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NAI/APS Lewis and Clark Fund for Exploration and Field Research in Astrobiology

Influence of high hydrostatic pressure on microbial communities from the Puerto Rico Trench

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Project Report

The focus of this proposal was to expand our current knowledge of psychropiezophilic ('cold- and pressure-loving') microorganisms through investigating the diversity of bacteria and archaea in the Puerto Rico Trench. Understanding high-pressure influences on microbial communities could lead to improved insights into the origin and evolution of life on Earth, as well as inform future exploration of life in the high-pressure ocean of Jupiter's moon Europa.

The NAI/APS travel grant funded a field expedition in October 2008 to the Puerto Rico Trench to collect seawater from a depth of 6,000 m (19,685 ft) for cultivation efforts and a variety of molecular analyses. In collaboration with the Bermuda Institute of Ocean Sciences (BIOS) and the Bermuda Atlantic Time Series (BATS), we successfully collected hadal seawater from the Puerto Rico Trench aboard the R/V Atlantic Explorer (**Fig. 1**). 288 l hadal seawater was collected using a CTD rosette (**Fig. 2**) with 24, 12 l niskin bottles. A suite of chemical measurements was taken for contextual data, as well as prokaryotic and viral counts (**Table 1**). 210 l was collected onto 142mm, 0.2μm polyethersulfone impact filters after a pre-filtration through a 3μm membrane to remove particle-attached microorganisms. Both 3μm and 0.2μm filters were stored in lysis solution at -80°C for community DNA extraction. Additionally, four 15 ml seawater samples were collected into polyethylene transfer pipets, heat-sealed, and pressurized to *in situ* pressure (60 MPa = 8,702 psi) for cultivation efforts.

Upon return to Scripps Institution of Oceanography, DNA was successfully extracted from both the 3μm and 0.2μm filters in collaboration with Dr. Shannon Williamson at the J. Craig Venter Institute. We are currently in the process of constructing large (384-well plates each) 16S rDNA bacterial and archaeal libraries from both the 3μm and 0.2μm

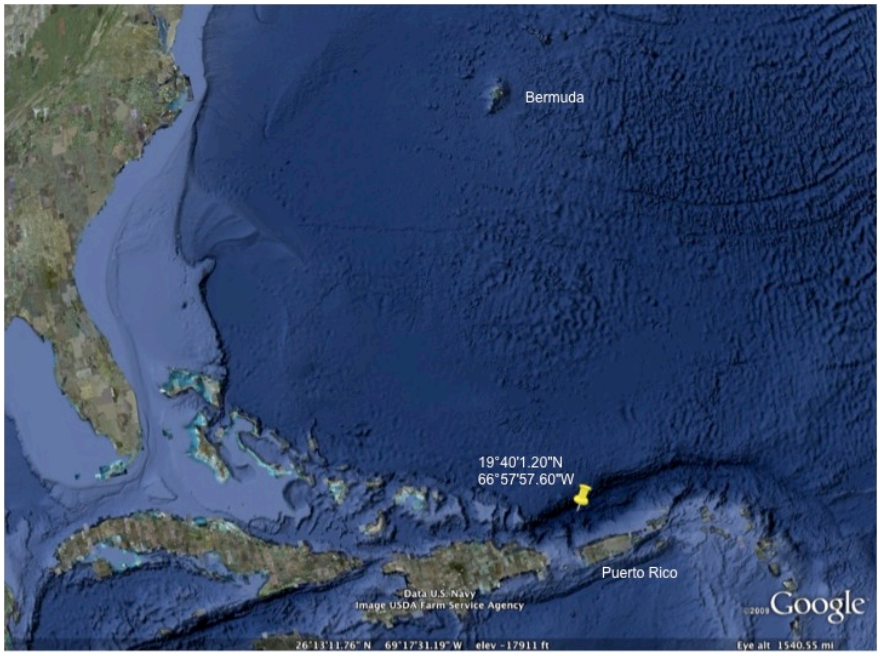
filters, as well as an 18S rDNA eukaryotic library from the 3μm filter. This will provide the first comprehensive bacterial and archaeal 16S rDNA libraries from a hadal environment. Additionally, we will have the opportunity to investigate the differences in microbial community composition between the particle-attached and free-living samples. We hope to gain insight into differentiating the authentic autochthonous microbial community from surface-derived microorganisms.

DNA extracted from the 0.2μm filter will also be used to construct a large-insert (~40 Kbp) environmental genomic library. This large-insert library will contain invaluable sequence data from the diverse microbial community in the Puerto Rico trench, allowing for detailed comparisons with surface seawater environmental genomic libraries. We hope to elucidate the evolutionary modalities and genomic constraints imposed by the unique physiochemical parameters in a hadal environment. The construction of a hadal environmental genomic library will be the first of its kind.

Lastly, the four 15 ml seawater samples collected for cultivation efforts were successfully transferred from Puerto Rico to San Diego on ice, under *in situ* pressure. A wide array of enrichment conditions has been established with these samples in an effort to isolate novel psychropiezophilic microorganisms. These enrichments are long-term endeavors since I am specifically targeting oligotrophic psychropiezophiles with low metabolic activity and slow growth. The successful isolation of diverse bacteria and archaea will be a resource for characterizing the physiology and adaptive properties that drive psychropiezophilic evolution.

The NAI/APS travel grant provided necessary funds to allow for the collection of these precious deep ocean samples from the Puerto Rico Trench. The funding supported equipment shipping, travel expenses, and supply costs. In particular, shipping costs for our high-pressure equipment and pressure vessels were covered with the generous award funding. I am truly grateful for the financial support through the Lewis and Clark Fund for Exploration and Field Research in Astrobiology.

(a)



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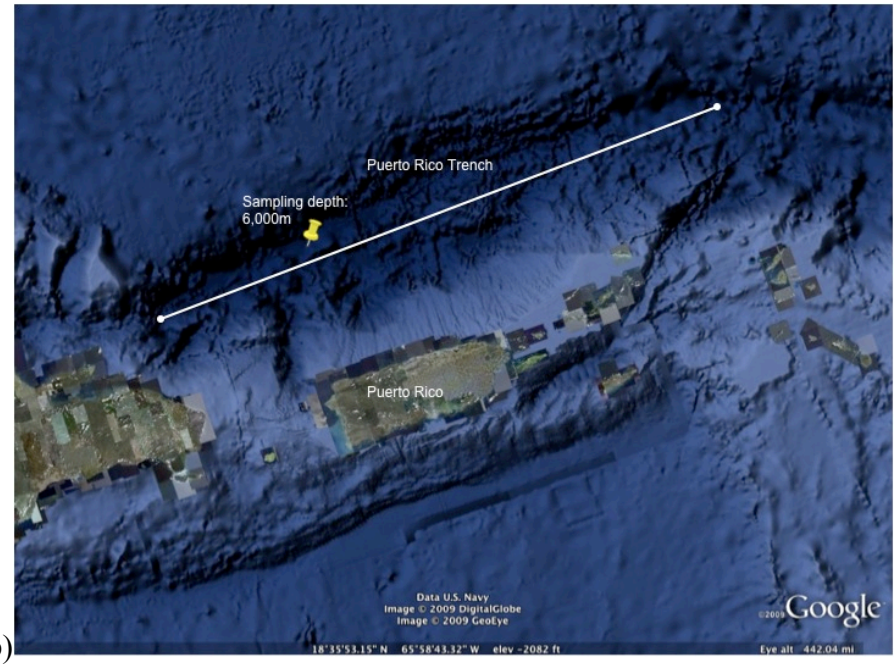

Figure 1. Sampling site. (a) Transect from Bermuda to Puerto Rico aboard R/V Atlantic Explorer (Bermuda Atlantic Time Series). (b) Hadal seawater was collected from 6,000m (19°40'1.20"N 66°57'57.60"W) within the Puerto Rico Trench.

Figure 2. CTD rosette aboard the R/V Atlantic Explorer used for collection.

Table 1. Chemical measurements for surface (5m) and hadal samples (6,000m).

Depth (m)	N+N (µmol/L)	PO_4 (µmol/L)	Silicate (µmol/L)	NO_2 (µmol/L)	NH_4 (µmol/L)	NO_3 (µmol/L)	O_2 (mL/L)	Salinity
5	0.03 ± 0.026	0.013 ± 0.0058	1.67 ± 0.058	0	0.08 ± 0.02	0.03 ± 0.026		
6,000	24.18 ± 0.70	1.67 ± 0.01	63.77 ± 0.25	0	0.043 ± 0.0058	24.18 ± 0.70	5.67 ± 0.0057	34.84 ± 0.0093

Depth (m)	Average Viruses (particles mL^{-1})	Average Prokaryotes (cells mL^{-1})
5	3.92×10^6	3.44×10^5
6,000	4.50×10^5	1.09×10^4