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Analysis of Microbial Communities Reflect Diel Vertical Migration in the Gulf of Mexico

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Graphical Abstract

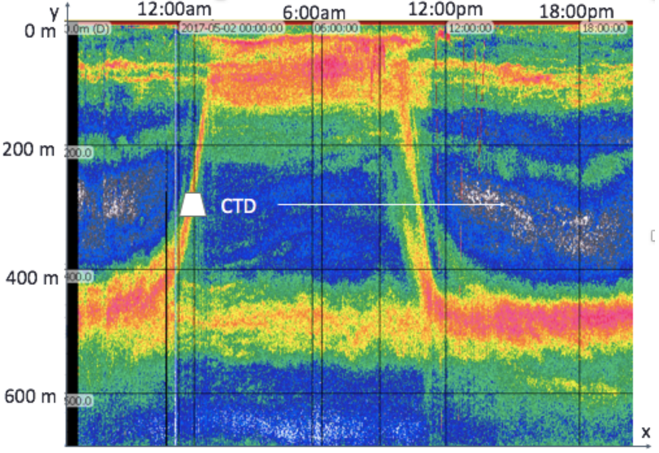

Figure 1. The figure shows the Vertical Migration occurring in real-time with the use of a multifrequency echosounder system that measures acoustic backscatter**.** Warmer colors represent higher densities of microzooplankton. However, we don't exactly know what all of these organisms are. The **Y-axis** is the depth, and the **X-axis** is time. (Image courtesy of Kevin Boswell, FIU)

Abstract: Marine Diel Vertical Migration (DVM), perhaps the largest movement of animals on Earth, is composed of mesopelagic species migrating vertically every night to feed in epipelagic depths and return to deeper water during the day. The objective of this study infers potential taxonomic identity of organisms in the DVM via their association with bacterioplankton signatures. High throughput sequencing of the 16S rRNA V4 region and the use of bioinformatics and statistics provided evidence to which bacteria appeared associated with either upward or downward vertical migration during two cycles. DNA was extracted from water samples and sequenced. Data analysis was performed using R Studio software. Our results confirm the vertical movement of bacterial taxa throughout the pelagic depths. The most abundant bacteria present during the Vertical Migration were of Genera Marinobacter, Alteromonas, Prochlorococcus, and class Gammaproteobacteria. These taxa occurred at depth of 320 meters which is the mesopelagic zone. Proteobacteria was only found during the vertical migration at mesopelagic depths, whereas Cyanobacteria was only found during the vertical migration at epipelagic depths. This indicates that these two phyla of bacteria are distinct to their respective zones.

Introduction

The earth's oceans hold vast amounts of water, with great depths and complex dynamics making them difficult to study. To better characterize the Gulf of Mexico, a relatively deep ocean basin, the DEEPEND consortium (www.deependconsortium.org) was formed. DEEPEND stands for Deep-Pelagic Nekton Dynamics. This consortium began after the Deepwater Horizon Oil Spill (DWHOS) in 2010. The BP/Deepwater Horizon (DWH) discharge in 2010 was the largest marine open water hydrocarbon discharge to date. The DWH well blowout at the seafloor discharged approximately 5 million barrels of oil and at least 250,000 metric tons of natural gas to the deep water (about 1,500m) of the Gulf of Mexico (Jove et al,).

Teams of DEEPEND scientists go on cruises to collect water and organismal samples and bring them back to the lab for molecular studies. The last cruise the team went on was in May, and they collected water samples for DNA extraction. However, this was not to check for oil contamination. This was to check another phenomenon of nature. This phenomenon is marine *Diel Vertical Migration* (DVM), perhaps the largest movement of animals on Earth. The Vertical Migration is composed of mesopelagic species migrating vertically every night to feed in epipelagic depths and return to deeper water during the day. The objective of this study infers potential taxonomic identity of animals in the DVM via their association with bacterioplankton signatures. High throughput sequencing of the 16S rRNA V4 region and the use of bioinformatics and statistics provided evidence to which bacteria appeared associated with either upward or downward vertical migration during two cycles.

Materials and Methods

Seawater samples were collected during a DEEPEND consortium research cruise in May 2017. Water samples were collected using a niskin bottle array from 0-326 meters depth. Realtime acoustic echosounder data was used to direct sample collection in order to capture seawater samples above, during, and below the vertically migrating organisms (Figure 1). Collected seawater was filtered through a 0.45um membrane on the ship, and preserved.

DNA sequencing and clustering

DNA was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (QIAGEN) PCR was used for amplification of the DNA targeting the V4 region of the 16S rRNA gene. An Illumina MiSeq sequencing platform using a V2 chemistry 500 cycle cartridge was used for sequencing. Initial processing of sequence data was performed in MacQIIME version 1.9.1("MacQIIME - Werner Lab," 2016). Raw sequences were quality filtered to remove all chimeric and low quality (quality score < 30) sequences. These sequences were then clustered into 97% similar Operational taxonomic units (OTUs) using a combination of open and closed reference OTU clustering strategies.

Data analysis

Data analysis was performed using the R Studio software (versions 3.3.2 and 3.4.0) with the use of the library "vegan".

Results and Discussion

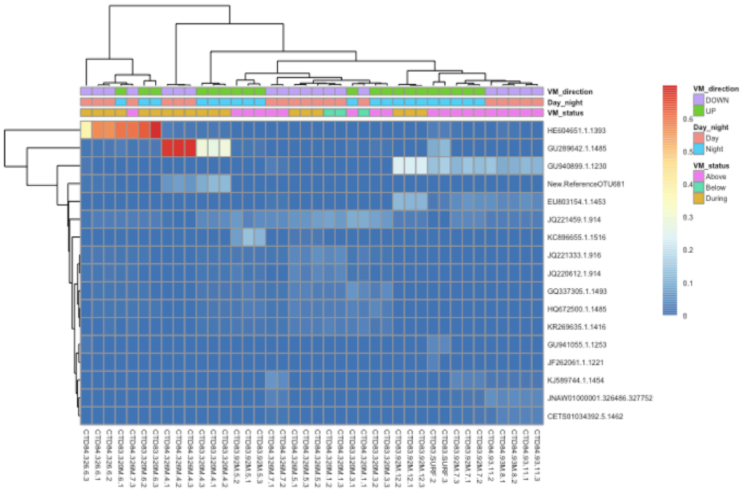

Figure 2. Heat map of abundant OTU's. Generated in R Studio version 3.4.0

The heat map above (Figure 2) shows the relative abundance of taxa with red indicating high and blue low relative abundance. The top three rows indicated vertical migration direction, time, and status, respectively. Two taxa at each depth (epipelagic and mesopelagic) were significantly more abundant in samples taken from within the vertically migrating layer. From the 40,872 different OTUs that were collected, we decided to observe in greater detail some of those OTUs that drove the change in our samples. The selected OTUs were those that were during the Vertical Migration and had greater abundance than the rest.

Table 1. Abundance chart and Taxonomy of OTU's. Generated in R Studio version 3.3.2.

Vertical Migration Status	Pelagic Zone	OTU ID	Taxonomy	Abundance	Depth
During	Epipelagic	GU940899.1.1230	Phylum - Cyanobacteria; Genus - Prochlorococcus	13.40%	92m
During	Epipelagic	EU803154.1.1453	Phylum - Cyanobacteria; Genus - Prochlorococcus	6.30%	92m
During	Mesopelagic	HE604651.1.1393	Phylum - Proteobacteria; Genus - Marinobacter	19.80%	320m
During	Mesopelagic	New.ReferenceOTU681	Phylum - Proteobacteria; Class - Gammaproteobacteria	2.90%	320m
During	Mesopelagic	GU289642.1.1485	Phylum - Proteobacteria; Genus - Alteromonas	14.80%	326m
Above	Mesopelagic	HE604651.1.1393	Phylum - Proteobacteria; Genus - Marinobacter	6.10%	326m

The abundance chart (Table 1) shows an abundance of Proteobacteria in the Mesopelagic zone, and an abundance of Cyanobacteria in the Epipelagic zone.

Conclusions

Diel vertical migration, perhaps the largest movement of animals on Earth is remarkable and well-organized. Every night both prokaryotes and eukaryotes come to mesopelagic and epipelagic depths to feed, and every morning they go back down to deeper depths. “The distinct diel vertical migration of scattering layers is believed to be due to changing ambient light conditions” (D’elia et al, 2016) as well as feeding and predation factors. The approach of extracting DNA from the water samples collected and analyzing the data into taxonomic categories of bacteria highlighted the vertical movement of these organisms throughout the pelagic depths.

We found that for prokaryotes there were a few distinct and abundant bacteria present during this phenomenon. The most abundant bacteria present during the Vertical Migration were OTU ID’s HE604651.1.1393, GU289642.1.1485, GU940899.1.1230, and New.ReferenceOTU681. The depth they were around was 320 to 326 meters, which is the mesopelagic zone. All of the bacteria that were distinct during the vertical migration and in the mesopelagic zone were proteobacteria. These were the most abundant bacteria in this pelagic zone. OTU HE604651.1.1393 was the most abundant bacterium that was found in the mesopelagic zone at 320 meters. Furthermore, the bacteria that were distinct during the vertical migration and in the epipelagic zone were cyanobacteria. These were the most abundant bacteria in this pelagic zone. OTU GU940899.1.1230 was the most abundant bacterium that was found in the epipelagic zone at 92 meters. With this information we can distinguish that these two phyla of bacteria are distinct to their pelagic zones. Proteobacteria is only found during the vertical migration at mesopelagic depths, whereas Cyanobacteria is only found during the vertical migration at epipelagic depths.

References

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- R package “picante”

Acknowledgements

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