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Discovery of a chemosynthesis-based community in the western South Atlantic Ocean

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ABSTRACT

Chemosynthetic communities have been described from a variety of deep-sea environments across the world's oceans. They constitute very interesting biological systems in terms of their ecology, evolution and biogeography, and also given their potential as indicators of the presence and abundance of consistent hydrocarbon-based nutritional sources. Up to now such peculiar biotic assemblages have not been reported for the western South Atlantic Ocean, leaving this large region undocumented with respect to the presence, composition and history of such communities. Here we report on the presence of a chemosynthetic community off the coast of southern Brazil, in an area where high-levels of methane and the presence of gas hydrates have been detected. We performed metagenomic analyses of the microbial community present at this site, and also employed molecular approaches to identify components of its benthic fauna. We conducted phylogenetic analyses comparing the components of this assemblage to those found elsewhere in the world, which allowed a historical assessment of the structure and dynamics of these systems. Our results revealed that the microbial community at this site is quite diverse, and contains many components that are very closely related to lineages previously sampled in ecologically similar environments across the globe. Anaerobic methanotrophic (ANME) archaeal groups were found to be very abundant at this site, suggesting that methane is indeed an important source of nutrition for this community. In addition, we document the presence at this site of a vestimentiferan siboglinid polychaete and the bivalve *Acharax* sp., both of which are typical components of deep-sea chemosynthetic communities. The remarkable similarity in biotic composition between this area and other deep-sea communities across the world supports the interpretation that these assemblages are historically connected across the global oceans, undergoing colonization from distant sites and influenced by local ecological features that select a stereotyped suite of specifically adapted organisms.

1. Introduction

Cold seep areas are widespread around the world's oceans, occurring at different latitudes and depths (Sibuet and Olu, 1998). They are characterized by the presence of fluxes of methane, oil, and/or other hydrocarbon components that reach the sediment–water interface from below via relatively focused conduits, such as faults (Arvidson et al., 2004). Chemosynthesis often occurs in

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these areas, supporting biotic communities sustained by gases such as methane and byproducts of its decompositions such as H_2S (Sibuet and Olu, 1998; Milucka et al., 2012), usually at temperatures of ~2–4 °C (Reed et al., 2006; Jørgensen and Boetius, 2007; Baco et al., 2010 and Das et al., 2011). Although such environments have been the focus of multiple studies for more than 20 years in the Northern Atlantic and Pacific Oceans (Paull et al., 1984; Barry et al., 2000; Sassen et al., 2004; Genio et al., 2008; Nunoura et al., 2008; Van Gaever et al., 2009a; Sibuet and Vangriesheim, 2009; Das et al., 2011; German et al., 2011; Hilário et al., 2011a; Vigneron et al., 2013a, 2013b), equivalent systems in the Southern Hemisphere have received less attention. A few studies have indicated that chemosynthetic communities also occur in western Africa and Peru, at equatorial latitudes, as well as further south off Chile and New Zealand (Olu et al., 1996a, 1996b; Sellanes et al., 2004; Ondreas et al., 2005; Cambon-Bonavita et al., 2009; Van Gaever et al., 2009b; Baco et al., 2010; Olu et al., 2010 and Rodrigues et al., 2010). Still, in spite of a previous study showing the existence of a faunal element (the polychaete *Amelinna* sp.) suggestive of a reducing environment in pockmarks off the coast of Brazil (Sumida et al., 2004), until now no clear documentation of such communities exists for the western South Atlantic Ocean. Such scarcity of information raises questions of whether these assemblages are also present in cold seeps found in this region, and if they have a similar composition to those identified so far in other areas.

Amongst the previous assessments of such ecosystems in other regions, some common features have emerged, such as the presence of the mat-forming vacuolated sulfur-oxidizing bacteria *Beggiatoa*, *Arcobacter*, *Thiothrix* and *Thiomargarita* (Olu et al., 1997; Levin, 2005; Portnova et al., 2011 and Vigneron et al., 2013a). These communities also tend to include representatives of specialized animal groups, including bivalve mollusks belonging to families Solemyidae (*e.g.* genus *Acharax*), Vesicomyidae (*e.g. Calyptogena*) and Mytilidae, which generally carry in their gills endosymbiont bacteria that are able to oxidize methane or hydrogen sulfide (Sibuet and Olu, 1998; Imhoff et al., 2003; Treude et al., 2003; Kalanetra et al., 2005; Levin, 2005; Cordes et al., 2010). Other animals that are typical of these habitats are highly specialized polychaete annelids belonging to the 'Vestimentifera' clade within the family Siboglinidae (*e.g.* genus *Lamellibrachia*) (Hilário et al., 2011a, 2011b). An interesting feature of these communities is the intricate interaction between such metazoan specialists and a complex and specialized microbiota, involving relationships ranging from direct predation to endosymbiosis. Across the range of such interactions, the ecological role of symbionts as well as free-living microorganisms is critically important and still poorly understood. Available evidence indicates that many of them are related directly or indirectly to the oxidation of hydrocarbon compounds by using oxygen, nitrate, ferric iron or sulfate as the terminal electron acceptor (Knittel and Boetius, 2009; Burgin et al., 2011). An important process affecting such dynamics (and influencing global deep-sea geochemistry) is the anaerobic oxidation of methane (AOM) using sulfate as a terminal electron acceptor (Zhang et al., 2011), a reaction which is mediated by a consortium of sulfate-reducing bacteria (SRB) and methanotrophic archaea (ANME) (Boetius et al., 2000; Orphan et al., 2002; Knittel et al., 2003; Knittel et al., 2005 and Knittel and Boetius, 2009). Among the former, the genera *Desulfosarcina*, *Desulfococcus* and *Desulfobolbus*, belonging to the class Deltaproteobacteria, are the most typical. The archaeal component of the consortium has been described on the basis of DNA sequences, and is presently allocated in one of three major ANME groups (ANME-1, ANME-2 and ANME-3) (Boetius et al., 2000). The ANME groups are related to the methanogens within the phylum Euryarchaeota, with ANME-1 being distantly affiliated with the orders Methanosarcinales and Methanomicrobiales, and ANME-2 and ANME-3 belonging to the order Methanosarcinales (Orphan et al., 2001; Knittel et al., 2005 and Lanoil et al., 2005). The mechanisms of the anaerobic oxidation of methane by ANME-1 and ANME-2 in the context of the archaeal–bacterial consortia are still unclear, but presumably involve a syntrophic association based on interspecies electron transfer. The archaeal member of the consortium apparently oxidizes methane and shuttles reduced compounds to the sulfate-reducing bacteria (Valentine, 2002 and Milucka et al., 2012).

Given the global biogeochemical relevance of anaerobic methane oxidation, as well as the role methane plays in atmospheric temperature regulation (Valentine, 2002), it is important to understand the structure and function of these deep-sea systems, which are likely critical components of the world's methane source-sink dynamics. More specifically, characterizing the biological diversity that occurs in each of these areas is necessary to understand their common as well as unique features, which may help unravel more completely their underlying biogeochemical properties.

In this study, we employ multiple approaches to document the occurrence of a chemosynthetic biotic community within a deep-sea pockmark off the coast of southern Brazil, located in the Rio Grande Cone area (Pelotas Basin). We show that methane seepage occurs in the area, and demonstrate that it harbors several biological components that are typical of cold-seep assemblages, opening up new avenues to comparatively characterize these systems across multiple sites in the world's oceans.

2. Material and methods

2.1. Site location and characterization

The study area consists of a pockmark named 'Pockmark 22', located at approximately 1200 m water depth on the northeastern flank of the Rio Grande Cone (Fig. 1). The latter is a large protuberance in the continental slope of the Pelotas Basin, western South Atlantic, where major bottom simulating reflectors (BSR) indicating the probable occurrence of gas hydrate deposits have been mapped both in Brazil (Sad et al., 1998; Fontana and Mussumeci, 1994; Clennell, 2000; Oliveira et al., 2010) and Uruguay (Tomasini et al., 2011).

To investigate the possible occurrence of shallow (*e.g.* a few meters below the seafloor) gas hydrates in this area, we performed two oceanographic campaigns in February/March 2011 (MR11 campaign) and in May/June 2011 (MD186 campaign). The MR11 campaign used the Brazilian research vessel *Marechal Rondon*, equipped with a deep-water Reson Seabat 7150 high-resolution multi-beam echo sounder (MBES; operating frequency of 24 KHz) and a SyQwest Bathy 2010 CHIRP sub-bottom profiler (SBP; central frequency of 3.5 KHz). The vessel was also equipped with a 6 m long piston corer. The MD186 campaign used the French research vessel *Marion Dufresne*, equipped with a MBES/SBP Thomson Seafalcon 11 (with operating frequencies of 12 KHz for MBES and 3.5 KHz for SBP), as well as a 40 m long, giant Calypso piston corer, and a 25 × 25 cm^2, 12 m long gravity box-coring device (CASQ core sampler).

2.2. Imaging of the pockmark using an autonomous underwater vehicle (AUV)

In addition to the two sampling campaigns, we also performed a separate research cruise that focused on the photographic documentation of the seafloor inside Pockmark 22. We used an autonomous underwater vehicle (AUV) model C-Surveyor II operated by the *R/V Rig Supporter*, which carried a high-resolution black-