The potential of radium-224 as a tracer of timescales of Gulf of Mexico crude oil exposure to the marine environment

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The potential of radium-224 as a tracer of timescales of Gulf of Mexico crude oil exposure to the marine environment

By
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Submitted in partial fulfillment of the requirements for the Degree of Master of Science in Coastal Marine and Wetland Studies in the School of the Coastal Environment
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October 2019

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Acknowledgments

I would like to extend my gratitude to the many people and organizations who have made this research possible. First and foremost, my advisor, Dr. Rick Peterson, whose efforts over the last ten years have formed the basis for this project and secured the funding. Your expertise, personal and professional guidance, and faith in me and my abilities have allowed me to grow as a person and as a scientist. Thank you for all your efforts on my behalf. I also would like to thank my committee members: Dr. Bill Burnett, Dr. Angelos Hannides, Dr. Mandy Joye, and Dr. Susan Libes. Whether through your expertise, classroom learning, or intriguing conversation, you have all helped me along this journey and contributed greatly to this research and my growth as a scientist.

This research would not have been possible without funding from the Gulf of Mexico Research Initiative and the Department of Coastal and Marine Systems Science. A special thanks to the captain and crew of the R/V Point Sur and the operating team of the ROV Odysseus at Pelagic Research Services for the unforgettable experience of deep sea research and sample collection.

I would like to thank my peers as well: Leigha Peterson, Elana Ames, Charlotte Kollman, Todd Rhodes, Doug Pastore, Christina Boyce, Mary Lee King, Cathryn Wheaton, Jessie Wingar, and all the graduate students I have been fortunate enough to work alongside. Your assistance, encouragement, and friendship means the world to me and I feel very fortunate to have met you all. Thank you for the memories and best of luck in all your future endeavors.

Finally, I want to acknowledge my family whose unwavering love and support has carried me through my academic career. Thank you for everything you have done and continue to do for me to help me along this journey.
Abstract

Petroleum pollution in the marine environment can be deleterious to coastal and marine ecosystems and can have sustained effects for years. While oil slicks on the surface of the ocean are tracked with relative ease using satellite-based technology, deep sea, neutrally-buoyant hydrocarbon plumes remain exceedingly difficult to track. We provide evidence for the utility of $^{224}\text{Ra}$ as a potential hydrocarbon tracer to determine the marine exposure time of crude oil. We employed time course incubations to constrain a time dependent $^{224}\text{Ra}$ release signature and tested a variety of timescales, temporal resolutions, oil sources, seawater, and experimental treatments to determine potential factors that contribute to the variability of $^{224}\text{Ra}$ release from hydrocarbons into seawater. Our results show quantitative release of $^{224}\text{Ra}$ from crude oil in contact with seawater and similar temporal variability (which increases with finer temporal resolution) in $^{224}\text{Ra}$ activity between two oil sources, regardless of the overall magnitude of release. The magnitude of $^{224}\text{Ra}$ release from crude oil is proposed to vary depending on the geochemistry of the source reservoir and biological activity therein as well as geochemical alterations as the oil flows through geologic conduits. Mechanisms of release are thought to be primarily associated with chemical degradation (i.e., photo- and bio-degradation) of the oil matrix and cation exchange processes. These interpretations warrant further investigation. However, our results provide the first evidence that release of $^{224}\text{Ra}$ from crude oil represents a disequilibrium from its particle-sorbed parent isotopes suggesting this isotope may be useful for examining the temporal dynamics of oceanic hydrocarbon plumes.
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1. Introduction

Hydrocarbons have been the dominant source of energy since the Industrial Revolution, yet scientists only began to realize their side effects as a pollutant in the 1970s (National Research Council, 2003). Widespread and frequent burning of hydrocarbons has led to considerable pollution from byproducts of hydrocarbon combustion (e.g., soot, ash, CO$_2$, SO$_X$, and NO$_X$) that have contributed to effects like heavy metal bioaccumulation, acid rain, and increased atmospheric inventories of CO$_2$ (Hangebrauck et al., 1964; Likens and Bormann, 1974; National Research Council, 2003). Additionally, the mechanisms employed for collection and transport of hydrocarbons have led to a multitude of large-volume discharge occurrences of liquid petroleum directly into the marine environment (National Research Council, 2003). These large-volume oil spills exert an immediate impact on local ecosystem health. The worst offshore oil spill resulted from the catastrophic Deepwater Horizon (DWH) blowout which began on April 20, 2010. The 87-day uncontrolled discharge of hydrocarbons injected an estimated 800 million liters of crude oil and at least 250,000 metric tons of natural gas into the Gulf of Mexico (Joye, 2015). The wellhead blew out at a depth of 1544 m (Edwards et al., 2011) resulting in a laterally-spreading hydrocarbon plume at the ocean surface (Lubchenco et al., 2012; McNutt et al., 2012) and another one at a depth of 1000-1200 m (Socolofsky et al., 2011).

Once in the marine environment, hydrocarbons are affected by a variety of physical and chemical processes (Saeed et al., 2011). Physical processes that influence oil distribution include evaporation, spreading, dispersion, and emulsification (Saeed et al., 2011). Chemical processes that influence oil distribution include dissolution, and photochemical breakdown by solar radiation (photodegradation; Fasnacht and Blough, 2002). Microbial biodegradation can transform toxic petroleum into harmless byproducts (Broojimans et al., 2009; Hazen et al., 2010; Edwards et al.,
Of all degradation mechanisms, photodegradation and biodegradation are most significant processes that remove oil from the environment (King et al., 2014 and references therein). Biodegradation may occur in plumes or sheens throughout the entire water column (Hazen et al., 2010; Edwards et al., 2011), whereas photodegradation is limited to surface water sheens.

Different types of hydrocarbons may be broken down by these mechanisms at different relative rates. Aliphatic (usually straight-chain) hydrocarbons are generally considered to be biologically labile compared with aromatic hydrocarbons, while the reverse is true for inorganic chemical processes. Aromatics, specifically polycyclic aromatic hydrocarbons (PAHs), are more biologically refractory due to their alternating double-bond ring structure. Due to this difference in bioavailability, various microbial communities have evolved to specialize in catabolizing specific hydrocarbon compounds.

Compared to surface oil slicks, deep hydrocarbon plumes are much more difficult to identify, trace, and constrain transport scenarios. While surface slicks are relatively easy to track using satellite-based synthetic aperture radar (SAR; MacDonald et al., 1993; Garcia-Pineda et al., 2010), deep hydrocarbon plumes are not visible from the surface and can only be detected using in-situ chemical sensors (i.e., dissolved oxygen anomalies; Camilli et al., 2010; Kessler et al., 2011). Furthermore, deep sea currents often differ in speed and direction from their surface counterparts (Schmitz et al., 2005; Bracco et al., 2016), rendering inferences of deep plume behavior based on surface measurements potentially misleading. Such a plume of dissolved and finely dispersed hydrocarbons may subsequently surface from upwelling or where the plume intersects nearshore regions, potentially harming downstream biota.

Surface oil slicks are exposed to a wider array of breakdown mechanisms than deep hydrocarbon plumes including photodegradation (Fasnacht and Blough, 2002), evaporation, and
dispersion from wave action (Saeed et al., 2011). The only two known mechanisms that can remediate a deep hydrocarbon plume are in-situ microbial degradation (Broojimans et al., 2009; Hazen et al., 2010; Edwards et al., 2011) and anthropogenic injection of chemical oil dispersants (i.e., COREXIT 9500 or COREXIT 9527; Seidel et al., 2016). While chemical dispersants are effective at atomizing liquid petroleum and promoting accelerated natural degradation processes (Paris et al., 2012; Seidel et al., 2016), their ecological effects are debated (Kleindienst et al., 2015; Prince, 2015). Hydrocarbons in the marine environment may also become buried through settling via adsorption onto sinking particles or contact with the seafloor (Chanton et al., 2014; Babcock-Adams et al., 2017 and references therein).

Marine oil snow sedimentation and flocculent accumulation (MOSSFA; Passow, 2016; van Eenennaam et al., 2016) is a recently characterized process which results in rapid sedimentation of oil and oil degradation products to the seafloor. Such deposition of oil and oil products may substantially increase the residence time of these compounds in the marine environment from weeks to decades (Prince, 2015; Fisher et al., 2016). Furthermore, a study by Hastings et al. (2016) suggests that marine oil snow deposition in deep sediments (800-1600 m) may alter natural redox conditions for years and significantly impair populations of benthic foraminifera. Deposited oil can also be reintroduced into the water column through resuspension (Diercks et al., 2018). The increased residence time of oil in the marine environment due to sedimentation emphasizes the need to understand the temporal dynamics of hydrocarbon residence characteristics.

The ability to accurately track oil spills through space and time is critical to evaluate the fate of the oil and thus reach decisions on mitigation strategies. The development of such a tool may substantially enhance our understanding of the spatiotemporal dynamics of hydrocarbon plumes. Preliminary evidence suggests that radium isotopes can be used to trace hydrocarbon
discharge and therefore may offer some promise as a tool to assess hydrocarbon exposure time in
the ocean (Peterson et al., 2013). Radium isotopes have proven to be useful tracers of various
processes, such as submarine groundwater discharge to the coastal zone and river plume mixing
(Moore, 1996; Peterson et al., 2008). The inherent temporal nature of the radioactive decay of
these isotopes allows investigators to constrain the temporal dynamics of spatially variable
processes. Among the four naturally-occurring isotopes of radium, $^{224}$Ra and $^{223}$Ra have relatively
short half-lives (3.66 days and 11.4 days, respectively), whereas $^{228}$Ra and $^{226}$Ra have much longer
half-lives (5.8 years and 1600 years, respectively).

Radium is constantly produced via decay of isotopes within the uranium and thorium decay
series that are sorbed to aquifer solids (Krishnaswami et al., 1982), so it often reaches high
concentrations in geologic fluids (Moore et al., 2008). The partition coefficient of radium in saline
waters suggests that while primarily remaining adsorbed to surfaces, some radium will desorb via
ion-exchange mechanisms and enter solution (Colbert and Hammond, 2008; Gonneea et al., 2008).
Radium has been well documented as being strongly enriched in saline formation fluids within
hydrocarbon reservoirs (Bloch and Key, 1981; Kraemer and Reid, 1984). In fact, radium is
generally considered to be one of the most prevalent naturally-occurring radioactive materials in
the oil and gas industry since scales inside pipes scavenge radium isotopes, often concentrating
them to dangerous levels (Smith, 1992).

We suspect radium may likewise be sorbed to the hydrocarbons themselves, such that upon
injection into the marine environment, radium isotopes may be released into the surrounding water.
This release would create a radioactive disequilibrium because the now dissolved radium would
no longer be supported by surface-bound parent isotopes. Ratios of these aqueous radium isotopes
may therefore offer a tool that can estimate the residence time of the hydrocarbons in the marine
environment. The goal of this work is to assess whether such a release of radium isotopes into the aqueous phase may occur as a function of the timing hydrocarbon presence in the water column. We employed a series of hydrocarbon incubation experiments using multiple oil sources in which we isolate various breakdown processes to examine whether hydrocarbon residence or breakdown preferentially injects radium into the aqueous phase. This approach allows us to characterize a radium isotope release signature and evaluate whether radium may be a useful hydrocarbon geochronometer. Radium release is also analyzed in the presence of chemical oil dispersants to investigate any influence they may have on the radium release signature.

2. Methods

Time-course incubations of oil in seawater serve as the primary experimental approach to constrain the radium isotopic signature from unique oil sources. Methods described below allow quantification of the signatures of the radium isotopes released into seawater. Exposure time is defined here as the amount of time the oil has been exposed to seawater, and therefore presumably to processes which chemically alter it (i.e., photodegradation and biodegradation). Time-course incubations were conducted using multiple oil samples from different sources. The first experiments were conducted using archived oil from the DWH oil spill. Freshly-discharged oil collected during two research cruises aboard the R/V Point Sur in the Gulf of Mexico in August/September 2018 and January 2019 was also used. Metadata for both archived DWH oil and oil collected during our two research cruises are available in Appendix 2.

2.1 Site Descriptions and Sample Collection
Freshly-discharged oil was collected from a well-documented seep field at Green Canyon lease block 600 (GC600) located approximately 120 nautical miles southwest of the Mississippi River Delta. The Green Canyon lease block lies on the Texas-Louisiana continental shelf in the north-central Gulf of Mexico (Figure 1). This lease block contains the two sampling sites visited on our research cruises, GC600 (oil seep) and GC699 (control). GC699 was selected as a control site as it lacks any known oil seeps and is distant from known seeps. The control site was where we collected seawater for the time-course oil incubation experiments. Seawater was filtered for particulate matter through glass microfiber filters followed by an acrylic fiber impregnated with MnO$_2$ (Moore and Reid, 1973) to remove ambient radium. Initial $^{224}$Ra activities of filtered seawater are reported in Figure 4 as the activity in Ra-free seawater blanks at T = 0 days. For Ra-free tap water, the same process was applied with the exception of particle filtration.

At GC600, the ROV Odysseus (Pelagic Research Services; Figure 2) collected actively discharging crude oil from two distinct features at the ‘Megaplume’ seep field (herein referred to as ‘Megaplume A’ and ‘Megaplume B’). These seeps are located approximately 19.5 meters from each other and are at a water depth of 1185 m. Discharged oil was collected into glass oil samplers housed within a plexiglass carousel. The manipulator arm of the ROV was able to index the carousel, remove and fill each oil sampler, and store it back in the carousel for the remainder of the dive.

During the first cruise, approximately 192 mL of oil was recovered from Megaplume A; approximately 340 mL of oil was collected from Megaplume A during the second cruise. Oil from Megaplume A from the first cruise will be referred to as Megaplume A1 oil while oil from the second cruise will be referred to as Megaplume A2 oil. Megaplume B was discovered during the second cruise and approximately 275 mL of oil was recovered for a total of approximately 615 mL
between both sources during the January 2019 cruise. Improved sampling methods during the second cruise allowed for more oil to be recovered. An appreciable amount of gaseous methane partially filled the oil sampler and froze out as hydrate during the first cruise. Therefore, only one sampler was used for each dive during which we learned that a slow, controlled ascent allowed the hydrate to sublime and degas. During the second cruise, a more careful oil collection approach was taken in which we allowed more methane gas release during oil collection. While some hydrate still formed, we filled all samplers during each dive resulting in more oil recovery.

2.2 Time Course Incubation Experiments – Experimental Design

A variety of treatments were utilized in the time-course oil incubation experiments to examine a change in radium isotope activity over time for various combinations of degradation mechanisms. For each treatment, a 1g:10L oil:seawater ratio was used to simulate oceanic hydrocarbon plume concentrations (as per Bælum et al., 2012). Oil was injected into seawater using a volumetric pipette (calibration information is available in Appendix 3). Oil was injected into 5 L of Ra-free seawater followed by an addition of another 5 L of seawater. Turbulence from the second seawater addition was relied on to mix fluid the oil and seawater to avoid inserting a mixing tool which would sorb oil. The two primary treatments include a surface ocean sheen simulation and a deep sea plume simulation with two additional treatments having live and poisoned microbial populations. These treatments are outlined in Figure 3 and briefly described below.

**Surface Sheen Conditions:** To simulate conditions at the ocean surface, oil-water mixtures were stored in open glass aquaria (70 cm L x 30.5 cm W x 40.6 cm H) in ambient sunlight for the duration of the incubation. The aquaria were placed in water baths to prevent excessive heating.
and evaporation of the inoculant waters. The two secondary treatments, live and poisoned, permitted differentiation of the effects of coupled microbial/photodegradation and photodegradation alone, respectively. Ra-free seawater blanks were placed alongside the outdoor oil-water mixtures to account for any potential contamination by rain, dust, dirt, or other sources of radium. These blanks were analyzed for radium content at each time point with any measurable radium activity being subtracted from the radium activity of the oil-water mixtures analyzed for that day. Treatments were prepared using crude oil and particle-filtered, Ra-free surface water as the inoculant water. Weather conditions (i.e., temperature, UV index, temperature, and cloud cover) and the temperature of the water baths were recorded daily.

**Deep Plume Conditions:** To simulate conditions in a deep ocean hydrocarbon plume, these oil-water mixtures were stored in glass carboys in dark, cold (5°C) incubators for the duration of the incubation period. Storing these carboys in the dark prevented photodegradation. During sampling, red light headlamps were used to minimize any effect of light on the sample. In the live treatments, native microbial populations in inoculant waters could have degraded the hydrocarbon compounds via catabolic respiration. The poisoned treatments should not have experienced any microbial degradation of the oil. Therefore, deep poisoned treatments are not expected to experience any degradation, thus represent the effect of the presence of oil in seawater. Treatments were prepared using crude oil and Ra-free deep (1100 m) water as the inoculant water.

Incubations were held for a maximum of three weeks, after which they were destructively sampled for $^{224}\text{Ra}$ activity at a predetermined time point. The original set of time points included an initial ($T = 0$ days) measurement, sampled immediately following oil injection, as well as terminations at 1 day, 3 days, 7 days, 11 days, 14 days, and 21 days. These time points were chosen strategically for three reasons: (1) we anticipated that most of the significant changes would occur
early; (2) these time points roughly represent 0.25, 0.5, 1, 2, 3, 4, and 6 half-lives of $^{224}$Ra; and (3) previous studies on hydrocarbon degradation show substantial oil breakdown over the course of several weeks. However, due to both scientific and logistical reasons, these time points had to be shifted somewhat between experiments. The original set of time points was used for the first DWH incubations. During the second set of DWH incubations, the 3 day time point was replaced with 2 day and 4 day time points to better resolve early changes. Incubations using freshly-discharged oil were extended out to 16 days for logistical reasons. Table 1 presents an overview of the conditions for each set of incubations. Section 2.3 provides a more detailed description of each experiment shown in Table 1.

2.3 Time Course Incubation Experiments – Treatment Details

The first set of these incubations (DWH-1) were preliminary experiments to test the efficacy of the experimental design. DWH oil was injected into Ra-free seawater collected in Long Bay, SC. For the poisoned samples, a 10 mM zinc chloride (ZnCl$_2$) concentration was used (as per Babich and Stotzky, 1978). The surface treatments in incubations were covered with plexiglass sheets to prevent contamination. Upon further investigation it was found that these plexiglass sheets had a very low UV transmittance, thus they were replaced with Ra-free seawater blanks for all other experiments.

The same oil and seawater sources were used for the second set of incubations (DWH-2) and ZnCl$_2$ was used again as a poison. Ra-free seawater blanks were placed adjacent to outdoor oil incubations and were analyzed at each time point to account for atmospheric contamination. For this second incubation experiment, the 3 day time point was replaced with two time points at 2 and 4 days in order to better resolve early changes in radium release.
The third set of incubations (GC600-1) was performed in Gulfport, MS directly after the first research cruise. For these incubations, Megaplume A1 oil was injected into GC699 seawater. GC699 seawater collected and filtered for particles and ambient radium at sea to be used as inoculant water. Water collected at the surface of GC699 was used in the surface sheen treatments while water collected at 1100 m was used in the deep sea plume treatments. These incubations also included Ra-free seawater blanks for outdoor samples. A 2 mM cadmium chloride (CdCl₂) concentration was used for poisoned treatments (as per Fulladosa et al., 2005). The timing of sample termination was altered due to logistical constraints. These altered time points can be found in Table 1.

A fourth set of incubations (GC600-4) was performed in April 2019 and used a combination of Long Bay, SC seawater and Gulf of Mexico seawater as inoculant waters. GC600 oil was used for these incubations with three different treatments: Megaplume A1, Megaplume A2, and Megaplume A2 with COREXIT 9527 (using a 1:1.67 dispersant-to-oil ratio as per Campo et al., 2013). Each of these treatments also included surface and deep ocean simulations as well as live and poisoned treatments within each depth simulation. Poisoned treatments contained a 0.05% HgCl₂ concentration (as per personal communication with Dr. Erik Smith, University of South Carolina). Time points of sample termination are the same as in GC600-1.

A fifth set of incubations (DWH-3) in July 2019 likewise examined the effect of COREXIT 9527 on radium release using DWH oil. Long Bay, SC seawater was used as inoculant water. The same dispersant-to-oil ratio as GC600-4 was used. Both surface and deep treatments were implemented comparing dispersant and non-dispersant oil incubations. Ra-free seawater blanks were again placed next to outdoor samples. Due to time and resource constraints, poisoned
treatments were omitted and timing of sample termination was altered. Time point changes are reflected in Table 1.

Short-term incubations were also performed, the first of which (GC600-2) was done in February 2019 – immediately upon return to Coastal Carolina University after the January 2019 research cruise – and the second of which (GC600-3) was done approximately six weeks later in March 2019. These short-term incubations were held up to 4 days, with an initial \((T = 0 \text{ days})\) measurement and termination time points at 0.5 days, 1 day, 1.5 days, 2 days, 2.5 days, 3 days, and 4 days. Megaplume A2 and Megaplume B oil were both used for these experiments. Finer temporal resolution allowed a more detailed examination of early changes in radium release. The replicate incubations in March 2019 were performed to examine any change in the radium signature of either oil source as a result of being removed from their geologic source and the marine environment for approximately 7 weeks (i.e., after decay of all excess \(^{224}\text{Ra}\)). Only live surface treatments were implemented for these experiments.

2.4 Variable Oil Concentration Experiment

We also tested the effect of differing oil concentration on the magnitude of radium release. Each sample contained 10 L of Ra-free GC699 seawater and Megaplume A1 oil injections of 1 g, 2 g, 3 g, and 4 g. None of these samples contained any poison. Two samples were also incubated using freshwater instead of seawater to test whether radium is released from oil via cation exchange processes. These incubations contained 1 g Megaplume A2 oil and 10 L Ra-free deionized tap water, one being terminated immediately after addition of the oil and the other after a 24 hour incubation period.
2.5 Analytical Methods

For each termination, the total volume of incubated fluids were emptied into a 1 L separatory funnel, connected by a short tube to a cartridge containing a 25 g acrylic fiber prefilter. The prefilter cartridge was then connected to another cartridge containing a 25 g dry acrylic fiber impregnated with MnO₂ (“Mn-fiber”; Moore and Reid, 1973). Acrylic fiber prefilters are highly lipophilic and sorb oil from the oil-water mixture but not any dissolved radium from the inoculant waters. As incubated fluids were not provided sufficient time to thoroughly separate in the separatory funnel, prefilters were relied on as the primary mechanism to separate oil and seawater phases. The Mn-fibers scavenge the dissolved radium from the oil-free inoculant waters, concentrating radium isotopes on the surface of the MnO₂ crystals embedded within the fibers. Flow velocities were maintained below 1 L/min to ensure efficient extraction of radium (Moore and Reid, 1973).

Short-lived radium isotope activities were measured using a Radium Delayed Coincidence Counter (RaDeCC) system, a closed loop system which measures ²²⁴Ra and ²²³Ra activities via their radiogenic daughters ²²⁰Rn and ²¹⁹Rn, respectively (Moore and Arnold, 1996). The RaDeCC requires that all fibers be within a certain humidity range (Sun and Torgersen, 1998) and that all salts be removed from the fibers before counting. Therefore, before the fibers were removed from the filtration rig, 1 L of Ra-free fresh water was passed through the fibers. The fibers were then partially dried using a compressed air stream (Sun and Torgersen, 1998).

Fibers were measured immediately after filtration. This first measurement on the RaDeCC provides the total ²²⁴Ra activity. After 3 weeks (i.e., after all excess ²²⁴Ra would have decayed away), the fibers were measured again on the RaDeCC to determine the amount of parent-supported ²²⁴Ra (from adsorbed ²²⁸Th) which is then subtracted from the total ²²⁴Ra activity to
calculate the excess $^{224}$Ra activity. Herein, the terms $^{224}$Ra and excess $^{224}$Ra will be used interchangeably. Acrylic fiber prefilters were likewise measured on the RaDeCC independently of the Mn-fibers to measure oil phase $^{224}$Ra.

Ra-226 activity was measured via emanation of its gaseous radon daughter, $^{222}$Rn (Peterson et al., 2009). Mn-fibers were sealed in gas-tight cartridges and held for three weeks to allow the $^{222}$Rn to grow into secular equilibrium with $^{226}$Ra to use $^{222}$Rn as a proxy (Peterson et al., 2009). Gas was then extracted and filtered with drierite (for moisture) and ascarite (for CO$_2$) to avoid collection of ambient radioactive gases. Lucas cells were then filled with radon gas, held for three hours to allow decay of short-lived radon isotopes, and measured for Rn-222 activity by alpha scintillation (Peterson et al., 2009).

Ra-228 activity was measured via $\gamma$-spectrometry using methodology outlined by Dulaiova and Burnett (2004). Mn-fibers were packed into custom-made crucibles fashioned using stainless steel tool-wrap type 321 (MSC Industrial Supply) and then ashed in a muffle furnace at 550°C for six hours. This process burns away the acrylic fiber and leaves ash composed of MnO$_2$ onto which radium isotopes remain adsorbed. These crucibles are then flattened and sealed with silicone caulking to ensure secular equilibrium between radium and radon daughters. Samplers were counted via gamma spectrometry on an Ortec germanium detector for approximately 48 hours. $^{228}$Ra activity is determined by proxy of its daughter, actinium-228 ($T_{1/2} = 6.1$ hours), at energies of 338 keV and 911 keV (Dulaiova and Burnett, 2004).

Excess $^{224}$Ra activities are reported in dpm (disintegrations per minute). These activities are typically normalized to sample volume or mass and therefore reported as dpm/L or dpm/g. However, given that all radium samples were obtained from the same volume of seawater and mass of oil (with the exception of the experiment described in section 2.3), we chose not to
normalize activity but to report the total activity recovered from the sample. We do not report $^{223}$Ra, $^{226}$Ra, or $^{228}$Ra activities as all samples exhibited large analytical uncertainties and were generally at or below our minimum detectable activities for these isotopes. Th-$^{228}$ activities were considered, however they were highly variable and exhibited no discernable temporal trends, thus are likewise not reported herein. Raw data for reported isotopes are available in Appendix 1.

3. Results & Discussion

3.1 Radium-$^{224}$ in Oil-Seawater Mixtures

Activities of $^{224}$Ra in oil-seawater mixtures were consistently elevated above Ra-free seawater blanks (Figure 4). For the DWH-2 incubation (Figure 4A), $^{224}$Ra activities averaged 0.223 ± 0.017 dpm for all oil incubations and 0.040 ± 0.005 dpm for seawater blanks and, for GC600-1, 0.161 ± 0.014 dpm and 0.005 ± 0.001 dpm for oil incubations and seawater blanks, respectively. While GC600-4 data is not shown in Figure 4, the average $^{224}$Ra activities for those oil incubations was 0.052 ± 0006 dpm with seawater blanks averaging at 0.007 ± 0.001 dpm. In all experiments employing Ra-free seawater blanks, oil incubations show a $^{224}$Ra enrichment of 1-2 orders of magnitude.

The source of the elevated $^{224}$Ra in these mixtures must be the crude oil as it represents the only additional variable (at least for live treatments) beyond the source water from which the Ra-free seawater blanks were derived. While poisoned treatments do have the addition of further chemicals, they exhibit similar magnitude $^{224}$Ra activities as live treatments containing only crude oil and seawater. Some possibility exists for contamination affecting the surface ocean treatments
as a result of being stored uncovered and outdoors. However, all deep sea treatments were stored in a controlled laboratory environment for the entirety of the incubation period and likewise showed $^{224}\text{Ra}$ activities well above those observed in seawater blanks, suggesting potential contamination in surface ocean treatments is unlikely to be a major contribution to observed $^{224}\text{Ra}$ enrichments. Additionally, the seawater blanks were also stored uncovered outdoors so they would have likewise been subject to any sources of atmospheric contamination. This evidence shows that $^{224}\text{Ra}$ must be released from crude oil into surrounding seawater.

3.2 Time Dependent Radium Release Signatures

The general release of $^{224}\text{Ra}$ from oil to surrounding seawater is further suggested from the temporal variability in aqueous $^{224}\text{Ra}$ activity. Peak $^{224}\text{Ra}$ activities in all live treatments are observed between 1-2 days followed by a decline in $^{224}\text{Ra}$ activities roughly following a theoretical radioactive decay curve. In some cases, we observe minor $^{224}\text{Ra}$ release at some time points as evidenced by higher $^{224}\text{Ra}$ activities than would be present due to radioactive decay from the maximum activity (Figure 5). These data show that $^{224}\text{Ra}$ activity varies significantly as a function of seawater exposure time. Perhaps more importantly, the fact that $^{224}\text{Ra}$ activity increases beyond that at $T = 0$ days (Figure 5) suggests further input of $^{224}\text{Ra}$ from oil.

While an initial injection of oil into seawater imparts a significant and immediate input of aqueous $^{224}\text{Ra}$, the fact that the activity further increases over the next 1-2 days suggests a continued supply mechanism. The question remains as to whether $^{224}\text{Ra}$ is injected into water simply as a function of oil presence (i.e., governed by some form of partitioning relationship), or whether the oil must be structurally broken down (i.e., photo- or microbial-degradation processes) to release the radium. A third possibility may be a combination of the two (i.e., structural
breakdown exposes new hydrocarbon surface area over which partitioning may then occur). While this question is explored later (Section 3.4), we can glean some insight by examining the temporal trends here.

For live treatments, we suspect the general behavior of early (1-2 day) $^{224}$Ra release followed by decay with minor additions afterward may be a result of the ‘boom-and-bust cycle of bacterial succession’ (Crespo-Medina et al., 2014). Hydrocarbon-bearing seawater experiences a boom of microbes specialized to consume a specific type of hydrocarbon compound (e.g., aliphatic or aromatic). After one specific hydrocarbon compound is largely catabolized, there would be a significant reduction in the population of that OMT. This bust is followed by a boom of another OMT, following a succession based on the lability of remaining hydrocarbons (Crespo-Medina et al., 2014). Our data (Figure 5) suggest this cycle may be occurring, perhaps with degraders of more labile compounds succeeding early followed by degraders of more refractory compounds later breaking down those compounds more slowly. For example, the radium release spikes at 1-2 days in Figure 5 may be a result of fast respiration of labile hydrocarbons followed by minor releases of more refractory compounds between days 10-15. Such a shift in microbial metabolism would represent a decreasing rate of structural breakdown of the oil over time, thus less new oil surface area exposed over time. However, these speculations require further investigation as biological activity was not measured or characterized in this study.

Poisoned treatments did not show the release signatures that would be expected if chemical degradation was the predominant factor in $^{224}$Ra release. In that case, surface poisoned treatments would follow a signature based on photodegradation of the oil while deep poisoned treatments should follow a radioactive decay curve following initial ($T = 0$) release of surface-bound $^{224}$Ra. While two of the deep poisoned treatments (Figure 5D and F) somewhat follow decay to near 0
dpm, we also observe cases of $^{224}$Ra release toward the end of the incubations (i.e., activities are observed above the theoretical decay curve from the peak activities). These radium release signatures may suggest an inefficacy of the poisons ($\text{ZnCl}_2$ for DWH-1 and DWH-2 incubations; $\text{CdCl}_2$ for GC600-1 incubations). However, we find this possibility unlikely as the literature suggests that our utilized concentration of each poison should have been sufficiently toxic to fully sterilize our samples (Babich and Stotsky, 1978; Fulladosa et al., 2005). In fact, Fulladosa et al. (2005) show that the CdCl$_2$ concentration used (2000 μM) is more than sufficient to reduce the population of *Vibrio fischeri*, a hydrocarbon-degrading bacterium native to the Gulf of Mexico, to near-zero. However, we cannot discount the possibility that the poisons worked early but did not sufficiently repress the populations throughout the entirety of the incubations.

Averages of all time series in DWH-1, DWH-2, and GC600-1 (*Figure 6*) show a general trend of initial ($T = 0$) $^{224}$Ra release followed by a peak in $^{224}$Ra activity after 1 day. Although all peaks in *Figure 5* differ in magnitude, the variability of $^{224}$Ra activity is similar across various seasons, latitudes, treatments, oil sources, and seawater sources. *Figure 6* thus represents the general time-variant $^{224}$Ra release signature from crude oil in seawater, separating the live treatments from poisoned treatments to differentiate more realistic environmental conditions from experimental treatments (i.e., addition of poisons). While the standard deviation is large (average $\sigma_{\text{all}} = 51.7\%$, average $\sigma_{\text{live}} = 47.3\%$), the average $^{224}$Ra release signature suggests some degree of predictability in the temporal variability of $^{224}$Ra activity in oil-seawater mixtures, though we do not observe nearly the enrichment in dissolved $^{224}$Ra after 1 day in the poisoned treatments as we do in the live treatments. Both sets of treatments indicate a relative enrichment after 10-12 days of incubation above that predicted by decay.
3.3 Potential Factors Affecting Time Dependent Radium Release Signatures

3.3.1 Temporal Resolution

Figure 7 illustrates the effect of temporal resolution on the observed variability of $^{224}$Ra time series in oil incubations. Performing time-course incubations on a timescale with finer resolution yields a more precise time series of $^{224}$Ra activity (Figure 7A). When the same data from GC600-2 is considered at a 1-day resolution (Figure 7B), we lose perspective of some variability. Pearson’s correlation coefficients between Megaplume A2 and Megaplume B time series at 12-hour and 24-hour resolutions indicate a weak correlation between the two oil sources at a 12-hour resolution ($R = 0.28$), yet a very strong positive correlation at a 24-hour resolution ($R = 0.95$). Visual analysis of Figure 7B alone suggests that Megaplume A2 and Megaplume B exhibit similar $^{224}$Ra release, yet at a finer resolution we observe significant differences. As these experiments were conducted outdoors, the 12-hour variability must be the result of day/night cyclicity in either temperature or UV irradiance.

3.3.2 Oil Source Geochemistry

Megaplume A2 and Megaplume B oil were collected at seeps located approximately 19.5 meters apart on the seafloor, therefore oil from both seep sites are presumed to be derived from the same subsurface hydrocarbon reservoir. Within five days of collection, their $^{224}$Ra release signatures from experiment GC600-2 are statistically distinct ($R = 0.28$) in terms of their variability over time, but their median $^{224}$Ra release is relatively similar with a difference of 17% (Figure 8, black boxes). After approximately seven weeks of holding time prior to a duplicate experiment to GC600-2 (GC600-3), the difference between the medians drastically increased to 136% (Figure
The overall magnitude of $^{224}$Ra release also decreased significantly between GC600-2 and GC600-3. Median $^{224}$Ra activities for Megaplume A2 decreased by more than an order of magnitude over the seven week hold time while activities for Megaplume B decreased by more than half an order of magnitude (Figure 8).

Specific seep site geochemistry may drive some of these differences. Assuming Megaplume A2 and Megaplume B oil are sourced from the same subsurface reservoir and that hydrocarbons can be considered homogenous within that reservoir, the primary factor that is difference between the two oil sources is the geologic pathway through which the fluids ascended prior to reaching the water column. All isotopes of radium are known to be sourced from the radioactive decay of particle-bound thorium isotopes in sediments (Krishnaswami et al., 1982). It is therefore likely that as liquid petroleum flows upward through a geologic matrix, dissolved radium would be entrained by the flow. Different pathways may have varying geochemical properties resulting in an alteration of the source $^{224}$Ra release signature. Thus Megaplume B oil may have sorbed more $^{228}$Ra, resulting in a less severe decrease in the inherent radioactivity of the source oil.

Data also suggest different reservoir geochemistry impacting $^{224}$Ra release. Between DWH and GC600 (Megaplume A1) oil incubations, mean $^{224}$Ra activities through the incubations decrease by roughly 50% in most cases (Table 2). The magnitude of maximum $^{224}$Ra releases between the two oil sources also show significant differences as peak $^{224}$Ra activities in DWH oil incubation time series are greater than those in GC600 oil incubation time series by a factor of 2-3 (Table 2). Low $^{224}$Ra activities in GC600 surface treatments could potentially be attributed to the oligotrophic Gulf of Mexico surface waters having repressed biodegradation (Edwards et al., 2011). However, GC600 deep treatments were incubated with deep-sourced seawater (which was
presumably not nutrient limited) and exhibited a similar magnitude of \(^{224}\text{Ra}\) release. DWH oil therefore appears to have a significantly higher level of releasable \(^{224}\text{Ra}\) compared to GC600 oil. This result was unexpected as DWH oil was held for approximately eight years between collection and experimentation, whereas Megaplume A1 oil was held for only a few days. Furthermore, Megaplume A2 and Megaplume B oil exhibited a similar magnitude of \(^{224}\text{Ra}\) release as Megaplume A1 oil after a few days of holding time. After an approximately seven week hold time, however, the released \(^{224}\text{Ra}\) of Megaplume A2 and Megaplume B oil declined significantly (Figure 8). Yet, after an approximately eight year hold time DWH oil exhibited a stronger \(^{224}\text{Ra}\) release signature.

We suspect that, in addition to impacts from transport pathway, the inherent radioactivity of an oil source is also controlled by geochemistry of the hydrocarbon reservoir. If \(^{224}\text{Ra}\) activities from DWH oil are sustained at the observed levels eight years after removal from the presumed geologic source of the radium, then the \(^{224}\text{Ra}\) must be supported by a radiogenic parent with a long enough half-life to still be in abundance after eight years. This source isotope is unlikely to only be \(^{228}\text{Th}\), the direct parent of \(^{224}\text{Ra}\), as it only has a half-life of 1.9 years and therefore would have decayed by ~94% over the four half-lives since collection. This source isotope may thus only be \(^{228}\text{Ra}\) (\(t_{1/2} = 5.8\) years) or primordial \(^{232}\text{Th}\) (\(t_{1/2} = 1.4 \times 10^{10}\) years). Independent laboratory analysis suggests that the activities of long-lived parent isotopes may support the measured \(^{224}\text{Ra}\) activities. Gamma spectrometry indicates that \(^{228}\text{Ra}\) and \(^{232}\text{Th}\) activities are \(1.86 \pm 0.98\) dpm per gram of DWH oil. It should be noted, however, that DWH oil was extracted directly from its subsurface reservoir and did not escape via a geologic pathway. Therefore, geochemical processes between the reservoir and the water column may have altered the inherent radioactivity of GC600 oil, which
would not have occurred with DWH oil. We also cannot discount the potential effect of different seawater sources between incubations.

Radium isotopes are significantly more soluble in marine settings than are thorium isotopes, so it may be that $^{228}$Ra is sorbed through contact with sedimentary radium sources through both the reservoir and transport pathway. Yet, is important to point out that our DWH oil was collected directly from the well during the blowout so it did not have contact with sediments between the reservoir and the wellhead. Thorium has been shown to complex with organic matter in the marine environment (Quigley et al., 2001) perhaps even more strongly than with inorganic matter (Langmuir and Herman, 1980) so it may be that the original organic matter (i.e., phytoplankton) that become these hydrocarbons sorbed additional amounts of $^{232}$Th while in the overlying waters before deposition.

The apparent difference in inherent radioactivity between DWH oil and GC600 oil may also be due to reservoir biodegradation. Anaerobic biodegradation of crude oil has been shown to occur in subsurface hydrocarbon reservoirs (Aitken et al., 2004; Paris et al., 2012), therefore it is reasonable to speculate that GC600 oil may discharge into the water column in a more degraded condition than DWH oil. Reservoir biodegradation could also explain the significant radium enrichments commonly found in saline formation fluids (Bloch and Key, 1981; Kraemer and Reid, 1984). Reservoir biodegradation could break the hydrocarbon matrix into smaller segments, thereby increasing surface area for exchange processes and would therefore release radium into surrounding saline formation fluids.

3.3.3 Chemical Oil Dispersant
In almost all cases of COREXIT 9527 addition to oil incubations, at least one secondary peak in $^{224}$Ra activity was observed later in the time series than the peak we commonly observe between 1-2 days (Figure 9). The most drastic of these peaks is shown in Figure 9C at 6 days and Figure 9D at 8 days. Secondary peaks in $^{224}$Ra activity are observed in dispersant treatments as late as 12 days (Figure 9a). The low $^{224}$Ra activities and high variability in all GC600-4 time series likely result from having completed these incubation ~5 months after collection so all of the excess $^{224}$Ra had decayed away. Therefore, the DWH-3 experiment was performed to determine whether the addition of COREXIT 9527 truly results in secondary peaks in $^{224}$Ra activity over time.

Ra-free seawater to be used for inoculant water in DWH-3 had higher $^{224}$Ra background activities than normal, thus a large correction had to be made which reduced the $^{224}$Ra activities significantly. Nonetheless, a $^{224}$Ra activity peak appeared relatively late in the dispersant deep treatment at 4 days, while the non-dispersant deep treatment showed no peaks (Figure 9F). There may have been an earlier peak in both treatments, but contamination corrections may have suppressed it in our data. Both surface treatments showed a slight $^{224}$Ra activity peak at 2 days (Figure 9E), but neither showed a secondary peak. However, significant $^{224}$Ra backgrounds in DWH-3 surface treatments were evident in the seawater blanks which may have affected the final $^{224}$Ra release signature. The high $^{224}$Ra background in all DWH-3 treatments makes it difficult to discern the true $^{224}$Ra behavior in these data, but speculations can be made based on the data available as well as visual observations.

DWH-3 dispersant surface treatments also experienced noticeable marine oil snow formation within the first four days of incubation, with more flocs and larger aggregates visible throughout the remainder of the incubation period (Figure 10). Neither other DWH-3 treatments nor any GC600-4 treatments exhibited marine oil snow formation. This phenomenon is not
necessarily indicative of active oil degradation as a study by van Eenennaam et al. (2016) shows that COREXIT in the presence of marine phytoplankton-associated bacteria is sufficient for the formation of marine snow regardless of crude oil presence. GC600-4 surface treatments likely did not exhibit formation of marine snow because offshore Gulf of Mexico surface waters are typically nutrient deficient (Edwards et al., 2011), thus likely lack the biomass necessary for visible marine snow formation. Our results, however, still cannot elucidate any effect of COREXIT 9527 on hydrocarbon-degrading microbes.

We believe the secondary peaks observed in oil-dispersant incubations may be a result of accelerated cycling of various microbial communities due to the increased oil surface area (i.e., a faster shift from aliphatic to aromatic hydrocarbon degrader communities). In that case, one would also expect accelerated overall biodegradation of the oil and therefore greater $^{224}$Ra release. However, the magnitude of $^{224}$Ra release in almost all dispersant treatments appears to be close to or less than that of non-dispersant treatments barring secondary peaks in $^{224}$Ra activity, especially in the DWH-3 time series (Figure 9). It is possible that a process that has not yet been considered could account for these later peaks in $^{224}$Ra activity, as it is still debated whether chemical oil dispersants accelerate biodegradation or inhibit hydrocarbon-degrader communities (Kleindienst et al., 2015; Prince, 2015). While our data suggest that COREXIT 9527 may have some measureable effect on $^{224}$Ra release from crude oil, the nature of the effect remains unclear.

3.4 Radium Release Mechanisms

Figure 4 demonstrates that crude oil releases $^{224}$Ra into the aqueous phase upon contact with seawater. The specific mechanism(s) for this release, however, remain(s) a matter of speculation. Comparing aqueous phase $^{224}$Ra and oil phase $^{224}$Ra (collected onto Mn-fibers and
acrylic prefilters, respectively), it is clear that nearly all of the measurable $^{224}$Ra in oil-seawater mixtures exists in the aqueous phase (Figure 11). Oil phase $^{224}$Ra activities in all time series remain at or below our minimum detectable activity. Since $^{224}$Ra cannot be simply created in these incubations, it must originate from the oil but may be imbedded deeply enough in the hydrocarbon matrix to remain undetectable when in that configuration. Analytically, $^{224}$Ra activity is determined by a proxy measurement of gaseous $^{220}$Rn, the radiogenic daughter of $^{224}$Ra, which must emanate from the sample so it can be swept into an alpha scintillation chamber by inert helium gas. In this case, our data imply that when in the oil phase, the $^{220}$Rn gas produced by decay of $^{224}$Ra cannot escape the hydrocarbon matrix to be available for detection. Since measurable and temporally dynamic $^{224}$Ra activities are only observed in seawater that has contacted crude oil, there must be some mechanism that releases the $^{224}$Ra from the oil matrix.

Therefore, it seems that some interaction between oil and seawater is necessary to release $^{224}$Ra from the hydrocarbon matrix. The solid:aqueous partition coefficient for $^{224}$Ra is significantly lower in saltwater than in freshwater (Gonneea et al., 2008) due to ion exchange processes in which other divalent cations readily available in saltwater (i.e., Mg$^{2+}$ and Ca$^{2+}$) can outcompete Ra$^{2+}$ for sorption sites on surfaces. We therefore conducted an experiment in which duplicate oil samples were placed in contact with seawater and freshwater. The $^{224}$Ra activity released from the oil in contact with seawater was significantly greater than that in freshwater (Figure 12), suggesting that ion exchange processes may be a factor in $^{224}$Ra release from crude oil. While intriguing, the low sample size (n=2) of these data suggest this to be an area in need of further research.

Additional evidence for ion exchange representing a significant radium release mechanism is shown in Figure 13. The highest $^{224}$Ra activities during the GC600-1 incubation (white boxes
in Figure 13) are consistently observed in treatments poisoned with CdCl₂. Since the cadmium ion in this molecule has a charge of 2+, it can compete with Ra²⁺ cations for sorption sites on the oil surface. As Fulladosa et al. (2005) show that the concentration used in these treatments should be more than sufficient to sterilize our samples, it is unlikely that hydrocarbon-degrading microbes persisted through or were stimulated by a 2 mM CdCl₂ concentration. The release of ²²⁴Ra in this case must therefore be a result of cation exchange. The addition of 2 mM CdCl₂ would have only increased the divalent cation concentration (considering Mg²⁺ and Ca²⁺ in seawater) by ~2%, but perhaps Cd²⁺ can more effectively compete for sorption sites that the other cations so would have a disproportionate effect on ion exchange.

DWH-1 and DWH-2 poisoned treatments exhibited ²²⁴Ra release signatures that more closely aligned with expectations: an initial (T=0) release of surface-bound ²²⁴Ra followed by a decay to near-zero dpm (Figures 13B and 13D). For both DWH experiments, ZnCl₂ was used as a poison instead of CdCl₂. Observations of white flocs forming in DWH-1 and DWH-2 poisoned treatments suggest that our addition of 10 mM ZnCl₂ was supersaturated and therefore the zinc may have precipitated. The reason for zinc precipitation is unknown since ZnCl₂ should have remained soluble at the concentrations and temperature ranges it was held (O’Neill et al., 2001). However, reprecipitation of the zinc would remove Zn²⁺ cations from solution and therefore render them unavailable for exchange with Ra²⁺ cations (and coincidentally, not effective at repressing microbial populations). We suspect this may be the reason why ion exchange did not appear to be a radium release mechanism until CdCl₂ was used.

Figure 13 also suggests that degradation of the crude oil matrix may represent a radium release mechanism. Significant peaks in ²²⁴Ra activity are observed at and around T₂ in live treatments (Figures 13A and B) where such peaks are more suppressed in poisoned treatments.
(Figures 13C and D). We believe these more significant peaks in the live treatments suggest that biodegradation was occurring in both surface ocean and deep sea treatments. Evidence for photodegradation is also apparent in these data, specifically between DWH-1 and DWH-2 surface treatments (Figures 13A and C). DWH-1 surface treatments were covered with plexiglass sheets to prevent contamination, but these covers were not used in later incubations as they were discovered to permit a low ultra-violet (UV) light transmittance. DWH-2 surface treatments exhibited greater $^{224}$Ra activities than those of DWH-1 at nearly every time point which is likely a result of allowing more solar radiation into the incubators by leaving them uncovered.

These data may therefore be indicative of $^{224}$Ra release resulting from photodegradation of the hydrocarbon matrix. Ion exchange effects discussed above may represent a mechanism by which radium enters the aqueous phase from the hydrocarbons, but would only impact surface-bound radium isotopes. Yet, these same surface-bound isotopes would also be able to produce $^{220}$Rn that would be able to escape the oil matrix and would be analytically measureable on the RaDeCC. Yet, oil phase $^{224}$Ra activities remained undetectable, implying the $^{224}$Ra must be more deeply imbedded in the hydrocarbon matrix. We suspect the mechanism of radium release must be dependent upon both photodegradation and ion exchange, where degradation breaks the hydrocarbon matrix into smaller parts, which then exposes new surface areas to the seawater and therefore subjects any newly exposed $^{224}$Ra to ion exchange processes.

Indeed, surface area of oil sheens seems to play a significant role in $^{224}$Ra release. For example, Figure 14 summarizes the results of an experiment in which we added different masses of oil to a constant water volume. In this experiment, we found that adding more oil actually decreased the amount of $^{224}$Ra released into the aqueous phase. These results are counter-intuitive but observations indicated a thicker sheen of oil on the water surface with more oil added. It
therefore seems that having too much oil coagulated together may have decreased the surface area available for radium release. Alternatively, excess oil may have complexed released radium, thus removing it from the aqueous phase and therefore rendering that radium undetectable by our methods.

We suspect that certain experimental conditions limited the amount of both photo- and microbial-degradation in our incubations relative to natural conditions. Photodegradation in surface ocean treatments may be inhibited by a reduction in UV transmittance through the walls of the aquaria. Therefore, solar radiation may only have impacted our oil sheens when nearly directly overhead – a condition unrepresentative of environmental oil sheens. Biodegradation in GC600-1 surface treatments could be inhibited by nutrient limitation due to use of oligotrophic Gulf of Mexico surface water as inoculant water (Edwards et al., 2011). In all surface treatments, microbes may be unable to survive through the production of toxic byproducts of UV-irradiated hydrocarbons (Saeed et al., 2011). Photo-oxidation of PAHs enhances their toxicity by a factor of 2-1000 (Barron and ka’Aihue, 2001). Such photochemical reactions likely occurred in our surface ocean treatments, the products of which may have poisoned microbes working to catabolize the hydrocarbons. The confinement of these oil-water mixtures in aquaria may have exacerbated this increased toxicity as the surface ocean has far more potential to disperse these toxic photoproducts. With no room to disperse, toxic products of PAH photo-oxidation would build up in the aquaria and may have poisoned our hydrocarbon-degrading microbes.

In DWH deep sea treatments, biodegradation may have been limited by a lack of specialized oil-degrading microbial communities in our inoculant waters collected from Long Bay, SC. Microbes native to Long Bay are likely only exposed to refined hydrocarbons in the form of pollution or terrestrial runoff, but not crude petroleum to which Gulf of Mexico microbes are
consistently exposed. It is therefore unlikely that hydrocarbon-degrader specialist species would readily exist in Long Bay, so microbes present in DWH incubations may have only been able to catabolize the most labile of the available hydrocarbon compounds. In GC600-1 deep sea treatments, we suspect that hydrocarbon-degrading microbes may have been largely inactive as a result of removal from their natural environment and filtration through 0.45 μm filters to remove suspended particles and therefore particle-affiliated microbial populations. Hydrocarbon degraders present in our deep-sourced inoculant waters would have been psychrophilic and thrived in the near-freezing temperatures of the deep sea. Many extremophiles are known to enter a viable but non-culturable (VBNC) state when removed from their native environment (De Maayer et al., 2014). De Maayer et al. (2014) point out that debate continues as to whether a microbial population in a VBNC state is actively implementing a survival strategy or continues to attenuate until the population loses the ability to be revived. Deep seawater collected at GC699 for our experiment was filtered and held at room temperature for nearly a week before use in GC600-1 oil incubations. Such a large volume of seawater was required for the experiment that it could not be kept cold. Therefore, it is possible that deep sea hydrocarbon-degrading microbes entered a VBNC state upon entering the low-pressure, high-temperature environment on the ship and were unable to fully rebound to their typical activities upon reintroduction to deep sea temperatures in our incubators.

4. Conclusions

The major finding of this study is that $^{224}$Ra is released from crude oil in contact with seawater which is evidenced by $^{224}$Ra enrichments in oil incubations relative to Ra-free seawater blanks. Radium is suspected to be embedded within the hydrocarbon matrix and sourced by long-
lived radiogenic parent isotopes, most likely $^{228}\text{Ra}$ and/or $^{232}\text{Th}$. We speculate that the inherent radioactivity of the oil, dictated by the concentration of long-lived source isotopes, is controlled primarily by source reservoir geochemistry and anaerobic biodegradation, with a secondary control being flow path geochemistry. This inherent radioactivity is believed to be different between oil sources and thus a plausible explanation for the variable magnitude of $^{224}\text{Ra}$ release between oil sources. Theorized mechanisms of radium release from the hydrocarbon matrix are chemical degradation of the hydrocarbon matrix (i.e., photo- and microbial-degradation) and cation exchange processes. While we suspect both processes represent potential radium release mechanisms, the rates at which either may do so remains unknown.

Further, we find some degree of predictability in the temporal variability of $^{224}\text{Ra}$ activities in oil-seawater mixtures with peak activities observed consistently within the first 1-2 days, followed by a decline in activity roughly following a radioactive decay curve. However, variability in $^{224}\text{Ra}$ activity does appear to be greater and less predictable in finer resolution time series. While we do not yet fully understand the specific mechanisms of radium release or the rates at which they occur, observed predictability in $^{224}\text{Ra}$ behavior suggests the potential of this isotope for use in an isotope-pair geochronometer.

Nonetheless, this study offers the first evidence that natural radioisotopes are associated directly with the hydrocarbon matrix and that radium becomes enriched in waters containing crude oil. This discovery offers the first step toward developing a geochronometer for crude oil exposure in the marine environment. However, in the ocean, any radium released from the hydrocarbon matrix would be subject to mixing processes (an effect that our experimental design excluded). Therefore, dissolved $^{224}\text{Ra}$ activity may change as both a function of oil exposure time and mixing processes, so we would need a long-lived radium isotope or stable analog of radium to normalize
\(^{224}\text{Ra}\) activities to for marine applications. Attempts during this study toward that end (data not shown) were unsuccessful due to the unacceptably high analytical uncertainties of \(^{228}\text{Ra}\), so future efforts should aim to find another suitable isotope to normalize \(^{224}\text{Ra}\) to in order to use this isotope as an oil geochronometer.
References


Smith, Erik. Message to the author. 4 April 2018. E-mail.


**Table 1.** Metadata for treatments and timing of various time-course oil incubation experiments.

<table>
<thead>
<tr>
<th>Experiment Name</th>
<th>Dates</th>
<th>Incubation Location</th>
<th>Termination Time Points (days)</th>
<th>Oil Source(s)</th>
<th>Inoculant Water Source(s)</th>
<th>Depth Simulation(s) Used</th>
<th>Treatments Used</th>
<th>Poisson and Concentration Used</th>
<th>COREXIT 9527?</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWH-1</td>
<td>March 2018</td>
<td>Conway, SC</td>
<td>0, 1, 3, 7, 11, 14, 21</td>
<td>DWH</td>
<td>Long Bay, SC</td>
<td>Surface/Deep</td>
<td>Live/Poisoned</td>
<td>10 mM ZnCl₂ (Babich and Stotsky, 1978)</td>
<td>No</td>
</tr>
<tr>
<td>DWH-2</td>
<td>July 2018</td>
<td>Conway, SC</td>
<td>0, 1, 2, 4, 7, 11, 14, 21</td>
<td>DWH</td>
<td>Long Bay, SC</td>
<td>Surface/Deep</td>
<td>Live/Poisoned</td>
<td>10 mM ZnCl₂ (Babich and Stotsky, 1978)</td>
<td>No</td>
</tr>
<tr>
<td>GC600-1</td>
<td>September 2018</td>
<td>Gulfport, MS</td>
<td>0, 1, 2, 4, 6, 9, 12, 16</td>
<td>Megaplume A1</td>
<td>GC699</td>
<td>Surface/Deep</td>
<td>Live/Poisoned</td>
<td>2 mM CdCl₂ (Fulladosa et al., 2005)</td>
<td>No</td>
</tr>
<tr>
<td>GC600-2</td>
<td>February 2019</td>
<td>Conway, SC</td>
<td>0, 0.5, 1, 1.5, 2, 2.5, 3, 4</td>
<td>Megaplume A2/Megaplume B</td>
<td>GC699</td>
<td>Surface</td>
<td>Live</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>GC600-3</td>
<td>March 2019</td>
<td>Conway, SC</td>
<td>0, 0.5, 1, 1.5, 2, 2.5, 3, 4</td>
<td>Megaplume A2/Megaplume B</td>
<td>GC699/Long Bay, SC</td>
<td>Surface</td>
<td>Live</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>GC600-4</td>
<td>April/May 2019</td>
<td>Conway, SC</td>
<td>0, 1, 2, 4, 6, 9, 12, 16</td>
<td>Megaplume A2/Megaplume B</td>
<td>GC699/Long Bay, SC</td>
<td>Surface/Deep</td>
<td>Live/Poisoned</td>
<td>0.05% HgCl₂ (Dr. Erik Smith, pers. comm.)</td>
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</tr>
<tr>
<td>DWH-3</td>
<td>July 2019</td>
<td>Conway, SC</td>
<td>0, 1, 2, 4, 7, 12</td>
<td>DWH</td>
<td>Long Bay, SC</td>
<td>Surface/Deep</td>
<td>Live</td>
<td>N/A</td>
<td>Yes</td>
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</table>
Table 2. Ra-224 activity statistics report with mean activities ± 1-σ standard deviation and activities at early (T = 1-2 days) peaks.

<table>
<thead>
<tr>
<th>*Experiment / Depth Simulated</th>
<th>**Maximum $^{224}$Ra Activity (dpm)</th>
<th>Mean $^{224}$Ra Activity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWH-1 / Surface</td>
<td>0.48 ± 0.02</td>
<td>0.19 ± 0.19</td>
</tr>
<tr>
<td>DWH-1 / Deep</td>
<td>0.59 ± 0.03</td>
<td>0.23 ± 0.20</td>
</tr>
<tr>
<td>DWH-2 / Surface</td>
<td>0.63 ± 0.03</td>
<td>0.28 ± 0.18</td>
</tr>
<tr>
<td>DWH-2 / Deep</td>
<td>0.45 ± 0.02</td>
<td>0.26 ± 0.15</td>
</tr>
<tr>
<td>GC600-1 / Surface</td>
<td>0.29 ± 0.02</td>
<td>0.14 ± 0.10</td>
</tr>
<tr>
<td>GC600-1 / Deep</td>
<td>0.26 ± 0.02</td>
<td>0.11 ± 0.10</td>
</tr>
</tbody>
</table>

*Only live treatment statistics are reported as they most accurately represent the desired depth simulated.

**Error represents 1-σ analytical uncertainty.
Figures

Figure 1. (A) Map of the north-central Gulf of Mexico showing various oil lease blocks (modified from BOEM, 2018). (B) Depiction of the location of GC600 (circle) and GC699 (square).
Figure 2. (A) ROV Odysseus on the deck of the R/V Point Sur. (B) Oil sampler carousel holding eight individual glass samplers. (C) Glass oil sampler with stainless steel T-handle and a hole at the bottom for oil bubbles to flow up into the sampler. (D) Methane hydrate formation during the first cruise. (E) Oil sampler actively collecting oil at Megaplume A during the second cruise.
Figure 3. Schematic of the experimental design. Collected hydrocarbons were homogenized and split between each treatment and subtreatment. Incubations were terminated at each time point (T).
Figure 4. Ra-224 activities in Ra-free seawater blanks compared to oil incubations for DWH-2 (A) and GC600-1 (B) incubations. Initial (T = 0 days) oil incubation activities (gray-striped white bars) are assumed constant across all treatments. The absence of a bar represents $^{224}$Ra activity below detection limits. Error bars represent 1-$\sigma$ analytical uncertainty.
Figure 5. Oil incubation time series with theoretical radioactive decay curves (solid lines) following peak $^{224}\text{Ra}$ activities. Shown are surface (left) and deep (right) treatments for the DWH-1 (top), DWH-2 (middle), and GC600-1 (bottom) incubations. Error bars represent 1-σ analytical uncertainty.
Figure 6. Time series of average $^{224}$Ra activity (for DWH-1, DWH-2, and GC600-1) taken at each time point for both live (A) and poisoned (B) treatments. Data were linearly interpolated at a 1-day resolution to ensure consistent length and resolution of time series compared. Closed circles represent averages in which at least one real data point was used. Open circles represent averages of only interpolated data. Error bars represent 1–σ standard deviation. Solid lines represent theoretical activity (based on pure radioactive decay) from the peak measured activity.
Figure 7. Results of GC600-2 experiment presented in 12-hour resolution (A) and 24-hour resolution (B). Experiment initialized at approximately 08:00 at $T = 0$ days. Error bars represent 1-$\sigma$ analytical uncertainty.
Figure 8. Box-and-whisker plot comparing range of values between oil sources Megaplume A2 and Megaplume B during incubation experiments GC600-2 (black) and GC600-3 (gray). Targets represent the median of the time series. Whiskers extend to the minimum and maximum of each time series. Bottom and top box edges are drawn at the 25th and 75th percentiles, respectively. Cross represents outlier.
Figure 9. Oil incubation time series of experiments using COREXIT 9527 dispersant with different oil sources under surface (left) and deep (right) conditions for GC600-4 live (top), GC600-4 poisoned (middle), and DWH-3 (bottom) incubations. Error bars represent 1-σ analytical uncertainty.
Figure 10. Images of marine oil snow in DWH-3 dispersant surface treatments. A) Marine oil snow flocs collected in a beaker. B) Close-up of a larger marine oil snow aggregate. C) Side-by-side comparison of a non-dispersant treatment (left) vs. dispersant treatment (right) in which small flocs and a large aggregate of marine oil snow is visible.
Figure 11. Oil incubation time series illustrating aqueous phase vs. oil phase $^{224}$Ra activities for surface (left) and deep (right) conditions for DWH-1 (top) and DWH-2 (bottom) incubations. Red horizontal lines are reference lines at 0 dpm. Error bars represent 1-σ analytical uncertainty.
Figure 12. Results of the salinity experiment using Megaplume A1 oil, Ra-free GC699 seawater, and Ra-free deionized tap water. Error bars represent 1-σ analytical uncertainty.
Figure 13. Oil incubation time series magnitude at each time point, T, which represent the index of the time series rather than a specific time in days. Results shown for DWH-1 (black bars), DWH-2 (gray bars), and GC600-1 (white bars) incubations under surface (left) and deep (right) conditions for live (top) and poisoned (bottom) treatments. Error bars represent 1-σ uncertainty.
Figure 14. Results of variable oil concentration experiment using Megapluume A1 oil and Ra-free GC699 seawater. Error bars represent 1-σ uncertainty.
Appendix 1

Raw Data

All data are available through GRIIDC.

For access to raw data files, please visit the following web link:

https://data.gulfresearchinitiative.org/pelagos-symfony/data-discovery

Data used in figures within this document as well as other data related to the project can be found under GoMRI RFP-VI, submitted by Dr. Richard Peterson, and under the project name “Radium Isotope Release from Oil Degradation: Development of an ‘Oil Clock’.”
Appendix 2

Sample Collection Metadata

Deepwater Horizon (MC252) crude oil:
Date/Time of Collection: 03:00 GMT on July 27, 2010
Geographic Coordinates: 28.736628°N, 88.365997°W
Vessel: Discoverer Enterprise drillship (Transocean Offshore Deepwater Drilling Inc.)
Storage: Stored in the dark by B&B laboratories
Received: October 13, 2016 (continued storage in dark)
Other information: Collected directly from the wellhead during the spill

Green Canyon lease block 600 (GC600) crude oil:
Vessel: R/V Point Sur (University of Southern Mississippi)
Other information: All oil collected by ROV Odysseus (Pelagic Research Services)

Megaplume A1:
Date/Time of Collection: (1) 18:02 GMT on September 1, 2018 and (2) 18:54 GMT on September 3, 2019
Geographic Coordinates: 27°22.1950’ N, 90°34.25211’ W
Water Depth: (1) 1184.3 m and (2) 1185.0 m

Megaplume A2:
Date/Time of Collection: 09:50 GMT on January 28, 2019
Geographic Coordinates: 27°22.19434’ N, 90°34.26785’ W
Water Depth: 1184.0 m

Megaplume B:
Date/Time of Collection: 02:30 GMT on January 29, 2019
Geographic Coordinates: 27°22.19434’ N, 90°34.26785’ W
Water Depth: 1185.0 m
Appendix 3

Determination of Oil Density and Pipette Calibration

Prior to the time-course incubation experiments, the density of the oil was found through a series of mass and volume measurements. The purpose of these measurements was to find the density of the DWH oil and to determine the volumetric equivalent of 1 g of the DWH oil. A 5 mL volumetric pipette was used for these measurements, pipetting the oil into a 10 mL glass graduated cylinder.

<table>
<thead>
<tr>
<th>Volume (mL)</th>
<th>Clean pipette tip mass (g)</th>
<th>Oily pipette tip mass (g)</th>
<th>Adsorbed Oil Mass (g)</th>
<th>Transferred Oil Mass (g)</th>
<th>Total Oil Mass (g)</th>
<th>% loss</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>2.8153</td>
<td>2.8631</td>
<td>0.0478</td>
<td>0.8653</td>
<td>0.9131</td>
<td>5.235</td>
<td>0.8301</td>
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<tr>
<td>1.15</td>
<td>2.8206</td>
<td>2.8698</td>
<td>0.0492</td>
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<td>0.9454</td>
<td>5.204</td>
<td>0.8221</td>
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<tr>
<td>1.2</td>
<td>2.8443</td>
<td>2.8724</td>
<td>0.0281</td>
<td>0.9254</td>
<td>0.9535</td>
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<td>0.7946</td>
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<tr>
<td>1.25</td>
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<td>2.8915</td>
<td>0.0542</td>
<td>0.9496</td>
<td>1.0038</td>
<td>5.399</td>
<td>0.8030</td>
</tr>
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<td>1.3</td>
<td>2.8500</td>
<td>2.8742</td>
<td>0.0242</td>
<td>1.037</td>
<td>1.0612</td>
<td>2.280</td>
<td>0.8163</td>
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<td>1.35</td>
<td>2.8241</td>
<td>2.8947</td>
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<td>1.1054</td>
<td>1.176</td>
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</tr>
<tr>
<td>1.4</td>
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<td>2.8973</td>
<td>0.0647</td>
<td>1.1693</td>
<td>1.234</td>
<td>5.243</td>
<td>0.8814</td>
</tr>
</tbody>
</table>

Table A1. Results of the first oil density and pipette calibration measurements, using a varying aliquot volume between 1.1 and 1.4 mL.

Varying oil aliquot volumes were used for the first measurements to determine a pipette volume setting that would transfer 1 g. of oil. A volume range of 1.1-1.4 mL was chosen to test based on an arbitrary estimate of oil density. The results of these first measurements are outlined in Table A1. The sum of the adsorbed and transferred oil masses represent the total mass of oil present in the given volumes. To calculate oil density, the total oil mass was divided by its respective volume in each trial. Based on the results, a pipette setting of 1.25 mL of oil delivers the closest value to 1 g. This was confirmed by using the average oil density (0.8312 g/mL) of
these measurements and the apparent optimal volume (1.25 mL), the product of which equals 1.039 g.

**Table A2.** Results of the second oil density and pipette calibration measurements, using constant 1.25 mL aliquot volume.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Clean pipette tip mass (g)</th>
<th>Oily pipette tip mass (g)</th>
<th>Adsorbed Oil Mass (g)</th>
<th>Transferred Oil Mass (g)</th>
<th>Total Oil Mass (g)</th>
<th>% loss</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.8139</td>
<td>2.8620</td>
<td>0.0481</td>
<td>1.0205</td>
<td>1.0686</td>
<td>4.50</td>
<td>0.8549</td>
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<tr>
<td>2</td>
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<td>2.8613</td>
<td>0.0460</td>
<td>0.9859</td>
<td>1.0319</td>
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<tr>
<td>3</td>
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<td>0.0477</td>
<td>0.9934</td>
<td>1.0411</td>
<td>4.58</td>
<td>0.8329</td>
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<tr>
<td>4</td>
<td>2.8217</td>
<td>2.8760</td>
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<td>0.94</td>
<td>0.9943</td>
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<tr>
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<tr>
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<td>0.9999</td>
<td>1.0490</td>
<td>4.68</td>
<td>0.8392</td>
</tr>
</tbody>
</table>

A second set of measurements (*Table A2*) was conducted using a constant 1.25 mL aliquot volume with six replicates to test the reproducibility of mass this volume would deliver using the same methods as the first set of measurements. In this case, the average transferred oil mass was calculated to be 0.9882 g. Since the transferred oil mass should be 1 g for our experiments, the volume was adjusted to 1.3 mL for adding oil to the incubators.