Summary of activities during the September CANON 12 experiment

The September CANON 12 experiment focused on finding high ammonia in the mesotrophic waters of the Coastal Transition Zone (CTZ) and elucidating the processes responsible for the formation and decay of the ammonia pool by following the dynamics in the water column over 6-8 days.

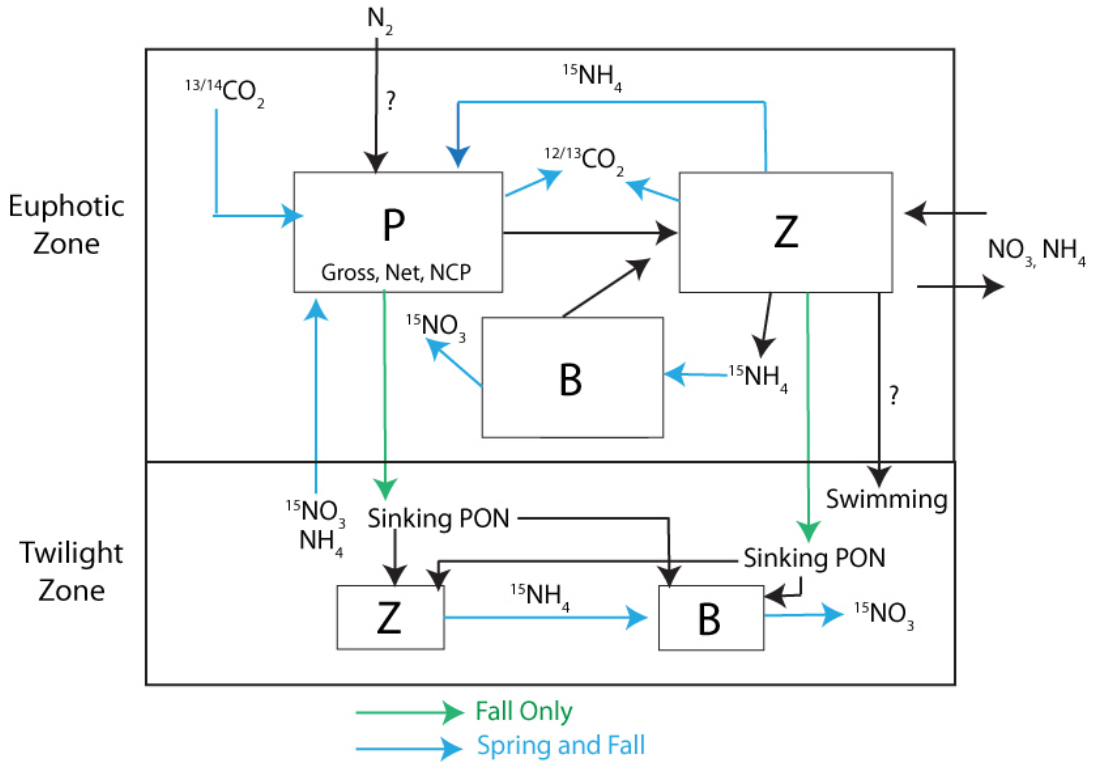
Autonomous Vehicle and Remote Sensing information prior to experiment

A Spray glider along Line 67, the Wave Glider MBARI and the Tethys Long Range AUV provided environmental information prior to the Western Flyer and Zephyr cruises (see http://odss.mbari.org/data/canon/2012_Sep/misc/sections/). Satellite remote sensing (sea level altimetry, sea surface temperature and chlorophyll by ocean color) on September 9th (http://odss.mbari.org/data/canon/2012_Sep/sst/SST_Chl_9Sep2012.png), a day before the cruises, provided further guidance as to where to focus the lagrangian experiment. A mixture of mature coastally upwelled and California Current waters that seemed appropriate for the experiment based on previous data were identified in the vicinity of CalCOFI stations 67-60 and 67-65.

Western Flyer and Zephyr cruises, ESP and AUV deployments

The Western Flyer and Zephyr departed Moss Landing on September 10, 2012. The Western Flyer began by making hydrographic measurements, including ammonia, along Line 67. High ammonia was found at 67-60 (http://odss.mbari.org/data/canon/2012_Sep/misc/sections/C0912-6760-NH4-091112.png) and the ESP and several smaller drifters were deployed at that location on September 11th. The Western Flyer continued a transect along Line 67 to 67-90 completing the large-scale distribution of properties including ammonia (http://odss.mbari.org/data/canon/2012_Sep/misc/sections/WF_nh4.jpg). Oligotrophic water was collected at 67-90 for mixing experiments by the Worden group (see below). The Zephyr remained with the ESP until the return of the Flyer. Strong winds impeded the deployment of the AUV Dorado until the evening of the 11th. Dorado was able to complete three surveys around the ESP drifter the evenings of the 11th, 12th and 13th (http://odss.mbari.org/data/canon/2012_Sep/dorado/). Tethys also began following ESP on the evening of September 12th (http://odss.mbari.org/data/canon/2012_Sep/misc/ESPdrift/sectionsESP_sal.jpg). During the drift scientists aboard the Western Flyer carried out a variety of assays designed at following the biogeochemical (figure below) and ecological dynamics in the waters around the ESP. The ESP remained in the mesotrophic waters until September 15th when it crossed into upwelled waters originating off Point Sur. The cruise then became a the tale of two (or three) chapters, (1) the relatively steady waters that ESP started in (a mix of old upwelled and California Current waters), (2) a brief interlude across a front between these waters and richer/but "older or more mature" coastally upwelled waters emanating from Point Sur, and (3) the higher productivity upwelled waters themselves. Salps and jellies were prevalent in both the "ammonia plume" waters and the mature upwelled waters. The interactions between warm more stable waters and the colder

upwelled ones were similar to those studied in MB by CANON in Spring 2012 but a few hundred kilometers away and offshore. The big exception was that the offshore waters are inverted in terms of biological productivity - the stable waters are less productive, the upwelled waters more in the offshore condition. In shore the freshly upwelled waters are low in chlorophyll since they have recently been at 50 m or below and the stable waters in MB are high in biological productivity. The ESP was recovered on 17th. During the period from 15 to 17th September the Flyer went back and forth between the stratified mesotrophic waters and the more eutrophic mature coastally upwelled waters. After recovery of ESP the Flyer returned to 67-60 for a 48 hour eularian occupation at that location.



Generalized schematic of the nitrogen cycle in the ocean showing measurements made via incubations with isotopes during the Fall CANON experiment. Samples to "sense" these and other enzymatic activities using molecular techniques for gene expression were also collected.

ESP activities on CANON 12

The ESP drifter collected and analyzed samples using an optical array and quantitative PCR (qPCR). The qPCR analysis last 16 hours and a list of primers assayed during PCR are given below. During this same phase an optical array of the probes shown below is developed. ESP also collects 7 archival samples for analysis in the lab during the 16 hour qPCR phase.

The ESP drifter was equipped with a Seabird 16 plus CTD sampling every 5 minutes, HydroRad hyperspectral spectrometer with upwelling radiance and downwelling irradiance collectors, ISUS spectrometer, and a 300 Khz ADCP.

qPCR primers

1) Positive control (IPC)
2) Assimilatory nitrate reductase (narBB, Synechoccus (targets Clade IV))
3) RuBisCO (cbbL, Synechococcus, targets ~85% of Syn in Monterey Bay)
4) Group I Archaea (16S rRNA gene (targets all AOA- Ammonia Oxidizing Archaea))
5) Ammonia monooxygenase subunit A gene (amoA targets AOA water column group A)
6) Ammonia monooxygenase subunit A gene (amoA targets AOA water column group B)

Optical array probes

1) SAR 11
2) SAR 86 subclades I and II
3) SAR 86 subclade III
4) OM60 (abundant phototrophic gammaproteobacteria)
5) SUP05-type sulfur oxidizing bacteria
6) Group I Crenarchaea
7) Group II Euryarchaea
8) Marine deltaproteobacteria (SAR324)
9) Marine cyanobacteria
10) Marine alphaproteobacteria (Roseobacter clades)

Robidart /UCSC/DeLong Samplin on CANON 12

Robidart: Transit (Line 67- Stations H3, 67-60, 67-80, 67-90): General sampling for molecular work (DNA/RNA)- likely Synechococcus gene expression (Fe, N limitation genes) and potentially microarrays (these are probably the lowest priority samples for microarrays)

Jimenez/Worden incubation collaboration (sampling *for* ***Irina Shilova/Jon Zehr UCSC***) - mixing experiment with water from 67-90 and 67-70. Sampled for microarray processing for 3 time points during incubation

ESP transit- **Robidart/ESP Team-** DNA/RNA- Verify ESP abundances for Synechococcus, AOA. Synechococcus abundances fluctuated during this transit so will likely characterize expression of cell stress and nutrient limitation genes. These samples can also be used for microarray analyses- would be great to match shipboard collection microarrays with ESP archive microarrays. Also would like to quantify bacteria at 5, 10, 20m in and out of salp-infested waters- water was "thick"- lots of colloidals- bacteria attach to colloidals and salps are good filter-feeders (I think they are indiscriminant). On the other hand, lots of colloidals means lots of DOM for bacterial growth…

During and end of ESP transit- DNA/RNA- Collected multiple (~16 samples) from several sites for general extraction optimization for ESP Gen 3 *for* ***Chris Preston***

During and end of ESP transit- DNA/RNA- Collected multiple (~8 samples) from two sites for zooplankton molecular work *for* ***Julio Harvey***- (I totally failed here- I should have collected more for Julio but I didn't have the energy…)

Robidart/Smith incubation collaboration- mixing experiment with water from the ammonia plume (1.2uM) with surface seawater (100nM ammonia). Comparison with surface seawater mixing with surface water enriched with ammonia (there are actually more details but not worth describing). Time points for ammonia, nutrients, flow cytometry, DNA/RNA and microarray analyses over 48 hours. Intent is to see the effects of increased light on the deterioration of the ammonia plume (incubation is at ~15% PAR), and to determine whether there are effects of the ammonia plume other than increased ammonia that stimulate the microbial community. (**Francisco:** I would have done an iron experiment but I didn't have access to TM-clean bottles this cruise- they have all been at sea in Hawaii all summer. I will have access to them next year- hint, hint.)

Transit (Line 67- Stations H3, 67-60, 67-80, 67-90): General sampling for molecular work (*for* ***Ed DeLong*** matched with Robidart/Zehr transit sampling mentioned above). *

ESP transit- DNA/RNA- Verify ESP archive metatranscriptomes *for* ***Ed DeLong***.*

- *All DeLong sampling was paralleled with Robidart/Zehr sampling: meaning any of these samples can be used for metatranscriptome/microarray comparisons.*

DeLong Metatranscriptomes Methods Summary

Microbes collected and preserved by an Environmental Sample Processor[7,8] suspended 10 m beneath a free-drifting surface float and additional seawater collected with the CTD will be processed as follows. Total community RNA is extracted, ribosomal RNAs removed by subtractive hybridization, and the remaining message linearly amplified and converted to cDNA using previously published protocols. Sequencing is performed using a GS FLX Titanium system.

Zehr et al. Microarrays

A description of the microarray concept can be found at https://sites.google.com/site/microtoolsii/. A full list of the probes on the microarrays used to analyze the CANON samples will be provided.

Worden Lab activities on CANON12

Worden group activities aboard CANON12 were in support of the larger cruise goal to understand nitrogen dynamics in the euphotic zone of mesotrophic waters in the vicinity

of more coastal portions of Line 67. Focused experiments and cell sorting were performed to understand how small eukaryotes (primarily picoplankton) and some archaea obtain their nutrients. The results will contribute to understanding of how more coastal populations of these organisms interact with other microbes and respond to environmental cues. Team participants at sea were Valeria Jimenez (MBARI), Jarred Swalwell (U. Washington, Temp. Employee MBARI), Noriko Okamoto (U. British Columbia), and Alyson Santoro (U. Maryland, Horn Point Laboratory).

Our goals for the cruise were to investigate the following:

1. Nitrogen/grazing **bottle incubation experiment** - sampling for FCM, DNA/RNA and population sorts. – Addresses question: how do communities shift with infiltration of low N water and reductions in grazing. Filtered 67-90 water was used to dilute water collected at 67-90 and the response of the communities followed over 48 hours.
2. **Natural populations and dynamics** with focus on ~67-90 (more oligotrophic) and ~67-60 (mesotrophic) with both euphotic and below euphotic zone sampling at the latter. Transition communities explored by SeaFlow and intermittent stations.
3. Evaluate whether protein additions enrich for protein degrading groups (e.g. euryarchaea) or unique protist –prokaryote interactions

The following samples were taken at every even numbered station along Line 67, and at one profile per day during the ESP drifter deployment, and during the re-occupation of station 67.60: a 4-7 depth profile of total DNA (>0.2 μm fraction), fixed samples for flow cytometric enumeration of phytoplankton groups, starting material for protist cultivation and live microscopic observation, and continuous underway measurements of surface phytoplankton communities using the SeaFlow flow cytometer. Samples were also taken along Line 67 for size-fractionated DNA samples (0.2, 0.8, 3.0, 10 μm).

A sorting flow cytometer was brought to sea, and live cell sorting (both populations and single cells) of chlorophyll-containing groups was conducted with samples from 67.90, 67.70, during the ESP drifter deployment, and during the reoccupation of 67.60. The latter contained many non-target, coastal organisms which restricted relevance to broader research goals, however a number of cell sorts were performed for later genomic characterization depending on screening results. Large volume filtrations for metagenomic and metatranscriptomic analysis were done at 10 m and 30 m depths at 67.60. These depths were chosen to compliment the drifting arrays and other experimental work by the Chavez group to understand microbial activity within the ammonium maximum. Lastly, selected profile stations were chosen for later dissolved urea concentration measurements to understand the distribution of simple organic nitrogen compounds in relation to both the ammonium maximum and eukaryote distributions along Line 67.

Chavez lab activities

The Chavez lab was responsible for drifter deployments and the standard hydrographic measurements on the cruise. In collaboration with Jason Smith at Stanford they carried out a series of incubation experiments on drifters and shipboard incubators. The incubations measured the incorporation of $C^{14}O_2$, $N^{15}H_4$ and $N^{15}O_3$ by primary producers, the production of $N^{15}O_3$ by bacteria from $N^{15}H_4$ and the production of $N^{15}H_4$ by zooplankton. The standing stocks of inorganic carbon and nitrogen were also followed over time. Water samples were collected for analysis of natural nitrogen isotopes in the lab of Karen Casciotti at Stanford. Samples were also collected for analysis of triple point oxygen isotopes in the lab of Paul Quay at the University of Washington. Samples for genomic analysis of nitrogen assimilation were collected for analysis in the laboratory of Chris Francis at Stanford. The team consisted of Chavez, Pennington, Blum, Alba Cobo (visiting graduate student from the University of Vigo, Spain), Jason Smith (Stanford) and Brian Peters (Stanford).