Rachel Parsons Intern Projects

2-4 student interns during the summer and 2-4 student interns in the fall on the following projects:

Project One

Project title: Marine bacterioplankton succession and subsequent overturn within seasonally hypoxic waters of a subtropical sound; Devil's Hole, Bermuda.

Programs: Bermuda Program, CABIOS, U.K. Associates of BIOS, Lehigh University Iacocca International Internship Program at BIOS, Princeton-BIOS Internship, Research Experiences for Undergraduates (REU), Fall Semester.

Collaborators: Dr. Nicholas Bates and Rebecca Garley.

Plan: Two students to cover the period of intense sampling (May through October) as the anoxic zone at Devil's Hole forms, changes, and is over turned.

Project summary: Oceanic oxygen minimum zones (OMZs) are expanding, especially in the tropical oceans. An oxygen minimum zone forms below the mixed layer depth as a result of net respiration by bacteria and other heterotrophs. The dominant bacterioplankton within OMZs were identified by small-subunit ribosomal RNA (SSU rRNA) gene surveys and identified as the SUP05-Artic96BD-19 group, the SAR11 and SAR324 clusters and marine group A (MGA). Devil's Hole is a submerged sinkhole in Harrington Sound, Bermuda that undergoes seasonal stratification and oxygen limitation from eutrophication. Devil's Hole's provides an ideal natural laboratory to study microbial lineages that play an essential role mediating sulfur, nitrogen, and carbon cycles. This project intends to determine the contribution of specific bacterioplankton lineages within Devil's Hole including SAR11, the SUP05 group, SAR324 cluster, *Thaumarchaea*, *Euryarchaea* and SAR202. The project will look at the creation of the anoxic layer and the subsequent overturn in 2017. Specific lineages will be quantified using fluorescent in situ hybridization (FISH) and catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH). The project also intends to analyze bacterial and archaeal 16s DNA sequences from 2016 and 2017 and determine the main lineages involved in carbon and nitrogen cycling. The resulting data will be put into the context of carbon and nitrogen chemistry at the site and then compared to past data sets in order to determine the spatial and temporal changes within this local OMZ.

Academic requirements: Undergraduate senior or junior in microbiology preferred. Courses in chemistry, biochemistry, microbiology, and microbial ecology.

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17 Biological Station, St. George's GE 01, Bermuda
T 441 297 1880 F 441 297 8143

Skills required: Working knowledge of microscopy and molecular techniques. Previous experience in epiflourescent microscopy and general molecular skills are an advantage. Working knowledge of Excel. Ability to follow protocols and work independently. Good attention to detail. Data Analysis. Field experience.

Skills learned: Microscopy analyses such as FISH and CARD-FISH. Computer programs including Advanced Excel, Image Pro Plus, PAST, Ocean Data View and the R programming language. Field sampling and scientific record keeping.

Additional information: The student will learn microscopy analyses such as FISH and CARDFISH and the image analysis protocols associated with this particular protocol. The student will gain field cruise experience and may be able to work with collaborators on the chemical analyses associated with the project. Since there are many different aspects and protocols involved with this project, it will be focused to the student's particular skill set and interests.

Project Two

Project title: The Spatiotemporal Distributions of SAR202 clades in the Sargasso Sea.

Programs: BIOS-SCOPE, CABIOS, U.K. Associates of BIOS, Lehigh University Iacocca International Internship Program at BIOS, Research Experiences for Undergraduates (REU).

Period: June through December.

Collaborators: Dr. Stephen Giovannoni (OSU) and Dr. Craig Carlson (UCSB).

Plan: One student to analyze samples from Bloom season in 2017.

Project summary: The oceanic carbon cycle is linked to the microbiology that both produce and consume the diverse carbon compounds that make up dissolved organic matter (DOM). SAR202 are ancient organisms that oxidize recalcitrant carbon. SAR202 are the most abundant and diverse bacteria in the dark ocean. Past research that measured SAR202 abundance in the ocean used probes that hybridize to the entire SAR202 clade and not to the diverse subgroups of SAR202. These subgroups are so diverse that they form class-level divergences. Recent BIOS-SCOPE research has shown that the SAR202 subgroups have different gene content and probably specialize in the oxidation of different recalcitrant compounds. Spatiotemporal variation has never been demonstrated among the SAR202 subgroups and few studies have looked at prokaryotic lineages at depths greater than 300 m. This study will quantify relative abundances of the SAR202 subgroups in depths ranging from 0-4000m using newly designed specific ribosomal RNA probes that target the specific subgroups. The study will focus on clades I, II and III and will use fluorescent in situ hybridization (FISH) methods that will be optimized to detect the subgroups. The resulting data will be investigated to identify vertical and temporal trends and correlations with variables such as dissolved organic carbon composition.

Academic requirements: Undergraduate senior or junior in microbiology preferred. Courses in

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chemistry, biochemistry, microbiology and microbial ecology.

Skills required: Working knowledge of microscopy, phylogeny and molecular techniques. Previous experience in epiflourescent microscopy and general molecular skills an advantage. Working knowledge of Excel. Ability to follow protocols and work independently. Good attention to detail. Data Analysis. Field experience.

Skills learned: Microscopy analyses such as FISH and CARD-FISH. Computer programs including Advanced Excel, Image Pro Plus, PAST, Ocean Data View and the R programming language. Ship board sampling and scientific record keeping.

Additional information: The student will learn microscopy analyses such as FISH and CARDFISH and the image analysis protocols associated with this particular protocol. The student may gain valuable research cruise experience provided that the timing works – BIOS-SCOPE Cruise in early July. The student may be able to work with collaborators on the chemical analyses and genomics analyses associated with the project.

Project Three

Project title: The microbial lineages associated with aggregate formation in the Sargasso Sea.

Programs: BATS, CABIOS, U.K. Associates of BIOS, Research Experiences for Undergraduates (REU).

Period: May to August.

Collaborators: Bianca Cruz (ASU) and Dr. Susanne Neuer (ASU).

Plan: One student to assist with microscopy protocols.

Project summary: This project focuses on the biological carbon pump, its relationship to plankton community composition, trophic links and surface productivity using DNA-based molecular techniques to help decipher the contributors to particle flux in the Sargasso Sea. In particular, how aggregates form in the smallest of the plankton organisms, the picoplankton, which includes the abundant cyanobacteria, *Synechococcus* and *Prochlorococcus.* Students will work with collaborators assisting with aggregate experiments and then focusing on determining which lineages are present in these aggregates using microscopy and molecular techniques including FISH, CARD-FISH and DNA extraction and sequencing.

Academic requirements: Undergraduate senior or junior in microbiology or chemistry preferred. Courses in chemistry, biochemistry, oceanography, microbiology and microbial ecology.

Skills required: Working knowledge of microscopy, phylogeny and molecular techniques. Previous experience in epiflourescent microscopy and general molecular skills an advantage. Working knowledge of Excel. Ability to follow protocols and work independently. Good attention

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to detail. Data Analysis. Field experience.

Skills learned: Experimental design. Microscopy analyses such as FISH and CARD-FISH. Computer Programs including Advanced Excel, Image Pro Plus, PAST and the R programming language.

Additional information: The student will learn microscopy analyses such as FISH and CARDFISH and the image analysis protocols associated with this particular protocol. The student may be able to gain research cruise experience and work with collaborators on experimental design and sequencing data associated with this project. The research cruises are planned for late May and early June.

Project Four

Project title: The microbiome of the macroalgae *Sargassum spp*.

Programs: Bermuda Program, CABIOS, U.K. Associates of BIOS, Lehigh University Iacocca International Internship Program at BIOS, Research Experiences for Undergraduates (REU), Fall Semester.

Period: June to August.

Collaborators: Dr. David Valentine (UCSB) and Dani Cox (UCSB).

Plan: One student to assist with microscopy and molecular protocols.

Project summary: Pelagic *Sargassum* are an ecologically important macroalgae that contains both macrobiota and microbial epiphytes. *Sargassum* thrives in the nutrient depleted conditions in the Sargasso Sea where dissolved phosphorus and nitrogen occur in very low concentrations. Nitrogen-fixing bacteria living on the *Sargassum* have been known to cleave the C-P of Methylphosphonate which is abundant in the Sargasso Sea. This study will investigate the *Sargassum* microbiome and specifically how methylphosphonate additions could promote growth of bacteria known to cleave the C-P bond. This process would provide phosphate to both the microbiome and *Sargassum*. Students will work with collaborators assisting with *Sargassum* experiments and then focusing on determining which lineages are present in the microbiome using microscopy and molecular techniques including FISH, CARD-FISH and DNA extraction and sequencing. Understanding this complex microbiome of *Sargassum* can help further knowledge of its crucial role in the Sargasso Sea's ecosystem.

Academic requirements: Undergraduate senior or junior in environmental sciences. Courses in chemistry, biochemistry, microbiology and microbial ecology would be useful.

Skills required: Working knowledge of microscopy, phylogeny and molecular techniques. Previous experience in epiflourescent microscopy and general molecular skills an advantage. Working knowledge of Excel. Ability to follow protocols and work independently. Good attention to detail. Strong organization skills. Some Field experience.

Bermuda Institute of Ocean Sciences
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Skills learned: Experimental design. Microscopy analyses such as FISH and CARD-FISH. Computer Programs including Advanced Excel, Image Pro Plus, PAST and the R programming language. Data Analysis and Statistics.

Additional information: The student will learn microscopy analyses such as FISH and CARDFISH and the image analysis protocols associated with this particular protocol. The student may be able to work with collaborators on experimental design and sequencing data associated with this project.

Project Five

Project title: Developing protocols for the Olympus IX83 inverted microscope.

Programs: CABIOS, U.K. Associates of BIOS.

Period: April to December.

Collaborators: Dr. Amy Maas (BIOS).

Plan: One student to develop microscopy protocols.

Project summary: The new inverted microscope at BIOS is capable of visualizing changes in organism development, calcification, and cell enumeration, and would greatly enhance the research capabilities of the institute. The new microscope provides the functionality to reduce the expected increase in user demand for the aging AX-70 microscope while substantially expanding the scope of the microscopy facilities to allow for a larger number of simultaneous filter cubes, the capacity to do live cell and larger organism imaging with lower intensity fluorescent signals and the ability to repeatedly measure growth and calcification of organisms on a range of slides, plates, and dishes. The student would be responsible for learning the cellSens software and moving current protocols to this new inverted microscope in order to expand the microscopy capabilities of BIOS.

Academic requirements: Masters student in microbiology or ecology.

Skills required: Experience in epiflourescent microscopy. Ability to learn new software and develop microscopy protocols. Ability to follow protocols and work independently. Good attention to detail. Strong organization skills.

Skills learned: Microscopy analyses such as FISH and CARD-FISH. Computer Programs including Advanced Excel, Image Pro Plus and cellSens. Data Analysis and Statistics.

Additional information: The student will learn microscopy analyses such as FISH and CARDFISH and the image analysis protocols associated with this particular protocol. The student will need to transfer protocols from Image Pro Plus to cellSense software and develop microscopy protocols for other scientists at BIOS should the need arise.

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Project Six

Project title: Growth, grazing, and virus-induced mortality of specific bacterioplankton lineages in the Sargasso Sea.

Programs: CABIOS, U.K. Associates of BIOS, Research Experiences for Undergraduates (REU).

Period: July to December.

Collaborators: Elizabeth Harvey (Skidaway).

Plan: Returning Masters student Zenaida Stead will have priority to complete this project in 2019. However, a second student would assist and help finish analyses for publication.

Project summary: Growth and mortality rates play a major role in microbial population dynamics, consequently affecting productivity and biogeochemical cycling in the ocean. Yet, little is known about regulation of mortality in marine bacterioplankton. This study will investigate bacterioplankton growth and mortality rates due to grazing and viral infection in the Sargasso Sea, by combining traditional dilution experiments, microscopy protocols using DNA-specific staining, and high-throughput flow cytometry cell counts, as part of the collaborative BIOS-SCOPE project. Dilution experiments will be performed with water collected in the day time, and at night. Incubations will be carried out for 24h using whole (undiluted) seawater and seawater that was 5-fold diluted either with grazer-free diluent water (0.22 µm filtered seawater) or with grazer- and virus-free diluent water (30 KDa filtered seawater). Growth and mortality rates will be determined using direct counts of bacterioplankton using both microscopy and flow cytometry. In addition, specific lineages will be quantified using fluorescent in situ hybridization (FISH) and catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH). Lineages of interest will be the bacteria, SAR11, SAR202, SAR324, *Rhodobacteracea* and *Alteromonas* as well as the archaea, *Euryarcheota* and *Thaumarcheota*.

Academic requirements: Undergraduate senior in microbiology and/or oceanography preferred. Courses in chemistry, biochemistry, microbiology and biological oceanography.

Skills required: Working knowledge of microscopy, flow cytometry and molecular techniques. Previous experience in epiflourescent microscopy an advantage. Working knowledge of Excel. Ability to follow protocols and work independently. Good attention to detail. Data Analysis. Field experience.

Skills learned: Microscopy analyses such as FISH and CARD-FISH. Computer Programs including Advanced Excel, Image Pro plus, PAST, Ocean Data View and the R programming language. Ship board sampling.

Additional information: The student will learn microscopy analyses such as FISH and CARDFISH and the image analysis protocols associated with this particular protocol. The student may gain valuable research cruise experience provided that the timing works – BIOS-SCOPE Cruise in early July.