Toxicity of oil and dispersant on the deep water gorgonian octocoral *Swiftia exserta*, with implications for the effects of the Deepwater Horizon oil spill

Janessy Frometa[1,*], Marie E. DeLorenzo[2], Emily C. Pisarski[1], Peter J. Etnoyer[3]

[1]JHT, Inc., 219 Fort Johnson Rd., Charleston, SC 29412, USA

[2] NOAA Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Rd., Charleston, SC 29412, USA

[3] NOAA Center for Coastal Monitoring and Assessment, 219 Fort Johnson Rd., Charleston, SC, USA

[*]Corresponding author. Email: Janessy.frometa@noaa.gov; 219 Fort Johnson Rd., Charleston, SC, USA

Abstract

Benthic surveys of mesophotic reefs in the Gulf of Mexico post Deepwater Horizon (DWH) showed that *Swiftia exserta* octocorals exhibited significantly more injury than in years before the spill. To determine the vulnerability of *S. exserta* to oil and dispersants, 96 h toxicity assays of surrogate DWH oil water-accommodated fractions (WAF), Corexit® 9500 dispersant, and the combination of both (CEWAF) were conducted in the laboratory. Fragment mortality occurred within 48 h for some fragments in the dispersant-alone and oil-dispersant treatments, while the WAF group remained relatively unaffected. The 96 h LC_{50} values were 70.27 mg/L for Corexit-alone and 41.04 mg/L for Corexit in CEWAF. This study provides new information on octocoral sensitivity to toxins, and indicates that combinations of oil and dispersants are more toxic to octocorals than exposure to oil alone. These results have important implications for the assessment of effects of the DWH spill on deep-water organisms.

Keywords: Deepwater Horizon, Anthozoa, Corexit, deep-sea coral, Gulf of Mexico, Median lethal toxicity

© 2017. This manuscript version is made available under the Elsevier user license
http://www.elsevier.com/open-access/userlicense/1.0/

1. Introduction

The Deepwater Horizon (DWH) oil spill was unique compared to historical oil spills, which have almost exclusively occurred at the surface or in shallow depths of nearshore waters, thereby simplifying the fate, transport and exposure pathways of oil to surrounding ecosystems (Peterson et al., 2012). In contrast, the blowout of the Macondo wellhead occurred in deep (1500 m), offshore waters and led to a variety of dispersed phases, including small oil droplets, gas bubbles, insoluble oil-gas mixtures and gas hydrates (Peterson et al., 2012), which led to multiple deep-water plumes (Camilli et al., 2010) and large surface slicks (NOAA, 2016). While half of the oil rose to the sea surface, the rest remained in the deep ocean (McNutt et al., 2012, Bagby et al., 2016).

Efforts to contain the spread of oil included the unprecedented injection of 0.77 million gallons of chemical dispersant (Corexit 9527 and Corexit 9500A) directly into the subsurface flow of oil at depth. An additional 1.4 million gallons were applied at the surface by airplane and small vessels over the course of two months (Hemmer et al., 2011; Kujawinski et al., 2011; Peterson et al., 2012). It is known that surface dispersants lead to the eventual degradation or dissolution of oil (Kujawinski et al., 2011). Prior to the DWH spill, dispersants had not been applied in deep water; very little is known regarding the efficacy of dispersants applied to oil at depth. Concentrations of dioctyl sodium sulfosuccinate (DOSS), one of the key ingredients of the spill dispersant, were elevated >1000-fold in surface sediments near the well (White et al., 2014; Bagby et al., 2016). A recent study found that these dispersants persist in subsurface oil-associated environments for up to four years (Kujawinski et al., 2011; White et al., 2014), much longer than previously anticipated.

The Pinnacles Trend Reefs is one of many regions of rocky biogenic structures in the Gulf of Mexico along the outer continental shelf between the Mississippi River and Cape San Blas, Florida that occur in the mesophotic zone (50-150 m; Gittings et al., 1992). These reefs represent drowned remnants of shallow-water coral reefs that formed over 18,000 years ago prior to rises in sea levels (Continental Shelf Associates and Texas A&M University, 2001). Today these reefs host important and diverse ecosystems of invertebrates (Gittings et al., 1992, Continental Shelf Associates, Inc. and Texas A&M University, 2001; Weaver et al., 2002; Lesser et al., 2009) and commercially valuable fish (Dennis and Bright, 1988; Weaver et al., 2002; Silva et al., 2016). Among the dominant invertebrate groups present at the reefs are sessile azooxanthellate, gorgonian sea fans (Continental Shelf Associates, Inc. and Texas A&M University, 2001; Silva et al., 2016). These heterotrophic, suspension feeders rely on the nutritional input from the surface (Sulak et al., 2008) and are therefore especially vulnerable to pollution.

After the DWH event, buoyant oil formed large surface slicks that spanned up to 40,000 km^2 of the Gulf of Mexico, and dispersed via winds, currents, and tides (NOAA, 2016). Several reefs in the 60-90 m depth range were situated under the oil slick for a period of 24-45 days (Figure 1; NOAA, 2014; Etnoyer et al., 2016). Post-spill surveys of these reefs showed that large octocoral colonies below the oil slick exhibited significantly more injury than in years before the spill, with 30-50% of large sea fans being injured (Etnoyer et al., 2016; Silva et al., 2016). Other studies in this region found order of magnitude declines in the abundance of demersal reef fish (Sulak and Dixon, 2015), polycyclic aromatic hydrocarbons (PAH) in coral tissues and surrounding sediments (Silva et al., 2016), and traces of DWH oil in sediments and water at nearby reefs (NOAA, 2016).

Isotopic depletion of carbon found in the mesoplankton and other suspended particulate matter suggested that fractions of DWH oil were transported northward in the shallow-water column, and that the oil carbon was transferred at least two trophic levels beyond primary consumers, thereby having substantial effects across the food web (Graham et al., 2010). When oil-derived marine snow becomes part of the pelagic food web, it becomes an important food source for zooplankton, fish and benthic organisms, such as heterotrophic corals (Passow et al., 2012).

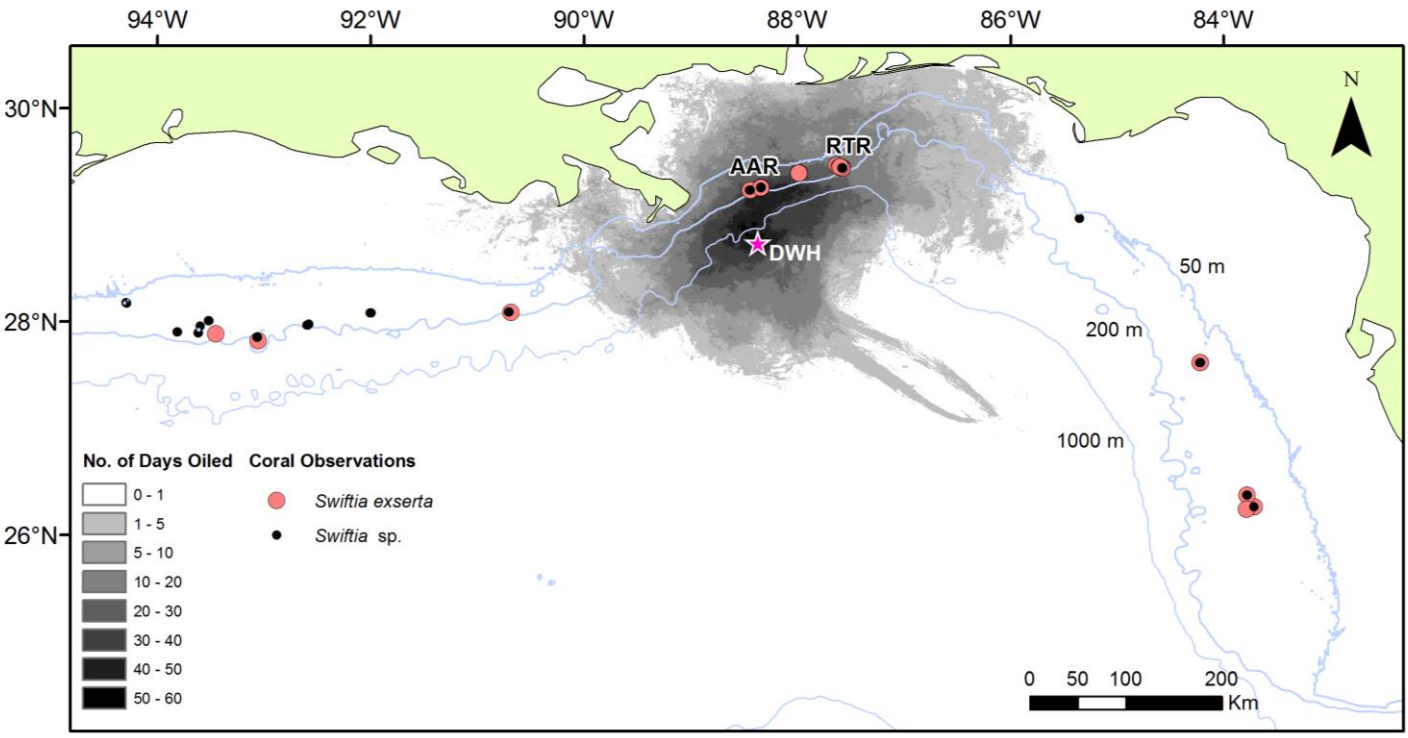

Figure 1. Map of surface oil slick following the DWH spill in the Pinnacles Trend Region, including the Alabama Alps Reef (AAR), and Roughtongue Reef (RTR), two mesophotic reefs located below the slick with documented injuries to *Swiftia exserta* colonies subsequent to the oil spill.

Although the presence of oil and dispersant was likely the cause of injury to sea fans in the Pinnacles Trend region, at the time limited information existed on toxicity thresholds for sessile heterotrophic corals, such as gorgonian octocorals. Some studies have shown that oil

exposure has detrimental effects on the growth, reproduction, feeding and other behavioral responses of heterotrophic corals (Loya and Rinkevich, 1980; Suchanek, 1993; Epstein et al., 2000; Goodbody-Gringley et al., 2013). In some cases the combination of dispersants and oil has been shown to be more toxic to corals than exposure to oil alone (Nelson-Smith, 1973; Epstein et al., 2000; Goodbody-Gringley et al., 2013; Rico-Martinez et al., 2013; DeLeo et al., 2016).

The primary objective of this study was to examine the acute (96 h) toxicity effects of surrogate DWH oil water-accommodated fractions (WAF), Corexit 9500 dispersant, and the combination of the two, known as a chemically-enhanced WAF (CEWAF), to *Swiftia exserta* (Ellis and Solander, 1786) in the laboratory. *S. exserta*, which occurs throughout the West Atlantic at depths between 10-200 m (Goldberg, 2001), is attainable through the aquarium trade. In the Pinnacles Trend Reefs, 40-50% of observed *Swiftia* spp. corals exhibited intermediate to severe injury post-spill, including overgrowth by hydroids, covering by sediment, and broken or bare branches (Figure 2; Etnoyer et al., 2016; Silva et al., 2016).

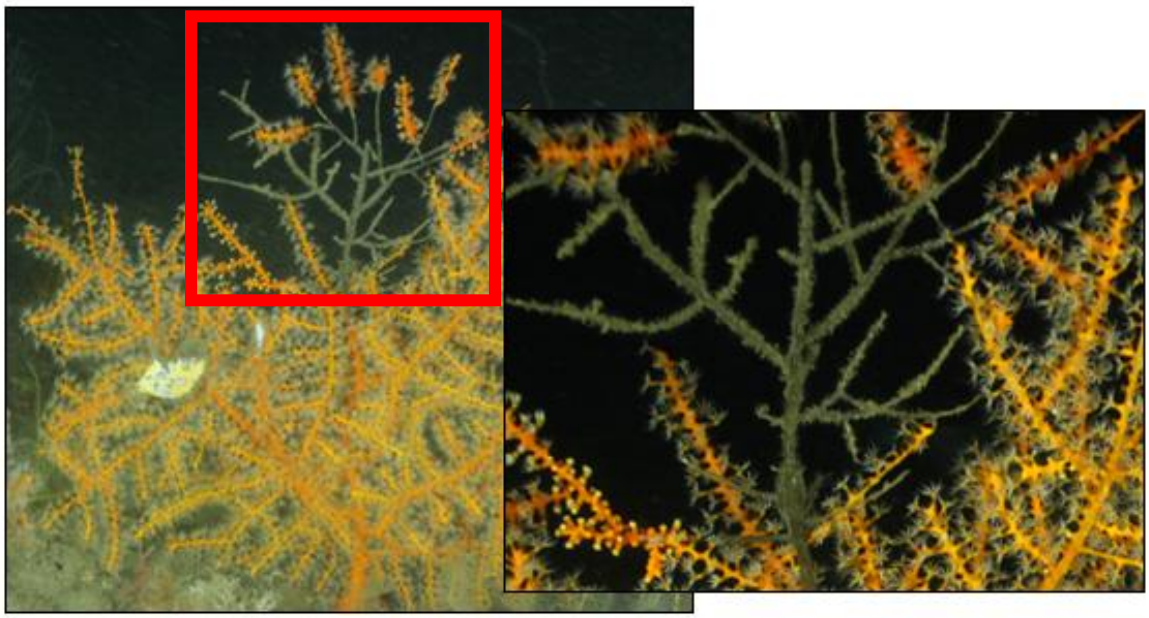

Figure 2. Close-up of unidentified *Swiftia* sp. colony at Alabama Alps Reef, showing large area of dead tissue surrounded by relatively healthy tissue. Polyps also show damage. Image credit: NOAA.

2. Methodology

2.1. Coral Collection and Maintenance

Live *S. exserta* colonies were collected off the southeast coast of Florida at 20-30 m using SCUBA, and shipped to Charleston, SC by Dr. Henry Feddern (Tavernier, FL, USA). Upon receipt, corals were slowly acclimated to seawater [filtered seawater from Charleston Harbor, SC, USA with artificial sea salts to raise salinity (Instant Ocean or Reef Crystals); 36 ‰; 19°C] in holding tanks, by replacing 10% of shipping water every 15 minutes. After approximately 4 h, whole colonies were transferred to a 150-gallon closed-system aquarium composed of a 130-gallon holding tank, small sump, protein skimmer, and small refugium. The refugium contained green macroalgae (*Chaetomorpha* spp.) to filter out nitrates and phosphates. Water quality was maintained using gravel biofiltration and protein skimmers, and continuously monitored. Corals were fed a combination of frozen brine shrimp (Hikari Sales USA, Hayward, CA), rotifers (Hikari Sales USA, Hayward, CA), cyclops copepods (San Francisco Bay Brand, Newark, CA, USA), and microblends of phytoplankton and zooplankton, 3-4 times a week.

2.2. Preparation of Chemical Treatments

2.2.1. Oil Water-accommodated Fractions (WAFs)

Water-accommodated oil fraction (WAF) and oil-dispersant chemically-enhanced WAF (CEWAF) treatments were prepared using the method of Hemmer et al. (2011) with some modification. Briefly, the full-strength WAF treatment was made in an aspirator bottle with a Teflon stir bar sitting at the bottom. The bottom outlet of the bottle was closed with Tygon

tubing and a glass stopper. Seawater (19 L, 36‰) was added to the aspirator bottle and stirred using a magnetic stir plate to create a small vortex. While stirring, 25 g/L of Louisiana Sweet Crude Oil (LSC) was added using a graduated cylinder. The final amount added into the solution was calculated using the difference in weight of the graduated cylinder before and after adding oil.

Stirring speed was then increased to create a vortex reaching approximately 25% of the height of the solution. The solution was stirred for 18 h and allowed to sit for 6 h. The glass stopper was then removed from the Tygon tubing and the solution under the top layer of oil slick was dispensed into a glass collection container. Dilutions of the full-strength solution (100%) were made with seawater, in order to achieve concentrations of 100%, 50%, 16.67%, 5.56%, and 1.85% WAF.

2.2.2. Chemically-enhanced Oil Water-accommodated Fractions (CEWAFs)

The CEWAF treatment was prepared in the same manner as the WAF treatment except for the addition of 1.25 g/L Corexit 9500 in order to achieve a 20:1 oil to dispersant solution. After mixing and stirring, the solution was dispensed into a collection container and dilutions were made with seawater in order to achieve concentrations of 100%, 50%, 16.67%, 5.56%, and 1.85% CEWAF.

2.2.3. Corexit 9500 Dispersant-Only

To test the dispersant alone, seawater and Corexit 9500 were combined in 1 L glass cylinders to produce five testing concentrations: 100, 50, 25, 12.5, 6.25 mg/L. The treatments were then poured into 1 L glass beakers.

2.3. Toxicity assays

2.3.1. Experimental Design and Fragmentation

Three short-term (96 h) toxicological assays were conducted in filtered seawater (FSW): (1) crude oil water-accommodated fractions (WAFs), (2) chemical dispersant alone (Corexit 9500A), and (3) the chemically-enhanced WAF (CEWAF). Four *S. exserta* colonies were cut into six smaller fragments of approximately 30-50 polyps and attached with super glue to small glass pegs with a drilled hole in the center (Figure 3). Within each assay, fragments were placed in one of six groups (control and five experimental treatments). The control group contained seawater alone. Experimental treatments were selected based on preliminary range-finding tests. Each treatment group contained four fragment replicates from four distinct colonies.

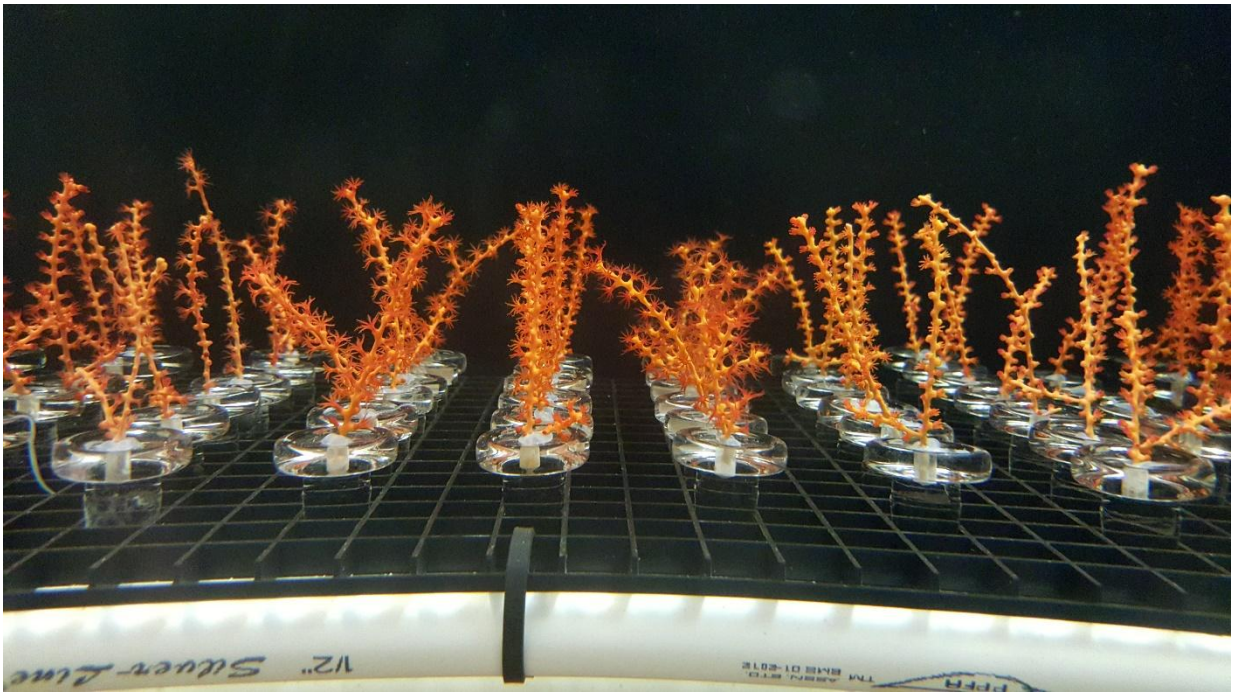

Figure 3. Image of healthy fragments of *Swiftia exserta* in aquaria prior to laboratory toxicity assays.

2.3.2. Testing Conditions

Individual fragments were placed in 1 L glass beakers (one fragment per beaker) containing 1 L of the treatment solution; the beakers were covered with aluminum foil to minimize evaporation. The fragments were kept upright by attaching the glass peg to a small plastic grid set at the bottom of the beaker. Exposures were all static and treatment solutions were not renewed except for the Corexit-alone assay, in which the treatment was renewed every 24 h to maintain dispersant concentrations and allow comparison to LC_{50} values determined for other species using the static-renewal method (DeLorenzo et al., in journal review). Cultures were kept in the dark at 19°C and 36‰ salinity with aeration.

2.4. Health Scoring

Coral fragments were photographed and their health was assessed at five time points throughout the assay (0 h, 24 h, 48 h, 72 h and 96 h). Health scores were based on methods developed by DeLeo et al. (2016) and modified for *S. exserta* based on prior observations while caring for the whole live colonies. Health scores (0-5) were assigned to each fragment based primarily on the percentage of live polyps and tissue remaining as follows: 4 or 5 = fragments with >50% live polyps and tissue, 3 = ~50% live polyps and tissue, 1-2 = <50% live polyps and tissue, and a score of zero was given to dead fragments with no remaining live polyps or tissue. Other signs of stress, which further refined scores of 1 or 2 and 4 or 5, were polyp retraction, mucus production, tissue sloughing, tissue discoloration and necrotic tissue.

2.5. Analytical Chemistry

2.5.1. Hydrocarbons

Water samples for all treatments (composite of all replicates) from the WAF and CEWAF assays were collected immediately after dosing (time = 0 h) and analyzed for total extractable hydrocarbons (TEH) and total PAH using methods from Reddy and Quinn (1999) and modified by DeLorenzo et al. (in journal review). Briefly, samples were acidified to pH 2, extracted via liquid/liquid extraction with dichloromethane and hexane, followed by clean-up with silica solid phase extraction. Samples were analyzed using gas chromatography mass spectrometry (GC/MS-Agilent 6890/5973N). The GC/MS contained a DB17ms analytical column (60 m x 0.25 mm x 0.25 um) and was operated in selected ion monitoring mode.

2.5.2. DOSS (dioctyl sodium sulfosuccinate)

Samples from the CEWAFs and Corexit-only treatments were analyzed for the concentration of DOSS, one of the key ingredients of the dispersant. After the initial 24 hours of the dispersant-only test, water samples were again collected for DOSS analysis, to quantify change in concentration, if any, within the first 24 h of the assay. Methods for the DOSS extraction followed those of Flurer et al. (2010) with a few modifications. Water samples were first diluted to calibration ranges. Samples were extracted using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe; AOAC Method 2007.01) and then filtered, concentrated, and analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS-Agilent 1100 HPLC/API 4000 Mass Spectrometer, equipped with electrospray ionization, operated in negative mode) on a C18 analytical column (2.5 µm, 2.1 x 50 mm).

2.6. Statistical Analyses

Health scores for each of the three assays were averaged for each treatment (control and experimental) at each of the five exposure times. To test for differences from the control at 96 h, Kruskal-Wallis tests were performed (JMP 12.1.0, SAS Institute, Cary, NC, USA) followed by Wilcoxon pairwise comparisons when results were significant ($p < 0.05$).

Where mortality was observed at the end of the 96 h assay, nominal and measured (using chemistry values at t = 0) median lethal toxicity concentrations (LC_{50}) and the concentrations to cause 10% lethality (LC_{10}) were calculated using SAS Probit analysis (PROC PROBIT, SAS 9.4, SAS Institute, Cary, NC, USA). For the Corexit-only and CEWAF tests, LC_{50} of Corexit was also calculated using measured DOSS chemistry values (17% DOSS in Corexit 9500, determined by mass).

3. Results

3.1. Exposure effects

3.1.1. Oil water-accommodated fractions (WAFs)

In the oil WAF assay, there was only one unexpected case of fragment death. This was observed in the 1.85% WAF group after 72 h, and this fragment was likely stressed before the experiment. However, even when that observation was included in statistical analyses, there were no significant differences ($p > 0.05$) among treatments at any time point (Figure 4). After 96 h, all fragments exposed to 100% WAF had their polyps fully retracted, and all fragments,

minus the one in 1.85% WAF, had a health score of 4 or higher. After 96 h, some fragments in experimental groups looked healthier than some in the control group (Figure 5).

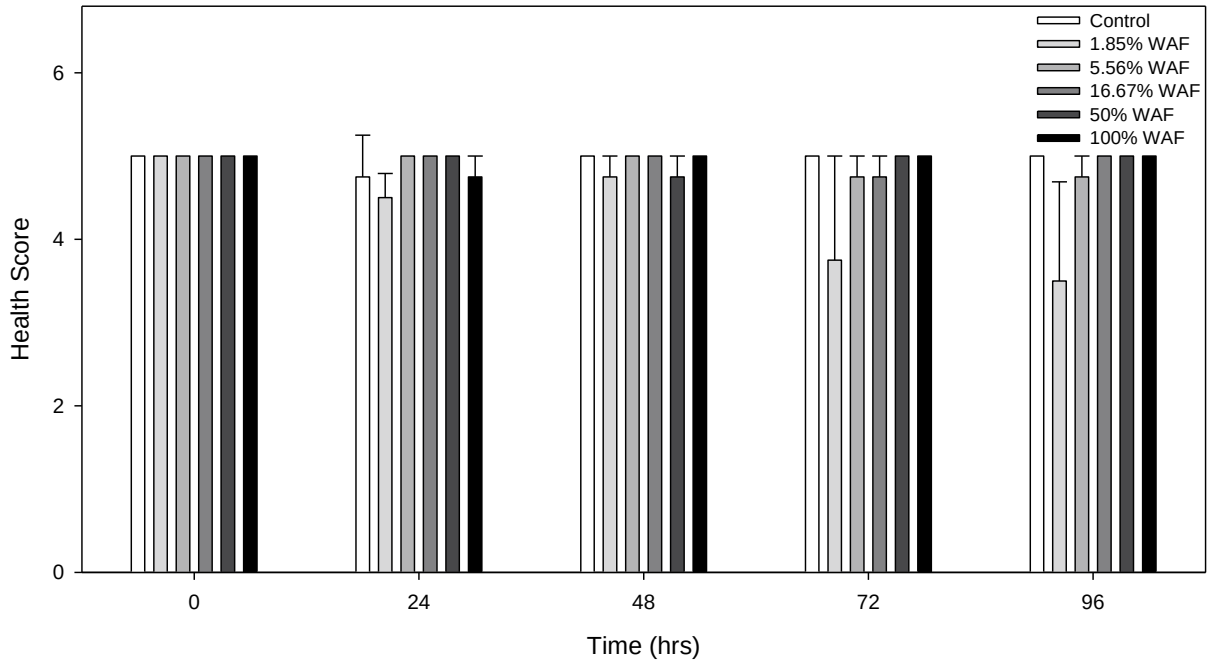

Figure 4. A plot showing results of *Swiftia exserta* exposure to LSC oil water-accommodated fractions (WAF). There was no significant difference among treatments (Kruskal-Wallis $p > 0.05$) at any time point.

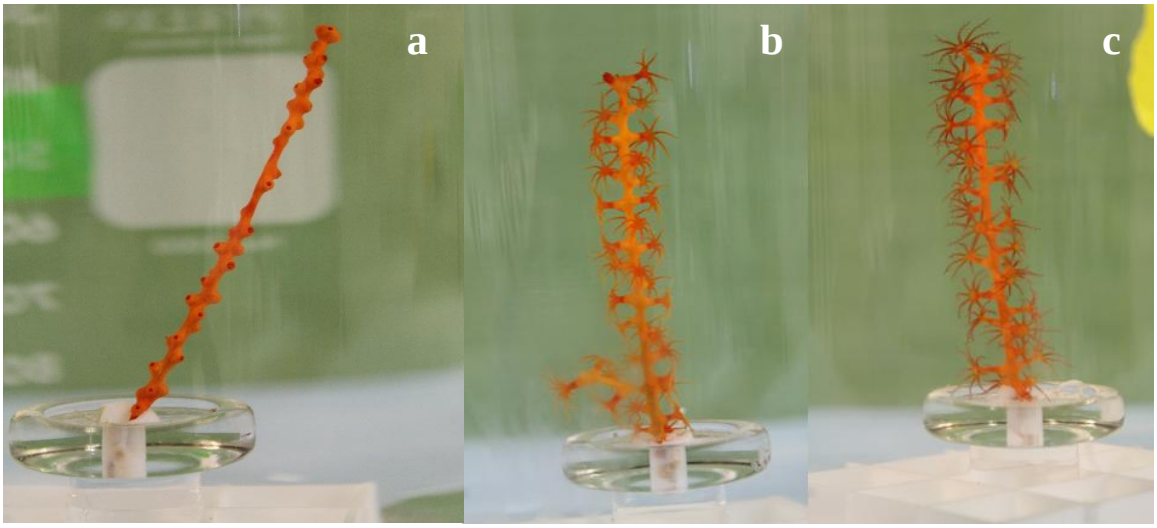

Figure 5. Images of *Swifta exserta* octocoral fragments in **a)** control group after 96 h duration, **b)** exposed to 1.85% water-accomodated fraction (WAF) for 96 h, and **c)** exposed to 16.67% WAF for 96 h. There were no significant differences amongst the treatments, other than retraction of polyps.

3.1.2. Corexit 9500 Dispersant

Severe health declines (health score ≤ 3) were first observed after 24 h in the 50 and 100 mg/L treatments (Figure 6). These corals exhibited tissue sloughing or loss but polyps kept their red color (Figure 7). Complete mortality (100% of all replicates in a group), was observed after 48 h in the fragments exposed to 100 mg/L Corexit. After 96 h, partial mortality (1 of 4 fragments) was observed in the group exposed to 50 mg/L, but fragments that were exposed to doses < 50 mg/L all retained a health score ≥ 4. There was a significant effect of treatment at all times except at t = 0 (Kruskal-Wallis p-values < 0.005). After 96 h, the 50 and 100 mg/L treatments were significantly different from the control (Wilcoxon p-values = 0.0256 and 0.0177, respectively).

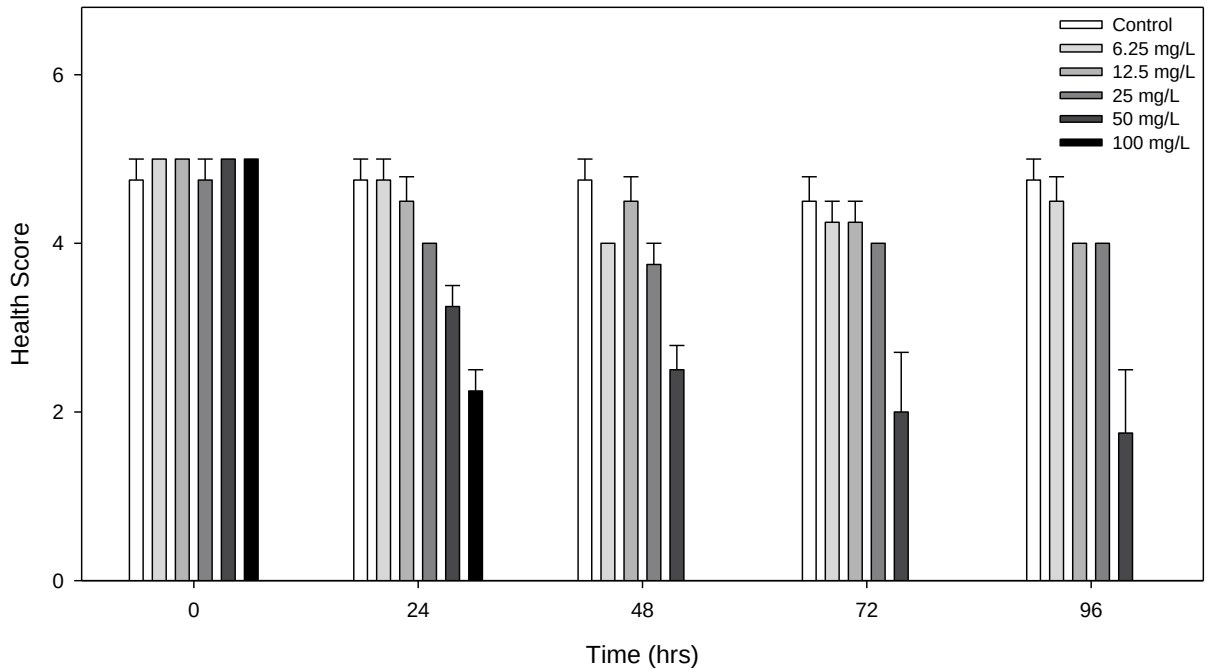

Figure 6. A plot showing results of *Swiftia exserta* exposures to Corexit 9500 dispersant. Severe health declines occurred in the two high-dose groups after 24 h.

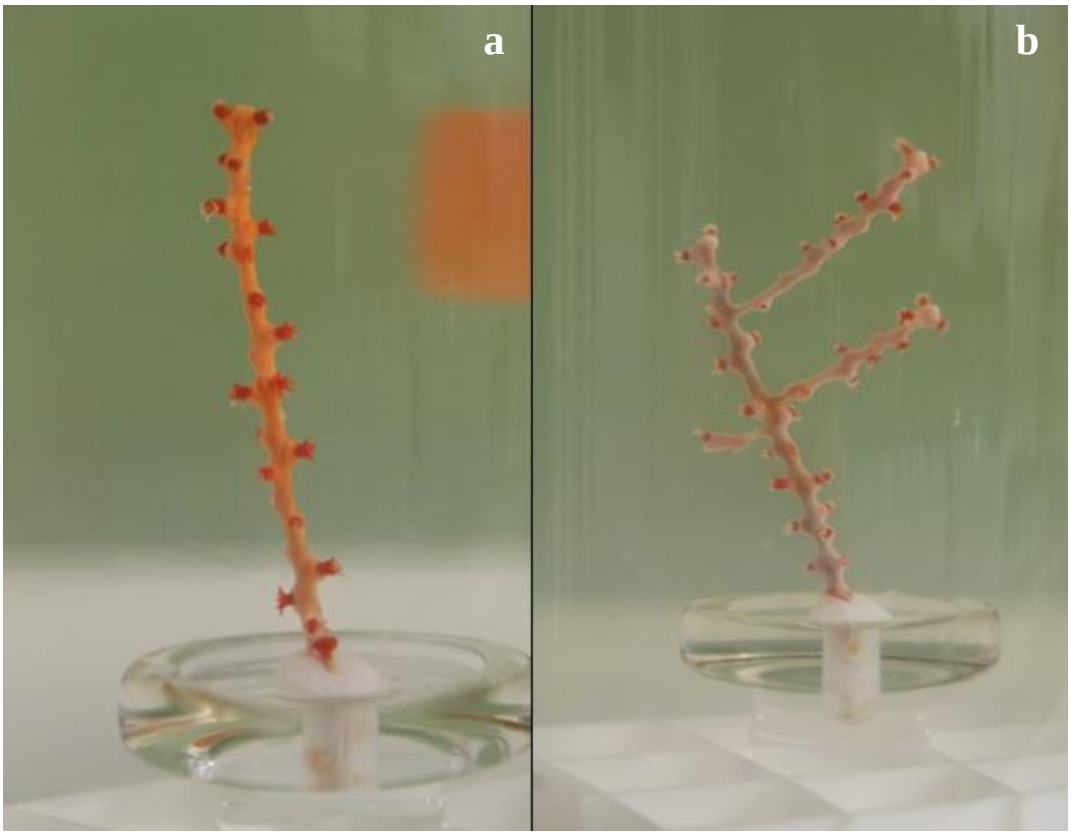

Figure 7. Images of *Swiftia exserta* fragments exposed to **a)** 50 mg/L Corexit for 24 h, and **b)** 100 mg/L Corexit for 24 h.

The nominal-based LC_{50} at 96 h (calculated using a Probit model of the log-transformed concentration data, distribution = Gompertz) was 51.17 mg/L Corexit (95% CI = 48.59, 53.90). The nominal LC_{10} was 48.69 mg/L Corexit (95% CI = 46.23, 51.29). The measured LC_{50} (Probit model, no transformation, distribution = Normal) was 70.27 mg/L Corexit. The measured LC_{10} was 64.23 mg/L Corexit. 95% confidence intervals could not be calculated at 96 h due to lack of partial mortality.

3.1.3. Chemically-enhanced WAF

Partial mortality was observed within 48 h, while complete mortality (all fragments) was observed within 72 h in both the 50 and 100% treatments (Figures 8-9). Control fragments remained healthy (health score ≥ 4), albeit showing minimal signs of stress (polyp retraction)

after 96 h (Figure 8). Significant treatment effects were observed in the CEWAF exposure at all times except t = 0 (Kruskal-Wallis $p < 0.005$). After 96 h, the 50 and 100% CEWAF treatments were significantly different from the control (Wilcoxon p-values = 0.0131 for both treatments).

The nominal-based LC_{50} at 96 h (Probit model, log transformation, distribution = Gompertz) was 45.58% CEWAF and the LC_{10} was 43.79% CEWAF (Table 1). The measured LC_{50} of TEHs (calculated using the Gompertz log model) was 45.86 mg/L and the LC_{10} was 44.31 mg/L the (Table 2). The measured LC_{50} of Corexit in CEWAF (Probit model, log transformation, distribution = Gompertz) was 41.04 and the LC_{10} was 40.28 mg/L Corexit in CEWAF (Table 2). 95% confidence intervals were not estimable.

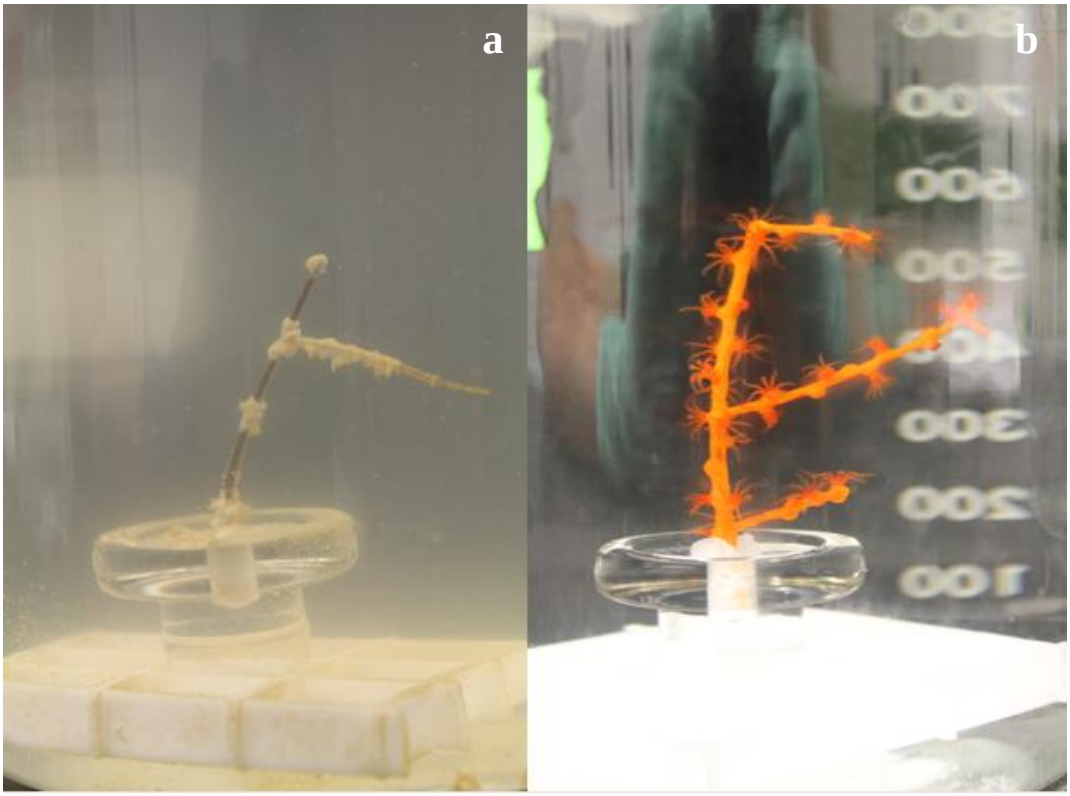

Figure 8. Images of *Swiftia exserta* fragments **a)** exposed to 50% chemically-enhanced WAF (CEWAF) for 48 h, and **b)** in control group after 96 h.

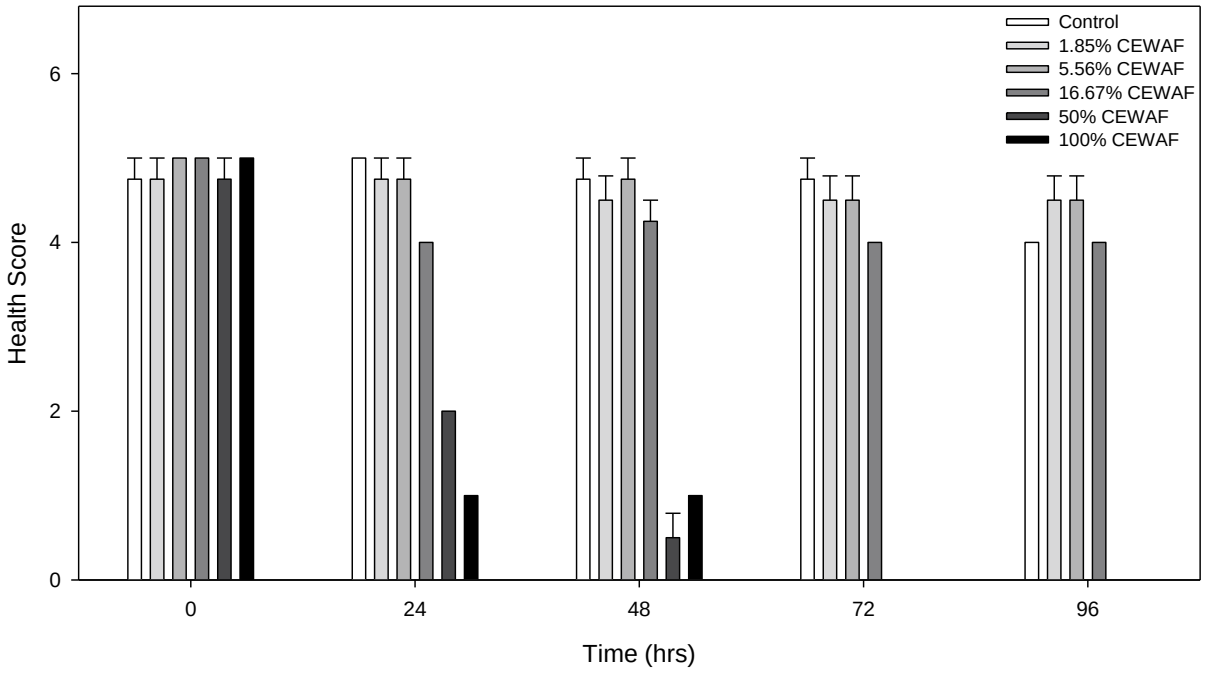

Figure 9. A plot showing results of *Swiftia exserta* exposures to chemically-enhanced WAF (CEWAF). Severe health declines occurred in the two high-dose groups after 24 h.

Table 1. Nominal-based toxicity values of *Swiftia exserta* octocorals after 96 h exposure to Corexit 9500 alone and in chemically-enhanced water-accommodated oil fraction (CEWAF). Confidence intervals for CEWAF treatments could not be calculated due to lack of partial mortality.

Test	**Nominal**				**Model Used**	
	LC_{50}	95% CI	LC_{10}	95% CI	Distribution	Data Transformation
Corexit-only (mg/L)	51.17	48.59-53.90	48.69	46.23-51.29	Gompertz	Log
CEWAF (%)	45.58	---	43.79	---	Gompertz	Log

Table 2. Measured Corexit and total extractable hydrocarbon (TEH) toxicity values (using concentrations at t = 0) of *Swiftia exserta* octocorals after 96 h exposure to Corexit 9500 alone and in chemically-enhanced water-accommodated oil fraction (CEWAF). Confidence intervals could not be calculated due to lack of partial mortality.

Test	Measured				Model Used	
	LC_{50}	95% CI	LC_{10}	95% CI	Distribution	Data Transformation
Corexit-only (mg/L)	70.27	--	64.23	--	Normal	None
CEWAF TEH (mg/L)	45.86	---	44.31	---	Gompertz	Log
CEWAF Corexit (mg/L)	41.04	---	40.28	---	Gompertz	Log

3.2. Measured chemistry concentrations

The total PAH_{50} and TEH concentrations measured in the full-strength (100%) CEWAF were 1.7 and 132 mg/L, respectively (Table 3). These concentrations were significantly higher than those measured in the WAF (total PAH_{50} = 0.28 mg/L; TEH was below detection limit). DOSS values and subsequent calculated Corexit concentrations for the CEWAF and Corexit-alone tests are shown in Tables 4 and 5, respectively. Measured concentrations of Corexit in the CEWAFs were, on average, 116% of the nominal concentrations (Table 4).

Measured Corexit concentrations in the Corexit-alone treatments (at t = 0), were 128% of the nominal, on average (Table 5). Additionally, DOSS concentration, which is only one component of Corexit (17% in Corexit), decreased minimally after the first 24 h of exposure for the 6.25, 12.5, and 100 mg/L doses, but increased in the doses of 25 and 50 mg/L Corexit (Table 5). These minimal decreases in DOSS may be due to chemical adherence to the container or adsorption or uptake by the coral fragment, while increases could be a result of further dissolution in the seawater within the first 24 hours of the dosing.

Table 3. Measured total polycyclic aromatic hydrocarbon (PAH_{50}) and total extractable hydrocarbon (TEH) concentrations oil water-accommodated fraction (WAF) and chemically-enhanced water-accommodated fraction (CEWAF) at t = 0.

Nominal (%)	**Total PAH_{50} (mg/L)**	**TEH (mg/L)**
WAF		
Control	0.001	Less than MDL*
1.85	0.004	Less than MDL*
5.56	0.015	Less than MDL*
16.67	0.044	Less than MDL*
50	0.123	Less than MDL*
100	0.280	Less than MDL*
CEWAF		
Control	0.000	Less than MDL*
1.85	0.056	2.64
5.56	0.123	6.75
16.67	0.151	19.6
50	0.868	49.6
100	1.70	132

*MDL = measured detectable limit (0.25 mg/L)

Table 4. Measured dioctyl sodium sulfosuccinate (DOSS) and Corexit 9500 concentrations in CEWAF treatments at t = 0.

Nominal	**Measured (t = 0)**		
CEWAF (mg/L)	**DOSS (mg/L)**	**Corexit (mg/L)**	**% nominal**
Control	Less than MDL*	Less than MDL*	100
1.85	0.305	1.8	97.0
5.56	1.56	9.18	165
16.67	4.47	26.3	158
50	7.28	42.8	85.7
100	15.4	90.6	90.6

*MDL = measured detectable limit (0.01 mg/L)

Table 5. Measured dioctyl sodium sulfosuccinate (DOSS) and Corexit concentrations in dispersant-only treatments at t = 0 and t = 24 h.

Nominal	**Measured (mg/L)**					
Corexit-alone (mg/L)	*DOSS (t = 0)*	*DOSS (t = 24 h)*	*Corexit (t = 0)*	*Corexit (t = 24 h)*	*% nominal (t = 0)*	*% nominal (t = 24 h)*
Control	Less than MDL*	Less than MDL*	NA	NA	100	100
6.25	1.45	1.30	8.52	7.65	136	122
12.5	1.55	1.31	9.13	7.71	73.0	61.7
25	6.33	6.55	37.3	38.5	149	154
50	11.4	17.0	67.1	100	134	200
100	30.8	28.9	181	170	181	170

*MDL = measured detectable limit (0.01 mg/L)

4. Discussion

This study found that exposure of chemically dispersed oil to *Swiftia exserta* octocorals was more detrimental to coral health than exposure to non-dispersed water-accommodated oil fractions. Mortality occurred quickly in both the dispersant-alone and chemically-enhanced WAF treatments, over the course of 96 hours for most of the fragments. Mortality (health score = 0) was observed after 96 h for 63% of the fragments exposed to high doses (50 and 100 mg/L) of Corexit 9500 and complete mortality was observed in fragments exposed to the two higher concentrations of CEWAF (50 and 100%). These data support the hypothesis that mesophotic octocorals are vulnerable to chemical dispersants and chemically dispersed oil at high concentrations over a short time period of just a few days.

These results are consistent with other studies that show that chemically dispersed oil exposures are more toxic than oil-alone treatments (Rico-Martinez et al., 2013; Goodbody-Gringley et al., 2013; DeLeo et al., 2016). The study is also consistent with others in that the dispersant-alone (measured LC_{50} = 70.27 mg/L Corexit) was slightly less toxic than the oil-dispersant mixture (measured LC_{50} = 41.04 mg/L Corexit). This result is expected due to the chemical dispersant's ability to increase the amount of oil that mixes into the water column, thereby increasing the hydrocarbon concentration (National Research Council, 2005).

Our study is the first to examine the effects of oil and dispersants on a gorgonian octocoral from the mesophotic depth range (50-150 m). Previous studies have shown effects of deep-sea octocorals (White et al 2012; DeLeo et al 2006). This is also the first to calculate median lethal toxicity values for LSC oil and Corexit 9500 for any deep water coral species. The nominal 96 h LC_{50} for Corexit 9500 alone was approximately 51.17 mg/L, which is comparable to that of juvenile clams and mysids (32.80 and 43.40 mg/L Corexit, respectively (DeLorenzo et al., in journal review). Octocoral colonies of *S. exserta* from our study were more sensitive to Corexit-only exposures than seven out of the twelve species of benthic invertebrates and life stage combinations tested by DeLorenzo et al. (in journal review). The 96 h LC_{50} for the Corexit-CEWAF based on measured TEH concentrations (45.86 mg/L TEH) was lower than several species of different life stages that were exposed to similar Corexit-LSC oil CEWAF preparations (DeLorenzo et al., in journal review). *S. exserta* fragments were more sensitive than embryo-larval and adult fish, adult snails, juvenile clams, juvenile polychaetes, and embryo, larval, and adult grass shrimp to Corexit-CEWAFs.

At high concentrations of CEWAF or Corexit-alone mixtures, the very thin coenenchymal tissue of *S. exserta* (Goldberg, 2001) breaks down, leaving the sclerites exposed.

Eventually, with the help of water flow, these sclerites disassociate from the central axis, leaving bare skeleton. Necrosis is proposed to be an extreme immune response to severe stress from temperature, pollutants, or disease (McClanahan et al., 2004; Silva et al., 2016).

These fine-scale effects were not consistent with the *in situ* trajectory of degradation to *Swiftia* spp. sea fans reported by Silva et al. (2016). Injuries reported for octocorals at mesophotic sites below the DWH oil slick were overgrowth by hydroids, covering by sediment, broken or bare branches (Etnoyer et al., 2016; Silva et al., 2016). The corals in our experiments were not fed and were isolated in beakers, so conditions were not conducive to flocculent material or overgrowth. Furthermore, the effect of oil and dispersants to these benthic organisms in a natural environment is possibly exacerbated by feeding on the oil-derived marine snow that rapidly sank to the sea floor (Daly et al., 2016; Passow, 2016).

It is important to recognize that the concentrations at which complete mortality occurred at 96 h in the experimental setting was higher than would be expected to occur over a large area of the seafloor. Yet, it is extremely difficult to estimate the fate of oil and dispersants after the DWH spill. A study by Silva et al. (2016) detected non-toxic levels of tPAHs (mean = 51 – 345 ppb) in the tissues of octocorals (n = 50) from the Pinnacles Trend. These tissue levels of hydrocarbons are similar to the aqueous exposure concentrations measured in our study (i.e., 280 ppb in the 100% WAF, 868 ppb in the 50% CEWAF). Although no mortality was observed in *S. exserta* corals exposed to 280 ppb of tPAHs alone, the dispersed oil solutions yielded approximately seven times the level of hydrocarbons in solution. We hypothesize that coral toxicity in the CEWAFs may be due to a combination of Corexit and higher bioavailability of PAHs.

Due to palpable limitations of the experimental setting, our study did not exactly replicate the conditions of the DWH oil spill. Many factors, like wind and waves, contributed to the mixture of LSC oil and Corexit throughout the water column of the Gulf of Mexico and those are difficult to simulate in the laboratory. Additionally, our experiments focused on quantifying the effects of short-term exposures of toxins, and it is likely that mortalities would have been higher had the experiments continued for a longer duration. The DWH oil slick affected mesophotic reefs sites in the Gulf of Mexico over a period of several weeks in 2010 (Etnoyer et al., 2016), while this study was relatively brief, conducted over a 96 h period.

The short-term assay was conducted to understand the toxicity thresholds to *S. exserta*, which had not been previously examined. The oil and dispersant concentrations tested in our study are comparable to those of other studies focusing on other aquatic or marine species (Hemmer et al., 2011; Goodbody-Gringley et al., 2013; DeLeo et al., 2016; DeLorenzo et al., in journal review).

This study is the first to quantify vulnerability of deep water gorgonian octocorals to chemical contaminants, and as such provides important information to inform management in the event of a future oil spill. With the rise of oil and gas production throughout the Gulf of Mexico, potential impacts to deep-water ecosystems, such as future spills, will remain a looming threat. Responding to such threats requires a better understanding of the sensitivity of contaminants to affected species, and we hope that our study will provide important information to improve management efforts of these important, yet vastly under-surveyed deep-water ecosystems.

Acknowledgements

Funding for research was provided by NOAA National Centers for Coastal Ocean Science and College of Charleston's Graduate Program in Marine Biology. We acknowledge Enrique Salgado, Carl Miller, and Paul Pennington for their gracious assistance with aquaria and laboratory maintenance, and Ed Wirth for assistance with chemical analyses. For their significant contributions, we also thank Erik Cordes, Mike Fulton, Thomas Greig, Andrew Shuler, Herman Wirshing, Daniel Wagner, Andrea Quattrini, and Ann Skradski.

Author Contributions

JF conducted the experiments, collected the data, analyzed and interpreted the data, and drafted the article. MED designed the work, interpreted the data, and critically revised the article. ECP analyzed chemistry samples and critically revised the article. PJE conceived the experiments, assisted with data collection, and critically revised the article.

References

Anderson, S. H., Peterson, C. H., Cherr, G., Ambrose, R., Anghera, S., Bay, S., Blum, M.J., Condon, R., Dean, T., Graham, W., Guzy, M., Hampton, S., Joye, S., Lambrinos, J., Mate, B., Meffert, D., Powers, S., Somasundaran, P., Spies, R., Taylor, C., & Tjeerdema, R. (2014) Understanding and Properly Interpreting the 2010 Deepwater Horizon Blowout. *Oil Spill Remediation: Colloid Chemistry-Based Principles and Solutions*, 19-57.

Bagby, S. C., Reddy, C. M., Aeppli, C., Fisher, G. B., & Valentine, D. L. (2016) Persistence and biodegradation of oil at the ocean floor following Deepwater Horizon. *Proceedings of the National Academy of Sciences*, 201610110.

Camilli, R., Reddy, C. M., Yoerger, D. R., Van Mooy, B. A., Jakuba, M. V., Kinsey, J. C., McIntyre, C. P., Sylva, S. P., & Maloney, J. V. (2010) Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science*, 330 (6001), 201-204.

Continental Shelf Associates & Texas A&M University, (2001) Mississippi/Alabama Pinnacle Trend Ecosystem Monitoring, Final Synthesis Report. U.S. Department of the Interior, Geological Survey, Biological Resources Division, USGS BSR 2001 0007 and Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA, OCS Study MMS 2001 080, 415.

Daly, K. L., Passow, U., Chanton, J., Hollander, D. (February 2, 2016) Assessing the impacts of oil-associated marine snow formation and sedimentation during and after the Deepwater Horizon oil spill. *Anthropocene*, 10.1016/j.ancene.2016.01.006.

DeLeo, D. M., Ruiz-Ramos, D. V., Baums, I. B., & Cordes, E. E. (2016) Response of deep-water corals to oil and chemical dispersant exposure. *Deep Sea Research Part II: Topical Studies in Oceanography*, 129, 137-147.

DeLorenzo, M.E., Key, P.B., Chung, K.W., Calvert, M., Good, J., Graham, E., Thomas, M., Wimberley, A., Pisarski, E., Shaddrix, B., Moore, J.G., Wirth, E.F., Fulton, M.H. (*In Journal Review*) Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms.

Dennis, G. D., & Bright, T. J. (1988) Reef fish assemblages on hard banks in the northwestern Gulf of Mexico. *Bulletin of Marine Science*, 43(2), 280-307.

Ellis, J. & Solander, D. (1786) *The Natural History of many curious and uncommon Zoophytes, collected from various parts of the globe by the late John Ellis, systematically arranged and described by the late Daniel Solander*. London, 208 pp.

Epstein N., Bak, R. P. M., & Rinkevich B. (2000) Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. *Marine Pollution Bulletin*, 40, 497–503.

Etnoyer, P., Wickes, L. N., Silva, M., Dubick, J. D., Balthis, L., Salgado, E., & MacDonald, I. R. (2016) Decline in condition of gorgonian octocorals on mesophotic reefs in the northern Gulf of Mexico : before and after the Deepwater Horizon oil spill. *Coral Reefs,* 35, 77-90.

Flurer, R. A., Boyd, B. L., Gamble, B., Gratz, S., Mulligan, K. J., Benner Jr, R. A., El Said, K. R., Jester, E. L. E., Burrows, D. G., da Silva, D. A. M., Krahn, M. M., Reichert, W. L., & Ylitalo, G. M. (2010). Determination of dioctylsulfosuccinate in select seafoods using a QuEChERS extraction with liquid chromatography-triple quadrupole mass spectrometry. US Food and Drug Administration, Division of Field Science.

Gittings, S. R., Bright, T. J., Schroeder, W. W., Sager, W. W., Laswell, S. J., & Rezak, R. (1992) Invertebrate assemblages and ecological controls on topographic features in the northeast Gulf of Mexico. *Bulletin of Marine Science*, 50(3), 435-455.

Goldberg, W. M. (2001) The sclerites and geographic distribution of the gorgonian *Swiftia exserta* (Coelenterata: Octocorallia: Holaxonia). *Bulletin of the Biological Society of Washington*, 10, 100-109.

Goodbody-Gringley, G., Wetzel, D. L., Gillon, D., Pulster, E., Miller, A., & Ritchie, K. B. (2013) Toxicity of Deepwater Horizon source oil and the chemical dispersant, Corexit® 9500, to coral larvae. *PloS one*, 8(1), e45574.

Graham, W. M., Condon, R. H., Carmichael, R. H., D'Ambra, I., Patterson, H. K., Linn, L. J., & Hernandez Jr, F. J. (2010) Oil carbon entered the coastal planktonic food web during the Deepwater Horizon oil spill. *Environmental Research Letters*, 5(4), 045301.

Hemmer, M. J, Barron, M. G., & Greene, R. M. (2011) Comparative toxicity of eight oil dispersants, Louisiana sweet crude oil (LSC), and chemically dispersed LSC to two aquatic test species. *Environmental Toxicology and Chemistry*, 30(10), 2244-2252.

Kujawinski, E. B., Kido Soule, M. C., Valentine, D. L., Boysen, A. K., Longnecker, K., & Redmond, M. C. (2011) Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental science & technology*, 45(4), 1298-1306.

Lesser, M. P., Slattery, M., & Leichter, J. J. (2009) Ecology of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology*, 375(1-2), 1–8.

Loya, Y. & Rinkevich, B. (1980) Effects of oil pollution on coral reef communities. *Marine Ecology Progress Series*, 3(16), 180.

McClanahan, T., McLaughlin, S., Davy, J., Wilson, W., Peters, E., Price, K., & Maina, J., (2004) Observations of a new source of coral mortality along the Kenyan coast. *Hydrobiologia*, 530-531, 469-479.

McNutt, M. K., Camilli, R., Crone, T. J., Guthrie, G. D., Hsieh, P. A., Ryerson, T. B., Samas, O., & Shaffer, F. (2012) Review of flow rate estimates of the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences*, 109(50), 20260-20267.

National Research Council. (2005) *Understanding Oil Spill Dispersants: Efficacy and Effects*. National Academy Press, 396 pp.

Nelson-Smith, A. (1973) *Oil pollution and marine ecology.* New York: Plenum Press.

NOAA (2014) Cumulative TCNNA SAR Oiling - Days of Oiling, Environmental Response Management Application. Web application. ERMA Deepwater Horizon MC252. National Oceanic and Atmospheric Administration. Accessed 20 Jan 2017. http://response.restoration.noaa.gov/erma/

NOAA Deepwater Horizon Natural Resource Damage Assessment Trustees. (2016) Deepwater Horizon oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. Retrieved from http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan

Passow, U., Ziervogel, K., Asper, V., & Diercks, A. (2012) Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environmental Research Letters*, 7(3), 035301.

Passow, U. (2016) Formation of rapidly-sinking, oil-associated marine snow. *Deep Sea Research Part II: Topical Studies in Oceanography*, 129, 232-240.

Peterson, C. H., Anderson, S. S., Cherr, G. N., Ambrose, R. F., Anghera, S., Bay, S., Blum, M., Condon, R., Dean, T. A., Graham, M., Guzy, M., Hampton, S., Joye, S., Lambrinos, J., Mate, B., Meffert, D., Powers, S. P., Somasundaran, P., Spies, R. B., Taylor, C. M., Tjeerdema, R., & Adams, E. E. (2012) A tale of two spills: novel science and policy implications of an emerging new oil spill model. *BioScience*, 62(5), 461-469.

Reddy, C. M., & Quinn, J. G. (1999) GC-MS analysis of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons in seawater samples after the North Cape oil spill. *Marine Pollution Bulletin*, 38(2), 126-135.

Reddy, C. M., Arey, J. S., Seewald, J. S., Sylva, S. P., Lemkau, K. L., Nelson, R. K., Carmichael, C. A., McIntyre, C. P., Fenwick, J., Todd Ventura, G., Van Mooy, B. A. S., & Camilli, R. (2011) Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences*, 109(50), 20229-20234.

Rico-Martinez, R., Snell, T. W., & Shearer, T. L. (2013) Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). *Environmental Pollution*, 173, 5-10.

Silva, M., Etnoyer, P. J., & MacDonald, I. R. (2016) Coral injuries observed at mesophotic reefs after the Deepwater Horizon oil discharge. *Deep Sea Research Part II: Topical Studies in Oceanography*, 129, 96-107.

Suchanek, T. H. (1993) Oil impacts on marine invertebrate populations and communities. *American Zoologist*, 33(6), 510-523.

Sulak, K., Berg, J., Randall, M., Dennis III, G. D., & Brooks, R. A. (2008) Dual-carbon sources fuel the OCS deep-reef community, a stable isotope investigation. *Proceedings 11th International Coral Reef Symposium*, 2, 945-949.

Sulak, K.J. & Dixon, P.M. (2015) Change in Gulf of Mexico Mesophotic Reef Fish Community Structure Across the Time Threshold of the Deepwater Horizon Oil Spill Event in 2010: Mesophotic Reef Fish Community Impacts of the DWH Oil Spill. (BEN_TR.01). DWH Deepwater Benthic NRDA Technical Working Group Report.

Weaver, D. C., Dennis, G. D., & Sulak, K. J. (2002) Community Structure and Trophic Ecology of Demersal Fishes on the Pinnacles Reef Tract, Final Synthesis Report (No. 2001-0008). US Fish and Wildlife Service.

White, H. K., Lyons, S. L., Harrison, S. J., Findley, D. M., Liu, Y., & Kujawinski, E. B. (2014). Long-term persistence of dispersants following the Deepwater Horizon oil spill. *Environmental Science & Technology Letters*, 1(7), 295-299.