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In situ grazing experiments apply new technology to gain insights into deep-sea microbial food webs

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ABSTRACT

Predation by grazing protists in aquatic habitats can influence prokaryotic community structure and provides a source of new, labile organic matter. Due to methodological difficulties associated with studies of deep-sea (below photic zone) microbiota, trophic interactions between eukaryotes and prokaryotes in mesopelagic and bathypelagic realms are largely obscured. Further complicating matters, examinations of trophic interactions using water samples that have been exposed to upwards of hundreds of atmospheres of pressure change prior to initiating experiments can potentially introduce significant artifacts. Here we present results of the first study of protistan grazing in water layers ranging from the euphotic zone to the bathypelagic, utilizing the Microbial Sampler-Submersible Incubation Device (MS-SID) that makes possible in situ studies of microbial activities. Protistan grazing in the mesopelagic and bathypelagic realm of the East Mediterranean Sea was quantified using fluorescently labeled prokaryotes (FLP) prepared from the naturally-occurring prokaryotic assemblages. These studies reveal daily prokaryotic removal due to grazing ranging from 31.3 ± 5.9% at 40 m depth to 0.5 ± 0.3% at 950 m. At 3540 m depth, where a chemocline habitat exists with abundant and active prokaryotes above Urania basin, the daily consumption of prokaryotes by protists was 19.9 ± 6.6% of the in situ abundance.

1. Introduction

Protists are unicellular eukaryotes that interact in several ways with bacterial and archaeal populations, shaping substantially the structure of these marine communities. Phagotrophy is an ancient mode of nutrition for protists that predates photosynthesis (Cavalier-Smith, 2002 and references therein), resulting in widespread distribution of this mode of nutrition among the major phyla in the protistan tree of life. Phagotrophic grazing can affect the quantity, activity, and physiological state of prey organisms, and sometimes protists exhibit prey preferences (Jezbera et al., 2006; Jürgens and Massana, 2008 and reference there in; e.g. Simek et al., 1997). Indeed, protistan assemblages may graze on the order of 25% to >100% of the measured daily production of prokaryote plankton (Sherr and Sherr, 1994), resulting in the consumption of prokaryote biomass at approximately the same rate as it is produced (Pernthaler, 2005). Through grazing, protists actively participate in the transfer of organic carbon from the dissolved fraction to higher trophic levels, in a heterotrophic food chain involving bacteria, nanoflagellates (2–20 μm), and ciliates, that has been termed the "microbial loop" (Azam et al., 1985). Protists are capable of inducing significant shifts in prokaryote community composition, of enhancing microdiversification, and of impacting prokaryote community evenness (Simek et al., 1997). Dominant groups and size classes of common protistan grazers (particularly small flagellated protists and ciliates) as well as some known ecological impacts of grazing were recently summarized (Jürgens and Massana, 2008; Pernthaler, 2005). Through their grazing activities, protists are known to regenerate nutrients and modify or re-mineralize organic matter (e.g., Jumars et al., 1989; Sherr and Sherr, 2002).

In recognition of their importance in marine aquatic communities, protozoa are considered in numerical models of carbon cycling and in paradigms of surface and deep-ocean microbial ecology (Arístegui et al., 2009). Indeed, as phagotrophic protists and viruses

Abbreviations: MS-SID, Microbial Sampler-Submersible Incubation Device; FLP, Fluorescently labeled prokaryotes

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are the main sources of mortality for marine microbes (Arístegui et al., 2009; Fuhrman and Noble, 1995), they are presumed to be important in the cycling of both particulate and dissolved organic matter, however their specific roles in carbon cycling are not well understood. A large portion of oceanic primary production is usually respired in the well-oxygenated mixed layer through grazing and microbial remineralization processes, and returned to the atmosphere as CO_2. Microbial processing of dissolved and particulate organic matter in the deep sea remains poorly constrained, particularly regarding the impact of protist grazing on prokaryotic communities, which aside from shaping prokaryotic communities at depth, could serve as a fresh source of labile organic matter in deep ocean.

With increasing exploration of deeper ocean mesopelagic (200–1000 m) and bathypelagic (1000–4000 m) realms, we now know that while overall microbial cell counts tend to decline with depth, protists not only are present, but their communities in the deep ocean, including Gulf of Mexico are diverse (Lopez-Garcia et al., 2001; Sauvadet et al., 2010, 2011). Furthermore, where oxyclines and redoxclines exist, protist numbers and diversity increase relative to the adjacent deep-ocean waters (Edgcomb et al., 2011b; Stock et al., 2012). Studies of activities of phagotrophic protists have been conducted mainly within the photic zone, using samples from up to 200 m depth (e.g. Christaki et al., 1999; Karayanni et al., 2008; Tsai et al., 2012). Little is known about the grazing impact of protists on prokaryotic communities below the upper mesopelagic zone and in other "challenging" environments such as hydrocarbon polluted sea water in the Gulf of Mexico, where grazing protozoa might be able to encourage re-mineralization of growth-limiting nutrients, and where predation can possibly result in enhanced prokaryotic growth and activity, fostering the utilization of hydrocarbons as a carbon source for the microbial communities (Beaudoin et al., 2014).

Grazing studies to date have relied upon hydrocasting operations for collection of water samples from the water column typically via Niskin-bottle hydrocast operations. These samples are brought to ship's deck, and then typically drawn from the Niskin bottle for subsequent studies. While ship-board grazing studies of near surface samples collected with Niskin bottles likely introduce few artifacts associated with the collection and transfer of ocean samples into experimental containers, similar handling of deeper samples may result in dramatic alteration of microbe physiological state, particularly when studying anoxic and/or deep-water column environments (Feike et al., 2012). Such perturbations can affect downstream studies of various microbial activities, including protist grazing. Many microbial eukaryotes are extremely sensitive to physico-chemical changes, particularly oxidation-reduction (redox) state, significant sudden pressure shifts, and possible temperature changes. Consequently, Niskin sampling may only recover a fraction of the total in situ eukaryotic community from anoxic waters or redox interfaces below ~250 m. This phenomenon was observed in water samples from oxycline (300 m depth) and 900 m deep anoxic water samples from the Cariaco Basin, Venezuela (Edgcomb et al., 2011a). The same may be true from oxic deep mesopelagic and bathypelagic waters, where pressure changes during sample retrieval can be significant. Regardless of the degree to which individual water samples from different sites and depths are affected by traditional Niskin bottle retrieval, it makes sense that for a comprehensive picture of in situ microbial community activity the best strategy is to conduct rate process measurements in situ in order to minimize potential artifacts associated with experimental methods and to interpret the data in the context of the local environment. For complete examinations of hydrocarbon impacts on mesopelagic and bathypelagic microbial communities in locations such as the Gulf of Mexico, it is important to include studies of microbial eukaryotes, as they participate in marine nutrient cycles both directly and indirectly through predation on Bacteria and Archaea. For assessing carbon turnover due to phagotrophy, technology is required that minimizes the artifacts associated with sample handling by facilitating in situ incubation studies.

Here we present the first in situ study of phagotrophic protist grazing activities using water samples collected and incubated at selected depths down to 3000 m in the Eastern Mediterranean Sea, and also one experiment performed in a deep-sea redoxcline at 3540 m. Normally, with shipboard incubations, each experiment for each water depth studied, would be replicated. Given the 4–16 h of wire time required for each study, it was not possible to replicate experiments. As a result, ecological interpretations of data presented here must be interpreted cautiously, and require future replication for depths of interest. Results of this study of protistan phagotrophy, including in the bathypelagic realm, provide a first demonstration of the use of in situ approaches for providing insights into the activities of deep ocean protists. The grazing impact of the protistan community was estimated by (i) short term grazing experiments, tracing the ingested fluorescently labeled prokaryotes (FLPs), as described in Sherr et al. (1987) and (ii) long term incubations, estimating the rate of disappearance of the FLPs, according to Vazquez-Domınguez et al. (1999).

2. Materials and methods

2.1. Site description

Mediterranean Sea is characterized by an eastward progression of increasingly oligotrophic conditions, and the Eastern basin is defined as an ultra-oligotrophic, phosphorus-depleted system (Sarmiento et al., 1988; Thingstad et al., 2005). The study was conducted in Libyan Sea, Eastern Mediterranean (35° 13.840′N, 21° 28.478′E) 200 km west of Crete on the Mediterranean Ridge (Fig. 1), at the boundary of the accretionary complex and the so-called Inner Plateau of the ridge. At this location lies the Urania Basin, a sea floor depression that is filled with anoxic and highly saline brine that is characterized by the highest concentration of sulfide ever encountered in the marine environment (12–20 mM). The Urania hypersaline basin, as all Mediterranean deep Hypersaline anoxic basins (DHABs), is separated from the water column by a halocline possessing a steep salt gradient (3.9% to 27% [w/w] salinity) between anoxic hypersaline brine and overlying seawater with high bacterial activity (Yakimov et al., 2007a). The data presented in this study derive from 3 oceanographic expeditions carried out in 2011 and 2012 aboard the R/V *Urania* of the Italian National Research Council (CNR) and in 2011 on the R/V *Atlantis* of the Woods Hole Oceanographic Institution (WHOI).

2.2. Preparation of the prey analogues, FLP

Water samples were collected from the Eastern Mediterranean Sea at 3000 m in September 2011. After pre-filtering through 0.8 μm pore size filters to exclude protists and larger eukaryotes, prokaryotic cells were concentrated by tangential flow filtration (Pellicon System, Millipore Co. equipped with a Biomax 500 K polyethersulfone membrane). The concentrated sample was used as inoculum into sterile seawater to which 0.1% Luria Broth (LB) medium was added and the enrichment cultures were kept at 12 °C. When they attained exponential growth at (as determined by microscopy counts), the cells were pelleted by centrifugation (20 min at 2000 g), resuspended into sterile seawater and grown at the same temperature for an additional time that ranged from 5 to 15 days. Prokaryotic cells from the enrichments were harvested and stained (as described below) when the size of the prey analogues was within the size range of the natural prokaryotic population along the depth transect. FLPs from enrichments