Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean

K. Harding

K. A. Turk-Kubo

RE Sipler

*Virginia Institute of Marine Science*

M. M. Mills

D. A. Bronk

*Virginia Institute of Marine Science*

Follow this and additional works at: [https://scholarworks.wm.edu/vimsarticles](https://scholarworks.wm.edu/vimsarticles)

Part of the [Marine Biology Commons](https://scholarworks.wm.edu/vimsarticles), and the [Oceanography Commons](https://scholarworks.wm.edu/vimsarticles)

**Recommended Citation**

Harding, K.; Turk-Kubo, K. A.; Sipler, RE; Mills, M. M.; and Bronk, D. A., "Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean" (2018). *VIMS Articles*. 1359.

[https://scholarworks.wm.edu/vimsarticles/1359](https://scholarworks.wm.edu/vimsarticles/1359)

This Article is brought to you for free and open access by the Virginia Institute of Marine Science at W&M ScholarWorks. It has been accepted for inclusion in VIMS Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean

Katie Harding*, Kendra A. Turk-Kubo*, Rachel E. Sipler*, Matthew M. Mills*, Deborah A. Bronk†, and Jonathan P. Zehr*†

*Ocean Sciences Department, University of California, Santa Cruz, CA 95064; †Department of Physical Sciences, The Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA 23062; ‡Department of Ocean Science, Memorial University of Newfoundland, St. John’s, NL A1C 5S7, Canada; §Department of Earth System Science, Stanford University, Stanford, CA 94305; and †Bigelow Laboratory for Ocean Sciences, East Boothbay, ME 04544

Biological dinitrogen (N₂) fixation is an important source of nitrogen (N) in low-latitude open oceans. The unusual N₂-fixing unicellular cyanobacteria (UCYN-A)/haptophyte symbiosis has been found in an increasing number of unexpected environments, including northern waters of the Danish Straight and Bering and Chukchi Seas. We used nanoscale secondary ion mass spectrometry (nanoSIMS) to measure ¹⁵N₂ uptake into UCYN-A/haptophyte symbiosis and found that UCYN-A strains identical to low-latitude strains are fixing N₂ in the Bering and Chukchi Seas, at rates comparable to subtropical waters. These results show definitively that cyanobacterial N₂ fixation is not constrained to subtropical waters, challenging paradigms and models of global N₂ fixation. The Arctic is particularly sensitive to climate change, and N₂ fixation may increase in Arctic waters under future climate scenarios.

Significance

Biological dinitrogen (N₂) fixation (BNF) is an important source of nitrogen in marine systems. Until recently, it was believed to be primarily limited to subtropical open oceans. Marine BNF is mainly attributed to cyanobacteria. However, recently an unusual N₂-fixing unicellular cyanobacteria (UCYN-A)/haptophyte symbiosis was reported with a broader temperature range than other N₂-fixing cyanobacteria. We report that the UCYN-A symbiosis is present and fixing N₂ in the Western Arctic and Bering Seas, further north than any previously reported N₂-fixing marine cyanobacteria. Nanoscale secondary ion mass spectrometry enabled us to directly show that the symbiosis was fixing N₂. These results show that N₂-fixing cyanobacteria are not constrained to subtropical waters and challenge commonly held ideas about global marine N₂ fixation.

Author contributions: K.A.T.-K., R.E.S., D.A.B., and J.P.Z. designed research; K.H., R.E.S., M.M.M., and D.A.B. performed research; K.H., K.A.T.-K., R.E.S., and M.M.M. analyzed data; and K.H., K.A.T.-K., and J.P.Z. wrote the paper with assistance from all authors.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

Data deposition: The raw UCYN-A nifH sequences reported in this paper have been deposited in the Sequence Read Archive, www.ncbi.nlm.nih.gov/sra (Bioproject ID PRJNA476143).

*To whom correspondence should be addressed. Email: zehrj@ucsc.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1813658115/-/DCSupplemental.

Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved November 14, 2018 (received for review August 8, 2018)

www.pnas.org/cgi/doi/10.1073/pnas.1813658115

December 26, 2018 | vol. 115 | no. 52 | 13371–13375
abundances (10^5 to 10^6 nifH copies L^{-1}) were comparable to those at subtropical latitudes (6, 11, 21) but were considerably lower in the Chukchi Sea, on the north eastern Chukchi shelf and in the Beaufort Seas (Fig. 1 and SI Appendix, Fig. S3 and Table S2). Both UCYN-A lineages were primarily found in surface samples in low salinity ice-melt waters (SI Appendix, Fig. S4 and Table S2). These results verified that two strains of the N2-fixing cyanobacterium UCYN-A are present in polar waters, consistent with recent findings (9, 10).

Our genomic and morphological characterization of the high-latitude UCYN-A symbioses show these strains are indistinguishable from those reported in subtropical oceans. The UCYN-A1 and UCYN-A2 nifH sequences from the Arctic samples were identical to the most common sequences reported from subtropical oceans (Fig. 2 and SI Appendix, Fig. S2). Visualization of the different haptophyte hosts and their respective UCYN-A1 and UCYN-A2 cyanobacterial symbionts using lineage-specific CARD-FISH probes (22, 23) (Fig. 3) showed that the symbioses were similar in size and morphology (~3 and 5 µm, respectively) to the symbioses in tropical and subtropical oceans (4, 7, 23, 24). Other globally distributed eukaryotic picoplankton have specific cold-adapted strains (25, 26), so the discovery of the subtropical strains of the UCYN-A symbiosis in the Arctic was unexpected. 

15N uptake experiments coupled with CARD-FISH and single cell scale secondary ion mass spectrometry (nanoSIMS) showed that the UCYN-A symbiosis was actively fixing N2. While previous Arctic research has identified diazotrophs and detected bulk rates of N fixation, single cell rates of a marine cyanobacteria (or any marine microorganism) in the Arctic region (72°N) and with low water temperatures (4 °C) has been lacking. The UCYN-A1 and UCYN-A2 symbioses in the Bering Sea had mean cell-specific N2 fixation rates of 7.6 ± 14.5 fmol N cell^{-1} d^{-1} (n = 6) and 13.0 ± 7.7 fmol N cell^{-1} d^{-1} (n = 8), respectively (Fig. 4). In the Chukchi Sea, the cell-specific N2 fixation rates of the UCYN-A2 symbiosis were considerably lower, but two out of six cells were detectable with an average of 1.1 ± 2.0 fmol N cell^{-1} d^{-1}. Surprisingly, the UCYN-A1 cell-specific N2 fixation rates measured in the Bering Sea at 10.1 °C are similar to rates reported from the warmer waters (>25 °C) of the subtropical North Atlantic [0.45–12 fmol N cell^{-1} d^{-1} (27, 28)]. UCYN-A N2 fixation in polar waters shows that low temperature does not limit the distribution or activity of N2-fixing cyanobacteria. 

N2 fixation by UCYN-A accounted for the total measured N2 fixation rates in the Bering Sea but not in the Chukchi Sea. UCYN-A N2 fixation rates were estimated to be 10.5 ± 25 nmol L^{-1} d^{-1} and 0.004 ± 0.007 nmol L^{-1} d^{-1} in the Bering Sea and Chukchi Sea, respectively, when per-cell rates were scaled to volumetric rates (SI Appendix, Materials and Methods and Table S3). Measured N2 fixation rates in the bulk water sample from the Bering Sea (Station 1) were 6.9 ± 3.8 nmol L^{-1} d^{-1}, indicating that N2 fixation by UCYN-A accounted for total bulk rates (within error). In contrast, extrapolated rates from UCYN-A2 cellular N2 fixation in the Chukchi Sea were two orders of magnitude less than the measured bulk N2 fixation of 0.2 ± 0.2 nmol L^{-1} d^{-1} (detected, not quantified). More research is needed to determine the quantitative significance of N2 fixation by UCYN-A in this region, but the presence of actively N2-fixing
unicellular cyanobacteria in Arctic waters is surprising from an ecological perspective and important for mathematical models that predict global N₂ fixation.

It is unclear whether the UCYN-A symbioses in the Bering Sea and Western Arctic are advected into the Western Arctic Seas through the Bering Strait or are endemic populations. Microbial community structure in Arctic waters is heavily influenced by the originating water mass (29), and Shiozaki et al. (10) suggests the UCYN-A symbiosis detected by DNA assays originates from Pacific waters transported to the Arctic in the Alaskan Coastal Current, as has been reported for other species (30). However, UCYN-A1, which is widespread in the North Pacific Subtropical Gyre, disappears at the front between the North Pacific Subtropical Gyre and the North Pacific Subarctic Gyre (9, 21), and the UCYN-A2 symbiosis is not commonly found in the oligotrophic North Pacific (12). This finding suggests that the UCYN-A populations may be maintained throughout the year in the Arctic and may be endemic populations.

Our results provide support for resource ratio theory-based predictions that Bering Sea waters would be favorable for N₂-fixers (31) and extend the biogeo graphical range of active UCYN-A symbioses into the Chukchi and Beaufort Seas. New models are needed for predicting the biogeography of N₂-fixing microorganisms and N₂ fixation in the world ocean, including other Arctic regions and the Southern Ocean. The results of this study also have implications for global N₂ fixation and global environmental change. Arctic ecosystems are rapidly changing. Predicted effects include increased Pacific inflow, phytoplankton growing season, stratification, nutrient limitation, and sea-surface temperatures (32), all of which may select for UCYN-A and other N₂-fixing species that are commonly found in warm, oligotrophic waters. The results of this study change the paradigm that N₂ fixation and N₂-fixing cyanobacteria are common only in warm tropical or subtropical waters, and these results are critical for understanding and predicting global patterns of N₂ fixation.

Materials and Methods

Samples were taken in the Bering Sea, Chukchi Sea, on the Chukchi Shelf, and in the Beaufort Sea in September 2016.

DNA Extraction, nifH Amplification, and qPCR. Samples (2–4 L) were filtered by peristaltic pump onto sequential 3 and 0.2 μm polyphenylene ether filters (0.2 μm, 25 mm; Supor-200; Pall Life Sciences) in Swinnex filter holders. DNA was extracted using a modified DNeasy Plant Mini Kit (Qiagen) protocol, described in detail in ref. 33. PCR amplification of the nifH gene used degenerate universal nifH primers YANNI/450 and up/down in a nested reaction (34), with the second round primers (up/down) modified to contain common sequence linkers (35). Library preparation was carried out by the DNA Sequencing Core Facility at the University of Illinois at Chicago (rrc.uic.edu/sequencing). Amplicons were sequenced using Illumina MiSeq, to a sequencing depth of 40,000 sequences per sample.

UCYN-A1 and UCYN-A2 abundances were estimated using TaqMan qPCR chemistry and primers and probes specific for UCYN-A1 (36) and UCYN-A2 (11) and their respective haptophyte partners, UCYN-A1 host (SI Appendix, Materials and Methods) and UCYN-A2 host (11), in samples positive for nifH amplification.

¹⁵N₂ Rate Measurement Incubations. N₂ fixation was assessed using a modified version of the ¹⁵N-bubble method (39). Water samples for rate-measurement incubations were collected from Niskin bottles into gas-tight 1-L glass media bottles (KIMAX model no. 611001000) capped with black open-top caps with gray butyl septa (model no. 240680). The caps and septa were preconditioned in saltwater brine for 60 d before use. The media bottles and caps were acid-washed (10% HCl) and rinsed with copious amounts of high-purity water (18.2 MΩ cm⁻¹). The glass media bottles were also combusted at 500 °C for 4 h before use.

Measuring Cell-Specific N₂ Fixation Rates Using NanoSIMS. To visualize and map both strains and their respective hosts (UCYN-A1/UCYN-A1 host and UCYN-A2/UCYN-A2 host), a double CARD-FISH protocol was used according to the protocols detailed in refs. 22 and 23. The full suite of HRP probes, competitor oligonucleotides, and helper probes are given in SI Appendix, Table 5.1. Before nanoSIMS analysis, cells were transferred to a gridded silicon chip (1.2 cm × 1.2 cm with a 1 mm × 1 mm raster; Pelco SFG12 Finder Grid Substrate) and imaged and mapped under epifluorescence on a Zeiss Axioplan epifluorescence microscope equipped with digital imaging at the

![Fig. 3. Morphologies of Arctic UCYN-A symbioses are indistinguishable from subtropical strains. Double CARD-FISH comparison of UCYN-A lineages from the Bering Sea (A and C) and water collected at the Scripps Institute of Oceanography Pier in La Jolla, CA (B and D) show similar sizes and morphologies. CARD-FISH images show the symbiosis is intact, with both the haptophyte host (green and blue) and cyanobacteria (red). (Scale bar, 5 μm.)](Image)

![Fig. 4. UCYN-A symbioses fix ¹⁵N₂ in western Arctic waters. UCYN-A cell-specific ¹⁵N₂ fixation rates (A) and ¹⁵N enrichment (B–D) from nanoSIMS measurements after incubating natural populations in seawater with ¹⁵N₂. Bars of the same color (A) represent rates measured in individual symbioses (UCYN-A with host alga) from a single station and lineage (notated in underlying cell image). (Scale bar, 2 μm.) Averages are shown by a horizontal black line. Error bars are the SD of the cell-specific rate between the host and UCYN-A. Note the colored axes differ in scale on nanoSIMS images (B–D).](Image)
University of California, Santa Cruz (UCSC). 15N measurements of individual cells were determined by NanoSIMS analyses performed at Stanford Nano Shared Facilities (https://nnf.stanford.edu) on a Cameca NanoSIMS 50L at Stanford University. Image planes were accumulated after first being aligned. Isotope data were taken as a sum of counts in each plane per pixel. Cell outlines and regions of interest (ROIs) were determined as the best fit based on original CARD-FISH image, electron microscopy image, and accumulated images in 14C-15N and 13C. Cell size was determined based on ROIs of the defined haptothyme or UCYN-A cell. Cell-specific N2 fixation rates were determined by calculating the carbon content per cell based on a spherical cell volume (V) from the measured cell diameter determined by the ROI following the calculations of ref. 27. The C:N ratio of 6.3 was measured in UCYN-A from the tropical North Atlantic (28) and was used in our calculation to estimate N content of the cell. The limit of detection (LOD) was determined to be three times the SD of 15N in unenriched samples (0.02 At%), similar to the LOD determination described by Jayakumar et al. (37). More detailed methods and calculations can be found in SI Appendix.

**Bulk N2 Fixation Rate Measurements.** Bottles were filled in triplicate and capped with ambient air bubbles removed. All bottles were immediately placed in mesh bags to mimic the light intensity at collection depth. Different depths received different levels of screening. The bottles were then amended with 15N-2 gas enriched samples (99.8% 15N) gas purchased from Cambridge Isotope Laboratories, Inc. (lot no. I-199168A). Higher volumes of 15N2 gas were used in all samples after Station 1 to obtain enrichment levels closer to 10% (average 15N2 enrichment of 5.8 ± 2.1%). Samples were incubated for 24 h in flow-through incubators on deck (surface samples) or environmental chambers (deep samples) set to 0 °C ± 1 °C. Before use in the incubations, subsamples of the 15N2 gas stocks were assessed for 15NH4+, 15NO3, and 15N2, and the gas stock composition according to the methods described in ref. 38. NO contamination was measured. Incubations were terminated after 24 h. A membrane inlet mass spectrometer (MIMS) was used to assess the level of 15N enrichment in each sample immediately upon incubation termination. The MIMS data for each individual bottle were used to calculate uptake rates. Size-fractionated bulk N2 fixation rates were determined by filtering in series through 3.0-μm silver filters and then precombusted (450 °C for 2 h) glass fiber filter (GF-75) with a nominal pore size of 0.3 μm. Filters were stored frozen at −20 °C in sterile microcentrifuge tubes until analysis. Filters were thawed and dried overnight at 40 °C and analyzed on a Sercon Isotope ratio mass spectrometer with an SL autosampler that had not been used. Blank natural abundance samples were analyzed on an Integra2 combined isotope ratio mass spectrometer coupled to oceanic circulation. Limnol Oceanogr Lett 2:159-166. The LOD (i.e., 3x SD) of 15N fixation was determined for each sample, and for look@nanoSIMS consulting, and Stefan Green and his staff (DNA Services Facility and the University of Illinois, Chicago) for sequencing consultation. We greatly appreciate Mick Follows (Massachusetts Institute of Technology) and Kevin Attrill (Stanford University) for helpful discussions. This research was funded by National Science Foundation Award from the Office of Polar Programs (OPP) 1503614 (to J.P.Z.), Division of Ocean Sciences Awards 1214093 and 1559152 (to J.P.Z. and OPP-1504307 (to R.E.S.); and Simons Foundation grant (OPP) 1503614 (to J.P.Z.); Division of Ocean Sciences Awards 1241093 for look@nanoSIMS consulting, and Stefan Green and his staff (DNA Services Facility and the University of Illinois, Chicago) for sequencing consultation. We greatly appreciate Mick Follows (Massachusetts Institute of Technology) and Kevin Attrill (Stanford University) for helpful discussions. This research was funded by National Science Foundation Award from the Office of Polar Programs (OPP) 1503614 (to J.P.Z.), Division of Ocean Sciences Awards 1214093 and 1559152 (to J.P.Z. and OPP-1504307 (to R.E.S.); and Simons Foundation grant (OPP) 1503614 (to J.P.Z.). Part of this work was performed at the Stanford Nano Shared Facility under Award ECCS-1542152.

**ACKNOWLEDGMENTS.** We gratefully acknowledge Mary-Kate Rogener (University of Georgia) for providing MIMS analyses and IMRS sample collection to the Virginia Institute of Marine Science for providing IMRS analysis, Rosie Grassoville (UCSC) for discussions about N2 fixation rate measurements, as well as Laurie Juraneck (Oregon State University) and the captain and crew of the Research Vessel Sikuligan for field logistical support. We also thank Chuck Hitzmann (University of Nova Scotia) for his support in conducting our NanoSIMS consultation, Lubos Polerecky for look@nanoSIMS consulting, and Stefan Green and his staff (DNA Services Facility and the University of Illinois, Chicago) for sequencing consultation. We greatly appreciate Mick Follows (Massachusetts Institute of Technology) and Kevin Attrill (Stanford University) for helpful discussions. This research was funded by National Science Foundation Award from the Office of Polar Programs (OPP) 1503614 (to J.P.Z.), Division of Ocean Sciences Awards 1214093 and 1559152 (to J.P.Z.) and OPP-1504307 (to R.E.S.); and Simons Foundation grant (OPP) 1503614 (to J.P.Z.). Part of this work was performed at the Stanford Nano Shared Facility under Award ECCS-1542152.

**Data and Materials Availability.** All data are provided in this article and SI Appendix, with the exception of the raw UCYN-A n/IH sequences, which have been deposited in the Sequence Read Archive (www.ncbi.nlm.nih.gov/ sra) under Bioproject ID PRJNA476143.
34. Zehr JP, McReynolds LA (1989) Use of degenerate oligonucleotides for amplification of the niFH gene from the marine cyanobacterium Trichodesmium thiebautii. Appl Environ Microbiol 55:2522–2526.
35. Moonsamy PV, et al. (2013) High throughput HLA genotyping using 454 sequencing and the Fluidigm Access Array™ system for simplified amplicon library preparation. Tissue Antigens 81:141–149.
36. Church MJ, Short CM, Jenkins BD, Karl DM, Zehr JP (2005) Temporal patterns of nitrogenase gene (niFH) expression in the oligotrophic North Pacific Ocean. Appl Environ Microbiol 71:5362–5370.
37. Jayakumar A, et al. (2017) Biological nitrogen fixation in the oxygen-minimum region of the eastern tropical North Pacific Ocean. ISME J 11:2356–2367.
38. Dabundo R, et al. (2014) The contamination of commercial 15N2 gas stocks with 15N-labeled nitrate and ammonium and consequences for nitrogen fixation measurements. PLoS One 9:e110335.
39. Montoya JP, Voss M, Kahler P, Capone DG (1996) A simple, high-precision, high-sensitivity tracer assay for N2 fixation. Appl Environ Microbiol 62:986–993.
40. Gradoville MR, et al. (2017) Diversity and activity of nitrogen-fixing communities across ocean basins. Limnol Oceanogr 62:1895–1909.