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Gene expression profiling reveals deep-sea coral response to the Deepwater Horizon oil spill

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Abstract

Deep-sea coral communities are key components of the Gulf of Mexico ecosystem and were adversely affected by the Deepwater Horizon (DWH) oil spill. Coral colonies exposed to oil and dispersant exhibited mortality, damage and physiological signatures of stress. Understanding how corals respond to oil and dispersant exposure at the molecular level is important to elucidate the sublethal effects of the DWH disaster and reveal broader patterns of coral stress responses. Gene expression profiles from RNAseq data were compared between corals at an impacted site and from a reference site. A total of 1,439 differentially expressed genes (≥twofold) were shared among impacted *Paramuricea biscaya* colonies. Genes involved in oxidative stress, immunity, wound repair, tissue regeneration and metabolism of xenobiotics were significantly differentially expressed in impacted corals. Enrichment among the overexpressed genes indicates the corals were enduring high metabolic demands associated with cellular stress responses and repair mechanisms. Underexpression of genes vital to toxin processing also suggests a diminished capacity to cope with environmental stressors. Our results provide evidence that deep-sea corals exhibited genome-wide cellular stress responses to oil and dispersant exposure and demonstrate the utility of next-generation sequencing for monitoring anthropogenic impacts in deep waters. These analyses will facilitate the development of diagnostic markers for oil and dispersant exposure in deep-sea invertebrates and inform future oil spill response efforts.

KEYWORDS

anthropogenic, biomarkers, dispersant, Gulf of Mexico, immune response, invertebrate, octocoral, oxidative stress, *Paramuricea biscaya*, transcriptomics

1 | INTRODUCTION

The *Deepwater Horizon* (DWH) oil spill is the largest accidental oil spill in history, releasing an unprecedented amount of oil and chemical dispersants into the deep sea. While recent studies assessing the consequences of the DWH spill have found both sublethal and lethal impacts in populations of various marine species (Dubansky, Whitehead, Miller, Rice, & Galvez, 2013; Lane et al., 2015; McCall & Pennings, 2012; White et al., 2012; Whitehead et al., 2012), efforts to assess impacts to deepwater fauna are primarily limited to visual surveys (Fisher, Hsing, et al., 2014; White et al., 2012) and experimental exposures (DeLeo, Ruiz-Ramos, Baums, & Cordes, 2016). Furthermore, no prior studies have examined in situ impacts of DWH contaminant exposure to deep-sea species at the molecular level.

The deep oceans, defined here as depths below 200 m, comprise the largest ecosystem on the planet and are highly diverse in terms of representative animal phyla (Snelgrove, 1999) or total numbers of species (Snelgrove & Smith, 2002). However, relatively little is known

about the evolutionary history and genomic diversity of these deep, cryptic fauna. Historically, the deep sea was considered isolated from anthropogenic disturbance, although increased industrialization and resource extraction challenge this paradigm (Cordes, Jones, et al., 2016), as dramatically highlighted by the DWH disaster (Joye et al., 2016). One consequence of this spill was a persistent plume of oil microdroplets at approximately 1,100 m depth (Reddy et al., 2012). In addition, deposition to the seafloor (Romero et al., 2015) occurred via the sinking of oiled particulate organic matter or "marine snow" (Passow, Ziervogel, Asper, & Diercks, 2012) and/or as the by-products of the surface oil burns (National Response Team 2011; UAC 2010). These pathways of oil and dispersant delivery to the seafloor led to impacts on deep sea and mesophotic coral habitats (Etnoyer et al., 2015; Fisher, Hsing, et al., 2014; Hsing et al., 2013; White et al., 2012), the extent of which is still under investigation.

Deep-sea coral communities impacted by the DWH disaster were first discovered at Mississippi Canyon (MC) 294, which is located 11 km southwest of the wellhead at a depth of approximately 1,370 m. Several coral species were covered to varying degrees with a brown flocculent material (floc) that contained hydrocarbons from the Macondo well (White et al., 2012). The floc also contained the anionic surfactant, dioctyl sodium sulfosuccinate (DOSS), a major component of the chemical dispersants applied on surface slicks and at depth at the wellhead source (White & Lyons, 2014). Since the initial discovery, three additional impacted deep-sea coral sites have been found at similar depths (1,550–1,850 m; Fisher, Demopoulos, Cordes, & White, 2014) and three impacted, mesophotic sites were discovered at shallower (60–90 m) depths (Etnoyer et al., 2015).

The most common and severely impacted deep-sea coral species was the octocoral, *Paramuricea biscaya* Grasshoff 1977 (DWH Natural Resource Damage Assessment 2016). At the time of discovery, colonies exhibited signs of stress including mucous production, tissue sloughing and necrosis (White et al., 2012). Insight into the mechanism underlying this response can be gained by examining the whole-organism physiological response to experimental oil and dispersant exposure, which has shown that the dispersant is at least as toxic as oil solutions at the same concentrations (DeLeo et al., 2016). Subsequent declines in the health of the corals in situ, associated with hydroid colonization and continued tissue loss, were observed in the first few years (Hsing et al., 2013) followed by limited recovery of lightly impacted colonies but also continued health decline and branch loss in most of the corals (Girard & Fisher, 2018). Because *P. biscaya* is long-lived (>600 years) and slow-growing, 0.34–14.20 μm/year (Prouty, Fisher, Demopoulos, & Druffel, 2016), this species is particularly vulnerable to anthropogenic disturbances.

Damage to these habitat-forming corals can negatively affect biodiversity and ecosystem functioning (Freiwald, Fosså, Grehan, Koslow, & Roberts, 2004; Husebø, Nøttestad, Fosså, Furevik, & Jørgensen, 2002). These corals form arboreal structures that provide shelter, feeding grounds and nursery areas for a diverse range of species including commercially important fisheries (Cordes, Arnaud-Haond, et al., 2016; De Clippele, Buhl-Mortensen, & Buhl-Mortensen, 2015). As the frequency of human-induced disturbances increases in deeper waters due to resource extraction, pollution, bottom-trawling and the ongoing threat of ocean acidification, further investigations into how deep-sea corals respond to stress will improve our understanding of their resiliency to these challenges. Understanding how corals respond to oil spills and dispersant application requires a more in-depth understanding of their molecular and cellular responses to in situ exposure.

Coral stress responses can be revealed by examining changes in gene expression, the variation of which can be detected through RNA sequencing (RNAseq) (Ekblom & Galindo, 2011; Whitehead et al., 2012). In this present study, we constructed a de novo transcriptome and compared gene expression patterns among *P. biscaya* colonies damaged during the DWH oil spill, with those found at a reference site. Comparisons were made to better understand the impacts of exposure on underlying cellular processes that ultimately manifested into the observations of physical damage at MC294 (White et al., 2012). Relative expression changes were indicative of cellular stress and immune responses as well as toxin processing. Elucidating the consequences of contaminant exposure on impacted deep-sea invertebrates at the molecular level advances our understanding of stress response patterns and the damage imposed by the DWH incident.

2 | MATERIALS AND METHODS

2.1 | Sample collections

Spill-impacted *P. biscaya* colonies partially covered in floc containing Macondo oil (the reservoir the DWH had drilled into) and dioctyl sodium sulfosuccinate (DOSS), a component of the dispersants applied during cleanup efforts (White & Lyons, 2014; White et al., 2012), were collected from site MC294, 1,370 m depth (November 2010; n = 2: samples "exp685" and "exp686"). Samples were preserved in RNAlater *in situ*, using the remotely operated vehicle (ROV) *Jason II* (Figure S1). Samples from colonies of *P. biscaya* (n = 2) without signs of visible impact ("control" colonies; samples "unexp43" and "unexp47") were also collected and preserved in RNAlater in situ from Mississippi Canyon (site MC344, 1,850 m depth) during the same season (December 2010), using the deep-submergence vehicle (DSV) Alvin. It was not possible to collect control colonies of *P. biscaya* at MC294 as all corals were believed to have been exposed to hydrocarbons and dispersant to some degree (White et al., 2012), and it was impossible to collect control corals from other sites during the November cruise since the MC294 site was discovered during the last ROV dive of the series. Instead, *P. biscaya* colonies were sampled from the nearest known site at a similar depth within the Mississippi Canyon region (Doughty, Quattrini, & Cordes, 2014) at the first opportunity to do so.

2.2 | RNA isolation and sequencing

Total RNA was extracted using a Qiagen RNeasy Kit or a modified Trizol/Qiagen RNeasy protocol (described in Burge, Mouchka,

Harvell, & Roberts, 2013; Polato, Vera, & Baums, 2011) in cases where tissue was limited. Concentrations were assessed using a NanoDrop® ND-1000, and RNA quality was evaluated through gel electrophoresis via the presence of intact 28S and 18S ribosomal RNA bands and using an Agilent 2100 BioAnalyzer (Agilent Technologies). Total RNA was sent for mRNA library preparation (TruSeq RNA Library Preparation Kit v2) and high-throughput Illumina sequencing (HiSeq2000; 100 base pair (bp), paired-end reads) at the University of Wisconsin-Madison Biotechnology Center (UWBC, Madison, WI). Due to the limited availability of viable, intact coral tissue from which high-quality RNA could be obtained, additional quantitative methods (e.g., real-time quantitative PCR) were not feasible for validating these expression values.

2.3 | Sequence processing

Reads were quality-assessed using FASTQC v0.11.5 (Andrews, 2010) and subsequently quality-trimmed and filtered using TRIMMOMATIC v0.36 (Bolger, Lohse, & Usadel, 2014) in paired-end (PE) mode. To remove pervasive low-quality bases at the 3′ end, identified from FastQC per-base sequence content plots, we cropped reads to a maximum length of 99 bases (CROP:99). Each read was scanned using a 5-base window and cut if the quality Phred score dropped below 20 (SLIDINGWINDOW:5:20). Leading and trailing bases were removed if quality dropped below a score of 3 (LEADING:3 TRAILING:3). Trimmed reads with resulting lengths shorter than 60 bases were excluded (MINLEN:60). We also utilized Trimmomatic, in combination with CUTADAPT v1.15 (Martin, 2011), to remove adaptor sequences from reads (Trimmomatic ILLUMINACLIP:2:30:10; Cutadapt: –overlap 10). To correct random sequencing errors from the reads, we utilized the k-mer-based method Rcorrector (Song & Florea, 2015), with the k-mer counter JELLYFISH v.2.2.6 (Marcais & Kingsford, 2011), using default parameters. Unfixable reads were discarded. We identified read sequences belonging to potential microbial associates and/or contaminants by performing taxonomic assignments using the program KRAKEN v1.0 (Wood & Salzberg, 2014) with the standard database (downloaded on October 18, 2017), which includes all complete bacterial, archaeal and viral genomes available in NCBI's RefSeq database. Kraken was executed with default parameters. All reads classified as microbial were excluded.

2.4 | Transcriptome assembly

Clean paired-end reads from all individuals were normalized and assembled with Trinity v2.4.0 using default parameters (Grabherr et al., 2011; Haas et al., 2013). As a second measure to remove non-coral sequences, assembled contigs were again passed through Kraken. Assembled transcripts were analysed with TransDecoder v5.0.2 (https://transdecoder.github.io/) to identify open-reading frames (ORFs) that were at least 100 amino acids long (TransDecoder.LongOrfs) and predict likely coding regions (TransDecoder.Predict). The remainder of the analyses were limited to transcript isoforms with the longest ORFs. CD-HIT v4.7 (Fu, Niu, Zhu, Wu, & Li, 2012) was used to cluster redundant transcript sequences using a similarity threshold of 0.95. Assembly completeness was assessed via Benchmarking Universal Single-Copy Orthologs (BUSCO v3.0.2, Felipe et al. 2015, Waterhouse et al., 2017) using the hierarchical catalog of orthologs (Waterhouse, Tegenfeldt, Li, Zdobnov, & Kriventseva, 2013) for metazoans OrthoDB v9 (http://www.orthodb.org/). Transcriptome assembly quality was evaluated with TRANSRATE v1.0.3 (Smith-Unna, Boursnell, Patro, Hibberd, & Kelly, 2016). Completeness was further assessed through comparisons to the published octocoral transcriptomes of *Gorgonia ventalina* (90,230 sequences, Burge et al., 2013) and *Corallium rubrum* (48,074 sequences, Pratlong et al., 2015a). Comparisons via tblastx were conducted with a minimum e-value cut-off of 1×10^{-5} (for more information regarding the inferred phylogenetic relationships among these taxa, see Quattrini et al., 2018). Transcriptome annotation was performed using DAMMIT v1.0 (https://dammit.readthedocs.io) using P-FamA, RFam, OrthoDB, BUSCO and uniref90 databases (Scott, 2016). Annotations were parsed using custom R scripts.

2.5 | RNAseq analyses

To identify gene expression responses to oil exposure, we compared RNAseq data between floc-exposed colonies and controls. We quantified the expression of transcripts in each individual using Salmon (Patro, Duggal, Love, Irizarry, & Kingsford, 2017) in quasi-mapping mode. Significantly differentially expressed genes or DEGs (adjusted *p*-value <0.05, absolute log2-fold change >1) between exposed and control groups were identified using DESeq2 (Love, Huber, & Anders, 2014); DESeq2 also clusters DEGs using Pearson correlation distances. Gene clusters revealing variable expression among biological replicates were further analysed using KEGG MAPPER v3.1 (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2015) to decipher molecular interaction networks (KEGG pathway mapping; reconstruct), based on KEGG Orthology (KO) assignments by dammit (Scott, 2016).

A rank-based gene ontology (GO) analysis with adaptive clustering was performed using the program GO_MWU (Wright, Aglyamova, Meyer, & Matz, 2015), which utilizes a Mann–Whitney U (MWU) test and a global-ranked list of all genes assembled to identify GO categories that are significantly enriched among over- and under-expressed genes. A continuous measure of significance, -log(*p*-value) calculated from DESeq2 analyses, was used to identify enriched GO categories. Hierarchical clustering of GO categories was based on number of shared genes (clusterCutHeight = 0.25). Each GO category had to contain at least five genes to be considered and could not contain more than 10% of all genes.

3 | RESULTS

3.1 | Paramuricea reference transcriptome

On average, 76.3 million (*SD* 2.79 million) read pairs were generated per sample. Raw sequencing data are available on the NCBI's

Sequence Read Archive database under BioProject PRJNA481028. An average of 54.9 million (*SD* 10.7 million) read pairs per sample passed filtering and error correction steps. Of these, an average of 47.1 million (*SD* 10.6 million) remained after contaminant removal. The de novo reference transcriptome assembly for *P. biscaya* contains 60,699 contigs with a mean length of 871.8 bp (Table 1; DeLeo et al., 2018). In total, 88.9% of universal single-copy metazoan orthologs were identified in the reference assembly indicating a relatively complete (C:88.9% [S:75.2%, D:13.7%], F:4.3%, M:6.8%, n:978) and high-quality assembly. Approximately 81.8% (49,679) and 74.7% (45,348) of *P. biscaya* contigs had significant matches ($<1e^{-5}$) to the transcriptomes of *C. rubrum* and *G. ventalina*, respectively.

3.2 | RNAseq analyses

In this study, we identified 1,439 putative genes that were significantly differentially expressed (DEGs) among *P. biscaya* floc-exposed colonies and controls, corresponding to five gene clusters (Figure 1, Table S1). The absolute log2-fold changes ranged from 1.2 to 11.1 (Table 2, Figure S2). Among the spill-impacted samples, 1,026 DEGs were overexpressed relative to control colonies, including but not limited to collagens and other skeletal organic matrix proteins, carbonic anhydrase, sodium/potassium/calcium pumps, peroxidasin-like proteins, tyrosinase, the immunity complex-complement C3 (Figure 1: top/red cluster) and tumour necrosis factor (TNF) receptor-associated factors (TRAF1: top/red cluster, TRAF2/4/5: second/blue cluster). Some variability in the degree of overexpression was observed among the impacted colonies (Figure 1, Table S1: second/blue, third/green clusters), though all genes were still overexpressed relative to controls. KEGG pathway maps indicated that these variable genes are primarily associated with metabolic pathways, translation, signal transduction, apoptosis/necroptosis, immune signalling and digestion (Table S2). The remaining 413 putative genes were significantly under-expressed relative to controls including cytochrome p450 (CYP), ABC transporters, multidrug resistance proteins (MDR) and glutathione-S-transferase (GST).

Comparisons to visualize the overall effect of exposure on the genome-wide expression patterns of *P. biscaya* via a principal component analysis revealed a variable response among impacted colonies (exp685 and exp686) relative to a more consistent pattern of gene expression among controls (Figure 2). The first component, which explained 63% of the variation in expression, separated impacted *P. biscaya* "exp686" from the other colonies, while the second component explained 31% of the variation and separated impacted *P. biscaya* "exp685" from the unexposed corals (unexp43 and unexp47). Some of this variability appears to be associated with increased xenobiotic and energy metabolism, TNF pathway/caspase signalling associated with apoptosis and immune signalling linked to mitochondrial dysfunction in colony-exp686 (Table S2). Alternatively, colony-exp685 showed relative elevations in glycan/lipid metabolism and TNF/TRAF signalling associated with cell survival.

TABLE 1 Assembly statistics for the reference assembly

Paramuricea biscaya transcriptome	
Number of transcripts	60,699
Mean length (bp)	871
Reconstruction size (bases)	52,915,767
Transcripts over 1k bp	15,088
Transcripts over 10k bp	52
Transcripts with ORFs	38,477
GC content	42%
N50 (bp)	1,143
Transrate score	0.1829

3.3 | Gene ontology enrichment

In terms of enriched biological processes in the impacted corals, the rank-based GO analysis revealed significant enrichment among overexpressed genes linked to metabolism and cell differentiation (FDR < 0.01; Figure 3). Significant enrichment among the overexpressed genes was also associated with hydrogen peroxide metabolism, transcription, cytoskeletal organization and development (FDR < 0.05), in addition to responses to oxygen compounds, protein regulation and ossification (FDR < 0.1). For genes under-expressed among impacted colonies, enrichment was found for biological processes associated with DNA replication and integration (FDR < 0.01).

Among the molecular GO categories (Figure S3) associated with overexpressed genes in impacted *P. biscaya*, significant enrichment was found for oxidoreductase activity, structural constituents of ribosomes, DNA binding and peptide regulation (FDR < 0.01), protein kinase activity (FDR < 0.05), metal and chitin binding and calcium release channels (FDR < 0.1). Enrichment was also found among under-expressed genes corresponding to DNA polymerase, nuclease and hydrolase activities (FDR < 0.01), monooxygenases and hydrogen ion transporters (FDR < 0.01) as well as ATPases and electron carriers (FDR < 0.05).

Among the cellular component GO categories (Figure S4), significant enrichments for overexpressed genes correspond to the ribosome, collagen trimmers and the extracellular matrix/region (FDR < 0.01). For under-expressed genes, enriched categories include the mitochondria (FDR < 0.01), cytoskeleton, nucleotide-excision repair and proteasome complex (FDR < 0.05).

4 | DISCUSSION

This study provides the first published transcriptomic data set for a deep-sea octocoral. While published RNAseq data exists for approximately nine (Burge et al., 2013; Fuess, Mann, Jinks, Brinkhuis, & Mydlarz, 2018; Hongo, Yasuda, & Nagal, 2017; Pratlong et al., 2015a,b; Romiguier et al., 2014; Zapata et al., 2015) of an estimated 3,000 octocoral species (Daly et al., 2007), the three published deep-sea coral transcriptomes to date are from distantly related calcifying, scleractinian corals from the Red Sea (Yum et al., 2017), with

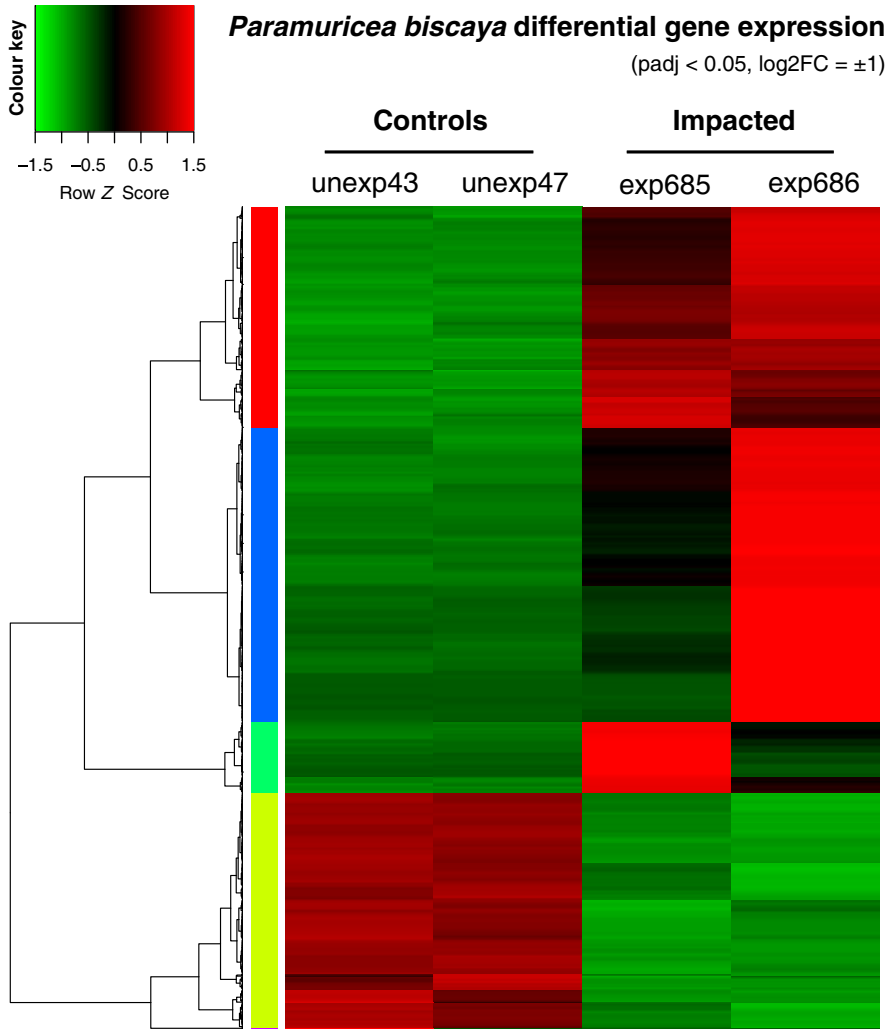

FIGURE 1 Heatmap showing 1,439 genes with significant differential expression among impacted *Paramuricea biscaya* colonies exposed to floc (exp685 and exp686) relative to expression in control colonies not exposed to floc (unexp43 and unexp47). Differential gene expression was considered significant if adjusted *p*-values (FDR) < 0.05 and absolute log2-fold change (FC) was >1. Hierarchical clustering (left) using correlation-based distances (Pearson) indicates five, colour-coded expression clusters. The red cluster (top) represents genes significantly overexpressed in both impacted corals relative to controls. The next two clusters (middle) also represent overexpressed genes among impacted corals, though this expression was variable among the floc-exposed colonies. Genes in the larger blue cluster (second from top) are highly overexpressed in colony-exp686, and to a lesser degree in colony-exp685 and vice versa for the smaller (third) green cluster. Genes corresponding to the yellow and pink (bottom two) clusters were significantly under-expressed in both impacted corals [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Number of differentially expressed genes (≥twofold) for impacted *Paramuricea biscaya* colonies, significantly over- or under-expressed (FDR = 0.05) relative to expression in "un-impacted" control colonies

	Overexpressed	Under-expressed
Total	1,026	413
Log2-fold change (FC)	1.3–11.1	1.2–7.8
Average Log2-FC	3.1	2.5

divergence estimates of approximately 600 MYA (Kumar, Stecher, Suleski, & Hedges, 2017). As the genus *Paramuricea* is widespread and has been impacted by several different types of anthropogenic stressors at a variety of depths (Cerrano et al., 2000; Coma, Pola, Ribes, & Zabala, 2004), this transcriptome will be useful in future investigations of anthropogenic impacts on corals and other invertebrates as the human influence on the deep sea expands and evolves.

Differential gene expression in this study is attributed to hydrocarbon and dispersant exposure via floc, among other conceivable exposure routes. The damaged well released oil and gas from April until late July 2010, and floc-exposed corals were sampled in November 2010. If the oil and gas release from the damaged well was the primary pathway of exposure, the initial contaminant response would have subsided long before the sampling point. However, the more likely pathway for the exposure of these corals and the generation of the flocculent material found covering them was via oil that rose to the surface and was transported to depth via oiled marine snow (Fisher, Demopoulos, et al., 2014; Fisher, Hsing, et al., 2014; Passow, 2014). Oil and dispersant exposure via oiled marine snow is far more likely to generate the patchy impacts that were observed among and within the coral colonies as opposed to exposure to relatively homogenous dissolved hydrocarbons or microdroplets from the deep-sea oil plume. It is possible that the variations in genome-wide expression patterns among spill-impacted *P. biscaya* colonies are correlated with the degree of exposure and the patchy nature of the floc. The relative amount of floc coverage on coral colonies has been linked to this species' ability to survive and recover after the floc is no longer visible (Girard & Fisher, 2018; Hsing et al., 2013) and would generate variable responses at the cellular level in these coral colonies.

We are confident that the significant differential expression observed between control and exposed corals is associated with

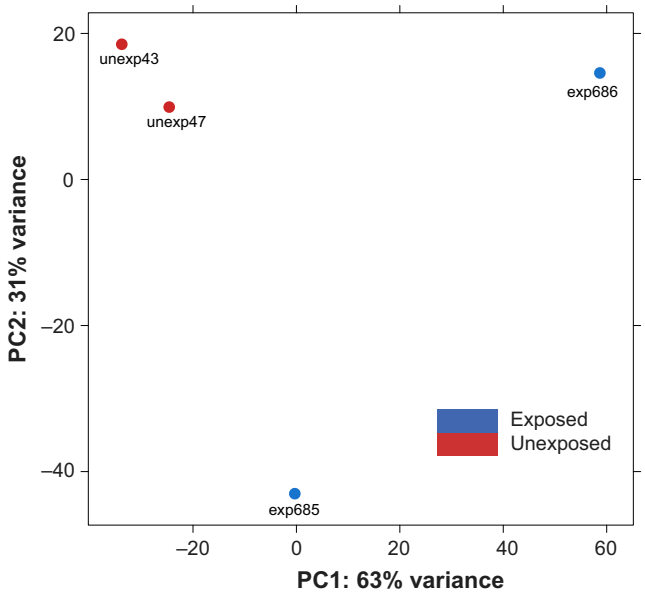

FIGURE 2 Principal component plot showing genome-wide expression patterns in floc-exposed, exp685 and exp686 (blue), and unexposed "control" colonies, unexp43 and unexp47 (red). Note that 94% of the total variation in expression is explained by the first two components [Colour figure can be viewed at wileyonlinelibrary.com]

responses to contaminant exposure. All corals at MC294 are believed to have been exposed to hydrocarbons and dispersants, and most of the colonies observed had significant amounts of floc coverage (Girard & Fisher, 2018; Hsing et al., 2013; White et al., 2012). In contrast, none of the observed control *P. biscaya* colonies at MC344 exhibited any signs of stress nor did they have significant amounts of floc coverage at the time of collection, although low levels of impact from the spill were documented in 2011 on a return visit to a different area of this lease block (Fisher, Hsing, et al., 2014; Girard & Fisher, 2018). It is possible that a portion of the genes differentially expressed was due to differences in depth or location between *P. biscaya* samples, but we do not believe this is significant as all corals were collected during the same season from the Mississippi Canyon region under similar environmental conditions (i.e., temperature and pressure).

4.1 | Wound repair and tissue regeneration

Floc-exposed deep-sea corals at impact site MC294 exhibited varying levels of tissue necrosis and excessive mucosal secretions (White et al., 2012). Processes associated with the production of structural molecules, including components of the extracellular matrix (ECM), were significantly enriched suggesting corals were undergoing

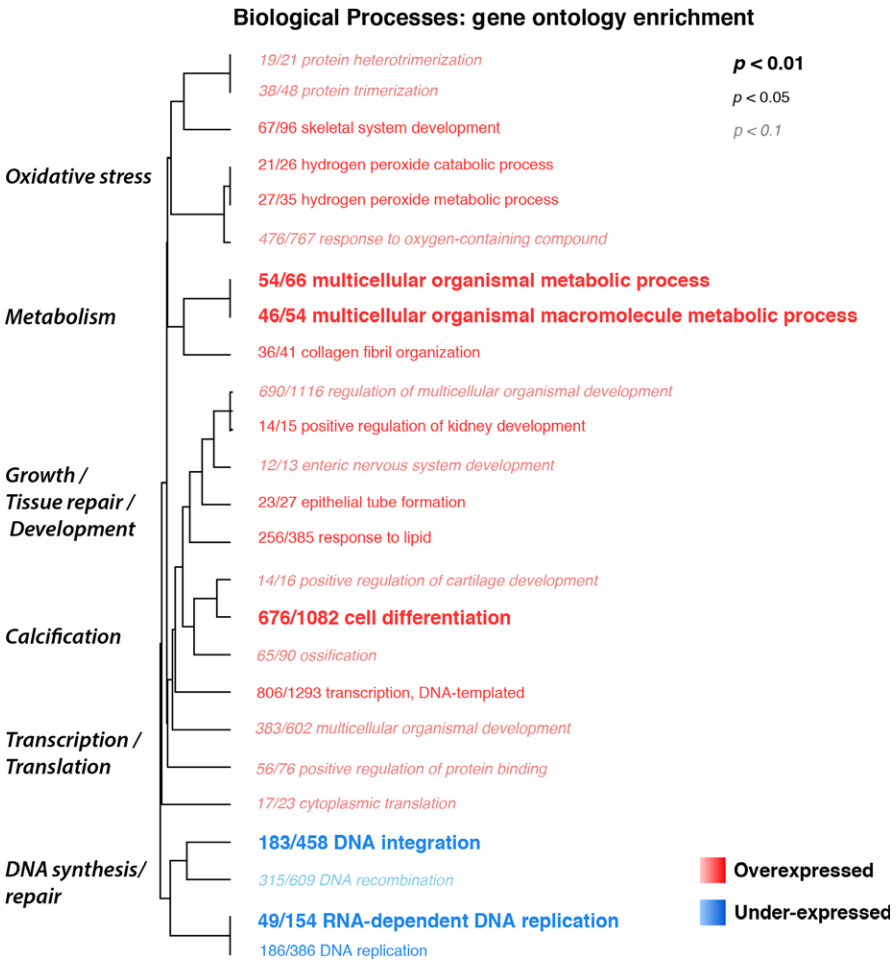

FIGURE 3 Hierarchical clustering of "biological process" GO categories based on the number of shared genes. Ontological categories in red are significantly enriched among genes overexpressed in floc-exposed corals relative to unexposed "control" colony expression. Significant enrichment among genes under-expressed in impacted colonies is labelled in blue (bottom cluster only). Differences in font size correspond to differences in FDR-corrected *p*-values. Fractions preceding category names represent the number of significant genes ($p < 0.05$) corresponding to each category relative to the total number of genes in that particular category [Colour figure can be viewed at wileyonlinelibrary.com]

inflammatory and wound repair processes. Numerous genes in the wound repair/inflammation and tissue regeneration pathways were significantly overexpressed including metalloproteases, peroxidasin and tyrosinase (see Table S1 for more details). Mesogleal or ECM metalloproteinases govern wound repair by functioning as key regulatory enzymes for cell composition (Massova, Kotra, Fridman, & Mobashery, 1998), though high misallocation is associated with cytotoxicity in certain tissues of vertebrates following acute stress (Sterchi, Stöcker, & Bond, 2008). Peroxidasin is also an important mediator of ECM organization, phagocytosis and defensive pathways, with elevated expression found in corals exposed to other environmental stressors (Barshis et al., 2013; Burge et al., 2013).

Phagocytotic granular amoebocytes within the epithelial tissue of the related octocoral, *G. ventalina*, have been found to activate pathways leading to melanin production in pathogen-infected epithelia (Mydlarz & Harvell, 2007), and it is likely that similar activity occurred in compromised *P. biscaya* tissue. Tyrosinase, a copper-containing oxidase that functions in the formation of pigments such as melanin (Palmer & Traylor-Knowles, 2012), was significantly elevated among impacted corals. Increased melanin synthesis has also been found in thermally stressed and diseased coral tissues (Mydlarz, Couch, Weil, Smith, & Harvell, 2009; Palmer, Mydlarz, & Willis, 2008). As "patchy" impact and mortality among colonies was observed at MC294 and other impact sites (Fisher, Demopoulos, et al., 2014; Fisher, Hsing, et al., 2014), this wound repair mechanism likely permitted partial colony survival and limited recovery in the years following the initial stress-induced polyp mortality (Girard & Fisher, 2018).

4.2 | Evidence for immune-related responses

Basal metazoans, including corals, have a basic, ancestral immune system from which other animals evolved their present immune functions (Goldstone, 2008; Reitzel, Sullivan, & Traylor-knowles, 2008; Shinzato, Hamada, Shoguchi, Kawashima, & Satoh, 2012; Venn, Quinn, Jones, & Bodnar, 2009). Invertebrates have innate immune defenses, with stress altering the expression of various immune components (Garcia et al., 2012). It is possible that floc-exposed *P. biscaya* became susceptible to colonization by foreign/harmful microbes as their immune status declined and tissue necrosis occurred. Impacted corals exhibited overexpression of immune-related genes such as complement C3, TRAFs and a putative tumour suppressor gene, DMBT1, thought to function in local inflammation and mucosal immune processes (Kang & Reid, 2003). This suggests that immunity plays a key role in invertebrate responses to oil and dispersant. Considering past transcriptomic investigations into pathogen exposure in the octocoral *G. ventalina* (Burge et al., 2013), our findings support the universal role of immunity in cnidarian stress responses. Likewise, our results imply a link between immune responses and the excessive mucosal secretions of impacted corals at MC294 (White et al., 2012). Coral mucus is thought to act as a crude defense against environmental stressors (Brown & Bythell, 2005), acting as a physiochemical barrier (Sutherland & Ritchie, 2004), though it can also function as a growth medium for bacteria and potential pathogens (Lipp et al., 2002).

4.3 | Impact on biomineralization and development

Enrichment of genes associated with development, cell differentiation and ossification indicates that impacted corals, or at least the portion of the colonies sampled, were undergoing a period of growth at the time of sampling. Whether this is linked to a period of development that began pre- or post-floc exposure is unknown. However, impacted colonies at MC294 exhibited enlarged and abnormally formed sclerites (skeletal components) that protruded from the outer epithelial tissue (White et al., 2012). Given their slow growth rates (<1 μM/year to ~14 μM/year, Prouty et al., 2016), and the elevated expressions of carbonic anhydrase which plays an important role in calcium carbonate precipitation (Bertucci et al., 2013) and solute carriers and bicarbonate transporters functioning in coral biomineralization (Zoccola et al., 2015), it is more probable that these responses are correlated with the developmental abnormalities and tissue regeneration observed at MC294.

4.4 | Cellular stress responses

Across all multicellular organisms, cellular stress responses include common mechanisms to react to macromolecular damage exceeding threshold levels, temporarily increasing tolerance limits to enable the stabilization of cellular homeostasis. When stress-induced protein or DNA damage occurs, the cellular stress response is induced, elevating the expression of appropriate response and repair pathways (Kültz, 2003). When the effects of the stressor exceed the cells' ability to maintain macromolecular integrity, apoptosis or programmed cell death is induced (Kültz, 2003; Kültz, 2005). Signatures of this response are apparent in impacted corals through enrichments in protein synthesis and regulation, and DNA binding and modification among the overexpressed genes. The overexpression of ribosomal proteins observed here has been previously reported in octocorals following periods of short-term stress (Burge et al., 2013), though underexpression was found in stressed invertebrates near death (Travers et al., 2010). Therefore, the elevated expression of ribosomal protein genes in impacted *P. biscaya* may indicate that the sampled corals, or at least the portion of the polyps directly examined within the colony, were still capable of responding to the stressful conditions with potential for partial colony survival, though prolonged exposure may have further increased negative impacts. Moreover, alterations in DNA binding and protein modification are known to occur during apoptosis (Shearer, Snell, & Hay, 2014), and prior genomic-scale approaches investigating heat stress (Edge, Morgan, Gleason, & Snell, 2005; Morgan, Edge, & Snell, 2005) and bleaching (DeSalvo et al., 2008; Voolstra et al., 2009) found similar gene expression patterns in shallow-water corals. Enrichment among this class of transcripts elucidates the involvement of common cellular defenses in response to the oil and dispersant exposure and possibly the timing and degree of inflicted impact beyond the observable "stressed" phenotype.

4.5 | Evidence for oxidative stress

Cnidarians have highly conserved immune and defensive pathways that may serve as potential biomarkers for stress (Elran et al., 2014; Goldstone, 2008; Goldstone et al., 2006; Reitzel et al., 2008; Shinzato et al., 2012; Venn et al., 2009). In particular, the "chemical defensome" consists of a network of evolutionarily conserved genes and proteins involved in various processes including metabolism, biotransformation (i.e., chemical modification) and/or removal of xenobiotic toxins (i.e., polycyclic aromatic hydrocarbons (PAHs) and components of oil) as well as protein homeostasis (Reitzel et al., 2008; Tarrant, Reitzel, Kwok, & Jenny, 2014). Antioxidant systems, a component of the "chemical defensome," are actively regulated to counterbalance potentially deleterious free radicals that can lead to oxidative stress (Lushchak, 2011). Significant enrichment in biological processes associated with hydrogen peroxide catabolism/metabolism, responses to reactive oxygen compounds and oxidoreductase activity—a process essential to intracellular biotransformation—indicates that the exposure to floc induced oxidative stress.

Prior studies on invertebrates have found oil exposure induces oxidative damage, the severity of which was composition dependent, with water-accommodated fractions (WAFs) inducing greater oxidative damage than crude oil (Solé, Buet, & Ortiz, 2007). Our results suggest impacted *P. biscaya* colonies underwent severe oxidative damage and were unable to appropriately regulate harmful free radicals (i.e., reactive oxygen species (ROS)). Further, oxidative stress, and ROS in particular, was shown to induce mitochondrial damage and potentially alter the metabolic rates of stressed animals (Finkel & Holbrook, 2000). This posits oxidative stress contributed to the mortality and/or partial colony survival observed among the remaining *P. biscaya* populations at MC294 during sampling and subsequent monitoring.

4.6 | Reduced ability to process toxins

Receptors of the chemical "defensome" in cnidarians also react to toxic substances (Goldstone et al., 2006). These receptors, most notably CYPs, are responsible for the biotransformation, detoxification and metabolism of most xenobiotics and are required for the efficient elimination of foreign chemicals from the body (Goldstone et al., 2006). Though elevated expression of CYPs appears to be a reliable biomarker for pollution (Devaux, Flammarion, Bernardon, Garric, & Monod, 1998; Porte, Biosca, Solé, & Albaigés, 2001) and oil exposure (Garcia et al., 2012; Han et al., 2014; Zhang et al., 2012) in aquatic animals, CYP was under-expressed among spill-impacted corals. Stifled expression of CYP1A1 in this study, as well as MDR1 which protects against cellular toxicity by excreting toxic compounds metabolized by CYPs (Bard, 2000; Borst & Elferink, 2002), could be indicative of high toxicity. Suppressed expression was previously linked to drug toxicity in vertebrates (Dean, Hamon, & Chimini, 2001; Piquette-Miller, Pak, & Kim, 1998) and in marine invertebrates exposed to high concentrations of anthropogenic biocides (Kingtong, Chitramvong, & Janvilisri, 2007). This implies that floc exposure was toxic at the cellular level and the portions of the impacted corals sampled were nearing irreparable damage.

5 | CONCLUSIONS

We identified genome-wide signatures of stress responses in the octocoral *P. biscaya* following exposure to *Deepwater Horizon*-associated oil and dispersant. The responses to this exposure include an altered expression of genes related to oxidative stress, immune response and wound repair mechanisms that may be linked to a surge in tissue regeneration and biomineralization, as well as a reduction in the corals' ability to process toxins.

Our findings suggest elevated stress proteins may have conferred a certain degree of resistance to cells and tissues that were not in primary contact with the floc and directly exposed to the crude oil/dispersant constituents. This, in conjunction with hypermelanization of damaged regions of the colony that established a barrier around compromised tissues, likely enabled the partial colony survival observed among in situ impacted *P. biscaya* colonies at MC294 (Girard & Fisher, 2018; Hsing et al., 2013). Our findings elucidate the cellular responses underlying the more obvious physical damages imposed by the DWH disaster. Evidence for significant differential expression of genes associated with immune (i.e., complement C3), wound repair (i.e., tyrosinase and peroxidasin) and toxin processing (i.e., CYP1A1 and MDR1) supports their use as potential biomarkers for future oil and dispersant exposure monitoring when impacts may not be immediately apparent. Similar to other environmental stress studies on invertebrates, the overexpression of TNF family members, specifically TRAFs, also make them likely candidates for use in future monitoring and conservation efforts as drilling increases in deep waters.

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DATA ACCESSIBILITY

Data used for these analyses are publicly available through NCBI's Sequence Read Archive database (BioProject ID: PRJNA481028) and through the Gulf of Mexico Research Initiative Information & Data

Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (R4.x268.000:0111); https://doi.org/10.7266/n75h7dwd. The assembly and associated metadata are available on the Dryad Digital Repository: https://doi.org/10.5061/dryad.9r3v1c3via.

AUTHOR CONTRIBUTIONS

D.M.D. wrote the manuscript with contributions from E.E.C., A.M.Q. and S.H. A.M.Q. and E.E.C. collected corals and consulted on bioinformatics with D.M.D., S.H., S.D.L. and R.J.K. D.M.D. performed laboratory work and contributed to bioinformatic design and analyses. S.H. designed and conducted bioinformatic analyses. A.M.Q., S.H. and R.J.K. edited the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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