Gene expression and stress response of the flatback mud crab Eurypanopeus depressus





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Following the Deepwater Horizon oil spill (DWH), the National Oceanic and Atmospheric Administration (NOAA) and the United States Geological Survey (USGS) reported that an estimated 205.8 million gallons of oil had been spilled, 8% of which was dispersed chemically (Lubchenco et al. 2010). Dispersant increases oil emulsification and therefore oil exposure to organisms living below the ocean's surface (Jernelöv 2010). Little is known of the physiological affects of oil and dispersant on marine organisms and the resulting ecological and environmental impacts (Perkins et al. 2005). Here, we used gene expression studies to characterize the effects of the DWH on decapod communities in the Gulf of Mexico. Flatback mud crabs, *Eurypanopeus depressus* (Figure 1), were collected from unaffected sites (Figure 2) and experimentally exposed to Macondo crude oil, as



Results

In an attempt to optimize RNA quality and quantity, RNA extractions were conducted with a variety of extraction kits and protocols (see Methods). Nevertheless, the Bioanalyzer results consistently showed degradation of total RNA in even the highest quality samples (Figure 4). It should be noted that the total RNA electropherograms also showed a lack of a 28S peak. This may be explained, in part, by a heatdenaturation step that is conducted prior to Bioanalyzer analysis, which fragments 28S rRNA along an endogenous "hidden break" sometimes found in arthropods, including decapod crustaceans (Winnebeck et al. 2010; Towle and Smith 2006). This results in 2 smaller 28S fragments, 1 or both of which may fall near the 18S peak. Total RNA concentrations that were measured using a NanoDrop were between 13.2 to 139.9 ng/ μ l for all samples.

Figure 1. Flatback mud crab, *Eurypanopeus depressus*.

as well as to combined oil and dispersant under laboratory conditions (Table 1). Analyses of next-generation RNA-Seq data are ongoing to identify and characterize genes that are differentially expressed, as well as quantify gene expression across treatment and control samples and identify rare transcripts that are correlated with hydrocarbon exposure and stress response. In addition to characterizing the genetic response of *E. depressus*, this study will be placed into the broader context of other crustacean communities and serve to identify the candidate genes suitable for further investigation in other invertebrate systems.



Figure 2. DWH oil spill area and sample collection site.

Figure 4. Electropherograms of total RNA preparations from muscle tissues of *E. depressus* produced by an Agilent 2100 Bioanalyzer using a Eukaryote Total RNA Pico Series II Chip System. There are 3 biological replicates per treatment.



Quantification of cDNA library concentrations using Qubit resulted in slightly low values between 9.58 to 39.4 ng/ μ l. However, these concentrations fall well within the library sample submission requirements for Beckman Coulter Genomics, which requires between 1 to 100 ng/ μ l for sequencing on the Illumina platform (beckmangenomics.com). Library fragment sizes range from 229 to 935 bp (Figure 5). This falls within the desired range of 100 to 1000 bp following fragmentation (Meyer et al. 2011; Meyer et al. 2013). Unfortunately, gel electrophoresis of cDNA libraries indicate that fewer than half of the libraries were of good quality, which is made evident by the absence of uniform smears (Islam et al. 2012) (Figure 5). However, average fragment sizes range from 273 to 460 bp in length. Size selection of libraries is recommended to be between 200-600 bp for sequencing on the Illumina platform, so cDNA libraries may prove adequate for future sequencing (Meyer et al. 2013; Islam et al. 2012).

Methods

Flatback mud crabs (*Eurypanopeus depressus*) were collected from areas not directly affected by the Deepwater Horizon oil spill (Figure 2). All specimens were collected at the same time and from the same area. After acclimating for 3 days in purified seawater, specimens were transferred into 1 of 4 possible treatments

Table 1. Experimental treatments of *Eurypanopeus depressus*.

Control	Treatment 1	Treatment 2	Treatment 3
Aerated	Not aerated	Not aerated	Not aerated
No Oil	No Oil	Oil	Oil
No dispersant	No dispersant	No dispersant	Dispersant
	H20 Only	Oil	Oil +

(Table 1). There were 4 experimental replicates of each treatment with 3 biological replicates in each experimental replicate (a total of 12 samples per treatment). Samples were harvested after 72 hours of exposure and placed immediately into liquid nitrogen. Specimens were then thawed and dissected in RNAlater Ice (Figure 3). Muscle, gill, and hepatopancreas tissues were preserved in RNAlater Ice at -80°C until RNA was extracted. Total RNA was extracted using the Qiagen RNeasy[®] Mini Kit, the Macherey-Nagel Nucleospin[®] RNA kit, and the Life Technologies PureLink[™] RNA Mini Kit. Total



RNA quality and quantity was verified using an Agilent 2100 BioAnalyzer and 12 samples of RNA extracted from muscle tissue was chosen for sequencing. Samples were sent to the Vanderbilt University Core Lab for sample preparation using the Epicentre Ribo-Zero[™] rRNA Removal Kit and cDNA library generation using the Illumina TruSeq Stranded Library kit. Library concentrations were verified with a Qubit[®] Fluorometer and library sizes were verified on an Agilent BioAnalyzer. Libraries were then sent to Beckman Coulter Genomics for RNA sequencing on an Illumina HiSeq Platform with an aim of 28 million reads per sample to quantify gene expression levels. *De novo* assembly will be conducted followed by a differential expression analysis. **Figure 5.** Electropherograms of cDNA libraries produced by an Agilent 2100 Bioanalyzer. The 2 large peaks at the beginning and end of each curve represent the lower and upper markers for quantitation.

Next Steps and Future Studies

The cDNA libraries are currently at Beckman Coulter Genomics awaiting further processing. Quantitative PCR (qPCR) of the libraries as well as spike-in quality control are expected to be conducted in the next few weeks followed by sequencing on an Illumina HiSeq Platform. Therefore, transcriptome assembly and differential expression analysis is currently pending. Within the next few months, we intend to identify genes involved in the response to crude oil and combined crude oil and dispersant exposure as well as to quantify the differential expression of these genes.

Figure 3. Dissection of sample in RNAlater Ice.

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