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Note

Can gelatinous zooplankton influence the fate of crude oil in marine environments?

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ABSTRACT

Gelatinous zooplankton are known for their capacity to excrete copious amounts of mucus that can be utilized by other organisms. The release of mucus is exacerbated by stressful conditions. Despite the recognized importance of cnidarian mucus to production and material flux in marine ecosystems, the role of gelatinous zooplankton in influencing the fate of oil spills is unknown. In this study we used laboratory experiments to observe the influence of mucus from the moon jellyfish (*Aurelia aurita*) on the aggregation and degradation of crude oil. The results show that jellyfish swimming in a dispersed solution of oil droplets produced copious amounts of mucus and the mucus aggregates that were shed by the animals contained 26 times more oil than the surrounding water. Incubation experiments showed that hydrocarbon degrading bacteria cell densities more than doubled in the presence of mucus and after 14 days, resulted in a significant increase in oil degradation. These results suggest that jellyfish can aggregate dispersed oil droplets and embed them within a matrix that favors hydrocarbon degrading bacteria. While this study lends support to the hypothesis that the presence of gelatinous zooplankton can impact oil spills large scale mesocosm studies will be needed to fully quantify the influence on a natural system.

1. Introduction

Oil dispersion begins immediately following a spill. This dispersal process facilitates bioremediation and eventually leads to the mitigation of the spill which enables ecosystems to recover. This process occurs naturally where oil is physically dispersed by waves or chemically through the use of chemical dispersants. Either way, a proportion of the oil ends up as small droplets in the water column (Delvigne and Sweeney, 1988; Gordon et al., 1973) either as oil alone or in association with other material that can be either biological (Lee et al., 1985) or inorganic (Lee and Stoffyn-Egli, 1998; Lee et al., 2002).

Gelatinous zooplankton can play pivotal roles in material flows through coastal planktonic communities during periods of high abundance. They directly impact the water column by the sheer volume of fluid they encounter during foraging (Hansson et al., 2005) and expend little energy to do so (Gemmell et al., 2015a; Gemmell et al., 2013). Large medusae forage by rhythmically pulsing their bells in order to generate a feeding current that consists of the formation of vortex rings (Gemmell et al., 2015b). This feeding current transports fluid around the bell and through trailing capture surfaces (Colin et al., 2012; Costello and Colin, 1995; Dabiri et al., 2005; Ford et al., 1997). Since feeding is coupled with swimming, these species swim nearly 100% of the time (termed cruising foragers; (Colin et al., 2003)) and, depending on their size, process tens of liters of seawater per hour through their capture apparatuses (Katija et al., 2011; Titelman and Hansson, 2006). In some coastal areas, *A. aurita* densities during bloom events can be >15 individuals m^{-3} (Gröndahl, 1988; Lucas, 1996) which would result in a large proportion of the water column be processed on a daily basis. In the Northern Gulf of Mexico populations of the jellyfish *Aurelia aurita* (moon-jelly) can also vary dramatically and have been observed at densities up to 0.4 individuals m^{-3} with an average population density of 0.08 individuals m^{-3} (size: 15–25 cm) in surface waters (Rakow and Graham, 2006). At these densities *A. aurita* populations in the Gulf of Mexico may process 8–38% of the water column every 24 h, based on clearance rate estimates of (Titelman and Hansson, 2006), during times of high abundance. As predators, gelatinous species can affect species composition of smaller zooplankton, such as copepods (Behrends and Schneider, 1995; Feigenbaum and Kelly, 1984; Lindahl and Hernroth, 1983; Matsakis and Conover, 1991), which also interact with suspended oil droplets (Almeda et al., 2014; Conover, 1971; Cowles and Remillard, 1983). Therefore, both directly, by fluid volume processing, and indirectly, by top-down ecological forcing of plankton community composition, gelatinous zooplankton are key conduits for material transformations in continental shelf waters when oil introductions occur.

The existing information also indicates that gelatinous species may tolerate relatively high concentrations of oil (Almeda et al., 2013; Barazandeh et al., 2009; Nelson-Smith, 1972) and continue functioning

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while other less-tolerant species decline in performance. Hence, the importance of gelatinous species may be enhanced relative to other species, as it is in O_2-depleted waters (Breitburg, 1994; Decker et al., 2004), during the physiological stress of high oil exposure. Consequently, there is strong reason to suspect, but little evidence to evaluate, a critical role for gelatinous species in oil transformations within planktonic communities. In addition to the physical interactions of oil droplets within feeding currents, medusae exude copious amounts of mucus that may associate with, and transform, oil droplets, creating regions of enhanced biodegradation. Gelatinous zooplankton release mucus as a means of excretion and as a defense mechanism (Arai, 1997; Heeger and Möller, 1987; Pitt et al., 2009; Shanks and Graham, 1988; Wild et al., 2010). These mucus exudates are rich in nitrogen, phosphorus and carbon (Ducklow and Mitchell, 1979). The release of mucus is exacerbated by stress (Pitt et al., 2009) and interactions with a plume of oil will likely induce high rates of mucus production. Mucus release has been most commonly studied in another group of cnidarians, the corals, and these adhesive mucus exudates function as particle traps and nutrient and energy carriers in these coral reef ecosystems (Niggl et al., 2010; Wild et al., 2004). Though less studied in medusae, some species of jellies have been shown to produce greater volumes of mucus than corals (Niggl et al., 2010).

During times of high jellyfish abundance, these animals have been shown to release high levels of nitrogen and phosphorus through the secretion of mucus (Condon et al., 2010; Pitt et al., 2009). During oil spills hydrocarbon degrading bacteria are often found to be nutrient limited (Lindstrom et al., 1991; Röling et al., 2002), which can result in much slower degradation of the oil and prolonged ecological consequences. Since biodegradation of oil by bacteria is very often nutrient limited, the presence of high abundances of gelatinous zooplankton may reduce microbial N and P limitation, leading to significantly increased biodegradation in these regions. Further, the formation of mucus aggregates by gelatinous zooplankton may act to concentrate oil and nutrients together for enhanced degradation. In this study we employ laboratory experiments to examine the role moon jellyfish (*Aurelia aurita*) may have on dispersed droplets after an oil spill.

2. Materials and methods

2.1. Observation of jellyfish swimming in the presence of crude oil

Ten *Aurelia aurita* with a bell diameter of 12 cm (s.d. 3.2) were collected from inshore waters of the Northern Gulf of Mexico, (27°50′19″ N, 97°3′8″ W) and maintained in a 300 L cylindrical aquarium at room temperature (21 °C) and a salinity of 33 ppt. Jellyfish were fed newly hatched (<24 h) *Artemia salina* daily. For experimental observations, one animal per trial was transferred to a 38 L aquarium and given 10 min to acclimate. Illumination was provided by a 75 W LED floodlight (Genaray SpectroLED). Two of the ten trials served as controls and aquariums contained only filtered seawater (33 ppt). After the 10 min acclimation period, an emulsified suspension of crude oil droplets was added to the aquarium for eight of the trials. This provided eight experimental replicates and two control replicates. The emulsification was created by mixing 4 mL of Light Louisiana Sweet crude oil in beaker containing 100 mL of filtered seawater using magnetic stir plate (Fisher Scientific) at 1000 rpm for 5 min. The contents of the beaker were added to the aquarium containing the jellyfish and created a dilute suspension of oil droplets with a final concentration of approximately 100 ppm. The oil emulsion was added using a long pipette and distributed throughout the aquarium to ensure an even distribution. Control treatments also had 100 mL of filtered seawater (FSW), devoid of oil, added in this manner.

Following the addition of oil, mucus aggregates were photographed as they were created and shed by the jellyfish using a Nikon D7100 DSLR camera with a 105 mm 1:1 macro lens, f 2.8. The observations were limited to 2 min after the addition of oil to ensure the concentration of droplets in the water column would not change appreciably during the course of observation. Photographs were analyzed using the ImageJ software (v. 1.51a). It should be noted that because mucus aggregates would appear in unpredictable locations in the aquaria depending on jellyfish location and images needed to be taken immediately without being disturbed before aggregates sunk or floated to the surface, all images of mucus/oil aggregates were shot free-hand and thus an accurate spatial scale was not obtainable. Therefore, analysis was based on relative measurements using camera pixels. Illumination was such that oil droplets appeared dark on a light background and the ImageJ particle analysis tool was used to determine the proportion of secreted mucus that contained crude oil and also how this compared to the relative proportion of crude oil in the surrounding water. Only in-focus oil droplets were quantified over a narrow focal depth of 9.5 mm to ensure only droplets within mucus aggregates and a discrete volume were measured. Data was compared statistically using a One-Way Analysis of Variance test (ANOVA).

2.2. Microbial growth experiments

Extruded mucus was collected from live *Aurelia aurita* (10–18 cm bell diameter) that were obtained from inshore waters of the Northern Gulf of Mexico, adjacent to the University of Texas Marine Science Institute (27°50′19″ N, 97°3′8″ W). Animals that were swimming within approximately 30 cm of the water's surface were carefully drawn into 20 L buckets whereby the act of this minor disturbance triggered a substantial release of mucus. Mucus was drawn into a wide bore pipette and placed into 50 mL scintillation vials.

The effect of jellyfish mucus on bacterial growth and oil biodegradation was evaluated using batch culture experiments. To obtain the bacterial consortium used in these experiments, coastal seawater from Mustang Island was enriched with 1000 ppm of light Louisiana sweet crude oil and incubated at 25 °C in an orbital shaker (120 rpm). After 10 days, an aliquot of the culture was transferred to fresh Bushnell Hass Medium (BHM) and incubated similarly (Bacosa et al., 2012). Enrichment was performed four times.

Prior to incubation, 100-mL glass amber bottles were autoclaved (120 °C for 30 min) and treated with Sylon CT (Sigma Aldrich) according to manufacturer's instruction in order to deactivate the glass and minimize oil adhesion. After the solvent was evaporated, artificial seawater (ASW) or natural seawater (NSW) was added in each bottle at desired volume (Table 1). The use of artificial seawater (Instant Ocean mixed in deionized water) was used as a control to determine the effect of any naturally occurring nutrients or material in the coastal seawater. Autoclaved jellyfish mucus (2 mL) was then added to treatment, and 0.5 mL aliquot of enriched bacterial consortium at late exponential phase was pipeted into each bottle. Finally, all bottles were amended with crude oil (light Louisiana sweet crude oil) at a final concentration of 1000 ppm (Bacosa et al., 2015a). Incubation was performed at room temperature in the dark, with shaking at 70 rpm (SBT30 1D Low Speed Orbital Shaker). Data was compared statistically using a One-Way Analysis of Variance test (ANOVA).

Duplicate bottles from each treatment were analyzed for bacterial density and total *n*-alkanes (C_9–C_{33}) at 7 and 14 days of incubation. Samples for bacterial count were preserved in formaldehyde at a final concentration of 2% and stored at 4 °C until analysis. After staining with SYBR Green, bacterial cells were enumerated using a BD Accuri

Table 1
Description of batch incubation. AS-Artificial seawater; NS-Natural seawater. Crude oil (Louisiana light sweet crude) was added at a final concentration of 1000 ppm.

	AS or NS (mL)	Mucus (mL)	Bacteria (mL)
AS + oil	19.5	0	0.5
AS + oil + mucus	17.5	2	0.5
NS + oil	19.5	0	0.5
NS + oil + mucus	17.5	2	0.5

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