

Church Lab PARAGON Sampling and Incubation Outline

Short Term Incubations

We plan to conduct a series of four short term particle remineralization experiments (24 hours). These experiments will aim to compare microbial dynamics with different nutrient availabilities during the remineralization of sinking particles. Particles will be collected at ~150m using the net traps and equal particle splits will be added to six 2L bottles containing whole seawater collected using the trace metal (TM) rosette from the same depth as the sinking particles. Nutrient additions will be made to three of these bottles and three will remain without nutrient additions. Each of the four experiments will test a different nutrient addition (Fe, N, P, and glucose). All six bottles will then incubate for 24 hours in the dark in an on-deck incubator set to the *in situ* temperature at the depth of water/particle collection. Separate T0 bottles will also be set up with equivalent treatments and sampled immediately for POC concentration, respiration, bacterial productivity based on ³H-leucine incorporation, metatranscriptomics, and bacterial abundance based on the quantification of 16S rRNA gene abundance using qPCR. At 24 hours, all six bottles (3 per treatment) will be sampled for bacterial productivity, metatranscriptomics, and bacterial abundance.

Logistical needs:

- Four dedicated 24 hour duration net trap collections of sinking particles at ~150 m each carried out ~2 days apart.
- Four dedicated TM rosette casts to ~150 m immediately prior to recovery of corresponding net trap
- Space in dark, temperature controlled incubator (~15-19°C) accommodating six 2L bottles (10.65" x 4.57" x 4.57" each) for 24 hours for each of four experiments
- Access to trace metal clean van
- Access to radioisotope van

Time-course Incubations

We plan to conduct a series of three time-course remineralization experiments (72 hours). These experiments will aim to determine the potential for iron limitation in the heterotrophic bacterial community under different scenarios of carbon availability. For each experiment, whole seawater from ~150 m will be collected with the TM rosette and distributed into 18-4L bottles. One experiment will be conducted with only whole seawater, the second will supplement POC with equal splits of particles collected at ~150 m with the net traps, and the third experiment will supplement labile DOC with added glucose. For each experiment, 9 of these bottles will be amended with Fe and 9 will remain unamended. Bottles will incubate in the dark in an on-deck incubator set to the *in situ* temperature at the depth of water/particle collection. Separate T0 bottles will be prepared equivalently to these treatments and sampled immediately for POC, TOC, respiration, bacterial carbon productivity based on ³H-leucine incorporation, iron uptake using ⁵⁵FeCl₃, metatranscriptomics, and bacterial abundance based on the quantification of 16S rRNA gene abundance using qPCR. At 24 hours, 6 bottles (3 per treatment) will be sacrificially sampled for TOC, respiration, bacterial carbon productivity, iron uptake, metatranscriptomics,

and bacterial abundance. At 72 hours, 6 bottles (3 per treatment) will be sacrificially sampled for TOC, respiration, bacterial carbon productivity, iron uptake, metatranscriptomics, and bacterial abundance. The remaining 6 bottles (3 per treatment) will be sacrificially sampled for POC.

Logistical needs:

- Three dedicated TM rosette casts to ~150 m each carried out ~4 days apart with the first cast as close to the beginning of the cruise as possible
- Space in dark, temperature controlled incubator (~15-19°C) accommodating 18-4L bottles (13" x 6" x 6" each) for the first 24 hours and 12-4L bottles for the remaining 48 hours for each of three experiments
- Access to trace metal clean van
- Access to radioisotope van
- A single dedicated 24 hour duration collection of sinking particles at ~150 m with the net trap recovered immediately following one of the TM rosette casts

Water Column Sampling

Zooplankton collection

- 3 integrated (0-45 m) vertical net tows in close succession (ideally at night)
- A single dedicated CTD cast to ~45 m in close proximity to net tows (all 24 bottles needed for this experiment)
- Access to a zooplankton collection net (ideally the 202 µm net used for HOT sampling or something similar with a ~1 m² net opening)

High vertical resolution sampling for macronutrients and DNA

- A series of 3 CTD casts targeting high vertical resolution sampling (~5 m) through the DCM and upper mesopelagic

Bacterial carbon productivity and iron uptake (³H-leucine and ⁵⁵Fe)

- 3 pre-dawn TM rosette casts to ~300 m spaced somewhat evenly throughout the cruise
- Deployment of in situ array following TM rosette cast from dawn to dusk with bottles attached at 6 depths (25 m, 125 m, 150 m, 175 m, 200 m, 300 m)
- Access to trace metal clean van
- Access to radioisotope van