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Response of deep-water corals to oil and chemical dispersant exposure

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ABSTRACT

Cold-water corals serve as important foundation species by building complex habitat within deep-sea benthic communities. Little is known about the stress response of these foundation species yet they are increasingly exposed to anthropogenic disturbance as human industrial presence expands further into the deep sea. A recent prominent example is the Deepwater Horizon oil-spill disaster and ensuing clean-up efforts that employed chemical dispersants. This study examined the effects of bulk oil–water mixtures, water-accommodated oil fractions, the dispersant Corexit 9500A®, and the combination of hydrocarbons and dispersants on three species of corals living near the spill site in the Gulf of Mexico between 500 and 1100 m depths: *Paramuricea* type B3, *Callogorgia delta* and *Leiopathes glaberrima*. Following short-term toxicological assays (0–96 h), all three coral species examined showed more severe health declines in response to dispersant alone (2.3–3.4 fold) and the oil–dispersant mixtures (1.1–4.4 fold) than in the oil-only treatments. Higher concentrations of dispersant alone and the oil–dispersant mixtures resulted in more severe health declines. *C. delta* exhibited somewhat less severe health declines than the other two species in response to oil and oil/dispersant mixture treatments, likely related to its increased abundance near natural hydrocarbon seeps. These experiments provide direct evidence for the toxicity of both oil and dispersant on deep-water corals, which should be taken into consideration in the development of strategies for intervention in future oil spills.

1. Introduction

The *Deepwater Horizon* (DWH) oil spill was one of the largest environmental disasters in history, releasing approximately 5 million barrels of crude oil at depth in the Gulf of Mexico (GoM) over a three-month period (Crone and Tolstoy, 2010; Camilli et al., 2011). In addition, nearly 7 million liters of oil dispersants were applied during the ensuing cleanup efforts. Dispersants are chemical emulsifiers that act to increase the rate of oil dispersion thereby increasing the amount of small oil droplets suspended in the water column, reducing oil slicks at the surface. Thus, dispersant applications affect the fate, transport and physical composition of oil. Of the 7 million liters of oil dispersants used, approximately 3 million liters were applied at depth for the first time in history (Barron, 2012), without a comprehensive understanding of how this subsea application might alter the fate of oil and impact benthic ecosystems (National Research Council, 2005).

Petroleum hydrocarbons released under high-pressure undergo a series of interconnected physical and chemical processes that affect their fate and transport in the deep sea (Camilli et al., 2010; Kessler et al., 2011; Reddy et al., 2012). Following the direct injection of disperant (Corexit 9527A and 9500A) to the Macondo well head at a depth of 1544 meters (m) (Hazen et al., 2010), a large oil plume persisted for months centered at approximately 1100 m depth, without substantial biodegradation (Camilli et al., 2010). Oil spewing from the wellhead encountered turbulent mixing and was emulsified as a result of its reduced buoyancy at depth and the application of dispersant (Fodrie and Heck Jr., 2011). Measurements of water-column samples collected from this deep-water plume (defined by Camilli et al., 2010) indicated that a significant portion of water-soluble hydrocarbon components were retained in deep waters, with unknown portions of insoluble hydrocarbons drifting to the sea floor (Reddy et al., 2012). Despite some emulsification of oil throughout the water column, surface waters were still polluted with oil slicks (Fodrie and Heck Jr., 2011). At the surface, some components of the oil were then transformed into aggregations of marine snow (and floc) by coagulation with suspended particulates and planktonic organisms. Although this marine snow disappeared from the surface layers of the GoM within a month, it is likely that it sunk into the deep sea as the oil weathered (Passow et al., 2012).

Recent studies have found both lethal and sub-lethal effects of the DWH blowout on species inhabiting pelagic and coastal environments (Barron, 2012; Silliman et al., 2012; Whitehead et al., 2012; Dubansky et al., 2013; Almeda et al., 2013). Prior studies have shown variable levels of crude oil toxicity on aquatic organisms with some fauna being more susceptible than others (Anderson et al., 1974; Bonsdorff et al.,

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1990; Coull and Chandler, 1992; Stark et al., 2003). Dispersant addition to the oil triggers a transient increase in hydrocarbon concentrations throughout the water-column (Pace et al., 1995), which can then lead to higher, more toxic exposures of dissolved and dispersed oil components upon contact with marine life.

Spill-impacted deep-sea coral communities were first discovered at a depth of approximately 1370 m, 11 km southwest of the Macondo well explosion, at the lease block site Mississippi Canyon (MC) 294 (White et al., 2012). Various species of coral, primarily *Paramuricea biscaya* (Grasshoff, 1977), were found covered with brown flocculent material (floc), exhibiting characteristic signs of stress and mortality, including excess mucus production, sclerite enlargement, and tissue loss. Further analysis of this floc revealed hydrocarbons from the Macondo well were indeed present (White et al., 2012). Whether the damage observed to the corals was induced by sinking oil-filled particulates, dissolved hydrocarbons, dispersants, or a combination of all of these sources is unknown. Subsequently, two additional sites were discovered to contain impacted deep-sea coral communities (Fisher et al., 2014).

Deep-sea corals alter the terrain of the sea floor and produce complex, heterogeneous habitat, which promotes benthic biodiversity (Cordes et al., 2008, 2010). In addition to reef-forming scleractinian corals, which generally occur at upper-slope depths (300–1000 m), octocorals and black corals (antipatharians) form large, tree-like structures from the subtidal to over 3000 m depth. These corals colonize hard substrata, and can form dense fields (Roberts et al., 2006). By increasing the complexity of the seafloor, they provide shelter, feeding areas, and nursery grounds for many fish and invertebrates.

Because deep-sea corals build the foundation for these communities, damage to them can impact biodiversity and ecosystem function (Husebo et al., 2002; Freiwald et al., 2004). Their longevity and slow growth rates make them particularly vulnerable to anthropogenic disturbance (Grigg, 1974; Emiliani et al., 1978; Druffel et al., 1990, 1995; Risk et al., 1998, 2002; Andrews et al., 2002; Adkins et al., 2004; Roark et al., 2009). As crude oil reserves are abundant in the GoM, with 1.5 billion barrels of oil extracted from the sea floor each day (Minerals Management Service, 2009), it is now a critical time for further examination of deep-sea coral response to oil and dispersant exposure.

Here, the effects of oil, dispersant and oil–dispersant mixtures were tested experimentally on three species of deep-sea coral living near the DWH oil spill site in the Gulf of Mexico, including *Paramuricea* type B3 (Doughty et al., 2014), *Callogorgia delta* (Bayer et al., 2014) and *Leiopathes glaberrima* (as re-described in Opresko and Baron-Szabo, 2001). *P. biscaya* was the most common of the corals impacted by the DWH oil spill (White et al., 2012; Fisher et al., 2014), and *Paramuricea* type B3 is the sister species to this coral (Doughty et al., 2014). *Paramuricea* type B3 was chosen because its shallower depth distribution (830–1090 m for *Paramuricea* type B3 vs. 1370–2600 m for *P. biscaya* with one individual collected at 850 m, Doughty et al., 2014) results in higher survivorship shipboard, and to avoid further impact to the relatively small populations of *P. biscaya* that have thus far been discovered. *C. delta* preferentially occupies habitats near natural oil seeps in the deep GoM (Quattrini et al., 2013), suggesting that the species may have evolved a tolerance for hydrocarbon exposure. *L. glaberrima* is slow growing and lives to very old ages, making it one of the oldest skeletal secreting organisms known to date (Roark et al., 2009). Slow growth rates make this species highly sensitive to natural and anthropogenic disturbances.

This study examined the effects of exposure to bulk oil–water mixtures, water-accommodated oil fractions (WAF), dispersants, and mixtures of hydrocarbons and dispersants using short-term toxicological assays ($\leq$ 96 h) that monitored phenotypic responses and survivorship. Specifically, we tested the hypotheses that oil/dispersant mixtures would be the most toxic to corals, and that *C. delta* would have a higher tolerance for hydrocarbons due to its affinity for natural seep habitats.

2. Methodology

2.1. Sample collection and acclimatization

All samples were collected from two sites in the GoM. *C. delta* and *L. glaberrima* were collected from the Viosca Knoll (VK) 826 site at a depth of approximately 500 m (29 °09.5′N, 88 °01.0′W; Cordes et al., 2008; Davies and Guinotte, 2011). *Paramuricea* type B3 colonies were collected from a large population of corals at approximately 1050 m depth at Atwater Valley (AT) 357 (27 °58.6′N, 89 °70.4′W; Doughty et al., 2014). At each site, corals were haphazardly collected with the remotely operated vehicles (ROV) Global Explorer MK3 or Hercules.

Samples were taken on multiple dives, with 5–6 colonies of both *C. delta* and *L. glaberrima* collected from VK826, and 5–6 colonies of *Paramuricea* type B3 gathered from AT357. Samples were collected several meters apart from conspecific colonies to reduce the likelihood of sampling clones. Corals were visually identified using live video stream from cameras attached to each ROV, before being collected with a manipulator arm and secured in an insulated “bio” box and or sealable collection quivers. When possible, branches of colonies were sampled to reduce impact.

At the surface, colonies were immediately transferred to containers with filtered seawater of the species-appropriate temperature and salinity (35 psu). *C. delta* and *L. glaberrima* were jointly maintained at approximately 8 °C and later, *Paramuricea* type B3 at 5 °C (the average in situ temperatures at depth) in a temperature-controlled room for the duration of the experiment. Temperature in holding vessels was continuously monitored using temperature probes (Hobo® Data Loggers). Corals were allowed to acclimate for 6–12 h prior to experimentation.

2.2. Preparation of bulk-oil treatments

For the bulk-oil experiment three stock solutions were prepared: crude oil (MASS oil collected from the Macondo well during the spill), dispersant (Corexit 9500A), an oil/dispersant mixture, and artificial seawater controls. All solutions were made with sterile artificial seawater (ASW, Instant Ocean™) at 35 psu, the average in situ salinity for both sites. ASW allowed us to accurately maintain desired salinity and temperature for large volumes of water without the potential for introducing contaminants from the ship's seawater system, and to avoid the unreliability of collecting buckets of seawater from over the side in variable sea states. We have used ASW to maintain other cold-water coral species alive in laboratory aquaria for extended periods of time without adverse affects (Lunden et al., 2014).

A stock bulk-oil solution was prepared at a concentration of 250 parts per million (ppm) by adding 50 µL of MASS oil to 199.95 mL ASW. The solution was mixed at room temperature for a 24-h period on an orbital shaker at approximately 500 rpm to achieve highest possible homogeneity. Oil dilutions were prepared from this stock solution. The subsequent oil concentrations were chosen in an attempt to determine the threshold for lethal toxicity, following preliminary toxicity studies on *L. glaberrima*. Dispersant concentrations were the same as the oil concentrations so as to examine the relative toxicity of oil vs. dispersant. The oil/dispersant-mixture stock solution was prepared with an initial targeted concentration of 250 ppm each of crude oil and Corexit 9500A by adding 50 µL of each to 199.90 mL of ASW. The dispersant stock solution was prepared by adding 50 µL Corexit 9500A to 199.95 mL ASW to achieve an initial concentration of 250 ppm. Serial dilutions were

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prepared from each of the three stock solutions to produce three target concentrations: 25 ppm (High), 7.9 ppm (Medium) and 0.8 ppm (Low).

All solutions were placed into sterile 50 mL glass vials. These were then incubated at 5 or 8 °C, dependent on species, and mixed continuously at low speeds for 24 h on an orbital shaker table to reduce separation and to encourage even oil distribution. Experiments were conducted between 8 and 27 November 2012 onboard the R/V *Falkor*.

2.3. Preparation of treatments using water-accommodated oil fractions (WAF)

For this experiment, stock solutions were prepared using only the water-accommodated oil fractions (WAF). For the WAF oil treatment, a higher oil volume (9.5 mL) of surrogate oil was added to 475 mL of ASW and mixed at high speeds (~350 rpm) in an attempt to produce a 1.2 mM WAF oil solution. The WAF was separated from the insoluble oil layer using a sterile separatory funnel, and used as a stock solution to produce experimental treatments with targeted initial total hydrocarbon concentrations of 250 μM (High), 150 μM (Medium) and 50 μM (Low) WAF. Target concentrations were chosen to find lethal doses, as none of the previous bulk-oil (only) concentrations proved to be lethal. This was done using a standardized WAF protocol (S. Joye, personal communication) and based on the highest concentrations of oil detected during the spill (~300 μM, Joye et al., 2011).

The oil/dispersant mixture treatment was prepared using the same oil volume, with 950 μL of Corexit 9500A added (one-tenth of the oil concentration) to produce a dispersant enhanced WAF (DE–WAF; oil/dispersant treatment), also mixed at high speeds (~350 rpm). As the dispersant concentrations in the bulk-oil exposures were not entirely lethal to *C. delta* in the short term and most of the observed health decline was seen towards the end of the exposures at the highest Corexit 9500A concentration, the range of dispersant concentrations was progressively increased from those used in the previous exposures to attempt to reveal the lethal concentration (LC50). The dispersant stock solution was made by adding 950 μL of Corexit 9500A to 475 mL of ASW, with an initial dispersant concentration of 848 mg/L (mixed at 200–300 rpm). All stock solutions were mixed at room temperature for 48–72 h. Experimental solutions were then made from these two treatments with targeted initial oil concentrations of 250 μM (High), 150 μM (Medium) and 50 μM (Low) and targeted initial total dispersant concentrations of 176.7 mg/L (High), 106.0 mg/L (Medium) and 35.3 mg/L (Low).

All solutions were placed into sterile 50 mL acid-washed glass vials prior to experimentation. There was an anticipated and unavoidable loss of hydrocarbons and dispersant due to the adhesion of hydrophobic components to the dilution containers with each sequential transfer, as well as the chemical and coral–microbial alterations of hydrocarbons and dispersant components over the course of the treatments. Therefore oil and dispersant concentrations are reported as conservative, initial targeted values only, and qualitatively designated as “High” “Medium” and “Low” in the analysis. Experiments were conducted from 23 June 2013 to 3 July 2013 onboard the R/V *Nautilus*.

2.4. Fragmentation and exposure experiments

For both bulk-oil and WAF experiments, four to six colonies of each species ($n=3$) were fragmented into similar sized (approximately 3–6 cm tall), genetically identical replicates, or “nubbins” ($n=11$) and placed into the oil, dispersant, oil/dispersant mixture and the control (ASW) treatments. *Paramuricea* type B3 had only three healthy colonies for the bulk-oil exposures. The number of polyps per nubbin varied for each species because of the wide range in polyp sizes and unique branching morphology. Samples were placed in 50 mL pyrex test tubes, mounted on a shaker table in a temperature controlled environment, and aerated every 24 h by bubbling air into the tubes and gently inverting each sample.

Each sample was photographed together with a scale and monitored for signs of stress at four time points (24, 48, 72 and 96 h) during the bioassay. Each experimental nubbin was assigned an overall health rating on a scale ranging from 0 to 5. The percentage of live polyps and tissue-covered skeleton primarily contributed to this rating: dead fragment (score of 0), ⩽50% (score of 1–2), ~50% (score of 3), ⩾50% (score of 4–5), while the other stress responses further differentiated between scores. Ratings were further refined based on the following phenotypic stress responses: percentage of polyp retraction and or inflation, presence and persistence of mucus discharge, dead or darkened tissue, sloughing tissue and exposed skeleton. While polyp mortality, polyp retraction, mucus release, loose tissue, and exposed skeleton were observed in all three species, swollen polyps were only observed in *L. glaberrima*, while darkened tissue was specific to *Paramuricea*. Tissue discoloration and whitening was only observed in *C. delta*. Furthermore, *C. delta* displayed a distinctive polyp coiling, ultimately forming node-like structures that eventually disintegrated, leaving behind exposed skeleton. Samples and treatments were randomized in an attempt to reduce health-scoring bias.

2.5. Survival analysis

Health rankings were averaged for replicate coral fragments in each experimental concentration and plotted over time to investigate health decline. This was done discretely for each round of experiments (bulk-oil or WAF), type of treatment (oil, dispersant and oil/dispersant) and species to determine the effect of concentration on fragment health over time. Health differences within the different treatments at the 96-h end-point were tested using a non-parametric Kruskal–Wallis test, and if applicable ($p<0.05$), non-parametric post-hoc, pair-wise comparisons were performed using the Wilcoxon method (using JMP® Pro 10.0.2).

To investigate fragment survival over time, a Kaplan–Meier (K–M) “time to event” survival analysis was performed separately for each experimental series (IBM® SPSS® Statistics v22, Kaplan and Meier, 1958). This test measures the fraction of fragments declining to a health status of 3 or below at each time point and generates a survival curve. To quantify differences amongst the survival curves for a given species and treatment, a Mantel–Cox log-rank test was used to evaluate statistical significance ($\alpha=0.05$); if significant, pair-wise comparisons were made, again using a Mantel–Cox log-rank test.

An additional K–M analysis was performed to compare survival across species in each treatment. Only “event” occurrences contribute to survival estimates; the remaining data becomes censored in the analysis. For this reason the ASW control treatments, in which all fragments maintained health ratings >3, were excluded from survival-estimate statistics during species comparisons. A similar percentage of censored cases were present in the oil, dispersant and oil–dispersant treatments for each species, and the pattern of censoring was similar.

Additionally, Cox regressions were performed to quantify the hazard (i.e. a decline in health) associated with (a) treatment (water, oil, dispersant/oil and dispersant), (b) concentration (High, Medium, Low, Zero), and (c) species (*C. delta*, *L. glaberrima*, *Paramuricea* type B3) for the two sets of experiments (bulk-oil and WAF). The “event” in the time-to-event analysis was reaching a health rating of 3 or below (3, 1, 2 or 0), as mortality was not observed in every treatment and concentration during the exposure. The hazard ratios were calculated for each factor with respect

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to control treatment (a), the zero concentration (b) and *C. delta* (c), as we had hypothesized this to be the species most likely adapted to oil exposure. Cox regression was performed in IBM® SPSS® Statistics v22.

3. Results

3.1. Exposure effects on Paramuricea type B3

3.1.1. Oil treatment

Complete fragment mortality was not observed for *Paramuricea* type B3 in the control, bulk-oil or oil–WAF treatments (Figs. 1A and 2A). In examining the effect of concentration on fragment condition at the end of the bulk-oil and WAF exposures, the Kruskal–Wallis test showed no significant differences among the 96-h health ratings across all oil concentrations and controls ($p > 0.05$).

3.1.2. Dispersant treatment

Whole fragment mortality was observed in *Paramuricea* type B3 nubbins exposed to the High dispersant treatment (Fig. 1D). This decline in health originated in the dispersant mixture within 48–72 h, with two of three colonies exhibiting complete fragment mortality at the end of the exposure period. The Kruskal–Wallis test revealed significant differences ($p < 0.05$) in health rankings for *Paramuricea* type B3 at the end of the exposure; pair-wise comparisons revealed significant differences between nubbins in the High dispersant relative to the control samples ($p < 0.05$).

High coral fragment mortality was observed in the dispersant treatment across all concentrations tested in the WAF experiments. One of six *Paramuricea* type B3 replicates died in the Low dispersant solution, with complete mortality observed in four of six replicates in the Medium dispersant treatment by 96 h. At High dispersant concentrations, four of six replicates were dead after only 48 h, with complete mortality of all fragments after 96 h (Fig. 2D). The Kruskal–Wallis test and pair-wise comparisons revealed significantly higher health ratings among the control *Paramuricea* type B3 nubbins relative to all levels of dispersant (Low, Medium and High; $p < 0.005$) as well as in the Low vs. High dispersant concentrations ($p < 0.005$).

3.1.3. Oil/dispersant treatment

Whole fragment mortality was observed in *Paramuricea* type B3 nubbins exposed to the High oil/dispersant treatment (Fig. 1G), with complete mortality in two of three fragments by 96 h. There were significant health differences among concentrations (Kruskal–Wallis, $p < 0.05$), and subsequent pair-wise comparisons revealed significant differences between fragments in the High oil/dispersant relative to the control samples ($p < 0.05$).

During the WAF exposures, complete mortality was observed in the oil/dispersant mixture (DE–WAF), for one of six *Paramuricea* type B3 samples in both the Low and High concentrations (Fig. 2G). The Kruskal–Wallis and post-hoc tests detected significant health differences in fragments exposed to all concentrations of the mixture relative to the controls ($p < 0.05$).

3.1.4. Comparisons between treatments for Paramuricea type B3

For comparisons made between treatments in the bulk-exposure series, the log-rank test revealed significant differences among the K–M survival estimates ($\chi = 7.62$, df = 2, $p = 0.022$); pairwise comparisons (Table 1) indicated these differences were between the oil and oil/dispersant treatments ($p < 0.0167$). The oil/dispersant treatment had the lowest mean estimated survival time of 87.6 h, compared to the overall mean estimate of 90.2 h (Table 2a, Fig. 3). In the WAF exposures there were also significant differences among time-to-event occurrences ($\chi = 57.3$, df = 2, $p < 0.001$), and pair-wise comparisons affirmed significantly different estimates between all treatments. The lowest time-to-event estimate was 82.5 h in dispersant compared to 96 h in oil and an overall average estimate of 90.8 h (Table 2b, Fig. 3).

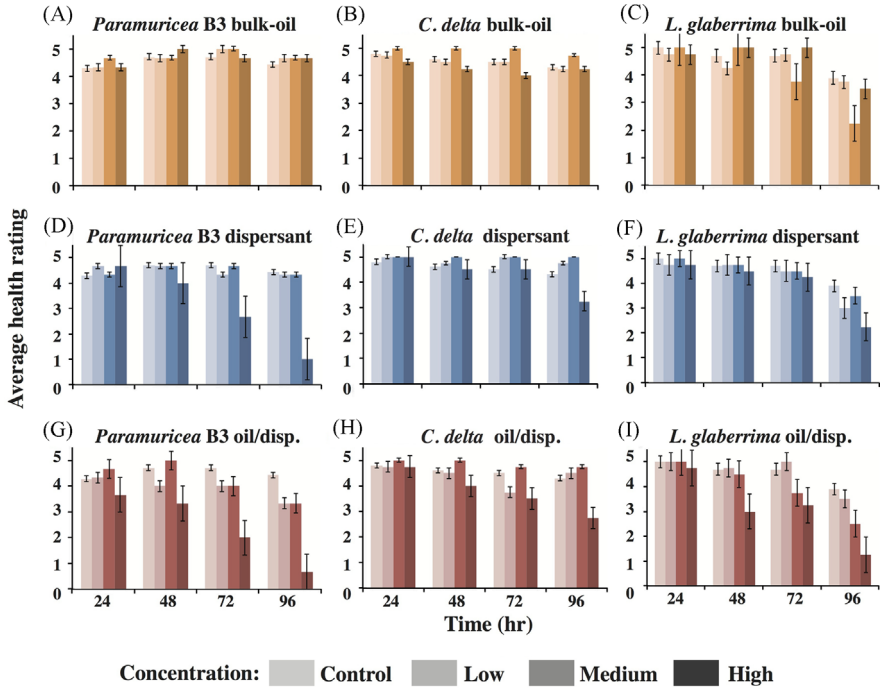

Fig. 1. Average health ratings over time for coral fragments exposed to various concentrations of bulk-oil mixtures (yellow/ top row), Corexit 9500A dispersant solutions (blue/ middle row) and oil–dispersant (oil/disp.) combination mixtures (red/ bottom row). Health rating scale 0–5. Bars show standard error.

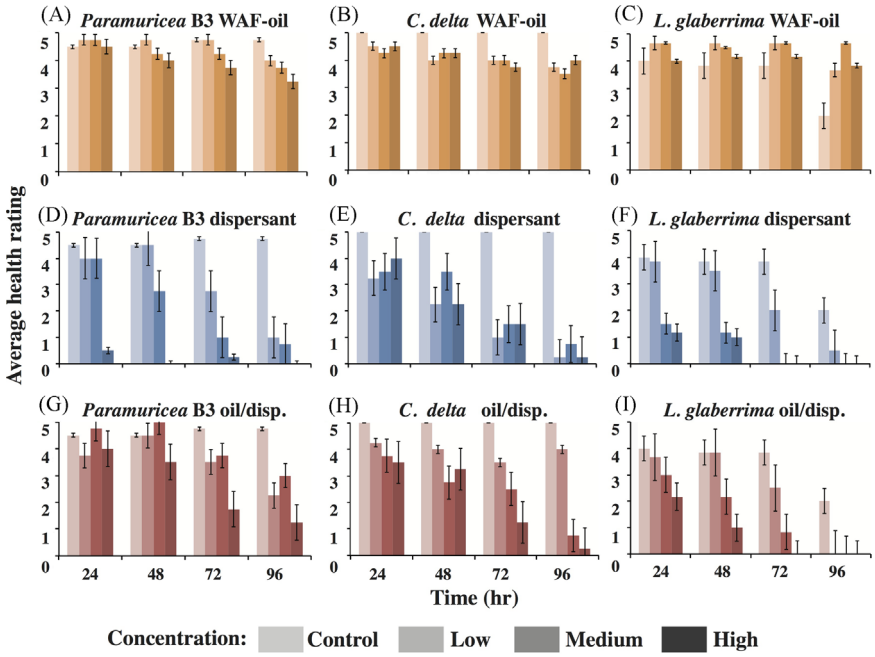

Fig. 2. Average health ratings over time for coral fragments exposed to various concentrations of water accommodated oil fractions (yellow/ top row), Corexit 9500A dispersant solutions (blue/ middle row) and water accommodated oil–dispersant (oil/disp.) combination mixtures (red/ bottom row). Health rating scale 0–5. Bars show standard error.

Table 1
Pair-wise comparisons of K–M survival estimates in oil, dispersant and oil/dispersant treatments within the bulk-oil and oil–WAF exposure series, using a Mantel–Cox log-rank analysis. Comparisons were done discretely for each of the three coral species: *C. delta*, *Paramuricea* (type) B3 and *L. glaberrima* (χ^2=chi-square, α=0.05). The event was a decline in health rating to 3 or below (bulk) or 1 and below (WAF). Bonferroni adjusted *p*-values for each within species comparison are $p < 0.0167$, with values in bold being significant.

Log-rank (Mantel–Cox)	Oil		Dispersant		Oil /dispersant	
	χ^2	*p*-Val	χ^2	*p*-Val	χ^2	*p*-Val
Bulk exposures						
C. delta						
Oil	–	–	1.766	0.184	0.284	0.594
Dispersant	1.766	0.184	–	–	3.594	0.058
Oil/dispersant	0.284	0.594	3.594	0.058	–	–
Paramuricea B3						
Oil	–	–	3.958	0.047	10.634	**0.001**
Dispersant	3.958	0.047	–	–	2.401	0.121
Oil/dispersant	10.634	**0.001**	2.401	0.121	–	–
L. glaberrima						
Oil	–	–	0.152	0.696	7.364	**0.007**
Dispersant	0.152	0.696	–	–	6.919	**0.009**
Oil/dispersant	7.364	**0.007**	6.919	**0.009**	–	–
WAF exposures						
C. delta						
Oil	–	–	14.127	**0.000**	4.788	0.029
Dispersant	14.127	**0.000**	–	–	3.651	0.056
Oil/dispersant	4.788	0.029	3.651	0.056	–	–
Paramuricea B3						
Oil	–	–	46.594	**0.000**	8.695	**0.003**
Dispersant	46.594	**0.000**	–	–	25.770	**0.000**
Oil/dispersant	8.695	**0.003**	25.770	**0.000**	–	–
L. glaberrima						
Oil	–	–	65.367	**0.000**	45.871	**0.000**
Dispersant	65.367	**0.000**	–	–	6.077	**0.014**
Oil/dispersant	45.871	**0.000**	6.077	**0.014**	–	–

3.2. Exposure effects on *C. delta*

3.2.1. Oil treatment

There was no complete fragment mortality in the control or bulk-oil treatments (Fig. 1B). However, one *C. delta* replicate in the Low oil–WAF died by the end of the exposure (Fig. 2B). The Kruskal–Wallis test showed no significant differences among the 96-h health ratings across all concentrations of bulk and WAF oil ($p > 0.05$).

3.2.2. Dispersant treatment

C. delta showed a decline in health in the High dispersant (Fig. 1E), though complete fragment mortality was not observed during the 96 h assay. The Kruskal–Wallis test revealed significant differences ($p < 0.05$) in health rankings, with the High dispersant showing a significantly greater decline in health than the Medium and Low concentrations ($p < 0.05$).

During the WAF exposures, 75% of *C. delta* fragments died in the Low dispersant, 25% in the Medium and 75% in the High dispersant after 96 h (Fig. 2E). Control fragment health was significantly higher relative to all concentrations of dispersant ($p < 0.05$).

3.2.3. Oil/dispersant treatment

Coral fragments also showed a decline in health within the High oil/dispersant treatment (Fig. 1H), but again complete fragment mortality was not observed. Significant differences were detected between nubbins in the High oil/dispersant relative to the control samples ($p < 0.05$).

During the DE–WAF exposures, mortality was observed in one colony in the Medium concentration and three of the four colonies in the High concentration (Fig. 2H). A Kruskal–Wallis test revealed significant health differences among treatments, with the Medium and High DE–WAF treatments significantly lower than the controls ($p < 0.05$), and the High DE–WAF also significantly lower than the Low treatment ($p < 0.05$).

Table 2
K–M means for time-to-event estimates for three coral species: *C. delta*, *Paramuricea* type B3 and *L. glaberrima*, in bulk-oil (a) and WAF (b) exposures using a Mantel-Cox Log-rank analysis. The event was a decline in health rating to: a) 3 or below, b) 1 or below.

Species	Treatment	Survival estimate	Std. error	95% confidence interval Lower bound	Upper bound
(a) Bulk exposure					
C. delta	Bulk-oil	90.3	1.88	86.6	94.0
	Dispersant	93.5	1.31	90.9	96.0
	Oil/disp.	90.5	1.77	87.1	94.0
	Overall	91.4	0.95	89.6	93.3
Paramuricea B3	Bulk-oil	91.9	2.25	87.5	96.3
	Dispersant	91.0	2.04	87.0	95.0
	Oil/disp.	87.6	2.50	82.7	92.5
	Overall	90.2	1.27	87.7	92.7
L. glaberrima	Bulk-oil	95.4	0.57	94.3	96.6
	Dispersant	96.0	0.00	96.0	96.0
	Oil/disp.	91.0	1.71	87.7	94.4
	Overall	94.1	0.62	92.9	95.4
Overall	Overall	92.1	0.54	91.0	93.1
(b) WAF exposure					
C. delta	Oil WAF	93.5	1.52	90.5	96.5
	Dispersant	89.1	2.05	85.1	93.1
	Oil/disp.	92.3	1.60	89.1	95.4
	Overall	91.6	0.99	89.7	93.6
Paramuricea B3	Oil WAF	96.0	0.00	96.0	96.0
	Dispersant	82.5	2.45	77.7	87.3
	Oil/disp.	94.5	0.88	92.8	96.2
	Overall	90.8	0.98	88.9	92.7
L. glaberrima	Oil WAF	96.0	0.00	96.0	96.0
	Dispersant	77.8	2.63	72.7	83.0
	Oil/Disp.	86.4	2.01	82.4	90.3
	Overall	86.4	1.23	84.0	88.8
Overall	Overall	89.3	0.64	88.1	90.6

3.2.4. Comparisons between treatments for C. delta

No significant differences were detected among K–M time-to-event estimates for *C. delta* fragments in all treatments within the bulk-oil series ($\chi=1.72$, df=2, $p=0.422$), with an overall time-to-event (health rating of 3 or less) estimate of 91.4 h (Table 2a, Fig. 3). However, significant differences were detected among treatment estimates in the WAF series ($\chi=12.5$, df=2, $p=0.002$); these differences were between the oil-only and dispersant-only treatments (Table 1). The lowest estimate was 89.1 h in the dispersant treatment relative to the 93.5 h in the oil, and an overall average time-to-event estimate of 91.6 h (Table 2b).

3.3. Exposure effects on L. glaberrima

3.3.1. Oil treatment

There was no complete fragment mortality for *L. glaberrima* nubbins in the control, bulk-oil (Fig. 1C) or oil–WAF treatments (Fig. 2C). However, the Kruskal–Wallis test detected significant differences ($p<0.05$) among fragment health ratings in bulk-oil mixtures at 96 h; this difference was due to lower rankings in the Medium oil compared to those in the Low oil ($p<0.05$) and the controls ($p=0.01$), although rankings were similar between the Medium and High oil concentrations.

There was a significant difference among *L. glaberrima* health ratings in the oil–WAF exposure ($p<0.001$); pairwise comparisons revealed that all concentrations of oil had significantly higher health ratings than control fragments ($p\leq0.005$). Ratings in the Medium oil–WAF were also significantly higher than the Low and High ($p<0.05$) oil concentrations.

3.3.2. Dispersant treatment

Whole fragment mortality was not observed for *L. glaberrima* samples, though there was a decline in health within the High dispersant treatment (Fig. 1F). The Kruskal–Wallis test also detected no significant differences among sample health ratings at 96 h ($p>0.05$).

During the WAF exposures, *L. glaberrima* samples in the High and Medium dispersant concentrations were dead by 72 h. By 96 h four of six fragments were also dead in the Low dispersant treatment (Fig. 2F). The Kruskal–Wallis test revealed significantly lower health ratings in all concentrations of dispersant: Low ($p<0.05$), Medium and High ($p<0.005$) relative to controls.

3.3.3. Oil/dispersant treatment

Whole fragment mortality was not observed in the bulk-oil/dispersant mixture (Fig. 1I). The Kruskal–Wallis test and pair-wise comparisons revealed significant health differences between *L. glaberrima* samples in the High and Medium oil/dispersant ($p<0.05$) and between both the High and Medium concentrations relative to the control samples ($p\leq0.01$).

For *L. glaberrima* samples in the DE–WAF, there was complete sample mortality in the High concentration by 72 h, with two of six colonies dead in the Medium DE–WAF (Fig. 2I). Health ratings for nubbins in the control and Low DE–WAF were significantly higher than those in the Medium and High concentrations ($p<0.005$).

3.3.4. Comparisons between treatments for L. glaberrima

The K–M analysis and log rank test revealed significantly different time-to-event estimates for *L. glaberrima* samples ($\chi=7.20$, df=2, $p=0.027$). Pairwise comparisons (Table 1) indicated this difference was between both the oil and dispersant treatments relative to the oil/dispersant mixture, which had the shortest time-to-event estimate of 91.0 h compared to an overall time-to-event estimate of 94.0 h (Table 2a, Fig. 3). Significant differences were also detected among time-to-event estimates in the WAF exposures ($\chi=61.7$, df=2, $p<0.001$) across all treatments. The lowest time-to-event estimate was in the dispersant treatment (77.8 h) with the highest estimate (96 h) in the oil treatment and an overall estimate of 86.4 h (Table 2b, Fig. 3).

3.4. Overall comparisons between treatments and concentrations

The Cox regression analysis for the bulk-oil series revealed significant differences ($\chi=57.8$, df=5, $p<0.001$) among rates in health decline (to health-rating 3, ~50% survival) among treatments (control, oil, dispersant and oil/dispersant) and concentrations (zero, low, medium and high). The High concentration significantly increased the hazard of reaching a health rating of 3 or below by 2.5 fold relative to control concentrations, but the Medium concentration did not significantly increase the hazard. Also, relative to controls, samples in the dispersant had an increased hazard risk of 2.3 fold, however the hazard increase in the bulk-oil and oil/dispersant mixture treatments were not significantly different from the control treatment (Table 3).

Similar regression analyses for the WAF exposures also revealed significant differences ($\chi=176.470$, df=7, $p<0.001$) among rates of health decline between treatments and concentrations. Relative to the controls, dispersant significantly increased the hazard of reaching a health rating of 3 or below by 3.4 fold, compared to 4.4 fold in the oil/dispersant treatment; being exposed to oil did not significantly increase the hazard. In addition, the medium treatment concentrations significantly increased the hazard by 1.3 fold relative to the control concentration, whereas the high concentration increased it by 1.6 fold (Table 4).

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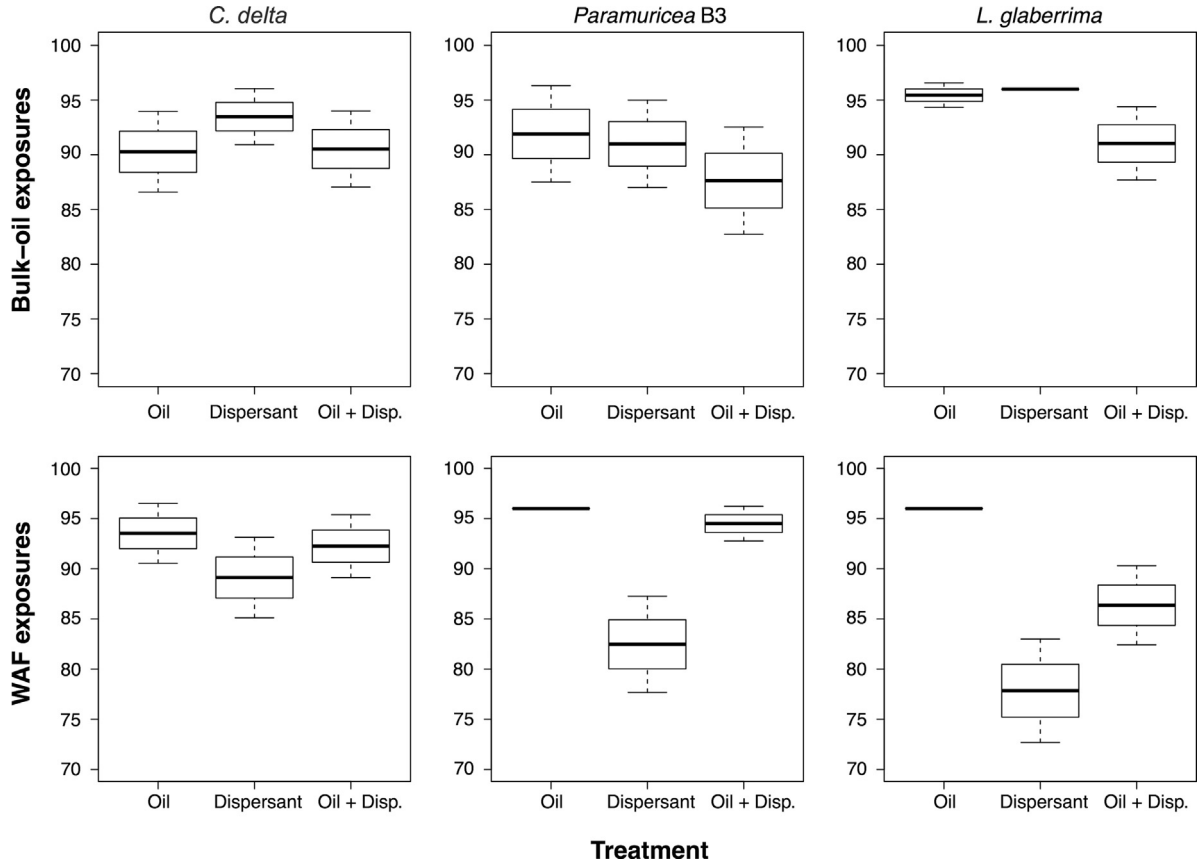

Fig. 3. Box-plots showing time-to-event estimates from the Kaplan–Meier survival analysis for coral fragments in three different treatments: oil, dispersant and oil/dispersant. (Top row represents bulk-oil exposures and bottom row represents oil WAF exposures.) The event was a decline in health rating to 3 or below (bulk) and 1 or below (WAF). Box ends represent standard error, line inside the box represents the mean and whiskers represent 95% confidence intervals.

Table 3
Log-rank tests of equality on survival distributions for the different levels of concentration in the bulk-oil and WAF exposure series. Significant *p*-values ($p < 0.05$) in bold.

Overall log-rank comparisons among concentrations				
Treatment		Chi-square	df	Sig.
Bulk-oil series				
Control	Log rank (Mantel–Cox)	–	0	–
Bulk-oil	Log rank (Mantel–Cox)	0.548	2	0.760
Dispersant	Log rank (Mantel–Cox)	15.635	2	**0.000**
Oil/dispersant	Log rank (Mantel–Cox)	21.793	2	**0.000**
WAF–oil series				
Control	Log rank (Mantel–Cox)	–	0	–
WAF–oil	Log rank (Mantel–Cox)	1.190	2	0.552
Dispersant	Log rank (Mantel–Cox)	33.246	2	**0.000**
Oil/dispersant	Log rank (Mantel–Cox)	20.061	2	**0.000**

3.5. Comparisons between species

Significant differences between K–M survival estimates were detected within the dispersant treatment as well as the control treatment during species comparisons for the bulk-oil series ($p < 0.05$; Table 5). The lowest time-to-event estimate of 90.3 h was for *Paramuricea* type B3 compared to an overall time-to-event estimate of 94.4 h. However, adding species into the Cox regression model did not improve fit, as species' rate of decline comparisons were not significantly different.

In the WAF exposures, these differences ($p < 0.001$) were also detected among species in the oil and oil/dispersant treatments. The lowest time-to-event estimate in oil was for *Paramuricea* type B3 at 68.8 h relative to 75.9 h for *L. glaberrima*, 85.2 h for *C. delta* and an overall time-to-event estimate of 76.3 h. *Paramuricea* type B3 (64.3 h) and *L. glaberrima* (65.3 h) had similarly low time-to-event estimates in the oil/dispersant treatment relative to 93.5 h for *C. delta* and an overall time-to-event estimate of 73.1 h. The Cox regression model also indicated that overall, *L. glaberrima* did significantly worse than *C. delta* by 1.3 fold but decline rates for *Paramuricea* type B3 were not significantly different from those of *C. delta* (Table 4).

4. Discussion

All three deep-sea coral species examined showed more severe declines in health in response to dispersant alone and the oil–dispersant mixtures than the oil-only treatments. The experiments reported here are the first ever to investigate the effects of oil and dispersant exposure on live, cold-water corals collected from the deep sea. Impacted corals have been observed at multiple sites in the deep GoM (Fisher et al., 2014), some covered with floc linked to oil from the Macondo well explosion (White et al., 2012). However, the unprecedented application of chemical dispersants in the deep-sea may have contributed to the observed pattern of impact. This exposure series provides crucial insight into the toxicological impacts of oil and dispersant release on three species of long-lived, habitat forming corals.

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Table 4
Predictor variables in Cox regression analysis and calculated hazard ratios of the odds of reaching health-ratings of interest (≤ 3) for the Bulk-oil and WAF–oil exposure series. Significant differences based on Wald test statistics; Low concentration is not present because values are constant or linearly dependent. Hazard ratios were calculated relative to the control water treatment, 0 mg/L concentration and the species *C. delta*, respectively. Significant *p*-values ($p < 0.05$) in bold.

Variable	Cox regression variables					
	Level	Wald	df	*p*	Hazard ratio	Standard error
Bulk-oil						
Treatment	Treatment	14.908	3	**0.002**	–	–
	Bulk-oil	0.011	1	0.918	0.963	0.355
	Dispersant	7.067	1	**0.008**	2.322	0.317
	Oil/disp.	0.123	1	0.725	1.133	0.367
Concentration	Concentration	19.279	2	< **0.001**	–	–
	Medium conc.	0.272	1	0.602	0.833	0.350
	High conc.	10.795	1	**0.001**	2.500	0.279
Species	Species	0.745	2	0.689	–	–
	Paramuricea B3	0.013	1	0.908	1.027	0.231
	L. glaberrima	0.645	1	0.422	1.212	0.239
WAF–oil						
Treatment	Treatment	95.263	3	< **0.001**	–	–
	WAF–oil	0.026	1	0.872	0.957	0.276
	Dispersant	27.407	1	< **0.001**	3.404	0.234
	Oil/disp.	41.745	1	< **0.001**	4.417	0.230
Concentration	Concentration	10.977	2	**0.004**	–	–
	Medium conc.	3.912	1	**0.048**	1.342	0.149
	High conc.	10.977	1	**0.001**	1.608	0.143
Species	Species	15.701	2	< **0.001**	–	–
	Paramuricea B3	1.807	1	0.179	0.817	0.137
	L. glaberrima	4.617	1	**0.032**	1.342	0.151

Table 5
Log-rank tests on equality of survival distributions for all species in the bulk-oil and WAF exposure series. Significant *p*-values ($p < 0.05$) in bold.

Overall log-rank comparisons among species				
Treatment		**Chi-square**	**df**	***p*-Value**
Bulk-oil series				
Control	Log rank (Mantel–Cox)	11.222	2	**0.004**
Bulk-oil	Log rank (Mantel–Cox)	2.496	2	0.287
Dispersant	Log rank (Mantel–Cox)	6.622	2	**0.036**
Oil/dispersant	Log rank (Mantel–Cox)	5.709	2	0.058
WAF–oil series				
Control	Log rank (Mantel–Cox)	60.353	2	< **0.001**
WAF–oil	Log rank (Mantel–Cox)	7.556	2	0.023
Dispersant	Log rank (Mantel–Cox)	2.584	2	0.275
Oil/dispersant	Log rank (Mantel–Cox)	22.22	2	< **0.001**

Regarding the components of the bulk-oil and WAF mixtures, hydrocarbon concentrations are likely an overestimate, given crude oil's variable and complex composition, containing thousands of compounds differing in hydrophobic and hydrophyllic tendencies (Clark Jr. and Brown, 1977; Singer et al., 2000; Di Toro et al., 2007). Dispersants also contain a variety of polar and non-polar surfactants and solvents (Singer et al., 1996). It is highly probable that there was adhesion of oil and dispersant constituents to the mixing flasks used during serial dilutions, as well as to experimental vials. Moreover, loss of water-accomodated oil fractions may have occurred through coalescence and surfacing throughout the exposure period (particularly in the bulk-oil exposure), volatilization during aeration, and/or biodegradation from the microbial communities associated with coral tissues (Couillard et al., 2005). Thus, it is difficult to determine the precise concentrations of oil and dispersant that each coral fragment may encounter at any given time during the course of the experiment but clearly actual exposures were lower than target values, making our results conservative estimates of the effects of oil, dispersant and oil/dispersant mixtures on deep-sea corals. Indeed, similar trends in health decline were observed within each treatment for all three species during four separate experimental trials.

The goal of this experiment was not to reproduce the exact conditions encountered by deep-water corals during the DWH spill, but rather to provide experimental evidence of their sensitivity to various concentrations of oil and dispersant. Reproducing exact conditions encountered by deep-water corals during the DWH spill is challenging because oil, dispersant and seawater mixtures form complex multiphase systems; an organism may then be exposed to many components of the oil and dispersant in various forms (National Research Council, 1989; Langevin et al., 2004). It is also important to note that corals within the vicinity of the DWH may have been exposed to these pollutants for longer than 96 h. Long-term exposures may see additional effects but were not feasible due to the time limitations of experimenting at sea. There is also a low survival rate when transporting deep-sea corals back to laboratory aquaria; *C. delta and L. glaberrima* only survive for approximately 1–3 months, whereas we have had no success keeping *Paramuricea* type B3 or *P. biscaya* alive over the long-term.

All three species of corals did surprisingly well in the oil treatments compared to the dispersant and oil/dispersant treatments (Figs. 1 and 2). In some cases, the corals appeared healthier in both the bulk-oil and oil–WAF treatments relative to the controls (e.g. *C. delta* and *L. glaberrima*, Figs. 1 and 2). Although corals can be negatively impacted when covered by oil particulates or floc (White et al., 2012), it is also possible that corals are deriving some form of nutrition from hydrocarbon components, a process that is likely to be mediated by their associated microbial communities. Anecdotal evidence for this linkage comes from the finding of at least one species of octocoral (*C. delta*) with increased abundances around natural hydrocarbon seeps (Quattrini et al., 2013). Previous studies of shallow-water octocorals also revealed non-selective hydrocarbon uptake of dispersed oil droplets into the gastrovascular cavity of the coral during water uptake (Cohen et al., 1977). Since additional food sources were not supplied during the exposure experiments, and most coral fragments within the oil treatments were frequently observed with a higher degree of polyp extension, similar uptake of dispersed oil components might have occurred.

Although our present study suggests MASS crude oil was not toxic over the range of concentrations tested in these experiments (Figs. 1 and 2), the effect of oil exposure on corals may be dependent on life-history stage. Crude oil (from the Macondo well) exposures of scleratinian coral larvae induced mortality within 24 h, while reducing settlement capabilities and post-settlement survival (Goodbody-Gringley et al., 2013). This suggests an increased vulnerability for coral planulae larvae and juvenile stages, although there was an influence of larval size on exposure tolerance. Other studies have shown premature ejection of planula larvae after exposure to water-soluble fractions of Iranian crude oil (Loya and Rinkevich, 1979) and sub-lethal oil damage to the female reproductive systems of scleratinian corals (Rinkevich and Loya, 1979). Similar sub-lethal impacts may have been imposed on cold-water corals exposed to oil released from the DWH disaster, although these effects may not be manifested for a number of years.

Treatments containing dispersants in both exposure experiments were the most toxic to the corals and induced the highest degree of overall fragment mortality (Figs. 1 and 2). As dispersants tend to increase the surface area of oil–water interactions, they may cause increased toxicological effects to marine organisms (Chandrasekar et al., 2006; Goodbody-Gringley et al., 2013). However, in the WAF exposure series, dispersant-only solutions were

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more lethal than the oil/dispersant mixture treatments (as compared to the bulk-oil exposure series), though both treatments resulted in some mortality (Figs. 1 and 2). Toxicity of dispersants is typically attributed to membrane disruption and impairment via surface-active compounds (Abel, 1974; National Research Council, 1989). Exposure results in increased permeability of biological membranes, loss of total membrane function and/or osmoregulation (Benoit et al., 1987; Partearroyo et al., 1990). Although Corexit 9500A was created in an attempt to reduce the toxicity of its predecessors while increasing effectiveness for dispersing more vicous oils, studies have shown that exposure effects are similar to older formulations, Corexit 9527 and 9554 (Singer et al., 1991, 1995, 1996), which are now considered toxic to a variety of marine organisms.

The results from this toxicological assay suggest that dispersant addition during the ensuing cleanup efforts following the DWH spill may have caused more damage to cold-water corals than the initial release of crude oil into the deep sea. Dispersants were toxic at the higher concentrations tested here, and dispersed oil solutions proved to be more toxic than untreated oil solutions (Figs. 1 and 2), as has been found in previous studies (Epstein et al., 2000; Mitchell and Holdway, 2000; Shafir et al., 2007; Bhattacharyya et al., 2003; Milinkovitch et al., 2011; Rico-Martinez et al., 2013). The ability of different types of dispersants to emulsify petroleum hydrocarbon components into the water column as well as the relative toxicity of the dispersants and crude oil, contribute to the overall toxicity of each solution (Epstein et al., 2000). The dispersant and oil/dispersant treatments were lethal to all three species in this study, particularly in the WAF exposure series where dispersant concentrations were higher.

It has been observed in several toxicology studies that dispersant additon increases the total concentration of polycyclic aromatic hydrocarbon (PAH) components in surrounding water (Couillard et al., 2005; Hodson et al., 2007). Specifically, it increases the concentration of less water-soluble high-molecular-weight PAHs, some of which induce enzymatic activity (i.e. cytochrome P4501A) that can metabolize PAHs into toxic forms causing a variety of detrimental effects (Henry et al., 1997; Billiard et al., 1999; Couillard et al., 2005). This could explain the more rapid decline in health for coral fragments exposed to the bulk-oil/dispersant and oil–WAF/dispersant mixtures, where it was likely that a larger proportion of crude oil compounds were made biologically available (Couillard et al., 2005; Schein et al., 2009). Larval exposure experiments on two species of shallow-water scleractinian corals, using BP Horizon source oil and Corexit 9500A, showed a significant decrease in survival and settlement in dispersant solutions and oil–dispersant mixtures, with complete mortality after exposure to 50–100 ppm solutions of dispersant (Goodbody-Gringley et al., 2013). In larvae of hard and soft coral species exposed to dispersants and Egyptian crude oil, all dispersant treatments were more toxic than the oil-only treatments with the highest toxicity observed in oil-dispersed solutions, which also resulted in abnormal development and tissue degeneration (Epstein et al., 2000).

Despite these results, it is unclear whether short-term exposures to oil and dispersant have long-term effects. Following brief (~24 h) exposures to Arabian crude oil or dispersed-oil (with Corexit 9527), there were no significant long-term effects on the yearly in situ skeletal growth of shallow water, hermatypic corals in the genus *Diploria* and *Acropora* (Dodge et al., 1984; LeGore et al., 1989). Though variability in growth rates during that year were not measured, similar experiments using a different scleractinian coral, *Porites furcata*, did reveal reduced growth in exposed fragments relative to controls (Birkeland et al., 1976). This indicates that although short exposure to oil and dispersant may not be lethal to these corals, additional sub-lethal impacts are possible, the extent of which need to be investigated further.

Oil transport to benthic sediments likely occurred through a variety of pathways after the DWH spill, including direct particulate sinking and absorption into marine snow (Passow et al., 2012). Exposure to oil-filled particulates may be more damaging to corals then the dissolved hydrocarbon components when additional stressors are present. As viscous particulates, such as flocs, settle onto benthic communities, the unavoidable exposure imposes many risks (Montagna et al., 2013) including the suffocation of sessile organisms. Floc was likely trapped in the mucous of corals (White et al., 2012) and may have also triggered the excretion of excess mucus in an attempt to remove the debris. This is an energetically costly mechanism, which may lead to reduced health when coupled to additional environmental stressors (Crossland et al., 1980; Riegl and Branch, 1995).

In conclusion, exposure to relatively high concentrations of crude oil does not appear to be as lethal to these species of deep-sea corals as dispersant and mixtures of hydrocarbons and dispersant. However, it is possible that a longer exposure to sub-lethal oil concentrations may cause adverse effects that could not be observed in this short-term toxicological assay. Further examination into the relative effectiveness of different types of dispersants, coupled to examinations of their relative toxicity, is required. To improve future response efforts, alternative methods of oil cleanup are needed, and caution should be used when applying oil dispersants at depth, as it may induce further stress and damage to deep-sea ecosystems.

Author contributions

Conceived and designed the experiments: DMD, DVR-R, IBB, EEC. Performed the experiments: DMD, DVR-R, IBB. Analyzed the data: DMD, IBB. Wrote the paper: DMD, IBB, EEC.

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