Report to SFI on BIOS-SCOPE

April 2018

Bermuda Institute of Ocean Sciences

BIOS



BIOS-SCOPE is a five-year multi-institutional research program for the study of microbial oceanography in the northwestern Sargasso Sea. 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.

In 2016 and 2017, the BIOS-SCOPE team participated in four research expeditions. While significant sample and data analyses are still underway, four peer-reviewed scientific papers have already been published. The team assembled in February 2018 for a data workshop and also presented their early findings at multiple international scientific conferences with nine BIOS-SCOPE abstracts accepted for presentation at the international Ocean Science Meetings.

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.

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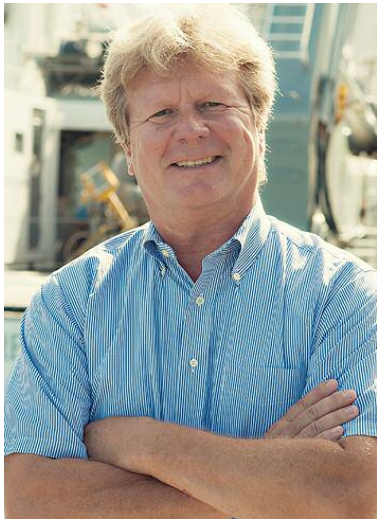
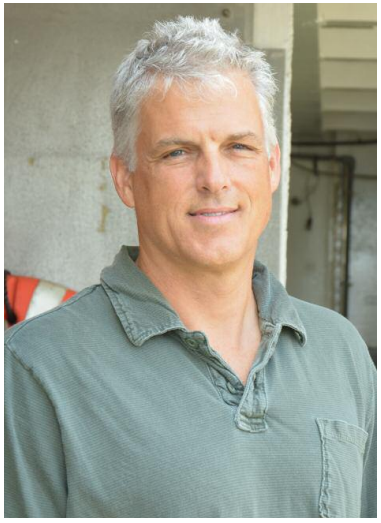
BIOS-SCOPE Team Members



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.

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Leocadio Blanco-Bercial, Investigator
Ruth Curry, Affiliated SFI Investigator
Amy Maas, Investigator
Rachel Parsons, Investigator
Rodney Johnson, Data Processing and Integration

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Stephen Giovannoni, Co-Principal Investigator
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Craig Carlson, Co-Principal Investigator
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Elisa Halewood, Research Support
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University of Exeter

Ben Temperton, Investigator
Joanna Warwick-Dugdale, Doctoral Student

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Woods Hole Oceanographic Institution

Elizabeth Kujawinski, Investigator
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Brittany Widner, Postdoctoral Fellow
Krista Longnecker, Research Support
Gretchen Swarr, Research Support

At-Sea Expeditions
& Research Progress

Fieldwork was the primary focus of activity for 2016-2017, with four at-sea expeditions and associated research projects carried out by BIOS-SCOPE investigators aimed at understanding how marine microbes control the production, removal and transformation of dissolved organic matter (DOM) within a web of ecological interactions in the open ocean waters near Bermuda.

Marine microbes comprise a remarkably diverse community in the ocean and account for more than 90% of the ocean's total biomass. The ecology of marine microbes is dictated by processes that occur on a variety of scales ranging from organism-compound and organism-organism interactions to large biogeochemical patterns on the ecosystem scale. Deciphering these processes requires a broad suite of genomic, ecological, oceanographic, and biogeochemical approaches applied to high-quality, high-resolution observational data acquired in different seasons.

CTD recovery on board the research vessel *Atlantic Explorer*. Photo by Jorge Sanchez.

Two expeditions were specifically funded by the SFI grant to capture the unique microbial communities and water chemistry conditions that develop in the North Atlantic Ocean each summer. Additional at-sea work was accomplished during federally-funded expeditions in the fall and spring, when colder temperatures and winter mixing disrupt microbial communities that prospered in the summer, redistributing dissolved organic matter to deeper waters where it may fuel the development of new microbial communities.

These four research expeditions are providing the crucial at-sea samples and data underpinning the research of the BIOS-SCOPE team:

- July 9-12, 2016
- September 7-9, 2016
- March 30-April 4, 2017
- July 8-12, 2017

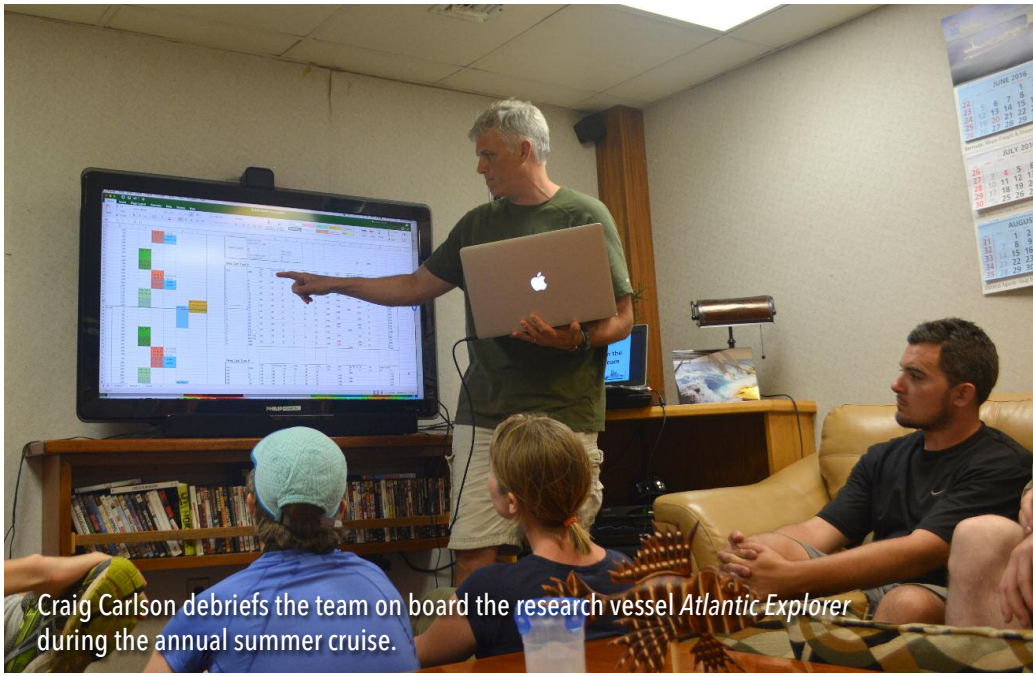
In the following sections, we review the research progress achieved to date on the various microbial interactions operating at the smallest scales to those at the larger scales.

Research Progress on Organism Compound Interactions:

The BIOS-SCOPE team uses a variety of chemical and genetic analyses to identify the species of bacteria that thrive on particular kinds of dissolved organic matter.

At the organism-compound scale, several collaborative experiments were conducted to assess the response of bacterioplankton—the bacterial component of plankton—to various compositions of dissolved organic matter in seawater. A major component of dissolved organic matter in the intermediate and deep ocean is a set of compounds called CRAM, or carboxyl-rich alicyclic matter. The interaction between bacteria and CRAM is being investigated collaboratively by the BIOS-SCOPE team, involving researchers from UCSB, OSU, BIOS, and WHOI.

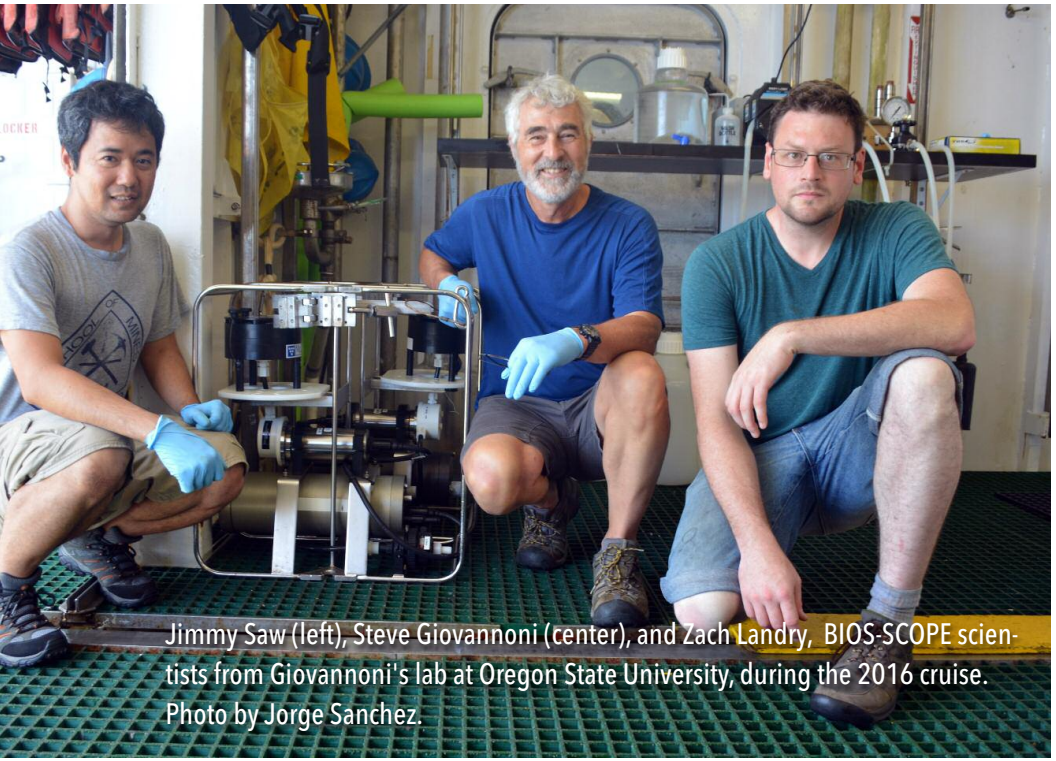
The team from WHOI is developing new high-resolution mass spectrometry methods to identify the chemical signatures of hundreds of unique molecules that make up dissolved organic matter. The WHOI team has also developed a new method to analyze metabolites in seawater. Metabolites are the small biomolecules produced by cells—an end-product of an organism's metabolic activity. The new detection method will facilitate the rapid and efficient analysis of BIOS-SCOPE field samples.



Craig Carlson debriefs the team on board the research vessel *Atlantic Explorer* during the annual summer cruise.

WHOI team leader Elizabeth Kujawinski recently received a new mass spectrometer with funding from The Simons Foundation; approximately 200 field and experimental samples from the BIOS-SCOPE program are now in queue at this facility. These samples represent a remarkable dataset that will allow the team to assess the temporal variability of organic matter.

Concentrations of organic compounds in seawater are a balance between production and consumption by marine microbes. In complementary work, Kujawinski's group identified metabolites that are produced by marine microbes in laboratory culture. During the March 2017 expedition, an experimental protocol



Jimmy Saw (left), Steve Giovannoni (center), and Zach Landry, BIOS-SCOPE scientists from Giovannoni's lab at Oregon State University, during the 2016 cruise. Photo by Jorge Sanchez.

was developed to quantify the loss of select metabolites in seawater. In the coming year, samples will be analyzed to quantify short-term (i.e., less than one day) changes in concentrations of select organic compounds within surface seawater.

High-resolution mass spectrometry was also utilized in combination with controlled incubation experiments to assess the chemical composition of dissolved organic matter released by zooplankton, as well as its influence on microbial communities, in a collaborative effort carried out by BIOS, UCSB and WHOI team members.

Research Progress on Organism Organism Interactions:

As with all living organisms, microbes grow and die but, remarkably, little is known about the processes that regulate mortality. To investigate microbial growth and mortality, the University of Georgia team conducted experiments on bacterioplankton during the July and September 2016 BIOS-SCOPE expeditions to quantify the rates at which bacterioplankton are eaten by other microscopic predators, and how many die from viral infections. The data reveal daily patterns in which bacterioplankton accumulated during the night and were lost from the system during the day. However, these initial data were not acquired over a full 24-hour cycle. To fill this data gap, the experiments were repeated during the July 2017 BIOS-SCOPE expedition; the additional data are currently being analyzed by the University of Georgia, UCSB, OSU, and BIOS teams.

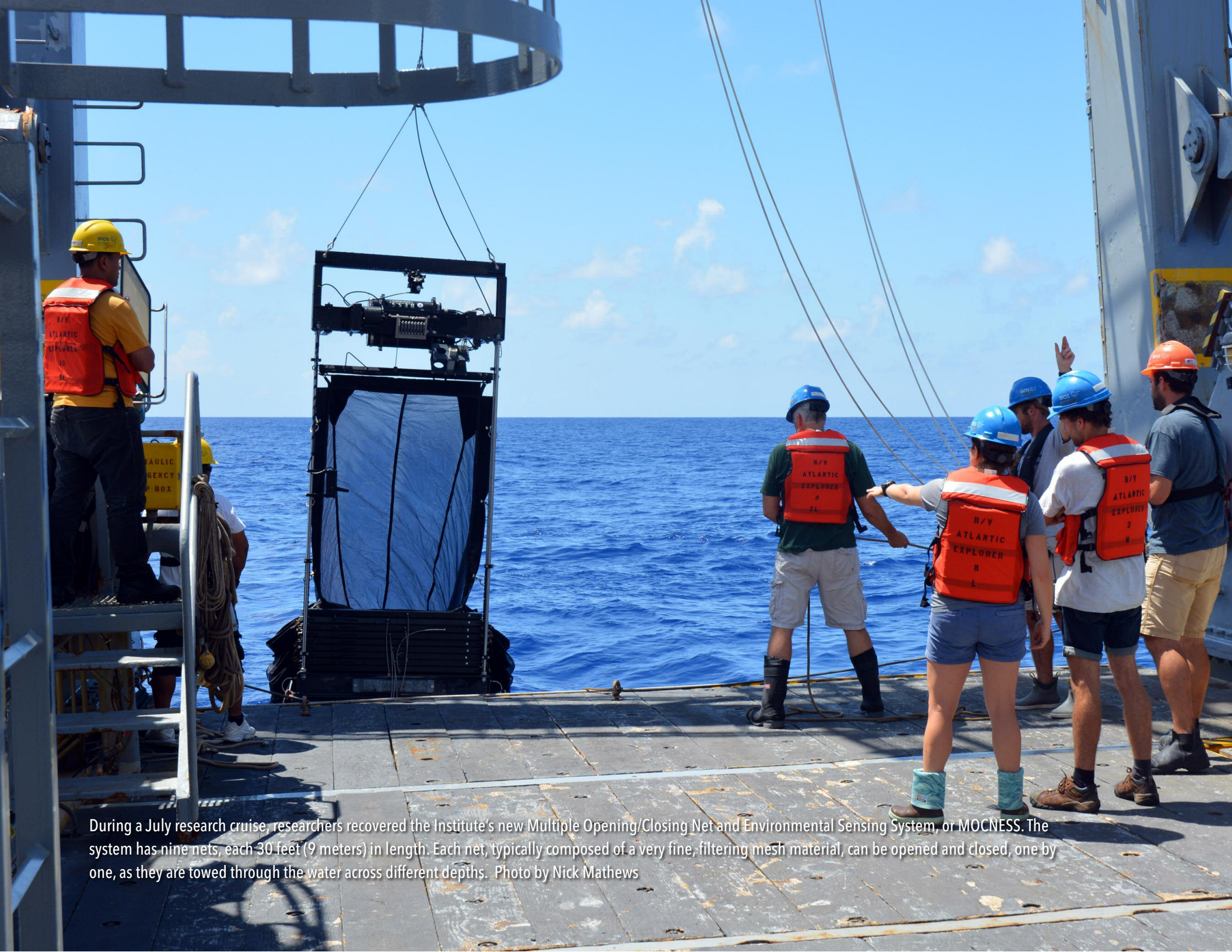
In 1990 BIOS-SCOPE co-principal investigator Stephen Giovannoni discovered a group of bacteria that turned out to be the most abundant organisms in the ocean. Giovannoni named the bacteria "SAR11" – the "SAR" being shorthand for Sargasso Sea, where the analyzed sample was acquired, and the very same location in the North Atlantic that is the focus of the BIOS-SCOPE program. SAR11 is the smallest known free-living cell and also has the smallest genome of any independent cell. Yet, because it is so abundant, it dominates life in the ocean and plays a huge role in the ocean's chemical cycles. Giovannoni's groundbreaking work on SAR11 continues under the BIOS-SCOPE program, with investigations on SAR11 genome microdiversity and its relationship to the oxidation of dissolved organic matter.

The most abundant and diverse bacteria in the dark ocean is SAR202, but many questions remain about the ecological role they play in the larger ocean system. Why are these cells so successful in the deep ocean? As part of the BIOS-SCOPE program, the team from OSU is investigating the genome of SAR202 to better understand how these bacteria oxidize dissolved organic matter in the absence of sunlight. Why are they capable of utilizing organic compounds that microbes in the surface ocean have trouble consuming? The answer may lie in the distinctly different metabolic strategies of microbes found in the surface waters versus the deep ocean. DOM molecules are metabolically expensive to use because they require complex arrays of powerful oxidative enzymes. Predation and competition for scarce nutrients in the surface may raise the cost of living for microbes living there, making it relatively unprofitable for cells to deploy this expensive metabolism. However, after DOM compounds are delivered through vertical mixing to the deeper ocean, where energy is scarce, the cost/benefit ratio changes, favoring SAR202 metabolism. Shipboard incubation experiments (conducted by the UCSB and OSU groups) have revealed that members of SAR202 became enriched upon amendment with DOM compounds.

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. While a bacterial genome can be reassembled from short strands of DNA, viral genomes have more variability and, therefore, require longer strands of DNA to be read by the sequencing device. Ultimately, viral ecologists would like to employ sequencing technology that can analyze the full length of the viral genome in a single read, but such technology does not currently exist.

In what promises to be a significant advancement, BIOS-SCOPE investigator Ben Temperton is developing the use of a long-read sequencing device called the MinION so it can be utilized on-site at BIOS. As part of the July 2017 expedition, the first high-resolution viral samples (obtained throughout the day and night) were obtained and analyzed to provide data on the relative abundances of viral populations and their infected hosts. In conjunction with other BIOS-SCOPE research, these data will provide the first comprehensive view of the role viruses play in shaping microbial communities and the ocean's biogeochemical cycles.

BIOS-SCOPE scientist Ben Temperton loads a DNA sample into the MinION for sequencing. Image courtesy of Ben Temperton



During a July research cruise, researchers recovered the Institute's new Multiple Opening/Closing Net and Environmental Sensing System, or MOCNESS. The system has nine nets, each 30 feet (9 meters) in length. Each net, typically composed of a very fine, filtering mesh material, can be opened and closed, one by one, as they are towed through the water across different depths. Photo by Nick Mathews

Research Progress on the Ecosystem Scale:

Genetic work is also underway on zooplankton, the small marine animals that consume organic matter in the sunlit surface waters at night and release organic and inorganic compounds as they return to deeper waters during the day. This daily migration through the water column shuttles microbes, genes, and nutrients throughout the ocean's depths and influences the community of microbes that live and die there. Populations of zooplankton can travel over 600 vertical meters every day and are thought to have an important effect on the entire ecosystem.

Thanks to a supplemental grant from SFI, BIOS acquired and deployed a special net system during the July 2017 expedition to collect zooplankton at various depths in the ocean. The net system, known as the Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS), has nine individual nets, each 30 feet (9 meters) in length, that can be opened and closed, one by one, at different depths as the system is towed behind the research ship. The deployment of the MOCNESS during the July 2017 expedition was integrated into a comprehensive sampling plan in order to evaluate a full suite of zooplankton-microbe-compound interactions. In addition, the BIOS team is combining a new machine-learning imaging system with metabarcoding analyses to measure the diversity and ecological impact of the zooplankton in their samples.

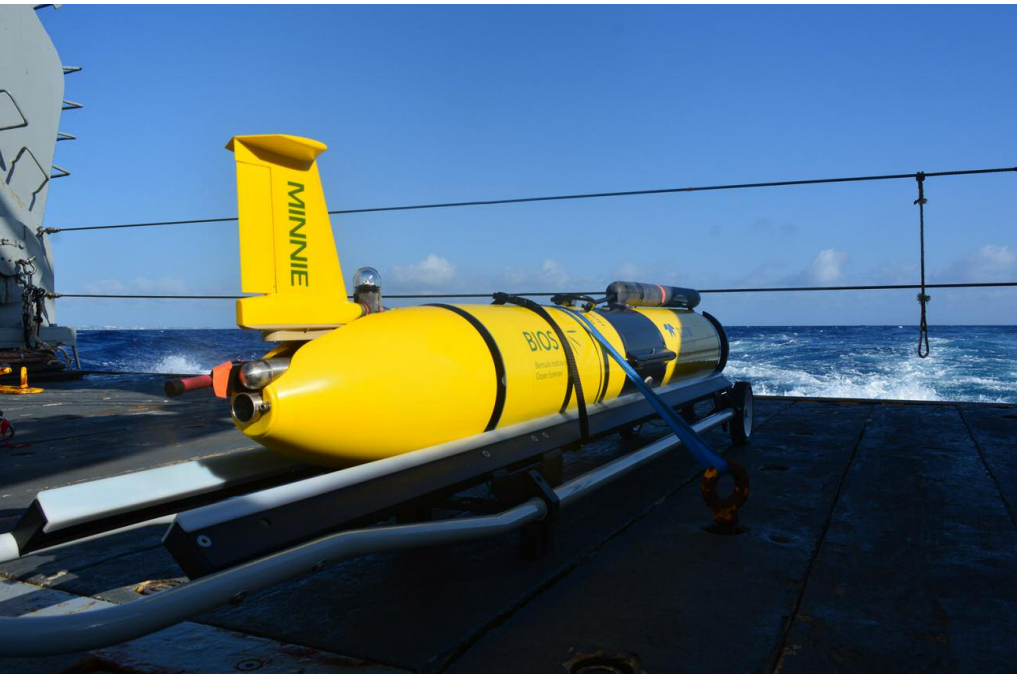
In addition to investigating the processes at work as they ripple up and down the food chain, the BIOS-SCOPE program is also diagnosing processes that operate on both short-term and longer-term time scales.

BIOS-SCOPE was designed to leverage the Bermuda Atlantic Time-series Study (BATS), which provides a monthly backbone of data that is crucial to the BIOS-SCOPE program. The additional ship days funded by SFI for BIOS-SCOPE provided the opportunity for more intensive sampling focused on the microbial ecosystem, as described in this report. The two SFI-funded expeditions were scheduled to take place in summer (July 2016 and July 2017); two additional expeditions, funded by other sources, were scheduled for fall (September 2016) and late winter/early spring (March/April 2017) in order to capture seasonal variations.

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. This redistribution of nutrients sets the stage for an annual burst of photosynthetic activity by phytoplankton known as the "spring phytoplankton bloom." As spring transitions to summer, the upper layers of the ocean are heated by the sun and both predators and prey reorganize

the structure of the various communities of organisms, giving the ocean a different structure that varies more strongly with depth. Concomitant with all of these changes are alterations to the microbial community, which are the primary focus of the BIOS-SCOPE program.

Coordinated field and experimental activities in 2017 provided high temporal resolution of measurements of DOM, nutrients, microbial genetics, organic chemistry and plankton ecology from February through July. BIOS-SCOPE also completed two expeditions at extreme biogeochemical states (i.e., cold deep-mixing in March 2017 and a warm stable period in July 2017). Between these expeditions, BIOS-SCOPE sampling was conducted on monthly BATS cruises. It is anticipated that data collected over the seasonal transition between biogeochemical states will provide detailed information regarding the fate of organic matter exported to intermediate depths, its chemical transformation, and the microbes responsible for its alteration.



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 2017, a major six-month field campaign was conducted from February to July 2017 utilizing all three gliders in BIOS's fleet, including the SFI-funded glider "Minnie." Spanning the winter-to-spring transition and the spring-to-summer transition, the gliders are providing a continuous data set that reveals a detailed picture of ocean physics and biogeochemistry that cannot be obtained from ship-based observations.

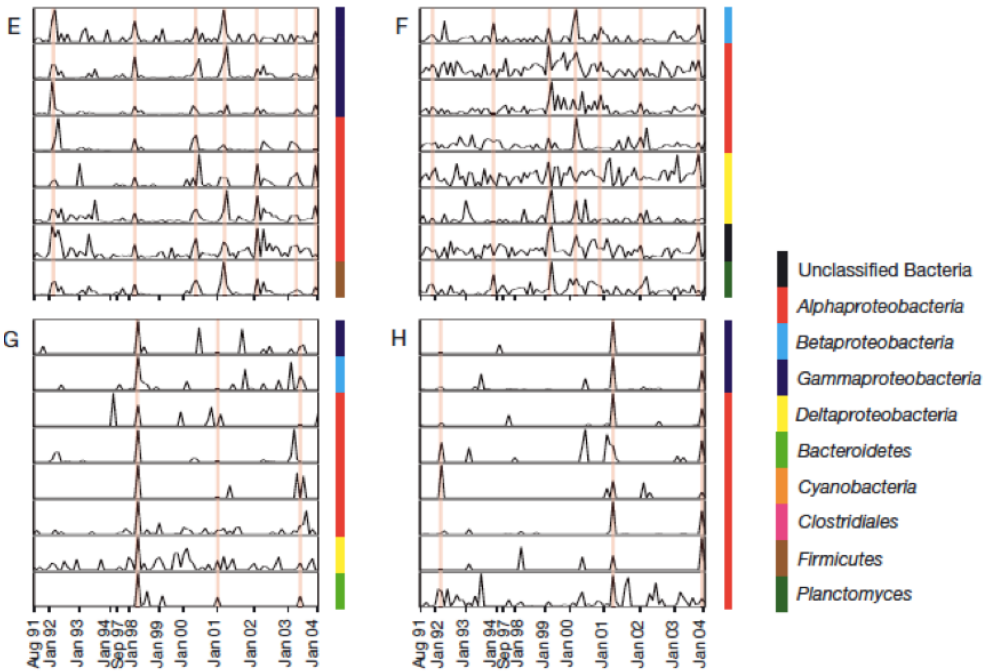
We anticipate that seasonal observations provided by BATS and BIOS-SCOPE, coupled with continuous glider observations, will allow BIOS-SCOPE investigators to gain a more detailed understanding of the fate of dissolved organic matter, its chemical transformation, and the microbial groups responsible for its alteration.

Data Workshop: February 9-10, 2018

Following the intensive 2016 and 2017 field seasons, the BIOS-SCOPE team spent several months processing the observational and experimental data and quickly recognized the need to convene a meeting to discuss data coordination and utilization.

Thanks to supplemental funding from SFI, the team assembled for a data workshop on February 9 and 10, 2018 in Portland, Oregon, just prior to the 2018 Ocean Sciences Meeting held February 11-16. The Ocean Science Meeting is an international gathering of approximately 4,000 ocean scientists and holding the BIOS-SCOPE data workshop in conjunction with this event was a practical option for the BIOS-SCOPE team members.

The format of the data workshop included a series of talks, poster presentations, and breakout discussions, during which the BIOS-SCOPE team further evaluated the data as a group, targeted and outlined emerging manuscripts, identified unknowns and exciting new directions, and designed strategies to address them in future field operations.



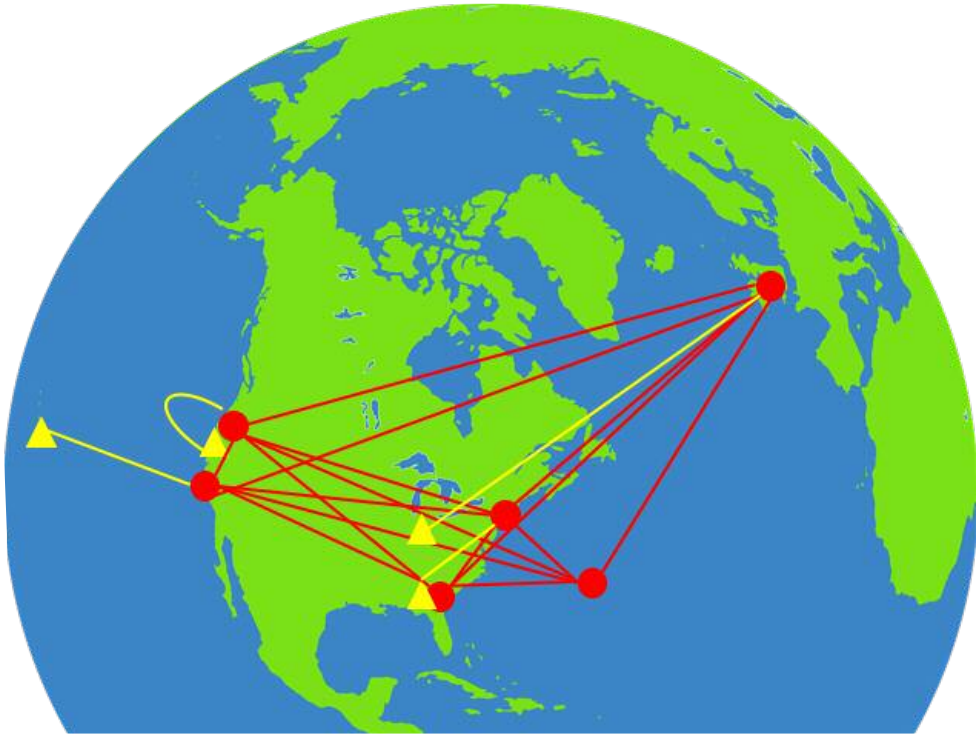
Data such as these, on the relative abundance of various taxonomic units, are among the variety of biological, biogeochemical, and biophysical datasets being analyzed by BIOS-SCOPE researchers.

New Collaborations

At its inception, BIOS-SCOPE was envisioned as a highly collaborative research program with BIOS, UCSB and OSU forming a core team that quickly integrated external investigators from the University of Exeter, University of Georgia, and WHOI into the fold. In 2017, additional collaborations were formed, expanding the academic network even further.

Craig Carlson formed a collaboration with Dave Karl, co-director of the SCOPE program and professor at the University of Hawai`i. Carlson and Karl are working together to analyze the biogeochemical cycling of organic matter at Station ALOHA, the location in the

The BIOS-SCOPE network has expanded beyond 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) (depicted as red circles below) to new academic collaborators at Monterey Bay Aquarium Research Institute (MBARI), The Ohio State University, University of Alabama, and University of Hawai`i (depicted as yellow triangles). Red and yellow lines depict the collaborations between the various teams as described in the text.



North Pacific Ocean that is the focus of the SCOPE program. Carlson is also collaborating with Matthew Church, a SCOPE investigator, on DOM bioavailability at Station ALOHA. These analyses will lay the groundwork for comparative studies between the ALOHA and BATS sites and is likely the beginning of future collaborations between SCOPE and BIOS-SCOPE investigators.

Stephen Giovannoni is collaborating with Alex Worden of the Monterey Bay Aquarium and Research Institute (MBARI) on an NSF-funded project to investigate the interactions between plankton cells and vitamin molecules. Giovannoni is also collaborating with statistician Tom Sharpton, also a professor at OSU, developing a new cell-culturing technology and applying this technology to deep-ocean bacteria. Both of these projects utilize BIOS-SCOPE sampling opportunities and data.

Elizabeth Kujawinski has been hosting Yuehan Lu of the University of Alabama for a sabbatical at WHOI. Together, they have been working to characterize the transformation of organic matter based on the BIOS-SCOPE data and the program's collaborative microbial growth experiments.

Ben Temperton has started a collaboration with Matthew Sullivan of The Ohio State University to develop a new molecular approach for evaluating host-virus interactions in microbial communities. A member of Sullivan's research group participated in one of the 2017 BIOS-SCOPE field expeditions.

Scientific Publications

Four papers have been published to date in peer-reviewed scientific publications:

- Landry Z., Swan B.K., Herndl, G.J., Stepanauskas, R., Giovannoni, S.J., 2017. SAR202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. *mBio* 8:e00413-17. https://doi.org/10.1128/mBio.00413-17.
- Vergin, K.L., Jhirad, N., Dodge, J., Carlson, C.A. and Giovannoni, S.J., 2017. Marine bacterioplankton consortia follow deterministic, non-neutral community assembly rules. *Aquatic Microbial Ecology*, 7.
- James, A.K., Passow, U., Brzezinski, M.A., Parsons, R.J., Trapani, J.N. and Carlson, C.A., 2017. Elevated pCO2 enhances bacterioplankton removal of organic carbon. *PloS One*, 12(3), p.e0173145.
- Johnson, W.M., Kido Soule, M.C. and Kujawinski, E.B., 2017. Interpreting the impact of matrix on extraction efficiency and instrument response in a targeted metabolomics method. *Limnology and Oceanography*: Methods 15: 417-428. http://dx.doi.org/10.1002/lom3.10181

Scientific Presentations

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.

1. Desmarais, M., Parsons, R., Johnson, E., Carlson, C.A., McLeod, K., Stevens, S. Distribution of bacterioplankton lineages within the twilight zone of the Sargasso Sea and their potential role in the biogeochemical cycle.
2. Kido Soule, M., Longnecker, K., Swarr, G.J., Fiore, C.L., Becker, J.W., Braakman, R., Dooley, K., Coe, A., Parsons, R., Chrisholm, S.W., Carlson, C.A., Kujawinski, E. Using metabolomics to characterize marine organic matter cycling by microbes.
3. Kujawinski, E., Carlson, C.A., Kido Soule, M.C., Longnecker, K., Parsons, R. Temporal dynamics of dissolved metabolites in the oligotrophic Atlantic Ocean.
4. Liu, S., Carlson, C.A., Parsons, R., Opalk, K., Huynh, N., Giovanonni, S., Bolanos, L., Kujawinski, E., Lu, Y., Longnecker, K., Landry, Z., English, C. Carboyl-rich alicyclic molecules (CRAM) proxy compounds select bacteria for oxidation of recalcitrant DOM in the mesopelagic Sargasso Sea.
5. Mass, A.E., Carlson, C.A., Kujawinski, E., Liu, S., Parsons, R., Widner, B. Dissolved organic matter composition of migratory zoo plankton excreta and its influences on prokaryotic communities.

Joanna Warwick-Dugdale, Doctoral Student at University of Exeter, on the R/V *Atlantic Explorer*.

Amy Maas, Investigator, in her lab at BIOS.

Shuting Liu, Postdoctoral Fellow, and Craig Carlson in the wet lab on board the R/V *Atlantic Explorer*.

Scientific Presentations, continued

6. Saw, J., Landry, Z., Nunoura, T., Stepanauskas, R., Carlson, C.A., Giovannoni, S. Diversity and abundance of flavin-dependent monooxygenases in SAR202 clade bacteria revealed by single-cell genomics and metagenomics.
7. Stewart, L.M., Mass, A.E., Blanco-Bercial, L. Beyond biodiversity: metabarcoding as a tool of ecological exploration.
8. Temperton, B., Warwick-Dugdale, J., Moore, K., Solonenko, N., Roux, S., Sullivan, M. VirION: toward long read marine viromics with the MinION sequencer.
9. Warwick-Dugdale, J., Solonenko, N., Moore, K., Chittick, L., Allen, M., Sullivan, M., Temperton, B. 'Loaves and Fishes' for marine virology: feeding >1000 nanopores with viral DNA.

Additional scientific presentations include:

1. Maas, A.E. What is the role of zooplankton on the midwater ecology in the Sargasso? Using new technologies and collaborations to answer old questions. National Academies Keck Futures Initiative Conference, Discovering the Deep Blue Sea, November 9-12, 2016, Irvine, CA.
2. Bittar, T., Bulski, K., Parson, R., Giovannoni, S., Carlson, C., and Harvey, E. Growth, grazing and virus-induced mortality of bacterioplankton in the Sargassa Sea. ASLO 2017 Aquatic Sciences Meeting, February 26 – March 3, 2017, Honolulu, HI.

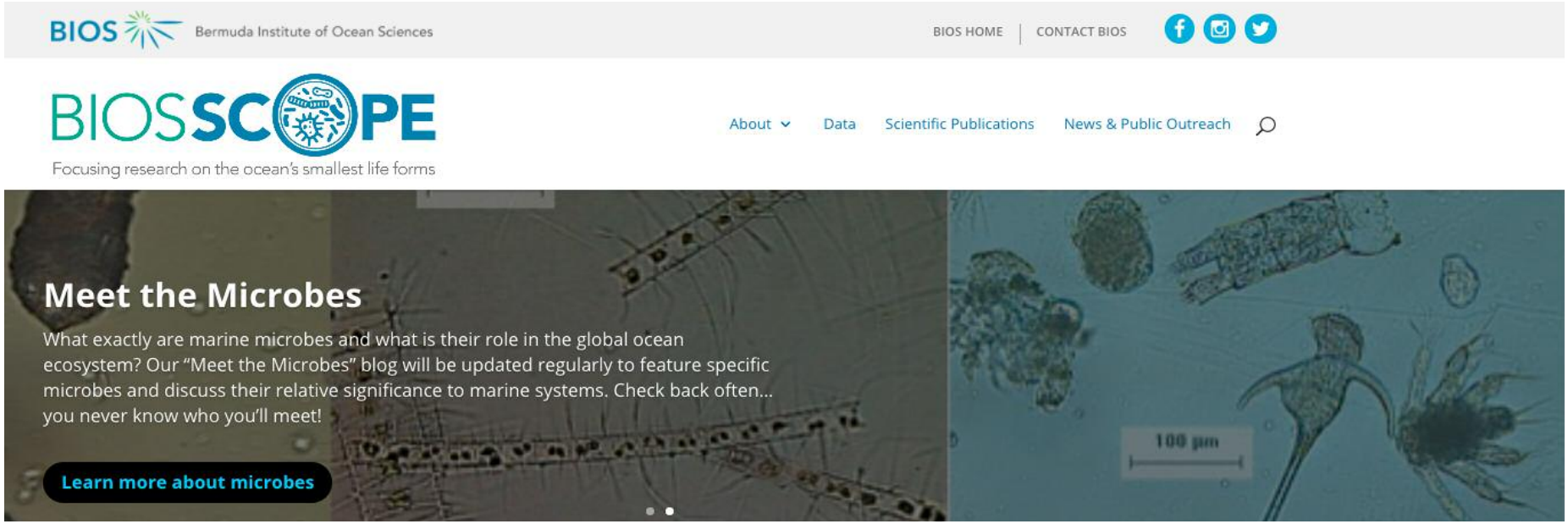
Website and General-Audience Publications

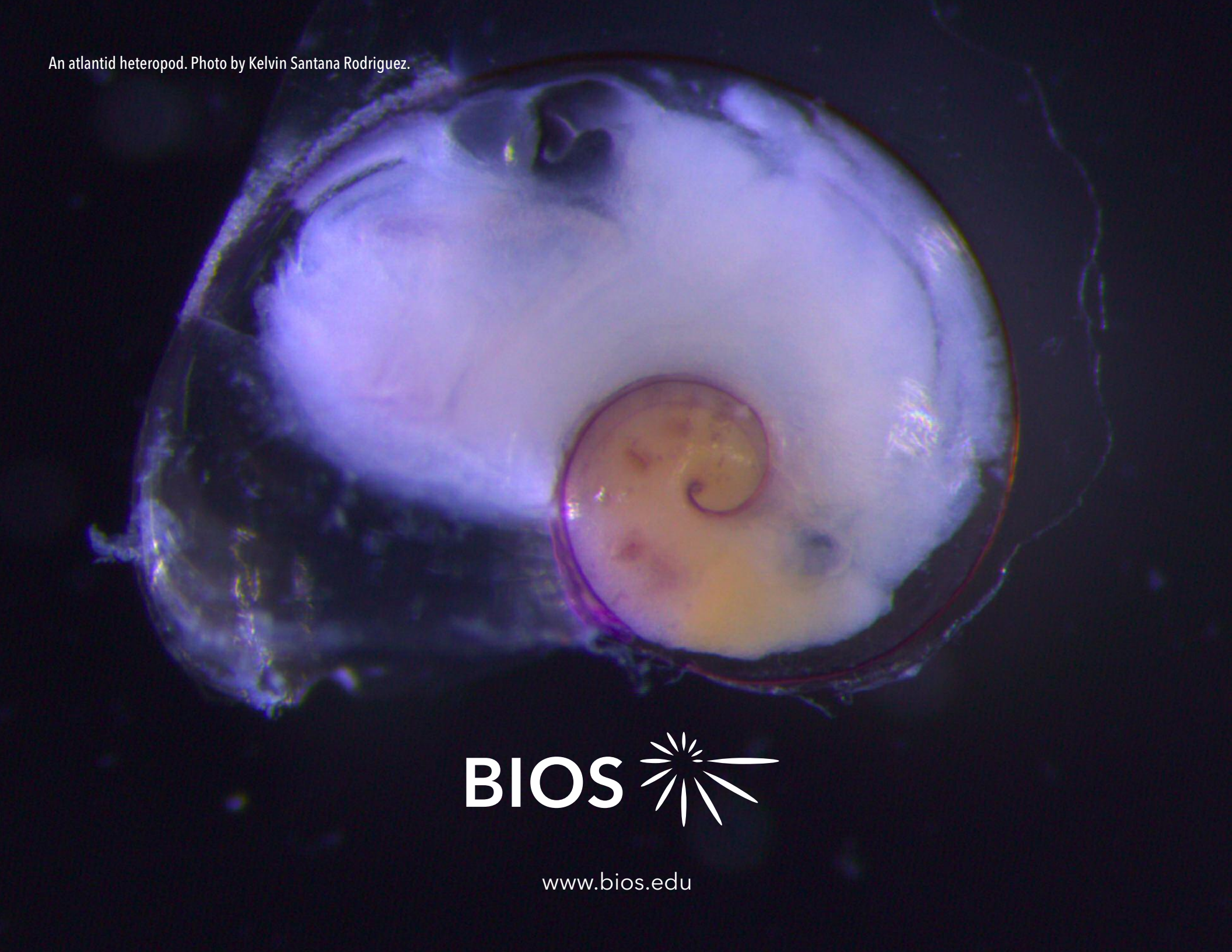
The BIOS-SCOPE program has a dedicated website that is being used for both scientific exchange and public outreach. Please visit http://scope.bios.edu

In conjunction with the development of the website, a unique logo and emblem for the BIOS-SCOPE were professionally designed and are being utilized by all investigators in their scientific presentations.

In an effort to share the importance of BIOS-SCOPE with the general public, four articles have been published to date in BIOS's online magazine *Currents*:

- A "Microbial Whodunit", July 2016. http://www.bios.edu/currents/a-microbial-whodunit/
- New Insights Bloom from BIOS-SCOPE's First Year of Data, August 2017. http://www.bios.edu/currents/new-insights-bloom-from-bios-scopes-first-year-of-data/
- A Special Net for Special Organisms, September 2017. http://www.bios.edu/currents/a-special-net-for-special-organisms/
- Predators and Puppeteers, September 2017. http://www.bios.edu/currents/predators-and-puppeteers





An atlantid heteropod. Photo by Kelvin Santana Rodriguez.

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