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Transcriptome-wide responses of aggregates of the diatom *Odontella aurita* to oil

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ABSTRACT: Diatom aggregates can play an important role in the formation of marine oil snow and the transport of oil from the sea surface to the benthos, yet their molecular response to oil has not been characterized. Here we use RNA-seq to analyze the transcriptome-wide responses of aggregates of the common Gulf of Mexico diatom *Odontella aurita* exposed to the water accommodated fraction of 2 different types of oil, Macondo surrogate and Refugio Beach oil. We identify a common set of 353 genes that are differentially expressed in response to both Macondo and Refugio oil exposure, relative to controls. Genes related to photosynthesis, and nuclear and ribosomal processes were all down-regulated in oil treatments, while genes related to repairing membrane damage, cellular stress, and the production of exopolymeric substances were up-regulated. Differential expression of genes was often greater in magnitude in the Refugio than the Macondo oil treatment, which may be due to differences in oil concentration in the treatments or to the physiochemical characteristics of the oils. Exposure to Refugio oil induced more severe nucleolar stress and more damage to chloroplasts than the lighter Macondo oil, which triggered an up-regulation of a more diverse suite of stress-response genes.

KEY WORDS: Diatoms · Marine oil snow · Transcriptome · Macondo surrogate oil · Refugio Beach oil

1. INTRODUCTION

Anthropogenic discharge of crude oil into the marine environment is a global-scale phenomenon that is responsible for introducing millions of liters into the oceans every year (Transportation Research Board and National Research Council 2002, Farrington 2013). Oil exposure is generally damaging to marine life (Beyer et al. 2016), although there is no consensus on the consequences for planktonic communities, particularly phytoplankton. Accumulated evidence suggests that oil exposure can lead to both increases (Cross 1987, Hu et al. 2011, Tang et al. 2019) and decreases (Paul et al. 2013, Brussaard et al. 2016) in net primary production. Sources of uncertainty arise from variability in effects on individual species (Özhan et al. 2014, Bretherton et al. 2018, 2019), interactions between oil exposure and environmental conditions (Karydis 1981, Özhan & Bargu 2014), changes in grazer behavior (Carls et al. 2006, Ortmann et al. 2012), and the type and concentration of oil (Hsiao et al. 1978, Gilde & Pinckney 2012, Garr et al. 2014).

The vertical export and sedimentation of hydrocarbons in association with phytoplankton aggregates has frequently been observed (Lubecki & Kowalewska

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2010, Parsons et al. 2014). Dissolved oil components and oil droplets can become incorporated into aggregates and form marine oil snow (MOS), which sinks and exports oil to the deep ocean. Following the Deepwater Horizon spill in April 2010, it was estimated that 21% (±10%) of the oil ended up on the seafloor (Chanton et al. 2015, Romero et al. 2017), though some of this oil may have been from natural oil seeps. This oil was found in association with large phytoplankton aggregates consisting mainly of chain-forming diatoms (Passow et al. 2012, Daly et al. 2016, Yan et al. 2016). Marine snow formation is an important part of the diatom life cycle, with spring blooms typically terminated by aggregation of cells into flocs followed by their sedimentation to the seafloor (Smetacek 1985, Kiørboe et al. 1994, Passow et al. 1994). Coagulation and aggregation rates are affected by factors such as diatom community composition (Tréguer et al. 2018) and life stage (Kahl et al. 2008). While there is an increasing understanding of the factors and processes that regulate how much oil is incorporated into aggregates (Passow et al. 2017, Suja et al. 2019, Quigg et al. 2020), little is known about how diatom aggregates respond to oil molecularly and physiologically, or how these responses vary with different types of oil.

Previous work on phytoplankton cultures has shown that oil exposure can damage DNA (Desai et al. 2010, Hook & Osborn 2012) and the photosynthetic apparatus (Aksmann & Tukaj 2008, Aksmann et al. 2011, Bretherton et al. 2018, 2019). Molecular studies on the model diatom *Thalassiosira pseudonana* have revealed that exposure to certain oil compounds (benzo(a)pyrene) can cause extensive up-regulation of genes associated with oxidative stress such as protein refolding (Carvalho et al. 2011a), while those involved in silica uptake, cell cycle regulation, and light harvesting are all down-regulated (Bopp & Lettieri 2007, Carvalho et al. 2011b). Regulation of genes associated with lipid synthesis and catabolism suggest that exposure to some hydrocarbons also requires maintenance of damaged membranes (Carvalho & Lettieri 2011, Carvalho et al. 2011a). It has been proposed that oil exposure may also increase the exudation of organic material, which may alter cell stickiness and facilitate the formation of MOS (Gutierrez et al. 2013, Xu et al. 2018, Passow et al. 2019). The exudation of exopolymeric substances (EPS) has been shown to have a protective role in preventing the penetration of toxic substances into the cytoplasm (Miao et al. 2009, Zhang et al. 2013), and thus EPS production in the presence of oil may reduce its toxicity. Increased exudation of EPS can in turn facilitate the formation of MOS (Gutierrez et al. 2013, Passow & Ziervogel 2016). However, few studies have focused on the influence of oil exposure on the genes and biochemical pathways associated with the release and exudation of such material in either free-living or aggregate-associated diatoms.

Here we use differential gene expression analyses to identify the physiological and molecular responses of diatom aggregates of the chain-forming diatom *Odontella aurita* to exposure to different oils. *O. aurita* CCMP816 is a common bloom-forming diatom in the Gulf of Mexico (Merino-Virgilio et al. 2013). We exposed *O. aurita* to 2 different types of oil: Macondo surrogate oil, which is representative of the light oil released in the 2010 Deepwater Horizon event, and higher viscosity Refugio Beach oil, which was harvested from the 2015 pipeline accident near Refugio Beach in Santa Barbara County, California, USA (see Passow et al. 2019). These 2 oils possess different chemical and physical characteristics that may alter how the diatoms respond at the molecular, biochemical, and physiological levels, including the formation of aggregates and MOS. For example, the Refugio oil contains more higher molecular weight aromatics than the Macondo oil, which are typically more toxic than lower molecular weight compounds (see Passow et al. 2019 for a detailed comparison). We exposed *O. aurita* to oil using the water-accommodated fraction (WAF), which is comprised of both the water-soluble fraction of oil as well as some particulate oil in the form of droplets (after Singer et al. 2001).

We identify differentially expressed genes and biochemical pathways (Gene Ontology analyses), including those thought to be associated with the exudation of organic material, between the control and oil exposure treatments and between the 2 different oil treatments. These analyses allow us to test the following hypotheses: (1) damage and stress from oil exposure will result in the differential expression of genes and pathways sensitive to oil, especially stress-related genes, and genes and pathways associated with photosynthesis; (2) exposure to oil causes an up-regulation of genes associated with exudation (EPS production); (3) both the magnitude and nature of transcriptomic responses will vary across oils due to their different physical and chemical characteristics. We define 'stress' as sub-optimal performance of metabolism (Pierce et al. 2005), with 'damage' being a particular type of stress that results in physical disruption of cellular structures such as membranes. Our goal is to gain insight into the physiological consequences of oil exposure through molecular analy-

sis, which may be helpful in developing models of the role of diatoms in MOS and particles exported from the surface ocean following an oil spill.

2. MATERIALS AND METHODS

2.1. Culture conditions

The culture conditions and roller table experimental design are described in Passow et al. (2019). Briefly, a semi-continuous culture of *Odontella aurita* CCMP 816 was acclimated over several months to 22°C under 70 µmol m^{-2} s^{-1} of white light with a 12 h light:12 h dark cycle in f/2-enriched seawater (Guillard & Ryther 1962, Guillard 1975). Seawater was collected from the University of California Santa Barbara seawater system from a source 800 m offshore at 18 m depth and treated with gravity sand filters, filtered twice (0.2 µm) and UV-treated for at least 30 min. Macronutrients were added to a final concentration of 58.9 µM nitrate, 3.6 µM phosphate, and 53.5 µM silicic acid.

2.2. Preparation of oil and control treatments

O. aurita was exposed to WAF prepared with Macondo surrogate oil (Marlin platform, Dorado source oil, density 0.865 g ml^{-1}), Refugio Beach oil (density 0.946 g ml^{-1}) and seawater only (control). Utilizing WAF for toxicity studies is advantageous because it ensures test organisms are exposed to toxicants and limits how much oil adheres to the sides of glass containers (Singer et al. 2001). The 2 WAF treatments were prepared using a modified version of the CROSERF (Chemical Response to Oil Spills: Ecological Research Forum) method (Singer et al. 2000). Briefly, oil was added to pasteurized seawater (1% vol:vol) in glass aspirator bottles with bottom spigots, leaving approximately 25% headspace. The mixture was stirred in the dark for 24 h with a magnetic stir bar. The resulting WAF was then harvested through the bottom spigot and mixed with dense *O. aurita* cultures sampled from late exponential/early stationary phase. The initial estimated oil equivalents (EOE) measured according to Wade et al. (2017) were 120 ± 27 and 367 ± 58 µg l^{-1} for the Macondo and Refugio treatments, respectively, At the end of the experiment, the EOE was determined to be 109 ± 72 and 200 ± 11 µg l^{-1} for Macondo and Refugio, respectively (as reported by Passow et al. 2019). Pasteurized (65°C, 4 h), unenriched seawater without oil was mixed with *O. aurita* for the control.

2.3. Roller table experiment and sampling of diatom aggregates

Roller tanks allow for the formation of diatom aggregates. WAF or seawater (for the control) was added to diluted diatom cultures in the ratio 1:4. The initial cell density in the experimental treatments was 22 000 cells ml^{-1}. Twelve glass roller table tanks (5.77 l) were used, with 4 replicate tanks per treatment. Tanks were filled, with no air bubbles remaining, incubated in the dark at 22°C and rotated at 2.6 rpm before sampling. The experiment was conducted in the dark to replicate the conditions marine snow aggregates experience once they sink out of surface waters. Low green light was used during sampling. Due to the effort required to sample aggregates from tanks, only 1 replicate from each treatment was sampled at a time. The 4 replicates were sampled in the morning and afternoon of Days 3 and 4. Aggregates were manually sampled by syringe or cut-off pipette, collected into a slurry, and then filtered onto 3 µm polycarbonate filters, flash frozen in liquid nitrogen, and stored at −80°C.

2.4. RNA extraction and sequencing

Total RNA was extracted from aggregates filtered onto 3 µm polycarbonate filters. Extractions were performed using organic extractions, as follows. Filters were bead beaten with 800 µl Trigent reagent using a FastPrep-24 and Matrix D beads (MP Biomedicals) 3 times for 45 s. The aqueous phase was mixed thoroughly with 300 µl of buffered phenol (pH 7.9) and incubated for 15 min at 4°C. Chloroform (150 µl) was added and samples were shaken for 15 s before centrifuging at 12 000 × *g* for 15 min at 4°C. The aqueous phase was recovered and extracted again with 150 µl chloroform, as above. Samples were isopropanol precipitated, DNAse treated (Turbo DNAse, Ambion AM1907), and dissolved in 40 µl RNAse-free water. Quality and quantity were assessed by nanodrop. mRNA was sequenced (Illumina HiSeq 2500) by the McGill University Genome Quebec Innovation Centre using the TruSeq mRNA stranded library preparation protocols (Illumina) for 125 bp paired-end reads. Within the TruSeq protocol, cDNA was synthesized using random hexamer primers from mRNA selected with magnetic beads bound to oligo-Ts and fragmented by divalent cations and heat (TruSeq Stranded mRNA Sample Preparation Guide 2013, protocol available at https://support.illumina.com).

2.5. Transcriptome assembly and annotation

The quality of the raw reads was checked using FastQC (version 0.11.5) (Andrews 2016). Raw reads were then mapped to the PhiX genome using Bowtie 2 (version 2.2.9) (Langmead & Salzberg 2012) and the unmapped reads were outputted using SAMtools (version 1.3.1) (Li et al. 2009). Kraken was used to remove the majority of potential bacterial, plasmid, and rRNA sequences with the NCBI bacteria and virus genome database and the Silva LSU and SSU ref rRNA sequence database (release 128) (Quast et al. 2013, Wood & Salzberg 2014). The first 12 nucleotides (nt) from the 5′ end of the reads and the 3′ adapter fragments were removed using cutadapt (version 1.10). Reads shorter than 25 nt were removed using cutadapt and paired using Trimmomatic (version 0.36). Unpaired reads were attached to the end of the left reads of the paired fragments for assembly. The left and right reads were merged and normalized using Trinity's (version 2.2.0) *in silico* normalization module, then assembled into contigs using Trinity's paired-end mode (Grabherr et al. 2011). The coding regions within transcripts were obtained using TransDecoder (version 3.0.0). The newly assembled transcriptomes were annotated using blastx for nucleotide sequences and blastp for peptide sequences generated from TransDecoder against the uniprot_swiss-prot database (release 2016.08). Pfam annotations were obtained using hmmscan (version 3.1b2). Blastx, blastp, and hmmscan results were systematically combined using Trinotate (version 3.0.1). KEGG identifications (IDs) and gene ontology (GO) IDs of each annotated gene were obtained from the uniprot database using uniprot IDs. KEGG IDs of each amino acid sequence were obtained from the GhostKOALA service from KEGG (www.kegg.jp/ghostkoala/).

2.6. Differential expression and GO enrichment

Cleaned and trimmed reads of each sample were mapped to the assembled transcriptome using Bowtie 2. Transcripts counts were obtained with Trinity's abundance_estimates_to_matrix module using eXpress (Roberts & Pachter 2013). Transcripts with counts > 0 in at least 2 of 4 replicates of each oil treatment and control were designated as genes and used for differential gene expression analysis. In order to evaluate the impact of different sampling times on transcriptome regulation, the differentially expressed gene analysis between Day 3 and Day 4, AM and PM, and oil and control were calculated using DESeq2 within the Trinity's run_DE_analysis module. Genes in Day 4, PM, and the 2 oil treatments with adjusted p-values less than 0.05 and log fold change unequal to 0 as compared to their expression level in Day 3, AM, and control, respectively, were determined as differentially expressed genes between Day 3 and Day 4, AM and PM, oil and control. Differential gene expression between different oils and control was also calculated. Genes in Macondo and Refugio treatments with adjusted p-values less than 0.05 and log fold change unequal to 0 as compared to their expression level in the control were determined as differentially expressed genes in Macondo and Refugio treatments, respectively. Principal component analysis for all genes and differentially expressed genes between different oil and control were done using the factoextra package in R (R Core Team 2020, version 4.0.3). Venn diagrams were made using the VennDiagram package in R. GO enrichment analysis was conducted using the GOstats (Falcon & Gentleman 2007) package in R (p-value < 0.01, size < 500, counts > 10). GOogy annotates genes and gene products using 3 domains: biological process, molecular function, and cellular component.

3. RESULTS

3.1. Influence of oil on aggregation

Passow et al. (2019) characterized the influence of oil (Macondo and Refugio) on aggregation and the incorporation of oil into diatom aggregates. In brief, a smaller proportion of *Odontella* cells aggregated in the oil treatments (Macondo 25%, Refugio 44%) compared to the control (57%) and less of the oil was incorporated in aggregates formed in the Macondo treatment (fraction of oil carbon measured as EOE, 19%) compared to the Refugio treatment (34%).

3.2. Sequences generated

Sequencing resulted in an average of 57.6 million paired-end reads for each of the 12 libraries (BioProject PRJNA505514, GoMRI database ID). We assembled 277 438 transcripts from the combined trimmed and filtered reads in all samples. Many of these transcripts had little support in the original reads; we identified 58 939 genes with non-zero counts in at least 2 of 4 replicates in at least 1 treatment.

3.3. Influence of sampling time on expression

Aggregate sampling was time-intensive so the 4 replicates for each treatment (control, Macondo, Refugio) were sampled at a different time or day (morning or afternoon on Day 3 or 4). A PCA of counts (transcripts per million, TPM) for all the genes indicated that samples show considerable variation across treatment and sampling time (Fig. S1a in Supplement 1 at www.int-res.com/articles/suppl/m671p067_supp1.pdf). Despite this variation at the gene expression level, the differential expression analysis clearly separated out the effects of oil treatment from sampling time (AM or PM and Day 3 or Day 4). The sets of differentially expressed genes were nearly disjoint for these 3 contrasts (oil, time, day) compared to the control (Fig. 1). The PCA of counts for just the genes differentially expressed between the oil treatments and control clearly distinguished the effects of Macondo and Refugio oils relative to the control (Fig. S1b).

3.4. Influence of oil on expression

A total of 1283 genes were significantly differentially expressed (DE) between the oil and control treatments (Fig. 2A). Of these, 353 DE genes were found in both oil treatments. There were more DE genes in the Refugio treatment (1000) compared to the Macondo treatment (636). Across both oil treatments, 40% of the DE genes were up-regulated and 60% were down-regulated for both the shared and distinct DE genes (Fig. 2B).

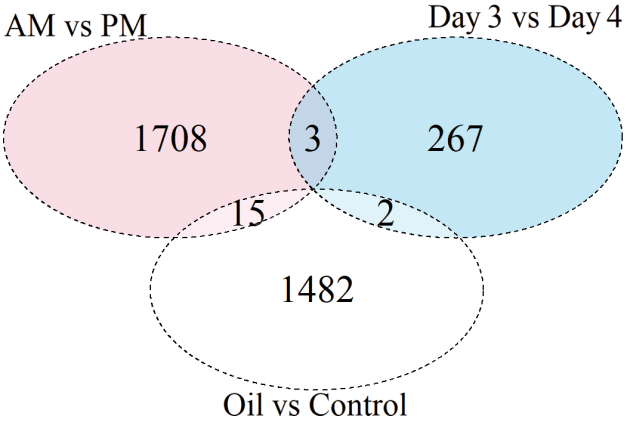

Fig. 1. Genes were differentially expressed (DE) in the oil treatments compared to the control and between different stages of sampling (time: AM vs. PM and Day: 3 vs. 4), but almost all DE genes were unique to only 1 of the 3 comparisons. The Venn diagram shows the number of DE genes in each comparison (272 to 1726) and the very small number of DE genes (20) arising in more than 1 comparison (2 to 15)

GO enrichment analysis showed that in both oil treatments, transcription of genes related to photosynthesis was down-regulated (Table 1). In the Refugio treatment, genes related to the nucleosome and glycolytic processes (carbohydrate metabolism) were also enriched in down-regulated genes. The up-regulated genes in both treatments showed no significant enrichment of any GO terms. We also observed down-regulation of photosynthesis-associated GO terms when the enrichment analysis was restricted to DE genes in both WAF treatments.

We found 40 DE genes with absolute log2-fold change ≥2.5 in both WAF treatments, with an additional 41 DE genes in the Macondo WAF treatment and 78 DE genes in the Refugio WAF treatment that might be potential biomarkers of oil exposure. Highly up-regulated DE genes were related to many processes including stress responses, signaling, and EPS production (Table 2). Highly down-regulated DE genes were related to photosynthesis and carbohydrate metabolism, among other processes. The details of all highly DE genes found in only 1 of the 2 WAF treatments can be found in Tables S1 (Macondo oil) and S2 (Refugio oil) in Supplement 2 at www.int-res.com/articles/suppl/m671p067_supp2.xlsx.

3.5. How the type of oil alters expression

Genes that are related to stress responses, the eukaryotic ribosome, signaling, biodegradation of aromatic compounds, pyruvate metabolism, apoptosis, amino acid transporters, the cytoskeleton, and base excision repair were uniquely up-regulated in the Macondo oil treatment (Table S1). Besides the genes that are involved in photosynthesis, 1 chaperone protein, 2 histones, 1 voltage-dependent calcium channel, 1 plastidial pyruvate transporter, and genes that are

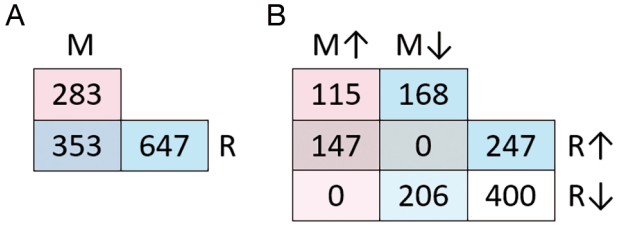

Fig. 2. The distribution of differentially expressed (DE) genes across the oil treatments. (A) The number of DE genes in Macondo (M), Refugio (R), or both treatments relative to control. (B) The same DE gene counts, separated according to whether they were (↑) up- or (↓) down-regulated. No genes were down-regulated in one oil treatment and up-regulated in the other

Table 1. Photosynthesis and photosynthesis-related (*) gene ontology (GO) terms enriched in down-regulated genes in the oil treatments relative to the control. All GO terms enriched in down-regulated genes in the Macondo oil treatment (M) were also enriched in the Refugio surrogate oil treatment (R), both indicated by x. BP: biological processes; CC: cellular component; MF: molecular function

GO ID	Category	GO term	Potential biological function	M	R
GO:0031976	CC	Plastid thylakoid	Photosynthesis	x	x
GO:0042651	CC	Thylakoid membrane	Photosynthesis	x	x
GO:0044436	CC	Thylakoid part	Photosynthesis	x	x
GO:0009535	CC	Chloroplast thylakoid membrane	Photosynthesis	x	x
GO:0015979	BP	Photosynthesis	Photosynthesis	x	x
GO:0009206	BP	Purine ribonucleoside triphosphate biosynthetic process	ATP metabolism*	x	x
GO:0009142	BP	Nucleoside triphosphate biosynthetic process	ATP metabolism*	x	x
GO:0046496	BP	Nicotinamide nucleotide metabolic process	NADPH/NADP+ metabolism*	x	x
GO:0072524	BP	Pyridine-containing compound metabolic process	NADPH/NADP+ metabolism*	x	x
GO:0006733	BP	Oxidoreduction coenzyme metabolic process	NADPH/NADP+ metabolism*	x	x
GO:0009168	BP	Purine ribonucleoside monophosphate biosynthetic process	ATP metabolism*	x	x
GO:0009144	BP	Purine nucleoside triphosphate metabolic process	ATP metabolism*	x	x
GO:0009124	BP	Nucleoside monophosphate biosynthetic process	ATP metabolism*	x	x
GO:0046034	BP	ATP metabolic process	ATP metabolism*		x
GO:0016168	MF	Chlorophyll binding	Photosynthesis		x
GO:0046982	MF	Protein heterodimerization activity	Protein heterodimerization		x
GO:0009523	CC	Photosystem II	Photosynthesis		x
GO:0030076	CC	Light-harvesting complex	Photosynthesis		x
GO:0000786	CC	Nucleosome	Nucleosome		x
GO:0018298	BP	Protein-chromophore linkage	Photosynthesis		x
GO:0009765	BP	Photosynthesis, light harvesting	Photosynthesis		x
GO:0006165	BP	Nucleoside diphosphate phosphorylation	ATP metabolism*		x
GO:0006096	BP	Glycolytic process	Carbohydrate metabolism		x
GO:0046031	BP	ADP metabolic process	ATP metabolism*		x
GO:0009135	BP	Purine nucleoside diphosphate metabolic process	ATP metabolism*		x
GO:0009185	BP	Ribonucleoside diphosphate metabolic process	ATP metabolism*		x
GO:0019363	BP	Pyridine nucleotide biosynthetic process	NADPH/NADP+ metabolism*		x
GO:0006090	BP	Pyruvate metabolic process	Carbohydrate metabolism		x
GO:1901292	BP	Nucleoside phosphate catabolic process	Nucleoside phosphate catabolism		x
GO:0022900	BP	Electron transport chain	Photosynthesis		x
GO:0016051	BP	Carbohydrate biosynthetic process	Carbohydrate metabolism		x
GO:0009165	BP	Nucleotide biosynthetic process	ATP metabolism*		x

involved in signaling, alternative splicing, the oxidation-reduction process, and the inhibition of lysosome fusion were uniquely down-regulated in the Macondo oil treatment (Table S1). Several eukaryotic ribosomal genes were uniquely up-regulated, while several chloroplast ribosomal genes were uniquely down-regulated in the Refugio oil treatment (Table S2). The *O. aurita* aggregates also uniquely up-regulated several genes involved in oxidation-reduction processes, signaling, and biodegradation of aromatic compounds, as well as nuclear proteins, a translation initiation factor, and cytoskeleton proteins in the Refugio oil treatment (Table S2). Besides photosynthetic and chloroplast ribosomal genes, *O. aurita* also uniquely down-regulated genes involved in pyruvate metabolism, stress responses, and signaling, as well as nuclear proteins, elongation factor Ts, and transporters in the Refugio treatment (Table S2).

4. DISCUSSION

Here we provide a first analysis of the impact of oil on the differential gene expression of marine diatom aggregates. It has been hypothesized that oil exposure may increase the exudation of organic materials (including EPS) from diatoms and that this could increase the formation of diatom aggregates and MOS (Passow et al. 2012). Here we found that oil exposure causes an up-regulation of several stress-related genes (e.g. chloroplastic pyridoxal reductase) and a down-regulation of photosynthesis-related genes, consistent with damage to cellular membranes. Although the amount of EPS exuded did not change with oil exposure (reported by Passow et al. 2019), there was an up-regulation of genes associated with carbohydrate exudation in both oil treatments, suggesting that a greater proportion of photo-

Table 2. Potential biomarkers of oil exposure, defined as genes with absolute log2-fold change ≥ 2.5 in both Macondo (M) and Refugio (R) treatments relative to the control (C). Trinity ID is the contig label generated during assembly. Sequences available at NCBI keyed by accession number (Acc. No.).

Trinity ID	NCBI Acc. No.	Log2-fold change M	Log2-fold change R	Annotation	Potential physiological functions	Role
TRINITY_DN161536_c0_g1	GHBW01304344	−6.2	−6.5	40S ribosomal protein S5-2	Ribosome	Ribosome
TRINITY_DN74698_c0_g2	GHBW01084020	−3.4	−7.2	60S ribosomal protein L18	Ribosome	Ribosome
TRINITY_DN173668_c0_g1	GHBW01316458	−5.0	−5.0	Cathepsin L 2	Silicatein α: cathepsin L-like protein in sponge biosilica	Biomineralization
TRINITY_DN83498_c0_g1	GHBW01099166	−4.2	−4.4	Ferredoxin	Photosynthesis	Photosynthesis
TRINITY_DN115159_c0_g1	GHBW01181778, GHBW01181779	−3.7	−3.1	Histone-lysine N-methyltransferase NSD1	Regulation of transcription	Transcription
TRINITY_DN112038_c0_g1	GHBW01167191	−3.6	−3.8	Cytochrome b5	Membrane-bound hemoprotein which functions as an electron carrier for several membrane-bound oxygenases	
TRINITY_DN95249_c0_g1	GHBW01122251, GHBW01122252	−3.4	−3.0	Nucleoside diphosphate kinase	Major role in the synthesis of nucleoside triphosphates other than ATP	
TRINITY_DN99514_c0_g1	GHBW01131753	−3.3	−3.0	Bilirubin oxidase	This enzyme participates in porphyrin and chlorophyll metabolism	Photosynthesis
TRINITY_DN100015_c0_g1	GHBW01132824, GHBW01132825	−3.1	−4.1	MFS transporter, NNP family, nitrate/nitrite transporter	Nitrogen metabolism	Nitrogen metabolism
TRINITY_DN65065_c0_g1	GHBW01068813	−3.0	−3.0	Non-histone chromosomal protein 6	Required for DNA binding by the FACT complex which is involved in multiple processes that require DNA as a template	Transcription/replication
TRINITY_DN107740_c0_g1	GHBW01152372, GHBW01152373	−2.9	−3.7	Ion channel-forming bestrophin family protein	A bestrophin-like protein, modulates the proton motive force across the thylakoid membrane	Photosynthesis
TRINITY_DN99247_c0_g2	GHBW01131127, GHBW01131128, GHBW01131129	−2.8	−3.5	L-ascorbate peroxidase	Redox reaction	Stress response
TRINITY_DN123535_c0_g2	GHBW01249510	−3.3	−3.5	Glyceraldehyde-3-phosphate dehydrogenase, chloroplastic	Carbon fixation in photosynthetic organisms	Carbon fixation
TRINITY_DN27530_c0_g1	GHBW01020980	−3.2	−3.3	Glyceraldehyde-3-phosphate dehydrogenase, chloroplastic	Carbon fixation in photosynthetic organisms	Carbon fixation
TRINITY_DN27530_c0_g2	GHBW01020981	−3.1	−3.3	Glyceraldehyde-3-phosphate dehydrogenase, chloroplastic	Carbon fixation in photosynthetic organisms	Carbon fixation
TRINITY_DN85400_c0_g1	GHBW01102592	−2.8	−3.2	Phosphoenolpyruvate/phosphate translocator 2, chloroplastic	Carbon fixation in photosynthetic organisms	Carbon fixation
TRINITY_DN82410_c0_g1	GHBW01097286	−2.6	−4.1	Ribose 5-phosphate isomerase A	Carbon fixation in photosynthetic organisms	Carbon fixation
TRINITY_DN57385_c0_g1	GHBW01058058	−2.6	−4.0	Ribulose-phosphate 3-epimerase	Carbon fixation in photosynthetic organisms	Carbon fixation
TRINITY_DN119112_c1_g1	GHBW01206435	−2.7	−2.7	Phosphoglycerate kinase, cytosolic	Carbohydrate metabolism	Carbohydrate metabolism
TRINITY_DN102565_c0_g2	GHBW01138763, GHBW01138764, GHBW01138765, GHBW01138766	−2.6	−2.5	Phosphoglycerate kinase	Carbohydrate metabolism	Carbohydrate metabolism

Table 2 continued on next page

Table 2 (continued)

Trinity ID	NCBI Acc. No.	Log2-fold change M	Log2-fold change R	Annotation	Potential physiological functions	Role
TRINITY_DN107127_c0_g2	GHBW01150546	−2.5	−3.1	Phosphoglycerate kinase	Carbohydrate metabolism	Carbohydrate metabolism
TRINITY_DN108784_c0_g1	GHBW01155602, GHBW01155603, GHBW01155604, GHBW01155605	−2.5	−2.9	3-oxoacyl-[acyl-carrier-protein] reductase FabG, chloroplastic	Fatty acid biosynthesis	Fatty acid biosynthesis
TRINITY_DN108432_c0_g1	GHBW01154544	−2.5	−2.6	Protein/nucleic acid deglycase HchA	Involved in conversion of methylglyoxal to a less toxic product	
TRINITY_DN79555_c1_g1	GHBW01092140	2.5	2.7	Elongation factor 1-alpha	tRNA transport	Translation
TRINITY_DN111828_c0_g2	GHBW01166330, GHBW01166331	2.5	2.6	SH3 domain-containing protein	Function unclear	
TRINITY_DN121316_c0_g1	GHBW01225466, GHBW01225467, GHBW01225468	2.6	2.8	Heat stress transcription factor A-2a, A-3	Transcriptional regulator that specifically binds DNA of heat shock promoter elements (HSE)	Transcription/stress response
TRINITY_DN100724_c0_g2	GHBW01134458	2.9	2.6	RNA-binding protein fox-1	Spliceosome	Spliceosome
TRINITY_DN117348_c0_g1	GHBW01194082, GHBW01194083	2.9	3.6	Ammonium transporter	Ammonium uptake	Nitrogen metabolism
TRINITY_DN115689_c0_g1	GHBW01184497, GHBW01184498, GHBW01184499	3.0	3.0	Calmodulin-binding transcription activator 1, 2	Associated with stress response	Stress response
TRINITY_DN96378_c0_g1	GHBW01124668, GHBW01124669, GHBW01124670	3.1	3.3	Polyamine oxidase	Maintenance of polyamine homeostasis	Stress response
TRINITY_DN89502_c0_g1	GHBW01110392	3.2	3.6	Sialate O-acetylesterase	Function unclear	
TRINITY_DN121984_c0_g2	GHBW01231806	3.6	3.9	Alpha-1,3-fucosyltransferase 10	May be involved in N-glycan production	EPS production
TRINITY_DN122121_c0_g5	GHBW01233221, GHBW01233222	3.7	4.0	Hybrid signal transduction histidine kinase G	Two-component systems: sensing environmental changes	Signaling
TRINITY_DN109444_c0_g2	GHBW01157658, GHBW01157659, GHBW01157660	4.2	5.5	Cytochrome c	Respiration	Respiration
TRINITY_DN89078_c0_g1	GHBW01109642	4.9	4.8	N1-acetylpolyamine oxidase, polyamine oxidase	Function unclear	
TRINITY_DN100709_c0_g2	GHBW01134424	5.1	4.5	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial	Branched chain amino acid degradation	Amino acid metabolism
TRINITY_DN25311_c0_g1	GHBW01018466	5.1	4.2	Single-pass membrane and coiled-coil domain-containing protein 4 homolog	Function unclear	
TRINITY_DN80842_c0_g1	GHBW01094457, GHBW01094458, GHBW01094459, GHBW01094460	5.1	5.3	Neurotrypsin	Function unclear	
TRINITY_DN52314_c0_g1	GHBW01051004	5.5	6.0	GDP-mannose transporter GONST3	May be involved in EPS production	EPS production
TRINITY_DN105765_c0_g1	GHBW01146789	7.1	7.2	Pyridoxal reductase, chloroplastic	Plays a role in oxidative stress responses	Stress response

synthate is allocated to EPS under oil exposure. As predicted, gene regulation varied across the 2 oils, with the heavier Refugio oil causing a higher degree of differential expression and more down-regulation of genes than the lighter Macondo oil; this could be due to the differences in either the oil composition or treatment concentration (EOE).

4.1. Oil exposure stimulates a down-regulation of photosynthesis-related genes

Exposure to oil caused a clear down-regulation of genes associated with photosynthesis, which would suggest that the photosynthetic apparatus of *Odontella aurita* is sensitive to oil (Table 1). The GO enrichment analysis indicates a down-regulation of chloroplast thylakoid membrane-associated genes, photosynthesis-related genes, and genes associated with ATP and NADPH generation. In this experiment, *O. aurita* was kept in the dark for 3 to 4 d before sampling, so some down-regulation of photosynthesis-related genes was expected. Differential expression was calculated relative to the controls (also kept in the dark), which means that the down-regulation observed in the Macondo and Refugio treatments was a result of oil exposure rather than light environment. There was a high log2-fold down-regulation in the expression of ferredoxin (Table 2), an important electron-accepting protein in photosynthesis. Down-regulation of ferredoxin in algae has most commonly been reported in response to iron starvation (Palenik et al. 2003, Pankowski & McMinn 2009), but these results suggest that this could also be an indicator of oil exposure. Reduced ferredoxin has been associated with decreased fitness and cell viability (Poncelet et al. 1998), and can stimulate cyclic phosphorylation (Holtgrefe et al. 2003). Typically, cyclic phosphorylation occurs to relieve over-reduction caused by down-regulation of PSII and other photosynthetic components (Heber & Walker 1992, Backhausen et al. 2000). Some components of oil are known to cause membrane damage or interruption to electron flow at PSII (Aksmann et al. 2011, Carvalho et al. 2011a), which could have caused a change in the redox potential across the thylakoid membranes in *O. aurita*. This would leave PSII vulnerable to oxidative stress via the build-up of electrons. Down-regulation of the photosynthetic components would slow down electron accumulation and could therefore be a way of preventing further damage.

4.2. Oil exposure and EPS production

Oil exposure may stimulate production and exudation of EPS, increasing the formation of MOS and promoting the export of oil from the surface of the water column (Passow et al. 2012). Generally EPS production is enhanced in diatoms under high light and low nutrient conditions (Staats et al. 2000, Thornton 2002). The molecular mechanisms that control exudation are still being characterized (Gügi et al. 2015, Shnyukova & Zolotariova 2015, Aslam et al. 2018), but it has been established that EPS is assembled initially in the Golgi apparatus, transported in vesicles to the cell membrane, and secreted to the environment, where the EPS undergoes further self-assembly (Wetherbee et al. 1998, Wang et al. 2000). Despite a down-regulation of photosynthesis, we found an up-regulation of several genes identified as potentially involved in EPS exudation in the oil treatments (Table 2). These include the GDP-mannose transporter GONST3 and alpha-1,3-fucosyltransferase 10 genes, which are involved in movement of GDP-mannose and fucose from the cytoplasm to the Golgi lumen. The production and breakdown of GDP-mannose and GDP-fucose is associated with the inclusion of mannose and fucose in EPS (Aslam et al. 2018). Increasing the mannose and fucose content of EPS changes its physical properties by enhancing gel stiffness (Zhou et al. 1998), and is a strategy that has been observed in sea-ice diatoms to alter the protective EPS coating in response to colder temperatures (Aslam et al. 2012, Steele et al. 2014). These patterns of differentially expressed genes could suggest that *O. aurita* has increased its allocation of photosynthate to exudates, and the chemical composition of its exudates, in response to oil exposure. These molecular responses in turn may influence aggregate formation and the formation of MOS. More work is required to characterize the genes associated with exudation and how oil exposure alters gene expression, exudate quality, quantity, and characteristics.

4.3. Potential biomarkers for oil exposure

Both oil treatments induced log2-fold changes greater than 7 in the gene for chloroplastic pyridoxal reductase (Table 2). This enzyme catalyzes the reduction of pyridoxal with NADPH and the oxidation of pyridoxine with $NADP^+$ (Herrero et al. 2011). Chloroplastic pyridoxal reductase plays an important role in salvaging B6 vitamins such as pyridoxal and pyridox-

ine (Rueschhoff et al. 2013). These vitamins are important in stress tolerance in higher plants (Bilski et al. 2000, Denslow et al. 2007, Havaux et al. 2009). Pyridoxine has a protective role against oxidative stress as it can prevent membrane damage by inhibiting lipid peroxidation (Kannan & Jain 2004). This could be a response to limit the membrane damage caused by certain components of oil. More work is required to confirm the function of this gene in diatoms, but the strong response in both treatments reported here suggests it is a potential biomarker for stress induced by oil exposure. Another notable biomarker is the gene for silicatein, which had a log2-fold change of −5 in both treatments. Silicatein is one of a suite of proteins involved in the silicification process. Genes involved in silica uptake and metabolism have previously been identified as biomarkers for polycyclic aromatic hydrocarbon (PAH) exposure in diatoms (Carvalho et al. 2011a,b) and are often strongly down-regulated. Changes to the silicification of the cell wall not only impact their contribution to silica cycling, but lighter frustules may also decrease sinking speed and affect the export of oil to the deep ocean.

In general, the molecular responses to both oil treatments were similar, making it difficult to identify unique biomarkers for diatoms exposed to a particular oil. Both oils induced a strong down-regulation of genes associated with pathways such as translation, transcription, and other genetic information-processing pathways (Tables 1 & 2). This could be due to some kind of nucleolar stress that is related to DNA damage. The magnitude of the change in gene expression (log2-fold change) and the number of GO enriched categories is generally larger in the Refugio treatment compared to the Macondo treatment (Table 2). These differences may be due to either the higher EOE in the Refugio compared to the Macondo treatment, or differences in the chemical composition of the oils. The Refugio oil is a much heavier and more viscous oil than the Macondo oil and contains more aromatic compounds and asphaltenes (Passow et al. 2019; their Table 2). However, more of the highly differentially expressed genes in the Macondo treatment are involved in oxidative stress responses compared to those in the Refugio treatment. The major PAHs found in the Macondo oil are low molecular weight compounds containing 2 to 3 rings (Wade et al. 2011), which tend to be more hydrophilic and therefore do not penetrate cell membranes as well as heavier PAHs with 5 rings or more. We speculate that the relative up-regulation of many more genes related to oxidative stress in the Macondo versus the Refugio treatment reflects a milder oxidative stress response to the lower molecular weight PAHs in Macondo versus heavier molecular weight PAHs in the Refugio oil (Tables S1 & S2).

The unique down-regulation of the chloroplast ribosomal genes and the photosynthetic light-harvesting genes in Refugio oil may be due to the presence of 5-ring PAHs. These PAHs can be converted into quinone-like compounds by bacteria and fungi (Juhasz & Naidu 2000, Kanaly & Harayama 2000, Carvalho et al. 2011a). These quinone-like compounds can, in turn, compete with plastoquinone for electrons and severely affect the light reactions of photosynthesis. In addition, the culture was not axenic, so co-cultured bacteria and fungi may be able to convert the 5-ring PAHs into quinone-like compounds or BaP-7,8-diol, which can be further converted into quinone-like compounds by the diatom itself using cytochrome P450 (CYP450) and glutathione S-transferase (GST) (Carvalho et al. 2011a). We observed the down-regulation of cytochrome b5 in both oil treatments (Table 2), which is known to augment many of the reactions carried out by CYP450 (Porter 2002). *O. aurita* may down-regulate the cytochrome b5 gene to weaken the conversion of BaP-7,8-diol into the quinone-like compounds that are more damaging to photosynthesis.

5. CONCLUSIONS

The transcriptomic regulation of *Odontella aurita* aggregates is consistent with several physiological studies on phytoplankton that have documented decreases in photosynthesis in response to oil and PAH exposure (Carvalho et al. 2011a, Özhan & Bargu 2014, Bretherton et al. 2019). The down-regulation of photosynthesis in response to oil exposure could be a potential mechanism to limit oxidative stress caused by changes to the redox potential in the thylakoid lumen, or due to oil overlaying the cell surface, decreasing CO_2 penetration to the cell. The down-regulation of photosynthesis may be a general response of all phytoplankton to oil. The up-regulation of chloroplastic pyridoxal reductase along with genes potentially involved in changes to EPS composition is possibly a strategy to protect the cell from membrane damage resulting from oil exposure, and its high log2-fold change makes it a potential biomarker for oil exposure. The differentially expressed genes in the treatment with the heavier Refugio oil suggest more severe nucleolar stress and more damage to the chloroplast, which may be attributed to its higher hydrophobicity and greater concentration of

higher molecular weight PAHs (Juhasz & Naidu 2000), while the lighter Macondo oil triggered an enhanced up-regulation of general stress response genes. Transcriptomics in conjunction with proteomic studies of different phytoplankton, and phytoplankton aggregates, exposed to a range of oils and environmental conditions will further improve our molecular understanding of the toxic effect of different oils and their impact on aggregate formation in the marine environment.

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