EXPORTS (RR1813)

EXPORTS: EXport Processes in the Ocean from RemoTe Sensing

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R/V Roger Revelle – Scripps Institution of Oceanography
Dates of Cruise: August 10-September 12, 2018

The following cruise report is a compilation of reports provided by each funded group funded by NASA or NSF to work within the EXPORTS framework. This report describes the work and preliminary results for the first field expedition to the Subarctic North Pacific near Station PAPA, conducted in a lagrangian framework with two research vessels and multiple autonomous assets. Reports here are for the work conducting aboard the R/V Roger Revelle August 10-September 12, 2018.

Zooplankton-mediated export pathways (Steinberg and Maas)
On cruise: Deborah Steinberg, Joe Cope, Karen Stamieszkin, Chandler Countryman, Andrea Miccoli

We quantified zooplankton-mediated carbon export via two main pathways: the production of passively sinking particles, particularly fecal pellets, and the active transport of carbon via diel and seasonal migrations. We conducted all of our experiments and quantitative sample collections as day-night pairs during each of the 3 epochs to capture diel variability. Thirteen incubations of individual, dominant mesozooplankton taxa (copepods *Neocalanus cristatus, N. plumchrus*, *Pseudocalanus*-like sp.; amphipods *Vibelia propinqua* and *Themisto pacifica;* krill *Thysonessa inspinata*; and the gelatinous salp *Salpa aspera*), and ten incubations of five different community size fractions provided fecal pellet production rates for estimating the total contribution of zooplankton egestion to the POC pool sinking out of the euphotic zone. While *Neocalanus* spp. often dominated the biomass, its contribution to fecal pellet flux was relatively low, as it all but ceases feeding and pellet production prior to descending to its diapause depth in late-Aug./early-Sept. The diel migrator *Salpa aspera* was present during epochs 1 and 3, but not 2. It was an important producer of large, rapidly sinking fecal pellets in the upper 100m, and in collaboration with others salp pellet sinking rates were measured. We also measured respiration, and with others, excretion (dissolved organic carbon and nitrogen, and ammonia), and a few mesozoop. grazing expts alongside the microzoop groip. Respiration and excretion experiments with individual dominant migrating taxa (*Neocalanus* spp., *Metridia pacifica*, *Thysonessa inspinata*, *Euphausia pacifica*, *T. pacifica*, *V. propinqua, Pneumoderma* sp.) simulated metabolism at depth waters after nighttime feeding in surface, with the two migrating euphausiids (*T. inspinata* and *E. pacifica*) having the highest respiration rates.

The six day-night pairs of discrete-depth net tows (MOCNESS tows) that we collected from 1000m to the sea surface for species abundance and size-fractionated biomass will be used to scale the above experiments up to the whole mesozooplankton community. Salps were migrating between ~400-600m during the day to the top 0-50m at night, and the large copepod *N. cristatus* migrated between the 50-100m layer during the day to the surface 0-50m at night. The size fractionated biomass samples -same as in community fecal pellet production experiments- will enable us to estimate fecal pellet production by the entire community, and we will use the abundance and taxonomy information from MOCNESS tows, alongside our metabolic experiments to estimate the active transport of dissolved nutrient pools by dominant zooplankton taxa. To estimate full-community respiration potential, we also collected samples for Electron Transport System (ETS) analysis. We will compare ETS to the respiration measurements scaled between the individual experiments and MOCNESS abundances samples, giving us bounds on zooplankton metabolism at depth. Finally, from the MOCNESS tows, we collected subsamples to analyze for gut fluorescence (grazing rates), and samples for H. Close and B. Popp for stable isotopes to examine feeding ecology/ trophic position. Given the importance of

the MOCNESS tows to our research objectives, we suggest that the correct MOCNESS calibration files be readily accessible, and that the nets themselves be better tended to (the nets came with a lot of small holes, for example). The EXPORTS cruise was very fruitful for the zooplankton group.

First steps - Linking remotely-detectable optical signals, photic layer plankton properties, and export flux

Cruise Report prepared by: Jason R. Graff, Emmanuel Boss & James Fox

Group Leaders (Oregon State University): Mike Behrenfeld, Kim Halsey, Jason Graff, Allen Milligan

Group Leaders (University of Maine): Lee Karp-Boss & Emmanuel Boss

Cruise Participants: Jason Graff, Emmanuel Boss, James Fox, Nils Haëntjens, Brian VerWey

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Objectives

Our primary objectives were to characterize phytoplankton abundances and community composition, the combination of total particulate and phytoplankton elemental stocks and pigments, rates of primary production and inherent and apparent optical properties (IOPs & AOPs) of surface waters from the process ship near St. P. This data set will also be used to address ecological questions concerning variability in community structure as well as to provide validation for remote sensing. We also examine vertical distribution of IOPs, plankton and large particles such as metazoan grazers and aggregates to better understand relations between surface processes that can be observed from space and the water column beneath it. Such detailed information has been identified as essential for quantifying stocks, composition, and export flux.

Measurements

Inline system

Continuous measurements (along the ship's track) of hyper-spectral absorption and attenuation (AC-S), backscattering (BB-3), CDOM fluorescence (WSCD), particle-size distribution and scattering in the near-forward direction (LISST), spectral fluorescence exited at 404 and 532nm including two fast-repetition-fluorometers (custom and WetLABS ALFA) and imaging flowcytometry (IFCB, for analysis of cell concentrations and species composition of phytoplankton and non-chlorophyll containing particles). Discrete measurements for phytoplankton carbon (C_{phyto}) and community composition (0.5-64 μm), total particular organic carbon (POC), and high performance liquid chromatrography (HPLC), and particle size distribution (Coulter) were also collected using the inline system.

Vertical profiles

Profiling video camera (UVP5-HD), mounted on the CTD rosette to examine vertical distributions and composition of large (> 100 m) particulate material.

Vertical profiles of cell abundances and composition of pico-, nano-, and micro-phytoplankton and PSD were collected from rosette Niskin bottles.

Optical profiles and surface radiometry

Daily profiles of particulate and dissolved absorption and attenuation (AC-S), backscattering (BB9), and CDOM fluorescence (FLCDRT) in the upper 100 m of the ocean using a Slow Decent Rate Optical Profiler and separately with a small cage for spectral backscattering (HydroScat-6).

Radiometry and AOPs: Downwelling irradiance and upwelling radiance using a surface buoy (HTSRB and HyperPro) and a ship mounted self-orienting system (Hyper-SAS includes cameras for QC) used primarily to derive remote-sensing reflectance.

Rate Experiments

^{14}C uptake experiments were conducted to investigate phytoplankton physiology and productivity. Ten 24 hr incubations were conducted to provide estimates of net primary production (NPP, units mg C m^{-3} d^{1}). Water was collected from 4/5 depths throughout the photic zone using the trace metal clean (TMC) rosette. This water was spiked with ^{14}C and incubated in the on-deck incubators at the appropriate light levels before begin filtered and analyzed. Fourteen short-term (2 hr) experiments were also carried out to measure photosynthesis vs irradiance (P vs E) parameters (α, P_{max}, E_k). Water for these short-term experiments was collected at different times (late morning and mid-afternoon) and depths (3, 10 and 30-32 m) to assess spatial and temporal variability. A fast repetition rate (FRR) fluorometer was using the line-line water system to provide continuous measurements of phytoplankton photophysiology (F_v/F_m, σ_{PSII}). Discrete FRR measurements were also made for samples taken from incubation experiments. Downwelling surface photosynthetically active radiation (PAR) was also measured continuously (15 second intervals) using a Licor PAR sensor.

Preliminary findings: NPP values in the top 30 m of the water column ranged from 2-4 mg C m^{-3} d^{1} during EPOCH 1. Below 30 m (70 m, 1% light depth) the values were significantly lower and ranged between 0.3-1 mg C m^{-3} d^{1}. A small increase (up >5 mg C m^{-3} d^{1}) was observed in the 5 & 10 m incubations towards the end of EPOCH two but values remained largely the same. At the start of EPOCH 3 a larger increase in NPP was observed at 10 and 20 m, measured at 5.5 and 8.4 mg C m^{-3} d^{1} respectively. These results are preliminary and need to be normalized to daily irradiance to provide a true measure of temporal changes in NPP. The values from the P vs E experiments are still to be interpreted.

The FRR data reveal characteristics typical of an Fe-limited system (nocturnal and midday suppression in F_v/F_m). This was confirmed by a series of bio-assay experiments (Jenkins group) which saw the F_v/F_m increase from ~0.25 to >0.5 after long (6-day) and short (24 hr) term incubations, following Fe-addition. The PAR data are being processed to provide time of sunrise and sunset, day length

and average daily irradiance. I will try and put these on the shared science folder before I leave but if you don't get access to them please e-mail me and I will make sure you get that data.

Photoacclimation experiments (with and without iron (Fe) additions) were conducted to evaluate 1) the growth irradiance to which phytoplankton are acclimated and 2) the extent to which Fe impacts the ability of phytoplankton to acclimate to changes in growth irradiance. These metrics are important in models of phytoplankton growth rate and net primary production.

Supporting/Collaborative measurements
We ran samples on the IFCB and BD Influx for a variety of experiments run by other groups: Jenkins, Steinberg, Marchetti, Santoro and Meden-Duer.

Highlights and initial results

Phytoplankton Community: BD Influx and Imaging Flow-Cytobot (IFCB)
Samples analyzed on the BD Influx revealed a community dominated in abundance by the pico-prokayote *Synechococcus* but once converted to biovolume or biomass will likely be dominated by the larger eukaryotic cells in the surface mixed layer. Cell abundance peaked below the surface mixed layer and often below the subsurface chlorophyll maximum depth at 50 and 65 meters. At these depths, *Synechococcus* was more abundant with eukaryotic groups contributing less to cell counts and biomass. Including the samples analyzed in collaboration with other groups, approximately 800 samples were run on the Influx.

The IFCB was analyzing 5 mL of the underway water ~ 22 min for particles (chlorophyll containing and others) ranging from ~3 to 150 um. Images from the IFCB were posted in almost real time on an intranet website (accessible on the ships network and automatically refreshed on a screen in the main lab). The phytoplankton population observed was dominated by small dinoflagellates and diatoms throughout EXPORTS with some larger diatoms observed less frequently. We collected > 2.4 millions individual images. Samples from both CTDs were ran to have a sense of the diversity and abundances with depth. IFCB runs were also used to document the effect of iron enrichment on local phytoplankton population in experiments performed by a variety of groups.

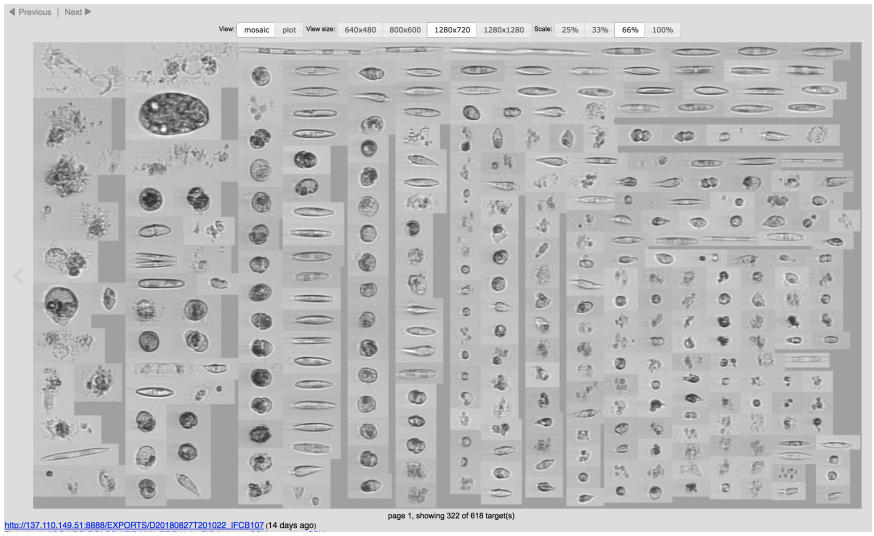


***Figure 3**. Typical IFCB mosaic of particles acquired in fluorescent mode (largest 322 out of 618 targets. Note pennate diatoms and a variety of dinoflagellates.*

Underwater Video Profiler (UVP5-HD)

The UVP5 provides high-resolution information on concentrations and compositions of large particles in the water column (> 100 **µ**m). We have obtained 84 vertical profiles with the UVP5 associated with all the SIO-CTD casts (including size distribution as function of depth), and, in addition, > 136,000 individual particle images. Images have been processed onboard and about half has been classified by taxonomic or functional group and that can, for example, be paired up with sediment trap and net tows performed by other groups. This is the first time we had a server with EcoTaxa on board a cruise which made the work of deriving vertical distributions of organisms significantly easier.

The dominating groups of particles included long diatom chains with thickness comparable to the UVP's pixel size (64 m), radiolarian (see figure, crustaceans (including copepods), pteropods and feces.

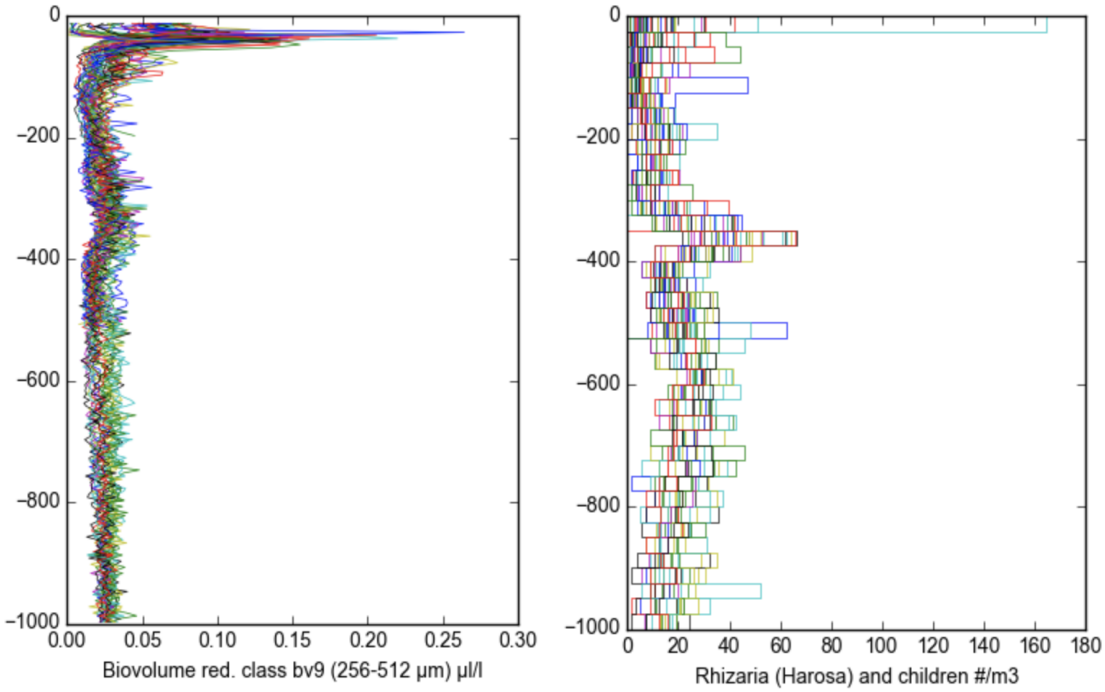


Figure 4. Depth distribution of Biovolume for particles in the 256-512 m size range (left panel) and number concentration of Rhizaria (primarily radiolarians, right panel) derived from the 84 profiles of the UVP..

In-Line IOPs

We have collected high-resolution measurements of IOPs along the trajectories to and from St. P (>600miles) and 26days at station. From these measurements, we can derive biogeochemical properties such as chlorophyll concentrations (derived from absorption measurements; Fig. 1 and size index (derived from particulate attenuation spectra). The diel cycle in cell size is clearly observed in these spectra.

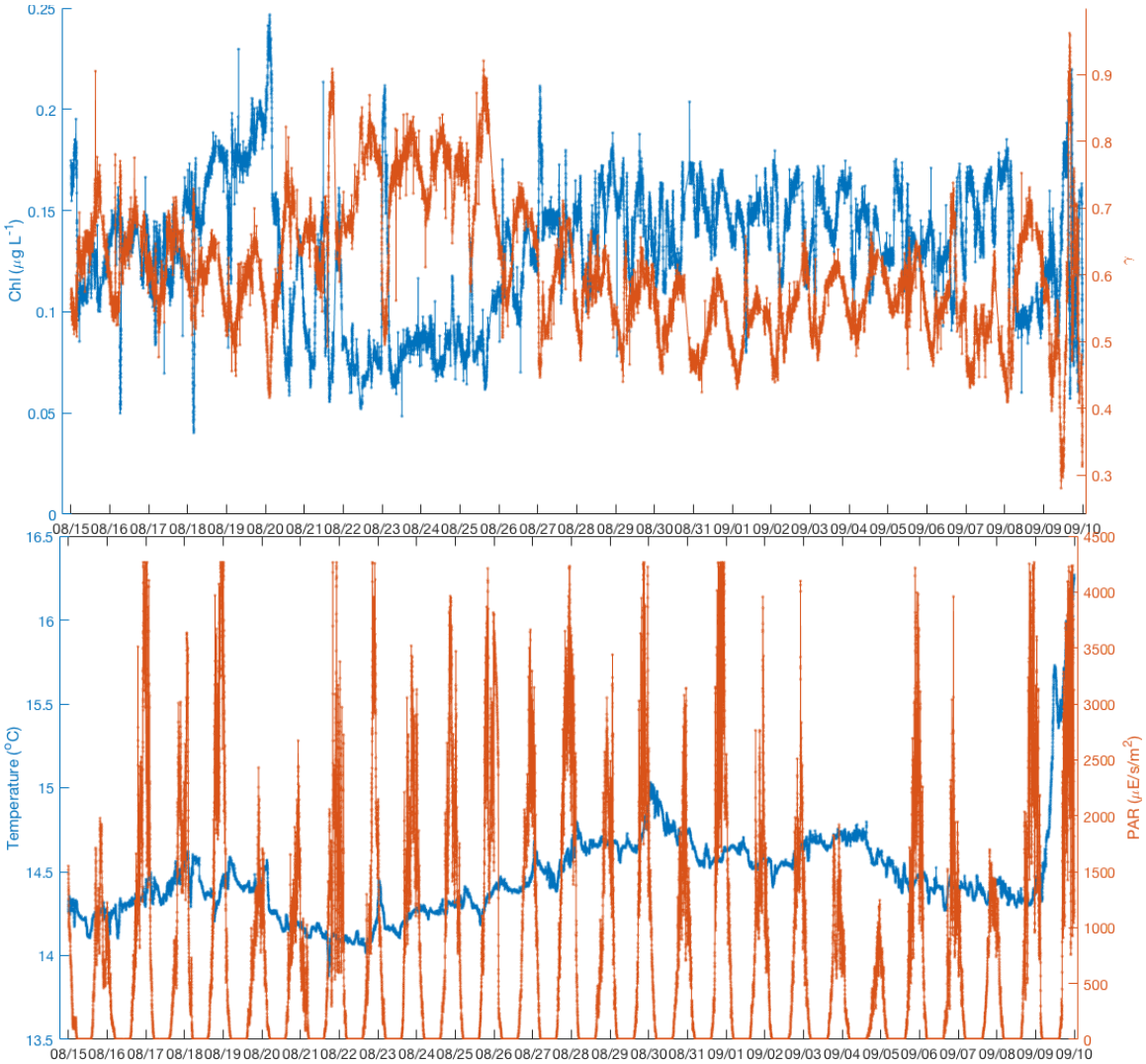


Figure 1. Near-surface chlorophyll concentrations (derived from particulate absorption line height at 676nm) and size-parameter (, derived from particulate beam attenuation, lower values means bigger particles) and associated temperature and PAR during the 3 epochs.

The differential particle volume distribution shows distinct double peaks in the PSD centered at 2.6 and 5.1 m throughout the Epocs (Fig. 2) with significant changes in magnitude correlated with chlorophyll.

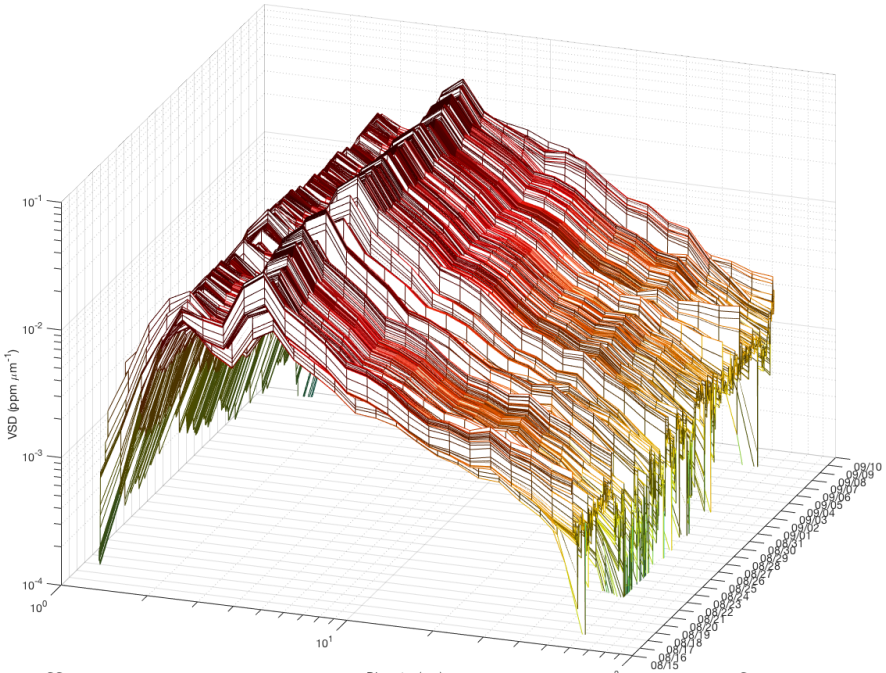


Figure 2. *Differential particulate volume distributions derived from the LISST in-line for the 3 Epochs. Note the double peak at* 2.6 and 5.1 m.

Slow Decent Rate Optical Profiler

We have obtained ~20 vertical profiles of the upper 100m characterizing both dissolved and particulate materials. Only the dissolved materials have been processed to date (Fig. 5). Note that typical uncertainties with this sensor are 0.01 m^{-1}. A combination of binning and careful calibration allows us to decrease this uncertainty by about a factor of 3.

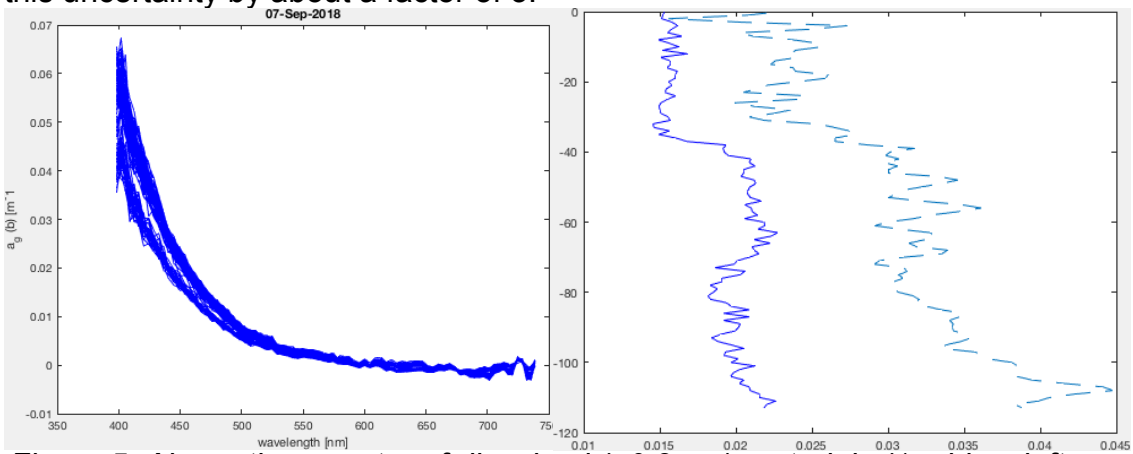


Figure 5. Absorption spectra of dissolved (<0.2 m) materials (1m bins, left panel) and absorption at 460nm as function of depth (right panel, solid line) measured with the SlowDROP. Dashed line represent fluorescence of DOM measured on the same package.

We collected and processed 35 profiles of spectral backscattering of the top 100m (e.g. Fig. 6) with the HydroScatt 6 which was on a different frame than the slow descent rate optical profiler.

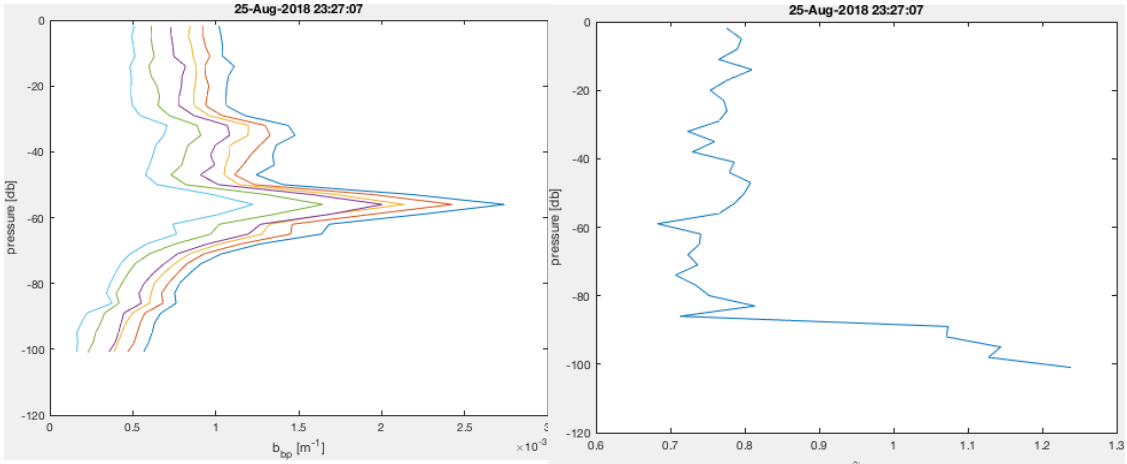


Figure 6. Values of backscattering at six wavelengths (400->700nm, left panel) and the spectral slope (right panel) as function of depth. For non-absorbing particles, the larger the spectral slope the smaller the associated particles.

Future analysis and collaboration with other teams

Most of the IOP measurements have been processed onboard and have been shared with other parties (up to the beginning of our ride back). They can provide a useful background of high resolution evolution of the upper-ocean.
Images from IFCB and UVP5 have been processed (i.e., background subtraction, blob identification and feature extraction) on board and uploaded to EcoTaxa on board. Upon return they will be shared with a wider audience on both the WHOI IFCB site as well as the Villefranche EcoTaxa website. The automatic and manual classification of individual particles was started on board and will keep going on shore.

We have already started a new investigation linking observed changes in time and depth observed with a spectral backscattering sensor (HS6) with changes in the community composition as observed with the BD Influx Flowcytometer.

EXPORTS Sub Arctic Pacific: R/V Revelle 1813

Cruise Report - Dissolved Organic Matter / Bacterial Interaction Group

Prepared by: Craig Carlson and Brandon Sephens

Principal Investigator: Craig Carlson
Group Participants: Brandon Stephens, Elisa Halewood, Keri Opalk
Cruise Participants: Craig Carlson, Brandon Stephens

Overview

Our group seeks to track and assess the temporal and spatial dynamics of dissolved organic matter, the flux of labile DOC through heterotrophic bacterial production, the compositional variability of DOM, as well as the mechanisms that control its accumulation, persistence and export in the subarctic Pacific. The overall cruise objectives are to:

•*Understand controls on the fractions of net primary production and net community production partitioned as DOC and DON*
•*Determine the flux of the most labile fraction of DOM required to meet carbon demand of heterotrophic bacterial production (i.e. bacterial carbon demand)*
•*Determine DOM bioavailability to microbes and the fraction of the seasonally accumulated pool that persists for weeks to months*
•*Assess DOM diagenetic state (compositional variations) over varying ecosystem / carbon cycling (ECC) states.*
•*Evaluate physical mixing of DOM out of the euphotic zone, its contribution to export and its fate*

The specific goals and activities include but are not limited to the following:

A. Resolve Temporal and spatial variability of DOC and DON: Bulk DOM profiles were collected daily from the surface 500 m and down to 1000 – 3000 m at least once per Epoch (~1,200 samples). These data will help provide high temporal resolution between ocean physics and suspended organic matter. The stocks of DOM will be merged with temporal data collected seasonally since 2016 from the Line P program. All data is pending sample analysis.

B. Understand controls on the fractions of net primary production and net community production partitioned as DOC and DON: DOC and DON must accumulate in order to be subsequently exported; predictability of the *complete* biological pump (POM + migrators + *DOM*) then requires predictive metrics for DOM accumulation. Temporal analyses of the observations from all cruises will quantify accumulation, which in turn will be tested for relationships against NPP, NCP, microbial community composition and biomass, nutrient status, and hydrographic conditions, which constitute the

probable ultimate controls on the accumulation of DOM. We will use data from this cruise as well as that collected seasonally from the Line P group to reconstruct an annual cycle of DOM accumulation, physical redistribution during mixing and subduction and subsequent removal by heterotrophic processes in the mesopelagic.

C. Determine the flux of the most labile fraction of DOM required to meet carbon demand of heterotrophic bacterial production i.e. bacterial carbon demand. Estimates of heterotrophic bacterioplankton production (BP) via 3H-Leu incorporation were performed during the daily afternoon cast to 500 m (~300 samples). Fig 1 is an example of 3H -Leu incorporation rates form EPOCH 1 and 2. BP estimates were elevated and variable within the surface mixed layer but remained elevated to ~100 m. There were occasional high BP rates measured at deeper depths which may correspond to vertical migrating organic matter flux or solubilization of sinking particles.

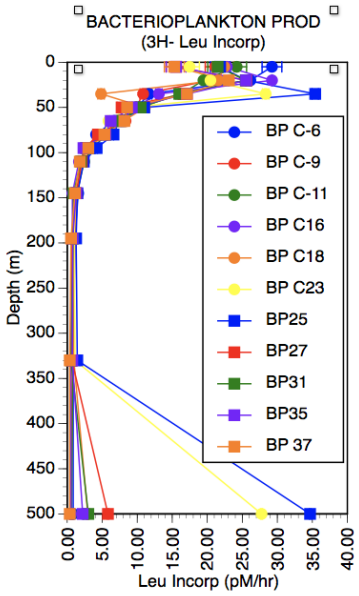


Fig 1. Vertical profile of 3H-Leu incorporation rates. This is a proxy for heterotrophic bacterial production.

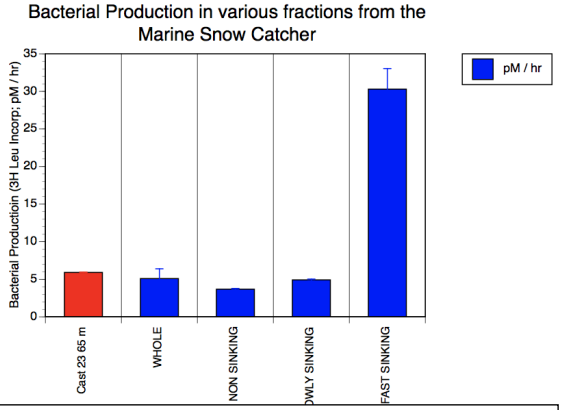


Fig 2. Estimate of BP from various sinking fractions of the marine snow catcher.

Estimates of BP were also performed on the various fractions of the marine snow catcher. We observed consistently higher rates of BP associated with the fast sinking fraction of the MSC fractions. Fig 2 is an example of elevated BP in the fast sinking fraction from 65 m. This elevated signature presumable is from activity associated with attached bacteria. In conjunction with the Passow Group we constructed a profile over 7 depth (20 – 500 m) of the various fractions of sinking and non-sinking particles.

Finally, BP rates will be combined with estimates of bacterial growth efficiency (BGE: see below) to provide estimates bacterial carbon demand which is a proxy of the flux of the most labile fraction of DOC through bacterioplankton.

D. DOM Lability and persistence: A total of 9 incubations of the natural microbial community were carried out for at least 7 and up to 18 days during the cruise to directly assess the fraction of accumulated DOM that is bioavailable to bacterioplankton on timescales of days to weeks, and to assess how much of the

accumulated DOM pool resists microbial degradation is available for horizontal or vertical export. Preliminary results from bacterial growth curves show that Surface (mixed layer depth) microbial communities tended to increase in cell abundance at the greatest rates and cell number (see Fig. 3, determined using a desktop flow cytometer courtesy of the Menden-Deuer lab). These experiments will ultimately be used, when coupled to organic carbon data to be measured on land, to determine microbial growth efficiencies on the naturally occurring dissolved organic matter. The Gifford lab also collected RNA samples during these incubations to identify the genes that were being expressed at key time points (e.g., exponential growth and stationary phases), which could provide invaluable insight into predominant pathways of the microbial communities during different phases of organic matter utilization.

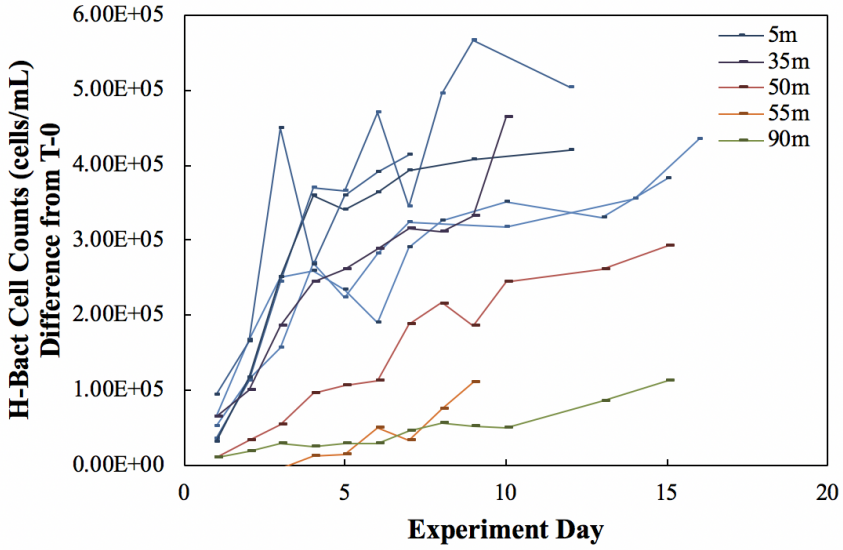


Fig 3. Bacterial growth curves from remineralization experiments performed at different depths on the EXPORTS cruise.

We also performed two additional microbial incubation experiments where we amended the natural surface communities with lysed cells and cellular exudates from two different organisms (synechococcus and chaetoceros, the latter of which were previously isolated from Line P and cultured by Adrian Marchetti) that were grown in ^{13}C-labeled media). The goal of these stable isotope probing experiments is

to track the labeled organic material to specific microbial lineages, but the immediate result from these experiments was enhanced microbial growth in the amendments relative to the natural control conditions.

The Carlson lab also carried out microbial incubations as part of collaborations with other groups in the science party. Several times during the cruise zooplankton and fecal pellets were provided by the Steinberg lab. One experiment involved zooplankton that we then incubated to test for dissolved organic carbon excretion. Another experiment involved isolating the fecal pellets of salps to quantify the change organic carbon concentration and organic matter composition of the fecal pellets.

Twice during the cruise material was taken from the Passow Lab's marine snow catchers. One such collaboration compared the microbial growth and carbon drawdown of material collected before and after settling. The intent of this experiment will be to test for influences of sinking processes on organic matter availability to the microbial community. A second incubation compared microbial abundances and organic carbon concentrations in the non-sinking particle fraction to the fast sinking particle fraction. The goal from this experiment will be to test for microbial growth efficiencies and community shifts on the fast sinking organic material. Preliminary results suggest cell numbers to have increased significantly with time on the incubations containing fast sinking particles.

E. **Chemical characteristics affecting lability**: Samples were collected during each EPOCH for analysis of DOM chemical characterization via pulsed amperometric detection HPLC (~125 samples) and high resolution mass spectrometry (~80 samples) analyses through collaborations with colleagues at Scripps Institution for Oceanography. Data Pending analyses.

F. **Contribution to export:** Estimates of the annual DOM export out of the surface 100 m and into the mesopelagic zone will be constrained by assessing changes in mesopelagic DOC inventories before and after deep convective mixing and will leverage DOM collection from EXPORTS asset deployment/ recovery cruises and seasonal samples collected during line P cruises.

G. **Microbial community structure and abundance**: DNA for amplicon sequence analyses were collected 2 -4 times per EPOCH through the upper 500 m (~80 samples). Samples will be archived until fund can be secured for sequencing analysis. Samples were also collected daily from profiles (5-500m) to estimate the abundance of the bacterial population (~300 samples). Several profiles were assessed by flow cytometry during the cruise using the Guava but DAPI slides were also prepared for quantitation by microscopy. DAPI counts pending analyses.

Table 1. of typical measurements collected at each station by UCSB DOM/Microbial interaction team.

						DNA 2- 4 X per

Nominal Depth	DOC / TDN	DOM Char. 2-4 X per epoch	Bacterial abundance	Bacterial production	Nutrients	epoch
5	X	X	X	X	X	X
20	X	X	X	X	X	X
35	X	X	X	X	X	X
50	X	X	X	X	X	X
65	X	X	X	X	X	X
80	X	X	X	X	X	X
95	X	X	X	X	X	X
110	X	X	X	X	X	X
195	X	X	X	X	X	X
330	X	X	X	X	X	X
500	X	X	X	X	X	X

Occasional samples to as deep as 3000 m were also collected for DOM and nutrients 1-2 X per EPOCH.

Our finalized results will be shared with the rest of the EXPORTS research team and be integrated with other ecosystem and biogeochemical measurements. They will then be disseminated to the broader scientific community via publications and presentations.

Many thanks to NASA for their support in this project, to Chief Scientists Debbie Steinberg and Jason Graff for their outstanding coordination, and to Captain Wes and the crew of the R/V Revelle for keeping the shipboard scientists safe.

Cruise Report RR1813: Menden-Deuer/Rynearson Group
PIs: Susanne Menden-Deuer, Tatiana Rynearson
Cruise Personell: Heather McNair, Françoise Morison, Ewelina Rubin

Objective and approach: The Menden-Deuer group's primary objective for EXPORTS was to quantify and characterize the loss of primary production through microzooplankton grazing. Three approaches were used to address microzooplankton grazing: dilution incubation experiments within the euphotic zone, size fractionated, long-term, incubations for depths below the euphotic zone, and genomic methods

Overview of work accomplished: Over the course of the cruise, the Menden-Deuer group accomplished all planned experiments.

Table 1 provides a summary of all dilution experiments performed. We followed two sampling schemes: 1) to quantify vertical structure in grazing pressure, water was collected throughout the euphotic zone at specific light-depths; 2) to isolate the effect of light on microzooplankton grazing and calculate an integrated, mixed layer loss that might be remotely sensible, water was collected from the surface and incubated at five light-levels. We performed eight vertical profiles experiments, and four surface-light experiments over the course of three epochs. In addition to these primary sampling schemes, we performed three experiments where water was collected from different depths within the mixed layer and incubated at a common light level to isolate the effect of community composition on grazing rate. To estimate rates of phytoplankton growth and grazer-induced mortality, samples were taken at the beginning and end of each experiment for both chlorophyll measurements and flowcytometry analysis of live samples.

We also worked with the Steinberg group to perform dilution experiments with additional macrozooplankton treatments, once with *Neocalanus* sp. copepods and once with euphausids.

Cell biomass samples for nucleic acid extractions (RNA and DNA) were collected at the beginning of each 24 h dilution experiment. These will be used to describe the composition of microzooplankton taxonomic units and to quantify gene expression related to microzooplankton grazing rates. In addition to in situ samples, microzooplankton isolation and culturing was attempted onboard to facilitate future lab experiments on grazing gene expression levels in individual microzooplankton species. To help characterize the diet of macrozooplankton, samples to analyze DNA of gut content of various zooplankton groups (including copepods, euphausids and salps) were collected from net tows with assistance from the Steinberg group.

Long-term deep-sea grazing experiments were performed at the beginning of each epoch to measure herbivory and bacterivory. Water samples from various depths from 50-300 m were split into two size fractions, >200 μm (whole water) and >1.2 μm (predator free) and incubated at 4 C in the dark for eight days. Differences in phytoplankton and prokaryote abundance within the two treatments were monitored using flowcytometry daily to see if removing predators

allowed bacteria abundance to increase and to see if phytoplankton abundance differentially decreased. Cell biomass samples were collected for DNA extractions to be used in SSU-amplicon sequencing to determine species community composition at the beginning and end of the incubations.

Preliminary Findings:

In situ chlorophyll concentrations (extracted) were relatively consistent across the three epochs, with surface (~5 m) averaging 0.25 (±0.06) μg L^{-1}. The contribution of the >5 μm cells to total chlorophyll varied between 16 and 44% and averaged 31%, with a relatively higher contribution of the >5μm in epoch 3 (Fig. 1).

Preliminary analysis based on bulk chlorophyll indicates that microzooplankton grazing rates were generally undetectable during epoch 1, with only 1/18 depth/light treatments showing grazing. During epoch 2, the occurrence of grazing increased with 10/24 depth/light treatments showing grazing. During epoch 3, 13/37 depth/light treatments showed grazing. The detection of grazing was patchy throughout the water column. Rates of grazing did not exceed that of phytoplankton growth overall, suggesting microzooplankton grazing alone was an insufficient mechanism to maintain the relatively constant chlorophyll concentrations observed throughout the epochs. However, these results are preliminary as chlorophyll data may need to be adjusted for potential changes in chlorophyll content of cells due to changes in light (photoacclimation) and/or iron exposure, using the flowcytometry data. Thus, phytoplankton growth and mortality rates may be revised. Flowcytometry analysis will also resolve rates of change in specific groups of nano- and pico-plankton and may reveal species specific differences in growth and/or mortality rates.

Additionally, plankton community species composition will be characterized using results from analyses of flowcytometry data, IFCB, and fixed (Lugol's) samples of whole seawater taken at the beginning of each dilution experiment for enumeration of microzooplankton.

Cursory examination of flowcytometry data from the long-term, deep-sea grazing experiments suggests that there was neither a difference in abundance of phytoplankton nor bacteria in the two size fractions. Further examination of flow cytometry data will provide specific rates of change in abundance for groups of phytoplankton, bacteria and predators.

The abundance of the microzooplakton species in collected water samples was too low to allow successful isolations. To obtain sufficient abundance of microzooplankton species for cell isolation, water samples were enriched with inorganic nutrients or specific prey-item (culture phytoplankton) and incubated for several days. These water samples will be transported to URI and used for further cell isolation and cultures of microzooplankton. Cell biomass samples will be used for nucleic acid extractions followed by quantitate PCR assay and Illumina SSU-amplicon sequencing

Table 1. Summary of dilution experiments performed during RR1813

Date	Epoch/Day	Event #	CTD #	Collection Depth	Incubation light

8/16/18	E1-D2	20180816.0905.001	SIO 007	73	1%
8/16/18	E1-D2	20180816.0905.001	SIO 007	35	10%
8/16/18	E1-D2	20180816.0905.001	SIO 007	23	20%
8/16/18	E1-D2	20180816.0905.001	SIO 007	8	65%
8/17/18	E1-D3	20180817.1145.001	SIO 010	7	0%
8/17/18	E1-D3	20180817.1145.001	SIO 010	7	20%
8/17/18	E1-D3	20180817.1145.001	SIO 010	7	40%
8/17/18	E1-D3	20180817.1145.001	SIO 010	7	65%
8/17/18	E1-D3	20180817.1145.001	SIO 010	7	100%
8/18/18	E1-D4	20180818.0911.001	SIO 013	73	1%
8/18/18	E1-D4	20180818.0911.001	SIO 013	34	10%
8/18/18	E1-D4	20180818.0911.001	SIO 013	23	20%
8/18/18	E1-D4	20180818.0911.001	SIO 013	6.5	65%
8/20/18	E1-D6	20180820.0906.001	SIO 020	9	65%
8/21/18	E1-D7	20180821.1104.001	SIO 024	22	20%
8/21/18	E1-D7	20180821.1104.001	SIO 024	8	20%
8/21/18	E1-D7	20180821.1104.001	SIO 024	8	40%
8/21/18	E1-D7	20180821.1104.001	SIO 024	8	65%
8/24/18	E2-D2	20180824.0901.001	SIO 033	73	1%
8/24/18	E2-D2	20180824.0901.001	SIO 033	40	10%
8/24/18	E2-D2	20180824.0901.001	SIO 033	23	20%
8/24/18	E2-D2	20180824.0901.001	SIO 033	13	40%
8/24/18	E2-D2	20180824.0901.001	SIO 033	5	65%
8/25/18	E2-D3	20180825.1243.001	SIO 036	10	0%
8/25/18	E2-D3	20180825.1243.001	SIO 036	10	20%
8/25/18	E2-D3	20180825.1243.001	SIO 036	10	40%
8/25/18	E2-D3	20180825.1243.001	SIO 036	10	65%
8/25/18	E2-D3	20180825.1243.001	SIO 036	10	100%
8/26/18	E2-D4	20180826.0903.001	SIO 039	74	1%
8/26/18	E2-D4	20180826.0903.001	SIO 039	41	10%
8/26/18	E2-D4	20180826.0903.001	SIO 039	24	20%
8/26/18	E2-D4	20180826.0903.001	SIO 039	6	65%
8/28/18	E2-D6	20180828.0931.001	SIO 046	58	5%
8/28/18	E2-D6	20180828.0931.001	SIO 046	28	20%
8/29/18	E2-D7	20180829.1147.001	SIO 051	60	5%
8/29/18	E2-D7	20180829.1147.001	SIO 051	21	20%
8/29/18	E2-D7	20180829.1147.001	SIO 051	6	20%
8/30/18	E2-D8	20180830.1202.001	SIO 053	8	0%
8/30/18	E2-D8	20180830.1202.001	SIO 053	8	20%
8/30/18	E2-D8	20180830.1202.001	SIO 053	8	40%
8/30/18	E2-D8	20180830.1202.001	SIO 053	8	65%
8/30/18	E2-D8	20180830.1202.001	SIO 053	8	100%
9/1/18	E3-D2	20180901.1009.001	SIO 060	50	5%
9/1/18	E3-D2	20180901.1009.001	SIO 060	35	10%
9/1/18	E3-D2	20180901.1009.001	SIO 060	20	20%
9/1/18	E3-D2	20180901.1009.001	SIO 060	10	40%
9/1/18	E3-D2	20180901.1009.001	SIO 060	5	65%

9/2/18	E3-D3	20180901.1206.001	SIO 064	50	1%
9/2/18	E3-D3	20180901.1206.001	SIO 064	45	5%
9/2/18	E3-D3	20180901.1206.001	SIO 064	40	5%
9/2/18	E3-D3	20180901.1206.001	SIO 064	35	5%
9/2/18	E3-D3	20180901.1206.001	SIO 064	30	20%
9/2/18	E3-D3	20180901.1206.001	SIO 064	25	20%
9/2/18	E3-D3	20180901.1206.001	SIO 064	20	20%
9/2/18	E3-D3	20180901.1206.001	SIO 064	15	20%
9/2/18	E3-D3	20180901.1206.001	SIO 064	10	20%
9/2/18	E3-D3	20180901.1206.001	SIO 064	5	20%
9/3/18	E3-D4	20180903.0903.001	SIO 067	75	5%
9/3/18	E3-D4	20180903.0903.001	SIO 067	35	10%
9/3/18	E3-D4	20180903.0903.001	SIO 067	20	20%
9/3/18	E3-D4	20180903.0903.001	SIO 067	7	65%
9/5/18	E3-D6	20180905.0917.001	SIO 074	9	0%
9/5/18	E3-D6	20180905.0917.001	SIO 074	9	20%
9/5/18	E3-D6	20180905.0917.001	SIO 074	9	40%
9/5/18	E3-D6	20180905.0917.001	SIO 074	9	65%
9/5/18	E3-D6	20180905.0917.001	SIO 074	9	100%
9/6/18	E3-D7	20180906.1138.001	SIO 078	58	5%
9/6/18	E3-D7	20180906.1138.001	SIO 078	16	20%
9/6/18	E3-D7	20180906.1138.001	SIO 078	6	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	50	1%
9/7/18	E3-D8	20180907.1201.001	SIO 080	45	5%
9/7/18	E3-D8	20180907.1201.001	SIO 080	40	5%
9/7/18	E3-D8	20180907.1201.001	SIO 080	35	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	30	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	25	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	20	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	15	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	10	20%
9/7/18	E3-D8	20180907.1201.001	SIO 080	5	20%

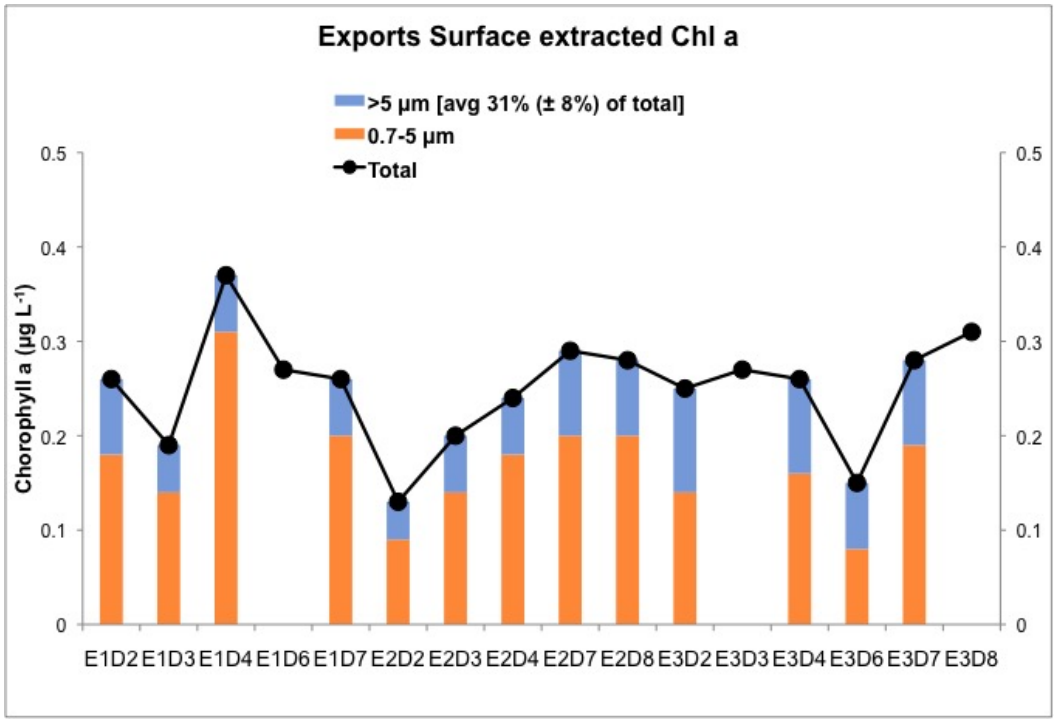


Figure 1. Size fractionated chlorophyll concentration (µg L^{-1}) from surface.

Many thanks and gratitude to NASA, members of the EXPORTS collaborating research teams and Captain Wesley Hill and the crew of the R/V Roger Revelle.

Cruise report for collaborative NSF funded team including PIs Bethany Jenkins (URI), Kristen Buck (USF), Mark Brzezinski (UCSB), with data and report contributions by collaborating PI Pete Morton (Florida State) and graduate students Kris Gomes (Jenkins) and Travis Mellet (Buck) and Postdoc Salvatore Caprara (Buck)

As OSP is a severely iron limited ecosystem, one of our team's broad goals was to facilitate trace element clean sampling in support of all of the EXPORTS research efforts on the Revelle. We conducted 28 casts with the trace metal sampling rosette (TMC) to facilitate water collection. Every other day of each sampling epoch (weather permitting) there was coordinated predawn water collection in the upper water column at predetermined depths matching 5 desired light levels (65%, 40%, 20%, 10% and 1 or 5% light). This water was utilized by several groups requiring trace element clean water for measurements requiring sample incubation that may be sensitive to trace element contamination (e.g. nutrient uptake rates, primary production, etc.). There were additional TMC casts to collect large volumes of water for groups conducting metatranscriptome profiling. We also conducted TMC casts for collection of vertical trace element profiles and intercalibration of measurements with the Canadian LineP program.

Our group's project focuses on diatoms, their contribution to export production at OSP, and being able to predict their likelihood of entry into an export pathway based on their physiological status. With our focus on diatoms, our objectives included measuring profiles of the rate of silica production at five depths across the euphotic zone to obtain integrated daily silica production rates (Brzezinski) and to determine the rate at which cells throughout the water column accumulate Fe (Buck group). Samples for biogenic silica (BSi) and nutrients were also taken by our team for all the in situ and experimental samples we collected.

Brzezinski conducted 7 profiles of Si production across the three EPOCHS and 8 experiments to assess the affects of added Fe and Si over 24 h on both silica production and carbon fixation rates at the 40% and10% light levels. BSi, POC and chl a concentrations were also measured on all experiments. All measures (profiles and experiments) were size fractionated in serial fashion through a 5 micron and 0.6 micron filter. DNA and RNA samples corresponding to the >5 μm and <5 and > 0.2 μm size fraction were also collected and archived (Jenkins group). The silica production profile work will be combined with profiles of carbon fixation and nitrate uptake to look at the stoichiometry (N:Si:C) of phytoplankton production. These data will be related to elemental fluxes in traps to examine differential remineralization rates of N, Si and C in the euphotic zone.

The short term experiments examined the effects of added Fe and Si on C fixation and Si uptake. Given the high concentrations of silicic acid at the OSP site we do not anticipate Si stress in this system. Fe stress is well known in the region. The only data we get at sea are preliminary values for the effects on C

fixation. No demonstrable effects of added Fe or Si on carbon fixation was apparent in either the >5 µm or 0.6-5.0 µm fractions in 8 experiments.

To measure Fe accumulation rates, the Buck group conducted nine 24-hour incubations with water from the pre-dawn trace metal casts occurring every other day during each epoch. For each of the five-targeted light depths, two 1 L polycarbonate bottles were incubated, one with ambient iron concentrations and another amended with 1 nM Fe^{57}. The goal of these short-term incubations was to determine the rate at which cells throughout the water column accumulate Fe. The use of the tracer in Fe^{57} coupled size-fractionation collecting > 5 µm and <5 µm and > 0.4 µm, can provide a first-order understanding of which organisms accumulate pulses of Fe to the environment by tracking it to the particulate phase on each filter. The response at each depth will be compared to the ambient concentrations of dissolved and particulate Fe at each depth to give context to the response observed.

Our collaborative team also conducted a series of experiments led by the Jenkins group to modulate diatom response to iron and silica addition over short and long time scales as well as longer time scale experiments to force the phytoplankton community into iron and silicate stress. One goal of these experiments is to follow the community level gene expression changes in response to perturbation using metatranscriptomics that will be valuable for interpreting the metatranscriptome profiles of the *in situ* communties sampled throughout the cruise.

Two short-term (24 hour) experiments were conducted with triplicate additions of Fe (1 nM), silicate (20 µM), or the co-addition of Fe and silicate along with unamended controls. The goal of the short-term experiments was to interrogate diatom genetic response to nutrient pulses over short time scales without shifting the composition of the community. Two long-term (6 day and 8 day) experiments were conducted where N and P were added to ensure sufficiency (20 µM NO_3^-, 1.25 µM PO_4^-) and either Fe (5 nM) or Si (20 µM) was added to drive the community into Fe or Si stress. In these long-term experiments, we anticipated a community shift and response to Fe addition and possibly Si addition. The first 6 day experiment we ran that spanned epoch 1 and 2 didn't seem to plateau in terms of chlorophyll response to iron addition (ultimately driving the community into Si stress) so a longer experiment (8 day) was conducted spanning epoch 2 and 3. In this experiment we followed response with 1 L bottles for chlorophyll measurements so we could pinpoint our time of harvest for maximal chlorophyll and nutrient drawdown. Experiments were conducted in deckboard incubators shaded to 50% light in 20L polycarbonate carboys to ensure enough biomass for downstream metatranscriptome profiling. We also conducted an additional two sets of long-term experiments in 4 L bottles in the deckboard incubators for the Buck group to follow Fe speciation and the organic ligand response in the various treatments. These experiments were also run for 6 and 8 days respectively with the following additions: 5 nM Fe, 20 µM Si, 5 nM Fe and 20 µM Si, and 1 nM Cu.

All experiments were initiated with water collected from the Buck lab surface towfish and incubations were set up and sampled using trace element clean procedures in our HEPA filtered bubble. Incubation experiment bottles were harvested for total, dissolved Fe, POC, nutrients and size fractionated chlorophyll, biogenic Si and particulate Fe (> 5µm, 0.6-5 µM) and rapidly filtered for size fractionated RNA and DNA (>5µm, 0.2-5 µM). We also followed the diatom community response with samples imaged on the IFCB in collaboration with Emanuel Boss and Nils Haëntjens and in archived samples preserved for microscopic enumeration in addition to DNA based measures.

The short term rate and grow out experiments did not show a clear response to Fe and it is not uncommon for short term nutrient uptake rates to be insensitive to added Fe, even in severely Fe-limited systems. In contrast, longer term grow out experiments conducted by Jenkins show a clear response to Fe and blooms of multiple *Pseudo-nitzschia* species. The >5 µM size fraction had the greatest chlorophyll increase (Fig. 1)

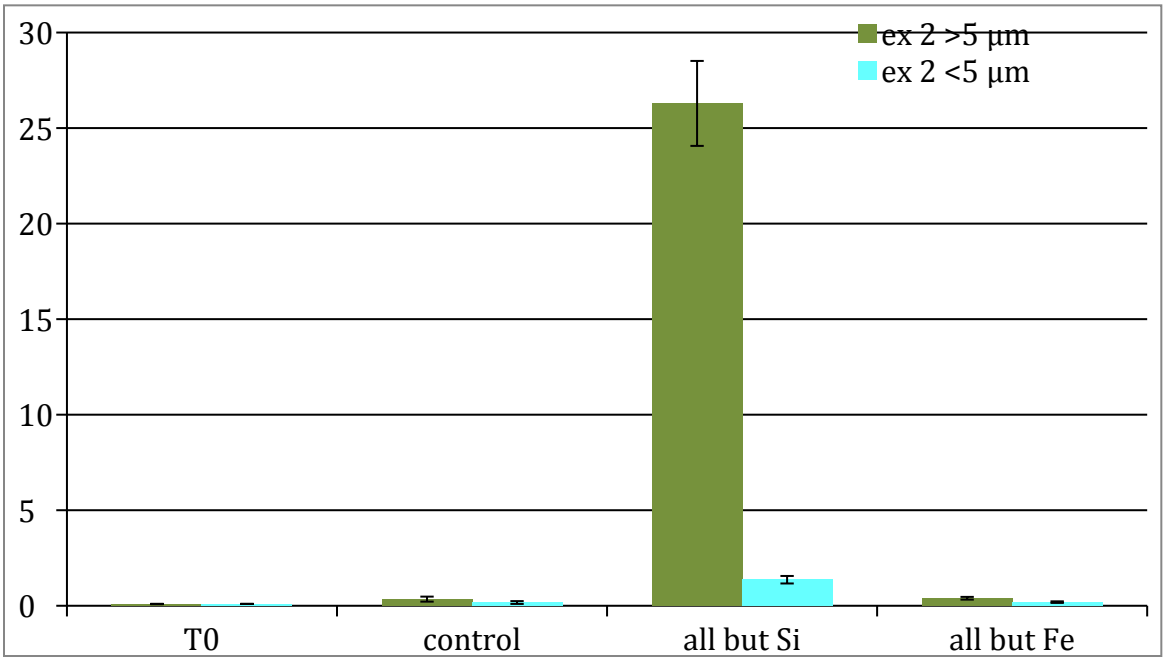


Figure 1. Response of phytoplankton community in the >5 and 02.-5 µm size fractions to 8 day additions of N, P and Fe (all but Si) and additions of N, P and Si (all but Fe). There is a pronounced growth response in the >5 µm size fraction to Fe addition and a modest response in the 0.2-5 µm size fraction. Very little growth in the control and all but Fe indicates our experiments were not contaminated with Fe.

Based on the observations that the large diatoms of the genus *Rhizosolenia* were abundant in trap samples we devised a way to use the snowcatcher samplers to quantify the abundance of these organisms in the euphotic zone. During two deployments samples were obtained at three depths and preserved for cell

abundance, genetic composition, BSi concentration and POC concentration. Similarly radiolarians proved abundant in traps. The trap team picked radiolarians from gel- traps for BSi analysis by Brzezinski. Those data together with the UVP enumerations of radiolarians in the upper 500 m will allow estimates of the standing stock of BSi in Rhizaria which can be compared to export in traps to obtain population turnover times.

As aerosol elements can be an important source of trace element addition, Pete Morton led aerosol sampling to quantify those inputs during our cruise period. Samples of aerosol particulate material were collected 14 August-9 September 2018, using two different filter substrates: Whatman 41 cellulose ester filter for major and trace element analysis (e.g., Al, Ti, Fe, Mn) and high purity silica QM-A for biological and organic analysis. Overall, six sampling events were completed, integrating ~30 hours of sampling time over 3-4 days per event. One event (3-6 September) collected 60 replicate Whatman 41 samples using all five samplers, in order to generate enough replicate samples to conduct an international aerosol intercalibration exercise (similar to that led by Morton et al. 2013). In addition, eight rain events were also captured using contamination-free rain samplers, which provided enough sample to analyze rain for major anions (e.g., nitrate, sulfate) and major and trace elements in unfiltered and sometimes (n=3) 0.2-um filtered rain waters. Overall, these samples will provide a time series of wet and dry nutrient deposition estimates to the HNLC eastern North Pacific Ocean.

The students (Gomes and Mellet) on our team wrote a blog post highlighting our work:
https://blogs.nasa.gov/earthexpeditions/2018/09/10/diagnosing-diatoms-do-anemic-diatoms-alter-north-pacific-food-webs/

EXPORTS Cruise Report

UNC Team Members: Adrian Marchetti (lead PI), Scott Gifford (co-PI), Weida Gong and Garrett Sharpe.

A. Scientific Achievements:

Marchetti Team (Adrian Marchetti and Weida Gong)

1. Routine sampling: During the cruise we collected productivity samples for estimates of gross and net primary productivity by measuring $H^{13}CO_3$ uptake following short (6 hr) and long (24 hr) incubations, new production by measuring $Na^{15}NO_3$ uptake following short and long term incubations, particulate carbon and particulate nitrogen and chlorophyll concentrations. All measurements were performed in triplicate at five depths throughout the euphotic zone corresponding to the light levels of 65%, 40%, 20%, 10% and 1% of incident irradiance (Io). Periodically, the 5% light depth replaced the 65% I_o light depth in order to sample at the particle maximum, as observed through flow cytometry measurements. Most samples were collected using the trace metal clean rosette however during Epoch 1, on days 4 and 6, productivity samples were collected using the conventional rosette due to rough seas preventing the deployment of the trace metal rosette. All measurements were also size-fractionated into >5 m and <5 m size classes. All chlorophyll analysis was performed on the ship (see figures and summaries below in section C). Isotope samples will be prepared at UNC and sent to the UC Davis Isotope Facility for analysis. Results are anticipated in the next four months.

In addition, at the shallowest depth in the mixed layer (either 65% Io or 40% I_o depending on cast) we collected samples for metagenomics, metatranscriptomics and 18S rDNA amplicon sequencing. During the days in which the 5% I_o depth was sampled, seawater for molecular analysis was collected at this depth. Sample preparation will take place at UNC along with sequencing of both DNA and RNA. Results are anticipated in the next four months. At both the surface depth and 5% I_o depth (depending on the cast), samples were also collected for analysis of viral abundance and composition to be performed by the Duhaime lab at the University of Michigan. Lugols preserved samples were also collected for large phytoplankton counts. Analysis of these samples will only occur on select samples depending on interesting features that are found in the sequence data.

2. Diel sampling: During epoch 1 and 3 we conducted higher frequency where over the span of 48 hours we collected samples every 6 hours to investigate diel patterns. Samples were collected for size-fractionated chlorophyll, metagenomics and metatranscriptomics, flowcytometry and large phytoplankton cell counts. Chlorophyll and flow cytometry measurements (by the Behrenfeld team) were performed on the ship. Molecular analysis will be performed back at

UNC. The time series was initiated on day 6 (epoch 1) and day 4 (epoch 3) with the routine cast. Subsequent sampling was performed from the ships underway seawater loop system. The time series ended following the routine sampling on day 8 (epoch 1) and day 6 (epoch 3).

3. Iron grow-out experiment: We performed one iron-grow-out experiment during Epoch 2. Seawater from a depth of 15 m was collected using the trace metal clean rosette. Seawater from four of the Go-flows was processed immediately to obtain the initial time point measurements. Remaining seawater was distributed into six, acid-cleaned 10L cubitainers where 5 nM $FeCl_3$ was added to three cubitainers and the remaining three served as unamended controls. The six cubitainers were then incubated in the 40% Io incubator for 4 days (96 hours). All size cubitainers were then harvested for all measurements as listed under the routine sampling measurements. Additional samples were collected for dissolved nutrients, biogenic silica and silica uptake (by the Jenkins team), photosynthetic efficiency, IFCB and flow cytometry (by the Behrenfeld team) and viral abundance and composition (Duhaime Lab). Chlorophyll measurements were performed on the ship. There was a 2.4-fold increase in the iron-amended treatment relative to the control. After 4 days, chlorophyll concentrations increased to 2.6 ug/L. The majority of the chlorophyll was in the >5 m size-fraction and primarily comprised of *Pseudo-nitzschia* spp. A similar time frame for this analysis is expected as with samples collected during the routine sampling.

Gifford Team (Scott Gifford and Garrett Sharpe)

1. Prokaryotic Genomics: DNA and RNA samples were collected from the euphotic zone to characterize the composition, functional potential, and activities of the prokaryotic microbial community. Sample collection occurred twice daily, primarily every other day of the epoch. This sampling schedule allowed us capture temporal variability over the course of the epochs as well as diel variability. While the major focus of our sampling was on the mixed layer, we also regularly took samples at the particle and chlorophyll maxima, and at several depths down to 110 meters. We expect this to be beneficial for characterizing the composition and activities of not only heterotrophic bacterioplankton, but also the diverse synechococcus populations that are abundant at these depths.

In addition to the water column characterizations, we collected molecular samples in conjunction with several other groups on board. In epochs 1 and 3, the Marchetti and Gifford groups collected samples from the inline filter every six hours for two days, which will be used to assess the diel transcriptional responses of the microbial community. Also with Marchetti lab, we collected samples during an iron grow out experiment to gain insight into how bacterial physiology and community composition might be effected by relief from iron limitation and substantial increases in phytoplankton production. Finally, we collected RNA samples from the Carlson lab remineralization experiments.

These samples will help identify the bacterial DOC activities behind the observed growth patterns and DOC drawdown. RNA was collected from four of these samples over the course of each epoch.

2. Community and Microbial Respiration: Microbial Respiration was quantified throughout the euphotic zone via oxygen drawdown assays. Similar to the molecular work, respiration measurements occurred twice per day, every other day of the epoch. Water samples were size fractionated when possible into community (whole water, no filtration) and free-living bacterioplankton (5 μm prefiltered) components. During the cruise we are able to obtain raw oxygen measurements, which will need to be processed post cruise to determine respiration rates. Preliminary analysis of one assay from 5m depth showed a community respiration rate of 0.7 μM O_2 day^{-1}, similar to surface oligotrophic values from Station ALOHA.

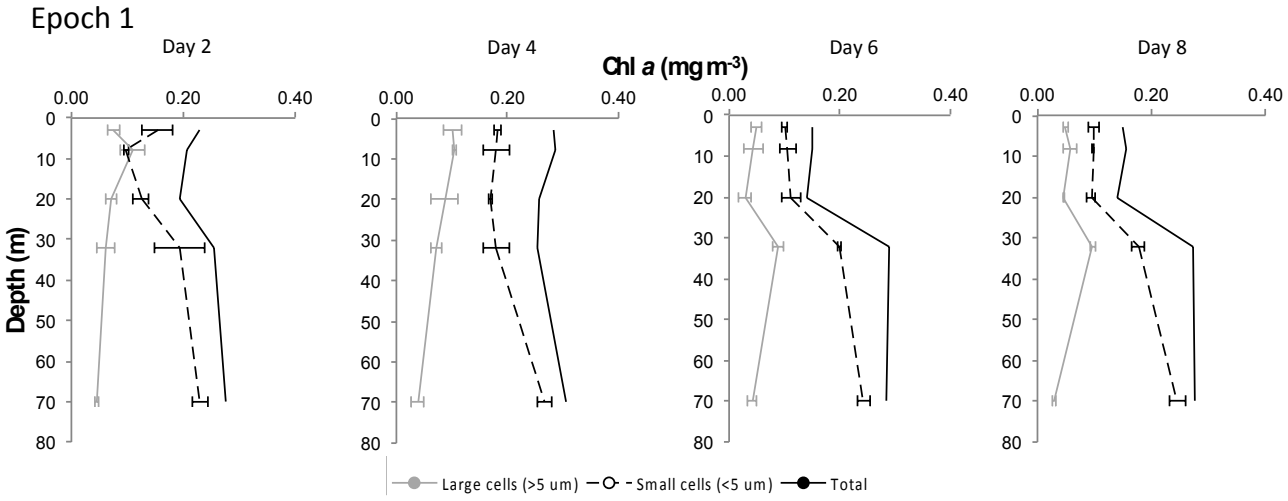
A major focus of this work will be combining the respiration rates with Carlson productivity and carbon demand measurements to provide a holistic picture of bacterial metabolism. Furthermore, we will also compare our measurements with NPP and NCP measurements being taken for EXPORTs. Preliminary discussions with James suggest that NPP can be around 0.8 μM C per day, which taken together with our preliminary respiration rate measurement of 0.7 μM O_2 day^{-1} suggest a tight recycling of primary production in this system although slightly autotrophic. These initial observations are supported by the Cassar EIMs measurements, which also suggest the system is slightly net autotrophic.

In addition to water column characterization, we also conducted respiration assays with the Passow marine snow catcher group on all three size fractions (SSP, NSP, and FSP) on day 6 of all three epochs. We also took respiration measurements from the RESPIRE trap water for the Santoro group. Both measurements will provide water column rates to compare to particle respiration rates, remineralization processes that may substantially influence export of carbon to depth.

Cassar Team (Weida Gong)

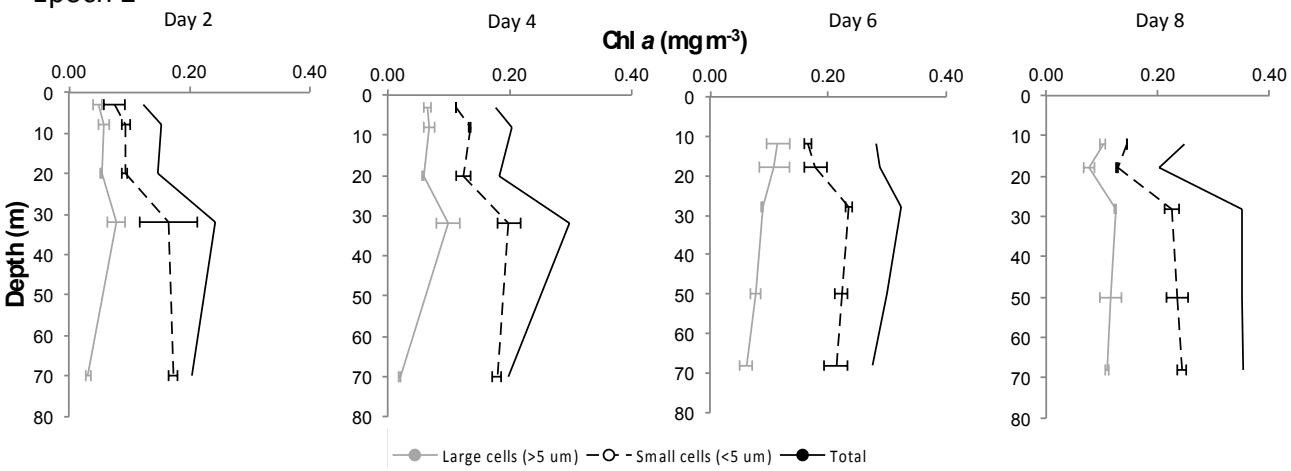
During the cruise, we have been measuring underway ΔO_2/Ar with Equilibrator Inlet Mass Spectrometry (EIMS) from ship's flow-through system. Because O_2 and Ar have similar physical properties, we can separate biological O_2 supersaturation from physical O_2 supersaturation to estimate net community production (NCP). Discrete O_2/Ar measurements were taken every 2~3 days from ~5m CTD Rosette to calibrate EIMS measurements and N_2O depth profiles were sampled twice per epoch to assess the influence of vertical mixing on O_2 concentration. Preliminary results from EIMS measurements suggest we have been sampling a water mass with slightly positive (1-1.2%) O_2 supersaturation over the past three epochs. NCP in units of mmol O_2 m^{-2} day^{-1} will be calculated after processing discrete samples back in the lab.

B. Preliminary data



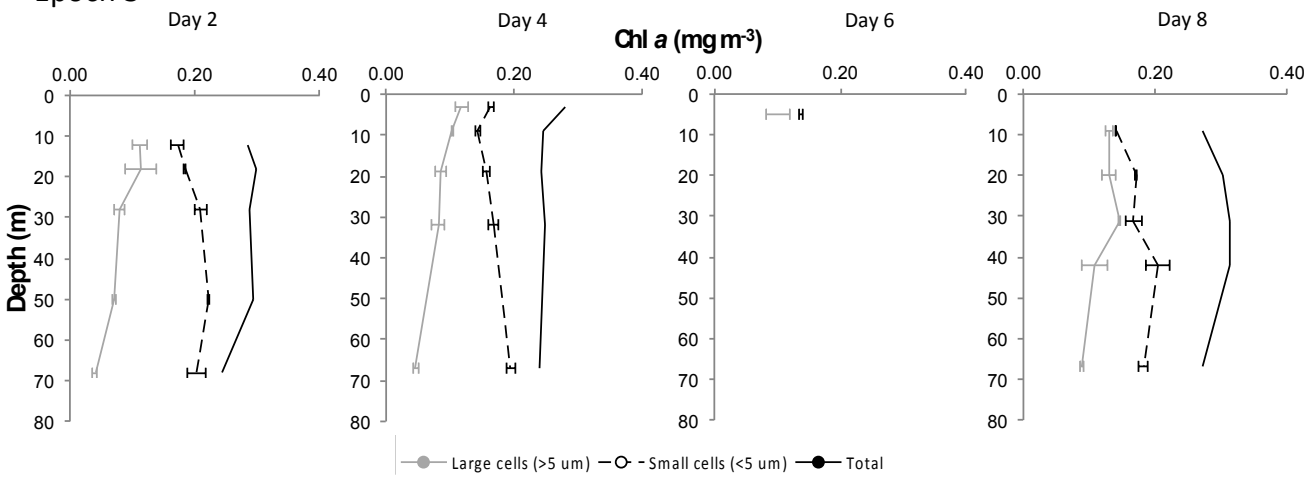
Epoch 1 Summary: We measure size-fractionated chlorophyll as a means to normalize our productivity rates (there are also other ways to normalize), although information can be gained from these measurements by themselves. The large size-fraction is typically made up of diatoms and dinoflagellates whereas as the small size-fraction is made up of eukaryotic flagellates (chlorophytes, haptophytes and perhaps some cryptophytes) and cyanobacteria (Synechococcus). In the mixed layer, the proportion of large cells was a third to half of the total and appeared to drop in the latter half of the epic. There is significant day to day variation in large cell contributions to total chlorophyll which is surprising given the rather constant mixed layer depth during this epoch. Perhaps this could be due to grazing variability? Below the mixed layer, but still within the euphotic zone, small cells dominate (as also seen with flow cytometry). Total chlorophyll in the mixed layer is below the historical average for Station P at this time of year which typically is between 0.3 and 0.4 mg m^{-3}.

Epoch 2



Epoch 2 Summary: During the first half of epoch 2, chlorophyll concentrations throughout the euphotic zone remained relatively low, with some of the lowest values obtained within the mixed layer throughout the entire cruise on days 2 and 4. The large size fraction continued to account for a small proportion of total chlorophyll concentrations, making up approximately one-third of the total chlorophyll concentrations. From day 6 onwards, chlorophyll concentrations slightly increased, particularly below the mixed layer and was dominated by small cells. Maximum chlorophyll concentrations were obtained on day 8 below the mixed layer and were mainly comprised of small cells.

Epoch 3



Epoch 3 Summary: During Epoch 3, chlorophyll concentrations were fairly uniform throughout the euphotic zone. In fact, on day 2, chlorophyll concentrations were highest in the mixed layer, although only slightly. Large cells contributed between one-third and one-half of total chlorophyll concentrations, with proportions increasing by the end of the epoch, especially in the mixed layer. Chlorophyll concentrations were highest on day following stormy weather on day 6, which restricted our sampling to the ships underway seawater system.

Cruise Report – Passow Lab

Water for TEP measurements was sampled, filtered, stained with Alcian Blue and frozen for later TEP analysis, on five days (8/21; 8/26; 8/30; 9/3 and 9/6) from 10-12 depths each, between the surface and 500 m (the midday/ afternoon SIO-CTD). TEP concentrations in the water column will be related to POC, Chl. a and heterotrophic activity measured from the same CTD.

The marine snow catchers (MSC) were deployed 11 times at 2-3 depths (between 20 and 500 m) to collect sinking velocity fractionated samples (thanks to Stuart and Nils for continued deployment help). After a two hour settling time, NSP (non-sinking particles), SSP (slow sinking particles) and FSP (fast sinking particles) were collected separately. The FSP fraction was scanned for marine snow sized (> 0.5 mm) particles, but none were ever observed in any of the samples, other than some artificially looking particles likely stemming from the ship (paint chip, incinerated pieces). Subsamples of the three fractions (NSP, SSP and FSP) were taken to measure POC/PON (always), fixed for enumeration (always, some initial measurements in FlowCam, Francoise); BSI, PIC, TEP (each 1-2 times per epoch), aggregation potential (1 time per epoch); bacterial production (1-3 times per epoch: Craig C.), respiration (1 time per epoch, Scott G.), prokaryotic and eukaryotic omics (each 1 time per epoch, Ewelina and Alyson), lithogenics (1 time per epoch, Phoebe). An additional cast of two replicate MSC was taken to collect enough water for a remineralization experiment (C. Carlson). In casts without production or respiration measurements, a polyacryl gel petridish was positioned in the tray to collect FSP and preserve their shape. Two further deployments of 3 MSC each were conducted to test the impact of settling times. All three MSC were deployed at the same depth (80m, 60m), but settled for 1, 2 and 4 hrs respectively. Samples for POC/PON, BSI, PIC and TEP in the NSP and sinking (SSP+FSP) fractions were collected and prepared. The MSC were also used 3-4 times as Mega-niskin bottles and the water drained, without prior settling, through a cod end (64 um) to enrich *Rhizosolenia* and other rare, large, but persistent diatoms important for flux (collaboration with Mark and Bethany).

Respiration rates and sinking velocities of individual salp fecal pellets were investigated to help estimate the importance of their flux. Pellets were photographed and sized before measurement of either respiration rate or sinking velocity. Respiration rate of was determined using the microoptode system from Unisense. Sinking velocity will be calculated using the orbit method from video footage of individual pellets in 1.2 liter rolling tanks, after solid body rotation was established. After the respective analysis each pellet was filtered onto a GFF for later POC analysis.

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Cruise Personnel: A. Santoro

Our research objectives on this cruise were to: (1) Quantify microbial respiration on sinking particles using an *in situ* particle capture incubation device (RESPIRE); (2) Determine changes in microbial community composition on sinking particles as a function of depth, sinking speed, and particle type and in comparison to non-sinking particles; and (3) Determine the role of sinking particulate matter as a source of nitrogen for nitrification.

In support of the first objective, RESPIRE traps were deployed on the drifting Surface-Tethered sediment Trap (STT) array during all three epochs at the three shallowest depths (approximately 95, 145, and 195 m). The RESPIRE traps employ an indented rotating sphere to collect sinking particles but exclude larger metazoan grazers (zooplankton). The sphere rotates at a pre-programmed interval for approximately 24 h, depositing particles in a lower chamber equipped with an oxygen optode to monitor oxygen drawdown associated with microbial respiration on the sinking particles. Technical issues during the first two deployments resulted in no oxygen data from Epoch 1 and Epoch 2, but did recover sinking particulate matter that will be used for molecular characterization of the particle-attached microbial community. During Epoch 3, we successfully collected oxygen data during the deployment at all three traps (Fig. 1). These data point to a strong biological respiration signal at the shallowest two depths, but an oxygen timeseries dominated by diffusion of surrounding low-oxygen water into the deepest trap. The data will be compared with changes in POC to evaluate the role of microbial respiration as a sink for carbon in the upper mesopelagic carbon budget. Splits of the 'live' (*i.e*. unpoisoned) sinking material from RESPIRE were given to several groups aboard the ship to evaluate bacterial production (Carlson), bacterial respiration (Gifford), lipid composition (Van Mooy), and particle size-spectra and fluorescence via flow-cytometry (Graff).

In support of the second objective, in collaboration with Colleen Durkin (MLML), over 100 RNALater-preserved samples were collected from the STT and the Neutrally-Buoyant Sediment Traps (NBSTs). These samples will be analyzed using 16S rRNA amplicon sequencing to characterize the particle-attached microbial community. The data will be compared with the 16S rRNA data from the RESPIRE-trap samples, which provide a window into how microbial communities on sinking particles change as the particles are degraded. Samples from the Marine Snow Catcher were also collected during every epoch, again to be analyzed using 16S rRNA sequencing to evaluate microbial community composition on particulate mater with different sinking speeds. Individual particles collected by Durkin were also preserved for microscopy to determine microbial abundances on sinking particulate matter and molecular analysis to example community structure on an individual particle level. All the particle-

associated samples (STT, NBST, MSC, and RESPIRE) will be compared to the microbial community of a concurrent set of samples (~70) collected from the mesopelagic water column.

Finally, to evaluate microbial processes contributing to the attenuation of nitrogen flux, nitrification rates were determined using ^{15}N tracer at 6-10 depths twice per epoch. Nitrification is the microbial oxidation of ammonia to nitrate and is the final step in the remineralization of organic nitrogen. The resulting 315 timepoint samples will be analyzed at UCSB using the denitrifier method for $\delta^{15}N_{NO3}$. Samples were also taken in collaboration with P. Rafter (UC Irvine) for analysis of natural abundance levels of $\delta^{15}N_{NO3.}$ As accurate low-level measurements of ammonium ($[NH_4^+]$) are required for calculating the nitrification rates, we also measured low-level $[NH_4^+]$ in the upper 500 m at least three times per epoch (Fig. 2). The $[NH_4^+]$ at OSP are consistent with historical data from the region and elsewhere in the stratified N. Pacific, showing low concentrations in the surface, a subsurface NH_4^+ maximum of ~330 nM near the base of the euphotic zone, and below the detection limit of 10 nM by 95 m. The depth-integrated nitrification rates will be compared to PON flux attenuation profiles from the water column and STT. Ultimately, we hope to construct a nitrogen budget for the upper mesopelagic. In support of this, we also conducted experiments in collaboration with the Steinberg group to measure rates of zooplankton excretion as an alternate source of N for nitrification. These measurements will also be useful to other groups on the cruise. Nitrification rates will be used to correct new production estimates based on NO_3^- uptake (Marchetti group), as any NO_3^- produced from nitrification results in an overestimate of new production. Nitrification is also a potential source of nitrous oxide (N_2O) in the mixed layer, thus our rates will help aid in the interpretation of N_2O profiles that are being used to estimate upwelling rates into the mixed layer (Cassar).

The late timing of the funding decision for our project created severe time constraints on the construction and testing of the RESPIRE traps, largely due to the lead-time required for the Aandera oxygen optodes. The instruments were delivered to UCSB approximately 10 days before the cruise shipment date and no in-water testing to simulate the deployment conditions was possible prior to the cruise. As a result, we were in the unfortunate position of deploying the traps for the first time on this cruise. We will ensure that technical issues related to the control software as well as operational issues associated with oxygen equilibration during the deployment.

FIGURES

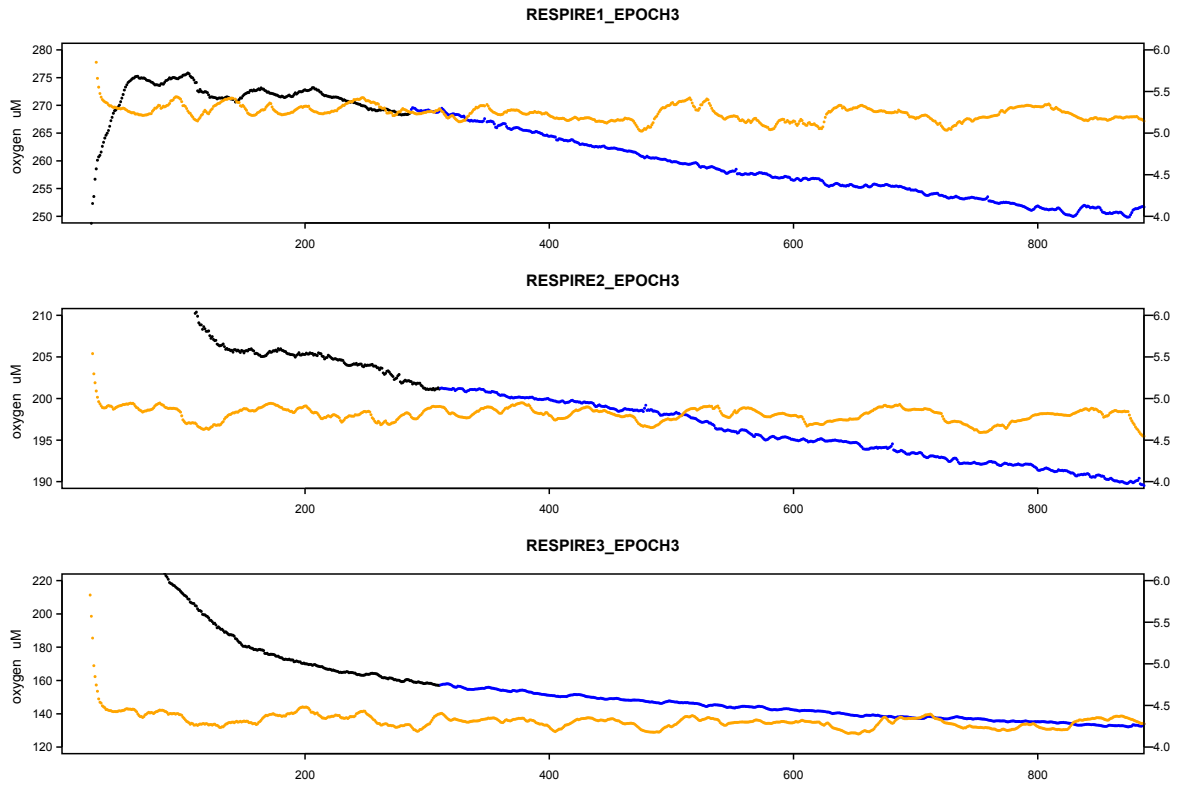


Figure 1. Timecourse oxygen during in situ incubations with sinking marine particulate matter using the RESPIRE traps during Epoch 3 at 105 m (top), 155 m (middle), and 205 m (bottom). The black trace indicates the particle collection phase and the blue trace indicates the incubation phase. Temperature is shown in orange. Oxygen concentrations in the trap were steady at the end of the collection phase at 105 and 155 m, suggesting the oxygen drawdown during the incubation phase represents microbial respiration. At the third depth, oxygen concentrations had not stabilized by the beginning of the incubation phase suggestion the drawdown over the deployment was due to molecular diffusion and/or mixing.

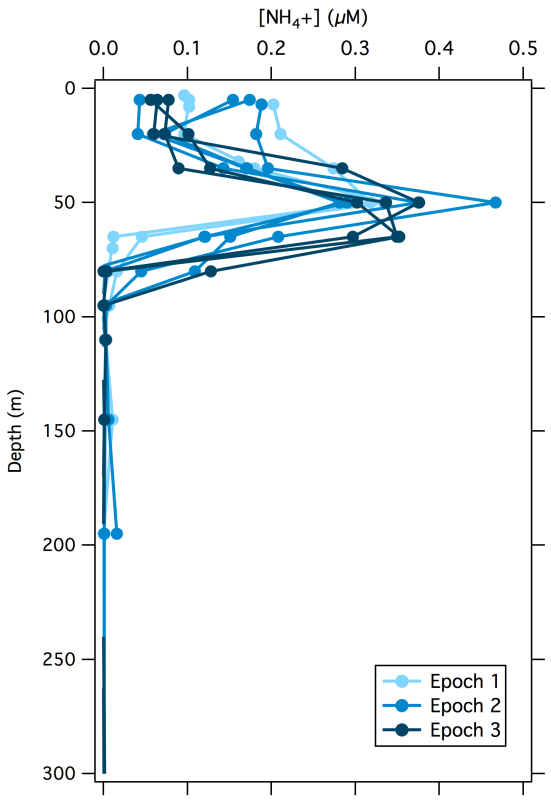


Figure 2. Shipboard low-level ammonium (NH_4^+) profiles measured during R1813 showing a characteristic subsurface maximum in NH_4^+ at the base of the euphotic zone.

Hydrograpy Team

Cruise Participants: Stewart Halewood, Jason Graff, Brian VerWey, James Fox
Report prepared by Stewart Halewood & Jason Graff

The Hydro Team performed multiple duties and sample collection in support of the EXPORTS science goals. The duties included: 1.) Perform freefall profiles with the AOP Profiler, recording Apparent Optical Properties (AOPs) at each station to match up with satellite ocean color observations and to provide local ocean light levels to allow others to tune their incubators. 2.) Collect samples for phytoplankton absorption (A_P), chlorophyll (Chl) and high-performance liquid chromatography (HPLC) samples. 3.) Oversee the regular CTD operations and record all SIO Rosette parameters. Do initial processing of CTD profiles during cruise to allow use of data by researchers during operations.

Results:

1.) 11 Full depth profiles of AOPs to 95m were performed when weather allowed. This data was recorded and Light levels at a range of percentages were provided so incubators could be set up to mimic ocean light level conditions. The data will be further processed back at UCSB.

On average the 1% light level was between 65-70m.
A chlorophyll max was apparent on every profile in the 470nm channel.

2.) As part of the Hydro team the following numbers of samples were collected, filtered, and frozen or stored at appropriate conditions until analysis at the designated labs.

- HPLC X 240
- CHL X 173
- AP X 244
- Colored dissolved organic matter X 244
- Total particulate organic carbon X 250
- Nutrients X 300

3.) In total 84 CTD profiles were taken with the Revelle SIO Rosette package. varying in depth from 500-3000m. All profiles were processed onboard and the data was made available to scientists daily. This data will be available for further use and can be reprocessed back at UCSB if required.

Cruise report – Sediment Trap and WireWalker group
Estapa, Buesseler, Durkin, Omand

Objectives and summary

Our project group's goals for this cruise were to sample sinking particles at 5 depths in the upper 500 m of the ocean during each of three 8-day sampling "epochs". To test the hypothesis that biology drives temporal variability in flux, we also characterized water column physical structure and particle properties at high vertical and temporal resolution using a WireWalker deployed alongside the trap array. We collected samples for bulk fluxes of particle mass, major bioelements (organic and inorganic C, N, biogenic Si), flux tracers (^{234}Th and ^{210}Pb/^{210}Po), lipids (for Van Mooy/NSF), stable isotopes (for Close/NSF), and lithogenic trace elements (for Lam/NSF). We also collected Optical Sediment Trap data to estimate the time-resolved flux variability, and collected imagery and sequencing samples from polyacrylamide gel traps to determine sinking particle identities and size distribution by microscopy. By linking the subsurface trap drift trajectories to measurements and models of the physical particle field, we will be able to connect our subsurface particle flux observations to simultaneous biological rate and ecosystem measurements in the euphotic zone and upper twilight zone, and to the ^{234}Th flux fields derived from the extensive CTD survey work on the R/V Sally Ride..

Cruise activities

During the North Pacific EXPORTS field campaign, we carried out three rounds of 5 day sediment trap deployments, with all successfully returning samples. We deviated somewhat from the pre-cruise trap schedule in response to conditions encountered at sea, shifting timings during Epoch 1 to avoid severe weather during recoveries, and lengthening deployments of shallower NBSTs during Epoch 2 in response to generally low flux conditions.

Surface tethered traps

The surface tethered trap (STT) array was successfully deployed and recovered three times. Minor issues with trap burnwires and rubber lanyards securing trap lids were encountered during Epoch 1 and remedied successfully for Epochs 2 and 3. A more significant challenge was that the wave-damping bungee near the top of the array parted during the severe weather in Epoch 1, likely due to chafing of the bungee by the parallel Amsteel backup line. The array was successfully recovered via the backup line. For subsequent deployments we swapped in our spare bungee, and replaced the original Amsteel backup with a spare length of Vectran cable. The upper termination of the 15 m cable immediately below the bungee was also damaged and had to be replaced with a 25 m cable, which increased the depths of all STT traps by 10 m for Epochs 2 and 3. The upper three STT frames (95/105 m, 145/155 m, and 195/205 m) also carried RESPIRE traps (Santoro/NSF), and prototype time-lapse cameras looking up into the gel trap tubes (Omand). The three cameras were successful during all Epochs, capturing a photo of the gel every 20 minutes. The cameras

also indicated that the arrays were tilted slightly by the off-center RESPIRE weight, which will be counter-balanced in future deployments.

Neutrally buoyant sediment traps
Neutrally buoyant sediment traps (NBSTs) were successfully deployed in all 3 epochs, but met with some challenges from adoptions of new equipment designs. Two NBSTs were of an older design based on the SOLO float platform (SOLO-NBSTs) and the remainder were of a new design, based on the APEX float platform (APEX-NBSTs). Newly-redesigned trap tube bottoms were found to leak rapidly in air when filled with cold (~4°C) water, probably due to mismatched thermal contraction properties of different plastics. This was remedied by filling the tubes with filtered surface seawater (~14°C) instead of water collected from the target deployment depth. However, the remedy caused NBSTs to linger at the surface after deployment, which was especially problematic for APEX-NBSTs. NBST-300 was unfortunately lost during pre-Epoch 1 test deployments, likely due to the float over-retracting while trapped at the surface, then descending too rapidly into cold water, where thermal contraction prevented the float from correcting itself. In subsequent deployments we attached a 46 g drop weight to each NBST via a 20-minute dissolving link which helped the traps descend safely and quickly from the surface.

In two instances APEX-NBSTs did not resurface as scheduled. In Epoch 2, NBST-304 was inverted by the ship's prop wash during deployment which probably fouled its 20 min drop weight. It was unable to resurface until after the weight fell off during its second 5 day cycle (cause unknown). In Epoch 3, problems with the Iridium message queue prevented NBST-302 and -303 from entering recovery mode for pickup after they resurfaced. They descended for another 5-day cycle and were picked up by the R/V Sally Ride on 9-Sept. In both these cases, samples were collected after trap recovery, and lessons learned will prevent these issues from arising again.

WireWalker
The Wirewalker ballasting was tested in port and observed to rise slowly to the surface. It was deployed (0600 Day 1) and recovered (1500 Day 8) from the ship without any problems for all three Epochs. On Epoch 1, the WW did 5 profiles but rose very slowly through the fresh/warm surface layer and eventually became suspended about 5-20m below the buoy. It is not totally clear why, but probably has something to do with being ballasted imperfectly. Between Epoch 1 & 2 another piece of buoyant foam was added, and this seemed to solve the problem. During Epochs 2 and 3 the WW walked throughout and made about 600 profiles total to 500m each time. During Epoch 1 & 2, a preliminary check of the data suggested that the sensors (CTD, O2, bbp, Chl F, CDOM F, PAR on profiler, beam attenuation, PAR on buoy) worked as expected. The O2 showed a linear, correctable drift. During Epoch 3, the O2 sensor gave some very strange (negative) numbers and did not show an expected pattern. There were three calibration (Inter-ship) casts conducted next to the WW, one per Epoch. Also the

WW sensors were attached to the SIO CTD for the final cast to 550m and will provide a sensor-to-sensor intercomparison.

Samples

Bulk fluxes – TC,OC,bSi,Mass, ^{234}*Th,* ^{210}*Pb/Po, Ba*

After collection, trap samples were split into 1/8 fractions using a custom made rotary splitter. This was done for 29 trap samples and 5 blanks, resulting in 280 separate samples for distribution for chemical analyses noted above. Prior to splitting, zooplankton "swimmers" were removed first by gravity filtering through a 335 micron screen, and then the screens were picked under a microscope to further separate swimmer material from flux components. Swimmer material varied greatly in terms of abundance, and types of organisms during each epoch and with depth, with quite high levels found in mid depths associated with amphipods. Later, on-board analyses included detection of ^{234}Th, with close to 100 samples (triplicates from each trap platform and depth) beta-counted at sea, which gave us our first indication of the variability in flux vs. depth.

Individual pellets and zooplankton

At sea, several samples consisting of sorted fecal pellets and zooplankton were provided to our group from Steinberg's team, for analyses of individual levels of ^{234}Th and C associated with specific particle types. The thought is to use these particle-specific Th and C contents (e.g., for individual salp pellets), and the number of such particles identified in the gel traps, to estimate the fraction of the total C and Th flux associated with specific particle types relative to the bulk material.

Gel trap imagery

All trap arrays carried one collection tube with a polyacrylamide gel layer in the bottom. All gel layers were imaged by light microscopy upon recovery. These images will be used to quantify the size distribution of sinking particles and the identity (i.e. fecal pellet, aggregate, organism) of each particle. Approimately ~18,000 images were collected in total.

Sequencing – bulk and individual particles

Individual particles were isolated from each gel layer and frozen at -80 degrees Celsius. These samples will be used for genetic analyses: the 18S and 16S DNA sequence fragements will be sequenced to identify the genetic remnants present within individual paritcles. Approximately 400 particles were isolated.

Preliminary observations

Bulk fluxes

Preliminary flux data during the cruise included initial counts of ^{234}Th fluxes from the traps (Figure 1). These showed modest fluxes during Epoch 1, decreasing to low flux during Epoch 2, and possibly higher again during Epoch 3. Agreement between trap types was good in Epoch 1, at all depths except 145 m. During

Epochs 2 and 3 the STT traps collected more than corresponding NBSTs. One possibility is that traps were increasingly spatially offset from one another as the epochs progressed (Figure 2).

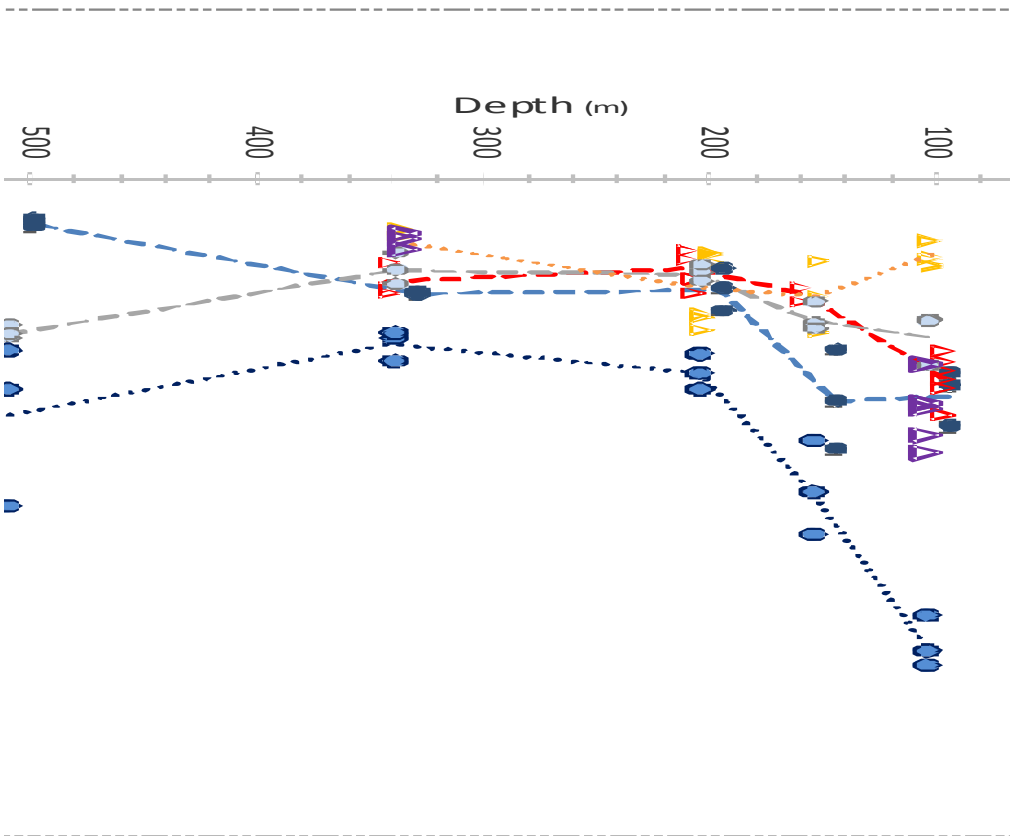
Gel trap observations

Particle flux during epoch one appeared to be dominated by large, long fecal pellets and salp fecal pellets. Rhizosolenia diatom cells could be observed inside the salp fecal pellets and as individual cells in the gel. Rhizaria cells (radiolarians and Phaeodarians) were abundant in the gel, as well as small “mini-pellets” which we think are formed by the feeding vacuoles of these rhizaria. Particle flux appeared to decrease during epoch 2, with far fewer fecal pellets and no salp pellets. Particle flux increased dramatically during epoch 3, with the reappearance of both pellet types and detrital aggregates. Rhizosolenia diatoms were still sinking and rhizaria were still very abundant in the gel traps. Particle flux appeared to attenuate rapidly with depth during epoch 3.

Wirewalker observations

The Wirewalker drifted in a tight pattern with the STT, generally staying within 1 mile or less. The drift was dominated by inertial circles and a north and eastward flow. The discontinuous data (see the decrease in deep Chl F between the end of Epoch 2 and the start of Epoch 3) suggest that the WW was seeing variability due—at least in part—to spatial gradients. There was evidence of diel cycles in all of the optical sensors, and variations with depth that were consistent with the observations made from the SIO CTD casts.

Figures


Depth (m)
500
400
300
200
100

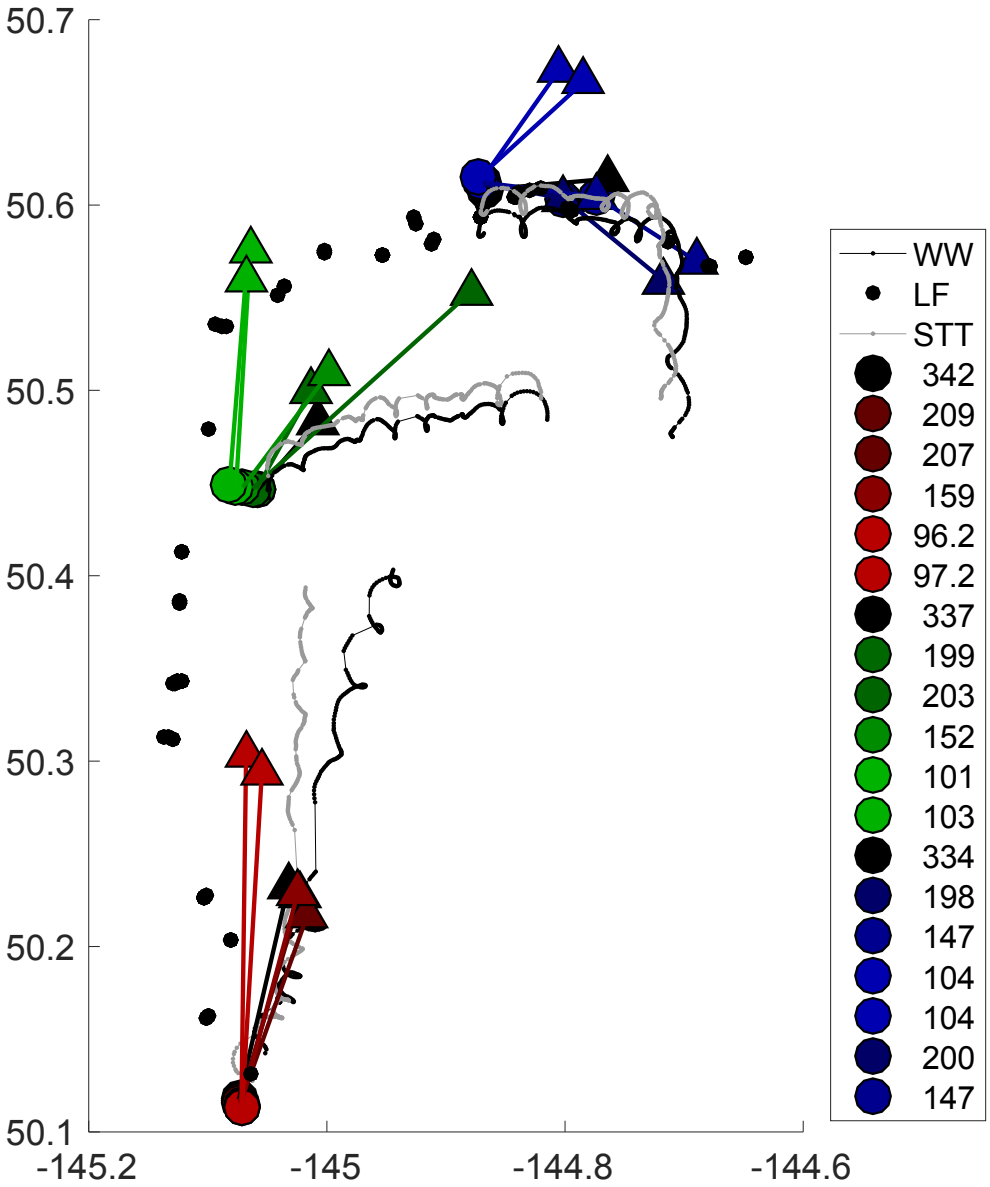


Figure 2. Trap and WireWalker drift trajectories for all three epochs. STT (gray line) and WW (black line) extended from surface buoys to 500 m. Lagrangian float (shown for reference) drifted at 95 m. Depths of individual NBSTs are shown in colors listed in the legend, with darker colors representing deeper depths. Epoch 1 NBST symbols are red, Epoch 2 symbols are green, and Epoch 3 symbols are blue. Deployment locations are shown by circles and recoveries by triangles. WW and STT tracks are limited to the corresponding 330 m NBST drift period, which was one day longer than shallower NBSTs in Epochs 2 and 3. The 203 m NBST in Epoch 2 and the 200 and 147 m NBSTs in Epoch 3 each drifted for two cycles.

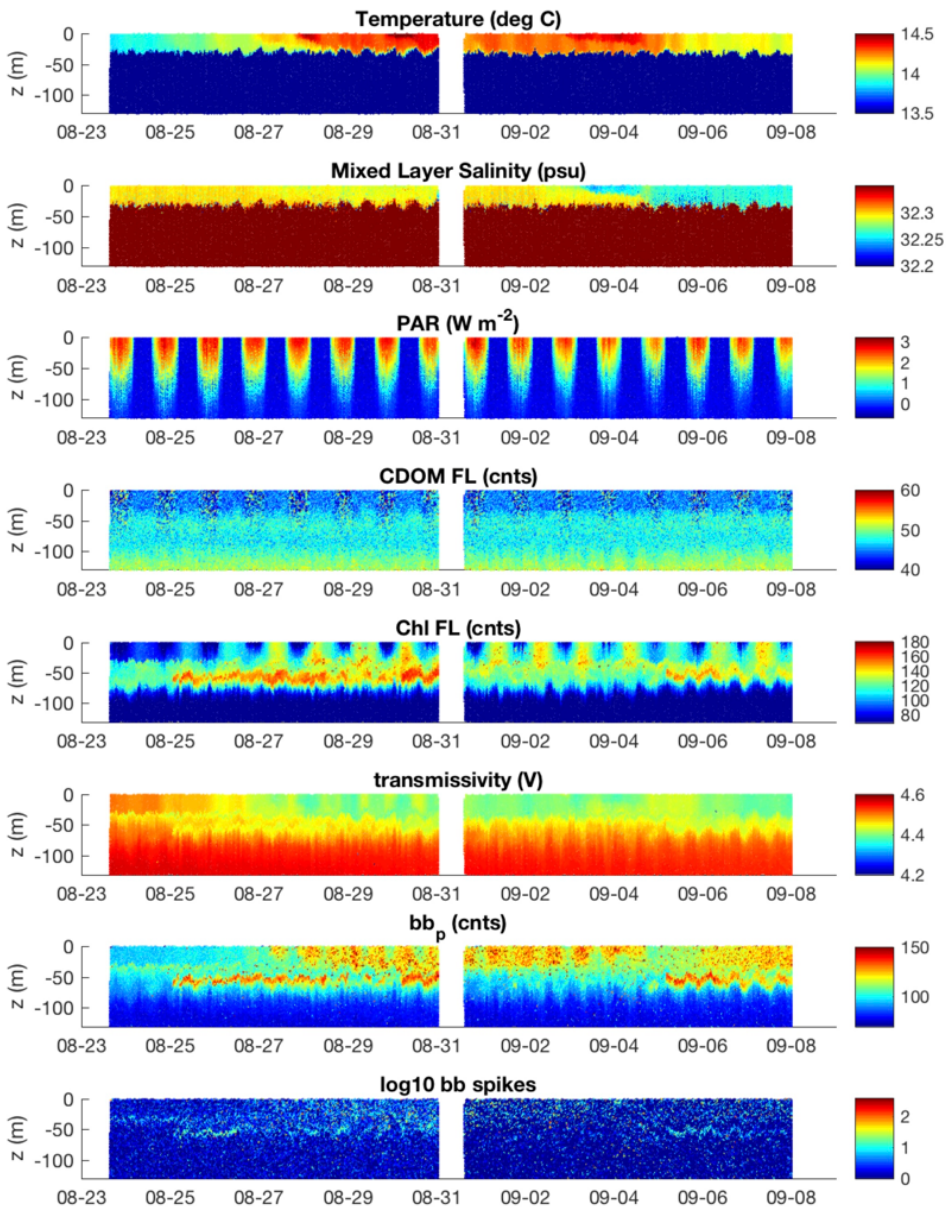


Figure 3: Epoch 2 & 3 Wirewalker timeseries over the upper 120m. Temperature and Salinity colorbars are saturated to show variations within the mixed layer. The optical data are given in raw values and will need qc and calibration.