

# Characterizing the microbial community structure in the methane maxima in the Gulf of Mexico



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## Oceanic methane paradox

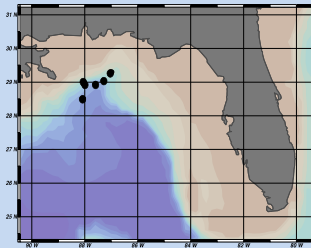
### Background

- Methane is a potent greenhouse gas, responsible for upwards of 20% of the Earth's warming in recent years.
- Understanding the source of methane in the marine environment is important given that the ocean accounts for up to 4% of global methane emissions (Karl et al 2008).
- In the Arctic Ocean, methane flux began increasing in 2007-through the present (Dlugokencky et al 2009).
- Methane production by microorganisms is considered an anaerobic process that is thermodynamically and kinetically not plausible in the aerobic open-ocean water column, given the near super saturating concentrations of sulfate.
- Its presence at supersaturated concentrations relative to the atmosphere in the global, aerobic ocean is termed the 'oceanic methane paradox.'

### Methane Source

- In the photic zone, methane is potentially produced as the result of microbial consumption of methylphosphonate (MPn), which is used when inorganic P is limiting.
- In microcosm experiments, methane was produced with the addition of MPn in light incubations, however, in dark oxic and dark anoxic water conditions, methane production is reduced 5 and 7-20 fold respectively (Finke et al 2011). These results suggest photosynthesis plays a role in methane production.
- Weller et al (2013) noted that within the upper 100m of an eddy, the abundance of *Synechococcus* sp. increased with depletion of  $^{13}C$  and an increase of methane concentration within the eddy.

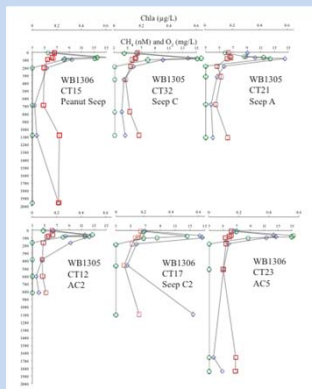
## Sampling



- Six open-ocean sites were sampled using a CTD rosette during Deep-C cruises (September and October 2012).
- Samples were preserved for geochemical and molecular analyses.
- Microbial samples were pre-filtered through a 2.7- $\mu$ m filter, then collected on a 0.2- $\mu$ m sterile filter.
- Microbial DNA and RNA were extracted from these filters.
- iTag sequencing of 16S rRNA genes was carried out using Illumina's MiSeq platform.

## Results

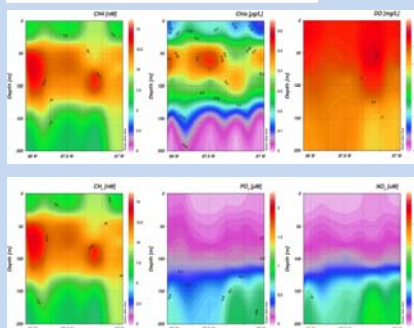
### Nutrient profiles



Methane (blue diamonds) was detected at higher concentrations near the seafloor when sampling at seep sites, decreased towards the surface, and reached a maximum in the photic zone (~60 m).

Oxygen (red squares) showed typical water column profiles.

Chlorophyll a (green circles) concentrations reached a maximum at the "deep chlorophyll max" which was coincident with the methane maxima.

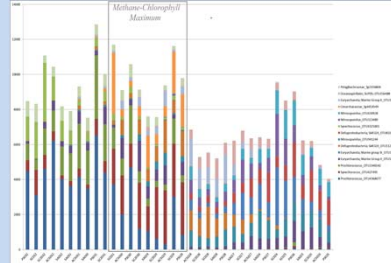


No correlation between methane and oxygen concentrations was observed.

Chlorophyll a concentrations were highly correlated with methane ( $R = 0.80$ ).

Phosphate and nitrate were inversely correlated with methane ( $R = -0.70$  and  $-0.68$ , respectively).

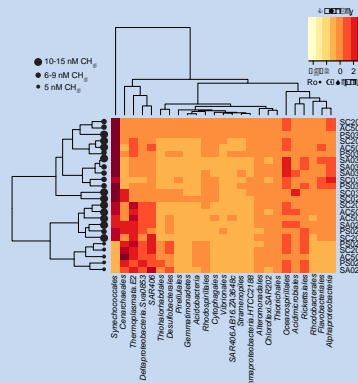
### Microbial community structure revealed by high-throughput, next-generation sequencing of 16S rRNA genes



iTag sequencing of 16S rRNA genes was used to characterize the microbial communities throughout the water column.

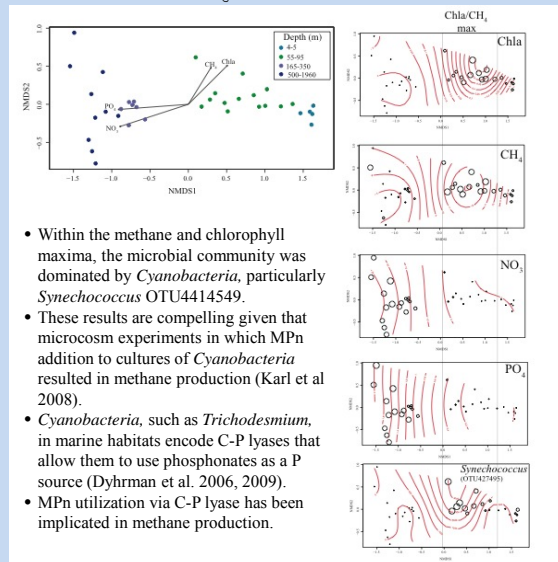
As expected, the microbial community varied with depth.

Of particular note was the increase in abundance of *Synechococcus*, which was highest at the methane and chlorophyll maxima.



The most abundant species in relationship to both methane and chlorophyll was the cyanobacterium *Synechococcus*, which we hypothesize may be involved in methane production.

Interestingly, no known methane oxidizers were found in the methane maxima, suggesting that methane would outgas to the atmosphere rather than be oxidized.



- Within the methane and chlorophyll maxima, the microbial community was dominated by *Cyanobacteria*, particularly *Synechococcus* OTU4414549.
- These results are compelling given that microcosm experiments in which MPn addition to cultures of *Cyanobacteria* resulted in methane production (Karl et al 2008).
- Cyanobacteria*, such as *Trichodesmium*, in marine habitats encode C-P lyases that allow them to use phosphonates as a P source (Dyhrman et al. 2006, 2009).
- MPn utilization via C-P lyase has been implicated in methane production.

## Conclusion & Future Work

- The increase in abundance of *Synechococcus* within the methane-chlorophyll maxima is compelling because its role in cycling MPn has been previously identified. For example, Ilikhyan et al (2009) reported that *Synechococcus* have genes encoding phosphonate utilization.
- Future work will be directed towards shotgun metagenomic and metatranscriptomic sequencing of samples collected in and near the methane-chlorophyll maxima.
- Additional sampling efforts will be carried out to analyze the relationship between photosynthetic processes and methane production by repeatedly sampling over a 24-h period.

## Acknowledgments

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