



Transcriptomic Responses of Deep-Sea Corals Experimentally Exposed to Crude Oil and Dispersant

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Resource extraction from the ocean comes with ecosystem-wide risks, including threats to its biota such as the habitat forming corals that support elevated biomass and biodiversity. Despite catastrophic incidents like the *Deepwater Horizon* oil spill (DWHOS) disaster that occurred in 2010, offshore oil and gas drilling continues to occur around the world. Previous work investigating the toxicity of oil and the chemical dispersant used in an attempt to mitigate the effects of the DWHOS revealed that the dispersant elicits a stronger, negative physiological response than oil alone. However, little is known regarding the specific ways in which these anthropogenic pollutants impact organisms at the cellular level. To investigate the impacts of each pollutant and their synergistic effects on corals, we analyzed the transcriptional responses of the deep-sea octocorals *Callogorgia delta* and *Paramuricea* type B3 following 12 h of exposure to oil, dispersant, and mixtures of oil and dispersant. Analyses revealed that the highest levels of significant differential gene expression were found among the treatments containing dispersant, which corresponds to the significant effects observed in physiological experiments. Functional analyses of annotated transcripts further suggest both species- and colony-specific responses to the exposures, likely due to underlying cellular and physiological differences. However, some commonalities were observed among the responses to chemical stress across treatments and species, including immune and cellular stress responses, altered energy metabolism, and oxidative stress, elucidating how corals respond to chemical pollutants. As resource extraction is an ongoing threat, this study demonstrates the importance of considering the varied and diverse responses of biota to anthropogenic disturbances and the implications of introducing chemicals into vulnerable ecosystems like those associated with deep-sea corals.

Keywords: gene expression, oil spill, Deepwater Horizon, octocorals, *Callogorgia*, *Paramuricea*, RNAseq, anthropogenic

INTRODUCTION

Marine ecosystems are increasingly threatened by resource extraction as oil and gas exploration expands offshore. Despite catastrophic incidents like the *Deepwater Horizon* oil spill (DWHOS) disaster which occurred in the Gulf of Mexico (GoM) in 2010, drilling continues in deep waters [>200 meters (m)] worldwide, with active ultra-deep (>1000 m) drilling advancing in the GoM (Fisher et al., 2014b; Cordes et al., 2016) and beyond. Drilling

activities can be detrimental to deep-sea communities in various ways including physical disturbances by mooring anchors and pipelines (Ulfsnes et al., 2013), exposure to toxic drill cuttings, muds, and fluids (Gray et al., 1990; Larsson et al., 2013), and accidental oil spills. In the United States alone there was an oil spill over 160,000 L on average every 1.75 years between 1971 and 2010 (Anderson et al., 2012). Oil and gas extraction in deeper waters also increases the likelihood of accidental spills (Muehlenbachs et al., 2013) like the DWHOS blowout of the Macondo well that occurred at approximately 1500 m depth. This was one of the worst environmental disasters in U.S. history, and the largest accidental oil spill world-wide, releasing approximately 5 million barrels of crude oil into the deep waters of the GoM (McNutt et al., 2012). During active well discharge, approximately 7 million liters of chemical dispersants (Corexit 9500A and 9527; Barron, 2012) were also applied in an attempt to mitigate the impacts of the oil release, both at the sea surface and at depth, without a comprehensive understanding of how these chemicals would affect deep-sea fauna.

Spill impacted deep-sea coral communities were initially found at a depth of 1370 m, approximately 11 km from the Macondo well at a site in the Mississippi Canyon (MC) 294 lease block (White et al., 2012). When first discovered, coral colonies were covered in a brown flocculent material (floc) containing Macondo well oil as well as dispersant constituents (White et al., 2014). Impacted coral species at this site, primarily the deep-sea octocoral *Paramuricea biscaya*, exhibited various phenotypic indicators of impact including abnormal skeletal (sclerite) development, excess mucous production, and tissue death (necrosis), as well as complete colony mortality (White et al., 2012). Corals showing signs of sublethal impacts exhibited subsequent declines in health (i.e., tissue and branch loss) and limited recovery potential (Hsing et al., 2013; Girard and Fisher, 2018). Additional impacted deep-sea coral communities were later identified based on their similarity in appearance to the impacted communities at the original site (Fisher et al., 2014a,b).

A recent study investigating the *in situ* transcriptional response of *P. biscaya* colonies collected in December 2010 at MC294, revealed further impacts at the gene expression level (DeLeo et al., 2018). These included elevated expression of genes associated with oxidative, immune, and metabolic stress responses, as well as wound repair mechanisms (i.e., hyper-melanization). Contrastingly, expression of the polycyclic aromatic hydrocarbon biomarker gene, cytochrome P450 (CYP) was depressed, which may indicate an inhibition of the corals' ability to process xenobiotics at the time of sampling. Further, the study revealed variability among the global expression patterns of the individual spill-impacted colonies (DeLeo et al., 2018).

Because *P. biscaya* occurs in the GoM between 1200 and 2600 m and rarely survives the trip to the surface, it is not amenable to controlled laboratory experiments aimed at testing the toxicity of crude oil and dispersants. A close relative of *P. biscaya*, *Paramuricea* type B3 (Doughty et al., 2014), is also found in the GoM at shallower depths between 800 and 1100 m. This species can survive careful collection and thus serves as the best available model to understand toxicity effects underlying the impacts observed in *P. biscaya*.

Laboratory toxicity tests with *P. type B3*, and with the more distantly related octocoral *Callogorgia delta*, revealed that exposure to dispersant or dispersant/crude oil mixtures is more toxic to these corals than is exposure to crude oil alone (DeLeo et al., 2016). This was particularly evident following exposure to the dissolved, water-accommodated fractions (WAFs) of the dispersants. Phenotypic responses observed in *P. type B3* were similar to those observed in *P. biscaya* at MC294, including excess mucous release, tissue damage, necrosis and mortality (DeLeo et al., 2016). The octocoral *C. delta* however exhibited less severe health declines than *P. type B3*, suggestive of taxon-specific responses.

The objective of this study was to investigate the transcriptional responses of the corals subjected to the laboratory toxicity tests performed by DeLeo et al. (2016) and compare them to the *in situ* transcriptional responses of *P. biscaya* (DeLeo et al., 2018). It is important to investigate the early transcriptional responses to oil, dispersant, and oil/dispersant mixtures in these corals, prior to the onset of visible damage and mortality in order to understand how deep-sea corals respond to, and potentially endure, acute pollution events from marine oil spills. The gene expression patterns of *P. type B3* and *C. delta* were examined after 12 h of exposure to (1) bulk oil and dispersant treatments (heterogeneous solutions, with dissolved and undissolved chemical components), and (2) treatments solely containing the dissolved, or WAF of oil and dispersant. As higher mortality was observed in the WAF exposures during the 96 h toxicity tests, we anticipated a greater degree of differential gene expression in response to the short-term WAF exposures, corresponding to more severely impacted biological processes. Expression profiles were analyzed to investigate the effect of treatment type – oil-only, dispersant-only, and oil/dispersant mixtures – on both coral species. We hypothesized that the corals would have a more pronounced genome-wide response following exposure to dispersant, relative to the crude oil, given the strong physiological response observed in the toxicity tests and the natural occurrence of crude oil in their habitat (Quattrini et al., 2013). Gene expression data were further explored to identify early onset genome-wide impacts underlying the health declines and mortality observed during the full 96 h toxicological assays described by DeLeo et al. (2016). These findings improve our understanding of how resource extraction impacts deep-sea biota, and will aid in the development of diagnostic biomarkers for future spill monitoring efforts.

MATERIALS AND METHODS

Sample Collection and Acclimation

All samples were collected from two sites in the GoM. *P. type B3* colonies were collected from a large population of corals at approximately 1050 m depth at Atwater Valley (AT) 357 (27°58.6'N, 89°70.4'W; Doughty et al., 2014). *C. delta* was collected from the Viosca Knoll (VK) 826 site at a depth of approximately 500 m (29°09.5'N, 88°01.0'W; Cordes et al., 2008; Davies et al., 2010). At each site, corals were haphazardly collected with the remotely operated vehicles (ROV) *Global Explorer MK3*

or *Hercules* for experimentation. For each species, five coral colonies were collected alive for the experimental exposures described in DeLeo et al. (2016). Three of the largest colonies were fragmented for use in this study. Samples were collected several meters apart from conspecific colonies to reduce the likelihood of sampling genetic clones. Corals were visually identified using live video from cameras attached to each ROV, before being collected with a manipulator arm and secured in an insulated biobox and/or sealable collection quivers. When possible, branches of large colonies were sampled to avoid whole-colony mortality. Collection of individual branches from large coral colonies produces little or no phenotypic response from the coral colony (EEC unpublished data). Additional coral specimens from the same sites, previously preserved *in situ* on the seafloor using collection quivers filled with RNALater, were used for RNA sequencing. This included fragments from two *P. type B3* (2011, ROV *Schilling UHD*) and four *C. delta* colonies (2010, ROV Jason II).

At the surface, live corals were immediately transferred to containers with filtered seawater of the species-appropriate temperature and salinity (35 psu). *P. type B3* was maintained at approximately 5°C and *C. delta* at 8°C (the average *in situ* temperatures at depth) for the duration of the experiment. Temperature in the holding vessels was continuously monitored using temperature probes (Hobo® Data Loggers). Corals were allowed to acclimate for approximately 12 h prior to experimentation.

Sublethal Oil and Dispersant Exposures

Partial coral colonies ($n = 3$ genets per species, per exposure series; six genets total) were fragmented into nubbins and exposed to oil and dispersant solutions (Figure 1) prepared according to the protocols described in DeLeo et al. (2016). In brief, corals were exposed to treatments made using either (1) heterogenous bulk oil, dispersant and oil-dispersant mixtures containing both dissolved and undissolved chemical components, hereafter referred to as the *Bulk-exposure series*, or (2) using only the dissolved or water-accommodated oil (and dispersant) fractions (WAF), referred to as the *WAF-exposure series*. In each set of exposures, fragments were placed in the following treatments: artificial seawater (ASW; control), oil-only, dispersant-only or an oil/dispersant mixture (to represent dispersed oil). ASW was chosen as the control for comparison to the oil and dispersant treatments as all mixtures (Bulk and WAF) were made using sterile ASW (made using Instant Ocean™ at the *in situ* salinity of 35 psu). Coral nubbins were exposed to sublethal concentrations of oil and dispersant for a duration of 12 h and preserved prior to the onset of visible tissue damage. For the *Bulk-exposure series* this corresponds to the “High” concentration of oil and dispersant (~25 ppm) described by DeLeo et al. (2016), and for the *WAF-exposure series* this corresponds to the “Low” concentration of oil (~50 μM) and dispersant (~35.3 mg/L). Nubbins in these concentrations were chosen for sequencing for several reasons: (1) there were clear phenotypic differences observed across treatments during the full 96 h exposures implying the mixtures elicited a physiological response, (2) the concentrations were deemed sublethal as severe stress responses and mortality were not induced across all treatment-types within

those 96 h, and (3) to minimize the differences between the target concentrations of the Bulk and WAF exposure series to facilitate comparisons. After the 12 h exposures, fragments were fixed in RNALater to preserve changes in gene expression. Samples were then frozen and returned to the lab at Temple University for further processing.

Sample Sequencing and Processing

Total RNA was isolated using a Qiagen RNeasy Kit or a modified Trizol/Qiagen RNeasy protocol (described in Polato et al., 2010; Burge et al., 2013), from both the *in situ* preserved samples and the experimental nubbins sampled after 12 h of exposure. RNA concentrations were determined using a NanoDrop® ND-1000 and RNA integrity was evaluated with gel electrophoresis and an Agilent Bioanalyzer. Library preparations, including a poly-A selection step to target primarily eukaryotic (coral host) RNAs, were performed by the sequencing facilities on high quality (RIN values > 7) RNA samples (Illumina Truseq RNA library kit). *In situ* preserved specimens were sequenced on an Illumina HiSeq2000 at the University of Wisconsin-Madison Biotechnology Center (UWBC, Madison WI, United States) to acquire 150 base-pair (bp) paired-end reads for use in assembling *de novo* reference transcriptomes for downstream analyses. The experimental samples were further multiplexed and sequenced across Illumina HiSeq2500 rapid flow cells at the Fox Chase Cancer Center (FCCC, Philadelphia, PA, United States) sequencing facility to acquire 100 bp single-end reads for use in gene expression analyses, at approximately 20–30 million reads per sample.

Raw reads were quality checked using FastQC v0.11.5 (Andrews, 2010). This output was further used to find overrepresented sequences, which were queried against GenBank using Blastx (e -value: e^{-15}) to determine whether they represented bacterial contamination. Trimmomatic v0.36 (Bolger et al., 2014) was then used to filter for quality, with a Phred score threshold of 30, leading and trailing bases dropped if the score was below 3 (LEADING: 3 TRAILING: 3), and excluding reads shorter than 50 bp (MINLEN:50). Cutadapt v1.15 (Martin, 2011) was used to trim adaptors and cut overrepresented sequences that appeared to be bacterial contamination with an overlap of 10 and a minimum-length of 60.

Transcriptome Assemblies

The filtered and cut paired-end sequences were further processed with Rcorrector (Song and Florea, 2015) using the k-mer counter Jellyfish v2.2.6 (Marcais and Kingsford, 2012), to remove erroneous k-mers. Read pairs deemed unfixable were discarded. Microbial contaminants were further identified and removed using the standard database in Kraken v1.0 (Wood and Salzberg, 2014). Sequences not classified as bacterial, microbial, archaeal, or viral in Kraken were used to generate *de novo* reference assemblies with Trinity v2.4.0 (Grabherr et al., 2011). Transcriptome quality was assessed with Transrate v1.0.3 (Smith-Unna et al., 2016) and the Benchmarking Universal Single-Copy Orthologs (BUSCO v3.0.2) program (Simão et al., 2015; Zdobnov et al., 2017) using the metazoa dataset ($n = 978$

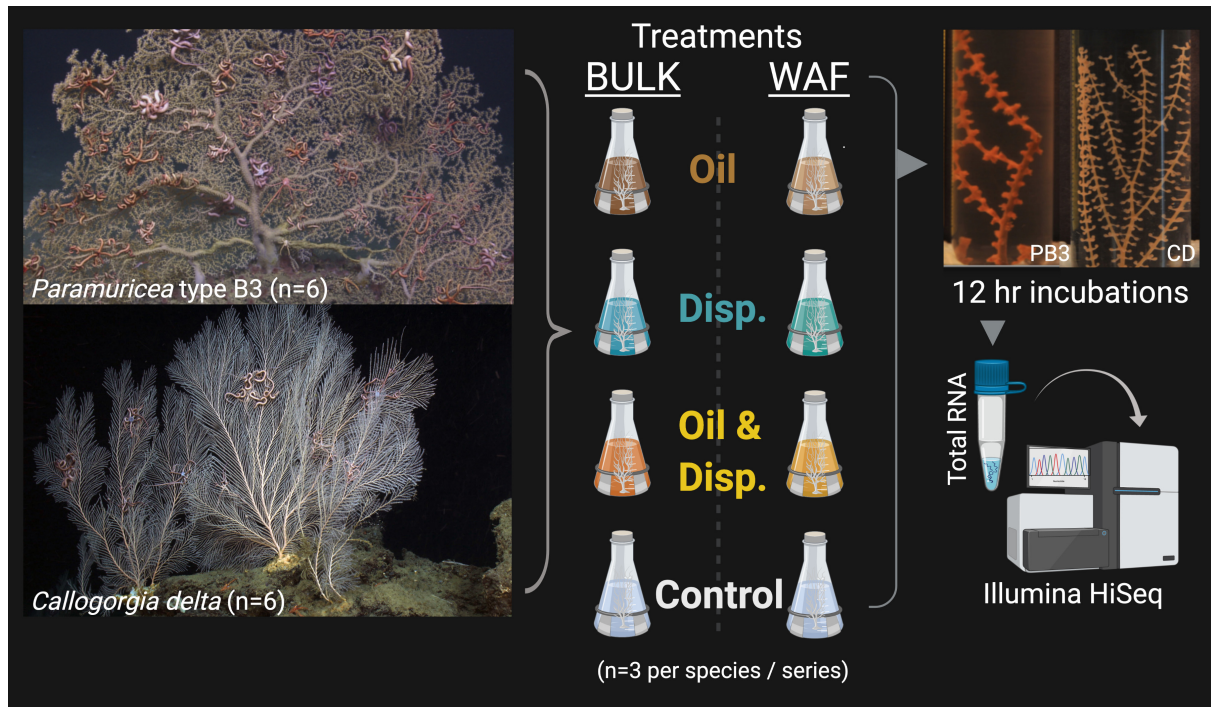


FIGURE 1 | Experimental setup for the deep-sea octocorals *Paramuricea* type B3 (PB3) and *Callogorgia delta* (CD) ($n = 6$ genets total per species, 3 genets per exposure series). Corals were exposed to both Bulk (heterogeneous) and Water-Accommodated Fractions (WAF) of oil and/or dispersant (center). Coral fragments were maintained at sub-lethal concentrations for 12 h, as determined in DeLeo et al. (2016) (right). For the Bulk exposure series, oil and dispersant concentrations each correspond to ~ 25 ppm. For the WAF exposure series, oil concentrations correspond to ~ 50 μM and dispersant ~ 35.3 mg/L. Control coral fragments were also incubated in the artificial seawater used to make the aforementioned treatments of each exposure series. Fragments were preserved (RNAlater) after 12 h incubations in the treatments, for total RNA extraction and sequencing (1×100 bp). Created with BioRender.com.

orthologs). Assemblies were further annotated via dammit v1.0¹ using the corresponding P-FamA, RFam, OrthoDB, BUSCO, and Uniref90 databases (Scott, 2016). Gene ontology (GO) annotations were also generated using standard parameters (seed ortholog e -value: 0.01; seed ortholog score: 60; min% of query cov.: 20; min% of subject cov.: 0) in eggNOG (Huerta-Cepas et al., 2016) using Github support scripts².

Gene Expression Analyses

Processed reads from *P.* type B3 and *C. delta* experimental samples were mapped to their respective reference transcriptomes using Salmon (Patro et al., 2017). The resulting count files were used as input for DESeq2 (Love et al., 2014), using R v3.6.2, to analyze the gene expression patterns of the corals in each exposure series (Bulk or WAF) and treatment. Expression was compared to the corresponding ASW control (of each species) using the tximport package (Soneson et al., 2015), which obtains gene-level expression by summing all transcript-level count estimates corresponding to a given gene. Transcripts were collapsed into genes for analysis at the gene level with a DESeq dataset design of colony + treatment, which was applied discretely to each exposure series. Transcripts were considered over-expressed if they had an adjusted p -value < 0.05

and a log-2 fold change (lfc) > 1 , and under-expressed if they had an adjusted p -value of < 0.05 and a lfc < -1 . An adonis (permutational multivariate analysis of variance using distance matrices) test, implemented using the Vegan package in R, was used to analyze the influence of each factor (colony, treatment, and the interaction of colony and treatment) on the variation in gene expression (Oksanen et al., 2013). A Principal Coordinate Analysis (PCoA) was also carried out to visualize how the samples grouped in multivariate space, and whether treatment-type and/or colony genet could explain their distribution.

KEGG Functional Analyses

In order to further understand the influence of each treatment on higher-level functions and biological processes, differentially expressed genes from each treatment were assessed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper v3.1 tool (Kanehisa et al., 2016). This was done to decipher the molecular interaction networks associated with the genes (KEGG pathway mapping; *reconstruct*), based on all available KEGG Orthology (KO) assignments designated by eggNOG (Huerta-Cepas et al., 2016). These data were compared to investigate cellular processes and pathways that were impacted in both species in the Bulk and WAF exposures and to elucidate species-specific differences in the early (12 h) responses to pollutant introduction.

¹<https://dammit.readthedocs.io>

²<https://github.com/z0n/annotatingTranscriptomes>

In order to compare the initial responses of the experimentally exposed corals to corals impacted by the DWHOS *in situ*, direct comparisons were made between the closely related octocorals *P. type B3* and *P. biscaya*. This was done by comparing the KEGG pathway annotations for the genes differentially expressed in this study to the KO assignments for genes differentially expressed among *P. biscaya* exposed to DWHOS oil and dispersant laden floc *in situ* (DeLeo et al., 2018) via KEGG mapper *reconstruct*. While KEGG pathways are comprised of various components, each with unique KO identifiers, only the overlapping KO identifiers were reported as shared impacts observed for *Paramuricea* spp.

Gene Ontology Analysis

To examine significantly enriched GO categories among the over- and under-expressed genes that correspond to either the biological process, molecular function, or cellular component sub-ontologies, a rank-based gene ontology (GO) analysis with adaptive clustering was performed using the Gene Ontology Mann-Whitney U (GO_MWU) program (Wright et al., 2015). This program utilizes Mann-Whitney U (MWU) tests and a global-ranked list of genes and their associated GO terms to identify enriched GO categories using a continuous measure of significance, $-\log(p\text{-value})$, calculated with DESeq2. Hierarchical clustering of GO categories was based on the number of shared genes (clusterCutHeight = 0.25). Discrete species-specific tests were carried out for the Bulk and WAF exposure series to analyze expression patterns related to all treatments (the factor “treatment” in the DESeq design matrix) as well as each treatment individually. GO terms significantly enriched among the over- ($p_{adj} < 0.05$, $\delta rank > 0$) or under-expressed ($p_{adj} < 0.05$, $\delta rank < 0$) transcripts were reported.

RESULTS

De novo Transcriptome Assemblies

The final *de novo* transcriptome assembly for *C. delta* contained 39,475 transcripts with a contig N50 of 1,467 bases (Table 1). Of the assembled contigs, 94.9% of metazoan orthologs (BUSCOs) were present, indicating a relatively complete reference assembly.

TABLE 1 | Summary statistics for the *de novo* transcriptome assemblies of *Callogorgia delta* and *Paramuricea* type B3.

		<i>C. delta</i>	<i>P. type B3</i>
Total transcripts		39,475	60,989
GC content		0.40	0.39
N50		1,467	1,451
BUSCO	% Complete	94.9	94.5
	% Singletons	85.1	86.0
	% Duplicates	9.8	8.5
	% Fragmented	1.1	1.9
	% Missing	4.00	3.60
Transrate	Score	0.20	0.29
	Optimal Score	0.20	0.32

The final reference transcriptome for *P. type B3* contained 60,989 transcripts with a N50 of 1,451 bases (Table 1). Among these contigs, 94.5% of metazoan BUSCOs were present. The TransRate scores were comparable to the optimal assembly scores, which considers only the “good” transcripts based on a learned cutoff value.

Gene Expression Analyses

The adonis analysis, conducted to investigate the influence of each factor – colony (or genet), treatment, and colony + treatment – on the variation in gene expression, was not significant. However, in both exposure series, “colony” explained the largest proportion of the variation in global gene expression patterns. Further, when analyzing global expression patterns using the PCoA, the samples grouped largely by colony (Figure 2).

Bulk Exposure Series

After 12 h in the Bulk exposure series, the greatest number of significant differentially expressed genes ($adj. p\text{-value} < 0.05$, $absolute lfc > 1$) was observed among *P. type B3*, with a total of 140 genes differentially expressed relative to the 66 genes differentially expressed in *C. delta* (Table 2 and Supplementary Table 1). For both species, the highest differential expression response was observed in the Bulk dispersant-only treatment. *P. type B3* had 2.9–34 times as many differentially expressed transcripts in the dispersant-only treatment, relative to the oil/dispersant mixture and oil-only treatments, respectively; *C. delta* had 4.8–6.0 times as many differentially expressed transcripts in the respective treatments. See Supplemental Information for more detailed results on gene expression and functional analyses.

Among the differentially expressed genes in both octocorals, 2 putative genes (annotated as *cartilage matrix protein-like* and *uncharacterized protein*) were shared between *C. delta* and *P. type B3* exposed to the Bulk-dispersant treatment (Supplementary Table 1). No putative genes with annotations were shared among the two species in the Bulk-oil or oil/dispersant mixture treatments.

WAF Exposure Series

After 12 h in the WAF exposure series, the greatest number of significant differentially expressed genes ($adj. p\text{-value} < 0.05$, $absolute lfc > 1$) was observed among *C. delta* with a total of 1,681 genes differentially expressed relative to the 133 genes differentially expressed in *P. type B3* (Table 2 and Supplementary Table 2). For *C. delta*, the highest differential expression response was observed in the dispersant-only treatment, though this number was comparable across treatments with at most 1.1 times as many differentially expressed transcripts relative to the oil/dispersant mixture and oil-only treatments. For *P. type B3*, the highest differential expression response was observed in the WAF oil/dispersant mixture treatment, with 1.2–36 times as many differentially expressed transcripts relative to the dispersant-only and oil-only treatments.

Among the differentially expressed genes, one putative gene (annotated as *lactoperoxidase-like*), was shared between *C. delta*

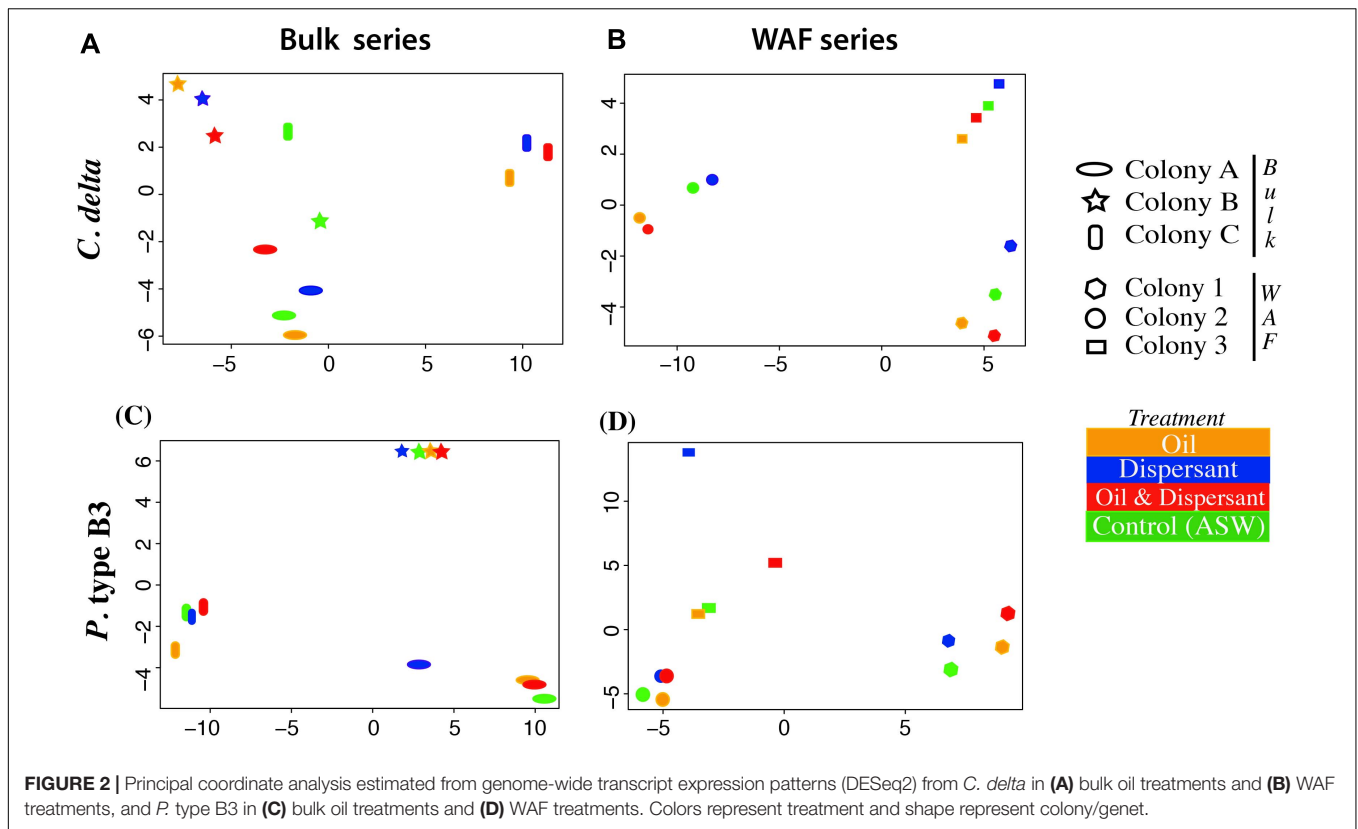


TABLE 2 | Summary of the differentially expressed genes in *C. delta* and *P. type B3* by treatment – oil, dispersant, and oil and dispersant mixtures- relative to control expression (seawater) in both the WAF (dissolved) and Bulk (heterogenous dissolved/undissolved) exposure series.

		Under-expressed	Over-expressed	Fold-change
<i>C. delta- bulk</i>				
Total transcripts with non-zero read counts: 26,306	Oil	0	8	1.2 – 3.1
	Dispersant	20	28	-4.0 – 3.4
	Oil/dispersant	4	6	-7.3 – 4.0
<i>C. delta- WAF</i>				
Total transcripts with non-zero read counts: 25,164	Oil	372	151	-4.1 – 3.1
	Dispersant	196	384	-4.0 – 8.6
	Oil/dispersant	295	283	-3.6 – 4.9
<i>P. type B3- bulk</i>				
Total transcripts with non-zero read counts: 26,850	Oil	1	2	-1.6 – 1.7
	Dispersant	19	83	-1.4 – 2.5
	Oil/dispersant	5	30	-5.5 – 2.9
<i>P. type B3- WAF</i>				
Total transcripts with non-zero read counts: 26,883	Oil	2	0	-5.2 – -4.7
	Dispersant	10	49	-4.7 – 7.1
	Oil/dispersant	52	20	-4.3 – 4.0

Genes were considered significant at an adjusted p -value ≤ 0.05 , under-expressed at a \log_2 -fold change ≤ -1 , and over-expressed at a \log_2 -fold change ≥ 1 .

and *P. type B3* exposed to the WAF-oil treatment, with an additional 14 putative genes shared among the two species in the WAF oil/dispersant mixture treatment, including but not limited to: a Slit homolog 1 protein involved in negative chemotaxis, a metalloendopeptidase involved in wound repair pathways, the growth factor bone morphogenetic protein 6 (BMP6) involved

in skeletogenesis and oogenesis, the interferon-induced protein (IFI35) involved in innate immune responses, and the tumor suppressor RNA-binding protein (RBM47) that responds to DNA damage (**Supplementary Table 2**). No putative genes were shared among the WAF dispersant-only treatment after 12 h of exposure.

TABLE 3 | Summary of the KEGG pathway analyses for *Paramuricea* type B3 (PB) and *Callorgia delta* (Cd) in the Bulk oil and dispersant exposure series.

KO term	BULK exposures					
	Oil		Disp.		Oil/Disp.	
	PB	Cd	PB	Cd	PB	Cd
Metabolism						
<i>Lipid metabolism</i>						
00100 Steroid biosynthesis					■	
00561 Glycerolipid metabolism					■	
00564 Glycerophospholipid metabolism					■	
<i>Xenobiotics biodegradation and metabolism</i>						
00983 Drug metabolism – other enzymes					■	
Environmental Information Processing						
<i>Signal transduction</i>						
04391 Hippo signaling pathway – fly					■	
04151 PI3K-Akt signaling pathway			■*	■*		
<i>Signaling molecules and interaction</i>						
04512 ECM-receptor interaction			■*	■*		
Cellular Processes						
<i>Cellular community – eukaryotes</i>						
04510 Focal adhesion			■*	■*	■	
Organismal Systems						
<i>Immune system</i>						
04610 Complement and coagulation cascades			■*	■*	■	
<i>Digestive system</i>						
04974 Protein digestion and absorption			■*	■*	■	
04972 Pancreatic secretion					■	
04975 Fat digestion and absorption					■	
<i>Nervous system</i>						
04725 Cholinergic synapse					■	

Processes that are associated with under-expressed (blue) and over-expressed (red) transcripts are highlighted for each species and treatment.

The asterisk (*) denotes transcripts that correspond to the same gene in both species. KEGG analyses were based on KO annotations via eggNOG. Reference **Supplementary Table 3** for more detailed information.

KEGG and GO Functional Analyses

Bulk Exposure Series

KEGG pathway maps revealed that the genes differentially expressed among both coral species in the Bulk dispersant-only treatment were associated with: the immune (complement and coagulation cascades) and digestive (protein digestion and absorption) systems, environmental information processing (intracellular signaling and ECM-receptor interactions), and cellular processes (focal adhesion) (**Table 3** and **Supplementary Table 3**). A response was also elicited in these pathways among *P.* type B3 fragments in the bulk oil/dispersant treatment, in addition to several metabolic pathways (e.g., xenobiotic biodegradation and metabolism). There were no KEGG assignments associated with the genes differentially expressed in the Bulk oil-only treatment or for *C. delta* exposed to oil/dispersant.

There were 104 enriched GO terms shared among both octocorals exposed to the Bulk dispersant treatment (**Figure 3**

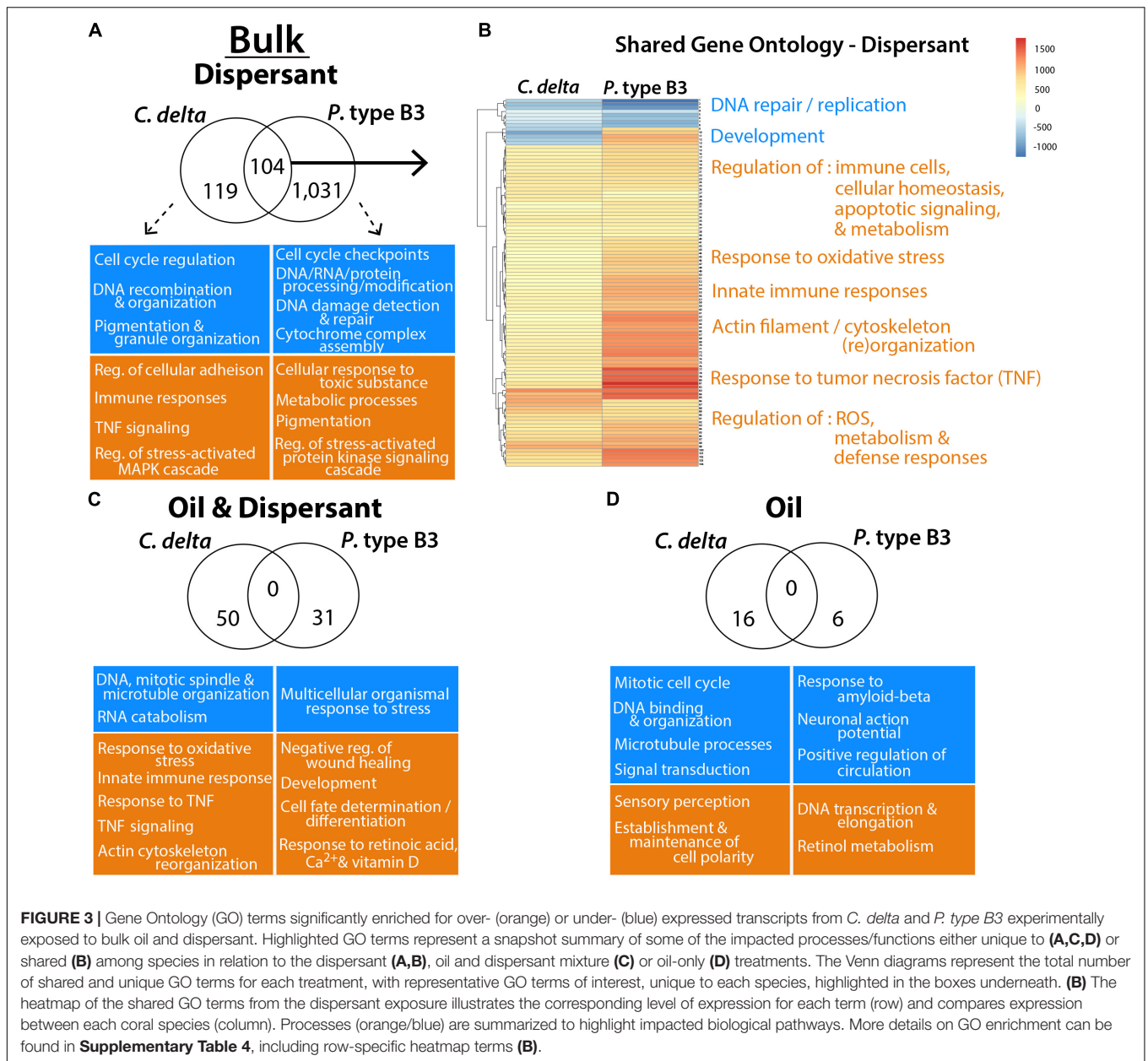
and **Supplementary Table 4**). Among the shared GO terms were “innate immune responses,” “response to tumor necrosis factor (TNF),” and “regulation of immune effector process,” further revealing elevated immune responses among *C. delta* and *P.* type B3 fragments exposed to dispersant. *C. delta* exposed to the oil and oil/dispersant treatments had additional enrichment of unique immunity-related GO terms including “positive regulation of immune system process” and “negative regulation of wound healing” (**Supplementary Table 4**). Shared GO enrichment analyses revealed additional impacts to cellular processes including, but not limited to, elevated “response to oxidative stress” and “regulation of apoptotic signaling pathway” and diminished “DNA repair.” There were no GO terms shared among *C. delta* and *P.* type B3 in the Bulk oil-only and oil/dispersant treatments. See **Supplementary Table 4** for additional details on GO enrichment and for details on the differences between species.

WAF Exposure Series

KEGG pathway maps indicated that the gene differentially expressed among both coral species in the WAF oil-only exposures was associated with metabolism (peroxidase) (**Table 4** and **Supplementary Table 5**). Common impacts were also observed in the WAF oil/dispersant treatment, which were further associated with genetic information processing (ribosome), environmental information processing (signal transduction), cellular processes (cell growth and death), and organismal systems (development and regeneration).

Among *C. delta* nubbins in the WAF exposure series, several KEGG pathways were similarly impacted by all three treatments, potentially indicative of a generalized, early (~12 h) response to anthropogenic pollutants. These pathways fell into four main categories: metabolism (carbohydrate metabolism), environmental information processing (signal transduction), cellular processes (cell growth/death and focal adhesion), and organismal systems (including the immune, endocrine and nervous systems, and environmental adaptation) (**Table 4** and **Supplementary Table 5**). KEGG analyses further revealed common impacts to crucial cellular stress response pathways (e.g., NF-kappa B, TNF, and chemokine signaling pathways) among the octocorals exposed to oil (alone or dispersed). Additional sub-lethal impacts were observed among corals exposed to chemical dispersant components (alone or with oil) including elevated calcium signaling and apoptotic processes. See **Supplementary Table 3** for full details on the KEGG analyses and **Supplementary Information** for detailed comparisons between treatments.

There were 10 GO terms shared among *C. delta* and *P.* type B3 in the WAF oil treatment, 64 terms shared in the dispersant-only treatment and 435 terms shared among the oil/dispersant mixtures (**Figure 4**). GO terms shared among the corals in treatments containing dispersant (alone or with oil) include “pigment cell differentiation,” “regulation of cytokine production,” “mucosal immune response,” and “regulation of inflammatory response,” which further revealed impairments to immune responses after



the 12 h exposures. GO enrichment analyses uncovered additional impacts to cellular processes following dispersant exposure including, but not limited to, elevated “ribosomal biogenesis” and “DNA repair/modifications” (Figure 4 and Supplementary Table 4).

Shared Impacts: DWHOS and Experimental

KEGG pathway analyses revealed that both *in situ* impacted *P. biscaya* and *P. type B3*, exposed to the Bulk treatments containing dispersant (alone or dispersed), exhibited similar impacts to environmental information processing pathways, specifically genes associated with signal transduction (i.e., Hippo and PI3K signaling pathways) and ECM-receptor interactions. Common

impacts were also observed among pathways involved in cellular processes including focal adhesion and protein digestion and absorption.

Similar impacts were likewise observed among DWHOS impacted *P. biscaya* and *P. type B3* experimentally exposed to WAFs containing dispersants. This includes impacts to metabolism, translation, protein folding and degradation, environmental information processing pathways (i.e., ABC membrane transporters) and various signal transduction pathways. Common impacts were also observed among pathways involved in crucial cellular processes including endocytosis and actin cytoskeleton regulation, as well as the digestive system (digestive secretions). See Supplementary Table 6 for more detailed information.

TABLE 4 | Snapshot summary of the KEGG pathway analyses for *Paramuricea* type B3 (PB) and *Callogorgia delta* (Cd) in the WAF oil and dispersant exposure series.

KO term	WAF exposures					
	Oil		Disp.		Oil/Disp.	
	PB	Cd	PB	Cd	PB	Cd
Metabolism						
<i>Metabolic pathways</i>						
01000 Enzymes – Peroxidase PXDN	*	*				
<i>Carbohydrate metabolism</i>						
00010 Glycolysis/Gluconeogenesis						
00620 Pyruvate metabolism						
00630 Glyoxylate and dicarboxylate metabolism		+		+		+
<i>Amino acid metabolism</i>						
00260 Glycine, serine, and threonine metabolism						
00270 Cysteine and methionine metabolism						
00380 Tryptophan metabolism						
00480 Glutathione metabolism						
<i>Xenobiotics biodegradation and metabolism</i>						
00980 Metabolism of xenobiotics by cytochrome P450						
Genetic information processing						
<i>Transcription</i>						
03040 Spliceosome						
<i>Translation</i>						
03010 Ribosome					*	*
03015 mRNA surveillance pathway						
03008 Ribosome biogenesis in eukaryotes						
Environmental information processing						
<i>Signal transduction</i>						
04010 MAPK signaling pathway						
04310 Wnt signaling pathway		-		-		-
04350 TGF-beta signaling pathway		-		-		-
04064 NF-kappa B signaling pathway						
04668 TNF signaling pathway						
04020 Calcium signaling pathway						
<i>Signaling molecules and interaction</i>						
04080 Neuroactive ligand-receptor interaction						
04512 ECM-receptor interaction						
Cellular processes						
<i>Transport and catabolism</i>						
04144 Endocytosis						
04145 Phagosome						
04142 Lysosome						
04140 Autophagy – animal						
04137 Mitophagy – animal					*	*
<i>Cell growth and death</i>						
04110 Cell cycle		+		+		+
04210 Apoptosis					*	*
04216 Ferroptosis					*	*
04217 Necroptosis						
04218 Cellular senescence						
<i>Cellular community – eukaryotes</i>						
04510 Focal adhesion		-		-		-
04520 Adherens junction						
04530 Tight junction						
04540 Gap junction		+		+		+

(Continued)

TABLE 4 | Continued

KO term	WAF exposures					
	Oil		Disp.		Oil/Disp.	
	PB	Cd	PB	Cd	PB	Cd
04550 Sig. pathways reg. pluripotency of stem cells						
<i>Cell motility</i>						
04810 Regulation of actin cytoskeleton						
Organismal Systems						
<i>Immune system</i>						
04610 Complement and coagulation cascades						
04620 Toll-like receptor signaling pathway						
04625 C-type lectin receptor signaling pathway						
04062 Chemokine signaling pathway						
<i>Endocrine system</i>						
04910 Insulin signaling pathway						
04920 Adipocytokine signaling pathway						
04921 Oxytocin signaling pathway						
04928 Parathyroid hormone synthesis/secretion						
04916 Melanogenesis						
<i>Digestive system</i>						
04970 Salivary secretion						
04974 Protein digestion and absorption						
04975 Fat digestion and absorption						
<i>Nervous system</i>						
04725 Cholinergic synapse						
04730 Long-term depression						
04722 Neurotrophin signaling pathway						
<i>Sensory system</i>						
04742 Taste transduction						
04750 Inflammatory mediator reg. of TRP channels						
<i>Development and regeneration</i>						
04361 Axon regeneration						
04380 Osteoclast differentiation						
<i>Aging</i>						
04211 Longevity regulating pathway						
<i>Environmental adaptation</i>						
04713 Circadian entrainment						
04714 Thermogenesis						

Processes that are associated with under-expressed (blue) and over-expressed (red) transcripts are highlighted for each species and treatment. Processes that were either under-expressed (–) or over-expressed (+) in all three treatments (oil, dispersant, and oil/dispersant mixtures) in a given species are further noted. The asterisk (*) denotes transcripts that correspond to the same gene in both species. KEGG analyses were based on KO annotations via eggNOG. Reference **Supplementary Tables 3, 5** for more details and impacted pathways.

DISCUSSION

This study provides novel transcriptomic data for two species of deep-sea octocorals, including a close relative of *P. biscaya* – the most severely impacted deep-sea species in the aftermath of the DWHOS (Deepwater Horizon Natural Resource Damage Assessment, 2016). Similar to *in situ* impacted *P. biscaya* (DeLeo et al., 2018), these results highlight the variability among coral responses to anthropogenic pollutant exposure, both inter- and intra-specific, and the utility of expression-level investigations of deep-sea fauna to elucidate sub-lethal impacts. These data add to

the sparse amount of high-throughput sequencing data available for octocorals and deep-sea species, and provide added resources for studying and monitoring coral and deep-sea ecosystems in the face of future anthropogenic impacts and global ocean change.

Past transcriptomic investigations of *P. biscaya* revealed genome-wide impacts following *in situ* exposure to oil and dispersant laden floe (DeLeo et al., 2018). However, the full extent and duration of the exposure to oil and dispersant during the spill is unknown. There were multiple conceivable routes of exposure as oil and chemical dispersants were released at depth (~1500 m; Hazen et al., 2010) and ultimately persisted

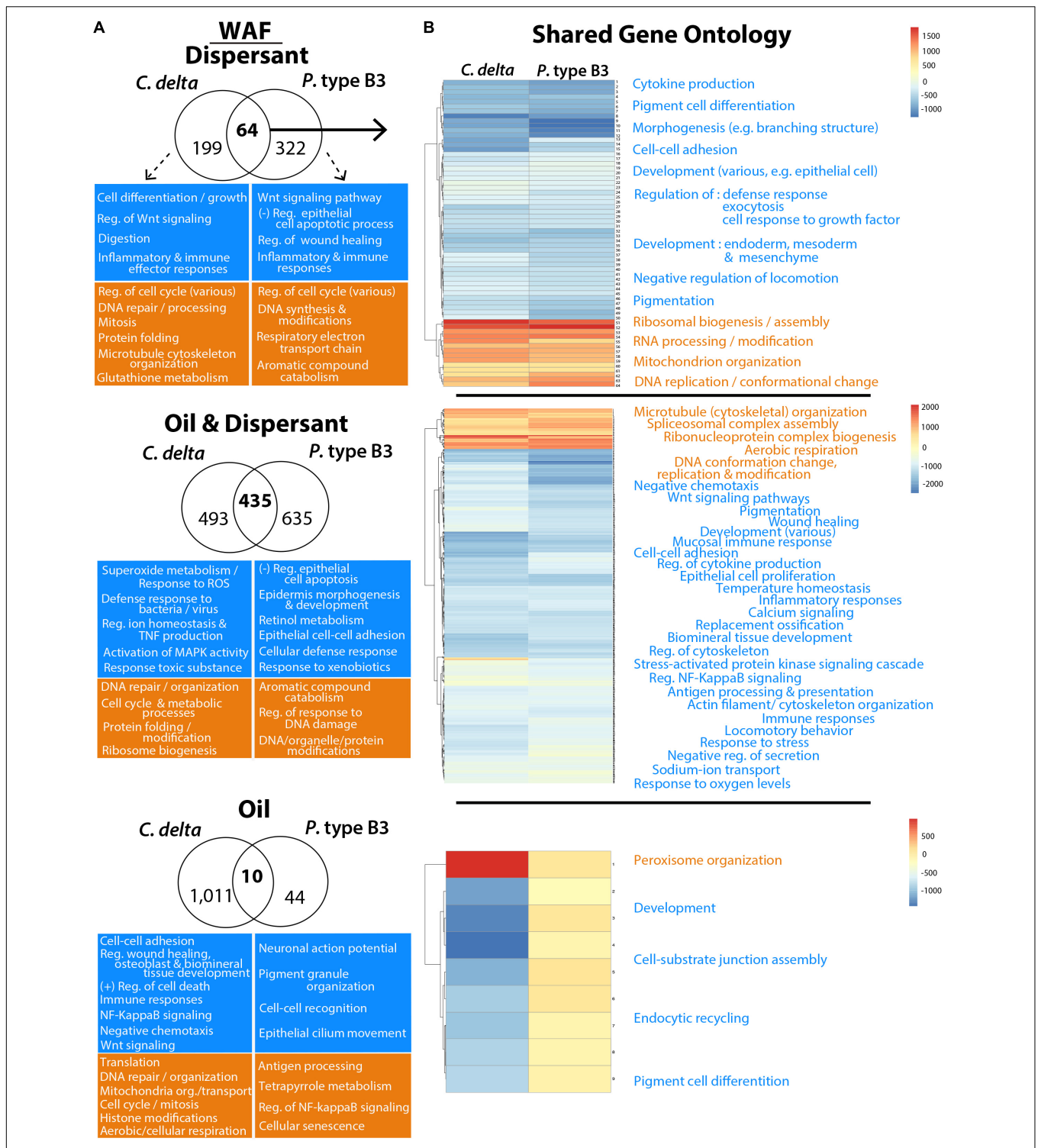


FIGURE 4 | Gene Ontology (GO) terms significantly enriched for over- (orange) or under- (blue) expressed transcripts from *C. delta* and *P. type B3* experimentally exposed to WAF oil and dispersant. Highlighted GO terms represent a snapshot of some of the impacted processes/functions either unique to (A) or shared (B) among species in relation to the dispersant (top), oil and dispersant mixture (middle), or oil-only (bottom) treatments. The Venn diagrams (A) represent the total number of shared and unique GO terms for each species in the different treatments, with representative GO terms of interest, unique to each species, highlighted in the boxes underneath. (B) The heatmaps of the shared GO terms from each treatment illustrates the corresponding level of expression for each term (rows) and compares expression between each coral species (column). Processes (orange/blue) are summarized to highlight various impacted biological pathways. More details on GO enrichment can be found in **Supplementary Table 4**.

as a deep-water oil plume around ~1,100 m (Camilli et al., 2010). These alternative exposure routes may have elicited early onset stress responses following initial pollutant contact, which may not have been detectable among flocc-exposed *P. biscaya* at the time of sampling. The transcriptional investigations of spill-impacted *P. biscaya* colonies by DeLeo et al. (2018) were also limited in terms of biological replication and temporal sampling due to restrictions on site access and sampling in the aftermath of the DWHOS. The experimental transcriptomic profiles presented here elucidate these early onset responses of deep-sea octocorals to oil and dispersants with increased replication, biological controls (genets), and interspecific comparisons. However, the authors note that limitations in obtaining and maintaining live deep-sea corals for laboratory experimentation at the time of sampling, as well as the partial colony sampling done to prevent adverse impacts *in situ* following the DWHOS, also limited the sample size, technical replication, and statistical robustness of this study. Regardless, these data show that the experimental oil and dispersant treatments that ultimately elicited mortality in these coral species during the full 96 h of exposure (DeLeo et al., 2016), particularly in the WAF treatments, also elicited large genome-wide expression changes of crucial stress response genes after just 12 h. The largest number of differentially expressed genes and the highest magnitude of gene expression change was found in treatments containing dispersants, relative to the treatments only containing naturally occurring crude oil. This study improves our understanding of how different species of corals respond to, and cope with, environmental contaminant exposure in the short-term, as well as the variability in these responses.

Common Responses to Pollutants

While gene expression profiles revealed the unique influences of different chemical fractions (Bulk/heterogeneous vs. WAF/dissolved) and treatments (oil-only, dispersant-only, and oil/dispersant mixtures) on the corals, they also revealed mutual responses elicited across various treatments in each exposure series.

Bulk Exposure Series

A common response observed among the bulk treatments, which contain both dissolved and undissolved oil and dispersant chemical constituents, involved altered energy metabolism and oxidative stress responses, both of which were observed among DWHOS impacted *P. biscaya* (DeLeo et al., 2018). Cytochrome c oxidase (CCO) activity, a key enzyme involved in aerobic metabolism (Simon and Robin., 1971) was diminished (*lfc* -1.6, *adj. p-value* 0.02) in *C. delta* exposed to treatments containing dispersant, alone or mixed with (dispersed) oil. As CCO catalyzes the terminal step in aerobic oxidative metabolism, CCO inhibition is suggestive of a hypoxic response among *C. delta* exposed to bulk dispersants. Likewise, CCO is a crucial enzyme regulating cellular energy production (Poyton et al., 1988), further suggesting impacts to tissue energy metabolism and cytotoxicity (Khan et al., 1990) following just 12 hrs of exposure. Conversely, CCO activity was significantly elevated among flocc exposed *P. biscaya* (*lfc* 1.9, *adj. p-value* 0.005; DeLeo et al., 2018),

and among the liver/gills of fish following longer exposures (24 and 96 h) to WAF-oil, alone or dispersed (Cohen et al., 2001; Mattos et al., 2010). Elevated CCO activity among these animals may be associated with ongoing oxidative stress responses during short-term exposures to oil WAFs, that could ultimately lead to the hypoxic signature observed in our study. It is possible that exposure to bulk oil and dispersant contaminants may cause a relatively rapid hypoxic response in *C. delta* as compared to exposure to dissolved WAF contaminants. Likewise, succinate-cytochrome c oxidoreductase activity (part of the succinate-oxidase complex of the respiratory chain system (Khan et al., 1990), was significantly elevated (*lfc* 1.1, *adj. p-value* 0.02) in *P. type B3*, in addition to genes associated with oxidative stress responses (GO terms). This suggests that the bulk dispersant exposures also induced oxidative stress among *P. type B3* after only 12 h, though to a different degree and possibly in a species-dependent manner.

WAF Exposure Series

The strongest genome-wide response was observed among both species exposed to WAFs, particularly WAF treatments containing dispersants. This suggests the coral species tested here are particularly sensitive to the WAFs of anthropogenic pollutants. The magnitude and severity of this response to WAF treatments containing dispersants was mirrored by the higher rates of health decline observed during the full 96 h exposure series (see DeLeo et al., 2016 for more details). These results support the growing body of evidence that chemical dispersants elicit strong, negative responses from marine invertebrates and can be more harmful than oil alone, as dispersants increase the surface area of oil-water interactions- potentially increasing toxicological impacts and bioavailability (e.g., Chandrasekar et al., 2006; Goodbody-Gringley et al., 2013; DeLeo et al., 2016). However, within the scope of this study, it is also probable that this response was inflated by concentration differences between Bulk and WAF solutions.

Dispersants are also known to impair cell membrane function (Abel, 1974; National Research Council, 1989). Therefore, strong early onset responses to dispersants at the genomic-level were not surprising, as exposure was shown to result in the increased permeability of biological membranes and loss of total membrane function and/or osmoregulating abilities (Benoit et al., 1987; Partearroyo et al., 1990). As genes associated with DNA repair, protein modification and apoptosis (programmed cell death) (Kultz, 2003; Kultz, 2005), were significantly elevated in WAF treatments containing dispersants, this further suggests that even short-term exposures can impact cellular integrity, leading to lasting impacts.

Common genome-wide responses were also observed among WAF treatments containing oil (alone or dispersed). These included an elevated expression of ribosomal proteins which was likewise observed among *in situ* impacted *P. biscaya* (DeLeo et al., 2018), and among shallow-water octocorals following periods of short-term pathogen stress (Burge et al., 2013). It has been suggested that the elevated expression of ribosomal gene products is a generalized response to environmental stressors

(e.g., Burge et al., 2013; DeLeo et al., 2018) and that ribosomal proteins play an important role in regulating metabolism in invertebrates during periods of stress (Travers et al., 2010). It is possible that the elevated expression of these genes following the sub-lethal exposures to oil and dispersant was an attempt to regain cellular homeostasis following stress induced DNA and/or protein damage (Kultz, 2003).

Various structural molecules and ECM components were also inhibited among the corals experimentally exposed to WAF oil treatments. These include fibrillins that confer structural integrity to tissues (Piha-Gossack et al., 2012) and are thought to play a role in stress tolerance, albeit in plants (Singh and McNellis, 2011). Therefore, it is possible that the dissolved, water-accommodated oil and dispersant constituents may both compromise coral tissue integrity to a certain degree.

Additional putative genes involved in wound repair and inflammatory responses (i.e., metallopeptidases and peroxidase; Massova et al., 1998), were inhibited among WAF treatments containing oil (alone or dispersed oil) as well, further suggestive of acute cytotoxicity and cellular damage (i.e., Sterchi et al., 2008). This may be linked to the tissue degradation and health decline observed during the 96 h experimental exposures (DeLeo et al., 2016). Conversely, genes coding for structural/ECM proteins and inflammatory/wound repair pathways (i.e., peroxidase) were significantly elevated in *P. biscaya* exposed to DWHOS floc *in situ* (DeLeo et al., 2018). It is probable that natural hydrodynamic flow *in situ* alters and dampens exposure impacts and enables sessile invertebrates, like corals, to withstand longer exposures and/or combat these stressors at the cellular level. It is also possible that the expression signatures observed in this study reflect the prioritization (elevated expression) of other homeostatic processes as an initial defense response against chemical stressors, which in turn reduced the energetic resources allocated to maintain processes such as tissue integrity and wound-repair in the short-term. The latter scenario is likely the case, as some of the genes under-expressed here (e.g., fibrillins and peroxidase) were over-expressed in the black coral *Leiopathes glaberrima* exposed to identical experimental treatments for 24 h (Ruiz-Ramos et al., 2017). While the functional role of peroxidase is not fully understood among invertebrates, it is believed to contribute to innate immune defenses (Gotenstein et al., 2010), responses to oxidative stress, and programmed cell death (Horikoshi et al., 1999). Based on our results, it is possible that some, if not all, of these processes are initially suppressed during early responses to anthropogenic pollutants to allocate resources to other homeostatic processes.

Many GO terms related to cellular adhesion were under-expressed in all treatments (i.e., cell-cell, cell-matrix, and cell-substrate adhesion). Cell adhesion molecules are functionally diverse and are integral in a wide array of cellular processes, such as cell-cell signaling, multicellular tissue development (Gumbiner, 1996), and immune responses (Johansson, 1999; Harjunpää et al., 2019). Reduced expression of cellular adhesion molecules could therefore have wide-reaching implications, and possible associations with impairments to immunity and delayed wound healing (Harjunpää et al., 2019), though

more rigorous investigation is needed to confidently draw these conclusions. Differential expression of genes associated with cellular adhesion were likewise detected among corals following temperature stress (Gates et al., 1992; Traylor-Knowles, 2019) and disease (Daniels et al., 2015; Young et al., 2020). Therefore, cellular adhesion processes appear to be commonly impacted by environmental stressors and could be used in future assessments to gain a snapshot of overall coral health/condition.

Species-Specific Differences

Our data suggest that the octocorals had distinct responses to the Bulk and WAF oil exposures after 12 h, as there was no major overlap among the differentially expressed genes that were annotated- though this may be linked to the limitations of our experimental design. It is possible that there was a delayed or altered reaction to the pollutants in one of the species. From a physiological standpoint, both *C. delta* and *P. type B3* did surprisingly well in both oil-only treatments during the full 96 h exposure series as compared to the treatments containing dispersants (DeLeo et al., 2016). Metabolic depression was primarily observed among the corals exposed to oil in this present study, including genes associated with xenobiotic biodegradation and metabolic pathways (i.e., ABC transporters and GST). Therefore it seems unlikely that these corals were metabolizing oil in the short term as was suggested for *L. glaberrima* (Ruiz-Ramos et al., 2017).

Cytochrome p450 (CYP), which functions in the biotransformation and detoxification of most xenobiotics (Goldstone et al., 2006), was also not significantly differentially expressed among the corals after 12 h. CYPs are required for the efficient elimination of foreign chemicals from the body (Goldstone et al., 2006) and have become a widely used biomarker for pollutant (Devaux et al., 1998; Porte et al., 2001) and oil exposure (Garcia et al., 2012; Zhang et al., 2012; Han et al., 2014). CYPs were significantly elevated in *L. glaberrima* experimentally exposed to oil for 24 h (Ruiz-Ramos et al., 2017) but CYPs, along with ABC transporters and GST, were depressed among *P. biscaya* exposed to oil and dispersant *in situ* (DeLeo et al., 2018), albeit for an unknown duration. Although CYPs were not significantly expressed here, components of this same functional pathway – xenobiotic metabolism by cytochrome P450 – were significantly suppressed in *C. delta* exposed to WAF oil and elevated in *C. delta* exposed to WAF dispersant. This suggests that this pathway is impacted after just 12 h of pollutant contact, but that these impacts are dynamic and may differ depending on the species and/or the duration of exposure.

C. delta and *P. type B3* experimentally exposed to WAF treatments, particularly those containing dispersants, each had a distinct overlap in gene expression with *in situ* impacted *P. biscaya*. Interestingly, this overlap appears to correlate with the treatments that elicited the strongest phenotypic response in the 96 h exposure series (see DeLeo et al., 2016). This is particularly apparent for *C. delta*, which had somewhat less severe rates of health decline in response to longer exposures to these treatments (DeLeo et al., 2016) and the strongest genome-wide response to the WAFs (after 12 h). It is possible

that this is linked to “frontloading” or constitutive expression of genes that respond to oil exposure and/or their increased capacity for gene expression plasticity of environmental stress response (ESR) genes. For shallow-water corals, it has been hypothesized that one or both approaches may impart a certain degree of resilience to environmental stressors (i.e., Barshis et al., 2013; Kenkel and Matz, 2016). As *C. delta* preferentially occupies habitats near natural hydrocarbon seeps (Quattrini et al., 2013), they have likely adapted to low levels of hydrocarbon exposure potentially by employing one of these approaches.

In the case of *C. delta*, plastic expression may be linked to “chemical defensible” genes, evolutionarily conserved genes and proteins that are involved in numerous processes including the metabolism and biotransformation of xenobiotic toxins [i.e., polycyclic aromatic hydrocarbons (PAHs), components of oil], antioxidant systems that respond to oxidative stress, as well as protein homeostasis (Reitzel et al., 2008; Lushchak, 2011; Tarrant et al., 2014). Many of the GO terms that were unique to *C. delta* compared to *P.* type B3 exposed to oil were associated with cellular stress responses, including the regulation of stress-activated MAPK and protein kinase signaling cascades, and multicellular organismal response to stress, potentially signifying an increased capacity to respond to the stresses of oil exposure. As *C. delta* exhibited somewhat less severe rates of health decline in response to longer exposures to oil (DeLeo et al., 2016), it is possible this species is more resilient to short-term pollutant exposure.

The heightened cellular level response observed for *C. delta*, in terms of the number of genes that were differentially expressed, suggests that short-term dispersant exposure can induce cell damage in as little as 12 h. Expression differences were observed for genes involved in necroptosis (i.e., toll-like receptors), a form of necrosis or inflammatory cell death (reviewed in Dhuriya and Sharma, 2018), and C-type lectin signaling cascades that induce the production of inflammatory cytokines and chemokines (reviewed in Tang et al., 2018). Tumor necrosis factor receptor-associated factors (TRAFs), immune system receptors that elicit immune and inflammatory (e.g., necrosis) responses (Palmer and Traylor-Knowles, 2012), were also significantly differentially expressed. TRAFs were over-expressed in the WAF dispersant-only treatment after 12 h but under-expressed among *C. delta* in the WAF oil/dispersant mixtures, as well as *P.* type B3 in WAF-oil. TRAFs were similarly over-expressed in *P. biscaya* exposed to oil and dispersant laden floc (DeLeo et al., 2018), indicating that over-expression may be linked to the dispersant constituents. These results suggest that even short periods of dispersant exposure may influence coral immune pathways and that immune/inflammatory pathway components make good candidates for future spill monitoring efforts.

Unlike *C. delta*, which exhibited a greater number of differentially expressed genes (and a larger average magnitude of gene expression change) when exposed to WAF treatments relative to Bulk treatments, *P.* type B3 had a comparable number of differentially expressed genes, albeit a higher magnitude of expression change in the WAF exposures. It is possible that this is linked to a reduced capacity for gene expression plasticity

of important ESR genes among *P.* type B3 relative to *C. delta*. Studies of shallow-water corals exposed to thermal stress revealed a higher degree of thermal tolerance and reduced bleaching impacts among populations of corals that exhibited a greater capacity for gene expression plasticity related to changes in environment/condition (Kenkel and Matz, 2016). It is possible that similar interspecific differences in plasticity also exist among deep-sea octocoral species. This would suggest that *C. delta* may be better able to mitigate intracellular damage resulting from anthropogenic stressors, and that *P.* type B3 is equally vulnerable to both undissolved (Bulk) and water-accommodated (WAF) components of oil and dispersant, though further investigation is needed to elucidate these differences.

Functional enrichment analyses for *P.* type B3 exposed to WAFs revealed that GO terms associated with Wnt signaling were significantly enriched among the under-expressed transcripts. Wnt signaling pathways are integral cascades associated with a myriad of cellular functions including development and cell-cell interactions. Down-regulation of Wnt pathways has also been detected in shallow-water corals in response to high temperature (Polato et al., 2013; Maor-Landaw et al., 2014) and low pH/high temperature conditions (Kaniewska et al., 2015). These signaling pathways could therefore be highly susceptible to environmental stress in corals and warrant further investigation.

CONCLUSION

As offshore drilling continues to increase worldwide, the need to understand species and ecosystem-level impacts has become paramount. Prior to the DWHOS there was no research into how chemical dispersants would react at depth and how prolonged exposure would impact deep-sea biota. As corals are important ecosystem engineers, creating complex structures that provide habitat to diverse suites of organisms, impacts to these species will have significant consequences. This and other studies provide evidence to suggest prolonged dispersant exposure is more harmful than oil exposure alone. Our findings indicate coral fauna have variable responses to anthropogenic environmental stressors, and some species and/or genotypes may be more resilient to environmental stress. These results further highlight the possible impacts of both dissolved and undissolved anthropogenic contaminants to corals, including metabolic and hypoxic consequences and impacts to immune and wound healing processes. Exposure to bulk/heterogenous contaminants appears to cause heightened oxidative stress and metabolic responses, while exposures to dissolved/WAF contaminants appears to elicit stronger impacts to tissue/skeletal development, immune responses and cellular damage/DNA repair. Similar processes were impacted among corals damaged *in situ* by the DWHOS following prolonged floc exposure among other probable routes of contaminant exposure (i.e., deep-water oil plume), highlighting both the early probable inception of this damage and the complex responses of corals to oil and dispersant constituents. This improved understanding of the ways in which oil and dispersant exposure affects corals is integral to improving

our ability to properly manage these ecosystems and respond to future exposure from standard operations and accidental releases.

DATA AVAILABILITY STATEMENT

The RNAseq data presented in this study can be found on NCBI's SRA database [BioProject ID: PRJNA708307].

AUTHOR CONTRIBUTIONS

DD and EC conceived and designed the experiments. DD performed the experiments and lab work with assistance from AB. DD and AG analyzed the data. SH consulted on bioinformatic analyses. DD wrote the manuscript with input from all co-authors. All the authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.649909/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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