Nereocystis luetkeana geographic patterns in physiology and microbiome from samples collected from 2019 to 2022 at nine sites spanning more than 200 kilometers in Washington state

Website: https://www.bco-dmo.org/dataset/1000920
Data Type: Other Field Results
Version: 1
Version Date: 2026-06-17

Project
» The role of microbes in driving productivity and carbon fixation in seaweeds (kelp and their microbiome)

Contributors	Affiliation	Role
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Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Canopy kelp are foundational species in coastal ecosystems and host diverse bacterial communities. We used these data to test the association between bull kelp (Nereocystis luetkeana) host traits, blade-associated bacterial taxa and seawater environmental features across nine sites spanning more than 200 kilometers in Washington state. The data collection spans the outer coast site Tatoosh Island, at the entrance of the Strait of Juan de Fuca. Data collection extended eastward to include sites within the Strait of Juan de Fuca and central and southern Puget Sound. Kelp traits and seawater features that were measured are contained within this dataset and include carbon fixation and DOC release, morphometrics of kelp, and nutrient content. Seawater features that were measured were nutrients, sea surface temperature, and salinity in the surrounding water column. Microbial community data based on 16S rRNA amplicon sequencing of kelp blades across all sites are archived at the National Center for Biotechnology Information (NCBI) under BioProject number PRJNA1301461. Bacterial taxa showed differentiation among sites, and blade-associated bacterial densities were higher at the outer coast site compared with the most inland site. Yet, 11 bacterial genera were present in at least 80% of the samples; these taxa probably serve as core members of the N. luetkeana microbiome and show both positive and negative correlations with host health and environmental features. The data indicated strong interrelationships between kelp traits, seawater features and bacterial community composition with implications for the health of this highly productive foundational species in coastal ecosystems. This dataset supports the 2025 Pfister, et al. paper published in Royal Society Open Science (DOI: 10.1098/rsos.250637).

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Coverage

Location: Washington state coastal waters
Spatial Extent: **N**:47.166522 **E**:-122.39833 **S**:47.166522 **W**:-124.73382
Temporal Extent: 2019-07-02 - 2022-08-14

Methods & Sampling

These are data on kelp traits, seawater features and 16S rRNA amplicon sequencing. All kelp measurements were

made in situ, often via kayak or at the shore using a measuring tape and calipers.

Kelp tissue was collected for elemental analysis by excising a 2 x 2 centimeter (cm) piece of tissue in the meristematic region. Tissue was ground to a fine powder in a GenoGrinder Spex (Metuchen, NJ), weighed in aliquots of 1.2 to 1.5 milligrams (mg) and packed into 3.5 × 5 millimeter (mm) aluminium tins (Costech, Valencia, CA). Packed tins were analysed on an elemental analyser isotope ratio mass spectrometer at Northwestern University Stable Isotope Biogeochemistry Laboratory (NUSIBL).

Carbon fixation was quantified using a section of the youngest, basal frond tissue extending 24 to 30 cm from the bulb. It was weighed (wet mass in grams (g)) with a Pesola scale and placed in a 1 liter (L) Nalgene container; the change in oxygen was measured for 1 hour with a Pyro Science contactless fibre-optic sensors (Oxygen Sensor Spots OXSP5) and an optical oxygen and temperature meter (FireStingO2 FSO2-4), with the sensor spots affixed inside each 1 L chamber.

DOC release and nutrient changes were quantified from seawater filtered through a GF/F filter and immediately frozen for analysis at the University of Washington Marine Chemistry Laboratory (methods from UNESCO), with DOC samples frozen in 40 milliliter (mL) glass vials with Teflon caps (Shimadzu, VOA) and other nutrients in acid-washed 60 mL high density polyethylene (HDPE) Nalgene bottles.

Using pulse amplitude modulated (PAM) fluorometry *in situ* (Diving Pam, Walz, Germany) we estimated fluorescence over a range of irradiances. PAM is a non-invasive, *in situ* method that quantifies Photosystem II fluorescence parameters across different light levels. Tissue was dark-adapted for 20 minutes and then rapid light curves were estimated with nine PAR levels from 0 to 1200. We estimated the maximum electron transport rate (ETR_{max}) and the photosynthetic efficiency at low light, both as the initial linear slope of ETR to irradiance (α) and as the quantum yield of photosystem II (Yield) on nine individual *Nereocystis* at both Tatoosh Island and Squaxin Island, the populations that span the greatest range of environmental conditions. Tatoosh Island was assayed on 9 and 20 June 2019 and Squaxin Island on 2 July 2019. At both sites, we selected individuals at least 1 meter (m) apart at low tide.

The salinity and seawater temperatures were assessed with a Castaway-CTD (Sontek) from a boat or kayak at all sites except Tatoosh Island where instrumentation is moored (Hach DS5).

Microbial density on individual kelp blades at either end of the geographic distribution, Tatoosh Island versus Squaxin Island, differed using 4,6-diamidino-2-phenylindole (DAPI) fluorescent staining of bacterial cells. We quantified bacterial cells from a section of the blade at approximately 20 cm distal from where the blade articulates with the bulb. We sampled a 3−5 mm slice from 10 individuals at each site (Tatoosh on 4 August 2019, Squaxin on 2 July 2019), preserving each slice immediately in Formalin, transferring to 50 : 50 EtOH : phosphate-buffered saline (PBS) after 1 hour, then freezing. In the laboratory, each piece was again sliced with a razor blade to approximately 0.5 mm, placed on a glass slide, and a solution of 1 microgram (µg) DAPI : 1 mL PBS was dropped on the slide-mounted slice. The slide was kept in dark for 20 minutes, chilled and rinsed with PBS prior to visualizing at 100× with an Olympus BX50 fluorescence microscope with a DAPI filter. We photographed 10 fields of view, selected haphazardly, for each individual slice, resulting in 100 images at each Tatoosh and Squaxin. In samples where bacteria were sparse or non-existent, we repeatedly adjusted the fine focus to verify no bacteria were present. We quantified the bacteria in ImageJ, enumerating the cells in the blue channel, adjusting the background and thresholds identically for all 200 images to eliminate autofluorescence by kelp cells. We calculated bacterial cell density as a percentage of the total imaged area.

Individual kelp was sampled for microbes by rubbing a cotton swab for 20−30 seconds over the mid-blade region. The swab was placed in a 2 mL Eppendorf tube, immediately chilled and transferred to a freezer within 4−6 hours. DNA from the swabs was extracted with a Qiagen DNeasy PowerSoil Kit (Qiagen). DNA was amplified, sequenced and amplicon sequence variants (ASVs) were identified by the Duchossois Family Institute Microbial Metagenomics Facility (DFIMMF) at The University of Chicago. The V4–V5 region within the 16S ribosomal RNA (rRNA) gene was amplified using universal bacterial primers and polymerase chain reaction (PCR) conditions described in Younker, et al. (2024). Approximately 412 bp region amplicons were then purified using a spin column-based method (Minelute, Qiagen), quantified and dual index adapters were ligated. Sequences were generated from the Illumina MiSeq platform using the QIASeq 1-step amplicon kit (Qiagen) for generating libraries and using 2 × 250 paired end reads with 5000 to 10,000 reads per sample. The default pipeline for processing MiSeq 16S rRNA reads was dada2 (v. 1.18.0) with minor modifications in R (v. 4.0.3). Reads were first trimmed at 190 bp for both forward and reverse reads to remove low-quality nucleotides, and chimaeras were detected and removed using the default consensus method in the dada2 pipeline. Then, ASVs with length between 320 and 365 bp were retained. Taxonomy of the resultant ASVs were assigned to the genus level using the RDP classifier (v. 2.13) with a minimum bootstrap confidence score of 80. Species-level classification used blastn (v. 2.13.0) and the refseq_rna database. We analysed ASVs with R, phyloseq and microViz (R version 2023.06.2+561). Chloroplast DNA that was not classified as Cyanobacteria were generally diatoms (Bacillarophyta) and were filtered out, as well as any other chloroplast sequence that did not match to the class Cyanobacteria. Sequences

of corn (Zea, in Plantae), in low numbers at some Puget Sound sites, were also removed.

All methods are described in the Pfister, et al. (2025) paper.

Data Processing Description

Basic statistical analysis was done with R, version 2023.06.2+561.

Packages used within R included phyloseq, microviz, ggplot, vegan, nlme, tidyverse

BCO-DMO Processing Description

- Loaded data from original file "Pfister_NereocystisGeography_RoySocOpen2025_BCODMO.csv" into the BCO-DMO system, treating "NA" as a missing data value (missing data values are empty/blank in the final CSV file).
- Converted date column from %m/%d/%y format to %Y-%m-%d format.
- Saved the final file as "1000920_v1_nereocystis_geography.csv".

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Related Publications

Hasegawa, Y., Mark Welch, J. L., Rossetti, B. J., & Borisy, G. G. (2017). Preservation of three-dimensional spatial structure in the gut microbiome. Plos One, 12(11), e0188257. https://doi.org/10.1371/journal.pone.0188257
Methods

Intergovernmental Oceanographic Commission (1994) Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements. Paris, France, UNESCO-IOC, 170pp. (Intergovernmental Oceanographic Commission Manuals and Guides: 29), (JGOFS Report; 19). DOI: https://doi.org/10.25607/OBP-1409
Methods

Jassby, A. D., & Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnology and Oceanography, 21(4), 540–547. Portico. https://doi.org/10.4319/lo.1976.21.4.0540
Methods

McCoy, S. J., Santillán-Sarmiento, A., Brown, M. T., Widdicombe, S., & Wheeler, G. L. (2019). Photosynthetic Responses of Turf-forming Red Macroalgae to High CO_2 Conditions. Journal of Phycology, 56(1), 85–96. Portico. https://doi.org/10.1111/jpy.12922
Methods

Pfister, C. A., Altabet, M. A., & Weigel, B. L. (2019). Kelp beds and their local effects on seawater chemistry, productivity, and microbial communities. Ecology, 100(10). Portico. https://doi.org/10.1002/ecy.2798
Methods

Pfister, C. A., Stanfield, E., Bogan, M., Weigel, B. L., Volbrecht, S., & Scorza, K. (2025). Foundational kelp species reveal links between host traits, the environment and the associated microbial community. Royal Society Open Science, 12(10). https://doi.org/10.1098/rsos.250637
Results

Wootton, J. T., Pfister, C. A., & Forester, J. D. (2008). Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. Proceedings of the National Academy of Sciences, 105(48), 18848–18853. https://doi.org/10.1073/pnas.0810079105
Methods

Younker, I. T., Molnar, N., Scorza, K., Weed, R., Light, S. H., & Pfister, C. A. (2024). Bacteria on the foundational kelp in kelp forest ecosystems: Insights from culturing, whole genome sequencing and metabolic assays. Environmental Microbiology Reports, 16(3). Portico. https://doi.org/10.1111/1758-2229.13270
Methods

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Related Datasets

IsRelatedTo

University of Chicago. Nereocystis luetkeana blade bacteria 16S 2022. 2025/08. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1301461. NCBI:BioProject: PRJNA1301461.

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Parameters

Parameter	Description	Units
year	Year	unitless
date	Date	Unitless
month	month	unitless
locale	within coastal Washington state; see Methods	unitless
region	locale divided into 3 regions in coastal Washington state	unitless
latitude	Latitude	decimal degrees
longitude	Longitude	decimal degrees
replicate	per site per year	unitless
wetmass_g	wetmass of Nereocystis tissue used in estimating carbon fixation, reported in grams	grams
drymass_g	drymass of Nereocystis tissue used in estimating carbon fixation, reported in grams	grams
mgCfixed_gdrymass_hr	the mg of Carbon fixed per g drymass of Nereocystis per hour	milligrams per gram drymass per hour
DOCmgl_release_gdrymass_hr_corr01	the mg of dissolved organic Carbon released per g drymass of Nereocystis per hour, with 0.01 added to all values to allow analyses of log values for all and to avoid dividing by zero (see below)	milligrams per gram drymass per hour

DOC_um_hr_drymass	the uM of Carbon dissolved organic Carbon released per g drymass of Nereocystis per hour	micromolar (uM)
to_po4	the initial P04 concentration in uM	micromolar (uM)
to_si	the initial SiO4 concentration in uM	micromolar (uM)
to_no3	the initial NO3 concentration in uM	micromolar (uM)
to_no2	the initial NO2 concentration in uM	micromolar (uM)
to_nh4	the initial NH4 concentration in uM	micromolar (uM)
to_DIN	the initial dissolved inorganic nitrogen concentration in uM	micromolar (uM)
uM_DOC_to	the initial dissolved organic carbon concentration in uM	micromolar (uM)
tf_po4	the final P04 concentration in uM	micromolar (uM)
tf_si	the final SiO4 concentration in uM	micromolar (uM)
tf_no3	tf_no3 - the final NO3 concentration in uM	micromolar (uM)
tf_no2	tf_no2 - the final NO2 concentration in uM	micromolar (uM)
tf_nh4	tf_nh4 - the final NH4 concentration in uM	micromolar (uM)
tf_DIN	tf_DIN - the final dissolved inorganic nitrogen concentration in uM	micromolar (uM)
no3_uptake_h_gdm	no3_uptake_h_gdm - the uM change in NO3 per hour per gram drymass of Nereocystis	uM change per gram drymass per hour
bulb_diam	bulb_diam - widest part of bulb, in mm	millimeters (mm)
blade_length	blade_length - blade length used in assay, in cm	centimeters (cm)
repro	indication of whether kelp is reproductive (R) or not (NR)	unitless

deln	the delta 15N, stable isotope ratio of Nitrogen, in Nereocystis tissue	per mil
delc	delc - the delta 13C, stable isotope ratio of Carbon, in Nereocystis tissue	per mil
pctn	pctn - the percent of tissue nitrogen (as dry mass) in Nereocystis tissue	percent
pctc	pctc - the percent of tissue carbon (as dry mass) in Nereocystis tissue	percent
c_n	c_n - the ratio of tissue carbon to tissue nitrogen (pctc/pctn)	ratio
temperature	temperature - in deg C	degrees Celsius
salinity	salinity - in ppt	ppt
ratioCfixtoDOCrel	ratioCfixtoDOCrel - the ratio of inorganic carbon fixed to dissolved organic carbon released (mcCfixed_gdrymasshr/DOCmgl_release_gdrymass_hr_corr01)	ratio

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Sequences were generated from the Illumina MiSeq platform.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	calipers
Generic Instrument Name	calipers
Dataset-specific Description	All kelp measurements were made in situ, often via kayak or at the shore using a measuring tape and calipers.
Generic Instrument Description	A caliper (or "pair of calipers") is a device used to measure the distance between two opposite sides of an object. Many types of calipers permit reading out a measurement on a ruled scale, a dial, or a digital display.

Dataset-specific Instrument Name	Qiagen DNeasy PowerSoil Kit (Qiagen)
Generic Instrument Name	DNA Analysis Kit
Dataset-specific Description	DNA from the swabs was extracted with a Qiagen DNeasy PowerSoil Kit (Qiagen).
Generic Instrument Description	A laboratory kit containing reagents and materials used to extract, purify, detect, measure, or otherwise evaluate DNA from a sample.

Dataset-specific Instrument Name	Olympus BX50 fluorescence microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Slides were visualized at 100× with an Olympus BX50 fluorescence microscope with a DAPI filter.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	pulse amplitude modulated (PAM) fluorometry in situ (Diving Pam, Walz, Germany)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Using pulse amplitude modulated (PAM) fluorometry in situ (Diving Pam, Walz, Germany) we estimated fluorescence over a range of irradiances. PAM is a non-invasive, in situ method that quantifies Photosystem II fluorescence parameters across different light levels.
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	GenoGrinder Spex
Generic Instrument Name	Homogenizer
Dataset-specific Description	Kelp tissue was ground to a fine powder in a GenoGrinder Spex (Metuchen, NJ).
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	elemental analyser isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Packed tins were analysed on an elemental analyser isotope ratio mass spectrometer at Northwestern University Stable Isotope Biogeochemistry Laboratory (NUSIBL).
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	measuring tape
Generic Instrument Name	Measuring Tape
Dataset-specific Description	All kelp measurements were made in situ, often via kayak or at the shore using a measuring tape and calipers.
Generic Instrument Description	A tape measure or measuring tape is a flexible ruler. It consists of a ribbon of cloth, plastic, fibre glass, or metal strip with linear-measurement markings. It is a common tool for measuring distance or length.

Dataset-specific Instrument Name	optical oxygen and temperature meter (FireStingO2 FSO2-4)
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	The change in oxygen was measured for 1 hour with a Pyro Science contactless fibre-optic sensors (Oxygen Sensor Spots OXSP5) and an optical oxygen and temperature meter (FireStingO2 FSO2-4), with the sensor spots affixed inside each 1 L chamber.
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	Oxygen Sensor Spots (OXSP5)
Generic Instrument Name	Oxygen Sensor Spot
Dataset-specific Description	The change in oxygen was measured for 1 hour with a Pyro Science contactless fibre-optic sensors (Oxygen Sensor Spots OXSP5) and an optical oxygen and temperature meter (FireStingO2 FSO2-4), with the sensor spots affixed inside each 1 L chamber.
Generic Instrument Description	Oxygen sensor spots allow oxygen measurements in closed sample containers with contactless read-out through a transparent window (glass, acryl glass) with special adapters and optical fibers. These patches often have a self-adhesive option.

Dataset-specific Instrument Name	Pesola scale
Generic Instrument Name	scale or balance
Dataset-specific Description	The youngest basal frond tissue extending 24 to 30 cm from the bulb was weighed with a Pesola scale.
Generic Instrument Description	Devices that determine the mass or weight of a sample.

Dataset-specific Instrument Name	Castaway-CTD (Sontek)
Generic Instrument Name	SonTek CastAway-CTD
Dataset-specific Description	The salinity and seawater temperatures were assessed with a Castaway-CTD (Sontek) from a boat or kayak at all sites except Tatoosh Island where instrumentation is moored (Hach DS5).
Generic Instrument Description	The Sontek CastAway-CTD (manufactured by Xylem) is a handheld castable instrument that provides instantaneous profiles of temperature, salinity, and sound speed. Each cast is referenced with both time and location using its built-in GPS receiver. The CastAway software displays profiles of the casts in addition to mapping the locations of the data collection points. The CastAway-CTD has a 5 Hz response and sampling rate, accurate to 0.1 (PSS-78), 0.05° Celsius. Conductivity range is 0 to 100,000 µS/cm. Temperature range is -5° to 45° Celsius. Pressure range is 0 to 100 decibars. Further specs and information can be found on the manufacturer's website: https://www.xylem.com/en-us/brands/wtw/wtw-products/castaway-ctd/

Dataset-specific Instrument Name	polymerase chain reaction (PCR)
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	The V4–V5 region within the 16S ribosomal RNA (rRNA) gene was amplified using universal bacterial primers and polymerase chain reaction (PCR) conditions.
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

Dataset-specific Instrument Name	Hach DS5
Generic Instrument Name	Water Quality Multiprobe
Dataset-specific Description	The salinity and seawater temperatures were assessed with a Castaway-CTD (Sontek) from a boat or kayak at all sites except Tatoosh Island where instrumentation is moored (Hach DS5).
Generic Instrument Description	An instrument which measures multiple water quality parameters based on the sensor configuration.

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Project Information

The role of microbes in driving productivity and carbon fixation in seaweeds (kelp and their microbiome)

Website: https://pfisterlab.uchicago.edu/basic-page/

Coverage: Northeast Pacific Ocean, coastal Washington state

NSF Award Abstract:
It is increasingly recognized that species in the ocean host a diverse set of microbes and that these microbes can determine the functioning of the host. Yet, the discovery of microbial taxa in nature far outpaces our understanding of their functions. Primary producers in the ocean show rich microbial communities and may contribute to the significant role that macrophytes play in coastal communities. This project focuses on a species of canopy kelp that is a foundational species along the shores of the northeast Pacific Ocean, the bull kelp *Nereocystis luetkeana*. The research explores the role of a diverse set of microbes in association with bull kelp. Using experimental manipulations of newly isolated microbial taxa from kelp, tests of whether interactions are positive, negative, or neutral are performed. Specific assays of whether microbes provision vitamins and enhance access to nutrients are carried out, as well as tests of whether kelp exudates benefit microbial metabolism and growth. Working directly with Tribal youth through an internship program, as well as with undergraduate, post-baccalaureate, and graduate students, helps train the next generation of ocean scientists. Communication channels with Tribal and State governments inform efforts to understand kelp declines. Host-microbe interactions are a component of the persistence of ocean species and this research informs the factors that underly these relationships.

The advent of DNA sequencing, imaging, and other molecular approaches have revealed that many key species in the ocean are a 'holobiome', and their fate is entwined with the microbes they host. Yet, this discovery of microbial taxa in nature far outpaces our understanding of microbial function. *Nereocystis luetkeana*, or bull kelp, and the bacteria the investigators have isolated from the kelp surface, present an opportunity to quantify host-microbe interactions, including how stressors of host health, such as ocean warming, alter these interactions. Experiments tracing stable nitrogen isotopes and manipulations with hosts and bacterial isolates are used to investigate possible exchanges and the currencies underlying interactions. Metabolomic, proteomic, and genomic analyses are used to quantify host-microbe interactions and linkage between taxa and their functions. Key microbial metabolisms being investigated include nutrient provisioning, vitamin synthesis, and the response to reactive oxygen species, among others. The demonstrated alteration of host-microbe interactions in the ocean when hosts are stressed suggest the findings of this study have application to understanding the factors that promote persistence of eukaryote-prokaryote partnerships.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2329475

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