Driving research questions and DRAFT plans for 2021 (Theme II)

Characterization of microbial assemblages associated with sinking & suspended particles

General approach: Thematic focus on characterizing community structure/activity of microbes associated with particles that vary in source/composition, depth of collection, type of capture mode (i.e. traps, nets, snow catcher, etc) and related to this, sinking versus suspended particle types.

Specific topics that have been previously identified for Theme II (somewhat modified):

a) Spatial and temporal variability (potentially various scales of each) and succession of microorganisms (prokaryotes, eukaryotes, viruses) on sinking and suspended particle size classes between upper ocean and mesopelagic waters.
b) Abundances, diversity, genomic and metabolic properties of all particle-associated microbial groups (prokaryotes, eukaryotes, viruses).
c) Microbial networks and interactions (e.g., trophic relationships, metabolic cross-feeding) of microbes associated with particles. Comparison with surrounding water.
d) Microbes and mechanisms associated with particle formation, sinking and dissolution.

Across-Theme connections: For Theme I, this component of the work will provide quantitative characterizations of microbial community structure and activities for the chemical/physical characterizations of particles that will be conducted within Theme I. For Theme III, this component will provide basis for understanding experimental studies of remineralization, decomposition and other rate measurements that will be conducted within Theme III.

NB: One key variable that must be determined is the type(s) and amount of particulate material that will be needed relative to amounts available by various collection approaches, as the amount available will dictate/limit the types of analyses that can be made. We might explore the potential for producing particulate material to provide additional material.

Task 1: Characterizing whole microbial communities by particle source/composition (direct splits of available material):

Questions: What sort and amounts of microbes characterize particles? How do they differ among different particle sizes and/or types? How do particle-associated microbial communities differ by depth? How unique are particle-associated vs. suspended microbial communities? Is there conclusive evidence of *Prochlorococcus* associated with particles in the field, and if so can they be isolated and cultured? Can models of community structure of particle-associated microbes reproduce the distribution/succession of microbial communities at different depths?

Approach: Examine an array of particles types (see possible list of particles below). Splits of samples among PI groups for analysis (PIs involved: Lindell, viruses; Chisholm, *Prochlorococcus* on particles; Dyhrman, *Trichodesmium* & diatom-diazotrophs; DeLong, other bacteria/archaea; Zehr, diazotrophs?; Caron, other protists and other euks, Weitz modelling). Potential particle types to be examined:

1) PIT material:
    a. Shallow (150 m) vs deep (175-500 m) PITs.
    b. Traditional ('live') vs preserved ('dead') PITs.

2) Van Mooy net trap collections.
3) Deep (4,000 m) traps material.
4) Snow catcher material.
5) SCUBA-collected material?
6) Suspended microbes and non-sinking particles (via traditional bottle casts).

Task 2: Characterizing the major metabolisms and trophic modes of particle-associated vs. suspended microbial assemblages.

Questions: How does trophic structure and microbial community metabolism compare between particle-associated and suspended microbe assemblages? Can we sort out dead/dormant microbes from metabolically active ones (and the processes associated with active ones) on particles of varying types, depth? What are the key/dominant microbe-microbe associations (and their ecological significances) among particle-associated microbes? How do microbial networks differ on and off particles? How do metabolite patterns map onto microbial community structure on and off particles? What are the genes associated with particle-bound *Prochlorococcus*, how do they compare with suspended *Prochlorococcus*?

Approach: (Lindell, cyanophage infection percentages; Lindell & Caron, relative importance of viral vs. protistan control of prokaryote abundances & community structure; Weitz, modeling microbial trophic structure on particles; DeLong, prokaryote and virus genomes and metabolic pathways; Chisholm, *Prochlorococcus* pangenome on particles; Dyhrman & Caron, *Trichodesmium* microbiome and eukaryotic metatranscriptomics; Ingalls, metabolite profiles; DeLong, Lindell, Caron, network analysis of microbial communities). Live/dead fluorescent assays via microscopy/flow cytometry? Partitioning and particulate microhabitats of diazotrophs? (Zehr?)

Task 3: Experimental studies of the succession of microorganisms (prokaryotes, eukaryotes, viruses) on sinking particles from upper ocean and mesopelagic waters (method TBD):

Questions: Does microbial community succession on particles explain depth-related differences in particle-associated microbial communities? That is, are microbial communities and processes appearing during experimental incubations more similar to microbial communities and processes associated with particles collected at greater depth (implying modification during sinking)?

Approach: Incubate (method TDB) various types of particles to investigate changes in microbial abundances as particles age. Time-series measurements of changes in viral, prokaryote and microbial eukaryote community composition and metabolic/trophic activity, and model-data integration by the Weitz group (DeLong, Lindell, Caron, Weitz). Identification of novel prokaryote-protist PA symbiotic associations via cell sorting and generation of eukaryote-associated prokaryotic MAGs (DeLong, Dyhrman, Caron, Zehr). Possible stable isotope probing experiments (DeLong). BonCAT type labelling experiments? nanoSIMs (Zehr)? Other thoughts?

FYI: Here are all the Themes as last outlined in prior summaries (I and III may have changed somewhat due to deliberations among the other leads):

1) Characterization of sinking particles over the upper 500 m
   a) Depth-dependent attenuation of energy content, elemental (C, N, P, H, O, Fe, etc.), and molecular composition of particles and DOM
   b) Vertical changes in particle size structure (suspended and sinking) and the role of predation/viruses as modifiers of particle size
   c) Source-tracking of export (e.g., where vertically in the water column does the material originate)
   d) Quantify absolute and relative contributions of living (e.g., *Prochlorococcus*, diazotrophs), dead, and dormant microbes to particle concentrations and export

   Organizers: Karl, VanMooy, White, Beatty

2) Characterization of microbial assemblages (prokaryotic, eukaryotic, viruses) associated with sinking and suspended particles:
   a) Time-varying succession of microorganisms (prokaryotes, eukaryotes, viruses) on sinking particles between upper ocean and mesopelagic waters
   b) Abundance, diversity, and metabolism of particle-associated microbes
   c) Quantify viral abundance, viral diversity, % infected cells on suspended and sinking particles; evaluate the role of viruses and predation as controls on particle microbiome populations
   d) Identify microbial networks and interactions (e.g., metabolic cross feeding) associated with particles

Organizers: Caron, Demory, Delong

3) Experimentally quantify rates, stoichiometry, and controls on organic matter remineralization
   a) Remineralization stoichiometry (C, N, P, O, H, Fe, etc.) at differing depths
   b) Adsorption/desorption of specific elements onto particles
   c) Experimentally examine nutrient/energy limitation of microbial decomposition and remineralization

Organizers: Church, Granzow, Karl