Marine bacterioplankton succession and subsequent overturn within seasonally hypoxic waters of a subtropical sound; Devil's Hole, Bermuda.

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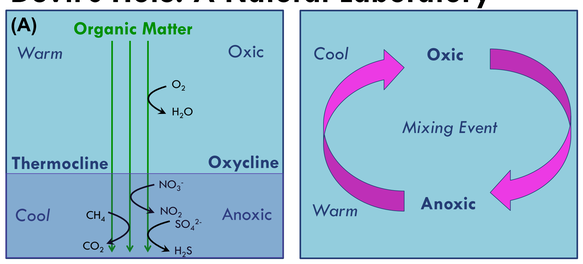
Background: Oxygen Minimum Zones

- Hypoxic marine environments are expanding due to increased sea surface temperatures, subsequent increased oxygen demand, reduced oxygen solubility and thermal stratification driven by anthropogenic climate change.
- These hypoxic marine environments are inhospitable to metazoan life, but support a diversity of microaerophilic and facultative anaerobic bacterial and archean life that perform cryptic metabolisms essential to the C, N, and S cycles.
- Here, we examined the dynamics of bacterioplankton communities endemic to the annually occurring oxygen minimum zone (OMZ) in Devil's Hole, Bermuda to understand the biogeochemical significance of OMZs.

Devil's Hole: A Natural Laboratory



(B)

N

Ammonia Oxidation:
$NH_3 \rightarrow NO_2^-$
$NO_2^- \rightarrow NO_3^-$
Marker Gene:
amoA
Lineage:
Thaumarchaeota

S

Sulfate Reduction:
$SO_4^{2-} \rightarrow H_2S$
Marker Gene:
aprA
Lineages:
SAR11
SAR324

C

Carbon Fixation:
$CO_2 \rightarrow CH_2O$
Marker Gene:
cbbL
Lineages:
Synechococcus
SAR324
Chlorobi
Methane Oxidation:
$CH_4 \rightarrow CO_2$
Marker Gene:
mrcA
Lineages:
Euryarchaeota

(C)

Favorability of Terminal Electron Acceptors
1. $O_2 \rightarrow H_2O$
2. $NO_3^- \rightarrow NO_2^-$
3. $Mn(IV) \rightarrow Mn(III)$
4. $Fe(III) \rightarrow Fe(II)$
5. $SO_4^{2-} \rightarrow H_2S$
6. $CO_2 \rightarrow CH_4$

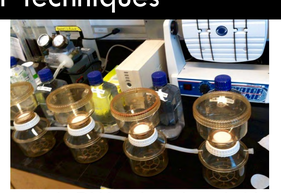
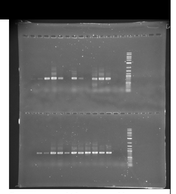
Fig. 1. *(A) A simplified diagram of summer stratification and fall turnover in Devil's Hole and the consequence of oxygen limitation on microbial metabolisms.* ***(B)*** *Table showing the specific microbial lineages and marker genes for nitrogen, sulfur, and carbon cycling.* ***(C)*** *Table of terminal electron acceptors in order of energy provided, adapted from Wright et al., 2012.*

Methods

Geochemical Techniques

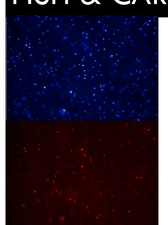
1. Salinity, dissolved oxygen (DO), temperature, and pH via YSI.
2. DO measured via Winkler titration.
3. pCO_2 and pH calculated at *in situ* temperature and salinity concentrations.

Molecular Techniques

1. Extracted genomic DNA using phenol chloroform with sodium acetate and Isopropanol DNA precipitation.
2. Quantified DNA with Qubit fluorescence and Nanodrop spectrophotometer.
3. Amplified genes of interest using PCR and measured band intensity of gel electrophoresis to calculate relative abundance.

FISH & CARD-FISH



Img. 1 *CARD-FISH SAR 11 from 08/25/16 0m.*

Probe Name	DNA Sequence 5' to 3'	Target
AC137R-Cy3 [1]	TGTTATCCCCCTCGCAAA	*Alteranomas* (genus)
Chlorobo441-Cy3 [2]	AAAACAGGATTCTTCCTCTAC	*Chlorobium* (genus)
Eury-806-HRP [2]	**CACAGCGTTTAACACCTAG**	***Euryarchaeota* (phylum)**
536R-Cy3 [1]	CAACGTAACCCCCTCCG	*Rhodobacteraceae* (family)
441-HRP [4]	**TACAGTCATTTTCTTCCCCGAC**	***SAR11* (order)**
103R-Cy3 [5]	GTTACTCAGCCGTCTGCC	*SAR202*
311R-Cy3 [3]	TGTCTCAGTCCCCCTCTG	(within *Chloroflexi* phylum)
1412-HRP [6]	**GCCCCTGTCAACTCCCAT**	***SAR324* (within *δ-proteo* class)**
Cren-537-HRP [3]	**TGACCACTTGAGGTGCTG**	***Thaumarchaeota* (phylum)**
338-Cy3 [3]	TGAGGATCCTCCGTCCGTCG	Non-specific
NON-HRP [3]	**ACTCCTACGGGAGGCAGC**	**Non-specific**

1. 2-3 ml aliquot of 10% paraformaldehyde water sample filtered through a 0.2 µm polycarbonate filter.
2. Tagged microbial clades of interest with specific probes using FISH (fluorescent in-situ hybridization) and CARD-FISH (catalyzed-reporter deposition FISH).

Biogeochemical Impacts of the Microbial Community

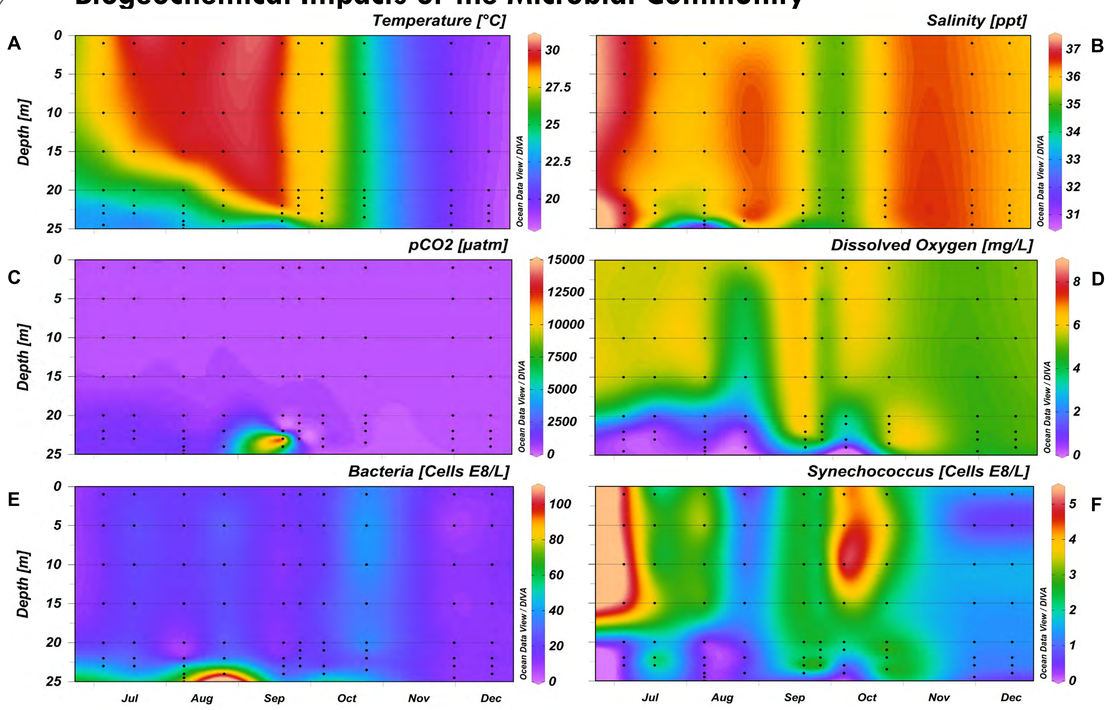


Fig. 2. *Contour plots over depth (m) on the y-axis and calendar date on the x-axis:* ***(A)*** *Temperature (°C),* ***(B)*** *Salinity (ppt),* ***(C)*** *Partial pressure of carbon dioxide (atm),* ***(D)*** *Dissolved oxygen (mg L-1),* ***(E)*** *Bacteria (cells x 10^8 L-1),* ***(F)*** *Cyanobacteria (cells x 10^8 L-1). Tropical storm Karl occurred on September 24, resulting in partial turnover; and Hurricane Nicole occurred on October 13, giving rise to complete turnover of the water column.*

Niche Development in the Microbial Community

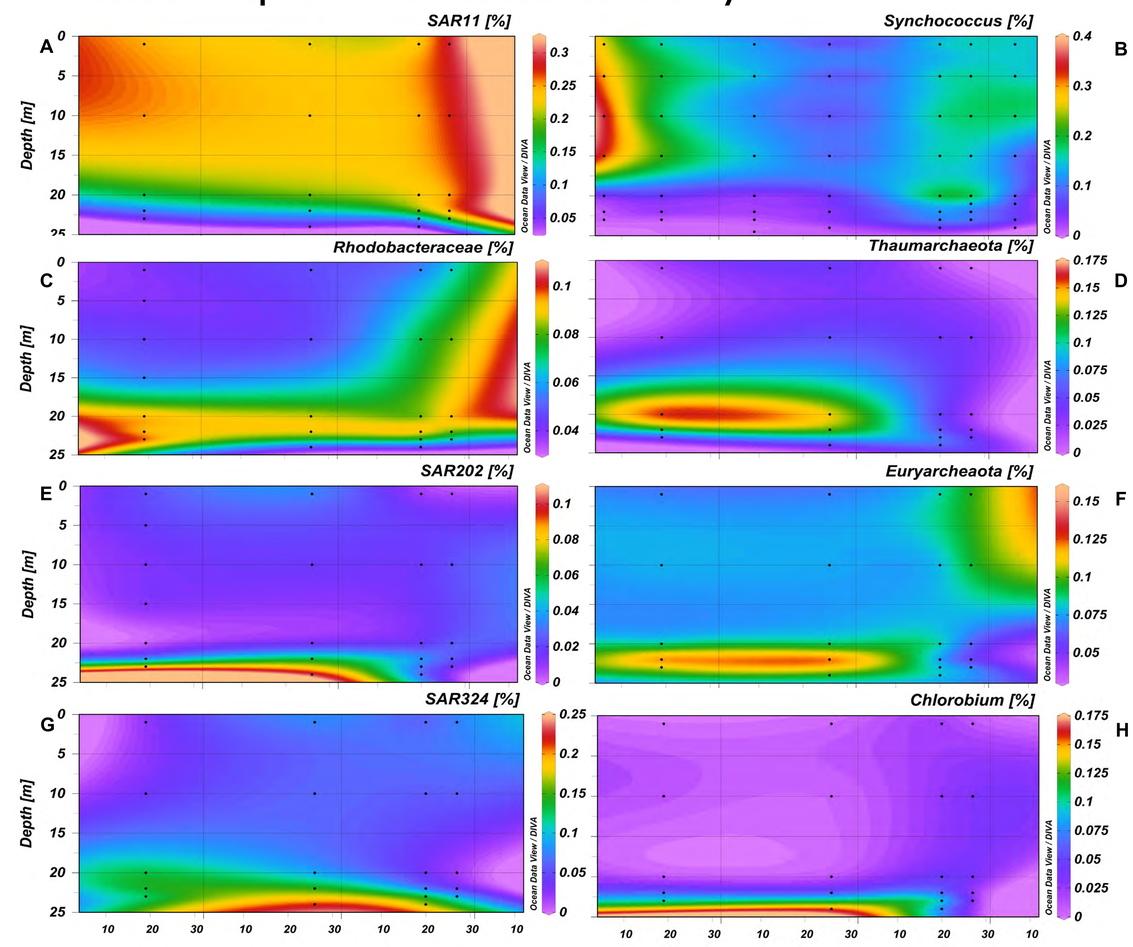


Fig. 3. *Contour plots over depth (m) on the y-axis and calendar date on the x-axis. Surface communities include* ***(A)*** *SAR11 (% abundance) and* ***(B)*** *Synechococcus (% abundance). Below, mid-layer communities at the oxycline include* ***(C)*** *Rhodobacteraceae (% abundance) and* ***(D)*** *Thaumarchaeota (% abundance). Lastly,* ***(E)*** *SAR202 (% abundance),* ***(F)*** *Euryarcheaota (% abundance),* ***(G)*** *SAR324 (% abundance), and* ***(H)*** *Chlorobium (% abundance) constitute the microbial community at depth.*

- The microbial community reaches peak abundance slightly before peak pCO_2, suggesting that the carbon dioxide build-up at depth is the result of microbial respiration (Fig. 2D, E).
- *Rhodobacteraceae, Euryarchaeota,* and *Thaumarchaeota* are abundant in the transitional zone (20-22m) (Fig. 3C, D, F).
- SAR202, SAR324 and *Chlorobium* are more abundant within the hypoxic zone (23 - 24m).
- When oxic conditions return after the partial and full turnover, the at-depth lineages, with the exception of *Rhodobacteracae*, dissipate, and the surface lineages (*SAR11* and *Synechochoccus*) dominate the water column.

Lineages & Gene Depth Profiles

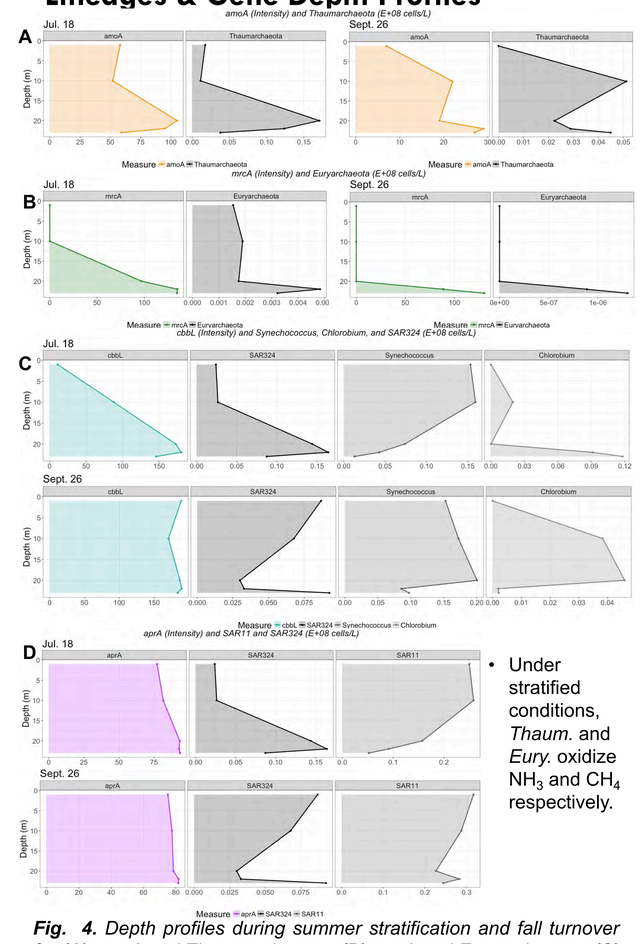


Fig. 4. *Depth profiles during summer stratification and fall turnover for* ***(A)*** *amoA and Thaumarchaeota,* ***(B)*** *mrcA and Euryarchaeota,* ***(C)*** *cbbL, SAR324, Synechococcus, and Chlorobium,* ***(D)*** *aprA, SAR11, and SAR324. amoA oxidizes NH_3, aprA reduces SO_4^{2-}, mrcA oxidizes CH_4, and cbbL fixes CO_2.*

- Under stratified conditions, *Thaum.* and *Eury.* oxidize NH_3 and CH_4 respectively.

Environmental Drivers

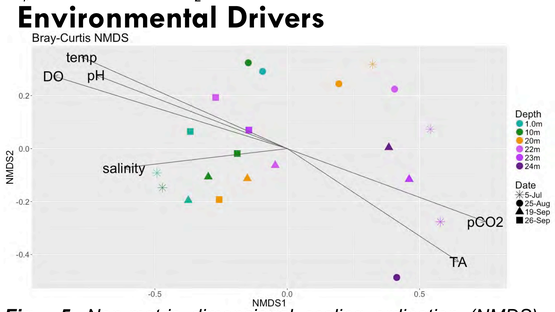


Fig. 5. *Non-metric dimensional scaling ordination (NMDS) with Bray-Curtis similarity between depths 0, 10, 20, 22, 23 and 24m, with environmental drivers including salinity, dissolved oxygen, pH, temperature, and carbon dioxide.*

- **CO_2 appears to play a strong role in structuring communities at depth, whereas turnover conditions, e.g. freshwater intrusion and DO, drive surface and turnover communities.**

Discussion

The suboxic bottom waters formed in late August, and transitioned to a mixed, ventilated profile in mid-September. Microbial biomass also peaked at depth under hypoxic conditions (1.03×10^{10} cells mL^{-1}). During stratification two ubiquitous marine bacterial clades, mixotrophic *SAR11* (≤22.97%) and oxygenic phototrophic *Synechoccous* (≤22.25%), dominated the upper oxygenated 20m of the water column (Fig.3). At the 20m oxycline, ammonia-oxidizing *Thaumarchaeota* (17.09%) dominated the water column, and we detected *amoA*, which mediated ammonia oxidation. In the anoxic photic zone, *Euryarchaeota* (≤16.38%), *SAR324* (≤15.23%) and *Chlorobium* (≤16.92%), dominated at 22m and 23-24m respectively. Under limiting oxygen availability, the microbial community exploited the changing redox potential of the water column. ***Future investigations into the microbial metabolic consortia between Desulfobacterales, Chlorobium, and Euryarchaeota will elucidate how multiple metabolic pathways work together to exploit the redox gradient under anoxic conditions.***

Acknowledgements

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