



## Identifying Labile DOM Components in a Coastal Ocean through Depleted Bacterial Transcripts and Chemical Signals

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### Introduction

Marine dissolved organic matter (DOM) contains one of the largest active organic carbon pools on Earth. A key step in the marine carbon cycle occurs when bacteria transport carbon from the oceanic pool and transform it chemically. This is a challenging link to understand, however, because of the thousands of compounds that make up the DOM pool, each with different biological turnover rates. Approximately 40% of the carbon fixed by marine phytoplankton is processed through heterotrophic bacteria within hours to days of fixation. This study sought to improve understanding of the molecules that support this link in the carbon cycle by using gene expression patterns in coastal bacterioplankton communities as biosensors of the most rapidly consumed substrates, and FT-ICR MS analysis for molecular-level information on changes in DOM composition.

### Experimental Procedure

Estuarine water samples from Sapelo Island (Doboy Sound, Georgia, USA) were collected at both low and high tides in July and October 2014. Estuarine samples were incubated in triplicates for 24 hours. Immediately after collection, samples were filtered using pre-combusted 0.7  $\mu\text{m}$  GF/F filters and pre-washed 0.2  $\mu\text{m}$  membrane filters. Filtrates were acidified to pH 2, and DOM was isolated using solid phase extraction (SPE) cartridges (Agilent Bond Elut PPL). Extracts were collected at day 0 (T0) and 1 (T24) for DOM analysis using a 9.4 T FT-ICR MS (passively shielded). Samples were collected simultaneously for functional genomics approaches (e.g., transcriptomics).

### Results and Discussion

By identifying genes whose relative expression decreased during a dark incubation when microbes were isolated from new organic matter sources, we generated a list of transport- and metabolism-related genes likely to be important in mediating flux of highly labile DOM. Compounds representing carboxylic acids, fatty acids, sugars, and organic sulfur compounds were indicated to be among the most bioreactive molecules at this coastal site, and taxonomic affiliations of depleted transcripts implied substantial variability among bacterioplankton groups with regard to which compounds were used as substrates. FT-ICR MS analysis of depleted molecular formulae provided information on higher molecular weight, semi-polar components of DOM, and suggested that functional groups in the size range of ~30-40 Da cleaved from larger molecules are also highly labile. Changes in molecular formulae were consistent with transformations of unsaturated aliphatic compounds with low oxygen to carbon ratios (which can include fatty acids) and compounds with sulfur heteroatoms. This untargeted survey of the most biologically labile components of DOM suggests that a diversity of low molecular weight metabolites along with functional groups from high molecular weight molecules, biased toward nitrogen and sulfur-containing compounds (**Figure 1**), are important in supporting bacterial heterotrophy in this coastal ocean.

### Conclusions

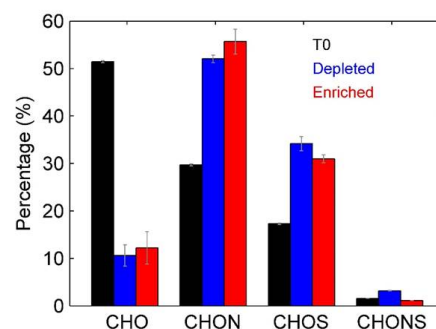
Small carboxylic acids, fatty acids, sugars, organic sulfur compounds, and organic nitrogen compounds emerged as important components of the labile DOM pool, along with functional groups cleaved from larger molecules. Querying DOM at the level of individual compounds using complementary biological and chemical methods is advancing understanding of one of the most important biological carbon fluxes on a global scale.

### Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490/1644779 and the State of Florida. In addition, we acknowledge NSF through the Sapelo Island Microbial Carbon Observatory (SIMCO, OCE-1356010). Support by A. McKenna, H. Chen and Y. Corilo with FT-ICR MS runs and PetroOrg software use are greatly appreciated.

### References (citation of the publication)

[1] Vorobev, A., *et al.*, *Environ. Microbiol.*, **20**, 3012-3030 (2018).



**Figure 1** – Elemental composition of compounds in the initial DOM (T0) and those changed in relative abundance (depleted or enriched) after a 24 h incubation experiment. Values shown are the fraction of the molecular formulae in each category (T0, enriched or depleted) that contained only CHO, CHON, CHOS or CHONS. The sum of each category equals 100%. n = 3.