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Molecular characterization of benthic foraminifera communities from the Northeastern Gulf of Mexico shelf and slope following the Deepwater Horizon event

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ABSTRACT

Benthic foraminifera are globally distributed protozoa in the world's oceans, which have been used as ecological indicators in both current and palaeo oceanography. The ecological properties and distribution of these organisms in various regions of the Gulf of Mexico (GOM) have been evaluated using microscopy; however molecular approaches for these purposes have been limited, especially in deeper regions. The BP Deepwater Horizon oil well failure in the northern Gulf of Mexico highlighted the need to better understand the distribution and abundance of these organisms relative to environmental factors and ecosystem perturbations such as the oil spill. Sediment samples were collected using a Shipek grab along transects on the northwest Florida GOM shelf (18–270 m depth). Clone libraries were developed from PCR amplified 18S rDNA genes for sequence analysis. Analysis of random clones from libraries were used as a proxy for community structure (presence and relative abundance) to document the spatial and temporal dynamics of benthic foraminifera on the Northwest Florida Shelf in the NE GOM shelf. Additional continental slope samples (200–1600 m depth) were obtained by a multicorer and treated in similar fashion. Mean species diversity in this study (H=2.49–3.36), agreed with pre-DWH event estimates, however the dominant agglutinated species in the deep-water samples did not match previous studies. Additionally, the dominant calcareous taxa from this study such as *Allogromida* sp. and *Psammophaga* sp., were inconsistent with previous reports. The dominant taxa in both coastal and deep-water sites include *Glabratellina* sp., *Trochammina hadai,* and *Trochammina* sp., and *Textularia sagittula* and *Bathysiphon argenteus* as well as members of genera *Astrammina, Bolivina, Cibicides* and *Cibicidoides*.

1. Introduction

Benthic foraminifera communities are distributed throughout the world's oceans, from the shorelines to abyssal depths (Sen Gupta, 1999). In addition to their biological importance in marine benthic food webs, (Lipps and Valentine, 1970) foraminiferal fossil records have been used for evaluating and establishing many geologic and stratigraphic correlations (Kennett, 1982). Sensitivity to changes in water quality and adaptations to specific water depths and sediment types makes them useful indicators of paleobathymetry, depositional environments, and environmental change .

The high abundance of benthic foraminifera in almost all marine environments and their short generation time, makes these organisms useful as bio-indicators of pollution impacts in coastal and transitional waters (Alve, 1995; Nigam et al., 2006), including oil spills (Morvan, 2004), hypoxia (Karlson et al., 2000; Strauss et al., 2012), urban sewage (Burone et al., 2006) and other types of anthropogenic impacts in coastal areas (e.g. Watkins, 1961; Seiglie, 1968; Rao and Rao, 1979; Setty and Nigam, 1984; Bhalla and Nigam, 1986; Nagy and Alve, 1987; Alve, 1995; Coccioni, 2000; Bergin et al., 2006, Martins et al., 2013, 2015b). They have been used to describe the geographical extent of anthropogenic impact as well as the recovery of benthic communities upon cessation of drilling activities (Denoyelle et al., 2012; Morvan et al., 2004; Ernst et al., 2006). Benthic foraminifera have recently been evaluated concerning the impact of oil exploration activities on the sea floor (Mojtahid et al., 2006; Schwing et al., 2015). The sedimentary strata of marine systems contain well-preserved and

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abundant tests of foraminifera. However not all the species have the same potential of preservation in the sedimentary record. Calcareous tests are generally well preserved in the fossil records, while specimens with organic and soft agglutinated tests are rapidly disintegrated after the death of the organism (Sen Gupta, 1999). Foraminiferal stratigraphy has been used to determine the compositional changes in foraminiferal fauna before and after the onset of oil exploitation activities and oil spills. Access to this type of record is useful in cases such as the BP oil spill in the GOM 2010, where little pre-oil spill baseline data for extant sediment surface communities was available. However, some components of the foraminifera community especially specimens with soft organic and agglutinated tests, most likely will be removed due to taphonomic processes.

Microscopic analysis capitalizes on high standing stocks for analysis of small sediment volumes, but requires expertise and time, and has to circumvent problems associated with cryptic species, soft-shelled foraminifera, and those that occupy empty shells of other foraminiferal species (Loeblich and Tappan, 1987; Habura et al., 2008; Gooday et al., 2013). Molecular techniques with DNA extracted from environmental samples can fill in gaps from the limitations of microscopic analysis, but has its own biases and relies on a database of sequences tied to identification by microscopy. Previously unknown forms can be discovered from environmental sequences by alignment with known forms, and can be used in ecological community analysis with an 'operational taxonomic unit' (OTU designation; Schloss et al., 2009; Lecroq et al., 2011).

The investigation herein was undertaken to provide basic information on the distribution and abundance of foraminifera in the NE GOM as related to environmental factors and impacts from the Deepwater Horizon oil spill in 2010. The event released over 4.9 million barrels of oil into the GOM from April to July of 2010 (Camilli et al., 2010). Estimates suggest that only 35% of the oil reached the water surface and 30–40% of the oil unaccounted for was deposited on the seafloor (Ryerson et al., 2011; Thibodeaux et al., 2011). Following the event, studies showed substantial (4–10-fold) increases in sediment accumulation rates (Brooks et al., 2014), as well as increased (2–3-fold) polycyclic aromatic hydrocarbons (PAH) concentrations (Romero et al., 2015). Studies have since correlated effects of the spill, regarding total petroleum hydrocarbon, PAHs to benthic faunal communities. A study evaluating the impacts on soft-bottom benthic macrofauna and meiofauna showed severe relative reduction of faunal abundance and diversity in a 3 km circumference from the wellhead, covering an area about 24 km^2 (Montagna et al., 2013). Moderate impacts were also observed multiple km toward the southwest and northeast of the wellhead, covering an area 148 km^2 (Montagna et al., 2013). Schwing et al. observed a 80–93% decline in total density of benthic foraminifera.

Typically, the GOM is an oligotrophic, turbulent basin with documented salinity levels higher than the world average with the exception of the Northern shelf where concentrations progressively drop due to large freshwater inputs. The gulf current in combination with close proximity to the equator yield tropical water temperatures. Water circulation and mixing in the GOM is driven by the force of the Loop Current which carries warm waters from the Atlantic while transporting nutrients and other particles and promoting water circulation throughout the entire basin (Sturges et al., 2005). The Northern continental shelf is subject to the influence of the Loop Current, freshwater river inputs, tropical storms and underwater topography effects such as those of the DeSoto canyon (Hamilton et al., 2005). These attributes of the GOM play a part in the biogeography of the foraminfera as well as their response to a nearly 10-fold increase over the average input of oil into the GOM (Ocean Studies Board and Marine Board; 2003). Preceding the event, many microscopy-based foraminiferal surveys were conducted throughout the GOM (Culver and Buzas, 1983; Denne and Sen Gupta, 1991; Buzas et al., 2007 and Bernhard et al., 2008). To complement recent studies, sequence analysis of clone libraries developed from DNA extracted from sediment samples was used to provide a molecular-based analysis of the community structure and distribution of species across the shelf and slope of the NE GOM.

2. Materials and methods

2.1. Location and sampling

Samples for this study were taken concurrently with a large study evaluating the fate of oiled sediment in the northern Gulf of Mexico (Fig. 1). Continental shelf samples were collected in July and December 2012, along two transects south of Pensacola and Destin, FL. Continental Slope samples were collected between August 16–28th, 2013 at variable distances from the failed BP well site. Samples were obtained on the R/V Weatherbird II and R/V Bellows, Florida Institute of Oceanography (FIO), on cruises supported by Gulf of Mexico Research Initiative (GMRI) research consortia. Precise locations, sample depths and sampling dates are listed in Table 1. For each cruise, hydrographic data were collected via a Sea-Bird CTD profiler, and data recorded at the bottom of profiles were assumed to represent near benthic conditions.

Shelf sediment was collected from the seafloor by using a Shipek® grab. The top centimeter of sediment was removed using a sterilized spatula and frozen at −20 °C on board ship. For deep stations, similar steps were taken to collect sediment retrieved using an Ocean Instruments MC-800 multicorer. The top 2 cm were removed from the cores and placed into sterile Falcon tubes. Additionally at deep water stations, the sediment-water interface (nepheloid layer) was collected by removing water overlying the cored sediments (WOC) retrieved by the multicorer. Upon multicorer retrieval, WOC (1–2 L/core; ~20–40 cm above sediment) was siphoned into polycarbonate bottles using pre-sterilized tubing. WOC samples were immediately filtered onto 0.22 μM Sterivex® filters and the filters stored at −20 °C on board ship. Care was taken not to disturb the sediment surface, as this material was being targeted for other analyses, including polycyclic aromatic hydrocarbon (PAH) content (see below). Between collection of samples, bottles and tubing were rinsed with 10% HCl, 95% EtOH and MilliQ water. Upon arrival to lab, all samples (sediment, filters) were kept at −80 °C until processing.

2.2. Nutrient analysis

Dissolved and total nutrient concentrations (nitrate-nitrite and orthophosphate) were determined using Environmental Protection Agency (EPA) standard procedures (300.00, 300.01, 300.03). Particulate matter was removed from ambient seawater for dissolved nutrient analysis by filtration through Whatman GFF filters. Filtrate was collected in 60-ml polypropylene bottles and stored at −20 °C until processing. Raw seawater (100 ml) for total nitrogen and phosphorous analysis was preserved with 0.4 ml of concentrated sulfuric acid (H_2SO_4). Nutrient concentrations were determined at the Wetlands Research Laboratory facility (UWF, Pensacola, Fla. [certification no. E71969]) with a BRAN+LUEBB Auto-analyzer. Chlorophyll samples were collected in 360 ml volumes on Whatman GF/F filters, extracted in 90% acetone and quantified fluorometrically (Welschmeyer, 1994).

2.3. PAH analysis

The top 0–4 mm of sediment cores was removed for extraction of organics and analysis of major PAH groups. We followed modified EPA methods (8270D, 3611B) (EPA, 2005, 2007) and QA/QC protocols for the analysis of PAHs. Sample preparation, use of internal standards, quantification of aromatic fractions, and calculation of diagnostic ratios of the major hydrocarbon sources was done as previously described (Moss et al., 2015).

2.4. Genomic DNA extraction

Sterivex units were opened with sterile cutting blades and forceps and the filter membrane removed with sterile forceps. The filters, as well as shelf and deep water sediment samples (composite of 3 per site; ~0.5 g per tube) were inserted into 2 ml bead-beading tubes (Powersoil; MoBio®). Lysis buffer from the kit was added and filters were subjected to 3 freeze-thaw (liquid nitrogen/75 °C) cycles. Tubes were agitated using a PowerLyzer homogenizer (MoBio®) using 90 s bursts at setting S3500. Genomic DNA extraction was done according to the manufacturer's instructions. Genomic DNA concentration was estimated using a Nanodrop 1000 and samples were stored at −20 °C until further analysis.