Title: Algal, bacterial and viral interactions as the backdrop to marine carbon and trace metal cycling

Description:

We are requesting 135 metatranscriptomes (after ribosomal RNA reduction) from temporal and manipulative samples collected at the Southern Ocean Time Series location as part of the Subantarctic Biogeochemistry of Carbon and Iron project (March 2018). Samples are microbial communities and thus a mixture of picoeukaryotes, bacteria and viruses. Metatranscriptomes will be used to describe the functional activity (and phylogeny) of the microbial community (algae, bacteria) from samples as well as provide information on members of the actively infecting portion of the virus community (ss/dsRNA and ss/dsDNA) that cannot be obtained from standard metagenomic analyses. Samples include water column profiles (32 metatranscriptomes, across 4 dates), experimental manipulations of trace metal availability (36 metatranscriptomes) and size-fractionation experiments designed to look at differences in processes occurring on particles collected in situ (i.e., bacteria/algal interactions from the >1 particulate size class, time course incubations of these, and controls). For 135 samples, the JGI allotment for the project should produce ~135 million reads per sample (conservative estimates) which should provide sufficient sequencing depth for this study.

The core component of this study is a collection of cleanly-sampled depth profiles (ranging from the surface layer to 500 m) which will be queried to specifically address aspects of trace element (with a focus on iron) and carbon cycling in this system. Yet interpretation of this type of information by itself is difficult as it often lacks context. Thus, to complement and constrain the above observations, experiments were completed onboard ship that controlled iron availability using previously outlined approaches [e.g., 1, 2, 3]. These experiments allow for the direct manipulation of cellular iron quotas for the entire in situ community [both increasing and decreasing quota, 1], establishing a framework to interpret both the unamended controls as well as the in situ water column samples across depth. We note that these experiments both increase and decrease the bioavailability of iron to the majority of the community (we are not aware of approaches to do this with other elements at this time). Finally, due to interest in how microbial communities on phytoplankton/particles in the ocean are physiologically different from communities that are “free-living”, and how these phytoplankton/microbe aggregates interact, samples were collected using in situ trace-metal clean McLane pumps and divided in to particulate (> 1 μm) or free (< 1 μm). These were maintained in on-deck incubators to allow for fraction-specific processes to occur. Time zero samples as well as a time course of these incubations under in situ conditions are available to address questions concerning inter-organismal (including virus, bacteria and algal) interactions.

1. Wilhelm, S.W., et al., Elemental quotas and physiology of a southwestern Pacific Ocean plankton community as a function of iron availability. Aquatic Microbial Ecology, 2013. 68: p. 185-194.
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iron in the HNLC waters of the subtropical Pacific Ocean. Aquatic Microbial Ecology, 2004. 35: p. 79-91.

Justification:

Metagenomic / metatranscriptomic approaches have been adopted to characterize the diversity of viral [4] and microbial communities [5] and their interactions [6, 7]. To date this includes studies we have completed in fresh waters [8-10] and marine systems [6]. Environmental genomics are particularly useful for studying microbial communities from extreme environments (or in this case difficult situations) where conditions are not conducive to traditional cultivation [11]. Interactions within microbial communities are difficult to "tease-apart" in lab studies: regardless of effort put into constructing artificial communities, it remains nearly impossible to rebuild and maintain reasonable facsimiles that capture all of the diversity (bacterial and phytoplankton, not to mention viral). To this end, microbial ecologists for more than a decade have turned to processing environmental samples with shotgun tools that capture snapshots of the entire community [12, 13]. Yet in spite of the incredible power of these tools, they often lack the information needed to test hypotheses that arise from in situ observations. To this end we have opted to focus on collecting both the in situ data needed to describe community diversity, interaction and function while simultaneously performing (what would typically be follow-up) experiments needed to ground truth water column profile information. What will result from this collection of data is an ability to generate new hypotheses from one data set while having the information immediately available to test these hypotheses in the other (we note that hypotheses will arise from both the water column and manipulative observations that require validation using the other type of data).
Along with validating our overall approach, analysis of samples will allow us to test specific questions generated over the last decade of collaboration between the PIs listed on this proposal (see Utilization). These questions are manifold, yet all require the data set as described to address. Ultimately we anticipate not only gaining major new insights into trace element and carbon cycles, but also bacteria-phytoplankton interactions (during both active phytoplankton growth and senescence/export) as well as ongoing virus-infection within the in situ community.

4. Breitbart, M., Marine viruses: truth or dare. Ann Rev Mar Sci, 2012. 4: p. 425-48.
5. Ottesen, E.A., et al., Ocean microbes. Multispecies diel transcriptional oscillations in open ocean heterotrophic bacterial assemblages. Science, 2014. 345: p. 207-212.
6. Moniruzzaman, M., et al., Virus-host infection dynamics of marine single-celled eukaryotes resolved from metatranscriptomics. Nature Communications, 2017. 8.
7. Stough, J.M.A., et al., Predicting lytic vs lysogenic states for Microcystis phage: metatranscriptomic evidence of lysogeny during large bloom events. PLoS ONE, 2017. 12: p. e0184146.
8. Steffen, M.M., et al., Ecophysiological Examination of the Lake Erie Microcystis Bloom in 2014: Linkages between Biology and the Water Supply Shutdown of Toledo, OH. Environmental Science & Technology, 2017. 51(12): p. 6745-6755.
9. Steffen, M.M., et al., Metatranscriptomic evidence for co-occurring top-down and bottom-up controls on toxic cyanobacterial communities. Applied and Environmental Microbiology, 2015. 81: p. 3268-3275.
10. Steffen, M.M., et al., Comparative Metagenomics of Toxic Freshwater Cyanobacteria Bloom

Communities on Two Continents. PLoS ONE, 2012. 7(8): p. e44002.
11. Tyson, G.W., et al., Community structure and meboism through reconstruction of microbial genomefrom the environment. . Nature, 2004. 428(37-43).
12. Venter, J.C., et al., Environmental genome shotgun sequencing of the Sargasso Sea. Science, 2004. 304: p. 66-74.
13. Angly, F.E., et al., The marine viromes of four oceanic regions. PLoS Biology, 2006. 4(11): p. e368.

Utilization:

We will query the data to address the following specific questions:
1. How do bacteria/algae physiologically regulate their response to trace metal availability and changes in cellular metal quota at the genetic level? How does this manifest itself across trace metal gradients seen with depth in the water column?
2. Do bacterial assemblages on phytoplankton and other particles help solubilize iron for phytoplankton in iron-limited environments?
3. Do pelagic bacteria associated with phytoplankton and other particles use different strategies for carbon assimilation than "free-living" bacteria, and do these strategies change with depth and particle nutrient ratios?
4. Are there differences in the sensitivity of bacteria to virus infection in particle associated cells vs bacteria that are "free-living"?
5. Can approaches to determine "who infects who" in large molecular data sets be extended from coastal systems to pelagic marine systems?
6. Are markers for lysogeny readily detectable in pelagic communities and do differences in community density (mimicked based on particulate vs free living) influence the lytic/lysogenic decision?

Questions 1-3 focus on elemental cycling, the role of heterotrophic bacteria in shaping these cycles and how bacteria-phytoplankton interactions drive trace metal availability in the upper mixed layer and scavenging / mineralization of elements during particle export. Data concerning water column chemistry (including trace metals, with Fe as an emphasis), productivity (primary and secondary), elemental quotas at the single cell level (synchrotron x-ray fluorescence), community abundance (flow cytometry), particle size distribution and quality (Flowcam and CHN analyses), electrochemistry for ligands and standard "metadata" are available. Evidence from our collaborations that trace back over a decade has demonstrated that particle associated communities in this region are dominated by a microbial subset, with a particular abundance of Roseobacter [14]. We have recently demonstrated that Roseobacter (consistent with these populations) can produce a siderophore. We have a preliminary siderophore structure, measured uptake kinetics and generated mutant libraries of over-producers and knockouts for this siderophore (manuscript in prep). Along with the availability of a recent review of trace metal homeostasis pathways in environmental microbes [15], we are set to address questions 1-3 (above) for iron and other elements as well as test the hypothesis that "bacterial siderophore production in the ocean occurs strictly in particle associated cells". PI Wilhelm helped usher in interest in Fe-organic interactions > 20 years ago [e.g., 16, 17, 18] and our observations remain consistent with this hypothesis. We note that this not only suggests bacteria help solubilize iron for actively growing phytoplankton in surface waters, but that bacteria work to mineralize iron

from senescent cells in aggregates.
Questions 4-6 target virus activity. Wilhelm, Buchan and LeCleir have interests in virus ecology that stretch back > 2 decades [19-22]. Available data for comparison include viral isolates (which form either lytic or strictly-lysogenic relationships) infecting Roseobacter in the Buchan lab: sequence information and pending transcriptomes of infected and naïve hosts (from current NSF-funding) are available for comparison. The Buchan lab is also analyzing 56 metatranscriptomes from coastal systems dominated by Roseobacter (CSP 2017). We also have virus-production estimates from all time zero and profile samples. Finally, as recent studies have raised questions concerning the importance of lytic / lysogenic interactions [23-25], we will tease these conditions apart based on comparisons of lytic and lysogenic infections in the lab with known viruses (above) and statistical approaches based on the expression of candidate lysogeny-associated genes and obligate lytic genes [7].

Community interest:

Data and information generated by this study will be of broad interest to the marine microbial community as well as researchers interested in microbe/host interactions, trace metal cycling and availability, virus-host interactions and virus ecology.
To our knowledge, the development of in situ sequencing with paired manipulative experiments is rather novel (especially with respect to deep-sequencing efforts). It is all too common for researchers to make a statement similar to "I wish I could go back and manipulate this and test that idea". We have attempted this by manipulating iron availability as well as particle-associated/free status in conjunction with sample collection: while not exhaustive, these approaches address many of the overarching functional observations from our ongoing efforts. These efforts will add a new level of context to our data that is currently not available in the "grab-and run" sampling approaches many of us have taken in the past. To this end, we feel the success of our approach (as much as that of the data itself) may garner broad interest from microbiologists on a global scale.

We also intend to develop data for the community in a user-friendly manner. To this end we have engaged a modeler (UT faculty member David Talmy), whom, along with exploring his own interests in drives of stoichiometry, will work with us to make the data more accessible to the community. Having paired cellular quotas, in situ chemistry and transcriptional information across samples will provide a potential first step towards the next generation of efforts, such as Earth Simulation Models, that can build across isolated observations and make them tractable on basin and global scales.

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15. Hogle, S.L., et al., Trace metal acquisition by marine heterotrophic bacterioplankton with contrasting trophic stratgies. Applied and Environmental Microbiology, 2016. in press.
16. Wilhelm, S.W. and C.G. Trick, Physiological profiles of Synechococcus (Cyanophyceae) in iron-limiting continuous cultures. Journal of Phycology, 1995. 31: p. 79-85.
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18. Wilhelm, S.W. and C.G. Trick, Iron-limited growth of cyanobacteria: multiple siderophore production is a common response. Limnol Oceanogr, 1994. 39(8): p. 1979-1984.
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21. Budinoff, C.R., et al., A protocol for enumeration of aquatic viruses by epifluorescence microscopy using Anodisc (TM) 13 membranes. BMC Microbiology, 2011. 11.
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23. Coutinho, F.H., et al., Marine viruses discovered via metagenomics shed light on viral strategies throughout the oceans. Nature Communications, 2017. 8: p. 15955.
24. Knowles, B., et al., Lytic to temperate switching of viral communities. Nature, 2016. 531: p. 466-470.
25. Weitz, J.S., et al., Lysis, lysogeny and virus-microbe ratios. Nature, 2017. 549: p. E1-E3.

DOE mission:

Development of an understanding of the tight coupling of bacteria and phytoplankton in marine systems is of critical interest to the entire marine microbial community (as readily apparent from the broad spectrum of presentations at the Ocean Sciences Meeting in Portland, Feb 2018). It is clear that Fe solubility and availability continues to be a major driver of carbon cycles in marine surface waters, yet community level interactions have been broadly overlooked in favor of drilling down on certain model organisms (e.g., diatoms). That the major colonizers of phytoplankton in this region are bacterial, and that these bacteria can readily produce powerful chelators (in this case actual siderophores, using the strict definitions of Neilands [26]) which constrain Fe availability, remains a nearly unresolved phenomena in the marine systems. Our efforts will begin to resolve this major geochemical relationship in terms of promoting phytoplankton activity (by solubilizing iron) in return for carbon (to the bacteria). Moreover, our efforts will illuminate the potential role of siderophores in driving iron scavenging in senescent cells which appear to be biologically attacked for specific elements while settling from the water column [27]. Ultimaltely, the coupling of in situ data with experimental data will allow for an understanding of the plasticity of microbial responses to shifts in biogeochemistry in situ, and help us link the transcriptional underpinnings to these shifts across populations.

Of special focus in the PIs laboratory is also the development of tool sets to characterize viruses (a stated priority in the 2018 RFP) in microbial systems with a particular focus on “who infects who” [6]. Our virus efforts will feed directly into DOE mission areas of “Carbon Cycling” and “Biogeochemistry”. Our research group is particularly interested in spatial and temporal variation in these communities (another stated focus for 2018) as it brings with it novel statistical power. It should be noted that, unlike many virus-ecology studies, our work is not limited to bacteriophage (although they are deeply examined): it includes ss/dsRNA viruses that infect protists as well as the dsDNA Nucleocytoplasmic Large DNA viruses now commonly referred to as “giant viruses” [28, 29]. Analyses of virus communities will focus on active infections

(transcripts) from the cell size class: this is potentially the closest link that can be obtained from molecular data to actual virus activity [30].

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29. Wilhelm, S.W., et al., Standing on the shoulders of giant viruses: 5 lessons learned about large viruses infecting small eukaryotes and the opportunities they create. PLoS Pathogens, 2016. 12: p. e1005752.
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Sample preparation:

On March 1, 2018, we engaged with the Australian research vessel RV Investigator to collect samples. All samples are in hand. Processing of samples for sequencing quality nucleic acids will now commence with the goal of having all samples processed and available for submission by June 1, 2018. We note that sample collection is described above.

Sample preparation for sequencing. RNA will be extracted using our previously successful protocols Sterivex™ [e.g., 9, 31] which are publically available[32]. We note that we are aware of JGI requirements and can ensure that all samples will meet or exceed specifications. We have full access to a NanoDrop (to characterize contaminants) and Qubit (to quantify RNA with PicoGreen). We also check every RNA sample by PCR with short-amplicon 16S rRNA gene primer sets (in our hands this approach is ~100 times more sensitive than Qubit).

Analysis of sample information. We start by assembling data from each sample into contigs to reduce complexity. Recruitment of reads back to contigs will establish relative quantitative distributions of reads within each sample. Contigs will be compared to reference taxa as well as via assignment tools available through IMG and other online portals (e.g., MG-RAST, iVirus). The PI's lab has also been developing workflows to specifically address biologicals not accounted for by standard pipelines: for example, we have developed databases to screen for ss/dsRNA and DNA viruses, with a particular focus on viruses that infect single-celled protists (Giant viruses) as well as RNA viruses [6, 33].

We note that a full time PhD student (Naomi Gilbert) has been recruited and has support to complete the work. To address individual questions above, we will return each time to the assembled contigs and recruited reads to extract specific information on the diversity or functions of interest. For example, for work on trace metal cycling on particles we will employ the information from our Roseobacter transcriptomics / siderophore projects and available literature [15]. In each case we initiate examination with statistical queries of spatial and temporal patterns and in contrasting experimental conditions (transcripts) with water column profiles. Much of this work will be done in Primer-e [34], which we also use to correlate

sequence abundance with environmental data to tease out putative drivers and even start to illuminate mechanisms. We look for variations in function (at the level of the individual population and across the collective community) in space and time against metadata to develop hypotheses that can be directly tested against the contrasting data sets.

To specifically examine virus diversity, distributions and effects, we will rely on combined online tools (e.g., VirSorter) and approaches we are developing in-house [6, 7, 33] that capture other members of the virus community. One goal is to continue to develop our "who-infects-who" approaches: by identifying putative virus-host partners based on statistical co-occurrences during active infection, we can improve approaches for the isolation of new virus-host models and begin to make specific predictions on virus effects. Having manipulative experiments at hand is particularly useful as these may have altered virus-host dynamics. The PIs are aware that several JGI scientists are interested in this area of research and we hope to be able to work together to drive this effort.

31. Krausfeldt, L.E., et al., Spatial and temporal variability in the nitrogen cyclers of hypereutrophic Lake Taihu. FEMS Microbiology Ecology, 2017. 93: p. fix024.
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