Life along the halocline: microbial diversity in the Bundera sinkhole, an anchialine system in the Indian Ocean

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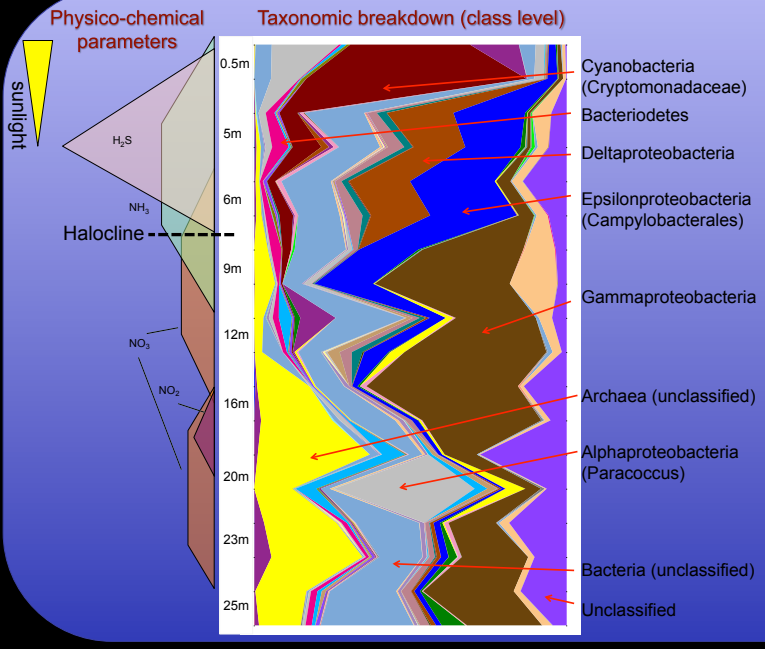
Background

Bundera sinkhole represents Australia's only deep anchialine environment: an inland freshwater pool connected to the ocean via a lengthy subterranean cave. Typical of anchialine systems, the Bundera sinkhole is characterized by a strong thermo-halocline, with hypoxic regions and layers of hydrogen sulphide. Such habitats generally contain a rich diversity of micro- and macrooganisms, many unique to anchialine environments, yet few studies have examined the microbial component of such systems in depth. The highly stratified water column within Bundera sinkhole was previously sampled at multiple depths down to 25 m to collect biological material and measure chemical and physical parameters (Seymour *et. al.* 2007).



Microbial diversity survey

•Samples from different depths contained distinct, structured microbial assemblages, reflecting the local hydrogeochemical environment.

•The surface layer was dominated by photosynthetic bacteria, however abundance tapers off by 5-6 m, fitting divers observations of low light levels below ~2 m.

•Deltaproteobacteria and Epsilonproteobacteria were predominant below the surface layer, including representatives from numerous sulfate-reducing genera including *Desulfobulbus*, *Desulfovibrio*, *Sulfurimonas* and *Sulfurovum*. This corresponds to the location of a strong H_2S layer between 1-7 m (concentrations up to 520 µM).

•Uncharacterized Gammaproteobacteria were abundant at intermediate depths. Divers reported observing filaments attached to sediments and cave walls tentatively identified as *Beggiatoa* and *Thiothrix* species.

•Archaeal abundance increased with depth.

•A large population of *Paracoccus* sp. (biochemically diverse genus, includes nitrate reducers) was observed in a single sample at 20 m.

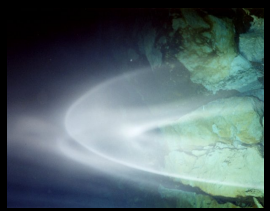
•Metagenome sequencing is planned to gain insight into differences in abundance of key functional genes at different depths.

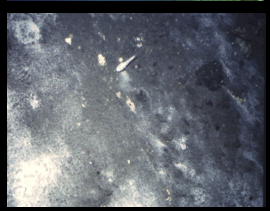
Methods

•In 2005 a physico-chemical profile (temp, salinity, dissolved O_2) and series of water samples for microbial and chemical (H_2S, DIN) analysis were collected from Bundera sinkhole at 1 m resolution down to 30 m (Seymour *et. al.* 2007). Samples for microbial analysis were collected on 0.4 um filters on FTA cards (Thacker, 2000) and maintained at -70°C.

•Punch samples of duplicate filters from 12 depths (from 0.5- 25 m) were selected for 16S amplicon pyrosequencing. For each sample PCR products were generated in triplicate (subsequently pooled) reactions using barcoded primers F515 and R816, which amplify a ~250 bp region of the 16S ribosomal RNA gene and provide broad taxonomic coverage for Archaea and Bacteria (Walters *et. al.* 2011). Pyrosequencing was performed using a 454 GS FLX instrument.

•Resulting amplicon sequences were quality filtered and subjected to phylogenetic analysis using the QIIME pipeline (Caporaso *et. al.* 2010). Reads were binned into OTUs at 97% sequence similarity using uclust. Taxonomy was assigned using BLAST with a maximum *e*-value of 0.001 against the Greengenes database.





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