

# Dallol: A unique study of life under multiple co-occurring physiochemical extremes

Vincent Rennie (1), Clare J. Warren (1) and Barbara Cavalazzi (2), Karen Olsson-Francis (1)

(1) School of Environment, Earth and Ecosystem Sciences, The Open University, Milton Keynes, MK7 6AA, UK (vincent.rennie@open.ac.uk). (2) Department of Biological, Geological and Environmental Sciences BiGeA, University of Bologna, Bologna, Italy.

### Abstract

While studies have been conducted on microbial communities inhabiting extreme environments, few studies have focused on sites with multiple physiochemical extremes. Dallol is a unique location for the study of the limits of life at the triple juncture of high temperature, low pH, and hypersaline extremes. This project aims to characterize the phylogeny and physiological adaptations of microbes inhabiting the Dallol hydrothermal system. Samples were collected from two outflows located in the main Dallol outcrop. Microscopy results from samples are inconclusive, with some very cell-like morphologies. Both anaerobic and aerobic enrichments have been carried out for the isolation of novel microorganisms. DNA has been successfully extracted and the amplified 16S rRNA gene will be sequenced.

#### **1. Introduction**

Studying environments exhibiting physiochemical extremes is key to understanding the limits of life. While studies have investigated physiochemical extremes in isolation, few have examined the effect of multiple co-occuring physiochemical extremes on microbial diversity [1]. Dallol represents a unique site to study the limits of life because it exhibits three physiochemical extremes simultaneously: low pH, high temperature, and salinity.

## 2. Geological Context

The main outcrop is located above a salt succession that is at least 900 meters thick [2]. Local fluids spring out at the surface (Figure 1,2) have mixed magmatic and meteoric origins [3]. As the water is heated, it passes through halite-dominated lithology that characterizes the surrounding area [2], becoming supersaturated in salts such as NaCl and MgCl<sub>2</sub>.



Figure 1: (a) Location 1 (L1) sampling site overview; (b) outflow source; (c) L1S4 site on Day 1 (D1)



Figure 2: An overview of the Location 2 (L2) sampling site on the main outcrop

The field pH meter could not measure pH values below zero, so pH was re-measured in the laboratory upon return, as was water activity. The temperature was measured in the field. Environmental parameters were measured for both sample locations (Table 1).

Sample	pН	Temp	DO (mmHg)	$A_{w}$
ID			(IIIIIIAg)	
Location 1				
L1S1D1	-0.30	68°C	51	0.714
L1S1D2	-0.33	-	-	0.710
L1S2	-0.34	44°C	30.5	0.710
L1S3D1	-0.34	40°C	200	0.709
L1S3D2	-0.37	-	-	0.708
L1S4	-0.40	35°C	150	0.710
Location 2				
L2 drip	-0.37	80°C	-	0.712
L2S1	-0.38	31.7°C	76.4	0.710
L2S2	-0.42	31.0°C	70.3	0.707
L2S3	-0.43	33.3°C	66.6	0.707

ICP-OES results indicate a NaCl-dominated, kosmotrophic environment with molar salt concentrations ( $\pm 10\%$  w/v).

#### 3. Cultivation

Given the inconsistent dissolved oxygen readings in the field, aerobic and anaerobic enrichments were set up parallel by adding yeast extract. After several weeks, the aerobic cultures began to differentiate with regards to colour and opacity. The differentiated cultures were subcultured into pH-adjusted media. the original sample material as well as subcultures was visualized (Figure 3).



Figure 3: Summary of the morphologies observed in original sample (a) and subcultures (b,c). White scale bar represents 100 nm

#### 6. Summary and Conclusions

The geochemical analyses carried out thus far confirm that the Dallol environment exhibits a triple

junction of extremes (pH <0, temperature  $\geq$  30°C, salinity  $\geq$  10%). Further geochemical characterization is planned to characterize the mineralogical composition of the salt crusts in order to determine its effect on the fluid composition.

While microscope analysis of the original samples from Dallol provided evidence for structures that were an order of magnitude larger than prokaryotic cells, these may be microbially-mediated, formed to create a more habitable microclimate under such extreme conditions. Future work will investigate whether these structures were formed under microbial influence. Microscope analyses of the subcultures showed cell-like structures between 1 and 15  $\mu$ m in diameter. However, given the lack of cell motility it is possible that these cells are not actively metabolizing. Sequencing of DNA extracted from the samples will determine which microbes are present in the samples collected.

Given the visualization of biotic and cell-like structures at various scales, it seems likely that there is microbial matter in these samples. This is increased evidence that life may be able to survive at the triple juncture of high temperature, low pH, and hypersaline conditions as seen in Dallol.

#### Acknowledgements

The laboratory work has been supported under the CENTA-DTP studentship. The fieldwork to Dallol was funded by Europlanet. Europlanet 2020 RI has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654208

#### References

[1] Harrison, J. P., Gheeraert, N., Tsigelnitskiy, D., & Cockell, C. S. The limits for life under multiple extremes. Trends in Microbiology, 21(4), pp. 204–212, 2013.

[2] Franzson, H., Helgadóttir, H. M., & Óskarsson, F. Surface Exploration and First Conceptual Model of the Dallol Geothermal Area, Northern. Proceedings World Geothermal Congress 19-24 April 2015, Melbourne, Australia, 2015.

[3] Darrah, T. H., Tedesco, D., Tassi, F., Vaselli, O., Cuoco, E., & Poreda, R. J. Gas chemistry of the Dallol region of the Danakil Depression in the Afar region of the northernmost East African Rift. Chemical Geology, 339, pp. 16–29, 2013.