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First evaluation of foraminiferal metabarcoding for monitoring environmental impact from an offshore oil drilling site

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ABSTRACT

At present, environmental impacts from offshore oil and gas activities are partly determined by measuring changes in macrofauna diversity. Morphological identification of macrofauna is time-consuming, expensive and dependent on taxonomic expertise. In this study, we evaluated the applicability of using foraminiferal-specific metabarcoding for routine monitoring. Sediment samples were collected along distance gradients from two oil platforms off Taranaki (New Zealand) and their physico-chemical properties, foraminiferal environmental DNA/RNA, and macrofaunal composition analyzed. Macrofaunal and foraminiferal assemblages showed similar shifts along impact gradients, but responded differently to environmental perturbations. Macrofauna were affected by hypoxia, whereas sediment grain size appeared to drive shifts in foraminifera. We identified eight foraminiferal molecular operational taxonomic units that have potential to be used as bioindicator taxa. Our results show that metabarcoding represents an effective tool for assessing foraminiferal communities near offshore oil and gas platforms, and that it can be used to complement current monitoring techniques.

1. Introduction

The demand for oil is expected to increase by 18.7 million barrels per day (mb/d) over the next 25 years, reaching 111 mb/d by 2040 (Organisation of the Petroleum Exporting Countries [OPEC], 2014). Moreover, the gas sector may represent the predominant source of energy beyond 2040 (International Energy Agency [IEA], 2012), and offshore and deep-water production will continue to rise as onshore reserves diminish (LUKOIL, 2013). Therefore, the need for effective benthic monitoring that can provide early detection of environmental changes will be increasingly important. Currently, benthic monitoring of these environments involve the sorting, identification and enumeration of macrofaunal assemblages using microscopy. This approach is laborious, costly, and relies on expert taxonomic knowledge (Borja et al., 2009; Fernandes et al., 2001).

Recent breakthroughs in high-throughput sequencing (HTS) technologies allow for species diversity to be estimated rapidly from small amounts (2–10 g) of sediment using a technique known as environmental DNA (eDNA) metabarcoding (Baird and Hajibabaei, 2012; Bourlat et al., 2013; Dowle et al., 2015a; Taberlet et al., 2012). Metabarcoding enables the identification of organisms without taxonomic expertise by matching short gene fragments (from HTS data) to a reference sequence library. Standardized protocols can be developed and the results are defendable and auditable (Ji et al., 2013; Valentini et al., 2009). These qualities make metabarcoding a cost-effective, reliable and rapid option to meet the increasing need for large-scale environmental impact assessments. Although the lack of reference sequences in barcoding libraries still represent an impediment to routine implementation of HTS methods, the continued improvement and accessibility of

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genomic tools is rapidly increasing the number of DNA barcodes available, which will reduce taxonomic assignment issues (Cristescu, 2014).

Metabarcoding studies of macrofauna have generally been limited to free eDNA (extra and intracellular DNA from dead cell [e.g. feces, urine, moult, mucus; Taberlet et al., 2012]). Conversely, studies on meio and microfauna have also investigated eDNA and eRNA in parallel (e.g. Dowle et al., 2015b; Lejzerowicz et al., 2013, 2014). Due to the persistence of DNA in the environment, eDNA samples may reflect living and dead assemblages, creating biases when performing ecological surveys (Lillis et al., 2009; Mengoni et al., 2005). Environmental RNA degrades rapidly and is therefore more likely to provide an accurate assessment of the living organisms in a sample (Lillis et al., 2009; Pawlowski et al., 2014a; Pochon et al., 2015).

Foraminifera are an abundant group of protists (up to thousands of individuals per 10 cm^3) that form an essential component of marine sediment communities (Murray, 2006; Sen Gupta, 2002). They are responsive to local conditions and have shorter life-cycles than macrofauna, making them good indicators of current and recent environmental perturbations, including organic enrichment, oil discharges, metal contamination and physical disturbances (Alve, 1999; Bergin et al., 2006; Casey et al., 1980; Ernst et al., 2006; Mojtahid et al., 2008). Foraminifera have also been shown to display slightly higher sensitivity to oil-based drilling mud than macrofauna (Denoyelle et al., 2010). Additionally, mineral-walled forms of foraminifera leave a microfossil record that can provide data for pre-pollution assessment in cases where no baseline studies are available (Ernst et al., 2006; Hayward et al., 2004; Hess et al., 2013; Schafer, 2000).

An extensive database of foraminiferal DNA sequences has been accumulated over two decades of research (Pawlowski et al., 2014a), providing a solid taxonomic framework for comprehensive metabarcoding. Diversity surveys using eDNA metabarcoding have dramatically changed traditional taxonomic notions of foraminifera. For example, eDNA analysis has revealed an unexpectedly high diversity of monothalamous (single-chambered) foraminifera in benthic ecosystems (Lecroq et al., 2011; Pawlowski et al., 2011). Foraminiferal metabarcoding has recently been applied to assess the impact of salmon farming activities in sheltered fjords, where communities and species-specific responses strongly correlate to organic enrichment, especially when using eRNA (Pawlowski et al., 2014b; Pochon et al., 2015). This provided the impetus to explore the use of this molecular monitoring tool for measuring the impact of a wider range of perturbations in the marine environment.

In this study, we investigated, for the first time, the use of foraminiferal metabarcoding to assess the impact of exploratory offshore drilling activities. The overarching aim was to determine whether foraminiferal metabarcoding can be used to detect shifts in overall communities and to identify the key bioindicator taxa that may respond with the variation in environmental gradients associated with this type of industrial marine activity.

Biological samples (foraminiferal and macrofaunal assemblages) and physico-chemical data were collected along transects radiating to the north and south of two oil wellheads (WHs) drilled off the Taranaki region, New Zealand. Both sites were characterized by coarser sediments, hypoxic conditions, low organic content, and higher concentrations of barium and arsenic at their WH (Skilton et al., 2015). We hypothesized that: (1) foraminiferal eDNA and eRNA assemblages would show stronger responses than macrofauna to shifts in environmental conditions caused by oil and gas operations, and (2) key foraminiferal taxa could be identified that were indicative of these environmental changes.

2. Material and methods

2.1. Field sampling

The study area was located 35 km off the west coast of New Zealand's North Island, in the South Taranaki Bight (Fig. 1A). In this region, the seabed is mostly uniform, consisting of soft sediment and with water depth ranging from 120 and 130 m. Between May and June 2014, well Oi-1 (39°21′019″S, 173°20′027″E) was drilled and 319 m^3 of water-based drilling mud and cuttings material were discharged at sea from the platform (Skilton et al., 2015) using the process described in Govier and Calder (2013). Due to drilling difficulties, another well, Oi-2 (39°20′955″S, 173°20′087″E), was spudded 150 m northeast of the former between June and July 2014, with the additional release of 243 m^3 of drilling material (Skilton et al., 2015).

The methodology used for sampling was based on the Offshore Taranaki Environmental Monitoring Protocol (OTEMP; Johnston et al., 2014a). It consists of a distance-graded sample station allocation. In this specific area, the north-south axis constitutes the main trajectory along which deposition of drilling mud and cuttings occurs (MetOcean Solutions Limited [MSL], 2013). Sampling stations were overlaid along this axis with the drilling rig located at the center (Fig. 1B). Twelve stations were sampled at Oi-2. One of these stations was directly adjacent to the drilling site. These samples provided a unique opportunity to study potential impact at sites close to WHs. Most monitoring programmes are unable to obtain samples within a 250 m radius from WHs. In this study, it was possible to obtain these samples because the sites were used for exploratory purposes only, and all activity ceased shortly after drilling. The remaining stations were located both northward and southward from the WH at approximately 100, 250, 500, 1000 and 2000, and one station situated at 4000 m northward from the WH. Two other stations were centered around Oi-1, one at the WH and one 100 m south. Two control sites were located 15 km south-east and 50 km south-west of the WH respectively (Fig. 1).

Using a modified stainless steel double van-Veen grab (Johnston et al., 2014), a total of 51 sediment samples were collected between August and October 2014, corresponding to 17 stations in triplicate (i.e., the grab was sent three times to the seafloor at each station; Table S1). To avoid creating a bow wake effect and disturbing the top sediment layer, the grab sampler was deployed and retrieved at a constant rate of 0.3 m/s. Upon retrieving the grab, the surface was inspected and samples only taken when the surface sediment was undisturbed. The grab is divided into two compartments. The full contents of the first compartment was sieved through a 500 μm mesh and preserved in 70% ethanol for analysis of macrofaunal communities. Subsamples (2 g) of undisturbed surface sediment (approximately 1 cm depth) were collected from the second compartment for foraminiferal eDNA/eRNA metabarcoding. These samples were placed in Life Guard™ Soil Preservation Solution (5 ml; MoBio, USA) using disposable gloves and spatulas, stored on ice during transportation to the laboratory and kept frozen (−20 °C) until further processing. The remainder of the second compartment was sampled for the analysis of sediment texture, organic content or ash-free dry weight (AFDW), trace metals (arsenic [As], barium [Ba], cadmium [Cd], chromium [Cr], copper [Cu], lead [Pb], nickel [Ni], zinc [Zn], mercury (Hg)), screen metals (manganese [Mn], iron [Fe]), polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbon (TPHs).

2.2. Laboratory analysis

2.2.1. Samples processing

Grain size analysis was performed by Resource and