Zooplankton community response to seasonality at BATS by metabarcoding

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Figure 1. Location of the BATS station.

METHODS

Zooplankton samples were taken during 2015 onboard the *RV Atlantic Explorer* during BATS cruises (Figure 1) using a 1-m² rectangular, 202 µm mesh net, during the day and night at each cruise. Samples were immediately fixed in 95% undenatured alcohol.

DNA was extracted from bulk samples following a modification of Corell and Rodriguez-Ezpeleta (2014). The 18S V9 region (Amaral-Zettler et al. 2009) was amplified and the product sequenced using a 2x200 bp Illumina MiSeq technology.

Bioinformatics were done in MOTHUR (Schloss et al. 2009), with 100% similarity threshold. All samples were standardized to 36,471 reads, and taxonomic units with global abundances < 2 removed from the table (global singletons).

A similarity matrix was built using Bray-Curtis index, and graphically represented using non-metric MultiDimensional Scaling (nMDS). Best non-hierarchical (kR) clustering level was established based on the rate of change of the R statistic between consecutive levels. A BEST analyses established which environmental factors were "best explaining" the resemblance matrix.

Mantle test was used to investigate the relationship between ordination of the samples based on community composition and the measured vertical flux at BATS (total biomass, C, N and P).

RESULTS & DISCUSSION

Hydrography; Figures 2 & 3.
Deep mixing early in the year, with a sudden shallowing of the Mixed Layer Depth (MLD) in April, with a thermal stratification increasing towards the summer. Mixing depth increases progressively during the fall, and thermal stratification weakens.
February spring bloom, followed by deepening of the Chl-*a* maximum layer, decreasing throughout the year, with minimums in December.

Community regimes; Figure 4.
The nMDS graph showed four groups, roughly corresponding to seasons, indicating four different community regimes. Color indicates the groups.

Chl-*a* and MLD would be the environmental variables best explaining the similarity between samples (**Table 1**).

Transitions between seasons were due to a mixture of common and uncommon OTUs. A mantel test comparing the community composition with the vertical particle flux measured at BATS (RELATE procedure) showed a significant statistical correlation (ρ = 0.348; p < 0.001). A direct correlation of the exported flux against zooplankton biomass was not found significant.

Table 1. Results from BEST analysis, showing which environmental factors were "best explaining" the resemblance matrix (Pearson correlation).

No. Variables	Correlation (ρ)	Variables
2	0.449	MLD, Chl-*a*
1	0.424	Chl-*a*
3	0.386	MLD, PP, Chl-*a*
1	0.368	MLD

References

Amaral-Zettler, L. A., E. A. McCliment, H. W. Ducklow, and S. M. Huse. 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PLoS ONE 4:e6372.

Corell, J., and N. Rodriguez-Ezpeleta. 2014. Tuning of protocols and marker selection to evaluate the diversity of zooplankton using metabarcoding. Revista de Investigación Marina, AZTI-Tecnalia 21:19-39.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75:7537-7541.

FOUR DISTINCT COMMUNITIES ARE DIFFERENTIATED, LINKED TO MIXED LAYER DEPTH AND CHL-*a*

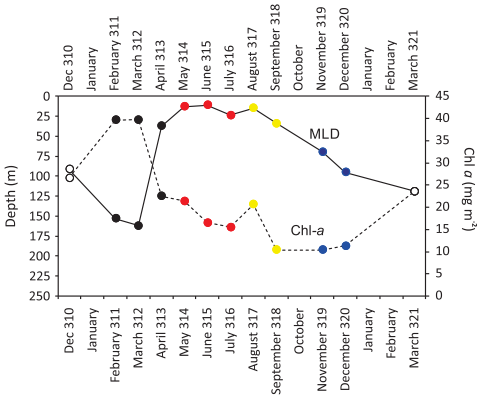

Figure 3: Mixed Layer Depth (MLD; solid line) and total Chl-*a* per square meter (from niskin bottle measurements; broken line). The combination of these two variables offered the best fit to explain the similarity between samples based on the community composition. Colors reflect the same grouping as in the nMDS.

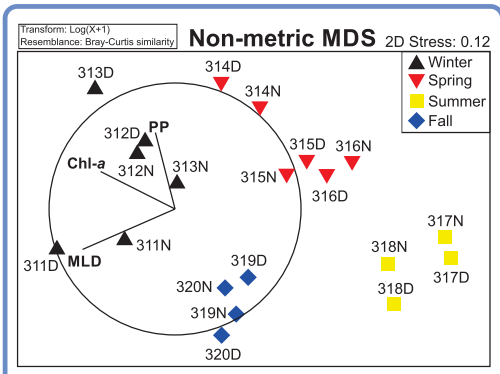

Figure 4: nMDS graph representing the grouping of the samples according to a clustering level of *K*=4. Roughly corresponding to seasons, it indicates four different community regimes. Color indicates the groups, and overlay represents the correlation (Pearson) between environmental factors (Mixed Layer Depth, Chl-*a* and Primary Production) and the ordination of the samples.

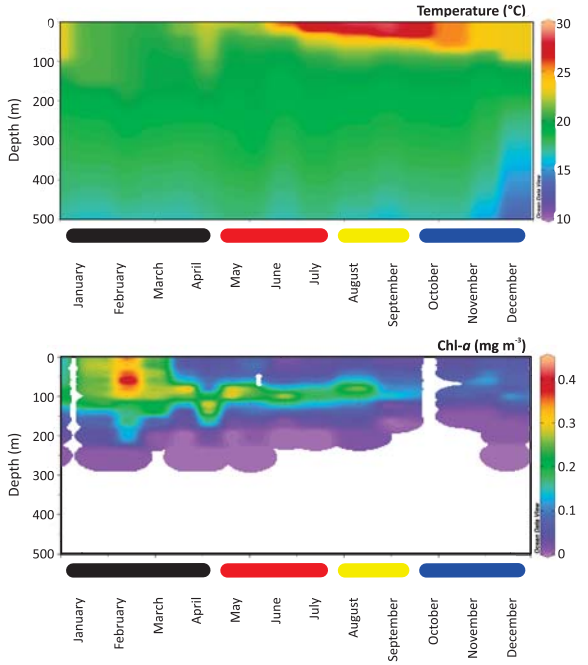

Figure 2. Contour plots representing temperature and total Chl-*a* per square meter (from niskin bottles) during 2015 at BATS. Missing Chl-*a* profiles were due to hurricane *Joaquin*. Temperature data was filled with glider data from the MAGIC program (http://magic.bios.edu/). Color bars below the plots indicate the different regimes based in the community composition (see Figure 4).

VERTICAL FLUX IS CORRELATED WITH COMMUNITY COMPOSITION, NOT WITH BIOMASS