

GEOCHEMICAL AND MICROBIAL DIVERSITY OF BUNDERA SINKHOLE, AN ANCHIALINE SYSTEM IN THE EASTERN INDIAN OCEAN

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The anchialine system at Bundera sinkhole, Australia, exhibits pronounced hydrogeochemical structure through depth that is reflected in the composition and distribution of the fauna. It is a strongly structured microbial ecosystem the components of which also change with depth and which is dominated by sulfur bacteria and chemolithotrophic microbial classes.

Key words: anchialine; chemocline; 16S amplicon sequencing; sulfur bacteria; chemolithotrophy

INTRODUCTION

Anchialine ecosystems contain assemblages of invertebrates the composition of which are broadly predictable however far apart in the world they occur. The most disjunct example is located in north-western Australia and is represented by the fauna of Bundera Sinkhole (WILSON & HUMPHREYS, 2001). This cave contains a number of genera endemic to anchialine systems and disjunct from those in the North Atlantic, as well as an array of genera endemic to this cave. Unlike the better studied anchialine systems in the tectonically stable Bahamian and Yucatan karsts, the Australian anchialine ecosystem will have been displaced inland by up to 100 km by the effect of Quaternary eustatic changes on the shore of the North West Shelf. These changes, superimposed on Late Tertiary orogeny, have likely been a driver of vicariance of the anchialine genera, at least five of which have speciated (e.g., *Stygiocaris*, PAGE *et al.*, 2007).

CHARACTERISTICS

The Bundera anchialine cave is located 1.6 km from the ocean on a 2.5 km wide coastal plain. The tidal range of 0.27 m is 14% of the amplitude of the semi-diurnal ocean tide with a temporal lag of 2.27 h. The sinkhole leads into a cave inclined ca 30° and the marine section is entirely within the cave. Anchialine systems have salinity stratified waters separated by a pycnocline. Organic matter accumulates at the density interface resulting in a redox gradient associated with hydrogen sulphide layers and a cascade of dissolved inorganic nitrogen speciation. Bundera has the most complex stratification of the anchialine systems considered by POHLMAN (2011), an attribute associated with the very gradual halocline, ca 10 m thick (HUMPHREYS, 1999).

PHYSICO-CHEMICAL PROFILE

The Bundera anchialine fauna was first recognised in 1993 and physicochemical, chemical and microbiological profiles have since been recorded. Environmental profiles of Bundera and surrounds were examined 10 times between 1994 and 2012, four times by divers and the remainder through recent bores. Physical and chemical profiles were made using various sondes and on occasion profiles of dissolved inorganic nitrogen species, H₂S and major ions sampled by divers (HUMPHREYS, 1999; SEYMOUR *et al.*, 2007).

The structure and location of the profiles over the first decade of sampling was highly stable although the amplitude of the measurements varied between years. Especially marked was the persistence of the double band of elevated H₂S marking the upper and lower boundary of the halocline (Fig. 1). The data in SEYMOUR *et al.* (2007) was recorded when the H₂S layer had migrated upwards and extended to the surface, venting H₂S, and the main features of the chemocline had become monophasic (Fig. 1). The vertical migration of the anoxic zone may have resulted from the addition of excessive organic matter 16 days prior to sampling in the form of an emu, *Dromaius novaehollandiae*, a species with an average mass of 36 kg. Such large organic matter additions may cause alteration and migration of the chemocline in meromictic Lake Shira, Siberia (DEGERMENDZHY *et al.*, 2005). Bundera has been hypothesised to contain a high diversity of microbial life on account of its hydrogeochemical structure and the morphology of the bacteria (HUMPHREYS, 1999). In addition, although microscale stratification through changing geochemical conditions and a complex microbial assemblage has been recorded, each dominated by heterotrophic, phototrophic or chemoautotrophic microorganisms (SEYMOUR *et al.*, 2007), and the taxonomic composition of this assemblage has not been determined.

16S AMPLICON SEQUENCING

Filtrand on 0.4 µm filters on FTA cards (THACKER, 2000) were maintained at -70 °C, the samples were duplicates of those presented in SEYMOUR *et al.* (2007). Duplicate 2 mm diameter punch samples of filters from 12 depths (0.5–25 m) were subjected to 16S amplicon pyrosequencing. The F515 and R806 primers provide broad taxonomic coverage for Archaea and Bacteria but are much less effective for Eukaryotes (WALTERS *et al.*, 2011). Resulting sequences were filtered through an automated pipeline in MG RAST and a broad outline is presented for 9 depths (0.5–25 m). This

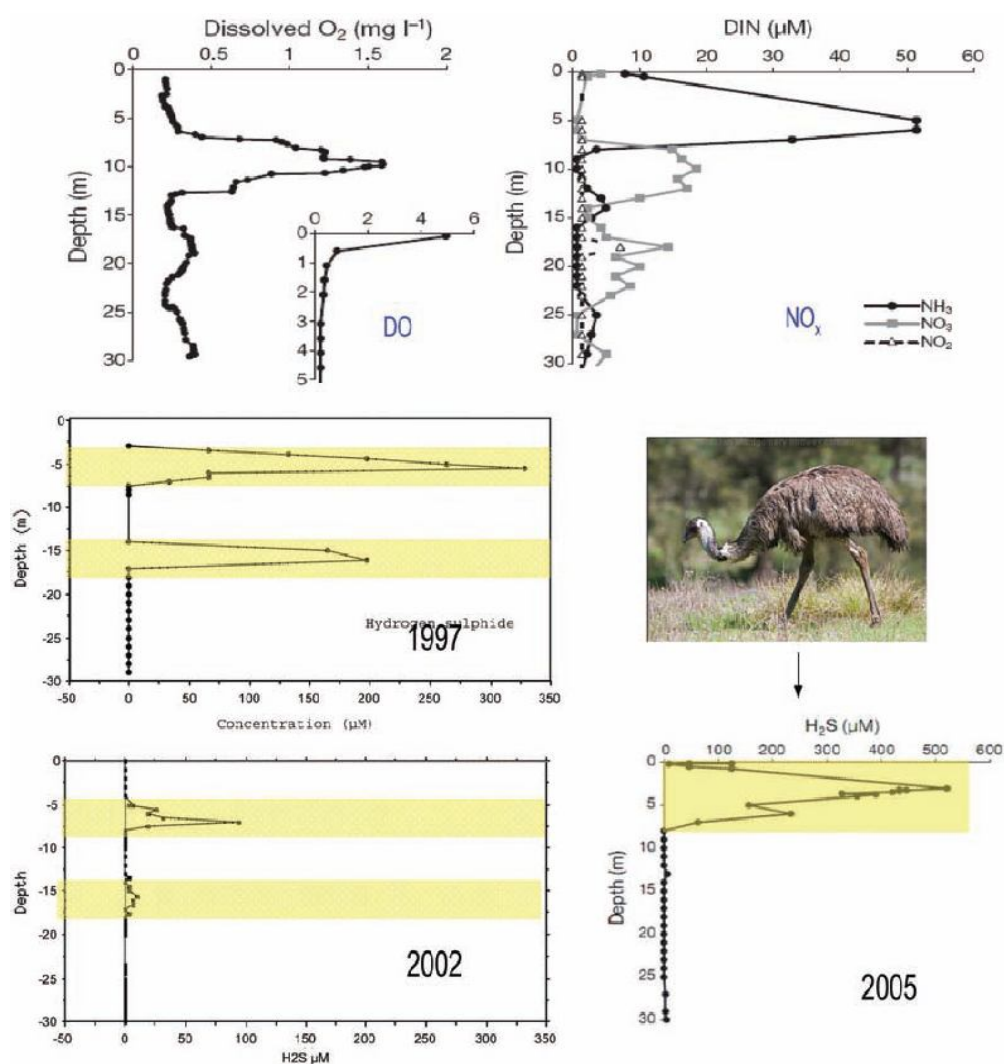


Fig. 1. Depth profile (Y m) in Bundera sinkhole of Upper: dissolved oxygen and dissolved inorganic nitrogen species from 2005 (from SEYMOUR *et al.*, 2007: Aquatic Microbial Ecology 50: 11-24). Lower rows: H₂S profiles for 1997, 2002 and 2005. An emu (*Dromaius novaehollandiae*) had died in the sinkhole 16 days prior to sampling in 2005.

yielded 50358 RNA reads from three Domains. There were 40625 Archaea and Bacteria reads, which included 34 Classes and 23 Phyla. Initial characterisation of the microbial assemblage shows that in broad composition it reflects the hydrogeochemical structure of the system.

About 65% of the Classes are Thaumarchaeota or chemolithotrophic or sulphur Eubacteria. Deltaproteobacteria were the most common SO_x reducers along with extremophile clades such as Deinococci. Thaumarchaeota are chemolithotrophic ammonia-oxidisers which were more abundant below 15 m depth, while Bacteroidia, many of which are halophilic anaerobes, increased with depth (Fig. 2). The proportion of Gammaproteobacteria, some of which oxidize H₂S, was highest at intermediate depths.

A plot of depth versus Class showed a strongly structured microbial ecosystem as the components of which also changed with depth and consequently with hydrogeochemical environment. The samples from 0.5m, in the oxic layer, had a high

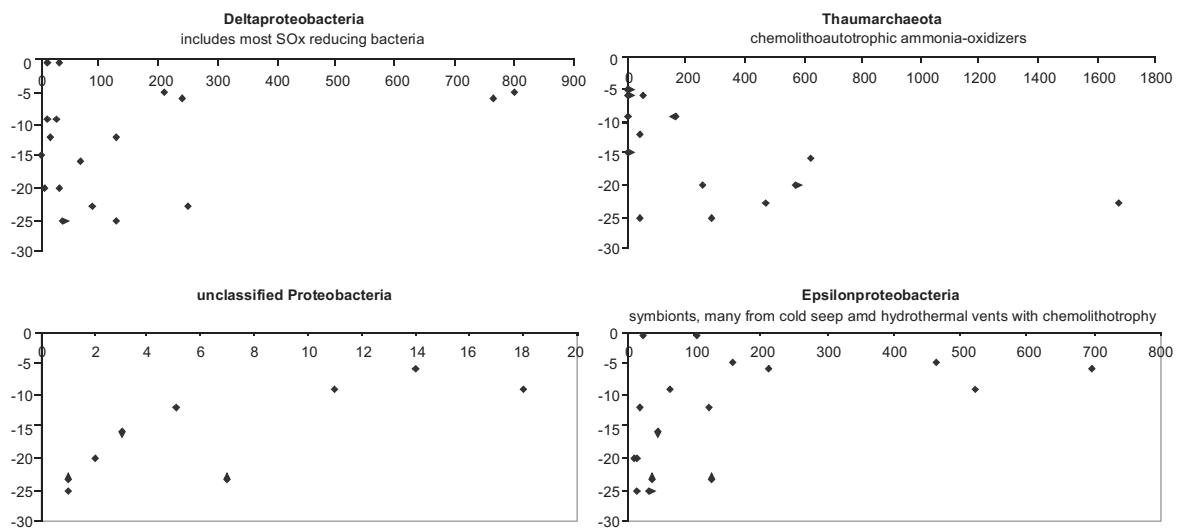


Fig. 2. Examples of depth profiles (Y m) in Bundera sinkhole of number of 16S rRNA reads predicted to be: top left) Deltaproteobacteria, includes most SO_x reducing bacteria; top right) Thaumarchaeota, chemolithotrophic ammonia-oxidisers; lower left) unclassified Proteobacteria; and lower right) Epsilonbacteria, many symbionts and in chemolithotrophic in cold seeps and hot vents.

proportion of eukaryotic 18S reads in the amplicon library which is surprising as the primers used are purportedly poor for eukaryotes. Later, we will use QIIME (Caporaso *et al.*, 2010) to more deeply analyse these samples.

DISCUSSION

While it is widely accepted that microbial assemblages are selected by the environment, the microbial assemblage itself is in many respects structuring the environment, with both factors contributing to the spatial distribution of microorganisms. Many studies dispute the idea that 'everything is everywhere' and indicate that broad biogeographic patterns could be maintained in the microbial world, the product of historical events (MARTINY *et al.*, 2006). In this regard, is the microbial assemblage reported here specific to Bundera or simply one example of a ubiquitous assemblage characteristically found in hydrogeochemically stratified communities, hypotheses that remain intractable owing, *inter alia*, to the problem of appropriate replication (FIERER, 2008). Alternatively, does the Bundera microbial assemblage represent a widely vicariant occurrence of a microbial assemblage specific to anchialine ecosystems that are elsewhere found on either side of the North Atlantic – the invertebrate assemblage in Bundera is widely separated from similar occurrences located on either side of the North Atlantic, elements of which diverged from each other in the Cretaceous or earlier (VON RINTELEN *et al.*, 2012).

Fuller analysis of the data outlined in this paper will enable us to report from where else members of the Bundera microbial assemblage have been identified. Similar assemblages imply similar biogeochemistries, so better studied systems where these microbes also occur could be used as analogues to understand how organic matter and dissolved nutrients are cycled in anchialine systems. Further, as many of these analogous habitats are difficult to access, the anchialine habitat may serve as living laboratories for habitats that are difficult to access, such as the deep sea.

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