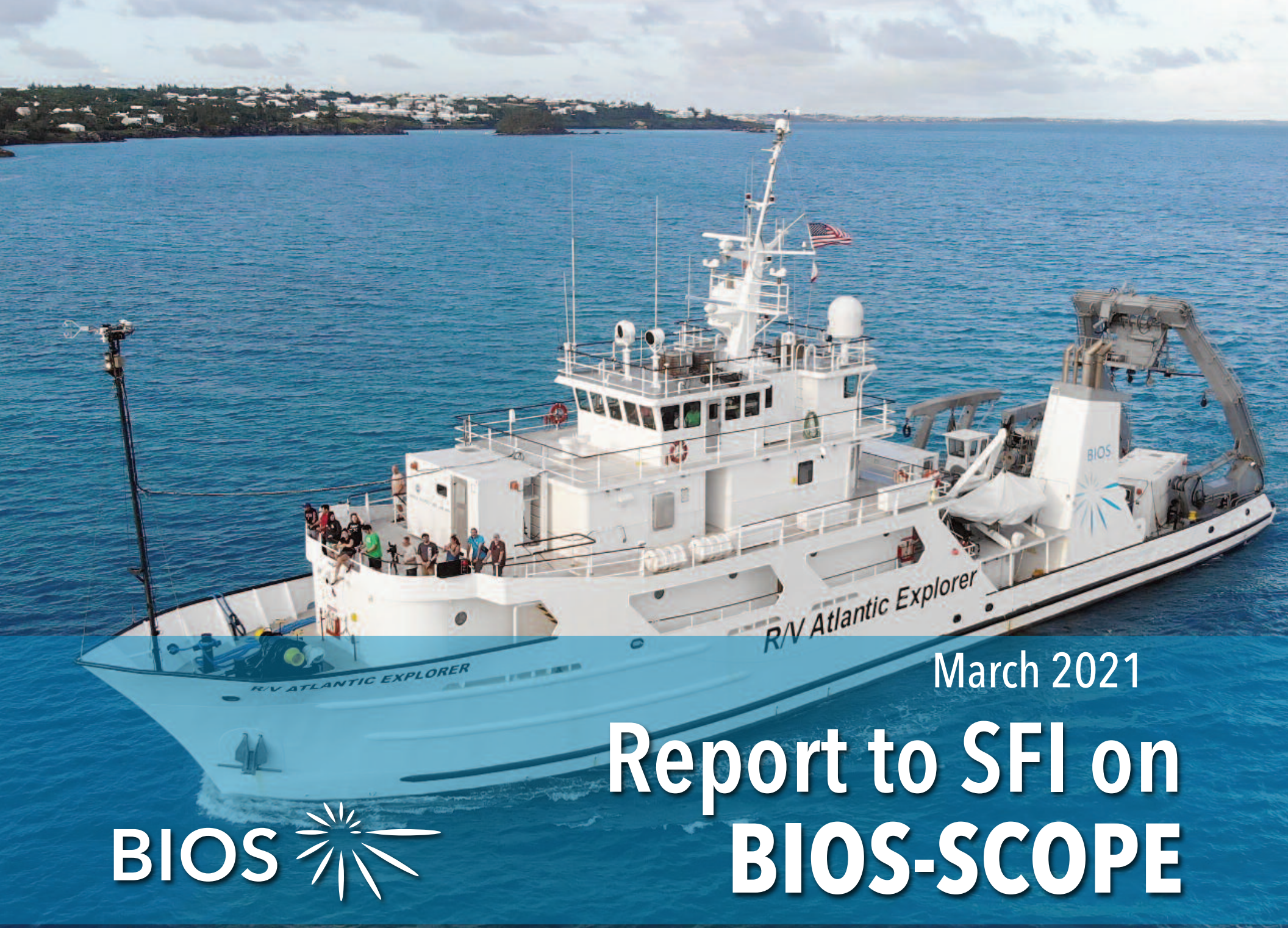


March 2021

Report to SFI on BIOS-SCOPE

BIOS

BIOS-SCOPE is a five-year multi-institutional and cross-disciplinary research program for the study of microbial oceanography in the northwestern Sargasso Sea. BIOS-SCOPE leverages combined expertise in microbial oceanography/dissolved organic matter (DOM) biogeochemistry, marine microbiology and bioinformatics, marine chemistry, micro- and mesozooplankton ecology, viral ecology, and agent-based modeling to evaluate microbial process, structure, and function on a variety of temporal and spatial scales. This project continues to benefit from its collaboration with the Bermuda Atlantic Time-series Study (BATS) and Hydrostation 'S' programs and the MAGIC glider program. Here we report major activities, progress, and findings from the Year 5 (Nov 1, 2019-Oct 2020) funding period.

The COVID-19 pandemic significantly impacted the BIOS-SCOPE field and laboratory-based activities planned for this funding period. However, despite these limitations, BIOS-SCOPE investigators have not been idle. The shutdown provided the team with a unique opportunity to organize and synthesize existing data into new manuscripts and data sets. Fourteen papers with full or partial support from this SFI grant were published in peer-review scientific journals or in various stages of the review process.

Additionally, numerous scientific talks and posters describing BIOS-SCOPE collaborative projects were presented at variety of national and international scientific venues. Some presentations were in-person before COVID restrictions took place, such as the 2020 Ocean Sciences Meeting in San Diego, while other meeting were virtual, including the Simons Collaboration director's talks and virtual microtalks, the Ocean Carbon and Biogeochemistry (OCB) meetings, and workshops and invited seminars at various universities around the country.

It is with deepest gratitude that we provide the following report to the Simons Foundation International, Ltd. on major activities, progress, and findings from the 2020-2021 funding period. The Foundation's support in establishing the program and its additional commitments to further enhancing the program have made this progress possible.

BIOSSCOPE
Focusing research on the ocean's smallest life forms

BIOS-SCOPE Team Members

The BIOS-SCOPE team comprises microbial oceanographers, molecular microbiologists, marine chemists, zooplankton ecologists, computational modelers, and physical oceanographers from BIOS and six other research institutions in the United States, United Kingdom, and Germany. 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.

BIOS-SCOPE Team Members

BIOS
Bermuda Institute of Ocean Sciences

Zachary Anderson, Research Support
Leocadio Blanco-Bercial, Investigator
Ruth Curry, Affiliated SFI Investigator
Cordie Goodrich, Hardware/Software Technician
Hannah Gossner, Research Support
Rod Johnson, Data Processing and Integration
Amy Maas, Investigator
Rachel Parsons, Investigator
Kevin Vergin, Visiting Scholar

Oregon State University OSU

Stephen Giovannoni, Co-Principal Investigator
Chih-Ping Lee, Postdoctoral Fellow

TU berlin

Ferdi Hellweger, Computational Scholar

University of Exeter

Ben Temperton, Investigator
Joanna Warwick-Dugdale, Doctoral Student

GEOMAR

Alexandra Worden, Visiting Scholar

University of Miami

Hilary Close, Visiting Scholar

University of New Hampshire

Elizabeth Harvey, Visiting Scholar

UC Santa Barbara

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

Woods Hole Oceanographic Institution

Elizabeth Kujawinski, Investigator
Krista Longnecker, Research Support
Erin McParland, Postdoctoral Fellow
Craig McLean, Graduate Student
Gretchen Swarr, Research Support

At-Sea Expeditions & Fieldwork

As previously mentioned, the COVID-19 pandemic and associated restrictions limited the fieldwork that could be conducted during the Year 5 period. In March 2020, the UNOLS Council recommended that the academic fleet, which includes the R/V Atlantic Explorer, stand down until COVID protocols could be implemented to ensure safe operations on all U.S. research vessels.

Time-Series Cruises: BATS cruises were reinstated in July 2020 after a four-month hiatus due to COVID-19. As such, BIOS-SCOPE variables (i.e., planktonic DNA, microbial biomass, and DOM composition over 1000m) were collected monthly from November 2019 to March 2020, and after BATS cruises resumed in July 2020 to present. In 2019, BIOS-SCOPE expanded the MOCNESS zooplankton tow efforts to seasonal tows (~every four months) on Hydrostation 'S' cruises.

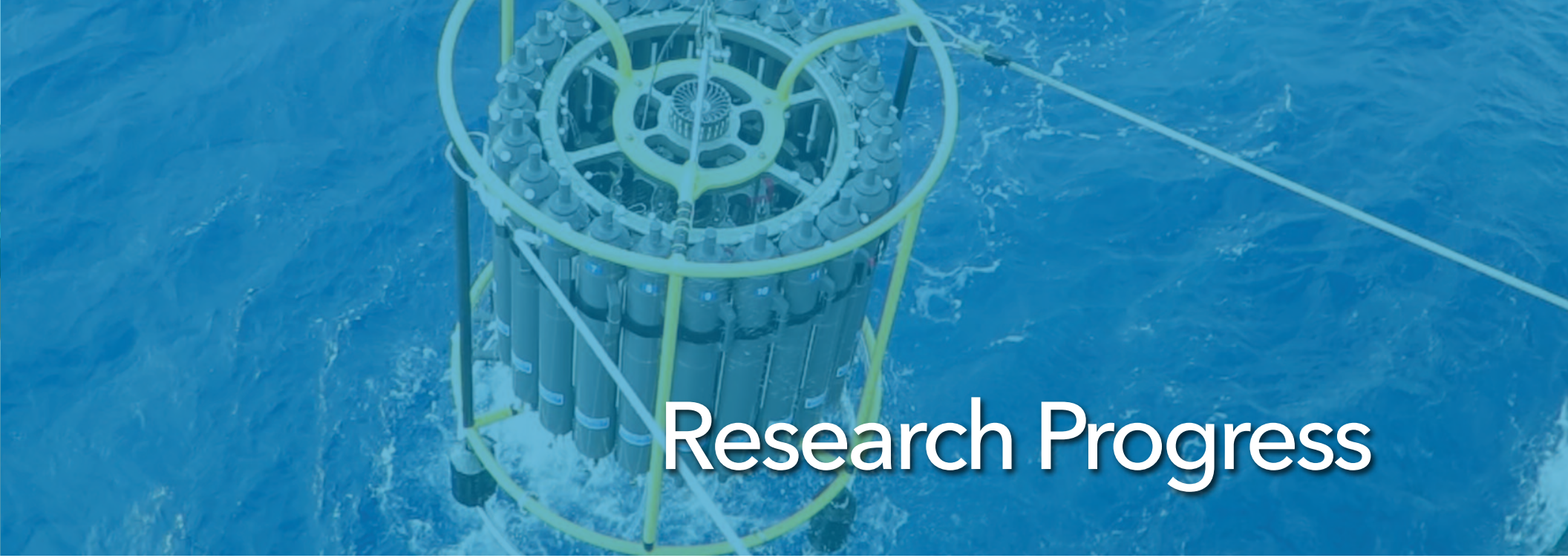
BIOS-SCOPE Process Cruise: When research cruise activity restarted in July, there were strict regulations that limited the number of personnel allowed to sail on UNOLS research vessels. These restrictions also reduced the number of over-the-side scientific operations that could be conducted at sea. At the same time, BIOS-SCOPE investigators experienced limited access to research facilities at their home institutions. The Bermuda Government took the necessary steps to reduce the local spread of the virus by mandating strict quarantine protocols upon arrival. Given these restrictions and the associated unanticipated costs, it was decided, after consultation with Marian Carlson and Bill Curry (President of BIOS), that it was in the best interest of BIOS-SCOPE field research to postpone the BIOS-SCOPE Process Cruise until Summer 2021.

Workshops, Synergies, Collaborations

The third data workshop was held February 14-15, 2020 in San Diego, CA ahead of the Ocean Sciences Meeting (OSM). Thirty-four researchers were in attendance contributing science talks that highlighted recent findings (see below). A significant portion of the workshop was dedicated to planning new research directions to be pursued as part of the BIOS-SCOPE II renewal proposal, which was submitted for consideration for funding in October 2020. In addition, a total of 11 abstracts describing BIOS-SCOPE research were also presented at the 2020 OSM.

The year also offered unique opportunities for synergies among the five Simons Foundation collaborations. Over a period of six weeks, from January through March 2020, the directors of the five Simons Foundation marine collaborations were invited to give programmatic overviews describing each team's major research activities and findings. This activity was conducted in preparation for the 2020 Joint Simons Marine Collaborations Meeting, which was originally scheduled to be held in-person on April 30 and May 1 in New York.

Due to COVID-19 restrictions, the meeting was, instead, held virtually October 5 and 6, 2020. The meeting consisted of virtual "poster" sessions narrated by 5-minute "microtalks." More than 40 "microtalks," including six from BIOS-SCOPE researchers, are still available to be viewed online by members of all collaborations, providing valuable resources to aid in the development of novel research pathways.



Research Progress

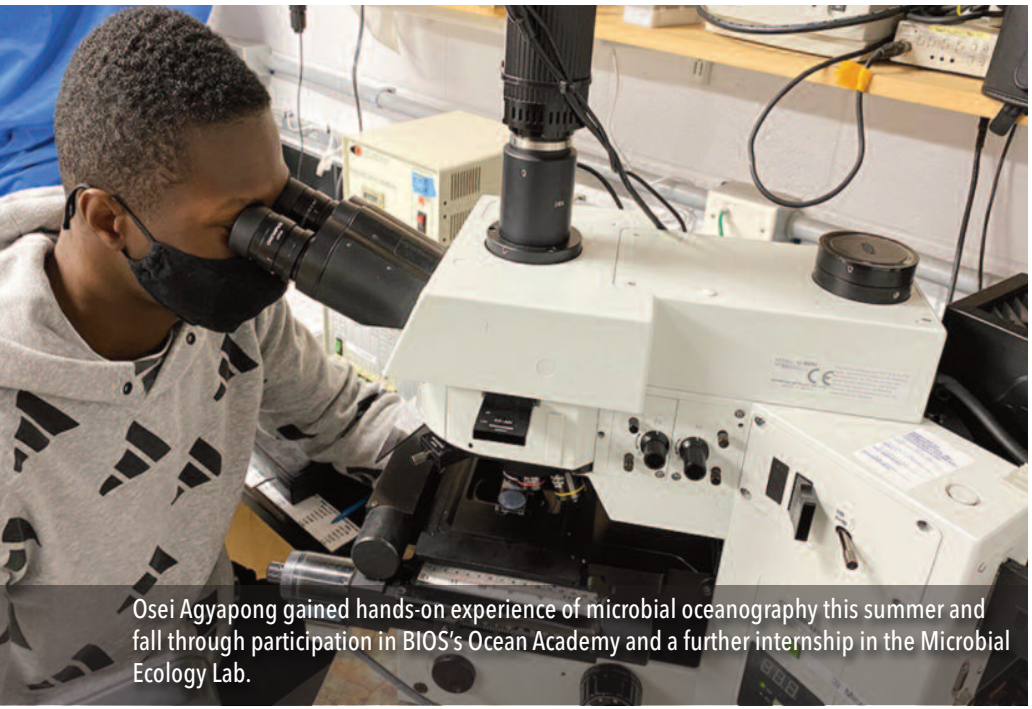
Through photosynthesis and subsequent food web interactions, a portion of the carbon fixed to organic matter is released as dissolved organic matter (DOM). DOM that is freshly released from phytoplankton, viruses, and zooplankton acts as fuel for bacterial growth. These bacteria either recycle DOM into carbon dioxide and nutrients, store it as microbial biomass, or transform it into compounds that accumulate as biologically resistant DOM (not available for use by organisms). Seasonally accumulated DOM is exported to depth as the water column mixes deeply in the winter months, becoming an important factor in the calculations of geochemical export of carbon from the surface to depth.

Once there, the exported DOM is oxidized by a unique assemblage of heterotrophic microorganisms that live in the dimly-lit upper mesopelagic zone (150-300m).

Understanding the source of DOM (phytoplankton, zooplankton or viral interactions), its composition, and how it interacts with bacterioplankton communities over time and depth, are among key research foci of BIOS-SCOPE. BIOS-SCOPE investigators aim to discover the fundamental mechanisms that govern the biogeochemical dynamics of organic matter by investigating the metabolic activity and genomic diversity of the plankton including phytoplankton, viruses, bacteria, protists and mesozooplankton.

Metabolites and DOM Chemistry

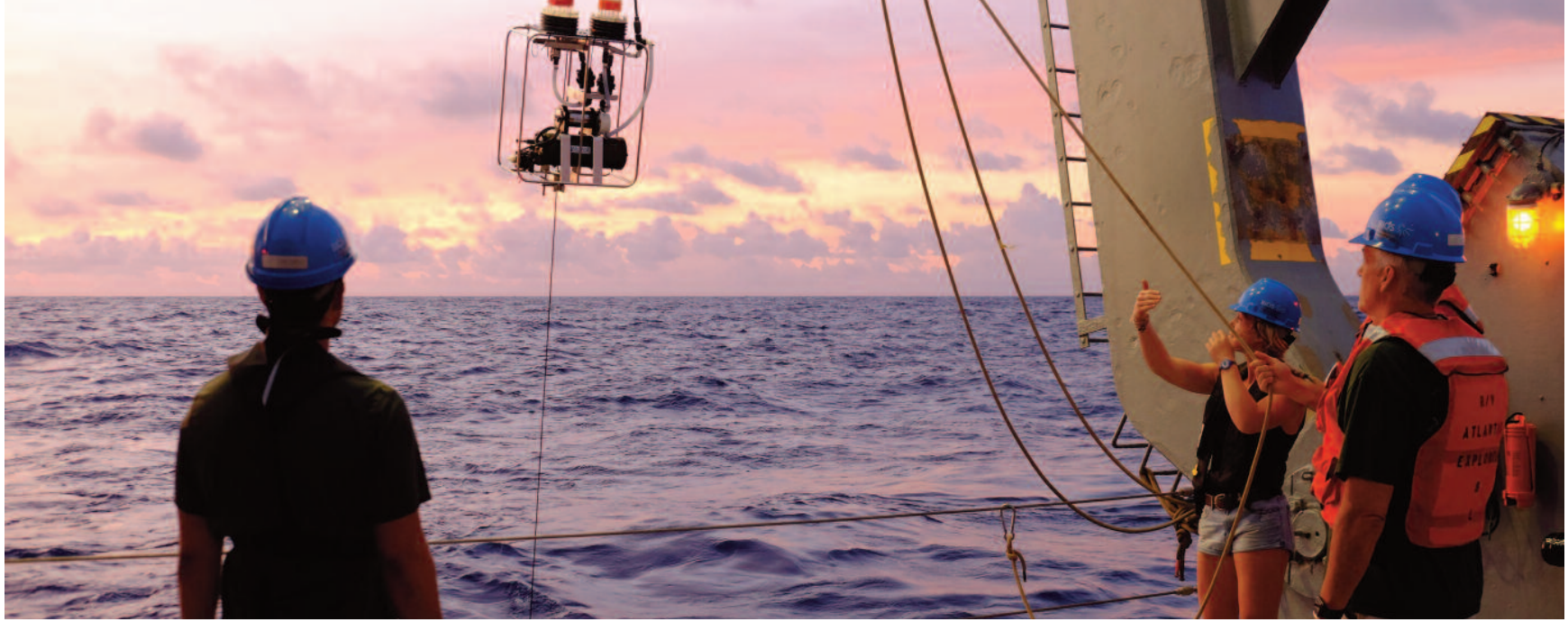
Dynamics of metabolites on multiple time scales: The DOM pool comprises a myriad of compounds. A component of these organic compounds, called metabolites, are actively produced and consumed by the resident plankton community. Until now, little was known about how metabolites varied in time or depth. The Kujawinski Lab (WHOI) collected the first multi-year (5 years) dataset of metabolite dynamics; developed advanced methods to expand our lexicon of molecule targets; and interacted with BIOS-SCOPE colleagues to integrate chemical data with biological mechanisms. No such metabolite datasets existed prior to this project and extensive data analysis is ongoing. Repeatable patterns of specific metabolite variability have now been observed on multiple time-scales, i.e., daily to seasonal. The full dataset of discovery-based analyses is being analyzed by Erin McParland, a new postdoc.



Osei Agyapong gained hands-on experience of microbial oceanography this summer and fall through participation in BIOS's Ocean Academy and a further internship in the Microbial Ecology Lab.

Development of new methodologies for quantifying metabolites: Brittany Widner, a postdoc in the Kujawinski Lab innovated a new method to isolate and identify dilute metabolites within a salty seawater. This new method has doubled the set of known organic compounds that can be recovered from seawater and will be implemented in future BIOS-SCOPE efforts.

Stable Isotope Probing (SIP) experiments: DOM released from phytoplankton consists compounds that span the range from biologically available to resistant. Linking specific bacterioplankton species to the incorporation of DOM of varying reactivity is important to understanding how microbial community structure is established. Shuting Lui, a postdoc in the Carlson Lab, led an experiment that used SIP to track DOM compounds of varying sources and reactivities directly into the DNA of microbes using these substrates (Liu et al., 2020). The team of researchers, which included the Giovannoni Lab (OSU) and the Parsons Lab (BIOS), developed an effective strategy for investigating organic molecule resource partitioning among the major bacterioplankton groups that will guide high-throughput cultivation in the future. SIP is part of a triad of approaches at the genome, cell, and ecosystem levels that BIOS-SCOPE investigators envisage are needed to build a more mechanistic understanding of the ocean carbon cycle.



Plankton & Systems Ecology

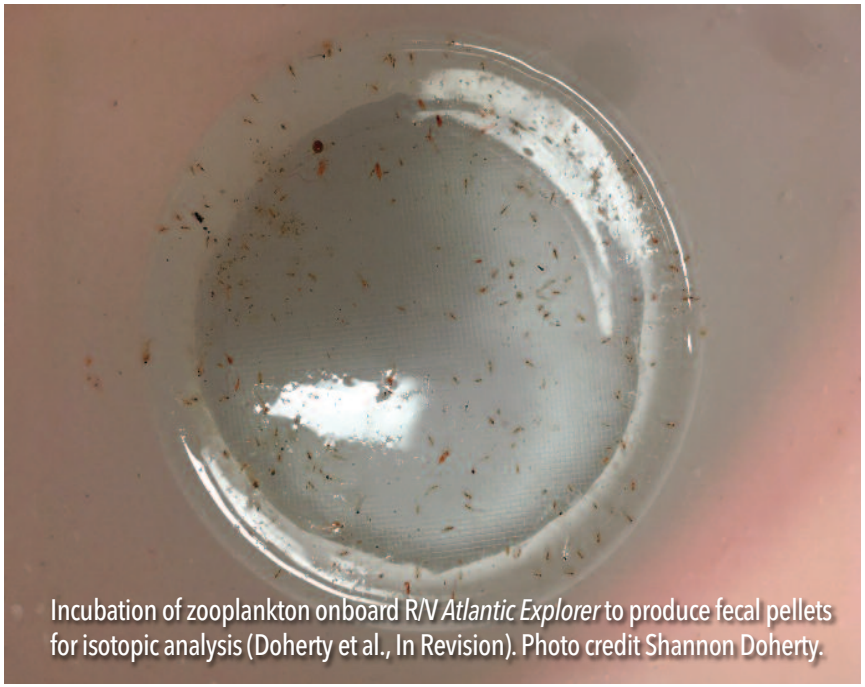
Effects of zooplankton excreta on prokaryotic communities: The Maas Lab (BIOS) conducted several collaborative experiments with the Carlson, Giovannoni, and Kujawinski groups to investigate how the physiological activity of vertical migrating zooplankton influenced the midwater bacterial communities. They demonstrated, for the first time, that the migratory copepod Pleuromamma xiphias—which transports surface-derived compounds including amino acids, vitamins, and nucleosides to depth on a daily basis—may act as important vectors of limiting organic nutrients for distinct mesopelagic microbial communities. These data support the hypothesis that vertical migrators' excreta acts as a mechanism for increasing niche diversity in the mesopelagic.

Zooplankton biodiversity; seasonality and distribution: To investigate how biogeochemical cycling is influenced by changing patterns of mesozooplankton and protistan plankton, the Blanco-Bercial lab investigates the changes in eukaryotic biodiversity. BIOS-SCOPE investigators are able to explore functional diversity at this ocean site by using molecular metabarcoding approaches for protists, fungi, zooplankton, integrated with image-based machine-learning approaches which, together, provide the necessary ecological statistics. Blanco-Bercial published the first Sargasso Sea study describing the seasonal variability of the zooplankton community structure in the context of particulate organic matter (POM) export and hydrographic and nutrient dynamics (Blanco-Bercial, 2020).

Computational approaches to understanding systems bioecology: The Hellweger Lab (Technical University of Berlin) is developing new computational approaches to better study systems bioecology (the combination of systems biology and systems ecology). The mechanistic microbial ecosystem model inference (MMEMI) integrates the extensive microbial, genomic, and environmental data collected by the BIOS-SCOPE team to explore mechanistic underpinnings of the observed dynamics.

Marine Microbiology & Microbial Physiology

Diversification of SAR202 bacteria's enzyme families: The Giovannoni Lab (OSU) uses comparative genomics to develop and test new hypotheses about how ocean plankton cells oxidize organic carbon. During this review period Jimmy Saw, in collaboration with other BIOS-SCOPE investigators, published a thorough analyses of the diversification of the SAR202 bacteria's enzyme families (Saw et al., 2020). Using a pangenomics analyses, the team revealed diversification of enzyme families and niche specialization of the globally abundant group of bacterioplankton called SAR202. They hypothesize that during Earth's mid-history, enzymes for degrading chemically recalcitrant organic carbon diversified in SAR202 bacteria, causing the oxidation of organic matter that had been accumulating in the oceans. Today, these organisms thrive and remain one of the dominant groups of deep ocean microbes. BIOS-SCOPE investigators continue to gather evidence to understand their role in catalyzing final stages of the carbon harvest.



Incubation of zooplankton onboard R/V *Atlantic Explorer* to produce fecal pellets for isotopic analysis (Doherty et al., In Revision). Photo credit Shannon Doherty.

Mixotrophy study at BATS: Mixotrophy is a nutritional strategy in which some microbes derive their energy from a combination of photosynthetic and heterotrophic metabolisms. New BIOS-SCOPE investigator Alexandra Worden (GEOMAR) used the BATS data to identify silicoflagellates as important phytoplankton that consume other cells at BATS during the summer when nutrients are highly limited (Choi et al., 2020). The observations that multiple uncultured mixotrophic groups are seemingly capable of photosynthesis and predation, and thrive during stratified periods, raises questions about potential shifts in trophic roles associated with seasonality and long-term changes in ocean conditions.

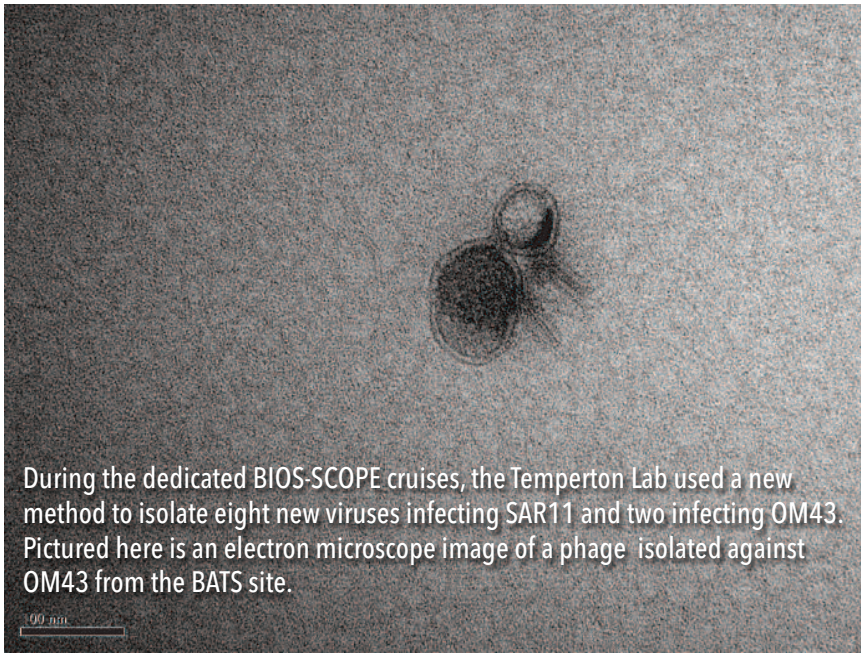
Interactions between microbial communities and particulate substrates: The Close Lab (Univ. of Miami) focuses on character-

izing the chemical transformations of organic particles in the water column that are mediated by bacteria and zooplankton. POM is important to the ocean carbon cycle as it sinks by gravity, thereby exporting carbon from the upper ocean into deeper waters where it is effectively sequestered on long timescales. Both microbes and zooplankton influence the relative quantities of carbon existing as non-sinking DOM, slowly-sinking (small) POM, and quickly-sinking (large) POM.

The Close Lab is using the naturally occurring nitrogen isotopic content of POM and how it changes over depth to decipher these complex microbial and zooplankton interactions. This is the first such geochemical tool to use ubiquitous compounds found within organic matter to distinguish the effects of microbial and animal heterotrophy. As part of this work, the Close Lab, in collaboration with the Maas Lab, made the first measurements of amino acid nitrogen isotope measurements of zooplankton fecal matter. These measurements will provide an end-member for the interpretation of the overall chemical composition of particles in the water column at BATS and is the subject of a recently submitted manuscript (Doherty et al. in review).

Marine Viruses & Viral Ecology

The Temperton Lab (Univ. of Exeter) focuses on how host-virus interactions affect organic carbon cycling at BATS. This group has a developed several new methods for resolving viral diversity in the open ocean and for isolating viral lineages. These isolates represent new model systems to evaluate viral–host models currently used in marine microbial ecology. Bacterial viruses (phages) are parasites that bind to their prey, injecting their genetic material into the host cell, and hijacking the host's metabolism for new phage synthesis that results in the eventual lysis (disintegration) of the host and release of viral progeny. The interactions between marine bacteria and phages are major drivers of both microbial community diversity and global carbon biogeochemistry. The Temperton Lab developed a new and efficient method for isolating viruses for fastidious taxa such as SAR11 bacteria. The method yielded 117 new viruses including the first ever viruses for bacterial taxa OM43, and new viral genera infecting SAR11 (Buchholz et al., 2020). During our dedicated BIOS-SCOPE cruises, they used this new method to isolate eight new viruses infecting SAR11 and two infecting OM43.



During the dedicated BIOS-SCOPE cruises, the Temperton Lab used a new method to isolate eight new viruses infecting SAR11 and two infecting OM43. Pictured here is an electron microscope image of a phage isolated against OM43 from the BATS site.

Synergies with Simons Marine Collaborations

- The research efforts of the BIOS-SCOPE program are both complementary and synergistic with the SCOPE program at Station ALOHA. Collaborations between the SCOPE-ALOHA and BIOS-SCOPE groups have resulted in three publications to date and are ongoing. The Carlson and Van Mooy Labs continue to collaborate on a study that uses an automated oxygen incubation system (AutoBOD) to resolve bacterial respiration at BATS. Temperton and Weitz's Labs collaborate to determine the viral infection model that best fits observations at the two sites. The BIOS-SCOPE team also participated in the Simon's Collaboration virtual conference in October 2020. This meeting spawned continued discussions with other Simons sponsored groups. In addition, BIOS-SCOPE investigators have interacted with investigators of C-BIOMES and the PriME teams. For example, Naomi Levine of the PriME group attended the BIOS-SCOPE data workshop in February 2020 and continues to interact with several groups within BIOS-SCOPE (Carlson, Kujawinski, Wordon).

Scientific Publications

In 2020, fourteen papers with full or partial support from the SFI grant were published or are currently in review in peer-reviewed scientific journals.

- Blanco-Bercial, L. (2020). Metabarcoding Analyses and Seasonality of the Zooplankton Community at BATS. *Frontiers in Marine Science* **7**: 173

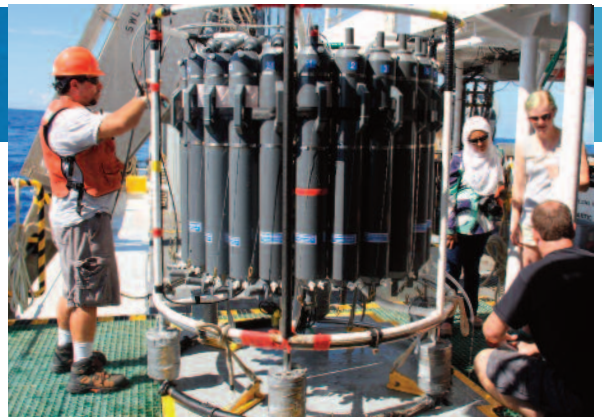
- Buchholz, H. H., M. L. Michelsen, M. J. Allen and B. Temperton (2020). Efficient Dilution-to-Extinction isolation of novel virus-host model systems for fastidious heterotrophic bacteria. bioRxiv

- Choi, C. J., V. Jimenez, D. Needham, C. Poirier, C. Bachy, H. Alexander, S. Wilken, S. J. Giovannoni, S. Sudek and A. Z. Worden (2020). Seasonal and geographical transitions in phytoplankton community structure in the Atlantic and Pacific Oceans. *Frontier in Microbiology* **11**:542372. doi:10.3389/fmicb.2020.542372

- Doherty, S., A. Maas, D. Steinberg, B. Popp and H. Close (Submitted). Distinguishing fecal pellets as a component of the biological pump using compound-specific isotope analysis of amino acids. *Limnology and Oceanography Submitted*

- Henson, M. W., V. C. Lanclos, D. M. Pitre, J. L. Weckhorst, A. M. Lucchesi, C. Cheng, B. Temperton and C.Thrash (2020). Expanding the diversity of bacterioplankton isolates and modeling isolation efficacy with large scale dilution-to-extinction cultivation. *Appl. and Environ. Microbiol*.**10**.1128/AEM.00943-20

BIOS



- Johnson, W. M., K. Longnecker, M. C. Kido Soule, W. A. Arnold, M. P. Bhatia, S. J. Hallam, B. A. S. Van Mooy and E. B. Kujawinski (2020). Metabolite composition of sinking particles differs from surface suspended particles across a latitudinal transect in the South Atlantic. *Limnology and Oceanography* 65: 111-127.10.1002/lno.11255

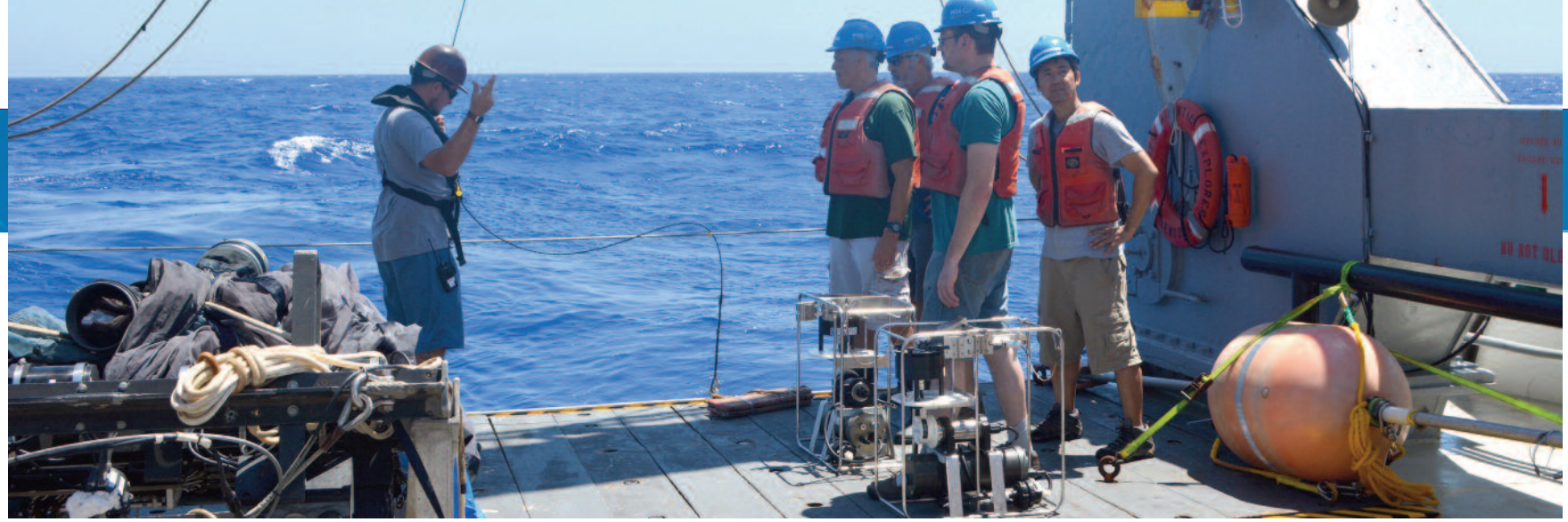
- *Liu, S., R. Parsons, K. Opalk, N. Baetge, S. Giovannoni, L. M. Bola.os, E. B. Kujawinski, K. Longnecker, Y. Lu and E. Halewood (2020). Different carboxyl-rich alicyclic molecules proxy compounds select distinct bacterioplankton for oxdation of dissolved organic matter in the mesopelagic Sargasso Sea. *Limnology and Oceanography* 65(7): 1532-1553.10.1002/lno.11405

- Liu, S., N. Baetge, J. Comstock, K. Opalk, R. J. Parsons, E. Halewood, C. English, S. J. Giovannoni, L. M. Bola.os, C. E. Nelson, K. Vergin and C. A. Carlson (2020). Stable isotope probing identifiesbacterioplankton lineages capable of utilizing dissolved organic matter across a range of bioavailability. *Frontiers in microbiology* **11:** 2364

- Maas, A. E., L. Blanco-Bercial and H. Gossner (in review). Use of Optical Imaging Datasets to Assess Biogeochemical Contributions of the Mesozooplankton. *Journal of Plankton Research*

- Maas, A. E., S. Liu, L. Bola.os, B. Widner, R. J. Parsons, E. B. Kujawinski, L. Blanco-Bercial and C. A. Carlson (2020). Migratory zooplankton excreta and its influence on prokaryotic communities. *Frontiers in Marine Science* **7**(1014).10.3389/fmars.2020.573268

- *McDonald, N, E.P. Achterberg, C.A. Carlson, M. Gledhill, S. Liu, J. R. Matheson-Barker, N. B. Nelson, R. J Parsons. (2019) The Role of Heterotrophic Bacteria and Archaea in the Transformation of Terrigenous Dissolved Organic Matter in the Open Ocean. *Frontiers of Marine Science* **6**: 743

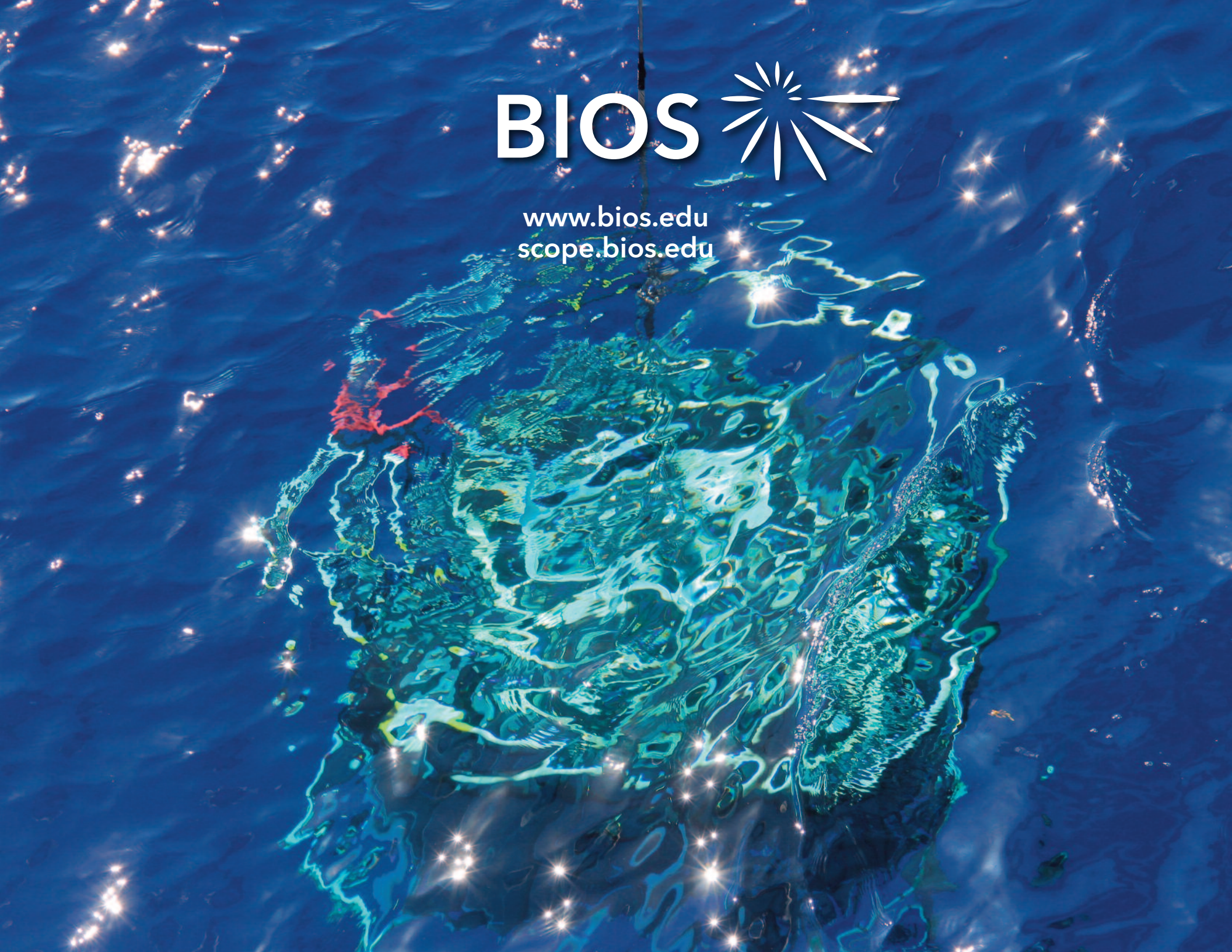


- Roux, S., E. M. Adriaenssens, B. E. Dutilh, E. V. Koonin, A. M. Kropinski, M. Krupovic, J. H. Kuhn, R. Lavigne, J. R. Brister, A. Varsani, C. Amid, R. K. Aziz, S. R. Bordenstein, P. Bork, M. Breitbart, G. R. Cochrane, R. A. Daly, C. Desnues, M. B. Duhaime, J. B. Emerson, F. Enault, J. A. Fuhrman, P. Hingamp, P. Hugenholtz, B. L. Hurwitz, N. N. Ivanova, J. M. Labont., K.-B. Lee, R. R. Malmstrom, M. Martinez-Garcia, I. K. Mizrachi, H. Ogata, D. P.ez-Espino, M.-A. Petit, C. Putonti, T. Rattei, A. Reyes, F. Rodriguez-Valera, K. Rosario, L. Schriml, F. Schulz, G. F. Steward, M. B. Sullivan, S. Sunagawa, C. A. Suttle, B. Temperton, S. G. Tringe, R.V. Thurber, N. S. Webster, K. L. Whiteson, S. W. Wilhelm, K. E. Wommack, T. Woyke, K. C. Wrighton, P. Yilmaz, T. Yoshida, M. J. Young, N. Yutin, L. Z. Allen, N. C. Kyrpides and E. A. Eloe-Fadrosh (2019). Minimum Information about an Uncultivated Virus Genome (MIUViG). *Nature Biotechnology* 37(1): 29-37.10.1038/nbt.4306

- *Saw, J.H.W., T. Nunoura, M. Hirai, Y. Takaki, R.Parsons, M. Michelsen, K. Longnecker, E.B. Kujawinski, R. Stepanauskas, Z. Landry, C.A. Carlson , S.J. Giovannoni. Pangenomics reveal diversification of enzyme families and niche. *mBio* **11**(1): e02975-02919.10.1128/mBio.02975-19

- *Wear, E.K., C.A. Carlson, M. Church. Differential bacterioplankton metabolism of phytoplankton lysates across a cyclone-anticyclone eddy dipole cycling of semi-labile organic matter in the photic zone. *Limnology and Oceanography* 65(7): 1608-1622.10.1002/lno.11409

**Publications previously listed as submitted in 2018-2019 progress report that were published in this reporting period.*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