Day 09: Saturday, June 01: Micrometers, Nanometers, Picometers, Oh My!

Land Ho! The rocky steep cliffs of Big Sur, California appeared beneath a blanket of early morning fog as PUPCYCLE 2019 completed the last round of the water sample filtrations and prep work from Incubation Site #1 (Broad shelf region). In the final hours of today, the scientists will begin collecting samples from Incubation Site #2 (Narrow shelf). The continental shelf at our current location is between 1 – 3 nautical miles (1.15 – 3.5 statute miles) from the shoreline as compared to the broad shelf region from Incubation Site #1 where the continental shelf extends from 23 nautical miles (nm) at the incubation site to 32 nm (26.5 – 36.8 statute miles; respectively) further out from the shoreline.

Figure 19 – Matt Hurst works inside the "Bubble Room" filtering water samples to measure iron (Fe) uptake during upwelling. [*Image credit: Miriam Sutton*]

Ken Bruland first noted the iron-limited marine environment of narrow shelf regions in 2001 when he was a researcher at the University of California – Santa Cruz. Since the publishing of Ken's findings, it has been well documented that narrow shelf regions lack enough distance from the shoreline to accumulate the concentrations of iron found along broad shelf environments.

Table #1 – Fractionalized Filtration by Particle Size	
Filter Size in microns(μm)	Particles Filtered Out of Sample
> 5.0	Large diatoms with Fe stored inside their cells
>0.8	Chlorophyll-size particles with Fe attached
>0.2	Particles Organic Matter (including bacteria)
<0.2	Dissolved matter (including ligands)
0.03-0.2	Organic Colloids
<0.03	Soluble (molecules)

Matt Hurst (Humboldt State University) is analyzing concentration levels of iron found in the seawater at each incubation site. Matt's experiment is measuring the concentration of iron collected during a relaxation phase and later comparing it to the concentration of iron found in the samples after upwelling has been simulated with the incubation system. This comparison allows him to measure the amount of iron being taken up or used by diatoms and other forms of phytoplankton. Filtered samples will be compared across the various PUPCYCLE 2019 study parameters: water depth (surface @15m vs. deep @90m); shelf width (broad vs. narrow); and upwelling phase (relaxed vs. active). Matt uses a filtration system designed to separate the particles found in his sample into five groupings according to size. The filtration system Matt is using onboard the R/V Oceanus is similar to a soil sampling sieve used to separate soil particles by size between gravel (>2mm), sand (>0.05mm), silt (>0.002mm), and clay (<0.002mm). Based on the phytoplankton scale, displayed on *Day 03: Sunday, May 26: The Phytoplankton Players*, gravel falls within the size range of mesoplankton such as copepods and isopods, which can be seen with the naked eye.

Larger particles include large chain-forming diatoms and other materials greater than 5.0μm are captured on the first set of filters. The next 2 filtration runs separate chlorophyll-sized particles with iron attached from particles of organic matter (POM) that include bacteria. These are followed by a fourth filtration run that separates the dissolved organic matter (DOM), including ligands from the remaining sample. A final filtration run separates the colloidal and soluble fractions left in the seawater sample. (See Table #1) During phytoplankton blooms, diatoms and other phytoplankton take up the iron found in seawater. Matt is able to isolate the amount of iron uptake by the diatoms using this fractionalized filtration method.

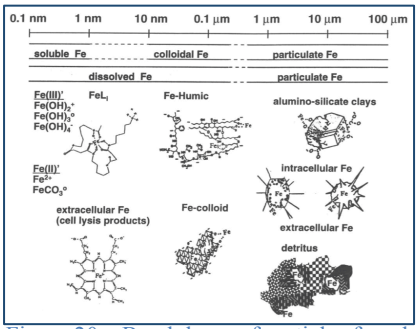

Figure 20 – Breakdown of particles found in seawater that Matt Hurst is investigating. [*Image credit: Matt Hurst*]

BIO INFO: Matt Hurst was born in Southern California and completed his undergraduate degree in Chemistry at Humboldt State University. He completed his Ph.D. in Chemistry from the University of California at Santa Cruz before returning to Humboldt State University. The PUPCYCLE 2019 cruise marks Matt's return to research at sea after 15 years.

Today's Certificate Challenge: Using the Phytoplankton scale on Day 3: May 26 and Table #1 showing the filtration particle size (above), in which category of plankton would bacteria be classified?

Day 10: Sunday, June 02: Game of Iron!

A few short hours after completing the filtering and storing of all the samples from Incubation Site #1: Broad Shelf, the researchers prepared their stations for the samples from Incubation Site #2. Seawater samples from Incubation Site #2: Narrow Shelf began arriving on deck shortly before midnight. Each research group secured their samples in the storage containers and placed them in the incubation systems strapped to the aft deck of the R/V Oceanus. Some of the scientists returned to their bunks around 3:00AM while others worked through mid-morning until the Deep Water Pumping System had completed its service to PUPCYCLE 2019 and was once again secured on deck.

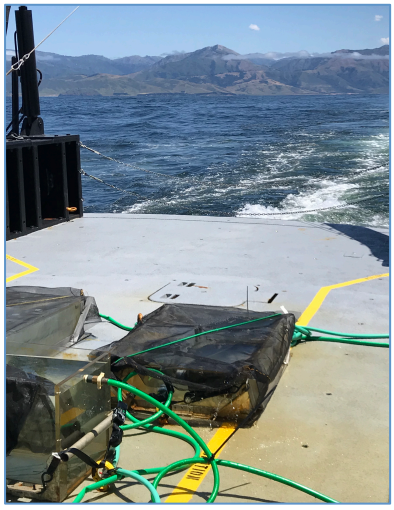

Figure 21 – Maite and Jian's diatoms have quite the view during their incubation period. [*Image credit: Miriam Sutton*]

The incubation system shown in Figure 21 is set up for experiments being conducted by Maite Maldonado and Jian Guo, both from the University of British Columbia (UBC). Their research focuses on iron transfer, or uptake, by diatoms. They are interested in determining if diatoms have the ability to take up iron from a certain compound, known as DFB (desferroxamine B). DFB is a type of siderophore ("iron carrying") compound that binds tightly to iron found in seawater, thereby making the iron unavailable to other microorganisms. While some bacteria (prokaryotic – containing no nucleus) may have the ability to produce another type of siderophore that can access the iron, it is unclear whether eukaryotic algae (containing a nucleus), such as diatoms, have this same capability. Iron is vital to phytoplankton's growth and survival and their research will provide insights into how much energy, if any, the diatoms are willing to expend in their effort to secure the iron they need.

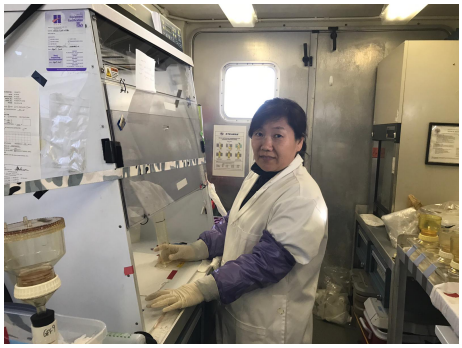

Figure 22 –Jian Guo prepares diatom samples for transcriptomics. [*Image credit: Maite Maldonado (UBC)*]

Researchers have learned that some species of diatoms are more adapted to low-nutrient environments while other species are more adapted to high-nutrient environments. When there is sufficient iron in their marine environment, both species are less interested in acquiring iron. Scientists describe this lack of motivation as "low affinity." When iron is limited, diatoms have less iron in their cells and their affinity increases ("high affinity") as they become desperate for any form of iron that might be available to them. As noted in Matt's research earlier (*Day 09: Saturday, June 01: Micrometers, Nanometers, Picometers, Oh My!*), iron can be found attached to other organisms and particles found in seawater besides DFB (i.e., chlorophyll, bacteria, ligands). Jian and Maite are investigating to see if diatoms living in an iron-limited environment might have the ability to take up the DFB compound and extract the iron for their cellular processes, which include photosynthesis. They, like many of the other PUPCYCLE 2019 researchers, will freeze and store their samples for transcriptomics back at UBC. The genes expressed by the diatoms will identify whether they are exerting energy to gain access to the iron bound to the DFB.

Jian Guo was born in China and immigrated to Canada to pursue her PhD at the University of British Columbia. She moved to California to complete her post-doctoral studies at the Monterey Bay Aquarium Research Institute (MBARI) before returning to the University of British Columbia, where she is completing a post-doctorate focusing on trace metal contaminants being released in the ocean by wastewater plants in Vancouver.

Today's Certificate Challenge: In which type of environment (Fe-limited or Fe-available) do diatoms possess the highest affinity for gathering iron?

Day 11: Monday, June 03: Heavy Seas, Anyone?

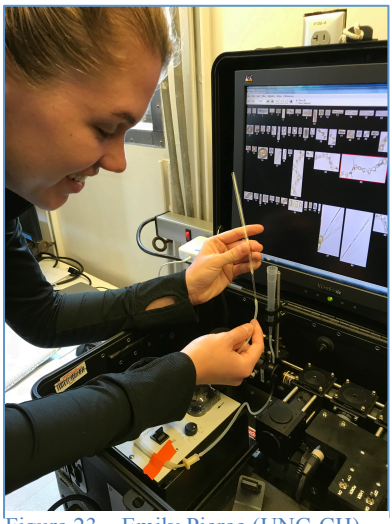

Figure 23 – Emily Pierce (UNC-CH) demonstrates how the FlowCAM® captures images of single cells. [*Image credit: Miriam Sutton*]

The R/V Oceanus rock and roll concert returned shortly after dinner as the captain charted our course northward to Newport, Oregon. Our hopes that the low-pressure center awaiting our return might have moved beyond our course were soon dashed as the pitch and roll of our research vessel increased in relation to our proximity to Cape Mendocino. While being rocked a bit too vigorously in my bunk, I began thinking of gene expression and how the genes being expressed by these tiny single-celled organisms provide the understanding for cellular responses to the environment. My pondering lead me to generate a new research question: What might the transcriptomics of some of the researchers' cells reveal if the scientists sequenced their own RNA prior to boarding the R/V Oceanus and compared them throughout the 2-week cruise? There are certainly different genes being expressed by the crew of the R/V Oceanus. The expression of the crew's "sea leg" genes is far superior to most of the scientists who boarded the vessel 10-days ago. There is one notable exception among the scientists: Emily Pierce (UNC-CH), who is also the youngest researcher on PUPCYCLE 2019. This is Emily's first experience on a research vessel and when I spoke with her during the first R/V Oceanus rock and roll concert less than a day out of port, she beamed and said, "I like this!" For Emily, the genes being expressed were more akin to euphoria than to the nausea responses endured by most of the scientists.

Emily is a rising senior at UNC-CH and is working with Chief Scientist, Adrian Marchetti's group while onboard. Emily spends many hours pipetting water samples into the FlowCAM® to capture images of individual species of phytoplankton. The images are saved to the computer for additional analysis and species sorting back in the lab. Once completed, Emily will have the relative abundance of each genus (Diatoms, Dinoflagellates, Haptophytes, and Chlorophytes) and species identified throughout the research area. Emily is also filtering and storing samples for chlorophyll analysis, as well as nutrient and toxin analyses. The commonality in her research protocol matches the other scientists' protocols with comparisons across broad vs. narrow shelf regions and deep water (90m) vs. surface water (15m).

Luckily for the other scientists still struggling with their own gene expression, today's schedule is quite light as they need only to prepare for tomorrow's 48-hour filtration from Incubation Site #2: Narrow shelf samples. Data from the second incubation site will assist the scientists in answering many of their research questions: Are certain species of diatoms better adapted in narrow shelf/iron limited environments? Do diatoms at the surface express different genes during upwelling than do diatoms found in deep water? While the scientists ponder these scientific questions, I continue to ponder whether my own RNA will adapt in a way that the euphoric gene expression I experience in the marshes back in Beaufort, NC will be transcribed to replace the nauseous gene expression I experience during each R/V Oceanus rock and roll concert.

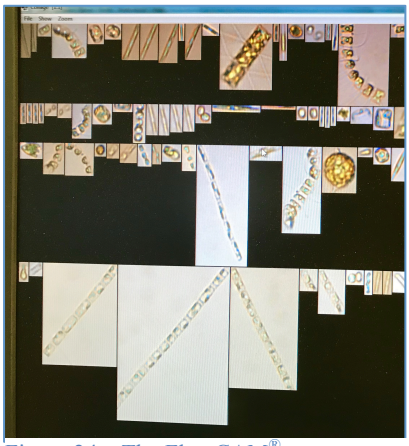

Figure 24 – The FlowCAM® captures images of large chain-forming diatoms as well as other phytoplankton. [*Image credit: Screenshot of FlowCAM® by Miriam Sutton*]

BIO INFO: Emily Pierce is from Durham, North Carolina and a rising senior at the University of North Carolina at Chapel Hill. She is pursuing a major in Environmental Science with a minor in Marine Science and plans to attend graduate school after completing her undergraduate studies.

Today's Certificate Challenge: Name the four different genera of phytoplankton being investigated during PUPCYCLE 2019.

Day 12: Tuesday, June 04: Building Glass Houses

Our transit northward continues, supported by the remnants of the R/V Oceanus rock and roll concert from yesterday. By 6:00AM, the scientists were filtering their 48-hour Narrow Shelf incubations and storing them for transfer once the ship arrives back in Newport, Oregon. Beyond the constant activity visible in the make-shift labs onboard the ship, there is so much behind-the-scenes work that must be completed with a research project like PUPCYCLE 2019 and the bulk of the responsibility for a successful research cruise lies on the shoulders of Chief Scientist, Adrian Marchetti. However, like any successful venture, Adrian must rely on others to facilitate the project's success. A recent UNC-CH graduate, Olivia Torano, holds one of the key support roles as the Marchetti Lab Manager. Olivia enjoys her multifaceted position at the lab. "I work with so many different scientists doing various types of research," said Olivia, "and learning more about what they are studying helps me to plan and prepare the lab to better meet their needs." She confirms the amount of preparation and planning for PUPCYCLE 2019 was mind-boggling. "Not only are you trying to order supplies and materials needed for data collection, but also the other items to prepare the test materials and equipment prior to and after the data is collected."

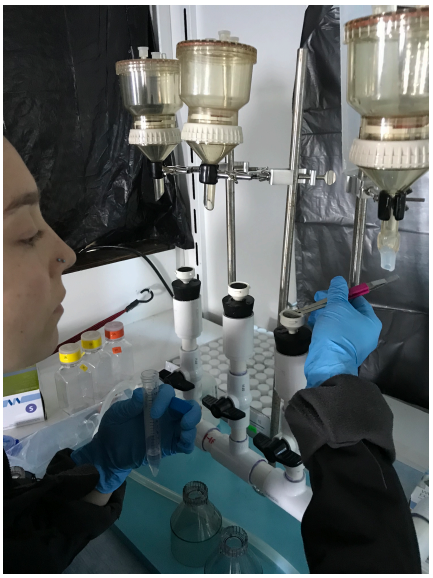

Figure 25 – Olivia prepares to seal a filter for cold storage. After the cruise, Olivia will analyze the nitrogen uptake by the diatoms. [*Image credit: Miriam Sutton*]

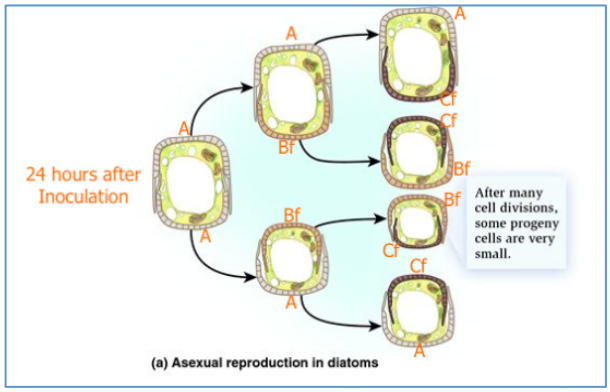

Figure 26 – Diatoms are treated with a fluorescent dye that reveals the number of divisions occurring during the incubation experiments Diatom offspring with an "f" indicate new cell growth. [*Image source: https://biology-forums.com/index.php?action=gallery;sa=view;id=782; modified by Miriam Sutton*]

Olivia is also conducting research during PUPCYCLE 2019. Her filtration system is set up to capture biogenic silica, which is the silica that diatoms (or other "glass house" organisms) use in the construction of their outer cell wall. In diatoms, this glass wall structure is referred to as a frustule. Each frustule has 2 parts that overlap and connect to form a wall of protection for the single-celled organisms. During reproduction, the cell divides and a new smaller frustule is produced using the silica dissolved in seawater. Olivia is investigating how much silica is being used to produce their shells during the timed incubation period and which species are growing fastest and using the most silica under the various environmental conditions, including: broad vs. narrow; Fe-limited vs. Fe-available; and surface vs. deep water communities. In order to measure the amount of silica being used, Olivia inoculates (or treats) her water samples with a fluorescent dye referred to as PDMPO (I'll spare you the long chemical name for this dye.). This treatment occurs 24 hours after their first incubation period. Once inoculated, the samples are returned to the incubation system for an additional 24 hours before Olivia runs her samples through the filters and prepares them for further testing back in Chapel Hill. Any new cell that was formed during the incubation period will fluoresce, or glow, when viewed under her microscope. This allows her to count the number of cell divisions for each species found in the sample. Whether she's counting cell divisions as they fluoresce beneath her microscope or the number of 0.2μm filter packs needed for the next filtration round, Olivia is supporting PUPCYCLE 2019 on deck and in the shadows.

BIO INFO: Olivia Torano was born in Michigan and completed her undergraduate degree in Ecology and Evolutionary Biology from the University of Michigan. She completed her Masters degree in Ecology in December 2019 from the University of North Carolina – Chapel Hill and now works as the Lab Manager for the Marchetti Lab at UNC-CH.

Today's Certificate Challenge: Diatoms build their cell walls, also known as ___, from silicates found in the ocean.

Day 13: Wednesday, June 05: Going with the Flow

The seas began to calm yesterday evening and the R/V Oceanus moved closer to the Oregon coast to begin another transect in search of an active upwelling zone. The previous shipboard experiments have been focused on locating "relaxed" non-upwelling zones, which occurs during the relaxation phase of the Upwelling Conveyor Belt Cycle (UCBC). During this time between active upwelling events, the water column becomes more stratified (or layered) with warmer water at the surface and colder water (filled with nutrients) toward the bottom. Previously, PUPCYCLE 2019 scientists collected surface (15m) and deep (90m) water samples during the relaxation phase and simulating an active upwelling event with incubation systems on deck. The incubation experiments allow them to observe the molecular (RNA-sequencing, gene expression, protein production) and physiological (nitrogen cycling, iron uptake, growth rate) responses of phytoplankton in a controlled environment. These incubation results will be compared to the data analysis from today's water samples, which will be collected during a naturally occurring active upwelling event. Today's active upwelling event was identified using updated satellite images and appears to be forming in the aftermath of the low-pressure system we endured the past 2 days. Monitoring the Underway System (which siphons a steady stream of surface water into computerized sensors onboard) allows the scientists to observe real-time changes in sea surface temperature (SST), salinity, and fluorescence. The search for cold (≤10*C) water at the surface that would indicate the start of an active upwelling cycle began after dinner last night. Three hours later, I stepped into the science lab after admiring a beautiful Pacific sunset and met Adrian Marchetti (Chief Scientist) coming from one of the shipboard computer monitors. (Figure 27) His eyes lit up as he announced, "We found cold water!" and he headed toward the Underway System to confirm the data with the additional onboard sensors. The transect route continued along the identified area and confirmed that the R/V Oceanus had indeed arrived at the start of an active upwelling event. By daybreak, the CTD and GoFlo® were being deployed for more water samples and Johnson Lin, graduate student at UNC-CH (Figure 28), and the other researchers began filtering and prepping actively upwelled phytoplankton samples for cold storage. Johnson prepares his samples for a Flow cytometer, which sorts and counts the different species of phytoplankton. According to Johnson, "Each phytoplankton group has a specific fluorescence pigment that the Flow cytometer uses to identify and count each group in the sample." This data will provide the PUPCYCLE 2019 scientists with a snapshot of the various types of phytoplankton found throughout the different test sites investigated during the cruise. As with many of the researchers onboard, Johnson has also donned a life vest and hardhat to assist in deploying the CTD equipment used to collect the samples for their research. Research at sea requires a helping hand from everyone onboard, whether you're a scientist or a crewmember.

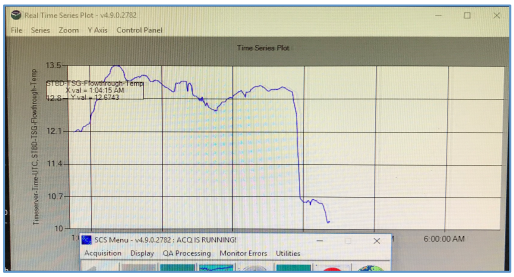

Figure 27 – A drastic decrease in the sea surface temperature (SST) indicated the location for an active upwelling event. *[Image credit: Miriam Sutton]*

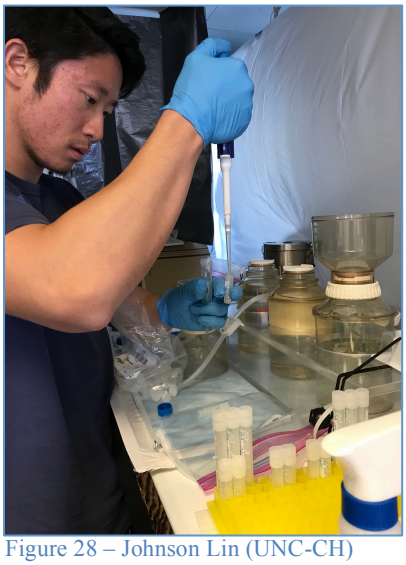

Figure 28 – Johnson Lin (UNC-CH) prepares his phytoplankton samples for the Flow cytometer he will use to identify and count each species. *[Image credit: Miriam Sutton)]*

Figure 29 – Johnson Lin (front) and Nataly Guevara (back) stand by to retrieve the CTD upon its return to the surface with their water samples. *[Image credit: Miriam Sutton)]*

BIO INFO: Johnson Lin was born in Los Angeles, California and completed his undergraduate degree in Aquatic Biology from the University of California at Santa Barbara. He is currently a graduate student in the Marchetti Lab at the University of North Carolina – Chapel Hill.

Today's Certificate Challenge: What does a Flow cytometer use to identify and count each species of phytoplankton?

Day 14: Thursday, June 05: PUPCYCLE 2019 Returns to Port

PUPCYCLE 2019 is a classic example of collaborative research among scientists. Twelve researchers investigating different interactions of phytoplankton within the Upwelling Conveyor Belt Cycle, all occurring in a world we cannot see. And, while each scientist has a different set of objectives, each aspect of their research is interrelated through the chemical and physiological responses of the phytoplankton they continue to filter from seawater samples on the final day of our 2-week research cruise. The schematic in Figure 30 illustrates the complexities of the marine environment in which these researchers are immersed. Labels have been added to identify each scientist with their investigative focus. No matter the differences, the commonality is the response of phytoplankton to the Upwelling Conveyor Belt Cycle that drives their microscopic ecosystem. Even more mind-boggling is to think that these scientists' research is focused on investigating the primary producers in the aquatic realm. Imagine all the interactions occurring in other segments of the food web: zooplankton (microscopic animals that feed on the phytoplankton) and the larger megafauna we see in our daily encounters with the creatures that call the sea home. From this amazing group of researchers we are learning how the creatures that we cannot see in the ocean are responding to changes in their environment. Previous studies have shown that iron is a key nutrient for phytoplankton, assisting to obtain the nutrients necessary for their cellular processes, including the photosynthesis we rely upon to draw down carbon dioxide levels while producing half the oxygen in our atmosphere. These earlier studies have also identified many regions throughout the world where iron is limited and regions where iron is readily available. Broad shelf regions allow more area for iron to accumulate and the presence of iron increases the primary productivity for these regions, thereby fueling the aquatic food web. As our oceans change with our climate, these organisms must adapt to survive. Using gene expression and other cutting-edge technology, these scientists can observe the cellular and physiological adaptations of these tiny organisms that play a significant role in the stability of our planet and ecosystem. One can hope that the microscopic responses being observed by the researchers will provide us with more insight into our own adaptability as our environment also continues to change.

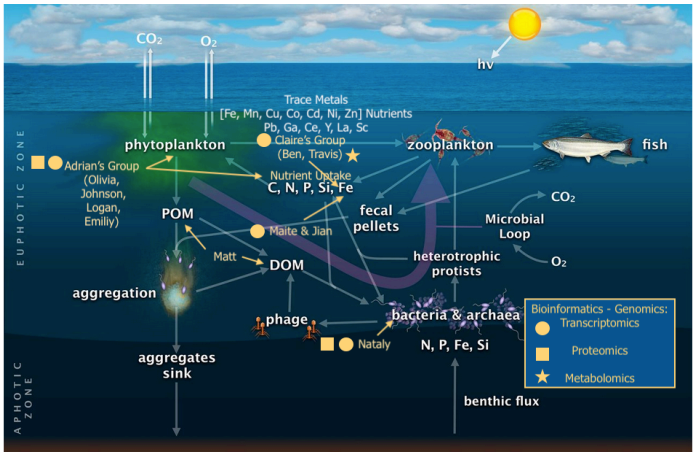

Figure 30 – The world of phytoplankton (and zooplankton) is very complex and PUPCYCLE researchers are exploring many components of this microscopic food web. *[Original image from: https://science.sciencemag.org/content/347/6223/1257594/tab-figures-data; modified by Miriam Sutton]*

As this 2-week expedition comes to a close, I am filled with gratitude for the researchers who allowed me to experience a small snapshot of their world as microbial oceanographers. I am thankful to the crew of the R/V Oceanus for their unwavering support during the expedition. And, finally, to those who participated in this research cruise through the virtual experience I worked to create with this blog and the various live feeds and posts through social media. For educators, this blog has been designed for classroom use with the goal of providing a virtual experience for students to gain an understanding of the marine technology used to explore our oceans and the life that lies therein. The blog and the supporting images, videos, and live feeds are available to educators as a stand-alone platform or as a supporting activity for standardized curriculum. Questions concerning this blog and its contents should be directed to *Science by the Sea®*.

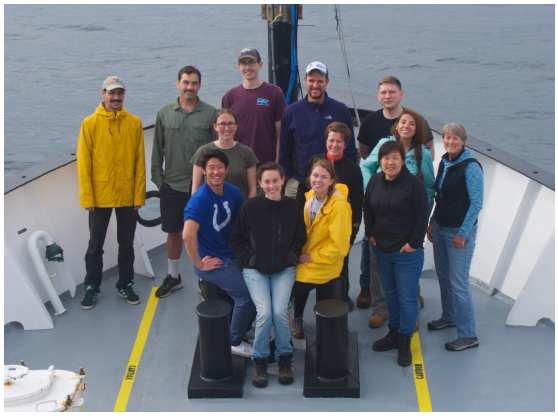

Figure 31 – PUPCYCLE 2019 Scientists from the University of North Carolina – Chapel Hill, University of British Columbia, and Humboldt State University. *[Image credit: Kate Kouba (OSU)]*

Today's Certificate Challenge: Complete the Google Form to receive the link to your Official PUPCYCLE 2019 Certificate.

Miriam Sutton, M.A., NBCT