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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 (≤ 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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