Transcriptomic effects of dispersed oil in a non-model decapod crustacean

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Background. Oil spills are major environmental disasters. Dispersants help control spills, as they emulsify oil into droplets to speed bioremediation. Although dispersant toxicity is controversial, the genetic consequences and damages of dispersed oil exposure are poorly understood. We used RNA-seq to measure gene expression of flatback mudcrabs (*Eurypanopeus depressus,* Decapoda, Brachyura, Panopeidae) exposed to dispersed oil. **Methods.** Our experimental design included two control types, oil-only, and oil-dispersant treatments with three replicates each. We prepared 100 base pair-ended libraries from total RNA and sequenced them in one Illumina HiSeq2000 lane. We assembled a reference transcriptome with all replicates per treatment, assessed quality with novel metrics, identified transcripts, and quantified gene expression with open source software. **Results.** Our mudcrab transcriptome included 500,008 transcripts from 347,082,962 pair-end raw reads. In oil-only treatments, we found few significant differences. However, in oil-dispersant treatments, over 4000 genes involved with cellular differentiation, primordial cellular component upkeep, apoptosis, and immune response were downregulated. A few muscle structure and development genes were upregulated. **Discussion.** Our results provide evidence that exposure to chemically dispersed oil causes a generalized cellular shutdown and muscular repair attempts. Our results suggest current oil-spill treatment procedures could be detrimental to crustaceans and indicate additional research is needed to evaluate the impact of oil spills in gene expression. Finally, traditional quality metrics such as N50s have limitations to explain the nature of RNA-seq compared to new methods in non-model decapod crustaceans.

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ABSTRACT.

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Methods. Our experimental design included two control types, oil-only, and oil-dispersant treatments with three replicates each. We prepared 100 base pair-ended libraries from total RNA and sequenced them in one Illumina HiSeq2000 lane. We assembled a reference transcriptome with all replicates per treatment, assessed quality with novel metrics, identified transcripts, and quantified gene expression with open source software.

Results. Our mudcrab transcriptome included 500,008 transcripts from 347,082,962 pair-end raw reads. In oil-only treatments, we found few significant differences. However, in oil-dispersant treatments, over 4000 genes involved with cellular differentiation, primordial cellular component upkeep, apoptosis, and immune response were downregulated. A few muscle structure and development genes were upregulated.

Discussion. Our results provide evidence that exposure to chemically dispersed oil causes a generalized cellular shutdown and muscular repair attempts. Our results suggest current oil-spill treatment procedures could be detrimental to crustaceans and indicate additional research is needed to evaluate the impact of oil spills in gene expression. Finally,

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INTRODUCTION

World energy demands make offshore oil drilling a recurrent environmental threat. Marine oil-spill cleanup includes mechanical removal with booms and skimmers, and non-mechanical responses such as on-site burning and chemical dispersion that emulsifies oil into droplets to speed bioremediation. Oil has documented detrimental effects on marine life (Peterson et al. 2003; Trustees 2016), including increased mortality and health decline (Barron 2012; Brown-Peterson et al. 2015; Whitehead et al. 2012; Yednock et al. 2015). However, the effects of employing dispersants on many biological mechanisms have only begun to be understood (Paul et al. 2013), particularly when used in massive quantities. For example, during the Deepwater Horizon Oil Spill (DWH) in April 2010, eight million liters of COREXIT® dispersants were applied to control 507 million liters of oil leaked into the Gulf of Mexico (Kujawinski et al. 2011). It took 87 days to contain DWH, affecting a large portion of the United States gulf coast causing major ecological and economic loss, in what is considered the worst environmental disaster in U.S.' history (Paul et al. 2013).

Understanding the effect of dispersed oil on organisms is critical as the most toxic compounds of crude oil, polycyclic aromatic hydrocarbons (PAHs), are released in large amounts after dispersion (NRC 2005). PAHs and other petroleum hydrocarbons accumulated in high concentrations following the DWH: 500 parts per million (ppm) in water and 200,000 ppm in sediments (Sammarco et al. 2013). While early toxicology tests have suggested that dispersant formulations used in DWH are less lethal than oil itself (Clark et al. 2001), few recent studies have revealed that dispersants have significant toxic effects on marine life (DeLeo et al. 2015; Paul et al. 2013; Wise & Wise

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2011) and can be more than 50 times more potent when mixed with oil than either oil or dispersant alone (Rico-Martínez et al. 2013). For example, corals show more severe health declines in both dispersant-only and oil-dispersant mixes, compared to oil-only treatments (DeLeo et al. 2015). Dispersant presence impaired survivorship, and muscle configuration and contractions showed sublethal toxicity in jellyfish (Echols et al. 2016). Likewise whale fibroblast show more chromosomal aberrations (Wise et al. 2014). Additionally, oil-dispersant toxicity can persist for months in the water column and sediments (Sammarco et al. 2013), perpetuating detrimental effects.

Transcriptomics allows exploring and assessing oil spill effects for genes expressed at the time of exposure. For example, if genomic references exist, microarrays and targeted quantitative polymerase chain reaction (qPCR) allow for screening hundreds to thousands of genes known to be affected by oil exposure (Bowen et al. 2016; Hansen et al. 2016b; Olsvik et al. 2011; Olsvik et al. 2012). While genomic references are lacking for most marine organisms, next generation sequencing (NGS) technologies allow for using transcriptomic approaches in any study system. RNA-seq, the direct massive-paralleled sequencing of all expressed genes at a given time (Wang et al. 2009), can thus be applied to understanding oil spill effects in non-model organisms. In recent years studies assessing immune response and other key biological processes at the gene level have employed RNA-seq to examine the effects of mechanically dispersed crude oil (Whitehead et al. 2012; Yednock et al. 2015; Zhu et al. 2016) but unfortunately there is limited information on the consequences of chemically dispersed oil exposure (Jenny et al. 2016; Liu et al. 2016; Bayha et al. 2017). Here, we examined the impact of oil and dispersant exposures on a non-model decapod crustacean to test whether chemically

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dispersed oil increases detrimental effects on gene expression beyond oil-only exposure. Due to the increased availability of harmful compounds to animal tissues after oil dispersion, we predict key biological processes will be severely impacted. Our objective was to find changes in gene expression in a controlled setting reducing variation sources, instead of aiming to replicate field conditions that are too complex to simulate *in vitro* and to avoid multiple sources of environmental variation.

We used RNA-seq to quantify gene expression in oyster bed-associated flatback mudcrabs (*Eurypanopeus depressus*) exposed to oil-only and oil-dispersant treatments (Fig. 1). We employed acute non-renewal exposures (Weber 1991) to generate changes in gene expression, albeit our objective was not to perform a comprehensive ecotoxicological study but rather a baseline transcriptomic comparison. Mudcrabs are an ideal system to test oil spill effects because they are associated to oysters and benthic sediments, for which larval stages and juveniles have shown detrimental effects from oil (Cucci & Epifanio 1979) and dispersal exposures (Anderson et al. 2014; Vignier et al. 2016). We collected crabs from sites outside of the DWH area (Fig. 1A) to avoid prior oil-spill exposure as a confounding factor. After a period of *in vitro* acclimatization, we exposed crabs to the following conditions, each with 3 replicates (Fig. 1C): non-aerated negative control (NC), non-aerated oil-only treatment (OO), and non-aerated oil-dispersant treatment (OD). Additionally, we included an aerated control (AC) to identify effects of oxygen stress, continuous oxygen supply, and experimental manipulation potentially present when using closed, static non- renewal exposures. As the impact of aeration appeared to have little effect on gene expression associated with oil or

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dispersant, further comparisons AC are not considered here. (See Supplementary Information *SI*.)

MATERIALS AND METHODS

Animals

We selected the flatback mudcrab *Eurypanopeus depressus* (hereto referred as crabs; Crustacea: Brachyura, Panopeidae) as the model species because of its association with the economically important Eastern oyster (*Crassostrea virginica*). Bivalves bioaccumulate environmental toxins through filter feeding (O'Connor 1998) and show great susceptibility to oil spills (Vignier et al. 2016). *E. depressus* and oysters are co-distributed throughout the North and South Atlantic coasts, and in the USA from Maine to Florida into the Gulf of Mexico as far as Texas (Williams 1984). Specimens were collected from Rollover Bay near Gilchrist, Texas (29.521667°N, 94.502100°W) on 9 July 2013. In the field, crabs were removed from *C. virginica* interstices. In the lab, collected specimens were re-determined and checked for good physical condition (i.e., no missing limbs, lesions, parasites). Specimens passing said criteria were placed in a 10-gallon tank with artificial seawater and acclimated *ex situ* for 3 days prior to treatments.

Experimental design, conditions, and treatments.

Treatments were mechanically enhanced water-accommodated fraction (WAF) and chemically enhanced water-accommodated fraction (CEWAF) following (Singer et al. 2000) using oil from the Marlin platform Dorado, an oil surrogate with no discernible differences in chemical composition to the MC252 oil from the DWH Macondo Prospect

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(Worton et al. 2015). The WAF was generated using a loading rate of 1g/l and mixed in a sealed container with minimal headspace and no visible vortex for 24 hrs before being allowed to settle for 30 minutes. We tried multiple loading rates of dispersant (1:10, 1:20, 1:30, 1:40, 1:100) to determine which concentration could produce a CEWAF with visible effects on crabs. Crabs exposed to concentrations lower than 1:50 did not show motility and behavioral differences compared to controls after gentle prodding with blunt forceps. Thus, the selected CEWAF had a final loading rate of 0.1g/l and a COREXIT® 9500 concentration of 1:50 to capture the responses of crabs to acute exposures. The CEWAF was mixed in a sealed container with minimal headspace and a vortex extending approximately 25% of the total depth of the container for 18 hours before being allowed to settle for 6 hours. After being allowed to settle, 3.5l of WAF and CEWAF were transferred to separate 3.75l vessels with care taken to not disturb the oil layer. We utilized a closed, static design with minimal headspace to limit loss of volatile compounds; vessels were sealed with foil-lined caps (OECD. 2002). Even though industry standard concentrations are about 1:20, dispersant concentration in our CEWAF (1000 parts per million - ppm) that generated an acute response falls within the lower bound of bioaccumulated total petroleum hydrocarbon (TPHs) concentrations found in Deepwater Horizon oil spill water and sediments (Sammarco et al. 2013). We did not verify exposure concentration analytically. Due to the lack of analytical data, our putative experimental concentration are likely lower than reported nominal loading rates due to multiple factors during exposures.

In addition to the WAF, CEWAF, and a sealed, non-aerated control, we included an aerated control to allow us to understand the potential impact of oxygen deprivation in

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the closed design to disentangle hypoxia genes from chemical-response genes. By having these two controls we were also able to assess potential gene expression effects due to experimental manipulation. These conditions are henceforth: Aerated control "AC", non-aerated negative control "NC", Oil-Only Treatment "OO" (non-aerated WAF), and Oil-Dispersant Treatment "OD" (non-aerated CEWAF). Each treatment and controls were replicated in separate exposure vessels per condition, 4 vessel replicates, and 3 crabs per vessel maintained at 22±2°C for 72-h static, non-renewal exposures (Weber 1991) for a total of 48 crabs. Crabs were not fed for the duration of the exposures. We were not able to include a Dispersant-Only Treatment due to limitations on read sequencing per lane (see Library Preparation) and budget constrains. Therefore the effects of dispersants independent from the effects of dispersed oil were not surveyed (see Discussion).

Tissue harvesting

After exposures crabs flash frozen in liquid N. Specimens were held in liquid N before dissection in RNA*later*-ICE (Life Technologies). Tissues from muscle, gills, and hepatopancreas were then frozen in RNA*later*-ICE at -80°C until total RNA extraction.

RNA extraction, quality control, and RNA-seq Libraries

We isolated total RNA from 48 individuals. Muscle was chosen over alternative tissue-types that were found to be high in RNAses (i.e. hepatopancreas) or that yielded insufficient amount of high quality RNA (i.e. gill). Tissues were lysed with a hand homogenizer PRO200 (BioGen, Pro Scientific). We used the column-based Nucleospin® RNA kit (Macherey-Nagel), and treated with DNAse (Clonetech) to extract RNA

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following manufactures' instructions. Total RNA was eluted in 50 ul of RNAse-free water (Sigma) and stored at -80 ºC. RNA integrity and concentration was determined in a Bioanalyzer 2100 (Agilent). Muscle RNA of 12 individuals with the highest concentration and quality were chosen for sequencing. These individuals comprise 12 samples in our experimental design: three repetitions of two controls (AC, NC) and two experimental conditions (OO, and OD) [Table S1].

Our 12 selected samples showed some RNA degradation. Thus, to ensure a high quality *de novo* transcriptome assembly we collected additional crabs from Florida (Fort Pierce; 27.436667ºN, 80.335556ºW) on 23 September 2014. These crabs were kept in 5-gallon tanks prior to RNA extraction. We extracted total RNA from muscle from three Florida crabs with the Trizol® Reagent (Life Technologies) method. Approximately 10-12 2mm ceramic beads were used in a MiniBead Beater (BioSpec) for homogenization in Trizol®, followed by chloroform-isopropanol/ethanol precipitation including the DNAse and elution steps mentioned previously following manufacturer's guidelines.

Library preparation

Samples containing 1ug of total RNA from the four experimental conditions were sent to the Vanderbilt University Core Lab in 2013 for sample preparation using the Ribo-Zero™ rRNA Removal Kit and cDNA library generation (Epicentre) using a TruSeq Stranded Library kit (Illumina). Library concentrations were verified with a Qubit® Fluorometer (Invitrogen) and library sizes were verified on a Bioanalyzer. The resulting 12 libraries were then multiplexed and sent to Beckman Coulter Genomics for RNA sequencing of paired-end 100 bps reads on one Illumina HiSeq 2000 lane aiming for 20

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202 million reads per experiment as recommended for *de novo* assemblies (Francis et al.
203 2013). We did not multiplex additional individuals (i.e. a Dispersant-Only treatment) in a
204 single sequencing lane as it would have reduced the number of reads per needed per
205 individual.

206 In order to increase sequencing depth for the *de novo* reference additional
207 libraries were generated from the three Florida crabs at the University of Georgia
208 Genomic Facility (GGF) in 2014 using 1 ug of total RNA a stranded Kapa RNA Kit
209 (Kapa technologies) and TruSeq adapters (Illumina). Quality control of these libraries
210 was assessed as previously mentioned. Libraries were sent from GGF to the University
211 of Texas Genomics Core Facility for sequencing on one Illumina HiSeq2000 lane. We
212 did not add any RNA spike-in control as they have been found to be not reliable enough
213 for normalization in Poly-A mRNA selection used by TruSeq and Kapa kits, and rather
214 normalized by biological replicates (see below; Munro et al. 2014; Risso et al. 2014).
215

216 **Bioinformatics, raw read processing and quality control**

217 Open source software packages and pipelines were employed for this project
218 bioinformatics' needs. All paired-end reads from 12 experimental and 3 additional
219 libraries, 15 total, were passed through quality control (QC) first by checking for
220 presence of adapters, indexes, repetitive kmers, and low-quality sequences in FASTQC
221 (Andrews 2010) to determine trimming parameters. Adapters, indexes, and low-quality
222 sequences were removed in TRIMMOMATIC 0.33 (Bolger et al. 2014) with the
223 following parameters: 30GB of total RAM memory, -six threads, a phred scores of 33,
224 cropping the first 9 bases, LEADING:30, TRAILING:30, SLIDINGWINDOW:4:15, and

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minimum kmer length of 36. FASTQC was run again on processed read files to verify that trimming was successful.

De novo transcriptome assembly and annotation

Due to the lack of an *E. depressus* reference genome we conducted a *de novo* transcriptome assembly in TRINITY 2.0.3 (Haas et al. 2013) using paired-end reads that passed QC from the 15 total libraries. All libraries were included to generate a single transcriptome, as it is necessary for a reference to include all individuals to detect differentially expressed genes across samples (Conesa et al. 2016; Haas et al. 2013). TRINITY was run on the FIU Panther Cluster in the High Performance Computer (HPC) environment with 24 cores and 256 GB of RAM with the following parameters: minimum kmer coverage of 4, maximum memory 252 GB, reverse single-stranded libraries, 24 CPUs, Butterfly maximum heap space 10GB, Butterfly initial heap space 10GB, Butterfly CPUs 24, and Inchworm CPUs 24.

We evaluated our assembly using traditional metrics including number of transcripts and N50 values, and supplemented them with the novel ExN50 (B. Haas op cit; https://github.com/trinityrnaseq/trinityrnaseq/wiki/Transcriptome-Contig-Nx-and-ExN50-stats) with TRINITY perl scripts. Recently, N50 values to assess quality have been deemed unreliable as they are often biased due to: (1) filtering artifacts and combinatorial isoform assembly that skew values upwards, or conversely (2) skewed downwards if assemblies are efficient at finding low-abundance rare splice variants particularly with deep sequencing. Thus, pondering the expression levels of each contig with the ExN50 is more appropriate (http://www.molecularecologist.com/2017/05/n50-

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for-transcriptome-assemblies/). We further checked for completeness according to conserved ortholog content in BUSCO 3.0.0 (Benchmarking Universal Single-Copy Orthologs; Simão et al. 2015) using annotated Eukaryota, Metazoa, Arthropoda, and Insecta databases (since there are no decapod crustacean databases) from ORTHODB v9 (Waterhouse et al. 2013; http://busco.ezlab.org/datasets/). These databases include an increasing number of orthologs as the taxonomic level gets more specific, i.e. there are fewer orthologs shared by all eukaryots compared to the number of orthologs shared between insects.

Additionally, we compared our *de novo* assembly with additional decapod crustacean and model arthropod transcriptomes to test how much deviated our assembly is from other taxa. For this purpose we downloaded and assembled nine single-sample Genbank SRA raw reads: four additional true crabs (*Eriocheir sinensis*-SRR1735536; *Portunus trituberculatus*-SRR768319; *Scylla olivacea*-SRR2440122; *Callinectes sapidus*-SRR2140752), five shrimp (*Macrobrachium nipponense*-SRR3196792; *Caridina rubella*-SRR1248238; *Neocaridina denticulate*-SRR1185328; *Penaeus monodon*-SRR1648423; *Fenneropenaeus mergiensis*-SRR1756093), one butterfly (*Bicyclus anynana*-ERR1022646) and two model arthropods (*Daphnia pulex*-SRR2350794; *Drosophila melanogaster*-SRR2930822). We repeated the exact same TRINITY parameters previously mentioned after read quality control on the same HPC cluster to reduce sources of variation. Lastly we also calculated traditional N50s, contig ('gene') number, contig length with TRINITY perl scripts, and created density plots of arthropod assembly contig variation in R 3.1 (R Core Development Team 2010).

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The resulting transcriptome was annotated with TRINOTATE 2.0.2 (http://trinotate.sourceforge.net) and TRINITY plug-in perl scripts on the same HPC node. The plug-in TRANSDECODER was used to obtain likely protein-coding regions and extract the longest open reading frames. Functional annotation was achieved by comparing transcriptome contigs to peptide and transcript databases with BLAST (Altschul et al. 1990), protein families with PFAM (Bateman et al. 2004) and HMMER(Finn et al. 2011), signal peptides with SIGNALP (Petersen et al. 2011), transmembrane proteins in TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), and remnant RNA with RNAMMER (Lagesen et al. 2007). Custom databases were downloaded for TRINITY from the Broad Institute's website (https://data.broadinstitute.org/Trinity/Trinotate_v3_RESOURCES/) for SwissProt, UniProt90, and PFAM. Annotated contigs were compiled and summarized in a SQLITE3 database by translating the transcriptome genes to a transcript map using TRINITY's TRANSMAP utility and loading those results into he SQLITE database. All annotations blasted to a record with a 1×10^{-5} identification E-value for gene ontology (GO) determination. Table 1 includes a brief summary of environmental and experimental conditions used to assemble the flatback mudcrab transcriptome compliant with the MIGS standard.

Differential gene expression statistical analyses

Reads passing QC for each replicate per condition (AC, NC, OO, OD) were included in the differential gene expression estimates. Libraries from Florida crabs were not included to avoid geographic, genetic, and sequencing artifacts (Gleason & Burton 2015). Pair-end reads were merged prior to alignment to the resulting *de novo*

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transcriptome. Pair-end reads with no overlapping regions were concatenated with a custom bash shell script to merged reads. Since single stranded libraries only contain the reverse read, all reads were reverse-complemented using SEQTK (https://github.com/lh3/seqtk). We did not incorporate RNA spike-ins as controls (see above), therefore our reported values are relative to normalization between biological replicates.

In order to separate effects of low oxygen and experimental manipulations from oil and oil-dispersant effects we first compared both controls to calculate abundance counts and differential expression analyses, and secondly we excluded the aerated control (AC) and re-estimated counts for NC, OO, and OD. Abundance and differential gene expression was calculated as follows. Transcript abundance was estimated for each experimental library using RSEM (Li & Dewey 2011) by preparing the transcriptome reference with a TRINITY utility perl scripts aligning experimental reads per treatment for transcripts and genes with BOWTIE (Langmead 2010), 7 cores, and 30 GB of memory. Raw count data from RSEM were normalized by determining fragments per kilobase of transcript per million mapped reads (FPKM) with similar TRINITY perl scripts.

Differential gene expression (DE) on normalized counts from the experimental reads was estimated on the BIOCONDUCTOR platform (Gentleman et al. 2004) in R 3.1 (R Core Development Team 2010) with packages edgeR (Robinson et al. 2010) and DESeq2 (Love et al. 2014). The transcript and gene normalized count matrices were used to calculate pairwise differential expression between each treatment from the mean and standard deviation of each biological replicate to generate Bland–Altman plots (Bland &

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317 Altman 1986) measuring logarithmic fold change in gene expression (M) over mean
318 average expression on log counts (A), known in microarray and RNAseq studies as MA
319 plots (Dudoit et al. 2002). Statistical significance was assigned on a $\log_2$ scale values
320 passing a False Discovery Rate (FDR) of 1%. Additionally, volcano plots were generated
321 to visualize gene expression fold change by their statistical significance (Cui & Churchill
322 2003). Parameters in edgeR were left as defaults. DESeq2 calculations were run with
323 comparisons per condition, a maximum sharing mode, and a local regression fit type.

324 Gene regulatory direction was determined in edgeR and DESeq2 for genes and
325 isoforms by comparing each experimental treatment replicate log-transform and zero-
326 centered normalized FPKM counts. Those counts were used to estimate Euclidean
327 distances between treatments and between genes to generate expression clusters by
328 similarity visualized as heatmaps with TRINITY perl and custom R scripts. Finally,
329 over and underrepresented GO terms were calculated with a statistical enrichment
330 analysis in GOseq (Young et al. 2010). We used DE pairwise comparisons of gene count
331 matrices between NC, OO, and OD estimated in DESeq2 and edgeR. We controlled for
332 gene size with weighted average gene lengths across all experimental conditions, and GO
333 annotations from the assembled transcriptome with TRINITY and TRINOTATE perl and
334 python scripts. We considered statistical significance when $P < 0.05$ after a 1% FDR
335 correction for multiple comparisons. Having two statistical methods, three possible
336 pairwise comparisons, and two possible patterns (overrepresented or enriched, and
337 underrepresented or depleted) per comparison pair, would produce 24 GO term
338 enrichments if every comparison has DE terms.

339

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RESULTS

We obtained 347,082,962 pair-end reads from our libraries with an average of 23 million reads per replicate. TRINITY (Haas et al. 2013) produces sequence contigs categorized as two *features:* '*genes*' represent unique contigs, and '*isoforms*' correspond to alternatively spliced transcripts in a given contig. Our TRINITY assembly yielded a transcriptome of 500,008 genes (i.e. unique contigs), 660,546 isoforms (i.e. alternatively spliced transcripts in a given contig), with an N50 of 421 base pairs (bps) and a mean length per contig of 428.03 bps. The total number of assembled bases was 282,733,223 (table S1). In contrast, between 60-80% ExN50 values were more than double the traditional N50 (Fig. 2A). The level of conserved single-copy orthologs ranged between 70.2-88.3% complete, 13.9-2.3% missing, and 15.9-9.2% fragmented BUSCOs for exclusive and inclusive taxonomic hierarchies, respectively (Fig. 2B).

Our comparative contig density plots revealed an unprecedented level of variability across single-sample Arthropoda assemblies and our *de novo* transcriptome (Fig. 3). TRINITY found fewest contigs in the model arthropods (*D. pulex* and *D. melanogaster,* Fig. 3C). In non-model arthropods (crabs, shrimp, and butterfly) the number of contigs ranged between 11,534-243,857. Shrimp ranged between 11,534-158,098 contigs (Fig. 3B), and crabs had the largest between 86,287-243,857 contigs (Fig. 3A). For every species the contig size distribution peaked between 400-600 base pairs (bps). However several N50 values were above the 1000 bps traditional benchmark inversely correlated with peaks' height, i.e. the higher the density peak, the lower the N50 (Fig. 3). Two crabs, three shrimp, and *D. pulex* had N50s <1000. For model arthropods and the butterfly, and in particular for *D. melanogaster,* a large portion of the density

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363 distribution exceeded 1000 bps. The contig size density for two shrimp (*F. mergiensis*
364 *and N. denticulata*) and two crabs (*C. sapidus* and *S. olivacea*) partially exceeded 1000
365 bps. Our *de novo* transcriptome had a similar contig size distribution to two crabs
366 (*Eriocheir sinensis* and *Portunus trituberculatus*) and three shrimp (*Macrobrachium*
367 *nipponense, Caridina rubella*, and *Penaeus monodon*). The number of contigs in our
368 transcriptome produced with 15 samples was approximately double compared that of
369 *Eriocheir sinensis* with a single sample, and its N50 30.5% smaller.

370 We compared NC and experimental treatments (OO, OD) to determined
371 differences in gene expression between crabs exposed to oil-only and oil-dispersant
372 mixes (*SI* Fig. S1, comparisons between controls [AC and NC, *SI* Fig. S2, *SI* Table S2]
373 can be found in Supplementary Information).

374 **Differential gene expression happened between oil-only and oil-dispersant.**

375 We measured differential gene expression between NC, OO, and OD using edgeR
376 (Robinson et al. 2010) and DESeq2 (Love et al. 2014). The main difference between
377 statistical methods was the amount of significantly expressed features, with DESeq2
378 detecting larger amounts with relatively lower significance magnitude. We report feature
379 numbers from DESeq2 herein (edgeR comparisons are found in the *SI*). Comparisons of
380 controls and treatments detected hundreds of genes and thousands of isoforms that were
381 differently expressed. Approximately 50% of these differentially expressed features in
382 our *de novo* transcriptome (*SI* DS1) could not be annotated and were left as unknown by
383 either low sequence similarity or by being putative proteins with missing gene ontology
384 (GO). Five differentially expressed features were shared between the controls and

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385 experimental treatments but did not blast to any GO (*SI* Fig. S1). Nevertheless, 99% of
386 differentially expressed genes and isoforms were detected in OO and OD.

387 Comparisons between NC and OO-OD identified 10s to 1000s of features with
388 significant differential expression (*SI* Figs. S3-S4, *SI* Table S3). Feature counts were as
389 follows: NC vs. OD identified 4388 genes and 3243 isoforms (Figs. 4, *SI* S3-S4, *SI* Table
390 S3); NC vs. OO identified 11 genes and 8 isoforms (Fig. 4, *SI* Table S3); OO vs. OD
391 identified 448 genes and 215 isoforms (Fig. 4, *SI* Figs. S3-S4, *SI* Table S3).

392 When comparing gene expression across replicates within treatments, replicates
393 within NC and OD had nearly identical heatmap profiles (Figs. 5, S4); however,
394 replicates within OO shared heatmap profile characteristics with both NC and OD (Figs.
395 5, *SI* S4). In 75% of heatmaps, 66% of OO replicates clustered more closely with OD (*SI*
396 Fig. S4) indicating that OO and OD replicates have similar gene expression profiles and
397 are statistically distinguishable from NC replicates.

398 Two clear feature clusters are present in all heatmaps: one cluster containing over
399 90% of differentially expressed features, upregulated in NC and downregulated in OD,
400 and a second cluster of less than 10% with the opposite pattern of regulation
401 directionality (clusters 1 and 2 respectively on Figs. 5, *SI* Fig. S4).

402 **Gene ontologies largely matched muscle components and immune response genes**

403 Gene ontology annotations allow for the identification of features and their functions.
404 Pairwise expression measured in log2 fold changes (logFC, *SI* DS14) between
405 experimental conditions ranged -16 to 12. Most differentially expressed features (logFC
406 > |8|) did not blast to a known protein, to an undescribed protein, or matched muscle

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components. Immune response features logFC ranged -4 to -2. Although gene expression studies typically show gene rankings per fold change limiting detailed description of the top 10-30 genes (See full rankings in *SI* DS14), we report herein transcriptome-wide GOs with differential gene expression instead of a handful of gen-per-gene comparisons. 70% of shared features detected as differentially expressed in NC and OD (*SI* Fig. S1) did not BLAST to a GO and were left as unknown (*SI* Figs. S5-8). Nevertheless, 19 features upregulated in OD (and thus downregulated in NC) were annotated with GO terms including: cell division, proliferation, and regulation, as well as muscle structure, attachment, development, and other functional components (*SI* Table S4, *SI* Figs. S5-S8). More specifically, upregulation of structural muscular elements included sarcomeres, I-bands, Z-discs, and muscle-associated actin binding responsible for muscle formation and contraction. Mechanisms involved in cell division, including chromatid cohesion and chromosome condensation, were upregulated. Lastly, we found one upregulated invertebrate immune response mechanism (hemocyte proliferation) involved in the protection against microbial infection (Schulz & Fossett 2005).

The largest GO annotation set included 151 features that were downregulated in OD. These features were associated with A) fundamental cellular components including the nucleus, membranes, cytoskeleton, organelles, and cytoplasm (*SI* Table S4, *SI* Figs. S5-S8) B) molecular functions such as energy transfer by ATP for ubiquitines, metal ion binding, mRNA processing, nucleotide binding, protein phosphorylation, stabilization, proteolysis, DNA synthesis, RNA processing and binding, transcription, translation and C) cellular processes such as apoptosis, apoptosis regulation, cell adhesion, cell migration and protein development, cellular response to hypoxia, glucose and glycogen

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metabolism, intracellular signal transduction, splicing speckles, transmembrane transport, and the mitotic cell cycle. We found several downregulated actin elements involved in microfilament formation for cytoskeleton structure and cell movement, locomotion, including filament binding and assembly. Downregulated components of the nervous system included dendrite morphogenesis, neuron projection, neuron apoptotic process, and neuronal cell bodies. Three metabolic/signaling pathways downregulated in OD included: A) general cell development and immune response pathway as indicated by cytokine signaling; B) cell growth, proliferation, survival, and apoptosis pathways including activation of mitogen-activated protein kinases (MAPK), SAPK/ Jun amino-terminal kinases (JNK), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), and transforming growth factor-β receptor (TGF-β); and C) protective response of the immune system pathways including Fc receptors (FcR), the IκB kinase (IKK) and NF-κB receptors, T cell receptors, and Toll-like receptors (TLRs). Lastly, in OD we found 3 and 19 downregulated isoforms of Tumor Necrosis receptors (TNF) and Cytochrome P450 (CYP), respectively (table S5). Importantly, TNFs work as natural defenses against tumors by killing abnormal cell growth (Fransen et al. 1985) and the CYP protein family of membrane receptors are widely used as hydrocarbon exposure biomarkers (Rewitz et al. 2006). These CYP transcripts belong to three protein clans (clans 3, 4, and 18). Unknown features, not matching a functional GO, constituted 30% and 8% of downregulated genes and isoforms, respectively.

Gene ontology terms were statistically over and under represented between controls and dispersed oil.

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GO term enrichment analysis tests for categories that dominate differential gene expression. Only 11 out of 24 possible pairwise enrichment tests included DE genes (DEGs; Fig. 6): 8 from DESeq2 and 3 from edgeR. In most cases Biological Processes (BPs) were the dominant ontology, followed by Molecular Functions (MFs), and lastly Cellular Components (CCs). Those DE GO terms were consistent with our cross-reference list (see above; *SI* Table 4). DE counts between NC and OO totaled 20 GO terms, and between OO and OD there were 470 GO terms. Pairwise DE counts between NC and OD were much higher, totaling 3578 GO terms. However, significantly enriched and depleted terms were only found in NC in NC-OD DESeq2 pairwise comparisons after the FDR correction, totaling 1832 and 75 respectively (Fig. 6). Statistically significant overrepresented GO terms included fundamental metabolic, developmental, and regulation processes, signaling, cellular differentiation, organization, and communication, and morphogenesis. Underrepresented GO terms included fundamental molecular activities, integration, transposition, DNA replication and recombination, in ribosomes and mitochondria (File DS 15). We found the same overall pattern in edgeR although none of the GO terms were significantly enriched.

DISCUSSION

Assembly quality and transcriptome variability in crustaceans

Our transcriptomic assembly yielded a relatively high number of contigs and relatively low N50s. These numbers would typically suggest a poor assembly, possibly stemming from the RNA degradation we reported. However, our ExN50s indicate the N50 based on 100% of contigs is biased due to short and lowly expressed isoforms. Further, 70-88%

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complete BUSCOs suggest that our assembly contains mostly complete genes present in all animals, and comparable to studies with seemingly no degradation and various degrees of divergence to references (57-99%; Levin et al. 2016). Crustaceans have many more splice variants compared to other metazoans, elevating contig count and low gene ontology annotation (~32%) due to highly divergent or novel sequences (Havird & Santos 2016). We were able to annotate ~50% of our transcriptome, suggesting that even though some gene fragmentation was present, our transcriptome has similar characteristics to other crustacean assemblies. Moreover, we found a little over ~4000 DEGs, similar to the average seen in previous studies (Havird & Santos 2016) indicating the number of contigs did not inflate detected DEGs. Our data supports recent observations that traditional metrics such as N50s are not a good proxy for RNA-seq data quality (https://github.com/trinityrnaseq/EMBOtrinityWorkshopSept2016/wiki/De-novo-Assembly,-Quantitation,-and-Differential-Expression; http://www.molecularecologist.com/2017/04/the-first-problem-with-n50/), in particular for non-model organisms where known metrics are not "one-size-fits-all" (Havird & Santos 2016).

Moreover, we showed an unprecedented amount of transcriptomic assembly variation in arthropods, particularly in non-model decapod crustaceans. The wide range of N50 values and contig numbers from uniform assembly and quality control further exposes how traditional expectations such as N50s do not accurately describe the nature of RNA-seq in many crustaceans. In all arthropods the bulk of the contig density was smaller than 1000 bps, implying "large" N50s are a misleading metric that does not represent contig size accurately. While many of our contigs ('*genes*') were shorter, their

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distribution fits with other crab and shrimp transcriptomes suggesting decapods could be more complex that model organisms and could have more, shorter genes (Havird & Santos 2016); not necessarily poorly assembled. Even if TRINITY overestimated the number of genes by a factor of two in fruit flies and water fleas (probably due to having a single sample per assembly), the number of contigs found in decapods is consistent with genomes at least an order of magnitude larger (0.17-0.2 Gbases *versus* >1-40 Gbases; http://www.genomesize.com/). Water fleas have high number of duplicated genes (Colbourne et al. 2011), thus it is possible that gene duplication could explain larger number of contigs in other crustaceans. Future research will help disentangle number of genes, assembly artifacts, and gene duplication in many non-model organisms.

Downregulated features dominate differential gene expression

We examined the effects of oil and oil-dispersant mixes on crab gene expression. Although numerous studies have examined acute oil-spill toxicity in animal systems (e.g., (Hemmer et al. 2011; Paul et al. 2013; Rico-Martínez et al. 2013; Wise & Wise 2011), there are relatively fewer studies that use genomic methods to investigate the response to oil-dispersant exposure (Bowen et al. 2016; Han et al. 2016; Hansen et al. 2016a; Hansen et al. 2016b; Jenny et al. 2016; Liu et al. 2016; Olsvik et al. 2011; Olsvik et al. 2012; Whitehead et al. 2012; Yednock et al. 2015).

Because our primary focus was on differential gene expression in response to OD exposure and methodological constrains, we did not include a dispersant-only treatment and cannot quantify the separate effects of dispersants. Nevertheless, acute toxicity tests on mysid shrimp (*Americamysis bahia*) and the inland silverside (*Menidia beryllina*) revealed that dispersant-only treatments had lower toxicity effects when compared to OO

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and OD conditions (Hemmer et al. 2011). Additionally, toxicity studies in decapod shrimp showed sensitivity increase of an order of magnitude in OD over OO (Fisher & Foss 1993). These studies suggest that crabs exhibit increased negative effects when exposed to dispersed oil.

In individuals exposed to OD, we found evidence for the downregulation of >4000 genes involved in fundamental cellular mechanisms, suggesting a generalized shutting down of crabs' cellular processes and functions (*SI* Table S3). Some of these included genes involved in actin binding, condensation, and folding in the cytoskeleton (*SI* Table S4). Failures associated with these mechanisms cause multiple diseases in humans (Lundin et al. 2010) and flight muscle alterations in *Drosophila* (Sparrow et al. 2003). For instance, downregulation of dendrite morphogenesis and neuron projection could be associated with actin defects in the nervous system. Another relevant finding was the downregulation of receptor genes involved in apoptosis, in particular TNFs (*SI* Table S5). When apoptotic mechanisms are suppressed, abnormal cell growth leads to tumors (Lowe & Lin 2000). Past studies have shown tumor growth after oil spills (Suchanek 1993), including evidence for neoplastic tumors in clams following the 1971 Maine oil spill (Barry & Yevich 1975). Genotoxic and cytotoxic effects, mainly chromosomal aberrations known to be associated with several types of cancer (Mitelman et al. 2007), have also been witnessed in whale fibroblasts exposed to oil-dispersant (Wise et al. 2014). The carcinogenic effects of PAHs are well documented (Phillips 1999), and our findings suggest there could a link between OD exposure in crabs, tumor necrosis receptor/apoptosis downregulation, tumor growth, and chromosomal abnormalities observed in previous studies.

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In addition to downregulated features, we identified a limited number of upregulated features related to muscular structure and development in OD. This suggests that crabs may be undergoing damage-repair mechanisms, or that muscle upkeep-related genes far outweigh immune system genes due to relative abundance normalization. Interestingly, the same features were downregulated in NC (Fig. 5), indicating upregulation is not involved in general muscle maintenance under control conditions.

Our GO enrichment analyses revealed a significant proportion of fundamental biological processes, cellular components, and molecular functions are overrepresented between controls and dispersed oil, further suggesting crabs could be experiencing a generalized shutdown following chemical oil spill cleanup. Although enrichment was not significant between controls and oil, and oil and dispersed oil, our results point towards an increase in differential gene expression after dispersion. On the other hand, after controlling for droplet size OO/WAFs and OD/CEWAFs have similar gene expression effects in North Atlantic cod larvae *in vitro* (Olsvik et al. 2012). This discrepancy between OO and OD effects could be due to multiple sources of variation, including biological response between different taxa, different oil, and different analytical methods. These contrasts showcase the need for more studies as generalized oil spill conclusions based on a handful of studies are quite limited.

The role of cytochrome P-4501 (CYP1As) proteins as universal proxy for hydrocarbon contamination

One known link between hydrocarbon exposure and signaling pathways is the production of cytochrome P-4501A (CYP1As) in clan-1, a protein crucial in the aryl-hydrocarbon receptor (AHR) signaling pathway. CYP1A is commonly used to test for toxic

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567 hydrocarbon exposure (Petrulis et al. 2001), because its expression is linked with AHR-
568 binding and metabolism of contaminants such as PAHs, polychlorinated biphenyl (PCB)
569 in vertebrate systems (Petrulis et al. 2001; Whitehead et al. 2012). Fish populations
570 naturally and experimentally exposed to DWH oil showed CYP1A upregulation (Brown-
571 Peterson et al. 2015; Whitehead et al. 2012). However, major differences exist between
572 vertebrate and invertebrate AHR pathways in that vertebrates have sophisticated aromatic
573 binding capabilities (Barron 2012). While AHR pathways exist in most animals, a past
574 review found dioxins and PAHs do not bind to invertebrate CYP1A-AHRs (Hahn 2002)
575 although there are recent evidences of CYP1A upregulation in oysters (Jenny et al. 2016)
576 and in copepods (Han et al. 2016; Hansen et al. 2016a; Hansen et al. 2016b; Olsvik et al.
577 2012). We found no evidence of CYP1A upregulation in crabs, congruent with past
578 studies using similar species (Yednock et al. 2015). The lack of AHR regulation mediated
579 by CYP1A in crabs could be due to a lower sensitivity to PAHs to those particular
580 receptors or, alternatively, CYP1A protein sequences in crabs may be too divergent to be
581 annotated. Moreover, CYP1A differential gene expression could be restricted to other
582 organs' specific responses not evident in muscle tissues. However, tissue specificity
583 artifacts seem unlike due to a similar absence of CYP1As in blue crab hepatopancreas
584 and gills exposed to oil (Yednock et al. 2015). Future research including dispersed oil and
585 multiple tissue types will help assess crabs' response to those seemingly universal
586 toxicology markers. Interestingly, we did find differential gene regulation in other CYPs
587 (clans 3, 4 and 18) used by invertebrates to metabolize aromatic toxins (*SI* Table S5,
588 (Rewitz et al. 2006); however, these proteins were downregulated in OD presence.
589 Nonetheless, in light of our findings, the continued use of CYP1A upregulation as a

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590 universal proxy for PAH, PCB, or dioxin exposure could have limited utility when used
591 in decapod crustaceans being important fisheries. We advocate for screening additional
592 CYPs as a complementary proxy for PAH exposure.

593 **Crustacean immune response pathway downregulation and dispersed oil**

594 In addition to AHRs signaling pathway, we identified the suppression of several immune
595 response receptors (FcRs, IKKs, T cells, and TLRs) known to be conserved across
596 animals (Ottaviani et al. 2007). Our findings identified nine TLRs that were
597 downregulated in OD, compared to a past study that found only three in an equivalent
598 OO (Yednock et al. 2015), suggesting increased immune repression in the presence of
599 OD in crustaceans. Conversely, we found upregulation for the hemocyte proliferation
600 pathway in OD. Hemocytes are the first line of defense to infection in invertebrates
601 (Barron 2012; Hamoutene et al. 2004), suggesting crabs are combating stress related to
602 pathogens. Several studies showed that exposure to oil resulted in an increased
603 susceptibility to disease and compromised reproductive, sensory, and neurological
604 systems. Therefore, exposure to chemically dispersed oil may exacerbate effects. For
605 example, fish species exposed to the Exxon Valdez spill had a 17-fold parasite load
606 increase (Khan 1990) and tuna fish impacted by DWH had impaired heart contractions
607 (Brette et al. 2014). In invertebrates (see references in (Suchanek 1993): hydroids and
608 jellies showed teratogenic effects and neurological changes; hermatypic corals had
609 decreased ovaria and less planula per polyp, ova degeneration, and lack of gonad
610 development; kelp crabs presented chemosensory-induced bradycardia and suppressed
611 chemoreception abilities; sea urchins showed delayed embryogenesis, asynchronism,
612 non-viable larvae, and functional loss of tube foot and spine movement; multiple bivalves

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613 exhibited reduced respiration and increased energy expenditures. Specifically in *E.*
614 *depressus*, occurrence of supernumerary and morphologically abnormal megalopal stages
615 was shown to be associated with crude oil (Cucci & Epifanio 1979). Our results identify
616 several immune response pathways that could be playing a role in the adverse effects
617 witnessed in other marine organisms (Peterson et al. 2003; Wise & Wise 2011) and
618 humans involved in oil spill clean-up (D'Andrea & Reddy 2013). This is of particular
619 importance because human lung cell cultures showed similar immune response impact to
620 gene expression when exposed to chemically dispersed oil (Liu et al. 2016). Therefore,
621 we suggest these receptors, such as TLRs, could serve as potential candidates as
622 complementary biomarkers to CYPs for aquatic toxicology.

623 We did not verify analytically concentrations of hydrocarbons prior and after
624 experiments. In acute non-renewal exposure it is possible than many toxic compounds
625 and oxygen are depleted due to consumption or transformation, leading to confounding
626 reasons for gene expression differences. All changes we document in this study are
627 consistent with lethal and sublethal effects of chemically dispersed oil in previous
628 toxicological (DeLeo et al. 2015; Echols et al. 2016; Wise et al. 2014) and transcriptomic
629 studies (Jenny et al. 2016; Liu et al. 2016; Bayha et al. 2017) where detailed analytical
630 data is available. Moreover, the lack of significant changes between aerated controls and
631 non-aerated treatments suggest that low oxygen is not driving gene expression. Future
632 transcriptomic analyses coupled comprehensive analytical testing and renewal exposures
633 will shed light into more precise points of concentrations and gene expression
634 correlations beyond the baseline changes we report presently.

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635 The need for developing less to non-toxic dispersants is pressing. Current and
636 future oil exploration contingency in the U.S.A. (Bureau of Safety and Environmental
637 Enforcement 2015; Shell Offshore Inc 2010) relies on the same dispersant,
638 COREXIT®9500, that our results and number of other studies (e.g., Hemmer et al. 2011;
639 Paul et al. 2013; Rico-Martínez et al. 2013; Wise & Wise 2011; Bayha et al. 2017), have
640 shown to be highly detrimental to animal health particularly in systems exposed to
641 xenobiotic bioaccumulation. Although COREXIT®9500 is one of the relatively less toxic
642 formulations vetted by the United States Environmental Protection Agency (Hemmer et
643 al. 2011), future research needs to find safer solutions for spill cleanup and explore the
644 longer-term consequences of oil and dispersant impacts on marine community health.

645 **CONCLUSIONS**

646 We examined changes in gene expression resulting from dispersed oil exposure using a
647 non-model decapod crustacean. We identified a large set of vital cellular functions and
648 components that are suppressed in the presence of chemically dispersed oil. Our results
649 suggest that exposure to mixtures of petroleum hydrocarbons and dispersants may lead to
650 generalized cellular shutdown. While exposure to oil-only and oil-dispersant mixes
651 resulted in similar effects in gene expression, samples exposed to oil-dispersant mixes
652 were generally an order or magnitude more intense. Our results indicate crabs retain little
653 ability to maintain fundamental cellular structure and defense against pathogens that
654 could help explain well-known health declines and mortality increases following oil
655 spills. Finally, finding a concordant suite and distribution of genes affected by dispersed
656 oil reported in previous studies supplies additional evidence for not relying solely on
657 traditional metrics to evaluate RNA-seq assemblies.

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658

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670 **Competing interests.**

671 Keith A. Crandall is an associate editor in PeerJ. The authors declare no competing
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673 **Additional Information**

674 All housing and experimental procedures were authorized and conducted following the
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677 **Author Contributions**

678 -Hernán Vázquez-Miranda performed laboratory procedures, analyzed the data, wrote the
679 paper with help of coauthors, prepared figures/tables, reviewed drafts of the paper.

-Brent P. Thoma and Juliet M. Wong performed experimental treatments and laboratory procedures, reviewed drafts of the paper.

-Brent P. Thoma, Darryl L. Felder, Keith A. Crandall, and Heather D. Bracken-Grissom designed experiments, secured funding, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

Data deposition

Annotated transcriptome assembly and additional data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org/data/R4.x257.228:0013. (DOI: http://dx.doi.org/10.7266/N71C1TZC). The raw data used in this study is available at the NCBI website under BioProject ID: PRJNA376168 (https://www.ncbi.nlm.nih.gov/bioproject/376168), BioSamples SAMN06351232-SAMN06351246. Transcriptome Shotgun Assembly (TSA) has been deposited at DDBJ/EMBL/GenBank under the accession GFJG00000000 (version 1.0: GFJG01000000). TSA file prepared with Transvestigator at http://doi.org/10.5281/zenodo.10471

Supplemental Information

Supplemental information for this article can be found online at (DOI: to be included)

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, and Lipman DJ. 1990. Basic local alignment search tool. *Journal of molecular biology* 215:403-410.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

703 Anderson JA, Kuhl AJ, and Anderson AN. 2014. Toxicity of oil and dispersed oil on
704 juvenile mud crabs, Rhithropanopeus harrisii. *Bulletin of environmental*
705 *contamination and toxicology* 92:375-380.
706 Andrews S. 2010. FastQC: A quality control tool for high throughput sequence data.
707 *Reference Source Babraham Bioinformatics*.
708 Barron MG. 2012. Ecological impacts of the Deepwater Horizon oil spill: implications
709 for immunotoxicity. *Toxicologic pathology* 40:315-320.
710 Barry M, and Yevich P. 1975. The ecological, chemical and histopathological evaluation
711 of an oil spill site: Part III. Histopathological Studies. *Marine pollution bulletin*
712 6:171-173.
713 Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Griffiths-Jones S, Khanna A,
714 Marshall M, Moxon S, and Sonnhammer EL. 2004. The Pfam protein families
715 database. *Nucleic acids research* 32:D138-D141.
716 Bayha KM, Ortell N, Ryan CN, Griffitt KJ, Krasnec M, Sena J, Ramaraj T, Takeshita R,
717 Mayer GD, and Schilkey F. 2017. Crude oil impairs immune function and
718 increases susceptibility to pathogenic bacteria in southern flounder. *PloS one*
719 12:e0176559.
720 Bland JM, and Altman D. 1986. Statistical methods for assessing agreement between two
721 methods of clinical measurement. *The lancet* 327:307-310.
722 Bolger AM, Lohse M, and Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina
723 sequence data. *Bioinformatics*:btu170.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

724 Bowen L, Miles AK, Ballachey B, Waters S, and Bodkin J. 2016. Gene Transcript
725 Profiling in Sea Otters Post-Exxon Valdez Oil Spill: A Tool for Marine
726 Ecosystem Health Assessment. *Journal of Marine Science and Engineering* 4:39.
727 Brette F, Machado B, Cros C, Incardona JP, Scholz NL, and Block BA. 2014. Crude oil
728 impairs cardiac excitation-contraction coupling in fish. *science* 343:772-776.
729 Brown-Peterson NJ, Krasnec M, Takeshita R, Ryan CN, Griffitt KJ, Lay C, Mayer GD,
730 Bayha KM, Hawkins WE, and Lipton I. 2015. A multiple endpoint analysis of the
731 effects of chronic exposure to sediment contaminated with Deepwater Horizon oil
732 on juvenile Southern flounder and their associated microbiomes. *Aquatic*
733 *Toxicology* 165:197-209.
734 Bureau of Safety and Environmental Enforcement. 2015. Chukchi Sea oil spill response
735 plan. Chukchi Sea regional exploration program. 3.0 ed.
736 http://www.bsee.gov/uploadedFiles/BSEE/BSEE_Newsroom/Publications_Librar
737 y/OSRPs/Arctic/2015_05_15_Revision%203_Redacted_Shell%20Chukchi%20Se
738 a%20OSRP%20with%20Cover%20Letters.pdf (accessed 16.12.01)
739 Clark JR, Bragin GE, Febbo EJ, and Letinski DJ. 2001. Toxicity of physically and
740 chemically dispersed oils under continuous and environmentally realistic
741 exposure conditions: Applicability to dispersant use decisions in spill response
742 planning. International Oil Spill Conference: American Petroleum Institute. p
743 1249-1255.
744 Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, Tokishita S,
745 Aerts A, Arnold GJ, and Basu MK. 2011. The ecoresponsive genome of Daphnia
746 pulex. *Science* 331:555-561.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

747 Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A,
748 Szcześniak MW, Gaffney DJ, Elo LL, and Zhang X. 2016. A survey of best
749 practices for RNA-seq data analysis. *Genome Biology* 17:13.
750 Cucci T, and Epifanio C. 1979. Long-term effects of water-soluble fractions of Kuwait
751 crude oil on the larval and juvenile development of the mud crab Eurypanopeus
752 depressus. *Marine Biology* 55:215-220.
753 Cui X, and Churchill GA. 2003. Statistical tests for differential expression in cDNA
754 microarray experiments. *Genome Biol* 4:210.
755 D'Andrea MA, and Reddy GK. 2013. Health consequences among subjects involved in
756 Gulf oil spill clean-up activities. *The American journal of medicine* 126:966-974.
757 DeLeo DM, Ruiz-Ramos DV, Baums IB, and Cordes EE. 2015. Response of deep-water
758 corals to oil and chemical dispersant exposure. *Deep Sea Research Part II:*
759 *Topical Studies in Oceanography*.
760 Dudoit S, Yang YH, Callow MJ, and Speed TP. 2002. Statistical methods for identifying
761 differentially expressed genes in replicated cDNA microarray experiments.
762 *Statistica sinica*:111-139.
763 Echols B, Smith A, Gardinali P, and Rand G. 2016. The use of ephyrae of a scyphozoan
764 jellyfish, Aurelia aurita, in the aquatic toxicological assessment of Macondo oils
765 from the Deepwater Horizon incident. *Chemosphere* 144:1893-1900.
766 Finn RD, Clements J, and Eddy SR. 2011. HMMER web server: interactive sequence
767 similarity searching. *Nucleic acids research*:gkr367.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

768 Fisher WS, and Foss SS. 1993. A simple test for toxicity of number 2 fuel oil and oil
769 dispersants to embryos of grass shrimp, Palaemonetes pugio. *Marine pollution*
770 *bulletin* 26:385-391.
771 Francis WR, Christianson LM, Kiko R, Powers ML, Shaner NC, and Haddock SH. 2013.
772 A comparison across non-model animals suggests an optimal sequencing depth
773 for de novo transcriptome assembly. *BMC genomics* 14:167.
774 Fransen L, Mūller R, Marmenout A, Tavernier J, Van der Heyden J, Kawashima E,
775 Chollet A, Tizard R, Van Heuverswyn H, and Van Vliet A. 1985. Molecular
776 cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression.
777 *Nucleic acids research* 13:4417-4429.
778 Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier
779 L, Ge Y, and Gentry J. 2004. Bioconductor: open software development for
780 computational biology and bioinformatics. *Genome Biology* 5:R80.
781 Gleason LU, and Burton RS. 2015. RNA-seq reveals regional differences in
782 transcriptome response to heat stress in the marine snail Chlorostoma funebralis.
783 *Molecular Ecology* 24:610-627.
784 Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB,
785 Eccles D, Li B, and Lieber M. 2013. De novo transcript sequence reconstruction
786 from RNA-seq using the Trinity platform for reference generation and analysis.
787 *Nature protocols* 8:1494-1512.
788 Hahn ME. 2002. Aryl hydrocarbon receptors: diversity and evolution. *Chemico-*
789 *biological interactions* 141:131-160.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

790 Hamoutene D, Payne J, Rahimtula A, and Lee K. 2004. Effect of water soluble fractions
791 of diesel and an oil spill dispersant (Corexit 9527) on immune responses in
792 mussels. *Bulletin of environmental contamination and toxicology* 72:1260-1267.
793 Han J, Won E-J, Kang H-M, Lee M-C, Jeong C-B, Kim H-S, Hwang D-S, and Lee J-S.
794 2016. Marine copepod cytochrome P450 genes and their applications for
795 molecular ecotoxicological studies in response to oil pollution. *Marine pollution*
796 *bulletin*.
797 Hansen BH, Jager T, Altin D, Øverjordet IB, Olsen AJ, Salaberria I, and Nordtug T.
798 2016a. Acute toxicity of dispersed crude oil on the cold-water copepod Calanus
799 finmarchicus: Elusive implications of lipid content. *Journal of Toxicology and*
800 *Environmental Health, Part A* 79:549-557.
801 Hansen BH, Lie KK, Størseth TR, Nordtug T, Altin D, and Olsvik PA. 2016b. Exposure
802 of first-feeding cod larvae to dispersed crude oil results in similar transcriptional
803 and metabolic responses as food deprivation. *Journal of Toxicology and*
804 *Environmental Health, Part A* 79:558-571.
805 Havird JC, and Santos SR. 2016. Here we are, but where do we go? a systematic review
806 of crustacean transcriptomic studies from 2014–2015. *Integrative and*
807 *Comparative Biology*:icw061.
808 Hemmer MJ, Barron MG, and Greene RM. 2011. Comparative toxicity of eight oil
809 dispersants, Louisiana sweet crude oil (LSC), and chemically dispersed LSC to
810 two aquatic test species. *Environmental Toxicology and Chemistry* 30:2244-2252.
811 Jenny MJ, Walton WC, Payton SL, Powers JM, Findlay RH, O'Shields B, Diggins K,
812 Pinkerton M, Porter D, and Crane DM. 2016. Transcriptomic evaluation of the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

American oyster, Crassostrea virginica, deployed during the Deepwater Horizon oil spill: Evidence of an active hydrocarbon response pathway. *Marine Environmental Research* 120:166-181.

Khan R. 1990. Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. *Bulletin of environmental contamination and toxicology* 44:759-763.

Kujawinski EB, Kido Soule MC, Valentine DL, Boysen AK, Longnecker K, and Redmond MC. 2011. Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental science & technology* 45:1298-1306.

Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, and Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic acids research* 35:3100-3108.

Langmead B. 2010. Aligning short sequencing reads with Bowtie. *Current protocols in bioinformatics*:11.17. 11-11.17. 14.

Levin M, Anavy L, Cole AG, Winter E, Mostov N, Khair S, Senderovich N, Kovalev E, Silver DH, and Feder M. 2016. The mid-developmental transition and the evolution of animal body plans. *Nature*.

Li B, and Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics* 12:1.

Liu Y-Z, Roy-Engel AM, Baddoo MC, Flemington EK, Wang G, and Wang H. 2016. The impact of oil spill to lung health—Insights from an RNA-seq study of human airway epithelial cells. *Gene* 578:38-51.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

835 Love M, Anders S, and Huber W. 2014. Differential analysis of count data–the DESeq2
836 package. *Genome Biology* 15:550.
837 Lowe SW, and Lin AW. 2000. Apoptosis in cancer. *Carcinogenesis* 21:485-495.
838 Lundin VF, Leroux MR, and Stirling PC. 2010. Quality control of cytoskeletal proteins
839 and human disease. *Trends in biochemical sciences* 35:288-297.
840 Mitelman F, Johansson B, and Mertens F. 2007. *Mitelman database of chromosome*
841 *aberrations in cancer*: Cancer Genome Anatomy Project.
842 Munro S, Lund S, Pine P, Binder H, Clevert D, Conesa A, Dopazo J, Fasold M,
843 Hochreiter S, and Hong H. 2014. Assessing technical performance in differential
844 gene expression experiments with external spike-in RNA control ratio mixtures.
845 *Nature Communications* 5:5125.
846 National Research Council (NRC). 2005. *Oil Spill Dispersants: Efficacy and Effects*:
847 National Academies Press.
848 O'Connor TP. 1998. Mussel Watch results from 1986 to 1996. *Marine pollution bulletin*
849 37:14-19.
850 OECD. 2002. Guidance Document on Aquatic Toxicity Testing of Difficult Substances
851 and Mixtures. OECD Series on Testing and Assessment,: Organization for
852 Economic Cooperation and Development.
853 Olsvik PA, Hansen BH, Nordtug T, Moren M, Holen E, and Lie KK. 2011.
854 Transcriptional evidence for low contribution of oil droplets to acute toxicity from
855 dispersed oil in first feeding Atlantic cod (Gadus morhua) larvae. *Comparative*
856 *Biochemistry and Physiology Part C: Toxicology & Pharmacology* 154:333-345.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

857 Olsvik PA, Lie KK, Nordtug T, and Hansen BH. 2012. Is chemically dispersed oil more
858 toxic to Atlantic cod (Gadus morhua) larvae than mechanically dispersed oil? A
859 transcriptional evaluation. *BMC genomics* 13:1.
860 Ottaviani E, Malagoli D, and Franceschi C. 2007. Common evolutionary origin of the
861 immune and neuroendocrine systems: from morphological and functional
862 evidence to in silico approaches. *Trends in immunology* 28:497-502.
863 Paul JH, Hollander D, Coble P, Daly KL, Murasko S, English D, Basso J, Delaney J,
864 McDaniel L, and Kovach CW. 2013. Toxicity and mutagenicity of Gulf of
865 Mexico waters during and after the Deepwater Horizon oil spill. *Environmental*
866 *science & technology* 47:9651-9659.
867 Petersen TN, Brunak S, von Heijne G, and Nielsen H. 2011. SignalP 4.0: discriminating
868 signal peptides from transmembrane regions. *Nature methods* 8:785-786.
869 Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, and Irons DB.
870 2003. Long-term ecosystem response to the Exxon Valdez oil spill. *science*
871 302:2082-2086.
872 Petrulis JR, Chen G, Benn S, LaMarre J, and Bunce NJ. 2001. Application of the
873 ethoxyresorufin-O-deethylase (EROD) assay to mixtures of halogenated aromatic
874 compounds. *Environmental toxicology* 16:177-184.
875 Phillips DH. 1999. Polycyclic aromatic hydrocarbons in the diet. *Mutation*
876 *Research/Genetic Toxicology and Environmental Mutagenesis* 443:139-147.
877 R Core Development Team. 2010. The R project for statistical computing. Vienna,
878 Austria.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

879 Rewitz KF, Styrishave B, Løbner-Olesen A, and Andersen O. 2006. Marine invertebrate
880 cytochrome P450: emerging insights from vertebrate and insect analogies.
881 *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*
882 143:363-381.
883 Rico-Martínez R, Snell TW, and Shearer TL. 2013. Synergistic toxicity of Macondo
884 crude oil and dispersant Corexit 9500A® to the Brachionus plicatilis species
885 complex (Rotifera). *Environmental Pollution* 173:5-10.
886 Risso D, Ngai J, Speed TP, and Dudoit S. 2014. Normalization of RNA-seq data using
887 factor analysis of control genes or samples. *Nature biotechnology* 32:896-902.
888 Robinson MD, McCarthy DJ, and Smyth GK. 2010. edgeR: a Bioconductor package for
889 differential expression analysis of digital gene expression data. *Bioinformatics*
890 26:139-140.
891 Sammarco PW, Kolian SR, Warby RA, Bouldin JL, Subra WA, and Porter SA. 2013.
892 Distribution and concentrations of petroleum hydrocarbons associated with the
893 BP/Deepwater Horizon Oil Spill, Gulf of Mexico. *Marine pollution bulletin*
894 73:129-143.
895 Schulz RA, and Fossett N. 2005. Hemocyte development during Drosophila
896 embryogenesis. *Developmental Hematopoiesis: Methods and Protocols*:109-121.
897 Shell Offshore Inc. 2010. Gulf of Mexico regional oil spill response plan. 6.1 ed.
898 http://housedocs.house.gov/energycommerce/Docs_06152010/Shell.Gulf.Oil.Spill
899 .Response.Plan.pdf (accessed 16.12.01)

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

900 Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, and Zdobnov EM. 2015.
901 BUSCO: assessing genome assembly and annotation completeness with single-
902 copy orthologs. *Bioinformatics*:btv351.
903 Singer M, Aurand D, Bragin G, Clark J, Coelho G, Sowby M, and Tjeerdema R. 2000.
904 Standardization of the preparation and quantitation of water-accommodated
905 fractions of petroleum for toxicity testing. *Marine pollution bulletin* 40:1007-
906 1016.
907 Sparrow JC, Nowak KJ, Durling HJ, Beggs AH, Wallgren-Pettersson C, Romero N,
908 Nonaka I, and Laing NG. 2003. Muscle disease caused by mutations in the
909 skeletal muscle alpha-actin gene (ACTA1). *Neuromuscular Disorders* 13:519-
910 531.
911 Suchanek TH. 1993. Oil impacts on marine invertebrate populations and communities.
912 *American Zoologist* 33:510-523.
913 Trustees DWH. 2016. Deepwater Horizon oil spill: Final Programmatic Damage
914 Assessment and Restoration Plan and Final Programmatic Environmental Impact
915 Statement. In: Trustees DHNRDA, editor: National Oceanic and Atmospheric
916 Administration. http://www.gulfspillrestoration.noaa.gov/restoration-
917 planning/gulf-plan/ (accessed 16.12.01)
918 Vignier J, Soudant P, Chu F, Morris J, Carney M, Lay C, Krasnec M, Robert R, and
919 Volety A. 2016. Lethal and sub-lethal effects of Deepwater Horizon slick oil and
920 dispersant on oyster (Crassostrea virginica) larvae. *Marine Environmental*
921 *Research* 120:20-31.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

922 Wang Z, Gerstein M, and Snyder M. 2009. RNA-Seq: a revolutionary tool for
923 transcriptomics. *Nature Reviews Genetics* 10:57-63.
924 Waterhouse RM, Tegenfeldt F, Li J, Zdobnov EM, and Kriventseva EV. 2013. OrthoDB:
925 a hierarchical catalog of animal, fungal and bacterial orthologs. *Nucleic acids*
926 *research* 41:D358-D365.
927 Weber CI. 1991. *Methods for measuring the acute toxicity of effluents and receiving*
928 *waters to freshwater and marine organisms*: Environmental Monitoring Systems
929 Laboratory, Office of Research and Development, US Environmental Protection
930 Agency.
931 Whitehead A, Dubansky B, Bodinier C, Garcia TI, Miles S, Pilley C, Raghunathan V,
932 Roach JL, Walker N, and Walter RB. 2012. Genomic and physiological footprint
933 of the Deepwater Horizon oil spill on resident marsh fishes. *Proceedings of the*
934 *National Academy of Sciences* 109:20298-20302.
935 Williams AB. 1984. *Shrimps, lobsters, and crabs of the Atlantic coast of the eastern*
936 *United States, Maine to Florida*. Washington, D. C.: Smithsonian Institution
937 Press.
938 Wise CF, Wise JT, Wise SS, Thompson WD, and Wise JP. 2014. Chemical dispersants
939 used in the Gulf of Mexico oil crisis are cytotoxic and genotoxic to sperm whale
940 skin cells. *Aquatic Toxicology* 152:335-340.
941 Wise J, and Wise JP. 2011. A review of the toxicity of chemical dispersants. *Reviews on*
942 *environmental health* 26:281-300.
943 Worton DR, Zhang H, Isaacman-VanWertz G, Chan AW, Wilson KR, and Goldstein AH.
944 2015. Comprehensive chemical characterization of hydrocarbons in NIST

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2977v1 | CC BY 4.0 Open Access | rec: 12 May 2017, publ: 12 May 2017

945 standard reference material 2779 Gulf of Mexico crude oil. *Environmental*
946 *science & technology* 49:13130-13138.
947 Yednock BK, Sullivan TJ, and Neigel JE. 2015. De novo assembly of a transcriptome
948 from juvenile blue crabs (Callinectes sapidus) following exposure to surrogate
949 Macondo crude oil. *BMC genomics* 16:1.
950 Young MD, Wakefield MJ, Smyth GK, and Oshlack A. 2010. Gene ontology analysis for
951 RNA-seq: accounting for selection bias. *Genome Biology* 11:1.
952 Zhu L, Qu K, Xia B, Sun X, and Chen B. 2016. Transcriptomic response to water
953 accommodated fraction of crude oil exposure in the gill of Japanese flounder,
954 Paralichthys olivaceus. *Marine pollution bulletin* 106:283-291.
955

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956 **Table1.** Environmental and experiment information for the flatback mudcrab
957 transcriptome compliant with the MIGS standard.

Item	Description
Investigation_type	Eukaryote
Project_name	Transcriptomic effects of dispersed oil in *Eurypanopeus depressus*
Collection_date	09-July-2013 and 23-September-2014
Lat_lon	29.521667 N 94.502100 W 27.436667 N 80.335556 W
Country	USA (Texas and Florida)
Environment	Oyster beds
Geo_loc_name	Rollover Bay, Texas (USA) Fort Pierce, Florida (USA)
env_biome	ENVO_00002030 (aquatic biome)
env_feature	ENVO_00000485 (sea shore)
env_material	ENVO_00002149 (sea water)
Ref_biomaterial	Primary transcriptomic report
biotic_relationship	free living
Rel_to_oxygen	Aerobe
Ploidy	Diploid
Sequencing_meth	Illumina HiSeq2000
Num_replicons	NA
Assembly	Trinity2.0.3

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Finishing_strategy	Draft
Estimated_size	2.80E9
Sample_material	muscle
Motility	Yes
Assembly method	Trinity2.0.3
Assembly name	de Novo
Sequencing technology	Illumina HiSeq2000

958

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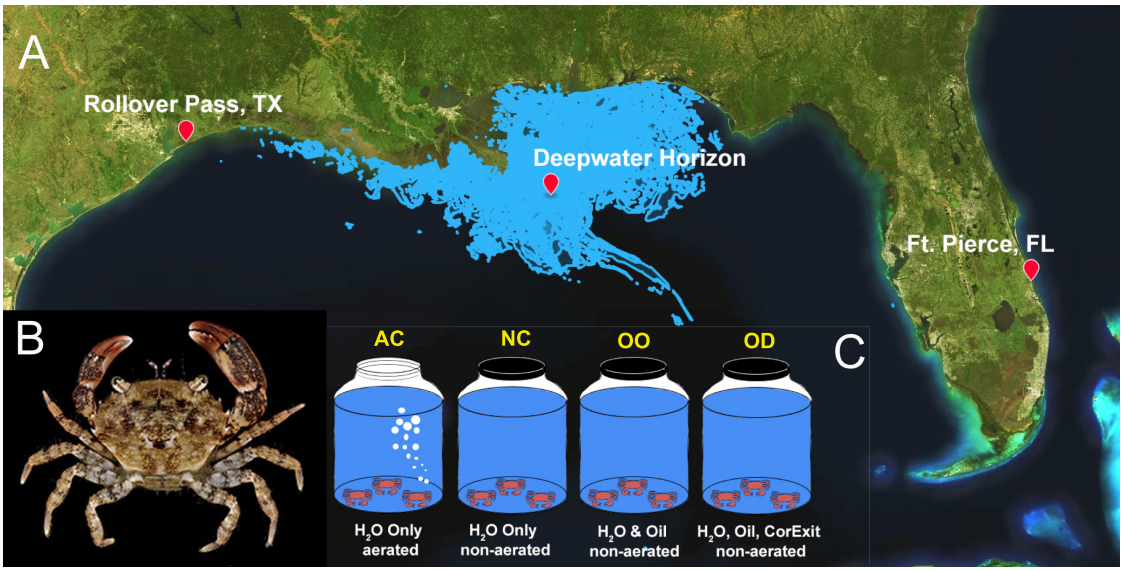
959



960

961 **Figure 1. Sampling sites, Deepwater Horizon Oil Spill (DWH) area, species of**
962 **interest, and experimental design. (A)** We collected samples in two sites not affected
963 by DWH [light blue area
964 (http://invertebrates.si.edu/boem/maps/BOEM_NMNH_GOM_collections.kmz); map
965 credit: created in Mapbox v2015-09-26-00-46-36 by B.P.T. (http://www.mapbox.com);
966 map data: OpenStreetMap© (http://www.openstreetmap.org/copyright)] of the **(B)**
967 flatback mudcrab *Eurypanopeus depressus* (Photo credit: B.P.T.). **(C)** Our experimental
968 design (Image credit: B.P.T.) consisted of two controls: aerated (AC), negative (NC); two
969 experimental treatments: oil-only (OO), oil-dispersant (OD).

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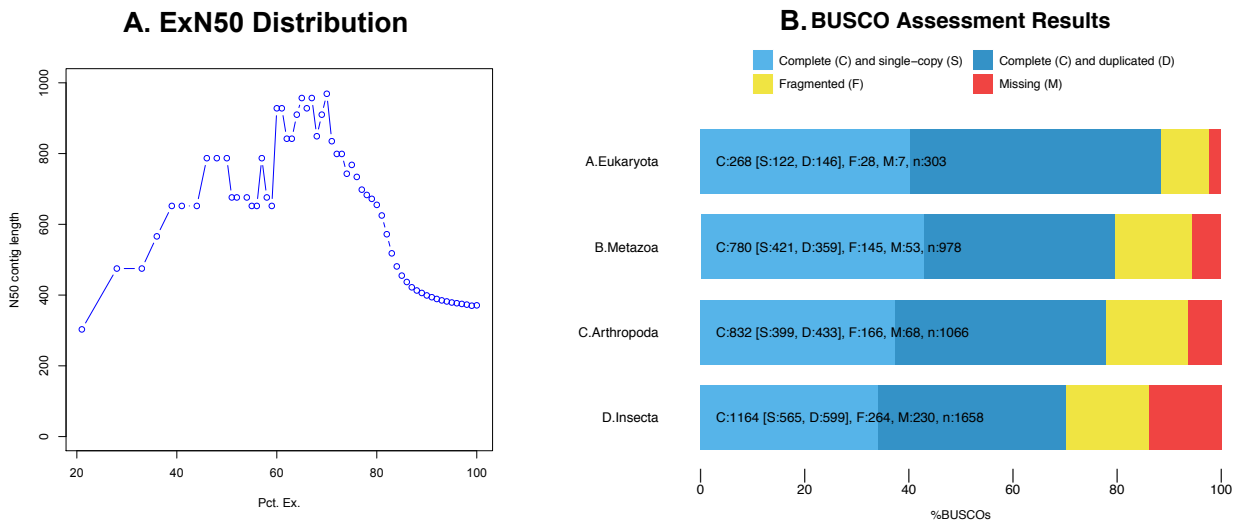

970

971 **Figure 2. Assembly quality and transcriptomic completeness. (A)** ExN50

972 distribution pondering N50 contig length by percentage expressed (Pct. Ex); and (B)

973 assembly completeness based on Benchmarking Universal Single-Copy Orthologs

974 (BUSCOs). Each bar represents ORTHODB ortholog assesment in a hierarchical

975 taxonomic order from Eukaryota to Insecta. Legend: complete genes in blue, missing

976 genes in red, and in yellow fragmented genes.

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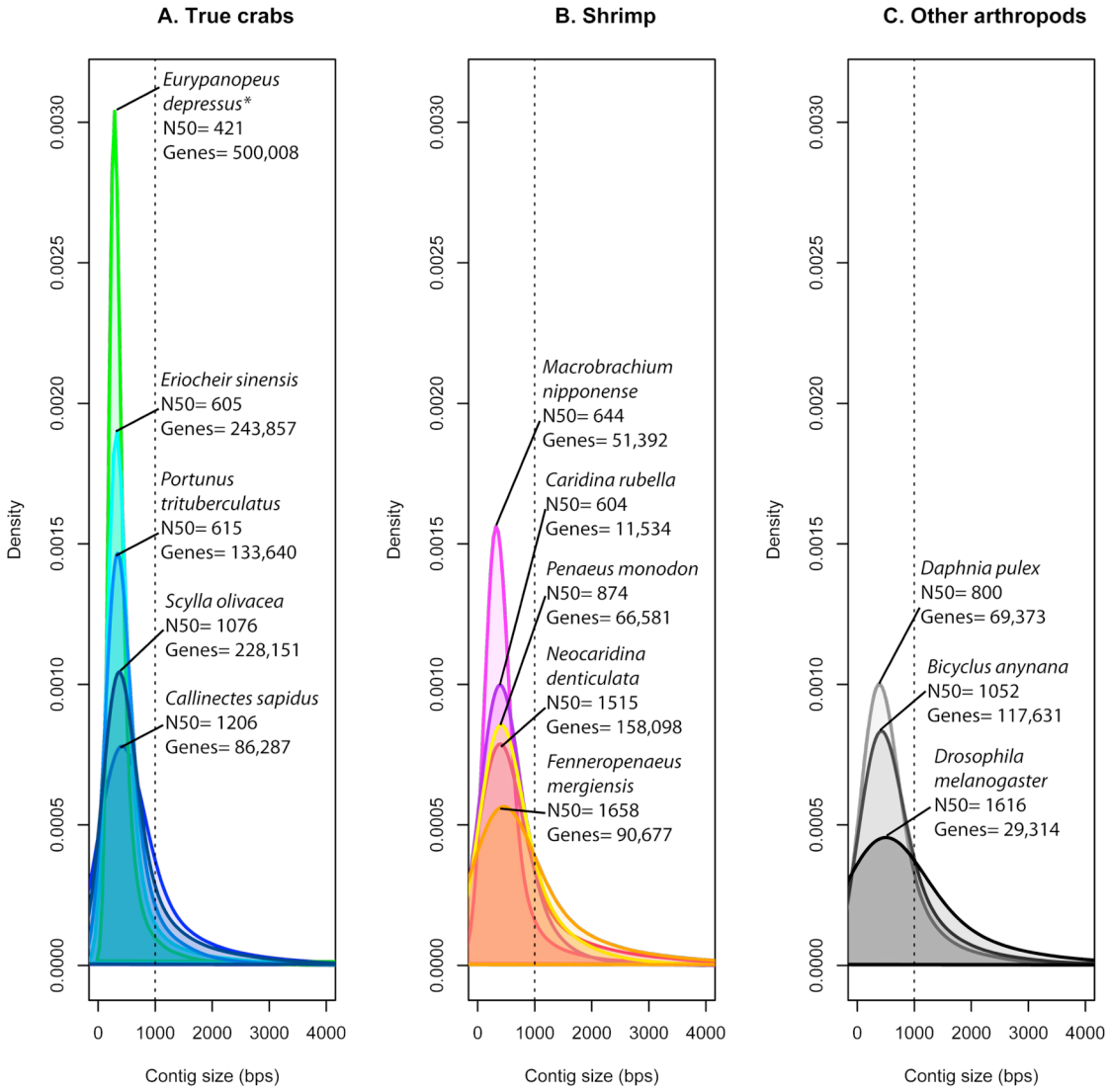

977

978 **Figure 3 Contig size density distribution and variability in decapod**

979 **crustaceans and model arthropods.** (A) Distribution of contig size in true crabs

980 [Decapoda: Brachyura], (B) Distribution of contig size in shrimp [Decapoda:

981 Dendrobranchiata & Caridea], and (C) in other arthropods including model

982 organisms fruit fly and water flea. Mudcrab assembly (*Eurypanopeus depressus*)

983 constructed with 15 samples is marked with and asterisk (*); every other assembly

984 includes a single Genbank SRA sample. All species were assembled in TRINITY with

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985 the exact same parameters in the same HPC cluster. N50 values represent at least
986 50% of contigs in an assembly are a given size in bps or larger. Dashed vertical lines
987 indicate a 1000 bps contig size threshold. Genes correspond to number of contigs
988 assembled by TRINITY.

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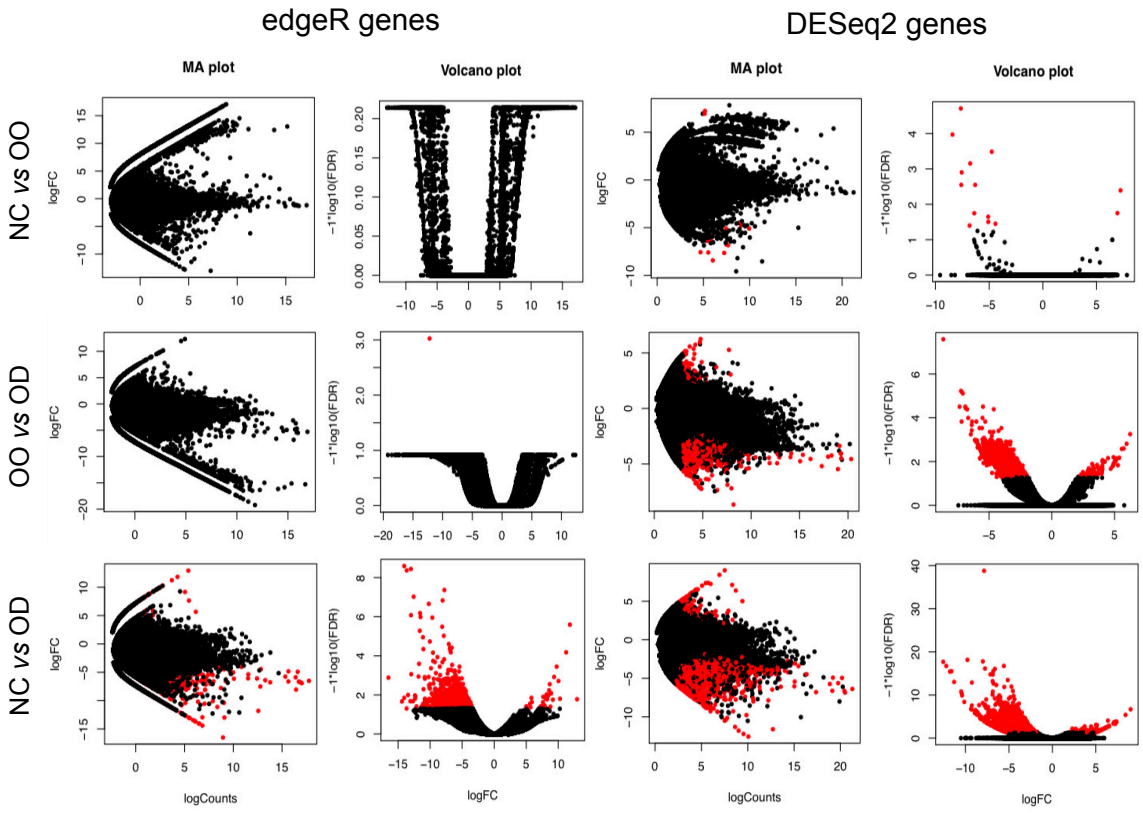

Figure 4. Pairwise differential gene expression MA and volcano plots between the negative control (NC) and experimental treatments (OO and OD). In MA plots X-axis represents mean average expression on counts on a $\log_2$ scale and Y-axis represents fold-changes in gene expression on a logarithmic scale. In volcano plots X-axis represents fold-changes in gene expression on a logarithmic scale, and Y-axis represents pairwise *t*-test negative $\log_{10}$ *P*-values Red dots represent significant comparisons passing a 1% false discovery rate (FDR).

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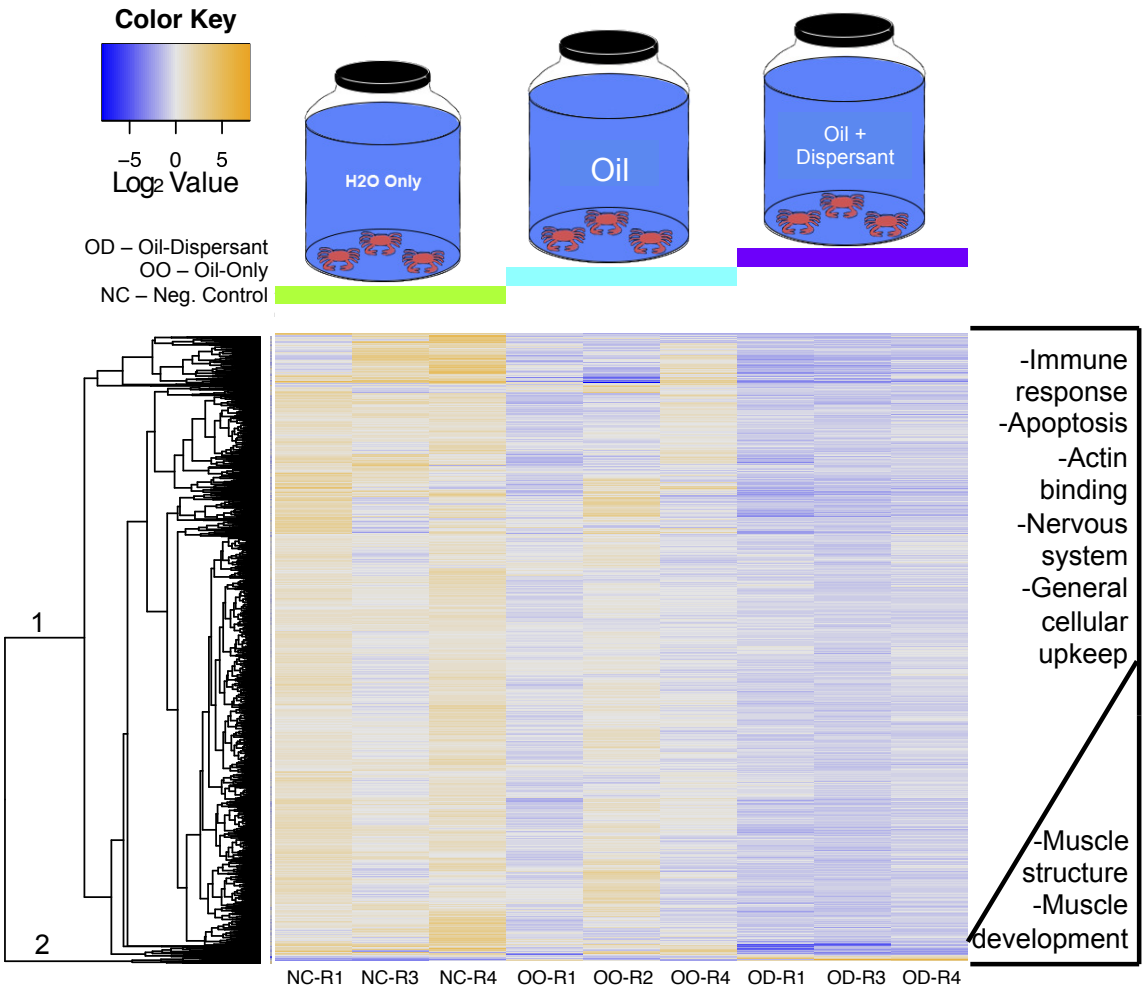

997

998 **Figure 5. Differential expression and regulation of genes estimated in DESeq2**.

999 Columns correspond to treatments: NC (negative control) in green, OO (oil-only) in cyan,

1000 and OD (oil-dispersant) in purple. The "R" label below columns represents replicates

1001 (Table S1). Rows correspond to significant genes passing a 1% FDR. Numbers on the

1002 left-side dendogram are feature clusters 1 and 2. Branch lengths on dendrogram

1003 correspond to Euclidean distances. Heatmap colors represent regulation direction from

1004 normalized, log-transformed fragments per kilobase of transcript per million mapped

1005 reads (FPKM) and zero-centered fold-change values: blue cells are downregulated genes,

1006 orange cells are upregulated genes, and grey values show no differential expression. For

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all comparisons see Figs. S7-8. List on right side are gene ontology (GO) terms discussed in text (full list: Table S4).

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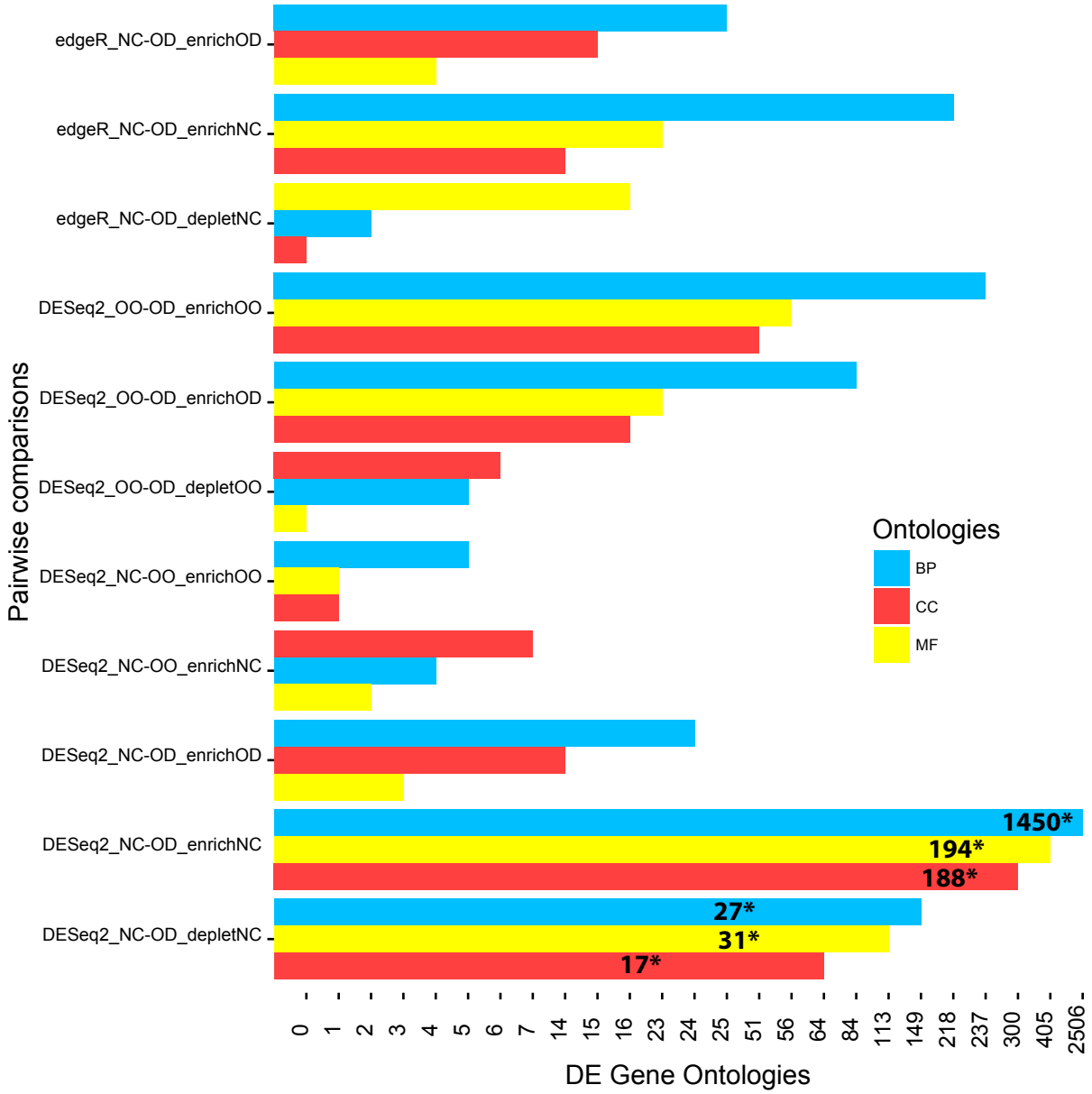

Figure 6. Gene ontology enrichment analyses of differentially expressed (DE) genes estimated in GOseq. Pairwise comparisons of DE gene enrichment tests between NC (negative control), OO (oil-only), and OD (oil-dispersant) treatments listed by statistical method (edgeR and DESeq2). Only 11 out of 24 possible comparisons included DE genes (DEGs) and thus were tested for enrichment. Each test includes method, pairwise comparison, and whether an experimental condition included overrepresented (enriched – enrich) or underrepresented (depleted – deplet) gene ontology (GO) terms. Ontology

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categories are: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Statistically significant enrichment counts ($P < 0.05$) passing a 1% FDR are marked with an asterisk (*). Bar plots with no count numbers were not significant.

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Supporting Information

Transcriptomic effects of dispersed oil in a non-model decapod crustacean

Hernán Vázquez-Miranda, Brent P. Thoma, Juliet M. Wong, Darryl L. Felder, Keith A. Crandall, and Heather D. Bracken-Grissom.

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Supplementary Results

Differential gene expression in aerated control (AC) and non-aerated negative control (NC). We found relatively few genes differentially expressed between aerated controls (AC) and non-aerated controls (NC; Table S2) potentially representing the effects of low oxygen and experimental manipulation. Only three genes passed the 1% false discovery rate (FDR) on the MA plots in edgeR. When replicates are counted separately in gene expression heatmaps, edgeR found no significant comparisons passing the 1% FDR (Fig. S2). With DESeq2 between 26 and 33 features were significantly expressed (Table S2, Fig. S2). The upregulated features under presumed low oxygen in DESeq2 were ATP, calcium, and metal ion binding on the A-band muscle component, glutathione metabolic process and transferase activities in the cytoplasm and cytosol, and chitin binding and metabolic processes. Calcium, metal ion and ATP binding are necessary for energy transfer and protein activation (Knowles 1980), glutathione is a critical antioxidant (Pompella et al. 2003), and chitin is an integral component of arthropod exoskeletons. Approximately 76% of upregulated features did not BLAST to a GO. GOs of downregulated features matched ribosomal binding proteins, endonucleases, tyrosine and calmodulin phosphatases and zinc ion binding on the membrane, cytoplasm, nucleus, and ubiquinone reductases on mitochondrial respiratory chain complex and mitochondrial membrane. Most of these processes are involved in multiple parts of cellular upkeep, signaling, protein activation and synthesis. Ubiquinone reductases are critical factors in the respiratory electron transport chain responsible for cellular energy production and antioxidant protection (Mellors & Tappel 1966). Close to 77% of downregulated features under presumed low oxygen conditions did not blast to a GO.

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Differential gene expression in non-aerated negative control (NC) and experimental treatments (OO-OD), and differences between statistical methods. We measured differential gene expression between the control, oil-only, and oil-dispersant treatments using edgeR and DESeq2. The main difference between those two statistical methods was the amount of significantly expressed features, with DESeq2 detecting larger amounts with relatively lower significance magnitude. We report numbers from DESeq2 first and numbers from edgeR second in this supplementary materials file SM (DESeq2-only numbers appear on the main text).

DESeq2.- Comparisons of controls and treatments detected hundreds of genes and thousands of isoforms that were differently expressed. Approximately 50% of these differentially expressed features in our *de novo* transcriptome (Database DS1) did not match any annotation and were left as unknown by either lack of database references or by being putative proteins with missing gene ontology (GO). There were only five differentially expressed features shared between controls and experimental treatments (Fig. S1). None of these shared features blasted to a GO. Most of the thousands of differentially expressed genes and isoforms were detected in the presence of oil-only and oil-dispersant treatments.

When comparing NC and experimental treatments (OO-OD), we detected a range of 11-4836 features with significantly differential expression (Figs. S3-S5, Table S3). When comparing NC to OD, we identified 4388 genes and 3243 isoforms (Figs. 2, S3-S5, Table S3). When comparing NC and OO (Fig. 2, Table S3) we found 11 genes and 8 isoforms, and 448 genes and 215 isoforms between OO and OD (Fig.2, Figs. S3-S5, Table S3).

edgeR.- Comparisons of controls and treatments detected hundreds of genes and tens of isoforms that were differently expressed. Similar to DESeq2 counts, approximately 50% of these

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differentially expressed features in our *de novo* transcriptome (Database DS1) did not match any GO annotation. There were only three differentially expressed features shared between controls and experimental treatments (Fig. S1). None of these shared features blasted to a GO. Most of the hundreds of differentially expressed genes and isoforms were detected in OO and OD.

Between NC and experimental treatments (OO-OD), we detected a range of 1-172 features with significantly differential expression (Figs. S3-S5, Table S3). When comparing NC to OD, we identified 172 genes and 87 isoforms (Figs. 2, S3-S5, Table S3). When comparing NC and OO (Fig. 2, Table S2) we found zero genes and zero isoforms, and 1 gene and zero isoforms between OO and OD (Fig.2, Figs. S3-S5, Table S3).

Although thousands of significantly expressed features were only detected by DESeq2, several hundred genes and isoforms were also found with edgeR (Fig. S6, Table S4).

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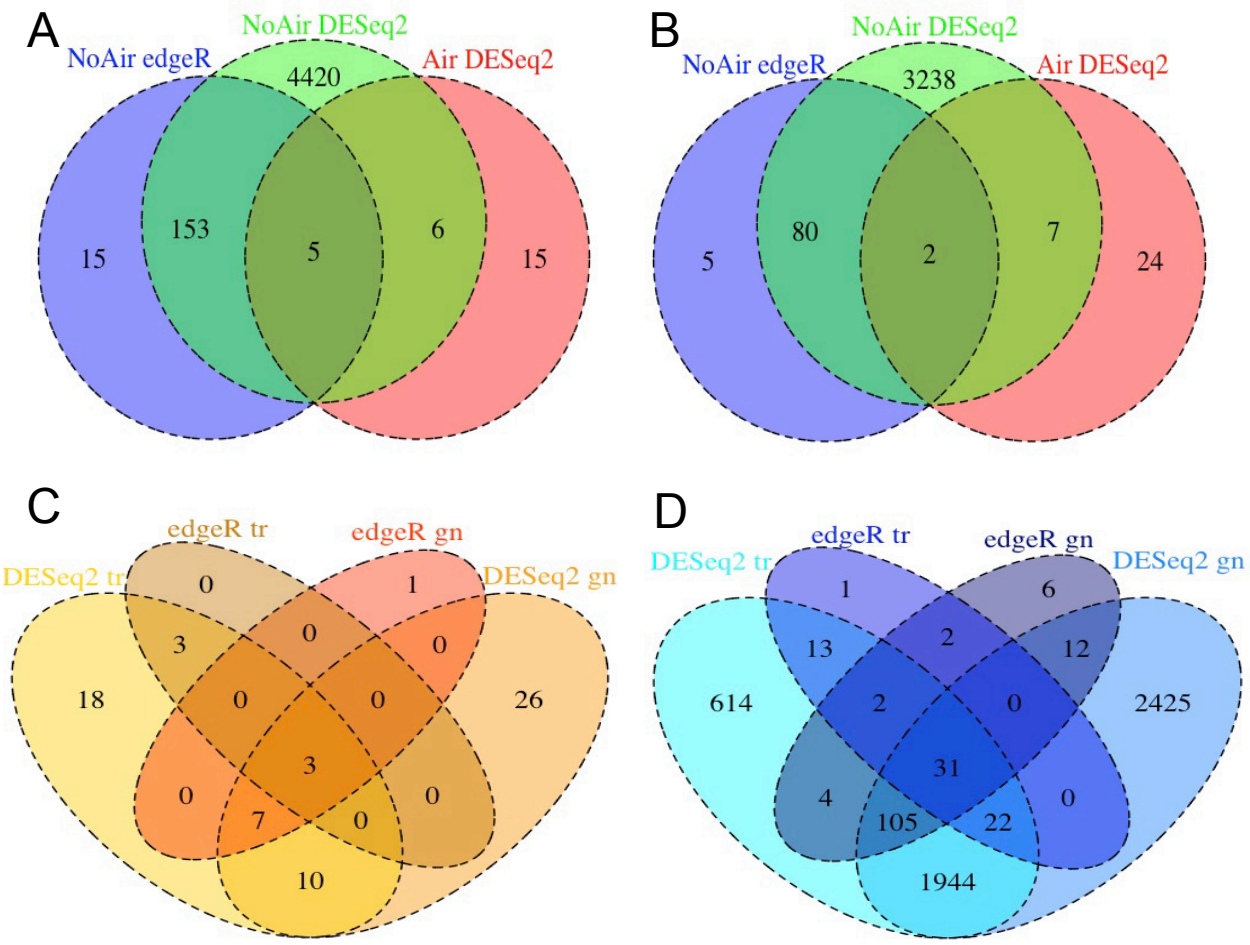

Figure S1. Venn diagrams of shared differentially expressed features between controls (AC-NC) and experimental treatments (OO-OD). (A) Trinity 'genes', (B) Trinity isoforms. AC is the aerated, negative control, NC is the non-aerated, negative control, OO is the non-aerated oil-only treatment, and OD is the non-aerated oil-dispersant treatment. Circles represent significantly expressed features passing an FDR of 1%, detected by edgeR in blue including non-aerated control and treatments NC-OO-OD, features detected by DESeq2 including non-aerated control and treatments NC-OO-OD in green, and features detected by DESeq2 including aerated, negative controls AC and non-aerated, negative controls NC in red. There were zero features

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passing the FDR of 1% including AC-NC controls in edgeR and thus were not plotted**.** Warm colors **(C)** correspond to upregulated features between NC and experimental treatments (OO-OD) across all statistical comparisons using OD as a reference, and cool colors **(D)** to downregulated features. Regulation direction occurs in the opposite sense (inverted colors) in features on NC (non-aerated control). The suffix "gn" corresponds to Trinity 'genes' and the suffix "tr" to isoforms. Color palettes match heatmaps in this paper.

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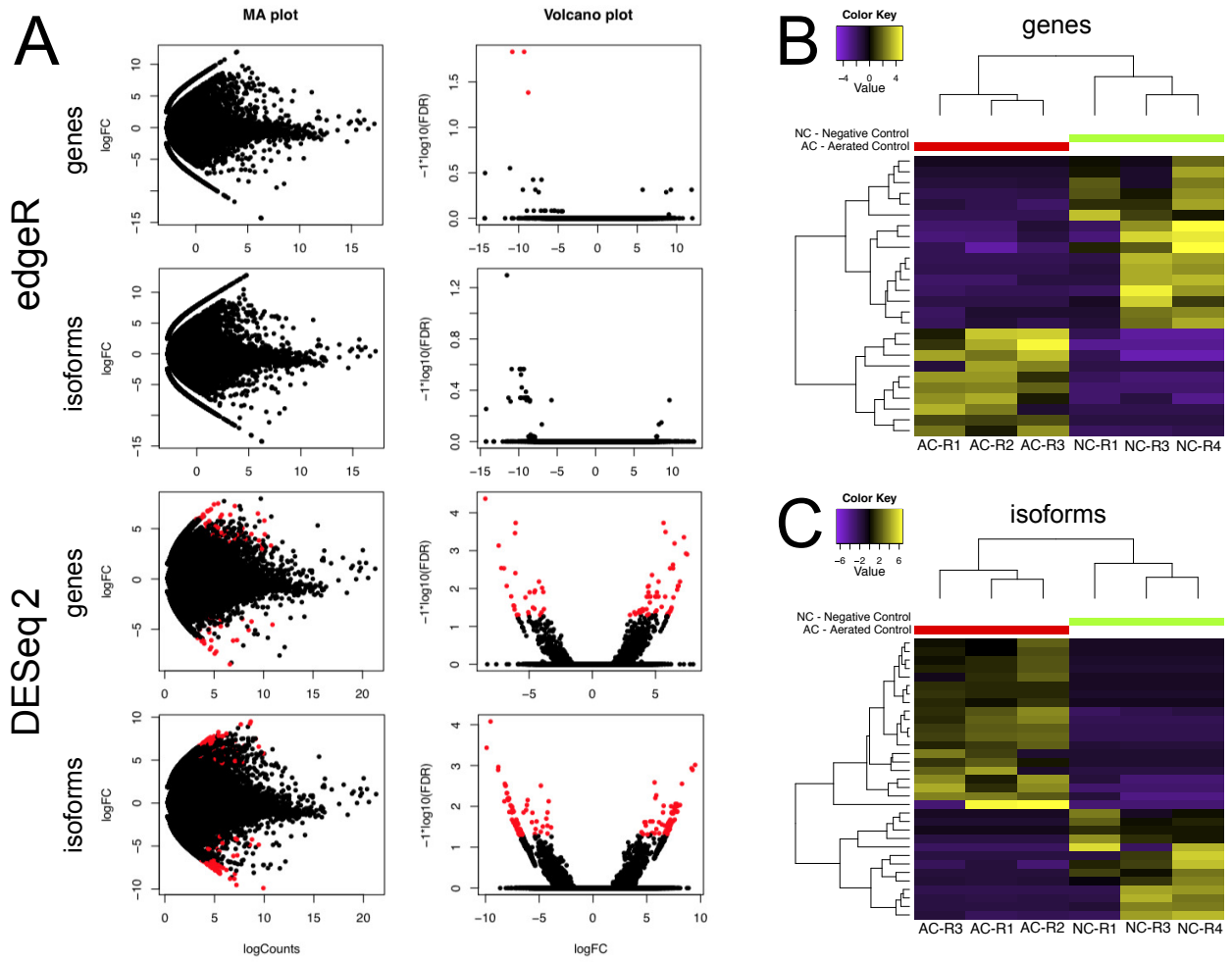

Fig. S2. Differential feature expression in low oxygen conditions between aerated (AC) and non-aerated (NC) controls. A) Left column boxes are pairwise MA plots where X-axes represent mean average expression on counts on a logarithmic scale Y-axes represens fold-changes in gene expression on a logarithmic scale. Right column boxes are Volcano plots where X-axes represent fold-changes in gene expression on a logarithmic scale and Y-axes represent the probability of each pairwise *t*-test gene comparison between treatments. Red dots represent significant comparisons passing an FDR of 1%. **B)** and **C)** are heatmaps representing direction of gene regulation for genes that passed the 1% FDR for Trinity 'genes' and isoforms respectively from DESeq2. "R" labels represent individual replicates (Supplementary

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Information Table S1). Warmer colors (gold) indicate upregulated features and cool colors (purple) downregulated genes for $\log_2$ fold-changes. Colors in black indicate zero-centered values. There were no significantly expressed genes found in edgeR passing the 1% FDR for heatmap generation.

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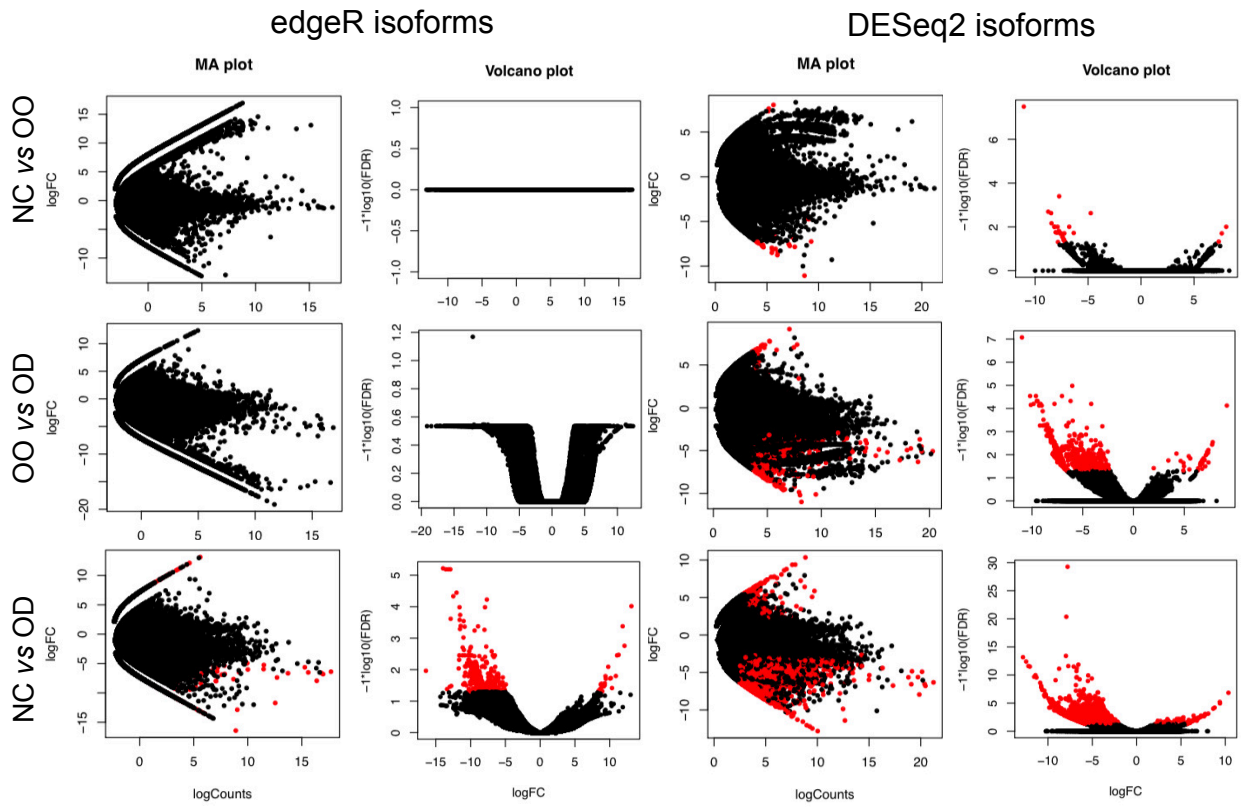

Fig. S3. Pairwise differential isoform expression MA and volcano plots between the negative control (NC) and experimental treatments (OO and OD). In MA plots X-axis represents mean average expression on counts on a $\log_2$ scale and Y-axis represents fold-changes in gene expression on a logarithmic scale. In volcano plots X-axis represents fold-changes in gene expression on a logarithmic scale, and Y-axis represents pairwise *t*-test negative $\log_{10}$ *P*-values Red dots represent significant comparisons passing a 1% false discovery rate (FDR).

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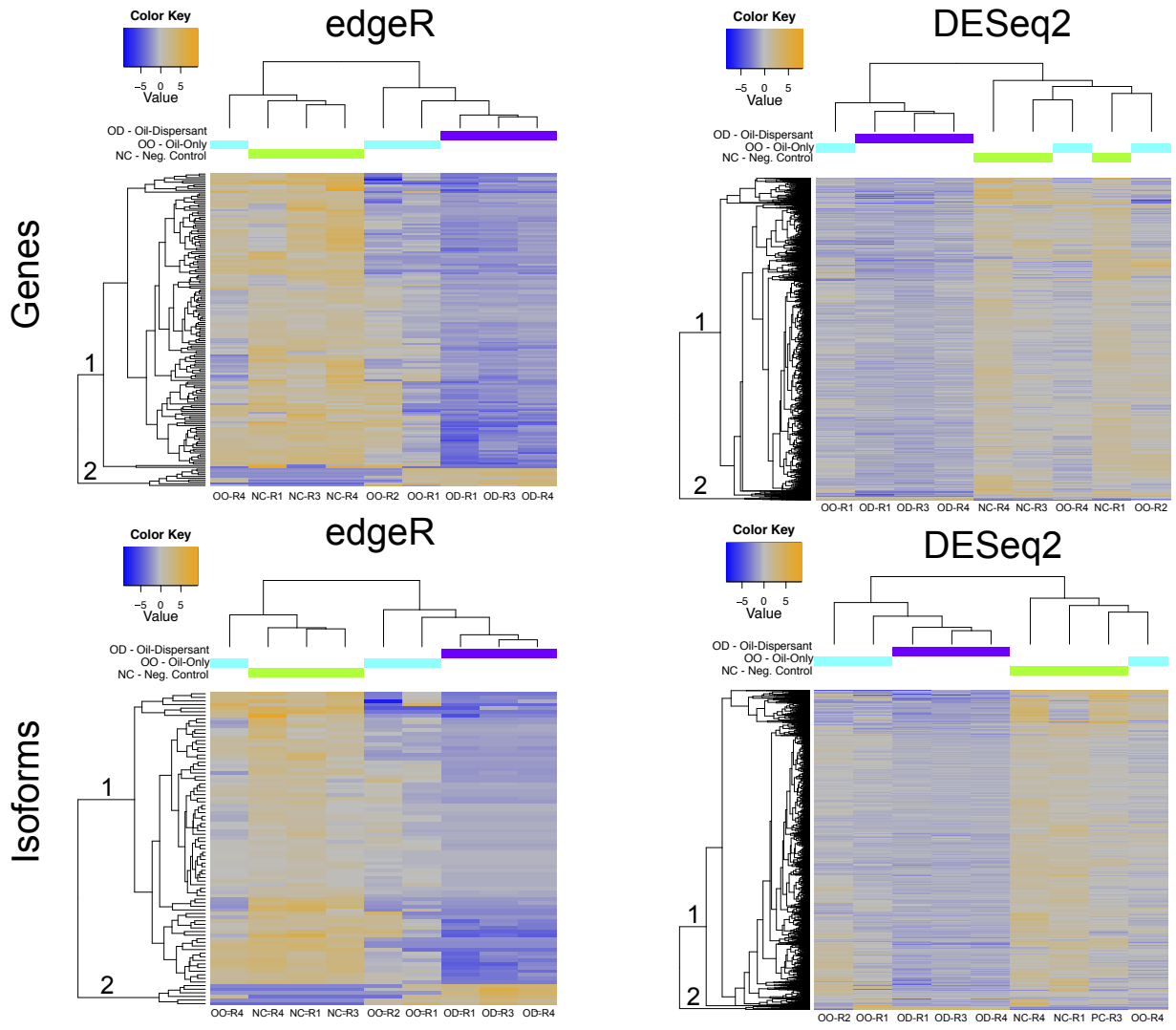

Fig. S4. Differential expression and regulation of features for all experimental treatments related to presence of oil and dispersant ordered by expression profile distances. Columns and top dendrogram correspond to treatments with three replicates each: NC (non-aerated control) in green, OO (non aerated with oil) in cyan, and OO (non aerated with oil and dispersant) in purple. "R" labels represent individual replicates (Table S1). Rows and left-side dendrogram correspond to features with statistical significance passing a FDR of 1%. Numbers on the left-side dendogram branches correspond to feature clusters 1 and 2 mentioned in the main text. Branch lengths on both dendrograms correspond to gene expression Euclidean

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distances. Heatmap colors represent regulation direction from normalized, log-transformed FPKM and centered fold-change values: blue cells indicate down-regulated genes, orange cells correspond to up-regulated genes, and grey values show no differential expression from a log2 count value centered at zero.

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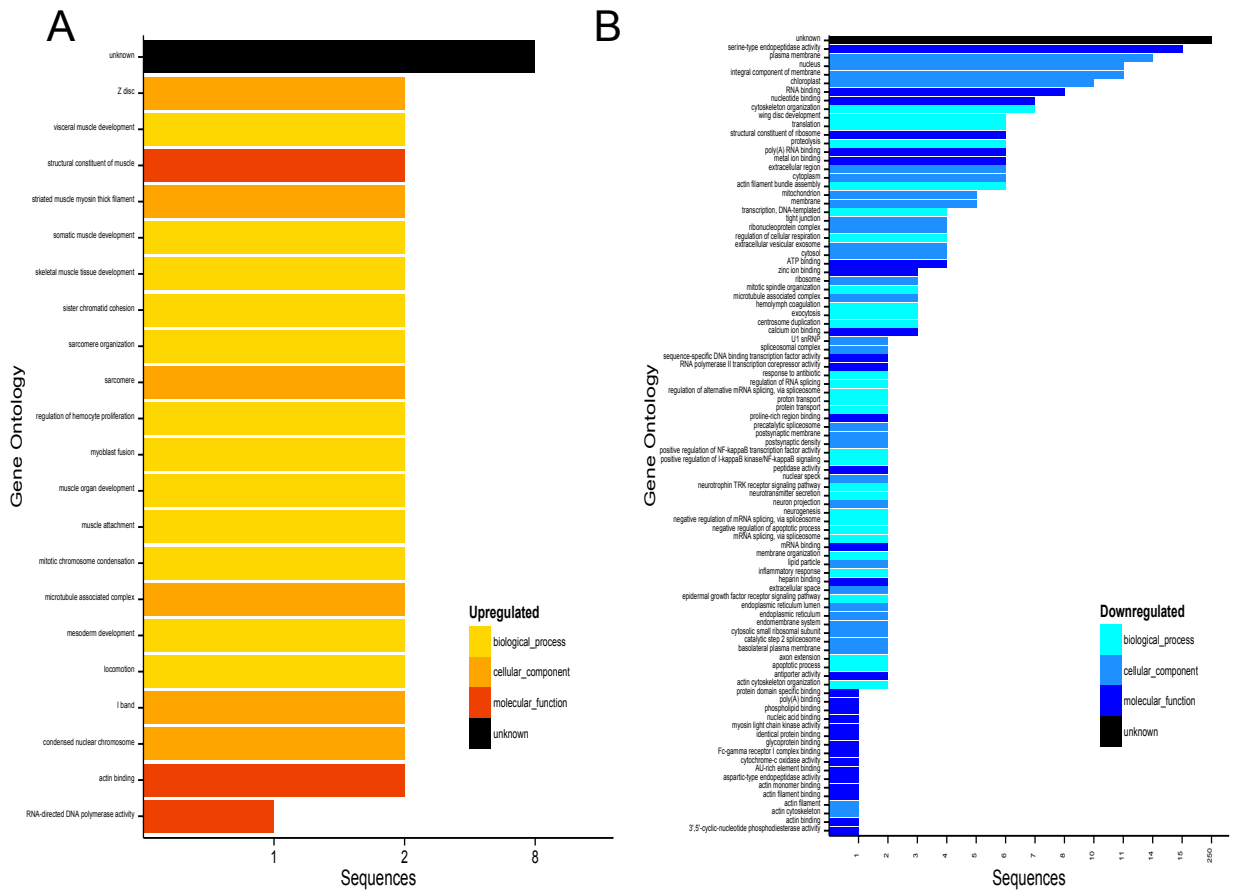

Fig. S5. Gene ontology annotations for genes from edgeR in oil-dispersant (OD). Colors in bar represent major upregulated (A; warm colors) and (B; cool colors) downregulated GO terms (see legend). X-axis indicates element count found in the database. Note last scale number is adjusted to the largest count value. GO detailed functions are arranged in reversed count number and then alphabetical order. Colors correspond to heatmaps in Figures 3, Extended Data Fig. S4. For full annotations see supplementary files DS2 DS6 and DS10.

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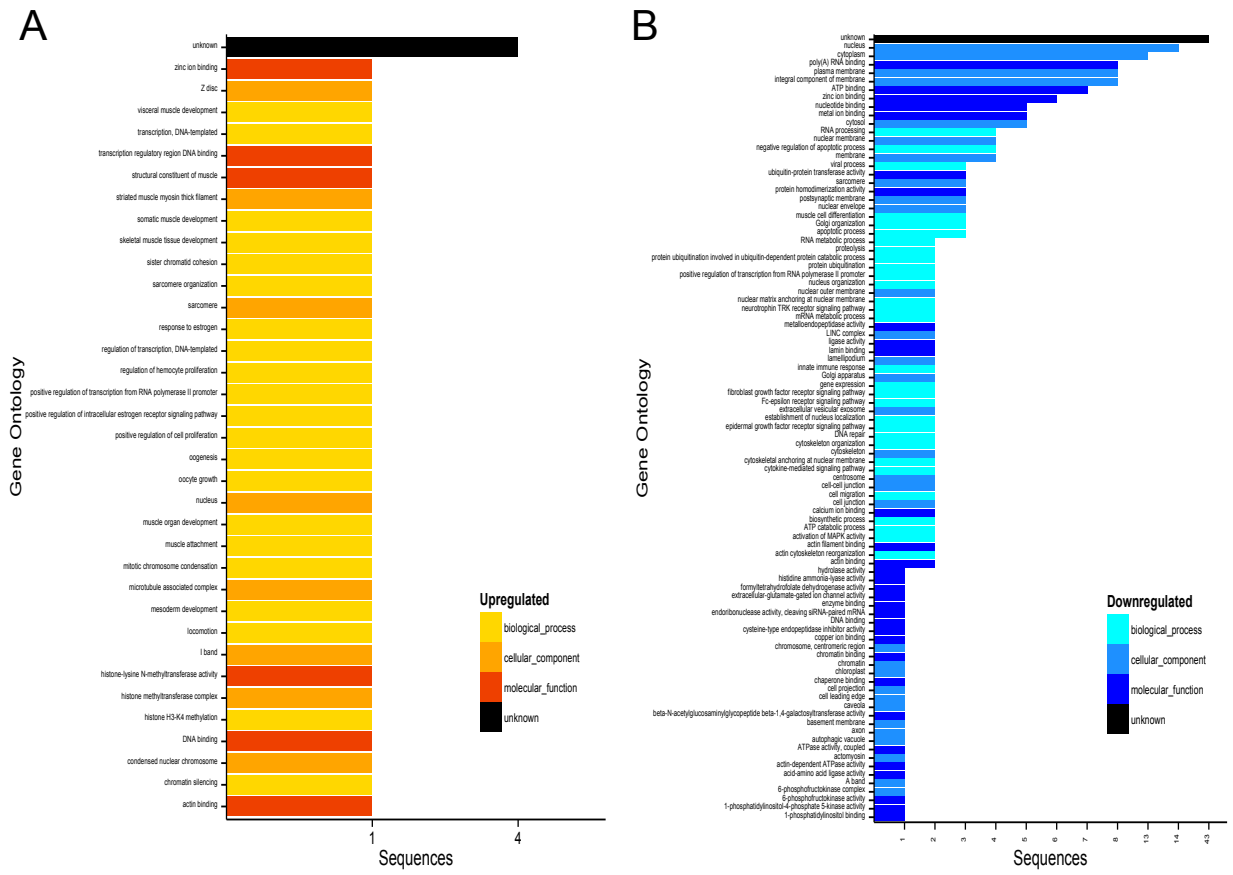

Fig. S6. Gene ontology annotations for upregulated isoforms from edgeR in oil-dispersant (OD). Colors in bar represent major upregulated (A; warm colors) and (B; cool colors) downregulated GO terms (see legend). X-axis indicates element count found in the database. Note last scale number is adjusted to the largest count value. GO detailed functions are arranged in reversed count number and then alphabetical order. Colors correspond to heatmaps in Figures 3, Extended Data Fig. S4. For full annotations see supplementary files DS3, DS7 and DS11.

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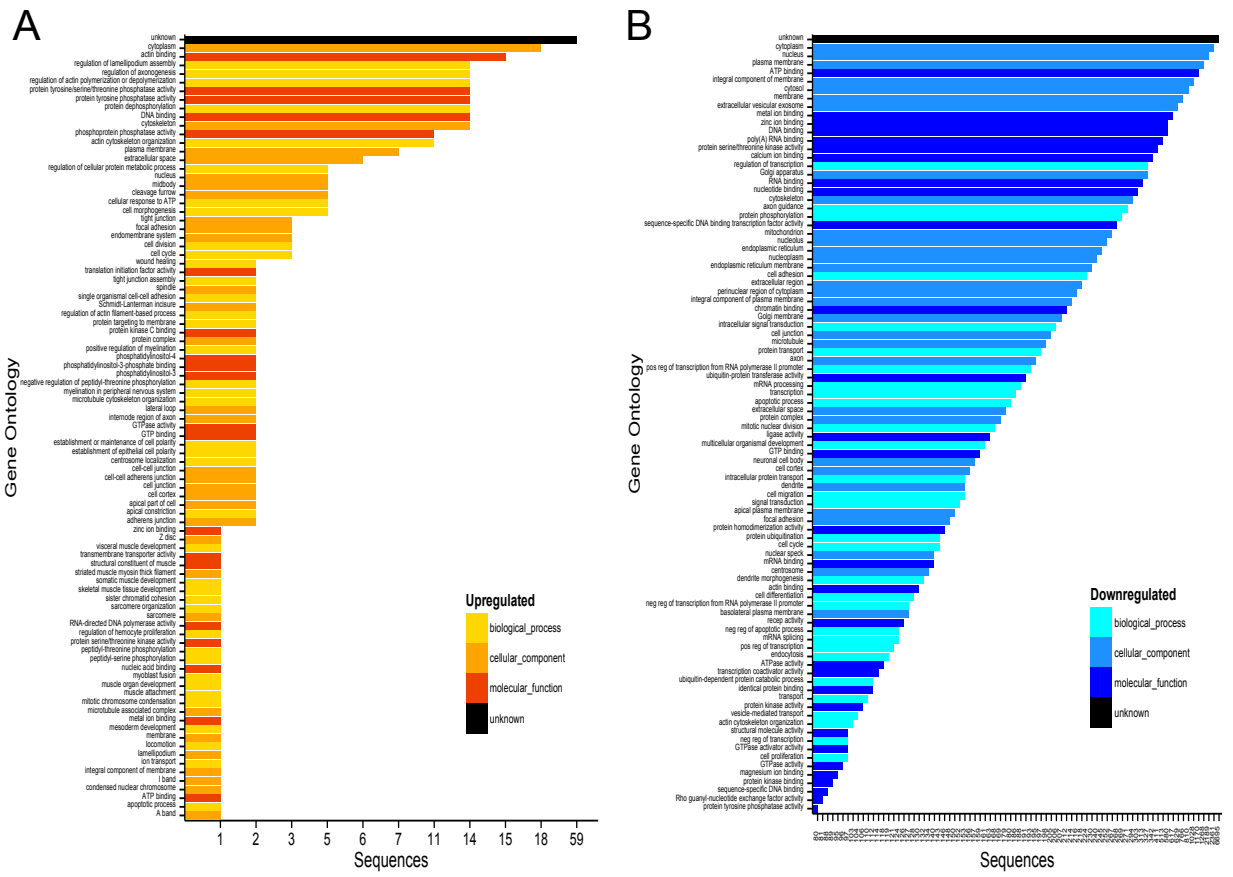

Fig. S7. Gene ontology annotations for genes from DESeq2 in oil-dispersant (OD). Colors in bar represent major upregulated (A; warm colors) and (B; cool colors) downregulated GO terms (see legend). X-axis indicates element count found in the database. Note last scale number is adjusted to the largest count value. GO detailed functions are arranged in reversed count number and then alphabetical order. Colors correspond to heatmaps in Figures 3, Extended Data Fig. S4. For full annotations see supplementary files DS4, DS8 and DS12.

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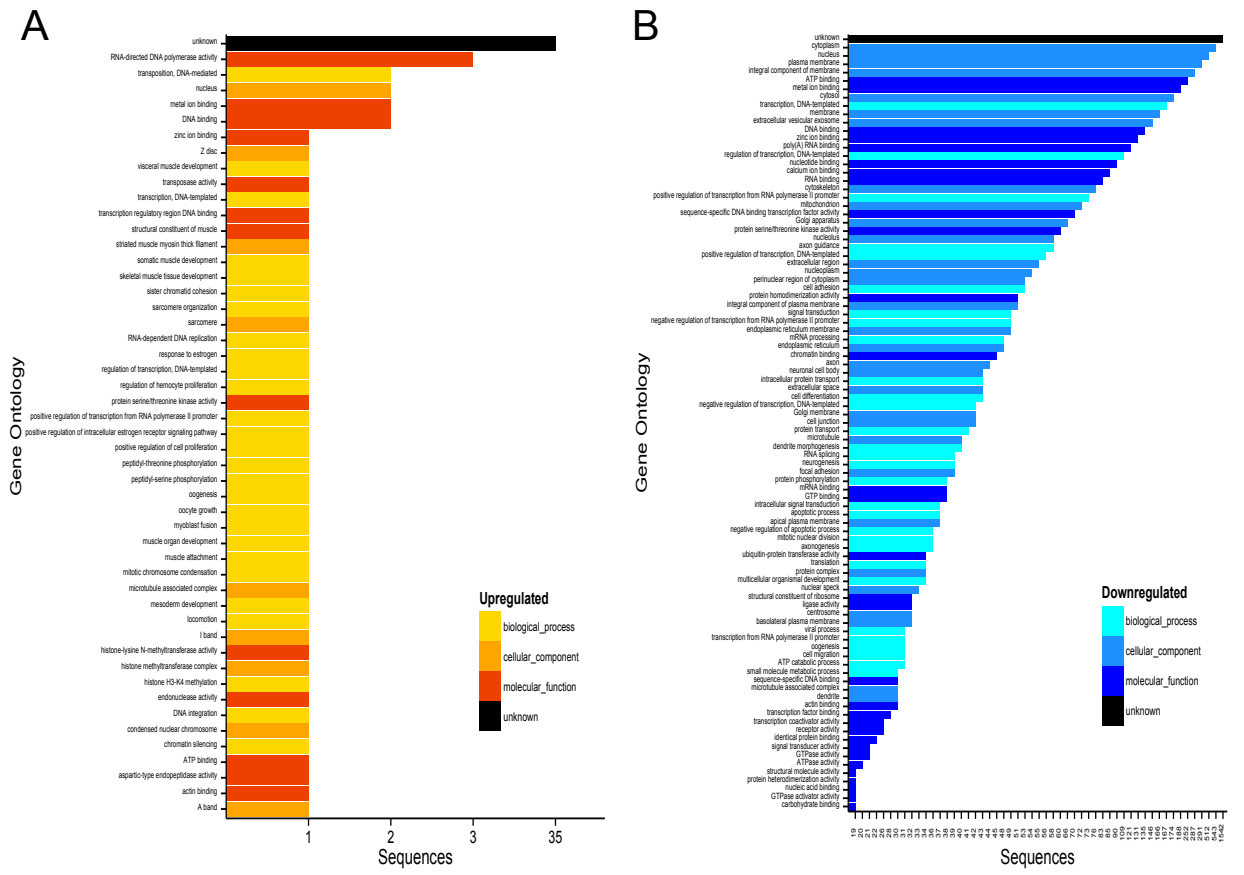

Fig. S8. Gene ontology annotations for upregulated isoforms from DESeq2 in oil-dispersant (OD). Colors in bar represent major upregulated (A; warm colors) and (B; cool colors) downregulated GO terms (see legend). X-axis indicates element count found in the database. Note last scale number is adjusted to the largest count value. GO detailed functions are arranged in reversed count number and then alphabetical order. Colors correspond to heatmaps in Figures 3, Extended Data Fig. S4. For full annotations see supplementary files DS5, DS9 and DS13.

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Supplementary information databases. Files and spreadsheets available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org/pelagos-symfony/dataset-submission?regid=R4.x257.228:0013). (DOI:10.7266/N71C1TZC.)

DS1 – Transcriptome assembly in Trinity format and spreadsheet with complete transcriptomic annotations

DS2 – spreadsheet with significant regulated genes from edgeR

DS3 – spreadsheet with significant regulated isoforms from edgeR

DS4 – spreadsheet with significant regulated genes from DESeq2

DS5 – spreadsheet with significant regulated isoforms from DESeq2

DS6 – spreadsheet with upregulated genes' GOs from edgeR

DS7 – spreadsheet with upregulated isoforms' GOs from edgeR

DS8 – spreadsheet with upregulated genes' GOs from DESeq2

DS9 – spreadsheet with upregulated isoforms' GOs from DESeq2

DS10 – spreadsheet with downregulated genes' GOs from edgeR

DS11 – spreadsheet with downregulated isoforms' GOs from edgeR

DS12 – spreadsheet with downregulated genes' GOs from DESeq2

DS13 – spreadsheet with downregulated isoforms' GOs from DESeq2

DS14 – spreadsheet collection of pairwise comparisons of feature counts ranked by log2 fold change for DESeq2 and edgeR

DS15 – spreadsheets with GO term enrichment analyses with GOseq, based on gene count matrices from edgeR and DESeq2

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References

Knowles JR. 1980. Enzyme-catalyzed phosphoryl transfer reactions. *Annual review of biochemistry* 49:877-919.

Mellors Aa, and Tappel A. 1966. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *Journal of Biological chemistry* 241:4353-4356.

Pompella A, Visvikis A, Paolicchi A, De Tata V, and Casini AF. 2003. The changing faces of glutathione, a cellular protagonist. *Biochemical pharmacology* 66:1499-1503.

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