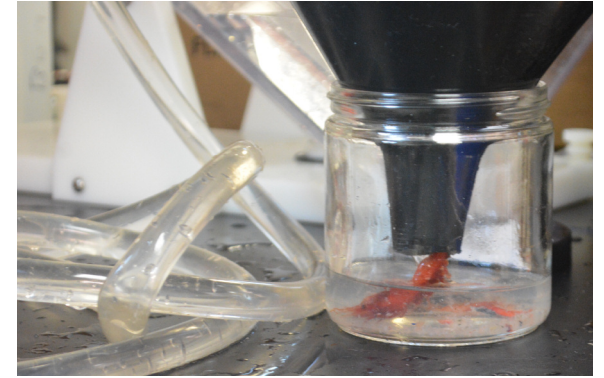


Report to SFI on BIOS-SCOPE

April 2019

Bermuda Institute of Ocean Sciences





BIOS-SCOPE is a five-year multi-institutional research program for the study of microbial oceanography in the northwestern Sargasso Sea, located within the North Atlantic Ocean. Established in 2015, BIOS-SCOPE leverages ocean measurements and ongoing research at the Bermuda Atlantic Time-series Study (BATS) site, bringing together new collaborations and technologies to study the ocean's smallest life forms.

The most powerful aspect of this collaborative project is its utilization of a broad suite of genomic, chemical, ecological, and biogeochemical approaches to evaluating microbial process, structure, and function on a wide variety of temporal and spatial scales. In our 2018 report, we organized the discussion of the progress achieved in 2016 and 2017 in terms of the spatial scales at which marine microbial ecology takes place—from organism-compound and organism-organism interactions to large biogeochemical patterns on the ecosystem scale. In this report on the progress achieved in 2018, we present the research activity and key findings to date in terms of the temporal scales of variability that take

place within the marine microbial ecosystem, ranging from diel (day-to-night) changes to those occurring over several seasons.

In 2018, the BIOS-SCOPE team conducted the program's fifth at-sea research expedition and carried out a wide range of research. Supplemental funding from SFI sponsored BIOS-SCOPE's first data workshop in Portland, Oregon in February of 2018. The workshop was used to identify collaborative datasets that could be advanced to publication. Five papers with full or partial support from the SFI grant were published or are currently in review; and nine science abstracts were presented at the 2018 Ocean Science Meetings in Portland, Oregon. In addition, seven abstracts describing BIOS-SCOPE research were presented at the ASLO Aquatic Sciences Meeting in Puerto Rico from February 23 through March 2, 2019.

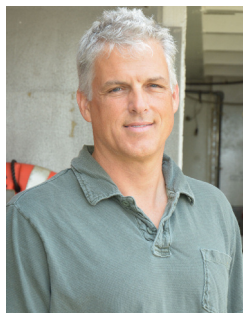
It is with the deepest gratitude that we provide the following report to Simons Foundation International LTD (SFI). SFI's support in establishing the program and its additional commitments to further enhance the program have made this progress possible.

BIOS-SCOPE Team Members

The BIOS-SCOPE team includes microbial oceanographers, molecular microbiologists, marine chemists, zooplankton ecologists, and physical oceanographers from BIOS and six other research institutions in the United States and United Kingdom. Each member brings unique expertise and novel technologies to the program.



Bill Curry, Program Leader
Curry is the President & CEO of BIOS and leads the multi-institutional BIOS-SCOPE program.



Craig Carlson, Program Director and Co-Principal Investigator
Carlson is a Professor at the University of California Santa Barbara (UCSB) in the Department of Ecology,

Evolution and Marine Biology and is a member of UCSB's Marine Science Institute. He is also a member of BIOS's Adjunct Faculty. As the Program Director of BIOS-SCOPE, Carlson oversees the overall science plan to ensure the research carried out is effective in its cross-disciplinary and integrative approach.



Leocadio Blanco-Bercial, Investigator
Ruth Curry, Affiliated SFI Investigator
Amy Maas, Investigator
Rachel Parsons, Investigator
Rod Johnson, Data Processing and Integration
Zachary Anderson, Research Support
Paloma Lopez, Research Support
Andrea Miccoli, Research Support



Stephen Giovannoni, Co-Principal Investigator
Zach Landry, Postdoctoral Fellow
Jimmy Saw, Postdoctoral Fellow



Craig Carlson, Co-Principal Investigator
Shuting Liu, Postdoctoral Fellow
Chance English, Research Support
Elisa Halewood, Research Support
Keri Opalk, Research Support



Ben Temperton, Investigator
Joanna Warwick-Dugdale, Doctoral Student



Hilary Close, Visiting Scholar



Elizabeth Harvey, Visiting Scholar



Elizabeth Kujawinski, Investigator
Winifred Johnson, Postdoctoral Fellow
Brittany Widner, Postdoctoral Fellow
Krista Longnecker, Research Support
Melissa Kido Soule, Research Support
Gretchen Swarr, Research Support

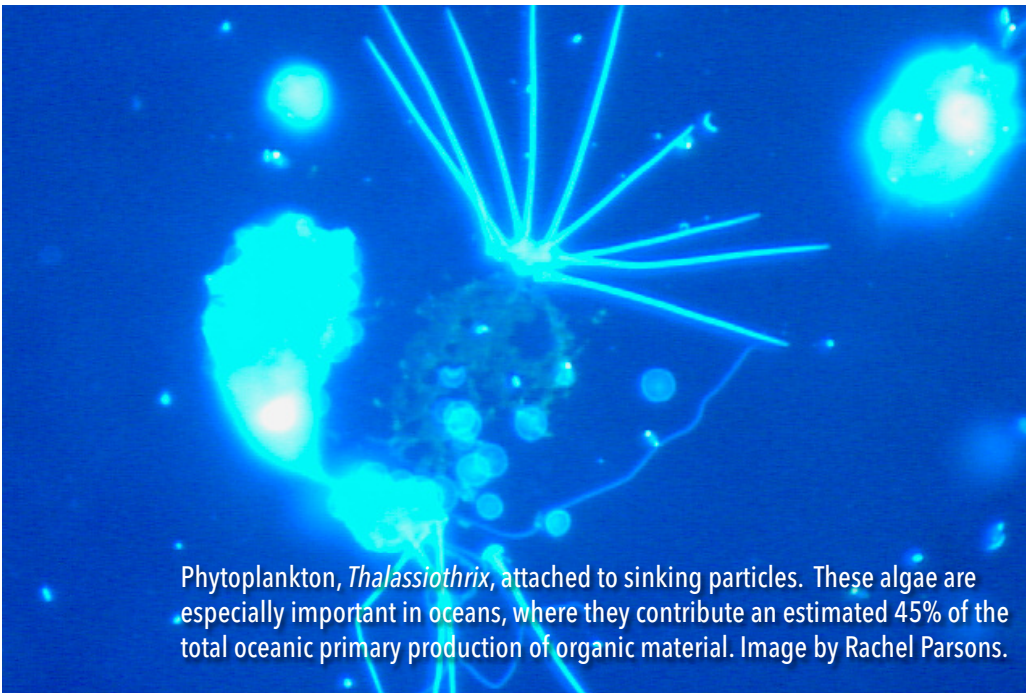
At-Sea Expeditions & Research Progress

The fifth at-sea research expedition for the BIOS-SCOPE program was conducted July 3-6, 2018, led by BIOS-SCOPE program director Craig Carlson. In addition to Carlson, 16 scientists participated in the expedition, as well as two undergraduate students. BIOS-SCOPE team members from BIOS, UC Santa Barbara (UCSB), Oregon State University (OSU), Woods Hole Oceanographic Institution (WHOI), and the University of Georgia (UGA) participated in the expedition; and were joined by collaborators from the Monterey Bay Aquarium Research

Institute (MBARI)—the result of a partnership formed in 2016—and from the University of Miami’s Rosenstiel School of Marine and Atmospheric Science (RSMAS), a new partner to the BIOS-SCOPE program.

The objectives of this 4-day expedition were to:

- explore if observed diel patterns in metabolites are coupled to vertically migrating zooplankton and other microbial and biogeochemical variables, as described in more detail below;
- conduct experiments on the interactions between microbes and dissolved organic matter (DOM), as described in more detail below;
- utilize the SFI-funded autonomous glider “Minnie” to provide continuous measurements of physical and biogeochemical properties from the ocean surface to depths of 1000 meters within 10 km of the ship’s operation, as described in more detail in BIOS’s November 2018 Report to SFI on the Mid-Atlantic Glider Initiative and Collaboration (MAGIC);
- acquire a comprehensive collection of particulate organic matter (POM) for molecular, organic, and isotopic characterization, and collect corresponding microbial DNA to evaluate how the microbes that are attached to particles transition between suspended and sinking size POM



Phytoplankton, *Thalassiothrix*, attached to sinking particles. These algae are especially important in oceans, where they contribute an estimated 45% of the total oceanic primary production of organic material. Image by Rachel Parsons.

fractions over depth. This avenue of inquiry is spurred on by BIOS-SCOPE's newest team member Dr. Hilary Close of the University of Miami, in collaboration with Craig Carlson's research group. This research aims to determine the fate of biomass produced in the upper vs. lower euphotic zone and how it contributes to carbon flux into the deep ocean, in situ respiration by bacteria, and respiration or packaging by zooplankton.

2018 Research Progress:

The overarching goal of BIOS-SCOPE is to better understand how marine microbes control the production, removal, and transformation of dissolved organic matter within a web of ecological interactions that take place on a wide range of time scales—from seconds to seasons. Deciphering these processes requires high-quality, high-resolution observational data and a broad suite of genomic, ecological, oceanographic, and biogeochemical approaches.

In the following sections, we review the research progress achieved to date on the various microbial interactions operating at both short (diel; day-to-night) and long (seasonal) time scales, as well as key findings to date with respect to specific bacterioplankton and viruses. We also include specific technological advancements that have been made during the pursuit of this research.



Craig Carlson (left) recovers a high-volume pump that samples microbial communities from different ocean depths.

Diel (Day-to-Night) Patterns

The team from WHOI has developed new high-resolution mass spectrometry methods to identify the chemical signatures of hundreds of unique molecules that make up DOM, as well as new methods to analyze metabolites in seawater.

Targeted metabolomics were used to analyze approximately 200 samples of DOM extracts collected during the first two years of this project. This analysis revealed the concentrations and dynamics of approximately 100 known compounds within the DOM pool at the Bermuda Atlantic Time-series Study (BATS) site, many of which have never been measured before in seawater.



Leo Blanco-Bercial collects samples from the MOCNESS during a cruise. Photo by Jorge Sanchez.

In a collaborative effort between the BIOS and WHOI teams, a correlation has been identified between diel patterns of metabolite distribution and the timing of vertical migrating zooplankton. The new metabolite data from the WHOI group reveals diel and vertical patterns in specific compounds. For example, thymidine was elevated at mesopelagic depths during the day and at the ocean's surface during the night. Thymidine was also produced by vertical migrating zooplankton in laboratory experiments. Since populations of zooplankton can travel over 600 vertical meters every day (and thereby shuttle microbes, genes, and nutrients

throughout the ocean's depths), we hypothesize that the diel signal in thymidine may be a direct result of its release by vertically migrating zooplankton. Experimental work is ongoing to further elucidate these linkages.

Laboratory experiments on zooplankton respiration also reveal diel patterns. Proteomic analyses were applied to compare the physiological state of the migratory copepod, *Pleuromamma xiphias*, immediately after arriving to the surface at night and six hours later.

These analyses suggest this copepod undergoes distinct physiological and metabolic changes over the diel cycle due to the exertion of its daily vertical migration.

The mortality of bacterial populations was measured during on-board experiments conducted as part of the BIOS-SCOPE expeditions and these, too, exhibit diel patterns. A clear diel pattern in the growth and mortality dynamics of the cyanobacterium *Synechococcus* sp. was revealed. A similar diel pattern has been previously reported for the cyanobacterium *Prochlorococcus* in the Pacific Ocean, but this is the first observation of *Synechococcus* diel dynamics in the Sargasso Sea.

Seasonal Patterns

During the winter, strong winds mix the upper portion of the ocean, transporting organic matter from the surface to depth, while bringing inorganic nutrients typically residing at deeper levels up toward the surface. These winter mixing events set the stage for an annual burst of photosynthetic activity by phytoplankton known as the "spring phytoplankton bloom," which affects the ocean's biogeochemistry and

ecology at all trophic levels. Concomitant with all of these changes are alterations to the microbial community.

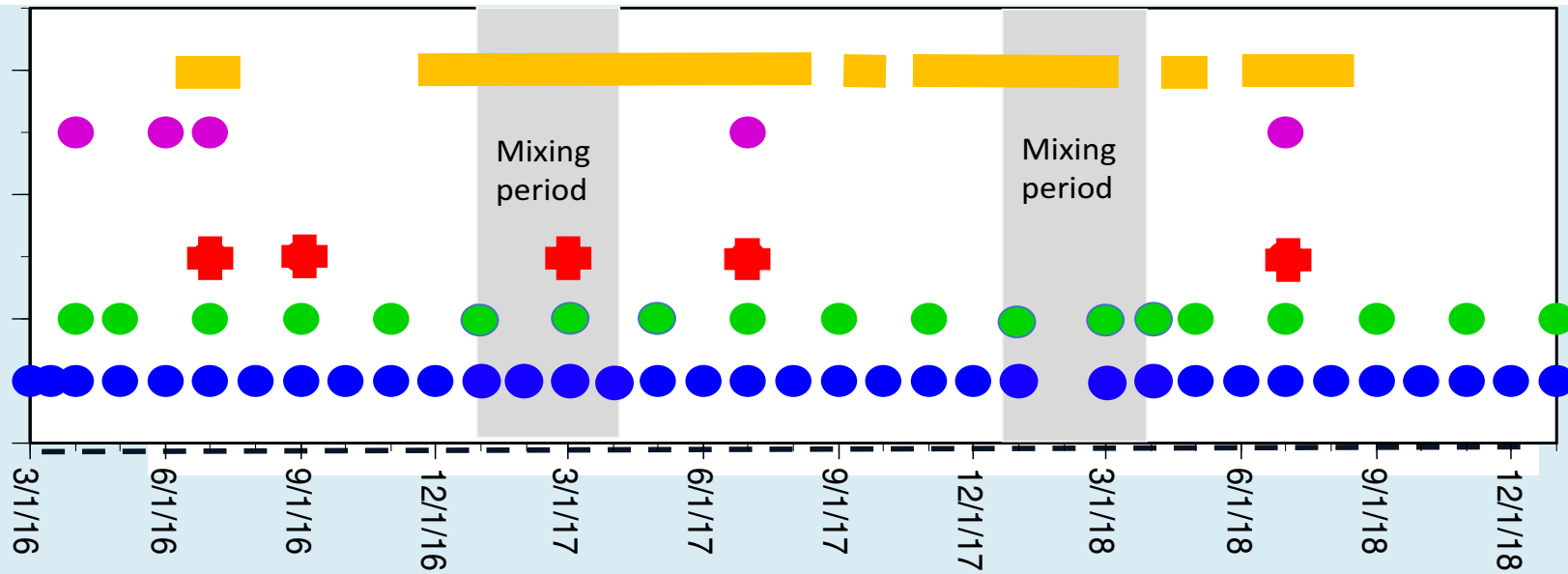
We know from the Bermuda Atlantic Time-series Study (BATS) that compounds within DOM exhibit seasonal variation due to microbial activity. Specifically, DOM compounds that are created through photosynthesis resist degradation by surface microbial communities, but later become available to—and fuel the growth of—deeper microbial communities after DOM is driven down to greater depths during winter mixing events.

As mentioned above, in 2018 approximately 200 samples of DOM extracts from the BIOS-SCOPE expeditions were analyzed to identify and quantify specific metabolites associated with the biogeochemical patterns observed from the winter to the summer. During the winter mixing events, surface bulk DOM is remarkably reduced, as high concentrations are mixed into deeper realms. In contrast, different responses to seasonal mixing were observed in the metabolite dataset. Some metabolite concentrations decreased at the surface during winter mixing events, some increased, and others showed no response. Analyses reveal seasonal variability of some metabolites as the system transitions from the winter mixing period to the summertime stable period. The targeted metabolomics analysis provides insight into the dynamics of metabolites that is otherwise obfuscated by analysis of DOM concentrations alone. Ongoing research will continue to investigate the linkage between metabolite dynamics and variability in microbial community structure.

As reported separately, BIOS's Mid-Atlantic Glider Initiative and Collaboration (MAGIC) is collaborating with the BIOS-SCOPE program, thanks to additional support from SFI. In 2018 MAGIC continued the field campaign initiated in 2017, extending an 8-month record of measurements offshore of Bermuda to one spanning 18 months. These high-resolution data detail the complex interplay between the ocean's biological productivity and its physical and biogeochemical properties on a daily, monthly, and seasonal basis, and thereby provide a rich contextual backdrop for BIOS-SCOPE investigations on microbial process, structure, and function.



Amy Maas and Andrea Miccoli record data during a research cruise.



BIOS-SCOPE benefits greatly from multiple sampling efforts in the waters southeast of Bermuda, providing excellent coverage before, during, and after the seasonal mixing period. The Bermuda Atlantic Time-series Study (BATS) provides a monthly data stream (shown in blue) to BIOS-SCOPE investigators, including samples of DNA and both microbial and biogeochemical variables. Targeted metabolite analyses on a subset of BATS data are shown in green. The dedicated ship time afforded to BIOS-SCOPE provides a seasonal platform (shown in red) upon which intensive water sampling and directed experiments can be conducted. Supplemental funding from SFI enabled MOCNESS deployments during BIOS-SCOPE and select BATS cruises (shown in pink). The near-continuous glider presence in 2017-2018 (shown in gold) provided a rich temporal backdrop that describes physical dynamics and some biogeochemical variability. The combination of these sampling efforts links data types across a number of time scales, from diel to seasonal, enabling the BIOS-SCOPE investigators to study the system in an unprecedented way.

Technological Advancements

As various research avenues were pursued, several technological advancements were made which, in-and-of-themselves, are significant achievements.

- Optofluidic single-cell genome amplification was used to obtain genome sequences from sub-micron cells collected during the BIOS-SCOPE expeditions. This work demonstrated the effectiveness of optofluidic technology for retrieving diverse single-cell bacterioplankton genomes, demonstrating the potential advantages in microbiology applications that require working with small sample volumes or targeting cells by their morphology. (Published in *Landry et al.*, 2018)
- Work on long-read viral metagenomics is now able to capture full-length viral genomes on single reads, negating the requirement for genome assembly and enabling high throughput, single-viral genomics from environmental samples. (Published in *Warwick-Dugdale et al.*, 2018)

Bacterioplankton

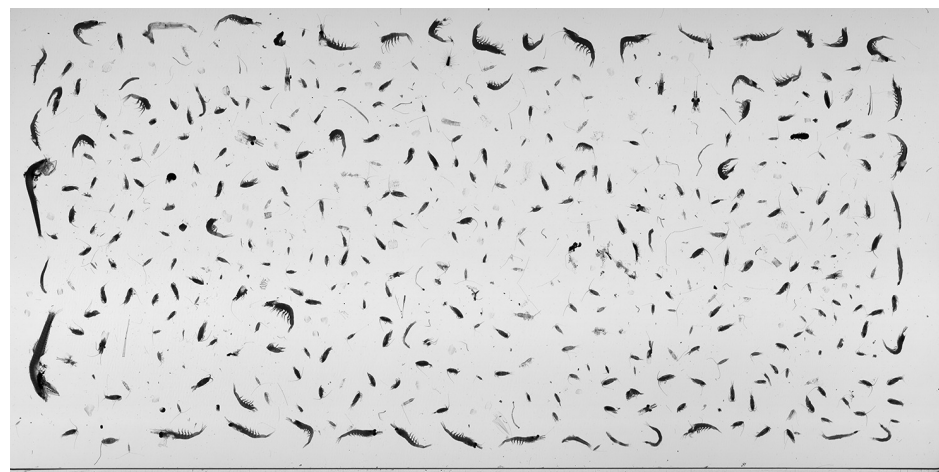
The UCSB team continued its research focused on the bacterioplankton SAR202, the most abundant and diverse bacteria in the dark ocean. Research has revealed that SAR202 comprises 5-30% of total bacterioplankton abundance in the dark ocean and that the clade has diverged into 4-5 subgroups. The vertical distribution of these different SAR202 subgroups is correlated to the expansion and diversification of different catabolic enzyme families, suggesting that SAR202 has diversified to exploit DOM resources that are spatially varying in the water column. This research shows in detail the patterns of genome evolution that support this hypothesis.

A major component of DOM in the intermediate and deep ocean is a set of compounds called CRAM, or carboxyl-rich alicyclic molecules. To better understand the interaction between bacteria and CRAM, a set of collaborative experiments were conducted by the UCSB, OSU, and WHOI groups. This experimental work demonstrated that specific subgroups of bacterioplankton—including SAR202 and SAR11 (the most abundant organisms in the ocean)—are capable of catabolizing recalcitrant CRAM proxy compounds. That is, these bacterioplankton are capable of utilizing organic compounds that other organisms are not. Furthermore, a series of experiments were conducted using stable isotope probing methods that enabled the research team to track the incorporation of recalcitrant DOM into SAR11, SAR202, and also SAR86 (another ubiquitous and highly abundant bacterioplankton), thereby increasing understanding of resource partitioning among bacterioplankton groups.

Viruses

As predators of marine bacteria, viruses are powerful players in the microbial ecosystem. Studying the genome of viruses presents a different set of challenges, however, due to their genetic complexity.

A collaborative effort between the research groups from OSU and Exeter has identified 24 complete or near-complete genomes for the first known viruses of the SAR202 clade. Analysis of the Global Oceans Virome dataset shows that these viruses are concentrated in the mesopelagic zone. Six of the auxiliary metabolic genes found in these viruses are homologous to those in the known SAR202 host genomes, suggesting an important role in host metabolism hijacking during infection.



Samples captured on the high resolution ZooSCAN imaging tool analyze zooplankton caught in a net tow during BIOS-SCOPE cruises.



New Collaborations



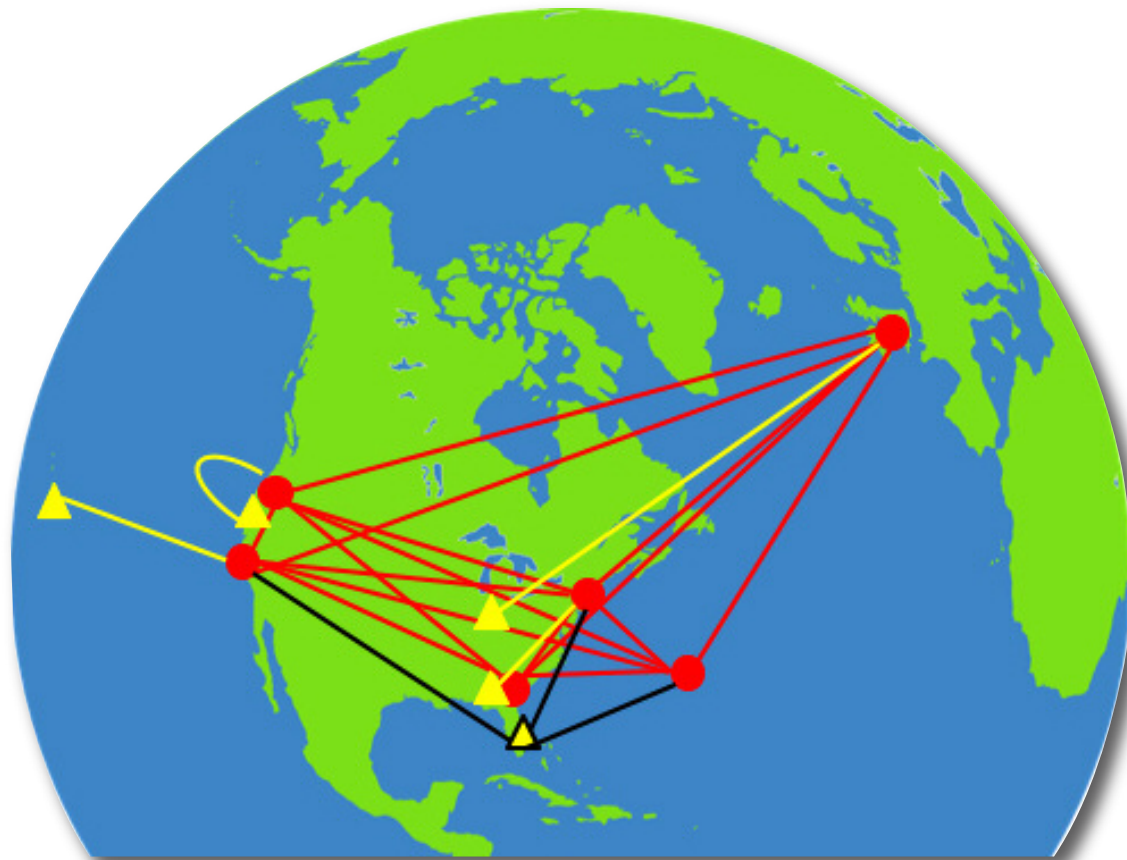
University of Miami visiting scholar, Dr. Hilary Close.

At its inception in 2015, BIOS-SCOPE was envisioned as a highly collaborative research program. In 2016 the core team of BIOS, UCSB and OSU was expanded to include investigators from the University of Exeter, University of Georgia, and WHOI. In 2017, collaborations with the University of Hawai'i, the Monterey Bay Aquarium and Research Institute (MBARI), the University of Alabama, and The Ohio State University were formed, expanding the academic network even further.

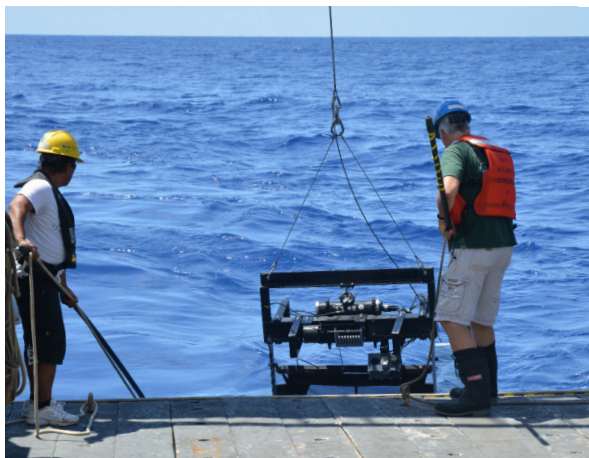
In 2018, Dr. Hilary Close of the University of Miami joined the project as a visiting scholar. Her research seeks to clarify the roles of microbes in the production and degradation of particulate organic matter. She is collaborating with Craig Carlson's group at UC Santa Barbara, Amy Maas at BIOS and Liz Kujawinski's group at WHOI to determine the fate of biomass produced in the upper versus the lower euphotic zone, and how it is transformed and contributes to carbon flux into the deep sea.

Synergies with SCOPE

- The first analysis of elemental content of C, N, and P and associated stoichiometry of SAR11 isolates has been published in *Limnology and Oceanography Letters* (White *et al.*, 2018). (White, Carlson, and Giovannoni groups)
- Optimizing the analyses of dissolved organic nitrogen at Station ALOHA has been published. (In press as *Foreman et al.*) (Carlson and Karl groups)
- North Pacific microbial remineralization experiments have been designed and conducted. Presented at the 2019 ASLO Aquatic Sciences Meeting. (Carlson and Church groups)



The BIOS-SCOPE network expanded in 2018 to include the University of Miami (depicted by the yellow triangle outlined in black). The core team of BIOS, Oregon State University (OSU), and the University of California Santa Barbara (UCSB) and the extended teams at the University of Exeter, University of Georgia, and Woods Hole Oceanographic Institution (WHOI) are depicted as red circles. The collaborators added in 2017, depicted as yellow triangles, include the Monterey Bay Aquarium Research Institute (MBARI), Ohio State University, University of Alabama, and University of Hawai'i. Red, yellow, and black lines depict the collaborations between the various teams as described in the text and previous reports.



Scientific Publications

In 2018, five papers were published or accepted for publication in peer-reviewed scientific journals:

- Foreman, R., K.M. Bjökman, **C.A. Carlson**, K. Opalk, and D.M. Karl (2018). Improved UV photo-oxidation system yields estimates for deep sea dissolved organic nitrogen and phosphorus. *L&O Methods in press*.
- Landry, Z.C., K. Vergin, C. Mannenbach, S. Block, Q. Yang, P. Blainey, **C. Carlson**, and **S. Giovannoni** (2018). Optofluidic Single-Cell Genome Amplification of Sub-micron Bacteria in the Ocean Subsurface. *Frontiers in Microbiology*, 9(1152).
- **Maas, A.E., L. Blanco-Bercial**, A. Lo, A.M. Tarrant, and E. Timmins-Schiffman (2018). Variations in copepod proteome and respiration rate in association with diel vertical migration and circadian cycle. *The Biological Bulletin*, 235(1):30-42. doi: 10.1086/699219. Epub 2018 Aug 16.
- Warwick-Dugdale, J., N. Solonenko, K. Moore, L. Chittick, A.C. Gregory, M.J. Allen, M.B. Sullivan, and **B. Temperton** (2018). Long-read metagenomics reveals cryptic and abundant marine viruses. bioRxiv.
- White, A.E., **S.J. Giovannoni**, Y. Zhao, K. Vergin, and **C.A. Carlson** (2018). Elemental Content and Stoichiometry of SAR11 Chemoheterotrophic Marine Bacteria. *Limnology and Oceanography Letters*, 4 (2): 44-51.

Scientific Presentations

As detailed in our April 2018 report, nine BIOS-SCOPE-specific presentations were given at the 2018 Ocean Science Meeting held February 11-16, 2018 in Portland, Oregon. The Ocean Sciences Meeting is a large international gathering, attended by approximately 4,000 ocean scientists.

In late February 2019, seven abstracts on the BIOS-SCOPE program were presented at the ASLO Aquatic Sciences Meeting in Puerto Rico:

- Blanco-Bercial, L. Annual Cycle of the Mesozooplankton Community in the Sargasso Sea.
- Harvey, E. A Matter of Time: Diel Rates of Phytoplankton Growth and Mortality in the Ocean.
- Kujawinski, E. Dissolved and Particulate Microbial Metabolites Reflect Distinct Metabolic Processes Across Spatial and Temporal Ocean Gradients.
- Liu, S., N. Huynh, J. Comstock, K. Opalk, E. Halewood, C. English, R. Parsons, S. Giovannoni, L. Bolanos, and C. Carlson. Stable Isotope Probing Identifies Bacterioplankton Lineages Capable of Utilizing DOM Across a Range of Bioavailability.
- Parsons, R., Z. Stead, K. McLeod, K. Bulski, T. Bittar, C. Carlson, and E. Harvey. Lineage Specific Mortality Rates of Bacterioplankton in the Sargasso Sea.
- Saw, J., T. Nunoura, R. Stepanauskas, K. Longnecker, E. Kujawinski, Z. Landry, C. Carlson, and S. Giovannoni. Pangenomics Reveal Diversification of Enzyme Families and Niche Specialization in Globally Abundant Deep-ocean Bacteria.
- Wear, E., C. Carlson, and M. Church. Bacterioplankton Metabolic Response to Phytoplankton Lysate Varies Across a Mesoscale Eddy Dipole in the North Pacific Subtropical Gyre.

Honors and Awards

- The AAAS honored Craig Carlson as a Fellow (elected November 2018).
- Jimmy Saw (former postdoc at OSU) was appointed Assistant Professor of George Washington University.
- Amy Maas was invited to the National Academies Keck Futures Initiative Conference entitled “Discovering the Deep Blue Sea 2016” and was subsequently awarded funding for a collaborative research project on zooplankton.

Chaetoceros microbe x1000. Image by Rachel Parsons.