

# Cruise Report

## *California Current Ecosystem* LTER Program



CCE-P2107 Cruise R/V *Roger Revelle*,

13 July – 13 August 2021



Compiled and submitted by: Katherine Barbeau, Chief Scientist

Scripps Institution of Oceanography, University of California, San Diego

Cruise ID: CCE-P2107 (= RR2105)

Master: Captain Wesley Hill

Depart: 13 Jul. 2021, 1000 (PDT), MarFac

Chief Scientists: Michael  
Stukel/Katherine Barbeau

Return: 13 Aug. 2021, 1030, MarFac

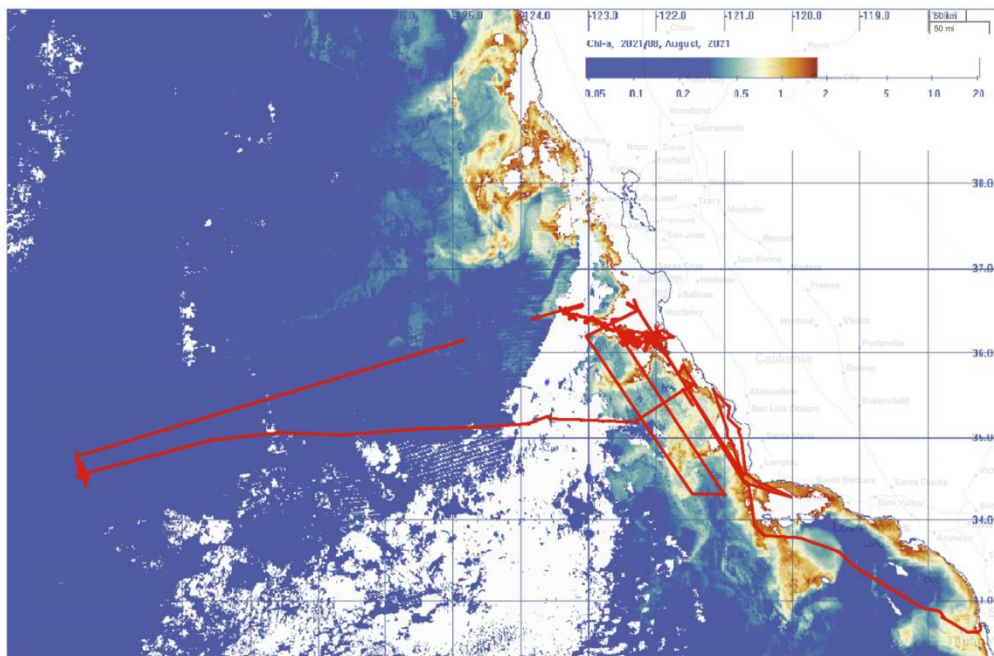
Science Technicians: Joshua Manger  
(SIO), Caitlyn Webster (SIO), Brent  
DeVries(SIO)

Vessel: R/V *Roger Revelle*

Operator: Scripps Institution of  
Oceanography

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Partial cruise track for CCE-LTER cruise P2107 off the coast of Central and Southern California, and spanning the California Current. While underway, continuous measurements were made of pH/pCO<sub>2</sub>, phytoplankton pigments and variable fluorescence by Advanced Laser Fluorescence Analyzer (ALFA), multi-frequency acoustic backscatter by EK80, and standard ocean (Temperature, Salinity, dissolved O<sub>2</sub>, Chl-*a* fluorescence, etc.) and meteorological variables.

## Cruise Participants

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SIO = Scripps Institution of Oceanography; FSU = Florida State University; JCVI = J. Craig Venter Institute; UCSC=UC Santa Cruz ; CSUSM= Cal State U San Marcos; UBO = Université de Bretagne Occidentale, France; ULCO= Université du Littoral Côte d'Opale, France ; STS = Shipboard Technical Support

## SCIENCE OBJECTIVES

This cruise, designated P2107 by CCE-LTER (or RR2105 for R/V *Roger Revelle*), was the third process cruise in Phase III of the *California Current Ecosystem* Long Term Ecological Research (CCE-LTER) program, supported by NSF. P2107 focused on cross-shore fluxes, plankton community changes, and biogeochemical export (of nutrients, organisms, nitrogen, and carbon) associated with coastal upwelling. P2107 employed a series of integrated approaches. The fundamental approach was quasi-Lagrangian experimental studies and in situ measurements carried out while following 3 discrete water parcels, the first 2 of which were part of a coastally upwelled water mass we identified off Pt. Sur, California. The third and contrasting water parcel was at the outer edge of the California Current and the inner edge of the Pacific gyre, much farther offshore. These quasi-Lagrangian series of measurements (each of which we term a "Cycle" of repeated measurements) were complemented by several related activities: deployments of multiple autonomous instruments (*Zooglider*, *Spray* glider, in situ incubation drift arrays, drifting sediment traps); a radiator survey that paralleled the coast; a diel study of an upwelled water mass south of Monterey Bay; an extended transect across the CA Current System; an alongshore sampling transect; detailed sampling in Santa Barbara Basin and the San Pedro DDT site; extensive remote sensing support (including satellite sensors and coastal high-frequency radar); and transect surveys of the Benthic Boundary Layer over the continental shelf in two locations.

Our specific objectives were to ***understand and quantify key mechanisms that transport coastal production and populations offshore in the CCE region, including the magnitudes and length scales of transport and their climate sensitivities.*** The principal hypotheses we sought to test were:

*H<sub>1</sub>: Lateral transport dominated by the interaction of Ekman transport and westward propagating coastal filaments provides a significant flux of nutrients and organisms to offshore waters.*

*H<sub>2</sub>: Carbon export associated with offshore transport is determined by in situ evolution of communities and nutrient regimes, and by subduction occurring largely at sharp frontal density gradients.*

The processes measured on this cruise included primary production, phytoplankton growth rates, nitrate uptake, silicic acid uptake, secondary production by bacteria, grazing by microzooplankton and mesozooplankton, iron limitation effects on phytoplankton growth, carbon and nitrogen cycling between dissolved and particulate phases, and elemental export in both particulate and dissolved forms. The pelagic food web was characterized by state-of-the-art measurement methods, including omics approaches (for prokaryotic and eukaryotic microbes), high-resolution imaging (phytoplankton to mesozooplankton), and multi-frequency acoustic backscatter (for mesozooplankton and nekton). Vertically stratified distributions of zooplankton were assessed by MOCNESS, complemented by in situ imagery from a UVP-HD mounted to the CTD-rosette and autonomous *Zooglider*. Several new ancillary programs were also represented on this cruise – the Lamborg/Schartup group from UCSC and SIO studied Hg biogeochemistry, and the Diaz group studied P biogeochemistry. Several new in situ visualization technologies were tested by the group of Tristan Biard. Most measurements were made in a Lagrangian reference frame while following discrete water parcels for 4-5 days at a time. Two of these water parcels were selected to represent different stages in the temporal evolution of a water mass

upwelled near Pt. Sur: i.e., nearshore in the freshly upwelled water mass and later as upwelled waters and entrained communities were advected offshore with Ekman flow and subducted, where export fluxes were expected to be elevated. The third water parcel was representative of the oligotrophic western edge of the CA Current and provided a strong contrast in community structure to the water masses influenced by coastal upwelling. A transect between these extremes was conducted to document the lateral transitions. A Benthic Boundary Layer (BBL) study in two regions (3 stations near Pt. Sur and 9 stations north of or near Pt. Conception) was conducted to understand the relationship between coastal iron supply in nearshore sediments and the flux of iron into the coastal ocean via upwelling and advection. An alongshore net sampling transect also documented changes in the zooplankton community along the mosaic of water masses in the upwelling-influenced region. We also sampled the DDT dump site in San Pedro Basin to document the potential impact of DDT on the local community, and explore the utility of DDT as a foodweb tracer.

Our **Broader Impacts** activities included providing seagoing research opportunities and training for 20 graduate students, two volunteers, and three postdoctoral investigators. A URM undergraduate also participated in the cruise as part of an ROA/REU supplement with Assistant Professor Darcy Taniguchi, a faculty member at CSUSM, a Minority-Serving, Primarily Undergraduate Institution. We communicated with the general public via an online blog created by graduate students Ralph Torres and Jamee Adams (<https://cce.lternet.edu/blogs/201908/>).

## OVERVIEW OF THE SCIENCE PLAN

Preparations for this cruise began with deployment of a *Spray* ocean glider on June 8, 2021 (Jeff Sherman and Dan Rudnick, IDG, SIO), to characterize cross-shore fluxes on a line extending from Pt. Conception in the south to Pt. Piños at the southern end of Monterey Bay. The glider profiled from 500-0 m. Concurrently with this deployment, and prior to departure of the R/V *Roger Revelle* from MarFac on July 13, 2021, satellite and high frequency coastal radar (CODAR, Marina Frants, SIO) images were generated daily to identify regions of filament formation. The remotely sensed variables analyzed included Chl-*a* (at 1.1 km and 300 m horizontal resolution, Mati Kahru, SIO); SST (Mati Kahru, SIO); Sea Surface Height and derived variables (Finite Size Lyapunov Exponents, Okubo-Weiss parameter; Alain de Verneil at NYU Abu Dhabi). Glider and remote sensing data were also examined daily at sea to guide site selection for Lagrangian Cycles.

After departure of R/V *Roger Revelle* from Scripps' MARFAC on July 13 at 1000, we calibrated the EK80 acoustic system in both broadband and narrowband mode. We then steamed to the test station site and completed a series of over-the-side instrument tests. We then steamed to the starting location of our radiator survey and began underway sampling for nutrients, Chl-*a*, HPLC, POC/PON, nutrients and <sup>234</sup>Th along three south-north lines extending from Pt. Conception to Pt. Piños, at successively greater distances from shore (see cruise track Pg 2 above), in order to provide measurements needed to calculate cross-shore fluxes of mass, nitrate (using a Temperature-NO<sub>3</sub> proxy), and Particulate Organic Carbon, between each pair of survey lines. From this survey and the glider measurements above (satellite unavailable due to cloud cover) it became apparent that there was little evidence of active

upwelling features along the coast, there having been very light winds for some time. While waiting for a wind event to occur, we carried out a diel sampling survey in an upwelling-influenced water mass just off Monterey Bay (referred to hereafter as the Diel Station). We also carried out benthic boundary layer (BBL) transect #1 to continue our time series sampling of these sites, from Cambria on the Big Sur coast south to just past Point Conception. This was followed by a sampling station in the Santa Barbara Basin, a biogeochemically interesting suboxic basin.

Starting around July 20, a forecast 2-day wind event began, reaching 20-25 knots in some coastal locations. After concluding the BBL transect, on the 21st we conducted an underway shipboard meteorological (MET) data and CTD survey back up the coast, looking for a fresh upwelling location. In this way, along with the *Spray* glider data, we identified a relatively cool, salty, and dense recently upwelled water mass just west of Pt. Sur. While not a filament per se, given the extended relaxation phase in the forecast we concluded that this water mass offered us the best chance in our study area to follow the development of an upwelled water mass as it moved offshore due to the influence of Ekman circulation. Thus we initiated Cycle 1 just off Pt. Sur, about 25 km from shore, deploying the sediment trap late on July 21<sup>st</sup> and the first drift array pre-dawn on July 22<sup>nd</sup>. *Zooglider*, a novel autonomous zooplankton imaging system and dual-frequency Zonar mounted on a glider together with CTD and fluorescence sensors, was deployed on Day 1 of Cycle 1 and piloted remotely to follow the movements of the drifters. After a 4-day Lagrangian cycle conducted in the upwelled water mass as it moved slowly offshore, early on July 26<sup>th</sup> we left an empty drift array in place to mark the water mass at the last drift array location and recovered the sediment trap, which had separated somewhat from the drift array. We left to perform BBL Transect #2 near Pt. Sur to characterize iron sources in the region. Late that night a diversion to Monterey was necessary to drop off Chief Scientist Mike Stukel, due to a family emergency. Kathy Barbeau assumed the Chief Scientist position at this point.

Returning to the marker drift array early on July 27<sup>th</sup>, based on observations of Chl-*a*, salinity and SST in the ship's flow-through system, as well as a CTD profile, we concluded that the main upwelled water mass was now to the northwest of our current drift array location, closer to our previous sediment trap location. We moved in the direction of our last sediment trap location and used the MET underway data to identify the location of the upwelled water mass based on higher Chl-*a*, higher salinity and lower SST. We initiated Cycle 2 in this water mass on the evening of the 27<sup>th</sup>, deploying the 2<sup>nd</sup> sediment trap outside of the Monterey Bay sanctuary and nearby shipping routes. We stayed in this cycle for 4 full days and witnessed a significant change in the community as the water mass continued to advect offshore to the northwest, eventually reaching over 120 km offshore. The chlorophyll maximum decreased greatly in magnitude and dropped lower in the water column as a layer of fresher water moved in at the surface. The sediment trap and drift array tracked together for Cycle 2. We recovered the sediment trap and concluded Cycle 2 on August 1<sup>st</sup>. Our final Lagrangian cycle, Cycle 3, was initiated about 800 km offshore, near the western edge of the California Current, in oligotrophic waters with a very deep chlorophyll maximum (~120 m). This provided a very contrasting community to the upwelling influenced waters and was an endmember of particular interest to many in the science party. The community remained stable and the drift array and sediment trap tracked together for the 4+ days of the cycle.

For each cycle, Globalstar-tracked sediment traps and an in situ incubation drift array were used to follow water parcels over repeated day/night measurements in order to quantify the temporal evolution of rate processes and plankton community composition. Both sediment traps and drift arrays employed a 3-m tall  $\times$  1-m diameter holey sock drogue centered at 15 m depth and telemetered positions ashore via Iridium every 10 min. All other measurements were made in these same water parcels in close proximity to the drift array (ca. 100-150 m away), or centered on the drift array (in the case of towed nets like the bongo and MOCNESS). Incubation bottles were suspended from the drift array at six depths spanning the euphotic zone in order to determine specific growth rates of phytoplankton and specific grazing rates of microzooplankton (using seawater dilution experiments), in situ rates of  $^{14}\text{C}$ -based primary production, Fe-limitation, and New Production from  $^{15}\text{NO}_3$  uptake. Additional measurements/samples at the drifter locations included Fe limitation incubations; vertical profiles of trace metals sampled with a trace metal clean rosette;  $^{14}\text{C}$ -based primary production;  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  based diel measurements of nitrate and ammonium uptake; Silicic acid uptake by rhizarians;  $^3\text{H}$ -leucine-based bacterial production and also an amino acid tagging-based method of assessing bacterial production; analysis of stable isotopes of N, C, and O; reactivity of DOC and DON; speciation and bioavailability of DOP;  $^{234}\text{Th}$ : $^{238}\text{U}$  disequilibrium; microbial diversity assessed by 16S and 18S ribosomal subunit genes; phytoplankton pigments by HPLC; size-fractionated Chl- $\alpha$ ; POC and PON; biogenic silica measurements; picoplankton samples for flow cytometry; microplankton samples for epifluorescence microscopy; mesozooplankton biomass and grazing (via gut fluorescence) in five size fractions from bongo tows; mesozooplankton community composition from bongo tows and shore-based Zooscan; mesozooplankton vertical distributions via MOCNESS samples for DNA metabarcoding and Zooscan; live zooplankton sampling via ring nets, bongos, and salp nets; Rhizarian community analysis and C and Si composition; and vertical profiles of macronutrients and standard hydrographic variables. In addition, continuous underway measurements were made on the ship's uncontaminated seawater system, including phytoplankton pigments, variable fluorescence ( $F_v/F_m$ ), and CDOM, all using an Advanced Laser Fluorescence Analyzer (ALFA; Chekalyuk and Hafez. 2008. LOM: 6:591); Imaging FlowCytobot (IFCB) samples; continuous  $\text{pCO}_2$ ; standard ship-provided measurements of ocean surface properties and meteorological variables (MET data); ADCP-derived currents at 75 kHz and later at 300 kHz (only from 8 Aug., 1545); and EK80 acoustic backscatter. In situ plankton and marine snow images were acquired on all CTD-rosette casts using a high definition Underwater Vision Profiler (UVP5-HD). Measurements are described in greater detail below in the group-specific entries.

Immediately following the conclusion of Cycle 3 on August 7, we initiated a transect eastward back across the California Current System, referred to as the California Current Transect (CCT). This consisted of 9 stations which, with the addition of the Cycle 3 Day4/5 samplings, covered the full range of hydrographic conditions and foodweb structure from the oligotrophic western edge of the CA Current/gyre interface to upwelling-influenced coastal waters. Sampling at each CCT station included a CTD with water column sampling for hydrography, biogeochemical parameters, and  $^{238}\text{U}/^{234}\text{Th}$  disequilibrium; a trace metal rosette cast; a standard oblique bongo tow for quantitative zooplankton sampling, and a salp net for live zooplankton sampling and associated rate measurements. *Zooglider*, having followed the general track of Cycles 1 and 2 and then continued out into the eastern edge of the CA Current for 18 days, was recovered via small boat on August 9 between CCT stations 6 and 7. The

eastern edge of the CCT intersected with another transect, the Alongshore Transect (AT), carried out on August 10<sup>th</sup> and 11<sup>th</sup>. The AT transect included 8 stations extending alongshore from San Simeon in the north to the Point Arguello region in the south. The CT and AT transects overlapped at AT Station 4 (= CCT Station 9). Each AT station included a CTD cast for basic hydrography, Chl-*a*, POC, and bSi sampling, followed by bongo and salp net tows for quantitative and live zooplankton sampling. Small boat zooplankton sampling was attempted near AT Station 5 on the evening of August 10<sup>th</sup>. Additional sampling for incubation studies was performed at various sites on both CT and AT transects. After completing the AT transect and a deep cast for <sup>234</sup>Th in Arguello Canyon on August 11<sup>th</sup>, we transited to the DDT dump site off Santa Catalina in San Pedro Basin, arriving on the morning of August 12<sup>th</sup>. At the DDT site we completed a CTD cast, McLane pump cast, EK80 survey, and deep MOCNESS tow before transiting back to MarFac, arriving around 10:30 am on August 13<sup>th</sup>, to conclude P2107.

P2107 was, in general, a successful cruise. We overcame several challenges in pursuit of our science objectives – we lacked a towed SeaSoar instrument platform that we had originally planned on having, there was essentially no real-time satellite Chl-*a* or SST data for the entire cruise due to cloud cover, weather conditions were not conducive to active upwelling and filament formation, and the original Chief Scientist had to depart less than halfway through the cruise. Despite these difficulties, we were able to complete our primary science mission and also address a number of ancillary questions which will foster new directions and new scientific collaborations within our program. The Captain, STS personnel, and crew were instrumental in maintaining a close schedule of round-the-clock operations, and addressing the sometimes impromptu nature of our scientific inquiries.



## Brief Chronology of Cruise P2107

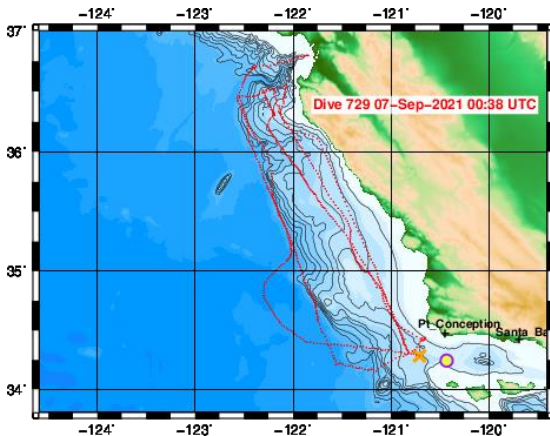
(see Daily Activity Schedule at the end of this document, and Event Log for details and accurate times)

13 July 2021 (1000 PDT)	Depart MarFac (departure delayed for high tide)
13 July (1700)	EK80 calibration
14 July (0830 - 2000)	Test casts and tows
15 July (0245) - 17 July (0130)	Radiator Survey (parallel to coast)
17 July (0630) - 18 July (1800)	Diel Station study at Radiator Survey station 1-20
19 July (1500) - 20 July (0800)	BBL Transect #1
20 July (0900 - 1400)	Santa Barbara Basin Station – CTD, TMCTD, McLane
20 July (2100) – 21 July (2130)	CTD Survey
21 July (2300)	Deploy sediment trap, Begin Cycle 1
22 July (1000)	Deploy <i>Zooglider</i>
26 July (0600)	Recover sediment trap, End Cycle 1
26 July (1200-1400)	BBL Transect #2
27 July (0000)	M. Stukel drop off, Monterey
27 July (1900)	Deploy sediment trap, Begin Cycle 2
1 Aug. (1000)	Recover sediment trap, End Cycle 2
1 Aug. (1200) – 2 Aug (2100)	Transit to offshore site
2 Aug (2300)	Deploy sediment trap, Begin Cycle 3
7 Aug (1200)	Recover sediment trap, End Cycle 3
7 Aug (2145) – 9 Aug (0940)	California Current Transect (CCT) Stations 1-6
9 Aug. (1200)	Recover <i>Zooglider</i> ; Annular survey, CTD
9 Aug. (1540) – 10 Aug (0100)	CCT Stations 7 & 8
10 Aug. (0700-2100)	Alongshore Transect (AT) Stations 1-5; CCT Station 9
11 Aug. (0400- 1420)	AT Stations 6-8
11 Aug. (2000) – 12 Aug. (0930)	Transit to DDT site, San Pedro Basin
12 Aug. (0930 - 2130)	DDT site sampling – CTD, McLane, EK80, MOCNESS
13 Aug (1030)	Arrive MarFac

## GROUP REPORTS

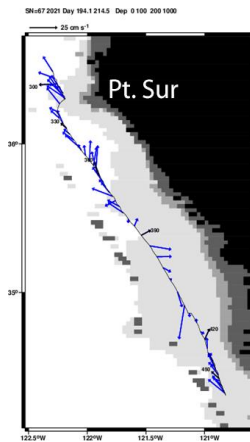
### *Spray* glider surveys (Mark Ohman, Sven Gastauer, Jeff Sherman)

A *Spray* glider (SN68) was deployed in order to sample prior to, during, and after the P2107 cruise on alongshore survey lines between Pt. Conception and Pt. Pinos. Its purpose was to identify regions of



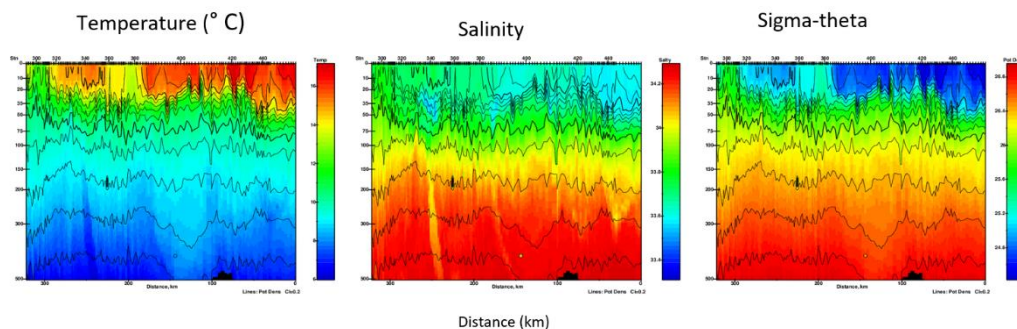
cross-shore flow along the coast and to conduct measurements suitable for the calculation of cross-shore fluxes of volume, mass, and nutrients (the latter via a temperature-NO<sub>3</sub> proxy relationship). This *Spray* was equipped with a Seabird CP41 pumped CTD, Seapoint mini-SCF Chl-*a* fluorometer, and 750 kHz Sontek Acoustic Doppler Profiler. It was deployed in Monterey Bay on 8 June 2021 and completed its survey of the region on 7 Sept. 2021, with over 730 dives. Dives were typically to 500 m depth at 2.5-3 hour intervals. The glider track is shown at left. Five survey lines were completed, 3 southbound and 2 northbound, prior to recovery off

Santa Barbara.



The second southbound leg (13 July – 2 Aug. 2021) began as R/V *Roger Revelle* departed the Nimitz Marine Facility. Average horizontal flows between 500-0 m revealed a strong flow toward the NW off Pt. Sur (blue vectors, left).

This region was associated with relatively cool (ca. 12° C), somewhat saltier, and denser surface waters, as seen in the upper left corner of the vertical section plots below. This signature is attributable to the influence of relatively recently upwelled waters west of Pt. Sur, which became the focal region for the location of Lagrangian Cycle 1.



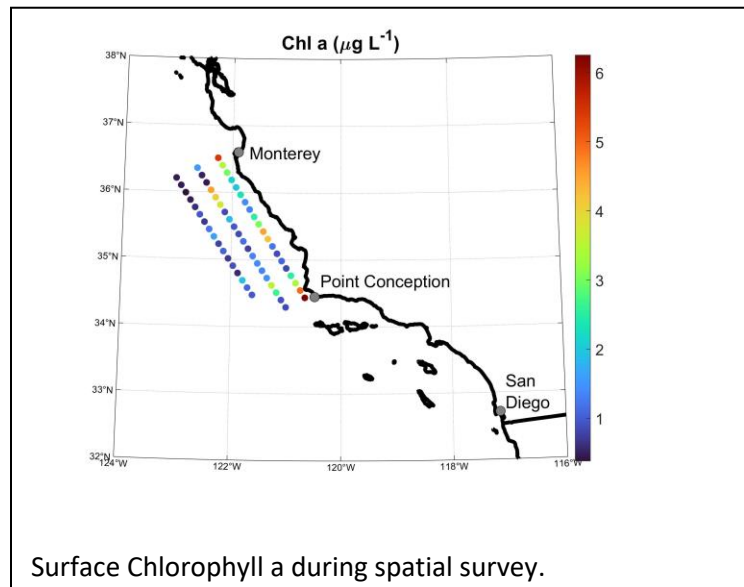
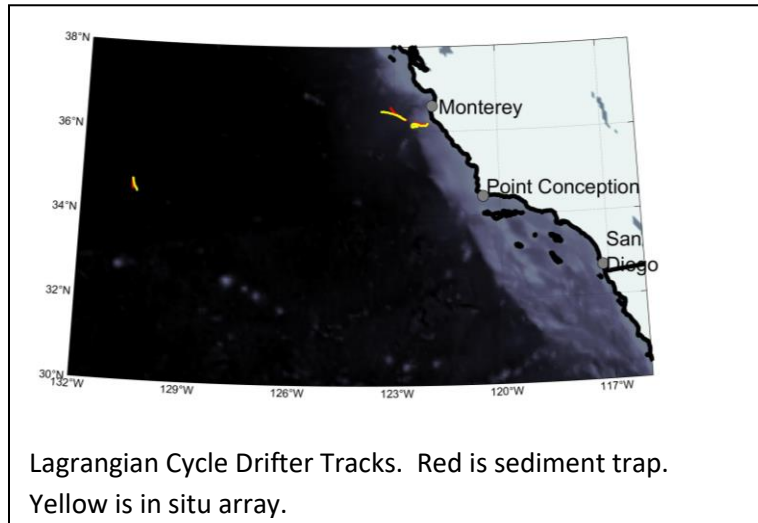
**Stukel Lab (M. Stukel, N. Yingling, J. Irving, C. Fender, K. Opeyemi, M. Roadman with substantial help from Sydney Plummer, Anissa Garcia, and Moira Decima)**

We had three goals for this cruise: 1) quantify the balance between new and export production between coastal and offshore regions, 2) determine the composition of sinking material and the processes driving the biological pump, and 3) quantify phytoplankton production and growth rates and protistan grazing rates. In addition to these primary goals, we also deployed an *in situ* array to track water parcels during Lagrangian experiments.

To achieve our first objective, we measured  $^{15}\text{NO}_3^-$  uptake, sediment trap C and N flux, and  $^{238}\text{U}$ - $^{234}\text{Th}$  deficiency.  $^{15}\text{NO}_3^-$  uptake was measured during 24-hour *in situ* incubations conducted on our experimental array at 6 depths on each day of our Lagrangian cycles. This yielded a total of 72 samples that will

be analyzed on land. We also conducted deckboard  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  uptake experiments to quantify diel patterns of nitrate and ammonium uptake and determine whether nitrate uptake was underestimated during 24-hour incubations. A total of three diel experiments were conducted, yielding an additional 24 nitrate uptake measurements and 24 ammonium uptake measurements.

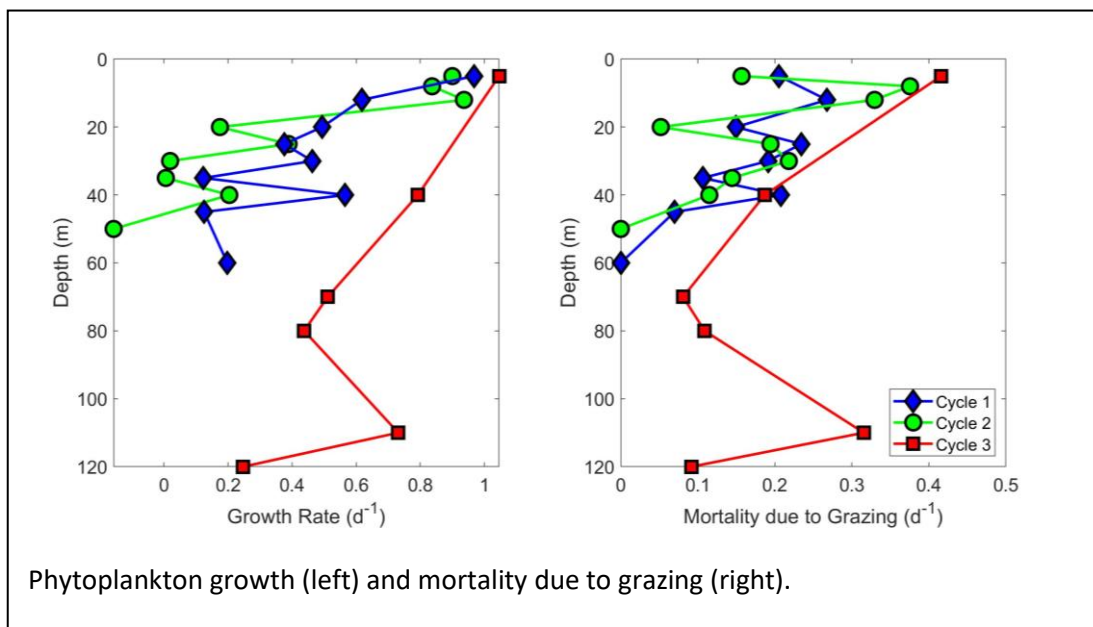
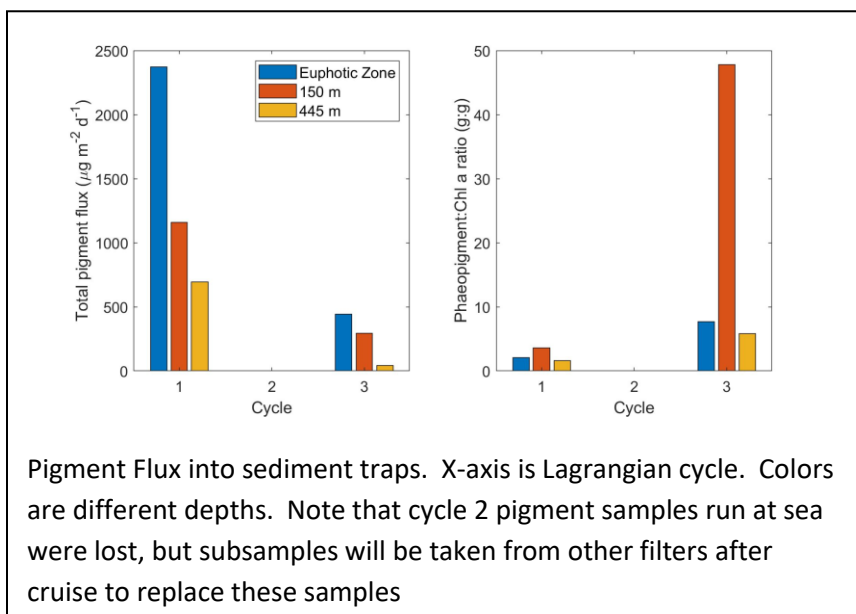
We deployed sediment traps during each of 3 Lagrangian cycles. Vertex-style sediment trap cross-pieces (8:1 aspect ratio, with a baffle on top of similar 8:1 aspect ratio) were typically shortly below the base of the euphotic zone (50 m on Cycles 1 and 2; 100 m on Cycle 3) and at depths of 100 m, 150 m, and 440 or 450 m. To quantify the spatial variability and extent of carbon flux, we used  $^{238}\text{U}$ - $^{234}\text{Th}$  deficiency measurements.  $^{238}\text{U}$ - $^{234}\text{Th}$  deficiency was measured at 12 depths on two profiles during each of our Lagrangian cycles for comparison to the sediment trap results (72 samples). It was also measured at 8 depths per CTD cast during a transect across the region. We also measured surface  $^{238}\text{U}$ - $^{234}\text{Th}$  deficiency in surface samples taken during a spatial survey



through the study region at the beginning of the cruise. These “spatial mapping” surface  $^{234}\text{Th}$  samples were paired with surface samples for chlorophyll *a*, nutrients, POC, and HPLC.

To achieve our second objective, we subsampled the sediment trap samples for a multitude of measurements including: C and N, particulate Si,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , C: $^{234}\text{Th}$  ratios, Chl *a* and phaeopigments, trace metals, organic molecules, microscopy, genomics, and transcriptomics (transcriptomics sediment trap tubes deployed with RNA Later in tubes to preserve RNA contained in organisms on sinking particles). We also deployed tubes containing

acrylamide gel to preserve aggregates and other sinking particles in their in situ shapes for microscopic analysis. Initial results (based on pigments and preliminary microscopic analyses) suggest that export was dominated by sinking fecal pellets on Cycle 1 and (especially) Cycle 3. Total pigment flux was



elevated in the coastal region (Cycle 1) and low offshore. To achieve our third goal, we conducted two-point microzooplankton grazing dilution experiments and  $^{14}\text{C}$  net primary productivity measurements. These experiments were conducted daily at six depths spanning the euphotic zone on the in situ array

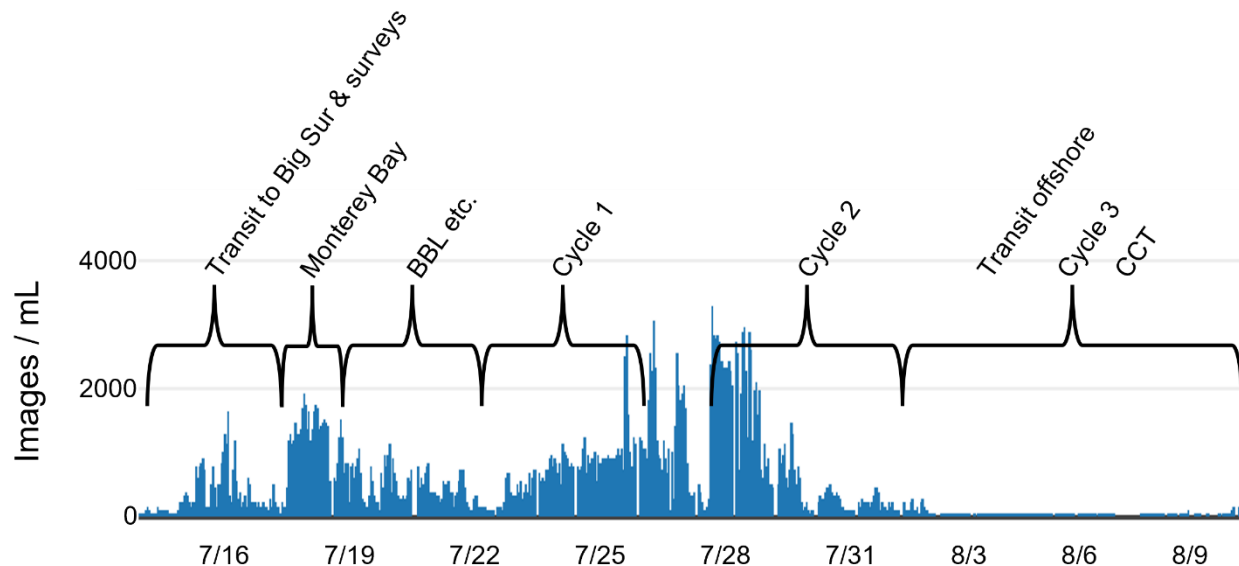
during our Lagrangian experiments. We conducted a total of 72 two-point dilution experiments and net primary productivity measurements. Samples from each of the dilution experiments were taken for bulk chlorophyll a and flow cytometry. We found that phytoplankton growth rates typically exceeded protistan grazing rates in the surface ocean by a substantial amount. This excess production was potentially available to mesozooplankton grazers. In addition, we conducted three full (five treatment) dilution experiments in deckboard incubators. The purpose of these experiments was to test the assumption of linearity in the two-point dilution experiments and to support measurements assessing diel variability in grazing, size specificity of grazers, the impact of reactive oxygen species on grazing, and the impact of wildfire ash byproducts on grazing rates.

**Full Dilution Incubations (Mike Stukel, Anissa Garcia, Sydney Plummer, Dante Capone, Anya Stajner, Monica Thukral, with additional help from Natalie Yingling and Christian Fender)**

A total of four 24-hour deckboard incubations were conducted to determine microzooplankton grazing and phytoplankton growth rates. These incubations were used to test the assumption of linearity in grazing rates obtained from two-point dilution incubations conducted on the in-situ arrays and to support additional science questions such as interrogating diel patterns in growth and grazing. Briefly, seawater was collected from the upper mixed layer (usually 12 m) before dawn and filtered (0.1  $\mu\text{m}$ ). Then, proportions of filtered and unfiltered seawater were gently mixed (20%, 40%, 60%, 80%, and 100% unfiltered seawater) to create a gradient of microzooplankton and phytoplankton encounter rates. Nutrients (ammonium, phosphate) were also added in select bottles to determine their impacts. In select experiments, additions of antioxidants, ash leachate, and jasmonic acid were added to determine their impacts on grazing and growth rates. Samples taken from these experiments included chlorophyll-a, flow cytometry (for abundance of major microbial populations, as well as size-spectrum analyses), DNA metabarcoding, epifluorescence slides, acid lugols preserved seawater, nutrients, hydrogen peroxide production and decay, RNA, and metabolite samples.

**Imaging FlowCytobot survey of phytoplankton communities (Tyler Coale, Allen Group)**

The Allen lab group deployed an Imaging FlowCytobot (IFCB) for the duration of P2107, sampling near-continuously from the uncontaminated seawater system and running discrete samples from the trace metal casts, conventional CTD rosette casts and incubation experiments. In keeping with best practices for underway sampling, a Graco Husky 1050e electric-operated diaphragm pump was installed to supply the ship's uncontaminated seawater system. The IFCB generated over 3 million images of fluorescent particles (<5–150  $\mu\text{m}$ ) over the course of the cruise (figure below). 98 distinct taxonomic groups were identified including 47 diatom and 28 dinoflagellate taxa. Additional groups imaged include Acantharea, Appendicularia, Bicocoeales, Ciliophora, Crustacea, Dictyochophyceae and Prymnesiophyceae.



Images per mL acquired by the IFCB sampling from the uncontaminated seawater system over the course of the cruise.

#### **Nitrogen and Organic Carbon parameters – Aluwihare Group (Ralph Torres, Lihini Aluwihare)**

(1) Suspended particulate organic matter (POM) and stable isotopes

Samples were collected on 25mm GF/F filters to measure particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations and the  $^{15}\text{N}$  and  $^{13}\text{C}$  content of suspended POM, as measured by isotope ratio mass spectrometry coupled to a CHN analyzer.

Samples were collected from the “noon” CTD casts for Cycles 1 – 3 (6 depths), at most of the BBL 1 and BBL 2 stations (3 depths), and during the “diel” sampling prior to commencing Cycle 1. Samples were not collected on the California Current Transect (CCT) due to CTD water budget considerations.

Volumes of 4L were drawn from the CTD Niskin bottles using Masterflex tubing into carboys (rinsed 3x) covered with black plastic bags to prevent influences due to light. Samples were drawn by vacuum pump at low vacuum and the filters were immediately stored at  $-80^{\circ}\text{C}$ . As noted below, these measurements serve to provide baseline information for interpreting carbon and nitrogen isotopes in different food web compartments.

(2)  $^{15}\text{NO}_3^-$

Samples were collected into 60ml HDPE bottles from a 47mm GF/F filter holder attached directly to the Niskin bottle’s nozzle and immediately frozen at  $-20^{\circ}\text{C}$  (within 30 min of collection). These samples were taken to measure the  $^{15}\text{N}$  and  $^{18}\text{O}$  composition of nitrate in the seawater, which serves as an important control on nitrate isotopes recorded throughout the food web (of broad interest to the CCE).

Samples were collected from the “noon” CTD casts for Cycles 1 – 3 (8 depths), during the “diel” sampling prior to commencing Cycle 1, and during the California Current Transect (CCT, 8 depths). Samples were

also collected on the 4000 m cast, at the Santa Barbara Basin site, and the San Pedro DDT Dumpsite Survey.

### (3) TOC/DOC

Samples were collected into 40mL pre-combusted clear borosilicate vials to later measure either the total or dissolved organic carbon concentrations. The primary intent from the TOC/DOC measurement is to quantify one of the largest reactive organic reservoirs in the ocean. 40mL volumes for DOC were collected by passing seawater through a pre-combusted 47mm GF/F filter holder attached directly to the Niskin bottle's nozzle. 40mL volumes for TOC were collected directly from the Niskin bottle nozzle. Samples were immediately acidified to a pH of 2 using trace metal grade acid upon collection (within 30 min).

Samples were collected from the first transect line during the Underway Survey, from the "noon" CTD casts for Cycles 1 – 3 (8 depths), during the "diel" sampling prior to commencing Cycle 1, during the California Current Transect (CCT), and during BBL Surveys 1 and 2. Samples were also collected on the 4000 m cast, the Santa Barbara Basin site, and the San Pedro, DDT Dumpsite survey.

### (4) FDOM

Samples were collected into 40mL pre-combusted amber borosilicate vials to later measure the fluorescent properties of DOM – which offers a coarse description of DOM composition. Samples for FDOM were collected from the "noon" CTD casts for Cycles 1 – 3 (8 depths), during the "diel" sampling prior to commencing Cycle 1, during the California Current Transect (CCT), and during BBL surveys 1 and 2. Samples were also collected on the 4000 m cast, the Santa Barbara Basin site and the San Pedro, DDT Dumpsite survey.

### (5) Metabolites

To analyze marine community metabolomes, we enriched metabolites from acidified seawater samples (and in the case of the San Pedro DDT dumpsite, from unacidified water as well) and separated them from salts using solid phase extraction (PPL cartridges) cartridges. After drying the cartridges with compressed N<sub>2</sub> gas, we stored them at - 20°C until further processing ashore. Samples for marine community metabolomes were collected from "noon" CTD casts for Cycles 1 – 3 (8 depths), during the "diel" sampling prior to commencing Cycle 1, during the California Current Transect (CCT), and during BBL surveys 1 and 2. Samples were also collected on the 4000 m cast (100 L volume), the Santa Barbara Basin site and the San Pedro, DDT Dumpsite survey (in addition to the traditional 1L samples we also collected 25 L of acidified and 25 L of unacidified seawater to be PPL extracted from near the basin sediments (~850 m)).

Ashore, we will elute metabolites with methanol from the cartridges and analyze them via Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Samples from the San Pedro DDT Dumpsite survey will be analyzed by Gas Chromatography Electron Capture Detector (GC-ECD), GC-MS or both.

(6) Bacterial Biomass and Production – (incl. Megan Roadman (FCM samples), Clay McClure (Bowman Group; Bacterial Production))

Samples for bacterial biomass (i.e., FCM) were collected by this group from “noon” CTD casts for Cycles 1 – 3 (8 depths), during the “diel” sampling prior to commencing Cycle 1, and during the California Current Transect (CCT). Other groups on the cruise also collected samples from other CTD casts and experiments. To measure bacterial cell abundance and size at each depth cryovials were filled with 3 mL seawater, fixed with 100  $\mu$ L of 10% paraformaldehyde, flash frozen in liquid nitrogen, and stored at -80°C. Samples will be analyzed by Karen Selph at the Univ. of Hawaii’s, SOEST Flow Cytometry Facility.

Samples for bacterial production rate were collected from “noon” CTD casts for Cycles 1 – 3 (8 depths), and during the California Current Transect (CCT). To estimate rates of bacterial protein synthesis, seawater was incubated with approximately 20 nM  $^3$ H-leucine for one hour at 9°C in triplicate with one control killed with final concentration 5% TCA. After the incubation was complete, all samples were killed with an addition of 100% TCA for a final concentration of 5% TCA. Samples were then pre-processed at sea by the centrifugation method. The dried pellets after the 80% EtOH wash were frozen and stored at -20°C. On shore, the pellets will be defrosted, scintillation cocktail added, and the tube assayed by liquid scintillation counter. Disintegrations per minute are converted to protein synthesis rates. This calculation is normalized by the corresponding cell abundances.

Comparative samples for bacterial production using BONCAT (Bioorthogonal non-canonical amino-acid tagging) methods were collected from the “noon” CTD casts for Cycles 1-3. To obtain an estimated rate of bacterial protein synthesis, seawater samples were incubated with 1 mM of L-azidohomoalanine for three hours at around 20°C in triplicate with one control sample killed with a final concentration of 4% paraformaldehyde (PFA). After incubation was complete, all samples were subsequently killed with an addition of 37% PFA for a final concentration of 4% PFA. Samples were then frozen and stored at -20°C to await processing. On shore, the samples will be defrosted and undergo strain-promoted DBCO (dibenzocyclooctyne) click chemistry steps. All samples will be assayed with flow cytometry to obtain fluorescence values to estimate production rates.

(7) Microbial Community Composition - (to be analyzed in collaboration with Andy Allen’s group at JCVI). Samples for bacterial biomass and production were collected from the “noon” CTD casts for Cycles 1 – 3 (8 depths), during the “diel” sampling prior to commencing Cycle 1, during BBL surveys 1 and 2, and during the California Current Transect (CCT). Samples were also collected on the 4000 m cast, at the Santa Barbara Basin site, and at the San Pedro Basin DDT dumpsite. Other groups also collected similar samples for their experiments.

For each sample, 4 L (and sometimes as little as 2 L) of seawater were filtered through a 0.22  $\mu$ m Sterivex filter using a peristaltic pump. Excess water from the Sterivex filters was removed using a syringe; the filters were then sealed with putty, flash frozen with liquid nitrogen, and stored at -80°C. On shore, the RNA from the filters will be extracted, converted to cDNA, and sequenced for 16S, 18S V4, and 18S V9. The sequence data will be analyzed to determine the microbial community composition.



## (8) Additional investigations

(a) *Two-day (48 hour) incubations at three cycles:* Acropak (0.2  $\mu\text{m}$ ) filtered seawater was collected from the chlorophyll maximum depth or at the surface and then incubated with microbial communities from the chlorophyll max (1:10 dilution with whole seawater inoculant), in the dark (covered in garbage bags) at 12 °C for 2 days. Two experimental and two control (no inoculant) conditions were set up in 9 L bottles, sampled at  $t=0$  and  $t=24$  hr for bacterial abundance (FCM) and TOC, and sampled at the incubation termination at  $t=48$  hr for FCM, TOC, 1 L metabolite samples and 2 L bacterial community composition samples. Sampling was typically done on the 19:00 CTD cast during cycles. Metabolites, community composition, TOC and DOC samples were also taken directly from the CTD at the chlorophyll max depth (and if different, the inoculant depth) during the  $t=0$  cast and 48 hours later (also from the “19:00” cast).

(b) *Santa Barbara Basin:* We have had a long-standing interest in nitrate isotopes and below sill-DOC dynamics at the Santa Barbara Basin. During the summer, oxygen in the basin is drawn down, and in addition to sediments being anoxic, the deep water column can also become anoxic. These conditions result in detectable DOC buildup (albeit 1-3  $\mu\text{M}$  C) and clear impacts on the isotope signature of water column nitrate. Learning the composition of DOC that is released from sediments under these conditions may shed light on the long-term DOC cycle, whereas the nitrate isotopes provide a snapshot into the complexity of nitrate reduction and re-oxidation in the basin. As such, we took the opportunity to collect a profile for nitrate isotopes, TOC, DOC, metabolites, and genetics at CalCOFI station 82.47 (81.8 46.9).

(c) *DDT dumpsite in San Pedro Basin:* In response to the recent resurgence of interest around the presence of two dumpsites in the Southern California Bight, where reports show that DDT waste from Montrose Chemical company was disposed, we took this opportunity to sample Dumpsite 2 in the San Pedro Basin on our return trip to San Diego. Previously, this site was mapped out by Eric Terrill and Sofia Merrifield (both at SIO) using side scan sonar data following a publication by Dave Valentine’s group at UCSB. A few days prior to our sampling, the R/V *Falkor*, during an expedition led by Lisa Levin, Paul Jensen and Greg Rouse (all from SIO), also stopped at Dumpsite 2 to do video surveys and collect push cores as well as benthic invertebrates from within the dumpsite. To complement these analyses, we took advantage of access to the MOCNESS nets, which is used to sample deep water column biota, and McLane pumps, that can filter particulate matter near the bottom sediments, and collected a small dataset that could provide insight into how DDT from this site could enter the pelagic foodweb. We sampled with the CTD to within 30-40 m of the bottom, near the location of high barrel densities as identified by the UCSB group, to collect oxygen and transmissometer data, as well as seawater for solid phase extraction. At this same site, we also lowered two McLane pumps, one to 850 m and the other to 600 m and pumped water onto 1  $\mu\text{m}$  quartz filters for approximately 1 hour at 7L/min (this was conducted by Carl Lamborg). Following this sampling, we travelled an 8 km route designed for us by Sofia Merrifield during which we did Multibeam and EK 80 surveys. We hope to use these latter data to determine community composition at depth at this site. Once this survey was completed, Stephanie Matthews assisted us with a MOCNESS tow that travelled across the San Pedro Basin (lengthwise) in a northwest direction (parallel to the coastline). We towed for approximately 2 hours between 790 and

650 m, and for another 1 hour between 650 and 550 m. The nets were dropped relatively quickly from 0 to 790 m; but the ascent from 550 m was slower. Samples were most comprehensive for the deepest nets as the upcast net closed prematurely and the downcast took place very quickly. The deepest tows travelled across the designated dumpsite (Dumpsite 2). Net samples were sorted into ziplock bags based on visual identifications (by chemists! – Max Fenton, Jamee Adams, Ralph Torres and Lihini Aluwihare) and frozen at -80 C.

(d) *4000 m cast*: We also took the opportunity during Cycle 3 to sample down to 4000 m with the CTD Rosette, with the goal of obtaining a DOM sample from the heart of the Pacific Deep Water water mass. Approximately 100 L of seawater from 3,500 m, was passed through an acid washed Acropak filter, acidified to pH 2, and then slowly extracted onto a total of 4, 5g PPL cartridges. This cast was also used to shrink some cups and heads!

(e) *Submersible Ultraviolet Nitrate Analyzer (SUNA)*: Ralph Torres, Jamee Adams (Diaz Group) and Max Fenton (Barbeau Group) maintained and monitored the SUNA mounted on the CTD Rosette frame.

(f) *Sediment trap samples*: We also obtained unpreserved sinking POM samples from Cycles 1, 2 and 3 for metabolite measurements. Depths varied with cycles but always included a 450 m sample.

(g) *Superoxide experiment (with Carl Lamborg's group)*: The goal of the superoxide experiment was to observe how organic matter composition and concentration changes due to the addition of a reactive oxygen species. 1.75 mL of a ~600  $\mu$ M superoxide solution (concentration determined by UV absorption) was injected into 1L bottles (triplicates) containing seawater from the Cycle 2 Day 2 18:00 CTD Cast at 1000 and 15 meters. Control samples (also triplicates) with no superoxide solution were also taken at both depths. DOC, TOC, metabolites, and fDOM were taken for the control samples. TOC, fDOM and metabolites were taken from the treated samples.

### **Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) production and decay (Sydney Plummer)**

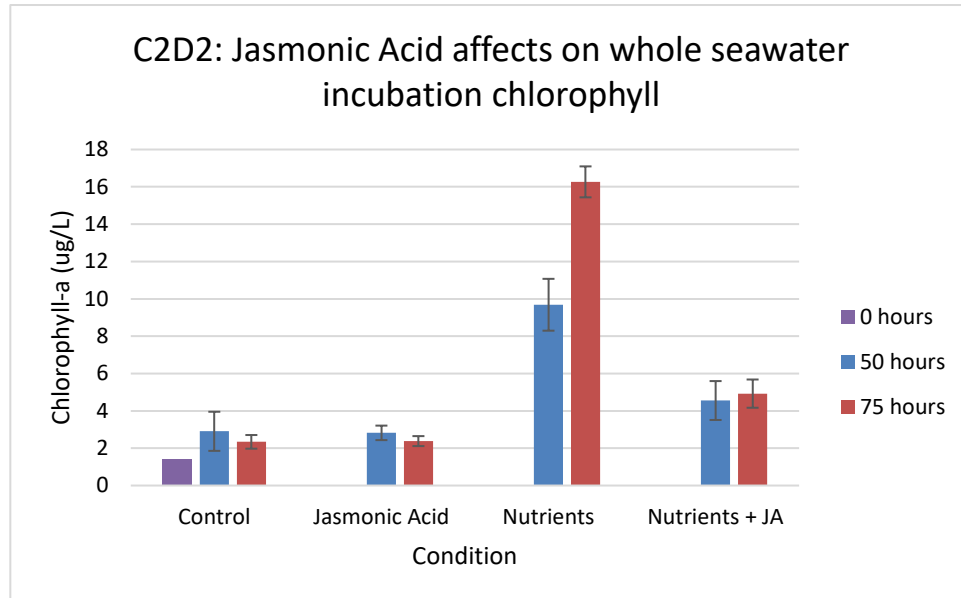
Microbial hydrogen peroxide production and decay rates were measured in seawater collected from the noon CTD casts on each day of the cycles to unravel hydrogen peroxide and DOM interactions. Briefly, seawater was collected at various depths throughout the water column, put into dark containers, and immediately analyzed using a colorimetric technique where horseradish peroxidase catalyzes the reaction between extracellular H<sub>2</sub>O<sub>2</sub> and the colorimetric probe Ampiflu Red. H<sub>2</sub>O<sub>2</sub> concentrations in samples were determined using absorbances of standard curves of H<sub>2</sub>O<sub>2</sub>. Biological production rates were calculated by determining the slope of H<sub>2</sub>O<sub>2</sub> concentrations over time. To measure H<sub>2</sub>O<sub>2</sub> decay rates, samples were incubated with known concentrations of H<sub>2</sub>O<sub>2</sub>, and measured at appropriate intervals using the colorimetric technique described above.

### **Jasmonic Acid Incubations (Monica Thukral (Allen group) with assistance from L. Aluwihare, S. Plummer, K. Barbeau, T. Coale)**

The ecological role of jasmonic acid in the California Current Ecosystem was investigated through two deck incubation experiments:

- 1) How does jasmonic acid (JA) addition affect growth, gene transcription, and small molecule production of whole sea water microbial communities?

This experiment was conducted during Cycle 2. Whole sea water incubations were conducted from surface water with and without nutrients (iron, silicic acid, nitrate, phosphate, vitamin B12) and with and without jasmonic acid. Significantly decreased chlorophyll was observed when jasmonic acid is



added to seawater, and this is only apparent under nutrient-amended (+Nu) conditions. This trend of decreased chlorophyll with JA addition is more pronounced after 75 hours. Using data from the imaging flow cytobot (IFCB), the phytoplankton community did not shift between +Nu and +JA+Nu conditions- there were fewer cells of the same organisms. Furthermore, the composition of the biomass was approximately 50% *Pseudo-nitzschia* diatoms. Samples were taken for the following analyses at 0 hour (initial) and 75 hour (final) time points: nutrients, flow cytometry, chlorophyll, RNA, metabolites; Chlorophyll was also measured at 50 hours.

- 2) How does addition of jasmonic acid to whole sea water communities affect grazing rates?

This experiment was conducted in conjunction with a coastal dilution experiment led by Sydney Plummer during the Alongshore Transect, near AT Station 5. Incubations were conducted from surface water with and without nutrients (ammonium, phosphate), with and without jasmonic acid, and with either whole sea water or 80% filtered sea water (from which grazers are removed). Samples were taken for the following analyses at 0 hour (initial) and 24 hour (final) time points: nutrients, flow cytometry, chlorophyll, RNA, metabolites, hydrogen peroxide production rate.

#### **Trace metal studies – Barbeau group (Katherine Barbeau, Kiefer Forsch, Max Fenton)**

Iron is a critical micronutrient that limits phytoplankton growth in significant areas of the ocean. The California Current upwelling region has been described as a mosaic of iron limitation with regions and features that range from iron replete, to iron co-limited, to iron-limited. We seek to understand how

iron supply shapes the composition and productivity of the phytoplankton community, and related impacts on biogeochemistry. The Barbeau group has also taken on biogenic silica sampling and analysis for cycles and transects, as previous cruises have shown that silica dynamics in this region are strongly impacted by the iron stress status of the diatom community.

Trace metal clean sampling activities –The Barbeau group's trace metal sampling activities on this cruise supported not only the Barbeau group, but also the activities of the Allen group, the mercury group (Lamborg et al.), and Viktoria Steck from the Diaz group. In addition, some collaborative trace metal work was carried out with the mesozooplankton group. We completed 42 casts with the trace metal rosette. Multiple trace metal rosette profiles were obtained at all cycles. Profiles were also obtained at Santa Barbara Basin, the Diel Station, and at all CCT stations. In addition to profiles, numerous stationary casts were made with the trace metal rosette to obtain water for incubation studies.

In addition to trace metal rosette casts, GO-Flo casts were carried out on two benthic boundary layer (BBL) transects. BBL transect #1, from Cambria to the Point Conception region, included 9 stations which have been sampled on several previous CCE LTER process cruises. BBL transect #2, 3 stations in the Point Sur region, was also sampled on P1908. One 30L GO Flo was destroyed during BBL transect #1 due to incorrect firing procedures.

Biogenic silica sampling - Biogenic silica (bSi) sample profiles were taken daily at all Cycles during the array setup casts, to coincide with the  $^{32}\text{Si}$  incubations carried out by Tristan Biard's group. Additional bSi profiles were taken at the Diel Station, during the CCT transect, and during the AT transect. The Barbeau group also received bSi samples from the sediment traps at each Cycle for processing, as well as pyrosome tissue and fecal pellet bSi samples from the mesozooplankton group. Additional bSi samples were taken from some incubation studies.

Incubation studies – Trace metal-clean incubation studies carried out by the Allen group, the Diaz group, and the Hg group using water collected by the Barbeau group are described in more detail below in their group-specific reports. In addition to these efforts, the Barbeau group also carried out several incubation studies:

- On-array Fe addition experiments (24 hrs, triplicate 1L bottles, incubated at chl max) were carried out at each cycle and sampled for size-fractionated mRNA ( $> 5 \mu\text{m}$ , Sterivex) in collaboration with Rob Lampe and Tyler Coale. These will be analyzed for prokaryotic mRNA to assess the response of free-living vs particle-associated bacteria to Fe addition. This data is also complementary to the Fe addition/Fe removal experiments described below in the Allen group report.

- Two Fe-addition experiments with mRNA size fractionation as described above were carried out for 48 hrs in the dark in the  $12^\circ\text{C}$  cold room using water from the deep scattering layer (DSL) at Cycle 3 (500 m) and AT Station 5 (225 m). DSL determined from EK80 signature.

- Kiefer Forsch set up a number of trace metal clean on-deck incubations to investigate Fe-Mn co-limitation and its potential impacts on P metabolism in the phytoplankton community. This research was carried out in collaboration with the Diaz group. We hypothesized that Fe/Mn co-limitation occurs in the offshore cycles (SCM, DCM), when both dissolved Fe and Mn concentrations are low. Incubations were initiated at four stations: Cycle 1 Day 2, Cycle 2 Day 2, Cycle 3 Day 2, and CCT Station #7.

Cycle 1 Day 2 incubation seawater was collected at the surface chlorophyll maximum during upwelling. Cycle 2 Day 2 was from a subducting chlorophyll maximum (25m). These two incubations were grown under 30% surface PAR. The following two experiments were from a deep chlorophyll maximum (DCM, 140m) and a subsurface chlorophyll maximum (SCM, 45m) and incubated at 1% surface irradiance and surface seawater temperature (13.5-15 degC).

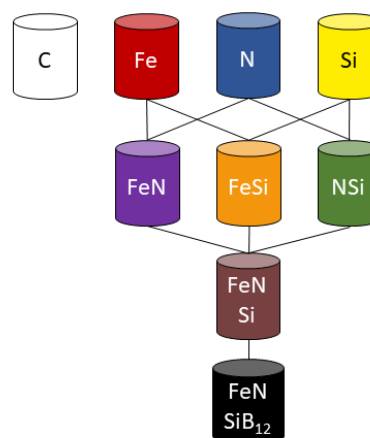
Incubations were subsampled for dissolved trace metals ( $t_0$ ), total dissolvable trace metals ( $t_0$ ), macronutrients, Chl-*a*, Phosphorous speciation (SRP, inorganic phosphate, organic phosphate), Alkaline phosphatase activity (APA), FCM (eukaryote and bacterial), 18S gene sequencing ( $t_0$ ), bSi ( $t_f$ ) and POC ( $t_f$ ). Preliminary findings from bulk Chl-*a* analysis indicated Fe-limitation at Cycle 2, in line with results from incubations carried out by the Allen group (see below).

#### **Allen group trace metal incubations (Rob Lampe and Tyler Coale, in collaboration with Barbeau group)**

*In situ* array iron addition/removal incubations - At Cycles 1 and 2, we exposed natural phytoplankton communities from the deep chlorophyll maximum to iron addition (+5 nM FeCl<sub>3</sub>) or iron removal (+100 nM of the iron chelator desferrioxamine B) and incubated them for 24 hours in their ambient environment on the *in situ* quasi-Lagrangian drifter. Evaluation of the mRNA and chlorophyll from these incubations will allow us to evaluate the phytoplankton communities' responses to changes in iron bioavailability under near-natural conditions. These experiments were also conducted on the 2014, 2017, and 2019 CCE LTER process cruises allowing us to compare across years.

Investigating nutrient co-limitation in the California Current Ecosystem - There is strong evidence for both nitrogen (N) and iron (Fe) limitation of phytoplankton growth in the California Current Ecosystem (CCE); however, these nutrients have largely been investigated as being the sole limiting nutrient where the addition of N or Fe alone leads to increased growth. Furthermore, silicic acid-limitation can be associated with Fe-limitation in diatoms as they increase Si uptake and/or reduce N assimilation relative to uptake rates during Fe-replete conditions. Vitamin B<sub>12</sub>, or cobalamin, is an important micronutrient that can be in very low concentrations in the region (< 1 pM) and may also play a role in limiting phytoplankton growth, particularly if N- and Fe-limitation are alleviated.

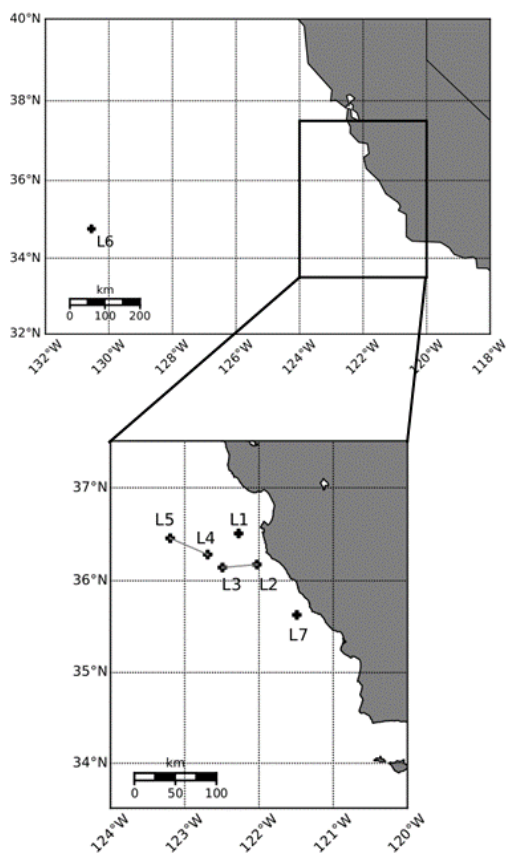
Simultaneous co-limitation, or where the addition of two or more nutrients is required to result in phytoplankton growth, has been observed in other regions and is suggested to be widespread in the ocean; however, it remains underexplored in the CCE. To characterize nutrient limitation patterns in the CCE and explore the existence of co-limitation as a bottom-up control on phytoplankton growth in the region, we conducted full-factorial trace metal clean incubations with Fe, N (nitrate) and Si (silicic acid) at seven locations during the cruise (see diagram at right, map below). An additional treatment with all the nutrients and vitamin B<sub>12</sub> was included to evaluate whether vitamin B<sub>12</sub> can limit phytoplankton growth once Fe, N, and/or Si limitation is removed.



Co-limitation experimental design.  
Each treatment consisted of triplicate 1L bottles.

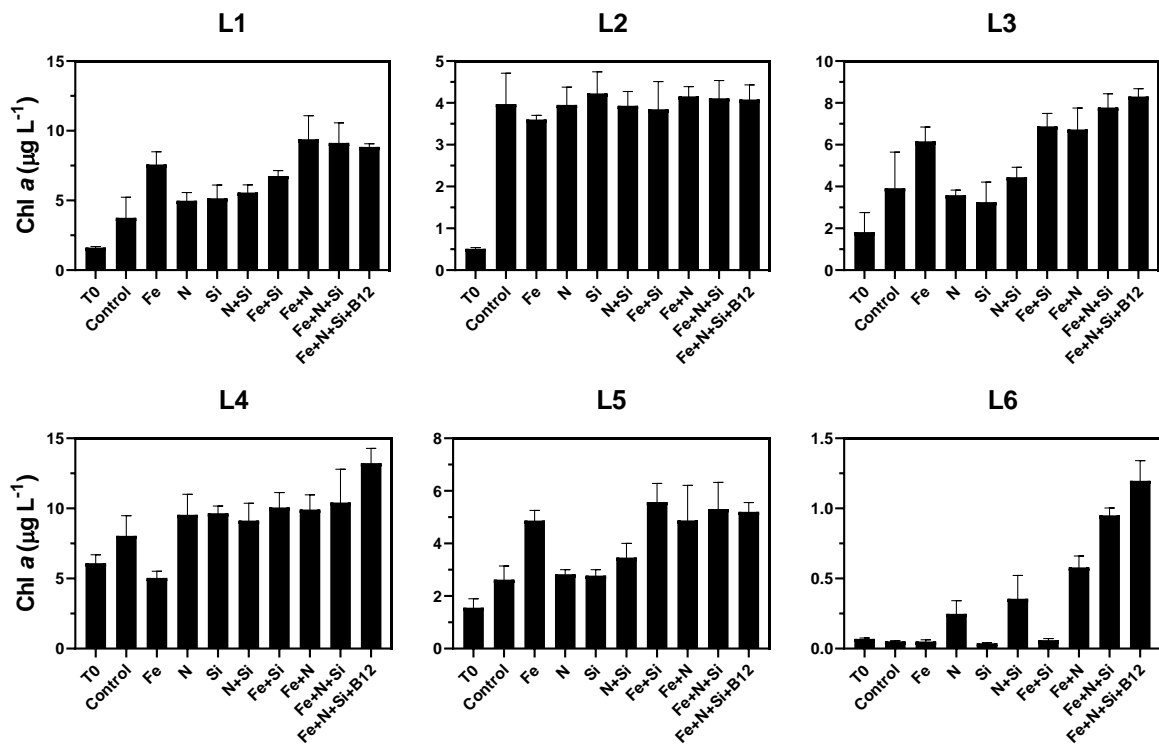
A range of nutrient limitation conditions was captured across the different experiments (bar graphs below). Sites L1, L3, and L5 showed a clear pattern of Fe being the primary limiting nutrient. It is likely that chlorophyll *a* concentrations at L4 were lower than the control due to the phytoplankton population quickly crashing after the addition of Fe resulting in rapid macronutrient drawdown. Site L6 was primarily limited by nitrate. The addition of multiple nutrients led to an even greater growth response at several sites, although these differences were often not statistically significant ( $P > 0.05$ ). Sites L1, L3, and L5 showed slightly higher chlorophyll *a* concentrations upon the addition of macronutrients, suggesting serial limitation of N and/or Si. Once N limitation was alleviated at site L6, the addition of Fe then Fe+Si led to even greater growth responses indicating serial limitation of Fe then Si. Interestingly, chlorophyll *a* concentrations were highest in the combined treatment with vitamin B<sub>12</sub> at sites L3, L4, and L6 indicating that vitamin B<sub>12</sub> limits phytoplankton growth under N, Fe, and Si-replete conditions.

Additional samples were collected from each bottle for macronutrient concentrations, flow cytometry, and RNA, and metabolomics. With the macronutrient samples, we will be able to evaluate potential differences in macronutrient drawdown. Flow cytometry will enable quantification and comparison of picophytoplankton abundances. The RNA samples will be used to evaluate community composition of both the prokaryotic and eukaryotic communities (16S and 18S rRNA) as well as gene expression (metatranscriptomics). Select treatments were also analyzed with the imaging flow cytobot (IFCB) to obtain a semi-quantitative assessment of the eukaryotic phytoplankton community. These assessments of community composition will enable us to evaluate the potential for different nutrients or combinations thereof to stimulate growth in different community members.



Left - Map of experimental locations numbered in chronological order. Locations connected by a line correspond to experiments conducted during the same cycle where the quasi-Lagrangian drifter was followed between experiments.

Below - Co-limitation experiments preliminary results. Chlorophyll *a* concentrations from sites L1-L6 in the initial conditions (T0) and each experimental condition. Data are not yet available for site L7. The y-axis scale is different for each experiment due to the wide-ranging conditions captured during the cruise.



## **Mercury Studies (Amina Schartup (in absentia) and Hannah Adams (SIO); Carl Lamborg and Xinyun Cui (UCSC))**

The Mercury Group (MG) set up analytical instrumentation in a “clean bubble” left in place by the Lam Lab (UCSC) on the previous R/V *Roger Revelle* leg. In the bubble, the MG was able to de-gas and immediately analyze samples for the two dissolved gaseous Hg species ( $\text{Hg}^0$  and  $(\text{CH}_3)_2\text{Hg}$ ). In addition, instrumentation that allowed the determination of total dissolved Hg was also set up and most of the subsamples for total Hg were analyzed on board as well. This arrangement allowed for methods testing and made possible the determination of the gaseous species which have no “shelf life” and must be de-gassed immediately. This cruise was also the first for SIO student Adams and the first in the US for UCSC student Cui.

Cycle Profiles - On 9 occasions during the three cycles, we received water generously provided by the Barbeau lab which facilitated the construction of vertical profiles of Hg species. This consisted of a total of 84 water samples, each to be measured for  $\text{Hg}^0$ ,  $(\text{CH}_3)_2\text{Hg}$ , total Hg, and  $\text{CH}_3\text{Hg}$  (upon return). The general trend revealed here was that dissolved  $(\text{CH}_3)_2\text{Hg}$  was higher in the waters that were more upwelling-influenced (colder, saltier, more nutrient-rich) and also followed trends of production in deeper waters as well. Another general trend was that  $\text{Hg}^0$  increased with depth, and total Hg had a nutrient-like profile (low at the surface and increasing with depth). We found that the Cycles in more productive waters (1, 2) had a higher total Hg concentration than the cycle in oligotrophic water (3).

Benthic Boundary Layer (BBL) Measurements – We received water samples from the Barbeau group to examine Hg speciation in the Central/Southern California coastline (Pt. Sur to Pt. Conception) benthic boundary layer. In total, we received 12 water samples to be measured for  $\text{Hg}^0$ ,  $(\text{CH}_3)_2\text{Hg}$ , total Hg, and  $\text{CH}_3\text{Hg}$  (upon return). We also asked for replicate samples (4) for  $(\text{CH}_3)_2\text{Hg}$  to test our analysis methods; specifically, differences between filtered vs. unfiltered water and using Carbotrap vs. Tenax traps to pre-concentrate the  $(\text{CH}_3)_2\text{Hg}$ . These samples were valuable in exploring sediments as a possible source of  $(\text{CH}_3)_2\text{Hg}$  to the water column. These samples revealed that the BBL is actually depleted in  $(\text{CH}_3)_2\text{Hg}$  relative to the water column, discouraging the view of it as an important source to the water column in general, and during upwelling events in particular.

California Current Transect Profiles - Returning in-shore to recover the *Zooglider*, the ship occupied several transect stations (CTD, Trace Metal Rosette (TMR), nets), and the MG received water from the Barbeau group at 7 of these to produce water column profiles at 8 depths per station (total of 56 samples) for  $\text{Hg}^0$ ,  $(\text{CH}_3)_2\text{Hg}$ , total Hg, and  $\text{CH}_3\text{Hg}$  (to be analyzed upon return). These stations added additional nuance to our currently existing Cycle stations by sampling water of intermediate nutrient status between the highly productive Cycle 1 and 2 stations and the more oligotrophic Cycle 3 stations.

Photochemistry Experiments - An assumed, but not fully tested, behavior of  $(\text{CH}_3)_2\text{Hg}$  is that it is photochemically labile. This is based on the observation that  $(\text{CH}_3)_2\text{Hg}$  is virtually absent from the ocean mixed layer during upwelling-unfavorable conditions. However, one published manuscript argued against this mechanism. So, we sought to test directly whether  $(\text{CH}_3)_2\text{Hg}$  was photolabile in ambient seawater. This was accomplished by exposing 4 samples of 400 m water in 2-L Teflon bottles from Cycle



3 Day 3 to ambient light conditions (quite clear and bright) on the forward 01 deck for a full daylight period, taking periodic subsamples. We found  $(\text{CH}_3)_2\text{Hg}$  rapidly disappeared, while a control sample held in the dark in the lab actually increased in  $(\text{CH}_3)_2\text{Hg}$  concentration. A subsequent repeat of the experiment showed much the same behavior, this time using bottles stored in the dark on deck with the exposed samples as controls. Under these high light conditions, the half-life for  $(\text{CH}_3)_2\text{Hg}$  appeared to be around 3 hours. This rate of decay, even if much slower due to lower in-situ light levels, indicates that  $(\text{CH}_3)_2\text{Hg}$  photodegrades faster than its loss to air-sea exchange, even under upwelling favorable conditions. This suggests substantial in-situ production, even in the mixed layer.

Diel Cycling Experiments (underway) - To test whether there is production of  $(\text{CH}_3)_2\text{Hg}$  at night in the absence of daylight (and photochemical degradation), we attempted a 24-hour experiment in which we sampled water from the underway system into 2-L Teflon bottles every hour and analyzed it for  $(\text{CH}_3)_2\text{Hg}$ . We conducted this experiment from 2pm on Cycle 3 Day 1 (8/3/2021) to 5am on the following morning (8/4/2021). This experiment was not fully completed because we did not measure any  $(\text{CH}_3)_2\text{Hg}$  during the entire period. This could potentially be due to being in low productivity waters during Cycle 3.

In an attempt to see if there is dark production of  $(\text{CH}_3)_2\text{Hg}$  in surface waters, we collected a total of 10 2-L surface water samples from the underway system into Teflon bottles before sunrise. 2 samples were analyzed immediately, 2 samples were placed on the forward 01 deck exposed to light for a full daylight period, 2 samples placed in the dark on the forward 01 deck for a full daylight period, 2 samples in the walk-in fridge in the dark for 24 hours, and 2 samples in the dark at room temperature. The samples were purged for  $(\text{CH}_3)_2\text{Hg}$  and measured immediately after the exposure periods (daylight period or 24 hours). Results seemed inconclusive, but we are still considering the data.

Analytical Experiments - We undertook several analytical related experiments at sea as well, taking advantage of the access to relatively high  $(\text{CH}_3)_2\text{Hg}$  water. These included:

- Differences between TMR and CTD for  $(\text{CH}_3)_2\text{Hg}$  analysis.
- Differences between different  $(\text{CH}_3)_2\text{Hg}$  sorption media analyzed immediately after purging as well as after several days of storage on the media.
- Differences between filtered and unfiltered water for  $(\text{CH}_3)_2\text{Hg}$  determination.
- Differences between underway water and other surface samples.

The results of these comparisons are still being looked at.

Superoxide Experiments - During the second BBL transect, we took some seawater samples and spiked with superoxide at micromolar levels, to see whether superoxide can get involved with Hg reactions, especially Hg reduction and demethylation. The data implied that superoxide might give rise to  $(\text{CH}_3)_2\text{Hg}$  production, which was unexpected.

Inspired by the results above, we conducted experiments on 7/27/2021 by sampling seawater from the underway system into 2-L Teflon bottles. 2 samples were bubbled immediately, while 2 other samples were spiked with superoxide and held in the dark for 1 h to leave sufficient time for reaction to take place, followed by bubbling. The control group was treated with superoxide distamuse (SOD) prior to the superoxide spikes. The results did not prove that superoxide could lead to increasing  $(\text{CH}_3)_2\text{Hg}$ . In

addition, we added superoxide into 2-L Teflon bottles filled with shallowest seawater on Cycle 2 Day 5, and compared the  $(\text{CH}_3)_2\text{Hg}$  value generated from same seawater without superoxide. It turned out that those values were basically same. Therefore, these superoxide experiments were inconclusive and will be explored back in Santa Cruz.

We made a high-concentration primary superoxide stock (~600  $\mu\text{M}$ ) for the Aluwihare group. This was used to see what sort of changes in DOC structures superoxide might induce.

McLane Pumping - For our own sampling and to facilitate sampling by the Decima and Diaz groups, we deployed McLane pumps 20 times during the cruise at a variety of depths and (for the Diaz group) using two filter types. These samples were processed in the clean bubble by Hg group personnel for the Decima and Hg groups and by Jamee Adams for the Diaz group.

We also sampled at two depths at the “DDT Site” off of San Pedro. One close to the bottom at about 775 m below the surface (about 50 m above bottom) and at 675 m below surface. Filters were passed on to the Aluwihare group.

Sediment Trapping - We received subsamples generously supplied by the Stukel group from their sediment trap array at two depths on each of the deployments. These were processed by Stukel group personnel and will be analyzed on-shore.

### **Phosphorus Studies – Diaz Lab (Jamee Adams, Viktoria Steck)**

Phosphorus is a vital nutrient of life, and essential for the proliferation and productivity of marine microorganisms. In certain regions of the global ocean, however, easily accessible orthophosphate is a biologically limiting nutrient that constrains marine primary production. In these areas, marine plankton express various metalloenzymes, such as alkaline phosphatase (AP), to acquire phosphorus instead from the less accessible pool of dissolved organic phosphorus (DOP). The Diaz lab studies transformations within the major phosphorus pools in the ocean as well as coupled cycling of phosphorus with trace metals.

Total Dissolved Phosphorus (TDP) - Samples were collected in 2L HDPE bottles and filtered in triplicate through 25mm GF/F filters into 60mL HDPE bottles. Samples were frozen at  $-20^\circ\text{C}$ . These samples were taken to measure dissolved organic P (DOP) by subtracting inorganic P from TDP. Samples were collected at the Diel Station (24-hour period), and Cycles 1-3, as well as at the Santa Barbara Basin CTD cast. Each set of samples was from an eight-depth CTD profile with samples taken at each depth.

Total Particulate Phosphorus (TPP) - Samples were collected in 2L HDPE bottles and filtered in triplicate through 25mm GF/F filters. Each filter sample (~2L of seawater) was frozen at  $-20^\circ\text{C}$ . Filtrate from these samples was used for TDP, and BAP samples. Samples were collected at the Diel station and Cycles 1-3, as well as at the Santa Barbara Basin CTD cast. Each set of samples was from an eight-depth CTD profile with samples taken at each depth.

Tripolyphosphate (3polyP) - Samples were collected in 2L HDPE bottles and filtered in triplicate through 25mm GF/F filters. Each sample received ~2L of particulate, and were frozen at -20°C. Samples will be used to assess the ratio of TPP:3polyP with depth. Samples were collected at the Diel station, and Cycles 1-3, as well as at the Santa Barbara Basin CTD cast. Each set of samples was from an 8-depth CTD profile with samples taken at the top six depths.

Bioavailable Phosphorus (BAP) - Samples were collected in 2L HDPE bottles and filtered through 25mm GF/F filters into one 1L HDPE bottle per depth. The top six depths were sampled from each 8-depth CTD cast to coordinate with APA measurements. Samples were collected at the Diel Station, and Cycles 1-3, as well as at the Santa Barbara Basin CTD cast.

Alkaline Phosphatase Activity (APA) -

*Kinetic plates:* Samples were collected from 2L HDPE bottles from the top 6 CTD depths during the Diel Station, Cycles 1-3, the Santa Barbara Basin, and at every station during the California Current Transect (CCT). Duplicate samples were run in black 96 well plates on a Molecular Devices multi-mode plate reader. Fluorescence was measured from the fluorogenic phosphatase substrate methylumbelliferyl phosphate (MUF-P), using a MUF-P gradient from 0-10uM for each depth. APA, Km, and Vmax kinetic parameters were measured for each depth.

*Competition plates:* Samples were collected from 2L HDPE bottles from the chlorophyll max during Cycles 1-3, the Santa Barbara Basin, and at every station during the California Current Transect (CCT). Duplicate samples were run in black 96 well plates on a Molecular Devices multi-mode plate reader. Fluorescence was measured from the fluorogenic phosphatase substrate MUF-P at a concentration of 500nM. Competing substrates were spiked into each well at a concentration of 0-10uM to assess competition between alternative substrates and MUF-P. Competing substrates included adenosine triphosphate, adenosine monophosphate, glucose 6-phosphate, pyrophosphate, tripolyphosphate, and 45-polyphosphate. A decrease in MUF-P APA compared to 0uM competing substrate concentration suggested competition by the alternative substrate.

Proteomics – McLane Pumps - Samples were collected from large volume McLane pumps onto a filter stack containing 50 µm Nitex, 3 µm Verapore, and 0.2 µm Supor filters for proteomic analysis. Each sample was from the chlorophyll max to optimize particle collection. Samples were flash frozen in liquid nitrogen and then frozen at -80°C. Samples were taken during days 1 and 3 of Cycles 1-3, and at one station during the CCT.

Shipboard Incubations - Phosphatases, including APs, are metalloenzymes, and their activity depends on the availability of trace metals, for example iron and zinc, in the surrounding seawater. In order to probe for potential metal regulation of DOP cycling in the marine environment, shipboard incubations were conducted at every cycle. Trace metal clean samples of planktonic communities from the near-surface or the deep chlorophyll maximum were spiked with a variety of potential metal cofactors (iron, zinc, cobalt, manganese) and incubated on deck for 2-5 days. At different timepoints during the incubation period, the effect of metal amendments on the bulk phosphatase activity was measured through AP

activity assays (single concentration DOP hydrolysis rates and competition plates). Certain incubation bottles were also sampled for flow cytometry, chlorophyll concentration, different phosphorus pools (soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), total particulate phosphorus (TPP)), and mRNA (16s/18s sequencing and metatranscriptomics).

In a second incubation experiment, the influence of trace metal availability on the hydrolysis of specific DOP compounds was assessed. In trace metal clean incubations, natural phytoplankton communities were amended with adenosine triphosphate (ATP), a DOP compound from the class of phosphoesters, with and without the addition of trace metals (iron, zinc, manganese). Treatments with orthophosphate served as a control. Compound-specific hydrolase activity was measured through collection of SRP samples at three timepoints during the 2-5 day shipboard incubation period, along with PP and flow cytometry samples. Altogether, these experiments will provide new insights into compound-specific and metal-dependent DOP biotransformations as well as intertwined metal and P cycling within marine microbial communities.

### **Acoustic Studies (Sven Gastauer)**

#### Simrad EK80 Calibration and Data Collection

Frequency modulated (FM - broadband) and Continuous wave (CW - narrowband) calibrations were successfully completed on the 13<sup>th</sup> of August 2021. Calibration procedures largely followed Demer et al. (2015). A 38.1 mm tungsten carbide sphere with a 6 % cobalt binder was used for all frequencies. The calibration sphere was moved inside the beam with a three point positioning system.

Calm weather conditions allowed us to complete the entire set of calibrations while drifting. The total of 10 runs of calibrations were completed within approximately 4 hours (~14:55 – 18:45 PDT). Calibrations were used to update the beam information of each transducer. Calibration results were saved as XML files.

All settings used during normal operation were calibrated (see table below).

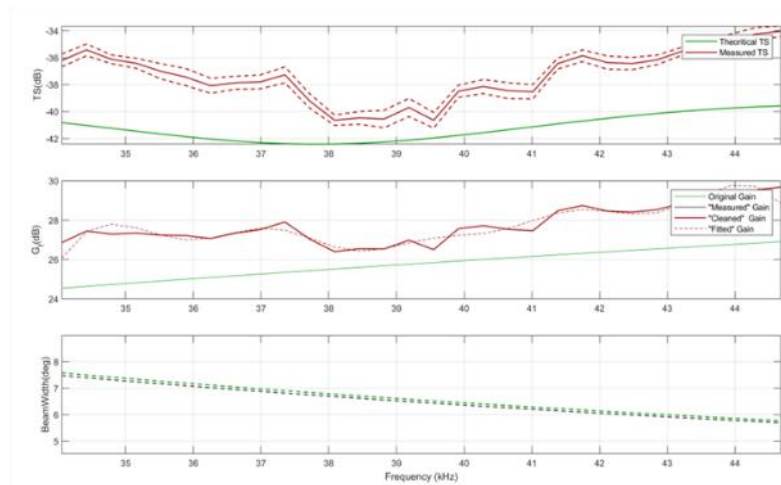
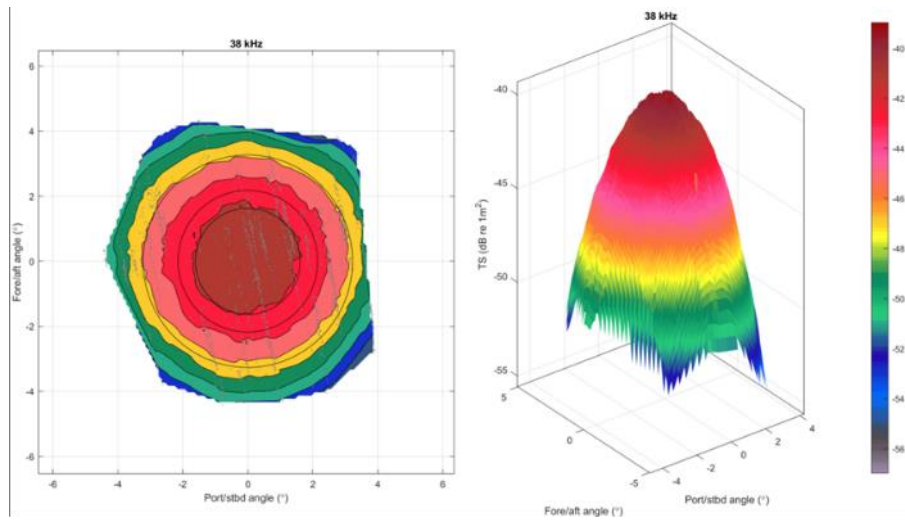
Normal operation CW and FM settings used and calibrated during CCE LTER 2105

Pulse	Pulse duration	Power	Frequency		Ramping	Direction
			Start	Stop		
CW	2.048	1000	18	18	Fast	Upward
FM	2.048	1000	34	43	Fast	Upward
FM	2.048	750	45	90	Fast	Upward
FM	2.048	250	90	160	Fast	Upward
FM	2.048	105	160	260	Fast	Upward
CW	1.024	1000	18		Fast	
CW	1.024	1000	38		Fast	
CW	1.024	750	70		Fast	
CW	1.024	250	120		Fast	
CW	1.024	105	200		Fast	

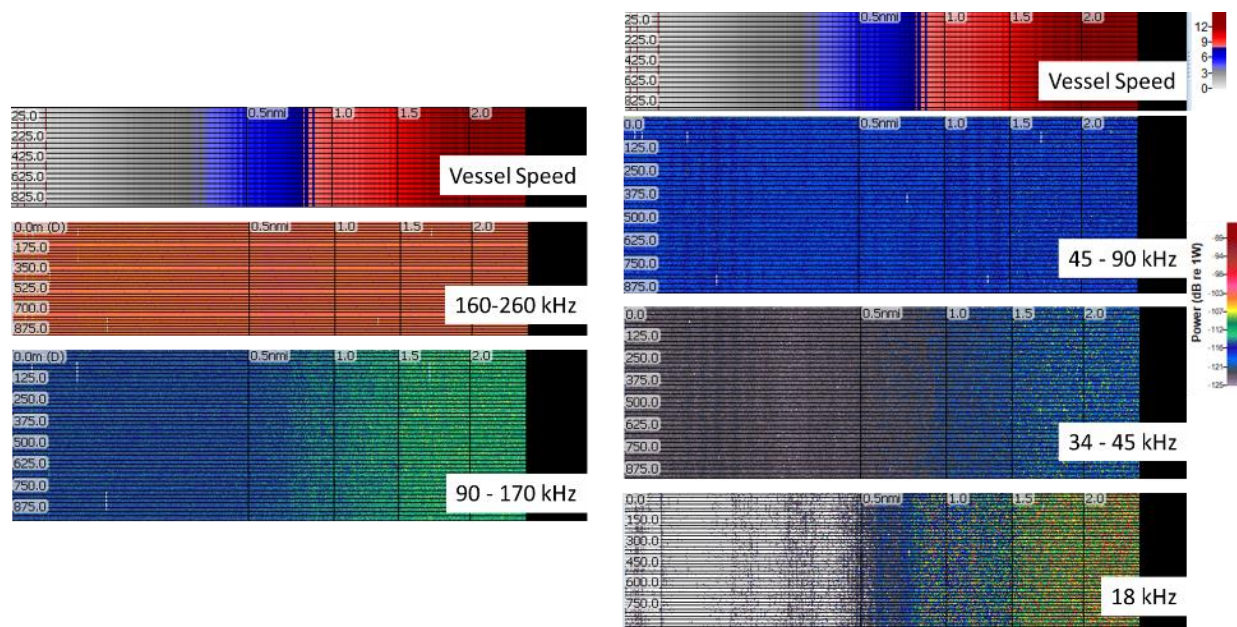
Once the calibration procedures were completed, an acoustic vessel noise test was performed. Over approximately 20 minutes, the vessel gradually sped up from drifting to 12 knots. All Simrad EK sounders were operated in passive mode. All, but the 18 kHz (for which no broadband license was available) were run in passive broadband mode with the settings described in the table above. A strong increase in background noise with increasing vessel speed was most noticeable in the 18, 38 and 120 kHz data (Figure below)

During the cruise all echosounders were operated in CW mode. Only at selected stations or at locations of special interest was the operation mode switched to broadband mode. The EK80 echosounders were synchronized with the ship mounted ADCP (75 and 150 kHz, operated in narrowband) through K-Sync (normal mode was EK80 – ADCP-ADCP-ADCP - EK80 with the EK80 runtime fixed at 1 second – or higher dependent on seabed depth and the ADCP at 250 ms). To operate the echosounders in broadband mode, a Mission Plan was defined in the Simrad software. To minimize nonlinear cross-talk of the different frequencies sweeps and their harmonics, two frequency groups (38 + 200 kHz and 18 + 70 + 200 kHz) were defined, following and after discussions with Khodabandeloo et al. (2021). Note that the pulse duration in broadband and narrowband mode were different, to maximize data resolution.

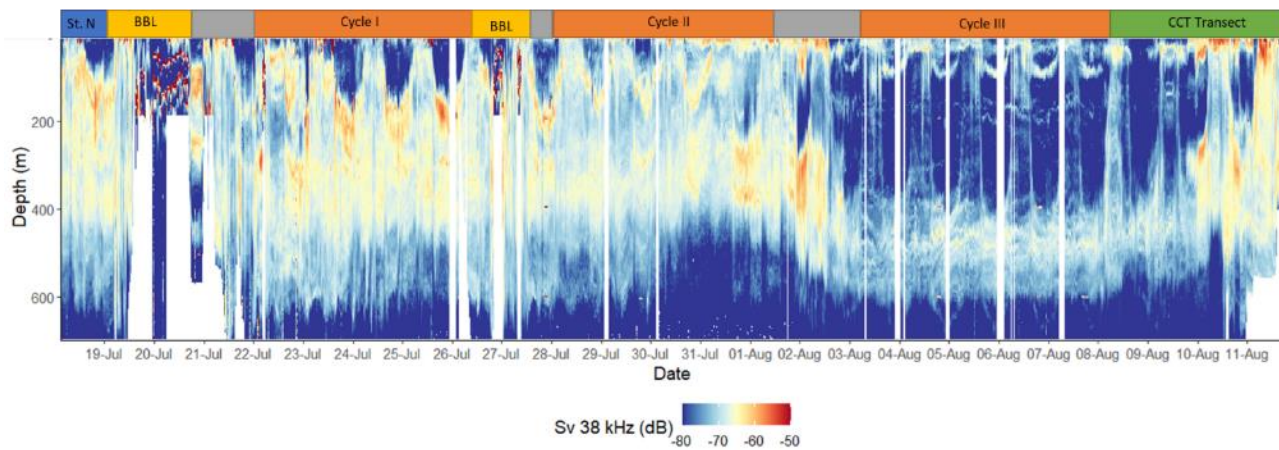
The entire recorded acoustic cruise recordings at 38 kHz are summarized below. Differences are evident in the intensity and depth of the DVM and the backscattering strength in the entire water column. Differencing the acoustic backscatter at different frequencies allows us to detect structural differences in the biological community and species group identification to a certain degree (see below).



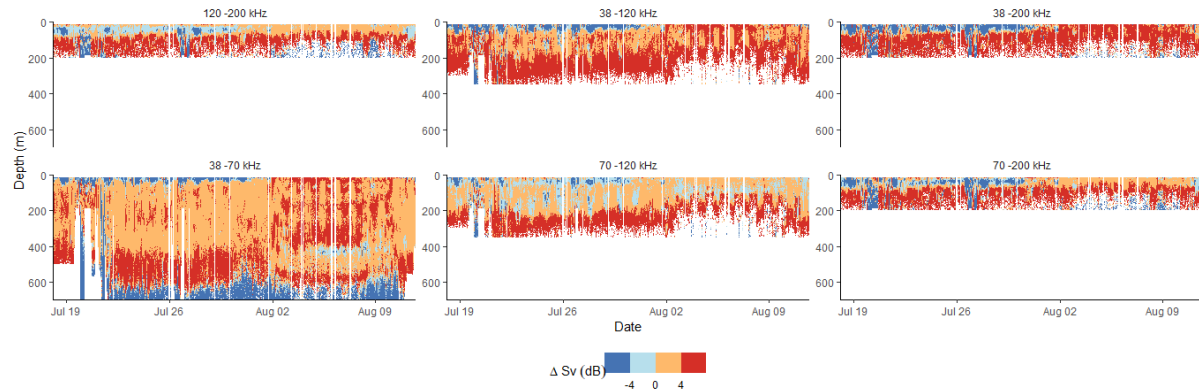
EK80 38 kHz beam pattern (top – left and right) and FM calibration results (TS, Gain and Beam width)



Noise pattern in Power (dB re 1W) at all available frequencies and corresponding vessel speed in knots (top)



Acoustic backscattering volume at 38 kHz (blank regions correspond to times at which broadband data was recorded). The top bar is a coarse indicator of the survey activity at a given time.



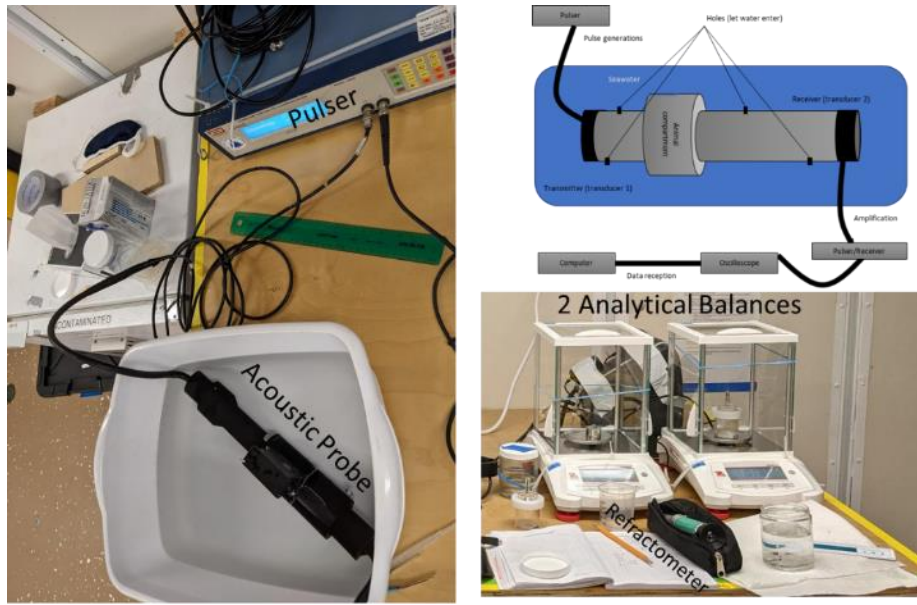
dB-Differencing of different frequencies, to gain some insights into structural differences in the biological communities as measured in multi-frequency acoustic data.

### Internal sound speed and density experiments

Understanding the backscattering properties of marine organisms is crucial to be able to translate the active acoustic backscatter into biologically meaningful metrics, such as size, length, biomass or abundance. The backscatter from a single organism is called target strength (TS). TS is largely dependent on the acoustic impedance, behavior or orientation and size or volume as well as the frequency at which the scattered sound wave was emitted. The acoustic impedance is generally defined through the material properties of the acoustic target, which can be described through the density and sound speed contrast inside the organism, compared to the surrounding fluid. For example, a fish with a gas filled swimbladder or a gas-bearing siphonophore have a very strong density and sound speed contrast compared to the surrounding ambient seawater, while a jelly fish is much more fluid-like in many ways. As a result, a swimbladdered fish will have a very strong TS while jellyfish and most other zooplankton taxa can be considered weak scatterers.

To improve our understanding of the scattering properties of zooplankton in the CCE LTER region, a sound velocity probe and a density measurement kit were used to conduct sound speed and density contrast experiments. Among others, krill, copepods, jellyfish, chaetognaths, ctenophores, salps, pyrosomes and pteropods were measured. Results from these experiments can be directly fed into modelling studies and contribute to an improved understanding and interpretation of the CCE acoustic data.





Sound speed and density contrast experimental setup. Left: acoustic probe connected to the signal generator/pulser; Top right: schematic of the acoustic sound speed probe; Bottom right: two Ohaus balances and refractometer used aboard R/V *Roger Revelle*

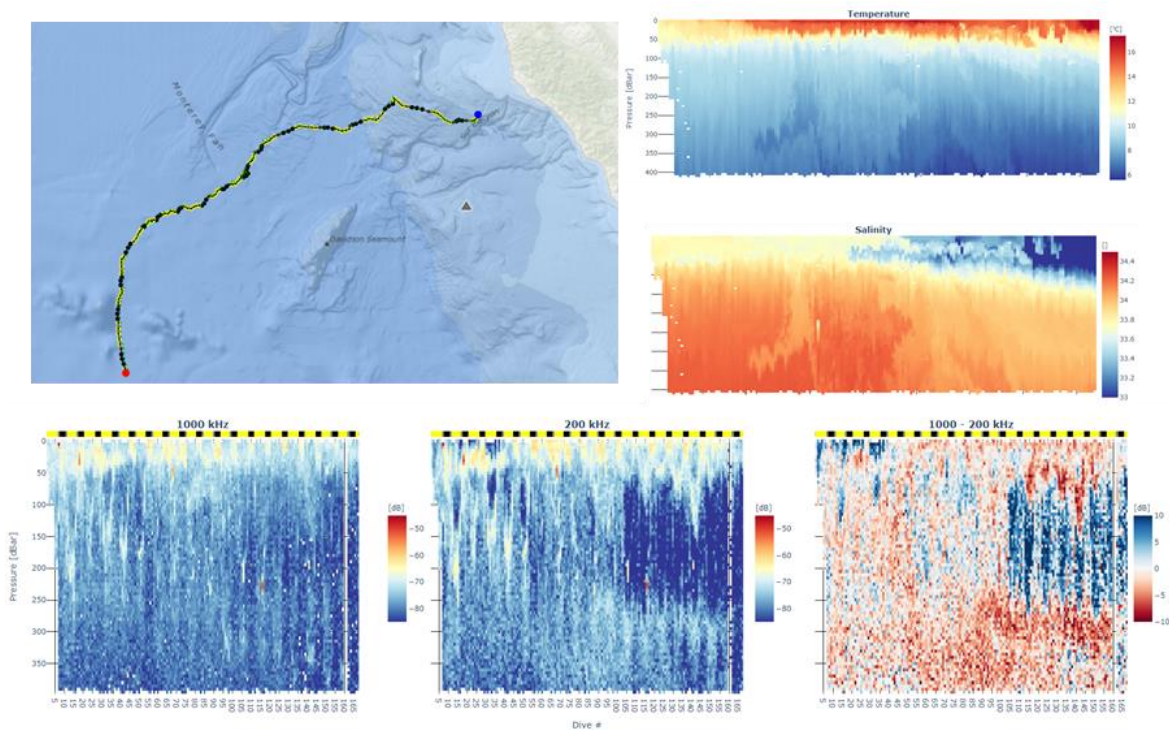
### Zooglider (Sven Gastauer, Mark Ohman, Jeff Sherman)



*Zooglider* deployment on the 22nd of July 2021 from R/V *Roger Revelle*

An autonomous *Zooglider* (Ohman et al., 2019) was deployed for 18 days, to assess frontal gradients in plankton communities and hydrographic communities. Measurements were done in a series of 167 *Zooglider* dives, from 400-0 m in which optical images of zooplankton and marine snow were recorded by Zoocam, acoustic backscatter at 200 and 1000 kHz (by Zonar) with concurrent CTD and fluorescence measurements.

*Zooglider* was deployed from R/V *Roger Revelle* on the 22<sup>nd</sup> of July at around 10 am within Cycle 1. At the time of *Zooglider* recovery, a brief annular survey was performed by R/V *Roger Revelle* around the *Zooglider* location, to allow for a comparison with the vessel mounted active acoustic data at 200 kHz. *Zooglider* information was a useful asset to improve survey planning, especially when satellite information was sparse due to cloud coverage. Summary information received by *Zooglider* can be seen below.



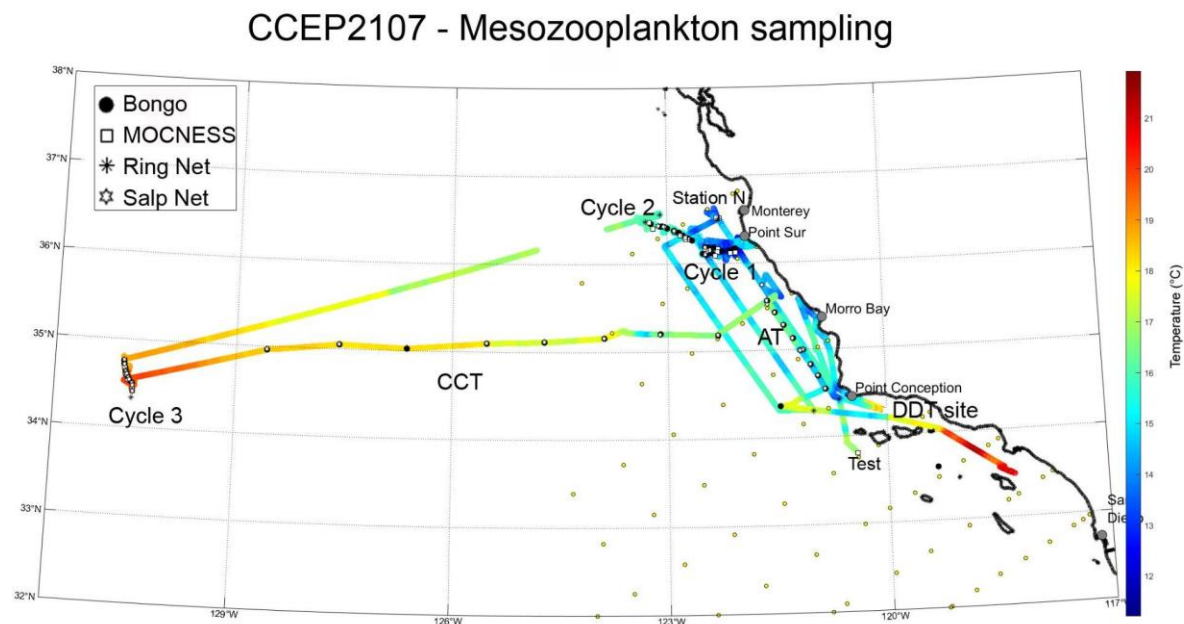
Summary information from the *Zooglider* mission taken from the near real-time Zoogallery webpage, showing: Top left: the mission track with an indication of day (yellow) and night (black) dives ; Top right: temperature and salinity; Bottom row: a summary of the acoustic Sv data at 1000 kHz, 200 kHz and the dB difference of 1000 – 200 kHz.

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- Ohman, M. D., Davis, R. E., Sherman, J. T., Grindley, K. R., Whitmore, B. M., Nickels, C. F., & Ellen, J. S. (2019). Zooglider: An autonomous vehicle for optical and acoustic sensing of zooplankton. *Limnology and Oceanography: Methods*, 17(1), 69–86.

**Mesozooplankton Studies (Moira Décima, Stephanie Matthews, Grace Cawley, Dante Capone, Anya Steiner, Minerva Padilla Villa, Julie Turko, Peter Fee)**

Sampling of the mesozooplankton community was carried out using Bongo nets (0.71m diameter, 202  $\mu$ m mesh), MOCNESS (1m<sup>2</sup>, 202 $\mu$ m mesh), Ring net (1m diameter, 303  $\mu$ m mesh) and a 'Salp net' (1m diameter, 202  $\mu$ m mesh). We divide the zooplankton sampling into 'Structured Sampling' and 'Experimental Sampling' sections. The former refers to the quantitative, repeated, and periodical sampling of the mesozooplankton community for stock and rate estimates, and the latter refers to the non-quantitative collection of specimens for a variety of purposes that will be detailed below. The figure below shows the location of all mesozooplankton tows.



Mesozooplankton tows conducted during P2107. Shape is coded by sampling type. Color is sea-surface temperature from the ship's underway system. Transects are California Current Transect (CCT) and Alongshore Transect (AT), cycles and stations as indicated. "Station N" here refers to the Diel Station.

Structured Sampling

*Integrated mesozooplankton biomass and grazing rates (Bongo tows)*

Oblique Bongo tows were conducted twice per day (between 0830-1000 and 2100-2230) for day and night estimates of mesozooplankton biomass and grazing rates on phytoplankton. A total of 28 tows were conducted during the Lagrangian experimental cycles: Cycle 1 (N=11 tows), Cycle 2 (N=9 tows), and Cycle 3 (N=8), with two additional tows collected at S.

Samples were collected using paired Bongo frames of 0.71m diameter equipped with 202  $\mu\text{m}$  mesh, and one General Oceanics flow meter on the port side net. Nets were deployed at 30 m  $\text{min}^{-1}$  to 300 meters wire out, with a towing angle of  $\sim 45^\circ$  (approximately to 210m depth). At 300m wire out nets were towed for 30 sec and subsequently retrieved at 30 m  $\text{min}^{-1}$ . A temperature-depth probe was attached to the frame to obtain exact sampling depths. Upon retrieval, the port side net was rapidly rinsed and the cod-end contents poured into a bucket and anesthetized with carbonated water. The sample was immediately taken into the lab and split using a Folsom splitter for the following analyses:

*Molecular probes:* 1/4 was filtered into a plastic cup with 202  $\mu\text{m}$  mesh bottom and immediately frozen in liquid nitrogen for future analyses.

*Gut fluorescence (GF):* 3/8 was size-fractionated through a five-filter sieve (0.2-0.5mm, 0.5-1mm, 1-2mm, 2-5mm, >5mm), and each size-fraction was transferred to a 202  $\mu\text{m}$  filter, placed in a petri dish, and frozen in liquid nitrogen for analysis ashore.

*Biomass:* 3/8 was size-fractionated through the same five-filter sieve and each size-fraction transferred to a pre-weighed 202  $\mu\text{m}$  Nitex filter, rinsed with ammonium formate isotonic with seawater to remove interstitial salts, placed in a petri dish, and frozen in liquid nitrogen.

*Formalin:* The starboard sample was rinsed into a bucket and preserved in 5% Formalin buffered with sodium tetraborate. Depending on the volume of the sample, a quart or pint size jar was used.

#### *Pyrosome biomass and abundance estimates*

Pyrosomes were present or abundant in most stations sampled. Because of their large size they are not well suited for quantification using the methods outlined above. Depending on the abundance of pyrosomes, either the entire sample or a subsample was imaged with a camera held in a PVC frame at a fixed distance from a sampling tray (with a ruler included in the image), in order to obtain abundance and size estimates (see figure below). A subsample was typically frozen for onshore analysis, and another subsample preserved in 5% Formalin. The aliquots preserved varied depending on the amount of pyrosomes and processing time required. We imaged pyrosomes in this way for both Bongo and MOCNESS tows, in order to estimate biomass and abundance.



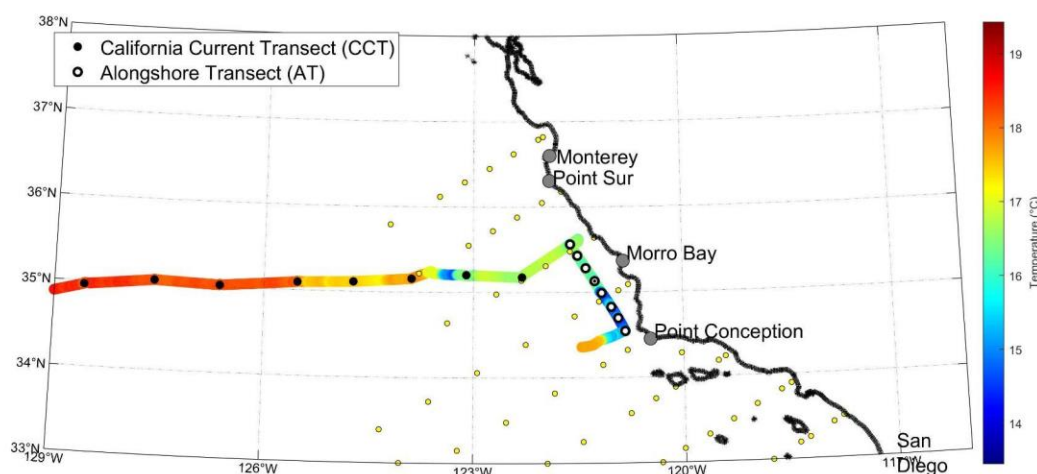
Pyrosome imaging set-up: example from Bongo Tow with GF and biomass aliquots.

#### *California Current Transect (CCT) and Alongshore Transect (AT)*

Oblique Bongo tows were conducted during the two transects: CCT (N=9) and AT (N=8). The CCT Bongos were conducted after the CTD and the Trace Metal cast, with timing of tows determined by station arrival. Sample processing during the CCT was the same as described for Bongo tows during the Lagrangian cycles ( $\frac{1}{4}$  molecular probes,  $\frac{3}{8}$  GF,  $\frac{3}{8}$  biomass).

The Alongshore Transect (AT) was conducted parallel to the coast to evaluate differences in the mesozooplankton and pyrosome community, and consisted of 8 stations, one coinciding with the CCT line (see below). Each station consisted of a CTD cast (to 300m when possible, 200m when bottom depth was shallower than 300m) to obtain a water column profile and for sampling at 3 depths (deep euphotic zone, mixed layer, and chlorophyll maximum) for Chl-*a*, biogenic silica and particulate organic material, followed by a quantitative Bongo (sample preservation protocol as detailed above, but without the  $\frac{1}{4}$  molecular probe aliquot to reduce processing time), and a live Salp net tow.





CCT and AT Bongo tows for mesozooplankton sampling.

#### *Vertically-stratified mesozooplankton sampling (MOCNESS tows)*

Quantitative paired day/night tows were conducted to 450m (4 tows, 2 day/night pairs per cycle) and 1000m (2 tows, one day/night pair per cycle) using a 1m<sup>2</sup> MOCNESS with ten 202  $\mu$ m nets, providing one integrated (surface to maximum depth) sample and 9 vertically-resolved samples. Typically 6 MOCNESS tows were conducted per cycle. However, issues with flow meter readings arose during Cycle 2. Because the hypothesized cause of these bad readings was pyrosomes clogging the flow meter, we conducted one additional tow to 450m where the position of the flow meter on the frame was changed. However, the change in flowmeter placement did not fix the issue. The flowmeter was then replaced and all tows during Cycle 3 have good flowmeter data, but the calibration factor needs to be evaluated. Volumes filtered for Cycle 2 will have to be estimated from distance towed, net angle, and estimated fishing surface area, where flowmeter data is lacking.

MOCNESS tows to 450m targeted the following depths: surface to depth (typically 0-475m), 450-350m, 350-300m, 300-250m, 250-200m, 200-150m, 150-100m, 100-50m, 50-25m, and 25-0m. MOCNESS tows to 450m were sampled in two ways. One set of day/night pairs was quantitatively split with a Folsom plankton splitter, with one half size-fractionated into the 5 sizes detailed above (but rinsed in Milli-Q instead of ammonium formate), and the other half preserved in 5% Formalin. The second set of day/night tows was split, and one half was preserved in 95% non-denatured ethanol with 5mM NH<sub>4</sub>OH for pH stabilization, and the other half was preserved in 5% Formalin.

Tows to 1000m targeted the following depths: surface to depth (typically 0-1025m), 1000-800m, 800-600m, 600-500m, 500-400m, 400-300m, 300-200m, 200-100m, 100-50m, 50-0m. MOCNESS tows to 1000m were processed in the following way. One half was preserved in 95% non-denatured ethanol stabilized with 5 mM NH<sub>4</sub>OH, ¼ was size-fractionated and the remaining ¼ was preserved in 5% Formalin buffered with sodium tetraborate for Zooscan and microscopy analysis.

## Experimental Sampling

### *Sampling for Rhizarians*

Net tows for rhizarian specimens and incubations were conducted to varying depths (N=19). Typically, a Ring net (303  $\mu\text{m}$  mesh) or Salp net (202  $\mu\text{m}$  mesh) was deployed to 50m to obtain intact specimens for incubations, followed by a Ring net (303  $\mu\text{m}$  mesh) to 300m to obtain specimens for POC measurements.

### *MOCNESS Tows for Acoustics and Rhizarian collections*

One MOCNESS per cycle (plus one at the Diel Station) was conducted to obtain specimens for experimental determination of density for use in acoustic algorithms, and for rhizarian specimen collections. Maximum depths varied according to the sampling location: Diel Station (1081m), Cycle 1 (630m), Cycle 2 (623m), and Cycle 3 (799m).

### *DDT sampling*

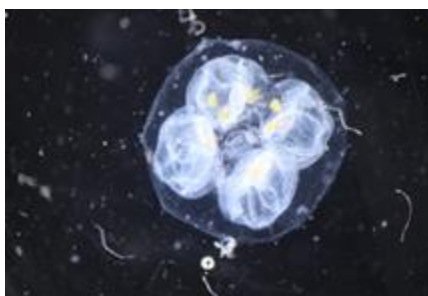
One MOCNESS tow was conducted to 794 depth at the DDT site, and towed at 2 depths: 789 to 657m for 125 minutes, and 627-546 for 75 minutes to obtain zooplankton biomass for DDT assessments.

### *Pyrosome sampling*

Pyrosomes were collected using the 'Salp net' to retrieve undamaged organisms to conduct grazing experiments in 20L plankton kreisels. Unfortunately, pyrosome behavior was never natural after collections, and organisms always sank to the bottom of the incubation tank. Different types of tanks were used, including 30L buckets, 120L drums, and 20L plankton kreisels with recirculating water, but none were successful. Young colonies (tetrzoooids and 'octo'-zoooids) were found in these live tows during Cycle 2 and Cycle 3, but these small colonies also did not survive in incubations.

Pyrosomes colonies collected with the Salp net were also incubated in buckets for fecal pellet production. Contents of the bottom of the containers were siphoned and filtered for POC and Chl- $\alpha$ .

Tetrzoooid with additional budding zoooids.



## Additional Zooplankton Group Activities

### *GoPro videography*

A GoPro was attached to either sampling nets or directly on the sampling line, with a dive light fixed to the side to obtain *in situ* footage of pyrosome colonies. Two videos with clear pyrosome sightings were obtained during Cycle 3, when abundances were high and the colonies were near the surface.



Images from GoPro footage taken during Cycle 3.

### *Grow-out experiments*

We conducted experiments to test whether iron in fecal pellets produced by zooplankton grazers could enhance phytoplankton growth. The experiment was repeated twice—once in productive waters (Cycle 2 and AT) and once in the oligotrophic offshore (Cycle 3). In productive waters, the euphausiid *Euphausia pacifica* was used as the model grazer, while the colonial pelagic tunicate *Pyrosoma atlanticum* was used offshore. Experiments involved the collection of zooplankton fecal pellets and subsequent incubation of local phytoplankton communities using trace metal clean water and methods. Incubation conditions and times varied, with the krill study lasting only 48 hours and the pyrosome pellet addition lasting 96 hours and involving the addition of nitrate, phosphate and silicate.

### *Wildfire/ash experiments - Dilutions and ash gradient*

Dilution experiments with ash leachate were conducted twice during the cruise and are included in the dilution section. In addition, two grow-out incubations using pure ash were conducted in nearshore productive waters. Experiments included three replicate controls, and three replicates per treatment (10mg L<sup>-1</sup> and 25mg L<sup>-1</sup> ash), and were conducted for 24 hours. Samples were taken for Chl-*a*, FCM and epi-fluorescence microscopy.

### *Aerosol sampling*

Two aerosol samplers were deployed during the cruise: the Coriolis Compact and a gast-pump sampler with a 47mm cellulose filter in an Advantec filter housing. Both samplers were deployed simultaneously for 24 hour periods 10 times throughout the duration of the cruise. Samplers were deployed no earlier than 4 hours following activity of the ship's incinerator and no burning occurred



during the deployment window. For 2 of the sampling events the power supply to the samplers was shorted, resulting in an early termination of sampling. We also intentionally sampled during a burning period as a control. The cruise trajectory did not intersect major smoke plumes, however during the final sampling satellite imagery and aerosol optical depth data from NASA revealed offshore transport of wildfire smoke from the inland Dixie fire in the San Francisco Bay area north of the study area. On shore the aerosol samples will be analyzed for particulate iron and black carbon to reference for future studies associated with more intense wildfire ash plumes.

### **Rhizaria Studies (Manon Laget and Natalia Llopis-Monferrer)**

#### Objectives

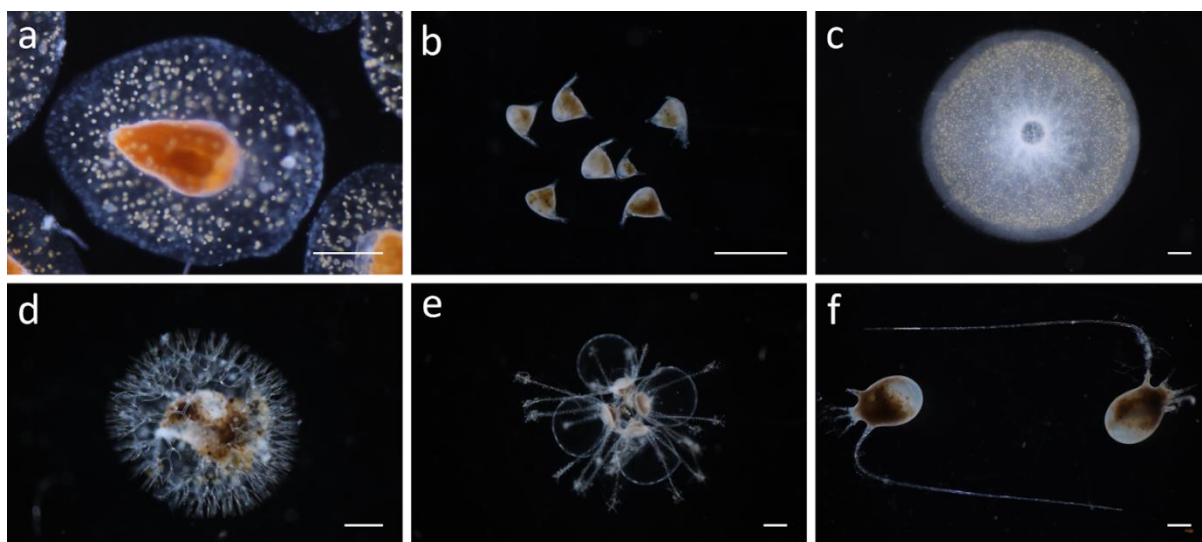
Rhizaria is a recently defined supergroup of eukaryotes, mostly single-celled and widely distributed in various kinds of environments. Among them, polycystine Radiolaria and Phaeodaria are present from the surface to the bathypelagic waters of all oceans. These protists, which range in size from a few micrometers to a few millimeters, can produce silica skeletons and are usually heterotrophic or mixotrophic feeders. Although widely studied by micro-paleontologists, the ecology and contribution of Rhizaria to the functioning of contemporary marine ecosystems has remained largely unexplored. The goal of our experiments is to assess the role of Rhizaria in the biogeochemical cycles of carbon and silicon.

Here we use imaging methods to assess the abundance of Rhizaria and their contribution to vertical fluxes of elements. We also collect samples of living Rhizaria to obtain estimates of particulate organic carbon and biogenic silica content, as well as production rates, from several study sites of the California Current Ecosystem.

#### Elementary composition (POC and bSi)

*Methods* - Rhizarian cells collected from various nets were isolated under microscopes and sorted by taxonomic group (see figures below). According to their size, from 1 to 40 cells were filtered onto a 0.4  $\mu\text{m}$  GF/F filter for POC or onto a 0.6  $\mu\text{m}$  polycarbonate membrane filter for bSi determination. In the meantime, we took photographs of individuals in order to obtain an estimation of their volume and relate it to elemental composition.

In total, 143 filters were made for POC and 42 for bSi, that were kept frozen and dried for incoming elemental analyses.



Representative examples of taxonomic groups found during the cruise (all scale bars 500  $\mu\text{m}$ ). (a) Nassellarian of the family Theoperidae, (b) Phaeodarians of the family Challengeridae (*Protocystis vicina*), (c) Solitary collodarian, (d) Phaeodarian of the family Coelodendridae, (e) Colonial phaeodarians of the family Medusettidae, (f) Phaeodarian of the family Tuscaroridae.

#### Rhizaria incubations

**Methods** - Cells were observed under an inverted microscope or a stereomicroscope and isolated with a Pasteur pipette to then be deposited in a 20 mL glass vial with filtered seawater. Cells were sorted according to a few targeted taxonomic groups (figure above). A total of 40 incubations were made. Glass vials containing between 1 to 40 cells of the same taxonomic group were spiked with the  $^{32}\text{Si}$  radioisotope and incubated on deck for 24h in a flowing-seawater incubator. After incubation, isotope samples were filtered by gentle (< 150 mm Hg) vacuum filtration onto 47 mm diameter, 0.6  $\mu\text{m}$  pore-size polycarbonate membrane filters (Nuclepore) and rinsed twice with filtered seawater to wash away non-particulate  $^{32}\text{Si}$ . Each filter was then placed in a clean 20 mL polypropylene liquid scintillation vial which was loosely capped to permit the sample to dry at room temperature for 48 hours. The vials were then capped tightly and returned to France for counting using the Cerenkov method. Once the counting is done, digestion will be performed to measure the particulate biogenic silica contained in the filters.

#### Seawater incubations

**Methods** - Biogenic silica production was determined as silicic acid uptake rates at 5 depths of the 2am cast (table below). Incubations were done in situ for 24 hours on the drift array. At the end of the incubation period, samples were filtered by gentle (< 150 mm Hg) vacuum filtration onto 47-mm diameter, 0.6- $\mu\text{m}$  pore-size polycarbonate membrane filters (Nuclepore) and rinsed twice with filtered seawater to wash away nonparticulate  $^{32}\text{Si}$ . Filters were placed in 20-mL polypropylene liquid scintillation vials loosely capped to allow the sample to dry at room temperature for 48 hours. The vials were then tightly capped and stored until analysis.

### *Seawater incubations*

Date	Cycle/Day	CTD cast	Depths sampled (m)
07/17/2021		9	5,10,25,45,75
07/18/2021		12	5,10,15,25,50
07/22/2021	1/1	33	5,12,20,40,60
07/23/2021	1/2	37	5,12,20,35,45
07/24/2021	1/3	40	5,12,20,35,45
07/25/2021	1/4	44	5,12,20,25,35
07/28/2021	2/1	54	5,12,20,25,30
07/29/2021	2/2	58	5,12,20,30,40
07/30/2021	2/3	61	5,12,20,30,40
07/31/2021	2/4	64	5,12,20,30,40
08/04/2021	3/1	70	5,40,80,110,120
08/04/2021	3/2	74	5,40,80,110,120
08/05/2021	3/3	77	5,70,80,110,120
08/06/2021	3/4	81	5,40,80,110,120

### DNA analyses

In addition to biogeochemical analyses, several specimens of two species of symbiotic *Nassellaria* (figure above) collected during Cycle 3 from 0 to 300 m were kept in ethanol and frozen for further DNA analyses.

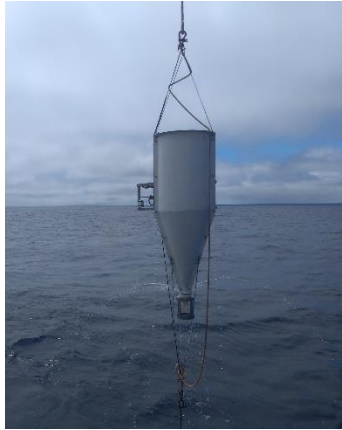
### Underwater Vision Profiler (UVP5)

*Instrument* - UVP5-HD, serial number sn200. Image volume: 1.14 L. Pixel size: 0.88 x 0.88  $\mu\text{m}$ .

*Data* - During the cruise 77 profiles were obtained. The raw images of particles were processed using Zooprocess software and the vignettes were imported to the Ecotaxa website to be later classified and validated.

#### VisuTrap deployment (UVP6 and sediment trap)

*Objectives and setup* - The VisuTrap is composed of a UVP6 camera mounted on a conical sediment trap in order to image the particles falling inside (figure below). It was deployed on the sediment trap line during each cycle, at 150 m and 450 m for cycle 1 and 3 and at 450 m for cycle 2. With this setup, we aim to quantify the *in situ* speeds of particles sinking inside the trap.



Deployment of the VisuTrap. The UVP6 camera is attached to the middle of the trap.

*Instruments and configuration* - 3 UVP6-LP were used during the cruise: 000141lp, 000143lp and 000147lp. Field of view is 180 x 151 x 23 mm (0.6 L) and pixel size is 0.73 x 0.73  $\mu\text{m}$ . During the deployment, the cameras switched each half hour between two modes of acquisition: a high frequency mode (2 Hz) keeping the entire images and a low frequency mode (0.2 Hz) keeping only the vignettes of the detected particles. The goal of the first mode is to record the trajectories of the particles sinking in the trap, whereas the second mode allows quantification of the particle concentrations.

*UVP6 data* - We obtained more than 12 days of acquisition at 450 m and more than 8 days at 150 m, half of each as entire images and half vignettes of individual particles. Vignettes will later be processed using the UVPapp software for identification and classification. Images will be analyzed with Python software to detect the trajectories of individual particles.

*Sediment traps material* - The material collected in the trap was frozen for further analyses.

## CCE-P2107 DAILY ACTIVITY SCHEDULE

(13 July – 13 August 2021) *R/V Roger Revelle*

*Listed times are estimates; consult Event Log for actual times.*

### 13 July

1000 Depart MARFAC  
1400 Calibrate EK-80  
2200 Transit to test station (33.605N 119.317W)

### 14 July

0830 Bongo Net Test Cast  
0930 CTD Test Cast  
1100 Trace Metal Rosette Test Cast  
1200 MOCNESS Test Cast – Aborted, terminated incorrectly  
Transit toward radiator survey start location  
1700 MOCNESS Test Cast

### 15 July

Continue to radiator survey start location (34.148N, 121.341W)

0100 Salp Net Tow

0145 CTD Cast

0245 Start Radiator Survey 1 (Underway sampling for nutrients, Chl, HPLC, POC/PN, <sup>234</sup>Th)

1-1	34.437	120.649
1-2	34.546	120.734
1-3	34.655	120.820
1-4	34.765	120.905
1-5	34.874	120.991
1-6	34.983	121.076
1-7	35.092	121.162
1-8	35.201	121.247
1-9	35.310	121.333
1-10	35.420	121.418
1-11	35.529	121.504
1-12	35.638	121.590
1-13	35.747	121.675
1-14	35.856	121.761
1-15	35.965	121.846
1-16	36.074	121.932
1-17	36.184	122.017
1-18	36.293	122.103
1-19	36.402	122.188
1-20	36.511	122.274
2-1	36.356	122.655
2-2	36.247	122.568
2-3	36.139	122.480
2-4	36.030	122.393
2-5	35.922	122.306
2-6	35.813	122.218

2-7	35.704	122.131
2-8	35.596	122.043
2-9	35.487	121.956
2-10	35.379	121.869
2-11	35.270	121.781
2-12	35.161	121.694
2-13	35.053	121.606
2-14	34.944	121.519
2-15	34.836	121.432
2-16	34.727	121.344
2-17	34.618	121.257
2-18	34.510	121.169
2-19	34.401	121.082
2-20	34.292	120.995
3-1	34.256	121.430
3-2	34.364	121.519
3-3	34.472	121.608
3-4	34.580	121.698
3-5	34.688	121.787
3-6	34.796	121.876
3-7	34.904	121.965
3-8	35.012	122.055
3-9	35.120	122.144
3-10	35.228	122.233
3-11	35.336	122.322
3-12	35.445	122.412
3-13	35.553	122.501
3-14	35.661	122.590
3-15	35.769	122.679
3-16	35.877	122.769
3-17	35.985	122.858
3-18	36.093	122.947
3-19	36.201	123.037

1600 CTD Cast (end of radiator survey line 1, station 1-20)

1700 Salp Net Cast

Continue radiator survey

### **16 July**

Continue radiator survey

900 CTD cast (end of radiator survey line 2, station 2-20)

1000 Ring net cast

Continue radiator survey

### **17 July**

0130 CTD (end of radiator survey line 3)  
Proceed toward radiator survey line Station 1-20 (Diel Station)

0630	CTD	(Diel study)
0730	Trace Metal Rosette	(Incubations)
0830	McLane Pump	(Deep Cast)
1200	CTD	(Diel study)
1300	MOCNESS	(Deep Tow)
1900	CTD	(Diel study)
2000	McLane Pump	(Shallow Cast)
2200	Bongo tow	
2300	CTD	(Diel study)

### **18 July**

0000	MOCNESS	(Deep Tow)
0630	CTD	(Aborted, electronics issues)
0700	Trace Metal Rosette	(Incubations)
0745	CTD	(Diel study)
0900	Bongo tow	
1000	MOCNESS tow	(Organism collection)
1530	CTD	(Diel study)
1650	Trace metal rosette	(profile)
1700	McLane Pump	(Shallow Cast)

Survey south on radiator survey line 1 to assess water temperature and search for filament, heading towards station 1-1.

### **19 July**

Start Benthic Boundary Layer transect #1 (BBL 1) ~1500

Station 1	Cambria	35°34.728'N	121°10.096'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 2	Pt. Estero	35°28.507'N	121°03.411'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 3	Morro Bay	35°21.621'N	120°55.541'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 4	Shell Beach	35°05.090'N	120°46.250'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			

## **20 July**

Continue BBL 1 transect

Station 5	Vandenberg	34°52.860'N	120°44.170'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 6	Santa Ynez	34°41.530'N	120°42.560'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 7	Pt Arguello	34°33.857'N	120°41.065'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 8	Line 80, Station 51	34°27.735'N	120°31.250'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			
Station 9	Gato	34°25.351'N	120°24.443'W
CTD Cast (Barbeau)			
GO-Flo Cast (Barbeau)			

Completed BBL 1 transect ~0800

0930	CTD	Santa Barbara Basin Station
1030	Trace metal rosette	Santa Barbara Basin
1130	McLane pump	Santa Barbara Basin

Transit to CTD survey location, toward Point Sur region

2100	CTD (profile only, 100 m from bottom)
2200	Ring net and salp net sampling

## **21 July**

0400	CTD (profile only, 100 m from bottom)
0500	CTD (profile only, 100 m from bottom)
0700	CTD (profile only, 100 m from bottom)
0930	CTD (profile only, 100 m from bottom)
1030	Net tow
1145	CTD (profile only, 100 m from bottom)
1230	Salp net
1700	CTD (profile only, 100 m from bottom)
1900	CTD (profile only, 100 m from bottom)
2000	CTD (profile only, 100 m from bottom)



**Cycle 1 Day 0**

23:00 Sediment trap deployment

**22 July****Cycle 1 Day 1**

0200 CTD (array setup, 200 m)  
0300 Trace metal rosette (array setup)  
0415 Array deployment  
0500 CTD cast (full dilution setup and thorium, 300 m)  
0600 Trace metal rosette  
0700 McLane Pump (shallow)  
0900 Bongo Tow (quantitative)  
1000 *Zooglider* deployment  
1130 Noon CTD cast (500 m)  
1230 Bongo tow  
1300 Shallow MOCNESS tow (Day #1)  
1800 Bongo tow (rhizarian collection)  
1900 CTD (incubations, 1000 m)  
2100 Bongo tow (quantitative)  
2200 Shallow MOCNESS tow (Night #1)

**23 July****Cycle 1 Day 2**

0200 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Transit away from station to dump waste  
0800 Bongo Tow (quantitative)  
0900 McLane Pump (deep)  
1200 CTD cast aborted, hydraulic leak  
1300 Deep MOCNESS tow (Day #1, pump waste while deeper than 500 m)  
1700 CTD cast (1000 m)  
1800 CTD cast  
2030 Bongo tow (quantitative)  
2100 Deep MOCNESS tow (Night #1, pump waste while deeper than 500 m)

**24 July****Cycle 1 Day 3**

0200 CTD (array setup, 200 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Leave drifter area to pump waste  
0900 Bongo tow (quantitative)

1000 McLane Pump (shallow)  
1200 Noon CTD (1000 m)  
1300 Shallow MOCNESS tow (Day #2)  
1600 CTD  
1700 Ring net (Rhizarian collection)  
1900 CTD cast (incubations, 1000 m)  
2000 Salp net (live organisms)  
2100 Bongo tow (quantitative)  
2200 Shallow MOCNESS (Night #2)

## **25 July**

### **Cycle 1 Day 4**

0200 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Leave drifter area to pump waste  
0900 Bongo tow (quantitative)  
1200 Noon CTD (1000 m)  
1300 Shallow MOCNESS (Rhizarians and Svens)  
1700 McLane Pump (deep)  
1930 CTD (incubations, 1000 m)  
2000 Salp net (live organisms)  
2100 Bongo tow (quantitative)  
2300 Ring net (Rhizarians and Svens)

## **26 July**

### **Cycle 1 Day 5**

0100 CTD (array finals, 1000 m)  
0200 Trace metal rosette  
0330 Array recovery – Re-deploy empty marker drift array  
0500 CTD at sediment trap location  
0600 Sediment trap recovery – END CYCLE 1  
0730 Proceed to BBL 2-1 location: 36° 11.6985' N, 121° 44.70402' W  
0900 CTD (to near the bottom, shallow water)  
0930 GO-Flo Cast  
1000 Proceed to BBL 2-2 location: 36° 13.69752' N, 121° 49.71684' W  
1100 CTD (to near the bottom, shallow water)  
1130 GO-Flo Cast  
1200 Proceed to BBL 2-3 location: 36° 16.2855' N, 121° 54.6969' W  
1300 CTD (to near the bottom, shallow water)  
1330 GO-Flo Cast  
1400 Return to marker drift array location + Bowtie survey (no sampling)

## **27 July**

0000 M. Stukel drop off, Monterey  
Transit to marker drift array location  
0700 Deep MOCNESS (Rhizarians)  
1000 Bongo tow (quantitative)  
1130 McLane Pump  
1400 CTD  
1600 Recover marker drift array  
1600-1900 Underway MET survey

### **Cycle 2 Day 0**

1900 Sediment trap deployment  
2200 Bongo tow  
2300 CTD (200 m)  
2330 Ring net

## **28 July**

### **Cycle 2 Day 1**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array deployment  
0500 CTD cast (full dilution setup and thorium, 300 m)  
0600 Trace metal rosette  
0700 McLane pump (shallow)  
0900 Bongo tow (quantitative)  
1000 Transit away from station to dump waste  
1200 Noon CTD cast (1000 m)  
1300 Shallow MOCNESS tow (Day #1)  
1900 CTD (incubations, 1000 m)  
2000 Ring net (live organisms)  
2100 Bongo tow (quantitative)  
2200 Shallow MOCNESS tow (Night #1)

## **29 July**

### **Cycle 2 Day 2**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette (3x, tech issues)  
0600 Transit away from station to dump waste  
0800 Bongo tow (quantitative)  
0900 McLane pump (deep)  
1200 Noon CTD cast (1000 m)  
1300 Shallow MOCNESS tow (Day #2)  
1700 Ring net tow

1800 CTD cast (incubation, 1000 m)  
1900 Salp net tows (live organisms)  
2100 Bongo tow (quantitative)  
2200 Shallow MOCNESS tow (Night #2)

### **30 July**

#### **Cycle 2 Day 3**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Leave drifter area to pump waste  
0900 Bongo tow (quantitative)  
1100 Noon CTD (1000 m)  
1300 Zooplankton sampling (salp and ring nets)  
1600 CTD (100 m)  
1630 Salp net  
1700 Trace metal test cast  
1800 McLane pump (shallow)  
2000 Bongo and ring net tows (live organisms)  
2200 Bongo tow (quantitative)  
2300 Salp net

### **July 31**

#### **Cycle 2 Day 4**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Leave drifter area to pump waste  
0900 Bongo tow (quantitative)  
1200 Noon CTD (1000 m)  
1300 Deep MOCNESS (Quantitative)  
1900 CTD (incubations, 1000 m)  
2000 Ring net (live organisms)  
2130 Deep MOCNESS (Quantitative)

## **1 August**

### **Cycle 2 Day 5**

0300 CTD (array finals, 1000 m)  
0430 Array recovery  
0630 Arrive at sediment trap location  
0700 CTD at sediment trap location (1000 m)  
0800 Trace metal rosette  
0900 Ring net  
1000 Sediment trap recovery – END CYCLE 2

Transit to Cycle 3 location offshore

## **2 August**

### **Cycle 3 Day 0**

2100 CTD  
2200 Sediment trap deployment  
2300 Salp nets

## **3 August**

### **Cycle 3 Day 1**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array deployment  
0500 CTD cast (full dilution setup)  
0600 Trace metal rosette  
0700 McLane pump (shallow)  
0900 Bongo Tow (quantitative)  
1000 Transit away from station to dump waste  
1200 Noon CTD cast (1000 m)  
1300 Shallow MOCNESS tow (Day #1)  
1700 Ring net (rhizarian collection)  
1900 CTD (incubations, thorium, 1000 m)  
2000 Salp nets (live organisms)  
2100 Bongo tow (quantitative)  
2200 Shallow MOCNESS tow (Night #1)

## **4 August**

### **Cycle 3 Day 2**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Transit away from station to dump waste  
0800 Bongo Tow (quantitative)

0900 McLane pump (deep)  
1200 Noon CTD cast (1000 m)  
1300 Shallow MOCNESS tow (quantitative, Day #2)  
1700 Ring nets (Rhizarians)  
1800 CTD cast (incubation, 1000 m)  
1900 Salp nets (live organisms)  
2130 Bongo tow (quantitative)  
2200 Shallow MOCNESS tow (quantitative, Night #2)  
2400 Salp nets

## **5 August**

### **Cycle 3 Day 3**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette (array setup)  
0330 Salp net  
0430 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Leave drifter area to pump waste  
0800 Bongo Tow (quantitative)  
0900 Deep CTD (4000 m)  
1200 Noon CTD (500 m)  
1400 MOCNESS for rhizarians, Sven  
1700 McLane pump cast (shallow)  
1900 CTD (shallow)  
2000 Salp nets (live organisms)  
2130 Bongo tow (quantitative)  
2230 Zooplankton sampling for rhizarians, Sven

## **6 August**

### **Cycle 3 Day 4**

0130 CTD (array setup, 1000 m)  
0300 Trace metal rosette  
0415 Array recovery and redeployment  
0500 Trace metal rosette  
0600 Leave drifter area to pump waste  
0800 Bongo Tow (quantitative)  
0900 McLane Pump (deep)  
1200 Noon CTD (1000 m)  
1300 Deep MOCNESS (Quantitative)  
1900 Ring net  
2000 CTD (incubations, thorium, 1000 m)  
2100 Salp nets (live organisms)  
2130 Bongo tow (quantitative)  
2230 Deep MOCNESS (Quantitative)

## **7 August**

### **Cycle 3 Day 5**

0400 CTD (array finals, 1000 m)  
0430 Array recovery  
0500 Trace metal rosette  
0600 Salp net  
0700 McLane pumps  
1030 Sediment trap recovery – END CYCLE 3

Transit to California Current Transect (CCT) Station 1

2200 CTD (1000m) – CCT1  
2300 Trace metal rosette – CCT1  
2330 Bongo/Salp net tows – CCT1

## **8 August**

0430 CTD (1000 m) – CCT2  
0530 Trace metal rosette – CCT2  
0600 Bongo/Salp net tows – CCT2  
1100 CTD (1000 m) – CCT3  
1200 Trace metal rosette – CCT3  
1230 Bongo/Salp net tows – CCT3  
1900 CTD (1000 m) – CCT4  
2000 Trace metal rosette – CCT4  
2030 Bongo/Salp net tows – CCT4

## **9 August**

0030 CTD – CCT5  
0130 Trace metal rosette – CCT5  
0200 Bongo/Salp net tows – CCT5  
0700 CTD – CCT6  
0730 Trace metal rosette – CCT6  
0800 Bongo/Salp net tows – CCT6  
1200 *Zooglider* recovery/annular survey  
1300 CTD  
1600 CTD (300 m) – CCT7  
1630 Trace metal rosette – CCT7  
1700 McLane pump – CCT7  
1800 Bongo/Salp net tows – CCT7  
2240 CTD (1000 m) – CCT8  
2340 Trace metal rosette – CCT8

## **10 August**

0010 Bongo/Salp net tows – CCT8

Transit toward Alongshore Transect (AT) Station 1

0500 CTD  
0530 Trace metal rosette (incubation)  
0700 CTD – AT1  
0730 Bongo/Salp net tows – AT1  
0930 CTD – AT2  
1000 Bongo/Salp net tows – AT2  
1215 CTD – AT3  
1300 Bongo/Salp net tows – AT3  
1530 CTD – AT4/CCT9  
1600 Trace metal rosette – AT4/CCT9  
1700 Bongo/Salp net tows – AT4/CCT9  
1900 CTD – AT5  
1930 Trace metal rosette (incubation)  
2000 Bongo/Salp net tows – AT5  
2200 Small boat sampling  
2300 Salp nets

## **11 August**

0400 CTD for S. Plummer dilution experiment at AT5  
0700 CTD – AT6  
0800 Bongo/Salp net tows – AT6  
1000 CTD – AT7  
1030 Bongo/Salp net tows – AT7  
1230 CTD – AT8  
1300 Bongo/Salp net tows – AT8  
Transit offshore  
1700 CTD (1500 m, thorium)  
1800 Ring net  
1900 Bongo tow  
2000 Salp net

Transit to DDT dump site, San Pedro Basin



## **12 August**

0945 CTD (870 m) – DDT site  
1045 McLane pumps – DDT site  
1300 EK80 survey – DDT site  
1530 MOCNESS (deep) – DDT site  
2200 Go Pro deployments

Transit to MarFac

## **13 August**

1030 Arrive MarFac