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Comparison of Niskin vs. *in situ* approaches for analysis of gene expression in deep Mediterranean Sea water samples

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ABSTRACT

Obtaining an accurate picture of microbial processes occurring *in situ* is essential for our understanding of marine biogeochemical cycles of global importance. Water samples are typically collected at depth and returned to the sea surface for processing and downstream experiments. Metatranscriptome analysis is one powerful approach for investigating metabolic activities of microorganisms in their habitat and which can be informative for determining responses of microbiota to disturbances such as the Deepwater Horizon oil spill. For studies of microbial processes occurring in the deep sea, however, sample handling, pressure, and other changes during sample recovery can subject microorganisms to physiological changes that alter the expression profile of labile messenger RNA. Here we report a comparison of gene expression profiles for whole microbial communities in a bathypelagic water column sample collected in the Eastern Mediterranean Sea using Niskin bottle sample collection and a new water column sampler for studies of marine microbial ecology, the Microbial Sampler – *In Situ* Incubation Device (MS-SID). For some taxa, gene expression profiles from samples collected and preserved *in situ* were significantly different from potentially more stressful Niskin sampling and preservation on deck. Some categories of transcribed genes also appear to be affected by sample handling more than others. This suggests that for future studies of marine microbial ecology, particularly targeting deep sea samples, an *in situ* sample collection and preservation approach should be considered.

1. Introduction

Microbial metabolic activities are the basis of almost every major biogeochemical cycle in the oceans, and as the research community transitions away from purely descriptive studies of marine microbes to system-level investigations of community activity and responses to changing environmental conditions, it is imperative that we obtain less biased samples for those studies. As a consequence of the fact that the majority of microorganisms are not amenable to existing cultivation approaches, many marine microbiologists and microbial ecologists have embraced culture-independent methods. Metatranscriptomics, or the isolation and sequencing of messenger RNA (mRNA) from an environmental sample, is one powerful method currently use for linking diversity with activity, and for examining microbial activities in response to changing conditions. Metatranscriptomics provides an overview of (at a minimum) the most highly expressed genes in a sample. These transcripts inform about the metabolic pathways that are utilized by microbiota in that sample at the time of sample preservation, and specific proteins that were expressed. Enabled by recent advances in high-throughput sequencing technologies and bioinformatics for processing datasets that can contain tens of millions of reads, metatranscriptomics has become one of the most powerful tools for examining microbial community activities. This approach has been used successfully to examine gene expression in varied marine habitats. Examples include deep subsurface sediments (Orsi et al., 2013), the North Pacific Subtropical gyre (Frias-Lopez et al., 2008a, 2008b; Poretsky et al., 2009), eastern tropical South Pacific oxygen minimum zone (Ulloa et al., 2012), coastal waters (Hollibaugh et al., 2010; Gifford et al., 2011), hydrothermal vent plumes (Li et al., 2013), microcosm experiments on mixed water layers from the NE Pacific Ocean (Marchetti et al., 2012). Metatranscriptomics was also recently used to study microbial responses to the Deep Water Horizon oil spill. Mason et al. (2012) found a rapid increased expression of genes associated with motility, chemotaxis, and aliphatic hydrocarbon degradation originating from members of the *Oceanospirallales* in hydrocarbon plume samples. Rivers et al. (2013) also applied

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metatranscriptomics to show increased activity (primarily associated with methane- and petroleum-degrading Gammaproteobacteria) within specific metabolic pathways for the degradation of alkanes, aromatic compounds and methane following the Deepwater Horizon spill. Metatranscriptomic studies in the future will be enhanced by sampling technologies that allow us to minimize potential artifacts that can be introduced due to sample handling.

For studies of gene expression it is necessary to minimize time between sample collection and chemical preservation. Historically, oceanographers studying marine microbiota have relied on ship-based hydrocasting operations whereby water samples from various depths in the ocean are brought to the surface via Niskin rosette samplers for shipboard water processing. This approach is likely not appropriate for mRNA-based investigations due to the typically large and variable lapses in time and accompanying physicochemical shifts samples are exposed to between collection and preservation. When working in the near-surface (top 1–200 m) up to 30 min may pass between when a water sample is collected and when it is returned to the surface, preserved and processed in the ship's laboratory. In addition, samples are exposed to pressure, and potentially temperature and redox changes before preservation. Pressure changes, potential physicochemical changes, and separation in time between sample collection and processing are exacerbated when working in the deep sea and/or collecting water samples from low-oxygen or anoxic zones. Impacts on transcription by microbes captured in water samples are also likely to vary between taxa depending fragility of cell structures, and on their strategies (or lack thereof) for responding to such changes. Given that average lifetimes of prokaryotic transcripts can be on the order of several minutes (Wang et al., 2002; Andersson et al., 2006; Steglich et al., 2010), the gene expression profiles of microorganisms can potentially be altered significantly. While delays in the preservation of DNA and rRNA (often used as a phylogenetic identifier of viable organisms) is less susceptible to such biases because of their significantly longer half-lives, delayed preservation of Niskin samples can still be an issue if cell integrity is lost due to unnatural and changing conditions during transport from the ocean to the ship's laboratory. Variable and unknown fractions of genetic material from lysed cells can be lost during filtration. This problem is particularly severe for microbial eukaryotes and potentially compounded when sampling greater depths (Edgcomb et al., 2011a, 2011b).

Numerous technical approaches have been undertaken for microbial sampling of the sea and include hydrowire deployed devices (Zobell, 1941; Nikin 1962; Lewis et al., 1963), devices that minimize exogenous contamination (e.g. Jannasch and Maddux, 1967; Taylor et al., 2006), samplers that preserve the conditions of the deep sea (e.g. Jannasch et al., 1973; Jannasch and Wirsen, 1977; Tabor et al., 1981; Bianchi et al., 1999), a sampler that can preserve a whole water sample *in situ* from 120 m depth (Feike et al., 2012), samplers that collect hydrothermal vent fluids (e.g., Malahoff et al., 2002; Phillips et al., 2003; Taylor et al., 2006), AUV (autonomous underwater vehicle) based water samplers (Bird et al., 2007; Ryan et al., 2010) those that conduct *in situ* molecular analyses, such as the environmental sample processor (ESP) (Scholin et al., 2006; Roman et al., 2007; Scholin 2010), assess phytoplankton assemblages via imaging flow cytometry (Olson and Sosik, 2007; Sosik and Olson, 2007) and that sample remote biospheres (Carsey et al., 2000; French et al., 2001; Blake and Price, 2002; Siegert et al., 2003; Cardell et al., 2004). A common limitation of available instrumentation for *in situ* preservation of deep-sea samples, or that might be adapted for this purpose, is the restriction to one or a few samples of limited volume. Sample replication is desirable when examining microbial diversity and activities, and when working in the deep sea, greater total volumes are often required due to low cell densities. Additionally, when working in the mesopelagic and bathypelagic zones, wire time is often precious, and the ability to sample multiple depths is an advantage.

Differences in microbial gene expression have been observed between samples preserved *in situ* vs. those recovered to the deck prior to preservation from suboxic samples collected from 70–120 m depth in the Baltic Sea (Feike et al., 2012). Additionally, we have documented that changing physicochemical conditions during Niskin sampling can cause lysis of some microbial eukaryotes (Edgcomb et al., 2011a, 2011b). Obtaining accurate information on *in situ* microbial activities is of fundamental importance to understanding microbially-driven ocean processes and responses of microbiota (and the major biogeochemical cycles that they mediate) to global climate change. To date, no data exist comparing the profile of microbial community gene expression in the deep sea using *in situ* vs. conventional Niskin-based approaches. Here we compare the profile of community gene expression by microbiota in waters from 2222 m depth in the Eastern Mediterranean Sea using conventional Niskin rosette collection vs. *in situ* filtering and sample preservation performed using a newly-developed oceanographic instrument for marine microbiological studies, the Microbial Sampler – Submersible Incubation Device (MS-SID) that allows for collection and *in situ* preservation of up to 48 filtered or whole water samples during a single hydrocasting operation. While not an ecological study of microbial activities at this location, the aim of this work was to conduct a general comparison of transcriptome results obtained using both methods, and to analyze the reproducibility of biological replicates collected sequentially using the MS-SID.

2. Materials and methods

2.1. Study site

The Ionian Sea extends from the Sicily Strait to the Cretan passage, in the Eastern Mediterranean Sea, which is characterized by an eastward progression of increasingly oligotrophic conditions (Sarmiento et al., 1988; Danovaro et al., 1999; Thingstad et al., 2005). The study was conducted using samples collected at a site named KM3 (36°29′98″N, 15°39′97″E) from 2222 m water depth in September, 2012 using the R/V *Urania* of the Italian National Research Council (CNR).

Niskin bottle water collection: Water was collected using 12 L Niskin bottles mounted on a General Oceanics rosette sampler equipped with conductivity-temperature and depth (CTD) and pressure sensors. Dissolved oxygen was measured with a SBE oxygen sensor mounted on the CTD, and nutrient concentrations were determined previously at this site using a nutrient auto-analyzer (La Cono, et al., 2010). After transferring water from Niskin bottles to a large sterile carboy, 30 L of water were pumped through a 0.22 μm Sterivex filter cartridge using a peristaltic pump operating around 125 ml/min containing a Durapore filter (Millipore, Millford, MA, USA), which was immediately filled with RNAlater (Life Technologies Inc., Grand Island, NY, USA) and frozen at −80 °C until extraction.

2.2. Use of the MS-SID

Water samples from the same depth and on the same day were also collected and preserved *in situ* using the MS-SID equipped with a CTD, two turbidity sensors, and an oxygen optode (Fig. 1). C. Taylor and McLane Research Laboratories developed automated micro-laboratories for conducting multiple tracer incubation studies during cabled or free-drifting deployments (Taylor and Doherty, 1990; Taylor et al., 1993; Taylor and Howes, 1994). This technology was recently modified by C. Taylor, V. Edgcomb, and McLane Research Laboratories into an instrument (Fig. 1) that conducts *in situ* tracer incubations in combination with *in situ*

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