/978-3-319-41073-9_1).
- Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22, 1658–1659. <https://doi.org/10.1093/bioinformatics/btl158>.
- Liang, Y., Koester, J.A., Liefer, J.D., Irwin, A.J., Finkel, Z.V., 2019. Molecular mechanisms of temperature acclimation and adaptation in marine diatoms. *ISME J.* 13, 2415–2425. <https://doi.org/10.1038/s41396-019-0441-9>.
- Mason, O.U., Scott, N.M., Gonzalez, A., Robbins-Pianka, A., Bælum, J., Kimbrel, J., Bouskill, N.J., Prestat, E., Borglin, S., Joyner, D.C., Fortney, J.L., Jurelevicius, D., Stringfellow, W.T., Alvarez-Cohen, L., Hazen, T.C., Knight, R., Gilbert, J.A., Jansson, J.K., 2014. Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. *ISME J.* 8, 1464–1475. <https://doi.org/10.1038/ismej.2013.254>.
- McNutt, M.K., Camilli, R., Crone, T.J., Guthrie, G.D., Hsieh, P.A., Ryerson, T.B., Savas, O., Shaffer, F., 2012. Review of flow rate estimates of the Deepwater Horizon

- oil spill. Proc. Natl. Acad. Sci. Unit. States Am. 109, 20260–20267. <https://doi.org/10.1073/pnas.1112139108>.
- Moss, D.N., 1962. The limiting carbon dioxide concentration for photosynthesis. Nature 193. <https://doi.org/10.1038/193587a0>, 587–587.
- Niimura, Y., Koh, E., Uchimura, T., Ohara, N., Kozaki, M., 1989. Aerobic and anaerobic metabolism in a facultative anaerobe Ep01 lacking cytochrome, quinone and catalase. FEMS Microbiol. Lett. 61, 79–83. <https://doi.org/10.1111/j.1574-6968.1989.tb03556.x>.
- O'Brien, P.Y., Dixon, P.S., 1976. The effects of oils and oil components on algae: a review. Br. Phycol. J. 11, 115–142. <https://doi.org/10.1080/00071617600650161>.
- Obruca, S., Sedlacek, P., Koller, M., Kucera, D., Pernicova, I., 2018. Involvement of polyhydroxyalkanoates in stress resistance of microbial cells: biotechnological consequences and applications. Biotechnol. Adv., Prospect. Biotechnol. 36, 856–870. <https://doi.org/10.1016/j.biotechadv.2017.12.006>.
- Pimentel, H., Bray, N.L., Puente, S., Melsted, P., Pachter, L., 2017. Differential analysis of RNA-seq incorporating quantification uncertainty. Nat. Methods 14, 687–690. <https://doi.org/10.1038/nmeth.4324>.
- Preusting, H., Nijenhuis, A., Witholt, B., 1990. Physical characteristics of poly(3-hydroxyalkanoates) and poly(3-hydroxyalkenoates) produced by *Pseudomonas oleovorans* grown on aliphatic hydrocarbons. Macromolecules 23, 4220–4224. <https://doi.org/10.1021/ma00221a007>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Quintero, M.J., Muro-Pastor, A.M., Herrero, A., Flores, E., 2000. Arginine catabolism in the cyanobacterium *synechocystis* sp. strain PCC 6803 involves the urea cycle and arginase pathway. J. Bacteriol. 182, 1008–1015. <https://doi.org/10.1128/JB.182.4.1008-1015.2000>.
- Rivers, A.R., Sharma, S., Tringe, S.G., Martin, J., Joye, S.B., Moran, M.A., 2013. Transcriptional response of bathypelagic marine bacterioplankton to the Deepwater Horizon oil spill. ISME J. 7, 2315–2329. <https://doi.org/10.1038/ismej.2013.129>.
- Robinson, A.M., Williamson, D.H., 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev. 60, 143–187. <https://doi.org/10.1152/physrev.1980.60.1.143>.
- Rughöft, S., Jehmlich, N., Gutierrez, T., Kleindienst, S., 2021. Comparative proteomics of *Marinobacter* sp. TT1 reveals corexit impacts on hydrocarbon metabolism, chemotactic motility, and biofilm formation. Microorganisms 9. <https://doi.org/10.3390/microorganisms9010003>.
- Rughöft, S., Vogel, A.L., Joye, S.B., Gutierrez, T., Kleindienst, S., 2020. Starvation-dependent inhibition of the hydrocarbon degrader *Marinobacter* sp. TT1 by a chemical dispersant. J. Mar. Sci. Eng. 8 <https://doi.org/10.3390/jmse8110925>.
- Schnarrenberger, C., Martin, W., 2002. Evolution of the enzymes of the citric acid cycle and the glyoxylate cycle of higher plants. Eur. J. Biochem. 269, 868–883. <https://doi.org/10.1046/j.0014-2956.2001.02722.x>.
- Sekiya, M., Hiraishi, A., Touyama, M., Sakamoto, K., 2008. Oxidative stress induced lipid accumulation via SREBP1c activation in HepG2 cells. Biochem. Biophys. Res. Commun. 375, 602–607. <https://doi.org/10.1016/j.bbrc.2008.08.068>.
- Suzuki, S., Kakuta, M., Ishida, T., Akiyama, Y., 2014. GHOSTX: an improved sequence homology search algorithm using a query suffix array and a database suffix array. PLoS One 9, e103833. <https://doi.org/10.1371/journal.pone.0103833>.
- Thompson, H., Angelova, A., Bowler, B., Jones, M., Gutierrez, T., 2017. Enhanced crude oil biodegradative potential of natural phytoplankton-associated hydrocarbonoclastic bacteria. Environ. Microbiol. 19, 2843–2861. <https://doi.org/10.1111/1462-2920.13811>.
- Transportation Research Board and National Research Council, 2002. Oil in the Sea III: Inputs, Fates, and Effects. The National Academies Press, Washington, D.C. <https://doi.org/10.17226/10388>.
- Wade, T.L., Morales-McDevitt, M., Bera, G., Shi, D., Sweet, S., Wang, B., Gold-Bouchot, G., Quigg, A., Knap, A.H., 2017. A method for the production of large volumes of WAF and CEWAF for dosing mesocosms to understand marine oil snow formation. Heliyon 3, e00419. <https://doi.org/10.1016/j.heliyon.2017.e00419>.
- Wade, T.L., Sweet, S.T., Sericano, J.L., Guinasso, N.L., Diercks, A.-R., Highsmith, R.C., Asper, V.L., Joung, D., Shiller, A.M., Lohrenz, S.E., Joye, S.B., 2011. Analyses of water samples from the deepwater Horizon oil spill: documentation of the subsurface plume. In: Liu, Y., MacFadyen, A., Ji, Z.-G., Weisberg, R.H. (Eds.), Geophysical Monograph Series. American Geophysical Union, Washington, D. C., pp. 77–82. <https://doi.org/10.1029/2011GM001103>.
- Wolfe, M.F., Schlosser, J.A., Schwartz, G.J.B., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L., 1998. Influence of dispersants on the bioavailability and trophic transfer of petroleum hydrocarbons to primary levels of a marine food chain. Aquat. Toxicol. 42, 211–227. [https://doi.org/10.1016/S0166-445X\(97\)00096-9](https://doi.org/10.1016/S0166-445X(97)00096-9).
- Wolfe, M.F., Schwartz, G.J.B., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L., 2000. Influence of dispersants on the bioavailability and trophic transfer of phenanthrene to algae and rotifers. Aquat. Toxicol. 48, 13–24. [https://doi.org/10.1016/S0166-445X\(99\)00028-4](https://doi.org/10.1016/S0166-445X(99)00028-4).
- Wood, D.E., Salzberg, S.L., 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol. 15, R46 <https://doi.org/10.1186/gb-2014-15-3-r46>.
- Zrenner, R., Sitt, M., Sonnwald, U., Boldt, R., 2006. Pyrimidine and purine biosynthesis and degradation in plants. Annu. Rev. Plant Biol. 57, 805–836. <https://doi.org/10.1146/annurev.arplant.57.032905.105421>.