VAAM2016 Abstract

Abstract topic: Environmental Microbiology

Title: **Investigating long-term preservation of RNA for qualitative surveys of aquatic microbial metatranscriptomes.**

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Abstract:

Microbial communities are the main catalysts of global biogeochemical cycling for multiple elements essential for life. The rapidity of their response to stressors and abrupt environmental changes implies that even fast and infrequent events can affect local transformations of organic matter and nutrients. Studying dynamics in microbial functionality at these subtle temporal- and spatial-scales is complicated by the rapidity of gene transcript turnover in cells, especially with respect to the inevitable delay between the sampling of seawater and the extraction of its RNA. This general obstacle underscores the need for an instrument that will allow the reliable sampling of microbial metatranscriptomes at frequent pre-established or event-triggered intervals, for refined temporal- and spatial-resolution. To advance the development of such a sampling tool, we examined the suitability of phenol fixation for long-term preservation of transcripts.

An artificial bacterial community was aliquoted to be either fixed with 10% v/v Stop Solution (5% phenol, 95% ethanol) or left untreated, and filtered at different time intervals over one month. Following fixation, cell numbers remained constant (~10^7 cells mL^{-1}) in contrast to increased cell counts in the unfixed aliquots. Both fixed and unfixed communities lost half their total RNA content after 48h but RNA Quality Number (RQN) revealed more extensive degradation in the fixed communities, at time of fixation and during the storage period. Interpreting this as an indication that 10% v/v Stop Solution is too aggressive on RNA molecules, we tested alternative fixation procedures on an *in situ* community in a second experiment. Fixation with 1% v/v Stop Solution provided higher RQN values for long-term storage. RNA-Seq was conducted on a selection of samples from the two experiments to ascertain that fixation efficiently conserves gene expression profiles in both artificial and environmental bacterial communities.