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Supplementary Information for

Symbiotic Unicellular Cyanobacteria Fix Nitrogen in the Arctic Ocean

Katie Harding[a], Kendra Turk-Kubo[a], Rachel Sipler[b,c], Matthew M. Mills[d], Deborah Bronk[b,e], Jonathan Zehr[a,1].

Jonathan Zehr
Email: zehrj@ucsc.edu

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Supplementary Information Text

Materials and Methods

Sample collection for DNA extractions - Samples were taken in the Bering, Chukchi and Beaufort Seas in September 2016 (Fig. S1). Water samples were collected from Niskin bottles attached to a CTD into clean polycarbonate bottles (washed 3x 10% HCl and 3x milliQ water) after 3x seawater sample rinses. Samples (2-4L) were filtered by peristaltic pump onto sequential 3 and 0.2 µm polyphenylene ether (PPE) filters (0.2 µm 25mm Supor-200, Pall Life Sciences, Port Washington, NY, USA) in Swinnex filter holders. Filters were transferred into

sterile bead beater polypropylene microcentrifuge tubes containing 0.5 and 1 mm glass beads (BioSpec) and immediately frozen in liquid nitrogen. Surface and the depth corresponding to chlorophyll maximum (DCM) were sampled at most stations and a near bottom depth was taken if the station depth was more than ~20m deeper than the chlorophyll maximum. Only surface samples were collected at Station 1 (off the coast of Nome, AK). Replicate samples were collected for DNA at each depth and station. Samples for molecular analysis were stored at -80^{o}C until processing.

DNA extraction and *nifH* amplification - DNA was extracted using a modified DNeasy Plant Mini Kit (Qiagen, Germantown, MD) protocol, described in detail in (1) that includes steps for disruption of cell walls and automation of wash steps. The *nifH* gene, a functional marker for nitrogenase enzyme in diazotrophs, was PCR-amplified in duplicate with one positive and at least two negative (water) PCR controls, using a degenerate universal *nifH* primers YANNI/450 and up/down in a nested reaction (2). Briefly, the first round of *nifH* amplification consisted of 1x buffer, 4 mM $MgCl_2$, 200 µM dNTP mix (Invitrogen, Carlsbad, CA), 0.5 µM YANNI primer, 0.5 µM 450 primer (Sigma-Aldrich Oligos, St. Louis, MO), 1 µl Platinum taq polymerase (Invitrogen), 1 µl DNA template and Ambion RT-PCR grade water (Invitrogen) for a total reaction volume of 15 µl. Thermocycler condition were as follows: initial denaturation at 95^{o}C for 3 min., followed by 25 cycles of 95^{o}C for 30 s 55^{o}C for 30 s 72^{o}C for 45 s and a final elongation at 72^{o}C for 7 min. The second round of *nifH* amplification used the up and down primers, each modified with common sequence (CS) linkers (3) using the same reaction conditions but with a final volume of 25 µl, and an additional 5x amplification cycles. Amplified products were checked with agarose

gel electrophoresis and replicates from samples with positive amplification were pooled at equal volumes. Library preparation was carried out by the DNA Sequencing Core Facility at the University of Illinois at Chicago (http://rrc.uic.edu/cores/genome-research/sequencing-core/) and included an additional ten rounds of amplification to add multiplexing oligonucleotides and sequencing adaptors according to protocols detailed in (4). Amplicons were sequenced using Illumina MiSeq, to a sequencing depth of 40,000 sequences per sample.

***nifH* amplicon sequence analysis and UCYN-A oligotyping** - Raw paired end *nifH* amplicon reads (2 x 250 bp) were merged using Paired-End read (PEAR) merging software (5) with a phred score of 20, a minimum length allowance of 300 bp, maximum length of 400 bp. Merged files were imported into QIIME (6) where chimeric sequences were removed using a *de novo* approach using UCHIME (7), and the remaining sequences were clustered into OTUs with 97% similarity using usearch6.1 (8). OTUs with less than ten sequences were removed. Representative sequences were imported into a curated *nifH* database (9) in Arb (10), where they were translated, non-*nifH* and sequences with stop codons were removed, then remaining sequences were aligned using a Pfam-curated multiple alignment of the NifH/frxC family (Fer4_NifH) in HMMer (11). The nearest closest cultivated relative (based on nucleotide sequences), and *nifH* cluster was determined using blastx against a curated list of genome-derived *nifH* sequences.

UCYN-A OTUs were identified in the blastx analysis described above, and unique UCYN-A sequences were recovered after clustering all UCYN-A sequences at 100% nucleotide similarity using usearch6.1 (8). UCYN-A sequences were aligned against a reference UCYN-A database using pynast (12) and trimmed in using the web-platform Galaxy (usegalaxy.org) to a length consistent with a global database of UCYN-A sequences (13). UCYN-A oligotypes – highly refined taxonomic units defined using nucleotide positions with high "entropy"– were defined using the alignment positions identified by Turk-Kubo et al. (13) and the oligotyping pipeline developed by Eren et al. (14). The following arguments were used in the oligotyping analysis: (i) a given oligotype was allowed to be present in only one sample (-s 1); (ii) a given oligotype was required to be present at a relative abundance of at least 0.1% in one sample (-a 0.1); and (iii) the most abundant unique sequence defining an oligotype was required to have a sequence count > 100 across the whole data set (-M 100).

Estimating UCYN-A abundances using qPCR – UCYN-A1 and UCYN-A2 abundances were estimated using TaqMan® quantitative PCR (qPCR) chemistry and primers and probes specific for UCYN-A1 (15) and UCYN-A2 (16) and their respective haptophyte partners, UCYN-A1 host (Thomspon, unpublished) and UCYN-A2 host (16), in samples positive for *nifH* amplification. All aspects of qPCR reaction conditions, thermocycling parameters, inhibition tests and standard generation and calculations to determine abundances are described in detail in (17), with the exception of a 64^{o}C annealing temperature for the UCYN-A2 assay. The limit of detection (LOD) and limit of quantification (LOQ), were 0.5 *nifH* gene copies/µL DNA extraction and 4 *nifH* gene copies/µL DNA extraction, respectively. DNQs (detected, not quantified) were abundance that

fell in between this range. When samples were below the LOQ (DNQs) but were above the LOD, they were given the minimum LOQ value and values at the LOD were given the minimum LOD value for visualization purposes. Abundances were quantified separately for the 3.0 and 0.3 µm size fractions and then averages were added to estimate total UCYN-A abundance in the sample.

CARD-FISH sample preparation and hybridizations - After 24-hour $^{15}N_2$ incubations, 100 ml of labeled sample was fixed with a final concentration of 1.8% formaldehyde (v/v) up to 48 hours at 4^{o}C to preserve cells for CARD-FISH and subsequent nanoSIMS. Fixed samples were gently filtered onto 25mm, 0.6 µm pore polycarbonate filters (Millipore Isopore, EMD Millipore, Billerica, MA, USA) with 25mm, 0.8 µm pore polycarbonate cellulose acetate support filter (Sterlitech Corp. Kent, WA, USA) using low vacuum (<100 mm Hg). Filters were stored in 2 ml cryovials (Nalgene, Millipore, Sigma, Burlington, MA), flash frozen in liquid nitrogen and kept at -80^{o}C until analysis. To visualize 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 (18, 19). The full suite of HRP probes, competitor oligonucleotides and helper probes are given in Table S1.

Measuring cell-specific N_2 fixation rates using nanoSIMs – Pieces of CARD-FISH were washed to remove mounting media according to (20). Briefly, filters containing targets hybridized using CARD-FISH as described above were transferred to silicon gridded wafers (Pelcotec Silicon

SFG12, Ted Pella Inc., Redding, CA) by dampening filter pieces with 10 µl water, placing them face down on silicon wafer and freezing at -80^{o}C for 5 min, then gently removing the filter while still frozen. In order to locate targets for nanoSIMS analysis, gridded wafers were mapped using bright field and epifluorescence on an AxioVersion Epifluorescence Microscope. NanoSIMS analyses were performed at Stanford Nano Shared Facilities (SNSF; https://snsf.stanford.edu) on a Cameca NanoSIMS 50L at Stanford University, CA, USA. Once a target cell was located, the cell was exposed to 2-3 min of large diameter, Cs^+ beam to saturate cell ions with Cs^+ before analysis. Cells were rastered with a 16 keV Cs^+ beam and a current between 2 and 4 pA over a 10 - 15 µm^2 area with an image size of 256 x 256 pixels and a dwell time of ~1 ms per pixel. Negatively charged secondary ions of carbon ($^{12}C^-$, $^{13}C^-$), nitrogen (as $^{12}C^{14}N^-$, $^{12}C^{15}N^-$), and a secondary electron microscope image (1AU) were collected simultaneously for 30-45 frames for each individual cell. Data was imaged and analyzed using the look@nanoSIMS image analysis software (21).

Cell-Specific nitrogen 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. Carbon content [c] was estimated using two equations for the different types of cells, UcynA_log[c]= -0.363+(0.863 x log(V)), haptophyte_log[c]= -0.422+(0.758 x log(V)), following the example of (*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. NanoSIMS measurements of $^{12}C^{15}N$:$^{12}C^{14}N$ correspond to the atom% enrichment within the cell. To determine true ion counts versus ratio noise the measured image was split in 100 equally sized

squares and the ion counts per pixel evaluated. Squares within the ROI had significantly higher counts per pixel compared to those outside the ROI. Imaged cell only includes ratio values inside the ROI as any ratio values occurring outside the cell were determined to be Poisson error due to low counts. After subtracting natural abundance of ^{15}N occurring at each station and factoring in the atom percent $^{15}N_2$ labeling in the enrichment (MIMS data), cell-specific nitrogen fixation rates were calculated (fmol N cell^{-1} day^{-1}). The limit of detection was determined to be three times the standard deviation of unenriched samples (0.02 At%), similar to LOD determination in Jayakumar*, et al.* (22). In other words, if the atom percent excess (At% of a cell minus the naturally occurring ^{15}N from bulk particulate nitrogen) was greater than three times the standard deviation of an average of unenriched samples (n=32) then samples were considered enriched above detection and cell specific rates were calculated. Due to limited Arctic samples, unenriched UCYN-A/haptophyte symbioses samples from subtropical latitudes were used to determine the limit of detection and were measured on the same nanoSIMS instrument. Cell specific rates are based on the two volumetric spheres of the haptophyte and cyanobacteria together. Using qPCR abundance data, the cell-specific N_2-fixation rates were extrapolated to estimate the total volumetric N_2-fixation by UCYN-A (nmol N L^{-1} day^{-1}) at a given station.

Supplementary Text

Chukchi and Beaufort Sea UCYN-A oligotypes - Oligotyping enables fine resolution of closely related phylotypes based on nucleic acid positions with high entropy (14). This approach has

been applied to UCYN-A diversity in several recent studies (13, 23), and there are now over 100 UCYN-A oligotypes defined across multiple studies. A total of 84,156 UCYN-A partial *nifH* sequences were recovered from 29 Arctic samples, and the analysis identified only two oligotypes, which represented 96.71% of the sequences submitted for analysis, with a perfect purity score of 1.00. The UCYN-A oligotypes found in the Arctic were oligo3 (ATTCTATTTTCTT), which is the dominant oligotype from the UCYN-A2 strain and oligo1 (ATCTCGCTTCTTT), which is the dominant oligotype from the UCYN-A1 strain (Fig. 1, Fig. S2; Turk-Kubo et al, 2016). Oligo1 is widely distributed throughout the subtropical oceans, while oligo3 is found at high relative abundances in high latitude samples from the Danish Strait but does not appear to be a dominant strain in the subtropical oceans (13). In high latitude waters, oligo1 was found at high relative abundances in the Bering Sea (Off Nome, Station 1) and, interestingly, in the proximity of ice algae (Station 14). It was detected, however, in 10 of the 29 samples. In general, oligo3 (UCYN-A2) was more widely distributed, being detected in 23 of the 29 samples.

UCYN-A abundances and distributions - The UCYN-A1 symbiont was quantifiable at a single station (Station 1; Fig. S3, Table S2) in surface waters with an abundance of 1.6×10^6 *nifH* copies L^{-1}, and although it was detected in samples in the Chukchi and Beaufort Seas, it was not above limit of quantification. The UCYN-A2 symbiont was detected at 10 surface stations with an average of 2.4×10^3 *nifH* copies L^{-1} (n=41) (Fig. S3, Table S2), and was below quantification in the DCM and deep samples. Interestingly, UCYN-A was detected throughout the water column as a combination of UCYN-A1 and UCYN-A2 at a single station (Station 14) which was in proximity to ice containing, ice algae. Higher abundances of UCYN-A1 were found in the 0.2 μm size fraction

(average of $3.9x10^4$ *nifH* copies L^{-1}, n=21), although it was also detected in the 3 µm size fraction (average of $2.0x10^4$ *nifH* copies L^{-1}, n=21). The majority of UCYN-A2 occurred in the larger size fraction (> 3 µm) (average of $4.2x10^3$ *nifH* copies L^{-1}, n=21), although it could also be quantified in the 0.2 µm size fraction (average of $5.1x10^2$ *nifH* copies L^{-1}, n=21). In general, the distributions and abundances of UCYN-A1 and UCYN-A2 are consistent with recent findings of Shiozaki et al., which reported similar abundances of both UCYN-A1 and UCYN-A2 in the Bering Sea in August 2014 (24), and the predominance of low abundances of UCYN-A2 throughout the Chukchi and Beaufort Seas in September-October 2015 (25).

The haptophytes associated with both UCYN-A strains were also quantified using qPCR. The UCYN-A1 host was not detected at any station or depth, but there is some evidence that the qPCR assay used in this study may not target a well-conserved region of the 18S rRNA gene (26). Further research is required to verify the 18S rRNA gene sequence of the UCYN-A1 host, but we can be confident that it is associated with a haptophyte that is very closely related to the host identified by based on the results from CARD-FISH analyses. The UCYN-A2 host was detected in 5 surface samples with an average of $1.1x10^4$ *nifH* copies L^{-1} (n=21). Consistent with reports from the western tropical South Pacific (26), the ratio of symbionts:host based on *nifH*:18S rRNA gene counts were greater than 1; the symbionts were detected in more stations than their respective hosts.

UCYN-A found primarily in late season melt water – Temperatures ranged from -1.6^{o} C to 10.2^{o}C, salinity ranged from 26.5 to 33.5 for the whole water column, and nitrate was below detection in all surface samples but one. Ice floes were present at many of the sampling locations, mostly in on the Chukchi Shelf, which created a low density freshwater layer on the surface of the water (<1 m). The depth of the chlorophyll maximum ranged from 17 to 38 m with an average of 28.4 m, but at certain stations disappeared entirely due to full mixing of the water column. UCYN-A was found, either in *nifH* libraries or via qPCR (or both), in samples that span the entire range of temperature and salinity. However, UCYN-A was primarily detected in surface water samples. No linear correlations were found between UCYN-A abundances and environmental factors presented. However, it is clear that UCYN-A was often found in late season melt water (27), based on temperature and salinity plots (Fig. S4). It must be noted, however, that this may be a result of sampling bias given that the majority of surface samples occurred in this water mass. The highest abundances of UCYN-A were found in the only sample in the Alaskan Coastal Current, which has been proposed to be the current by which subtropical UCYN-A populations are advected from the North Pacific to the Bering and Chukchi Seas (25).

Verification of active N_2 fixation in UCYN-A using CARD-FISH/nanoSIMS - NanoSIMS analysis allows the direct measurement of ^{15}N uptake by individual UCYN-A cells. This unique method of measuring N_2 fixation rates enables making a direct link between the amount of ^{15}N uptake and the identity of the cell responsible for that uptake. Sequencing or qPCR data can be used to find the most probable diazotroph(s) responsible for observed rates based on abundances or

transcripts, however, presence or transcription of *nifH* alone does not verify that a given cell is fixing N_2.

We used nanoSIMS to measure ^{15}N uptake into individual cells to quantify nitrogen fixation rates. Isotopic enrichment of ^{15}N in host or UCYN-A cells is attributed to N_2 fixation by UCYN-A, as the host lacks the ability to fix N_2, but the transfer of UCYN-A derived fixed N to the host cell and host-derived fixed C to the UCYN-A cell has been well established (28-30). Eight individual UCYN-A1/haptophyte symbioses from the Bering Sea (station 1) were measured, and symbioses had an average cell specific nitrogen fixation rate of 7.6 $\pm$ 14.5 fmol N cell^{-1}d^{-1}. A total of six UCYN-A2/haptophyte symbioses from the Bering Sea were analyzed with an average cell specific rate of 13.0 $\pm$ 7.7 fmol N cell^{-1}d^{-1}. A total of six UCYN-A2/haptophyte symbioses from the Chukchi Sea (station 2) were analyzed, two were above the limit of detection and resulted in an average cell specific nitrogen fixation rate of 1.1 $\pm$ 1.95 fmol N cell^{-1}d^{-1}. Summarized in Table S3.

The UCYN-A1 cell specific rates found here are similar to cell specific rates found in the tropical north Atlantic of 0.45 - 12 fmol N cell^{-1} d^{-1} (29, 30). To our knowledge these are the first reported cell-specific rates for the UCYN-A2/haptophyte symbioses. Rates for UCYN-A/haptophyte symbioses that were larger than the UCYN-A1/haptophyte symbioses were reported (31), but these are likely to be a third strain, UCYN-A3, based on their size class

(intermediate between UCYN-A1 and UCYN-A2 symbioses) and the emerging biogeography of the UCYN-A2 symbioses (13).

Based on UCYN-A abundances cell-specific N_2 rates were extrapolated to volumetric N_2 fixation rates (Table S3). UCYN-A1 in the Bering Sea (station 1) could be responsible for fixing up to 9.3 ± 17.6 nmol N l^{-1} d^{-1} in the surface water. UCYN-A2 abundances at the same station in the Bering Sea (station 1) could result in 1.2 ± 0.7 nmol N L^{-1} d^{-1}. The bulk nitrogen fixation rate in the surface waters of the Bering Sea was measured at 6.8 ± 3.8 nmol N L^{-1} d^{-1}. Extrapolated UCYN-A rates from nanoSIMS data can account for up to 100% of particulate bulk nitrogen fixation rates in the Bering Sea. UCYN-A2 abundances in the Chukchi Sea result in volumetric N_2-fixation rates of up to 0.004 ± 0.007 nmol N L^{-1} d^{-1}. Comparing these values to the bulk particulate N_2 fixation at this station (0.2 ± 0.2 nmol N L^{-1} d^{-1}, DNQ), other N_2-fixing organisms could be responsible for the average bulk N_2-fixation measured at this location. Interestingly, UCYN-A2 *nifH* sequences at this station (station 2) accounted for almost half (46%, Table S2) of the total *nifH* sequences recovered, and from sequence data alone UCYN-A could have been presumed as (one of) the primary contributors to N_2 fixation, showing the importance of cell-specific analysis.

Supplemental Figures S1 - S4

Figure S1. Station Map. Stations from the cruise in 2016, stations are organized from the Bering Sea to the Chukchi Sea and Beaufort Sea, from nearshore to offshore.

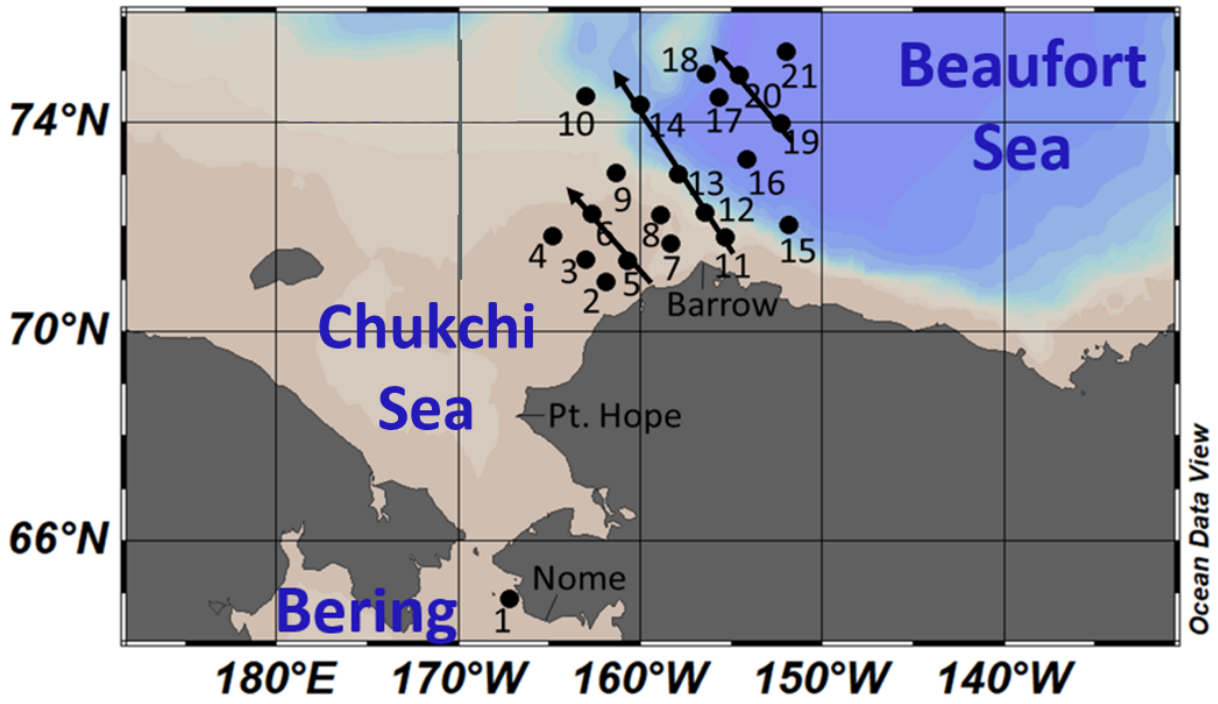


Figure S2. UCYN-A *nifH* oligotype distributions. Stations numbered according to the above station map. The dominant UCYN-A1 (oligo1) is shown in blue and the dominant UCYN-A2 (oligo3) is shown in black.

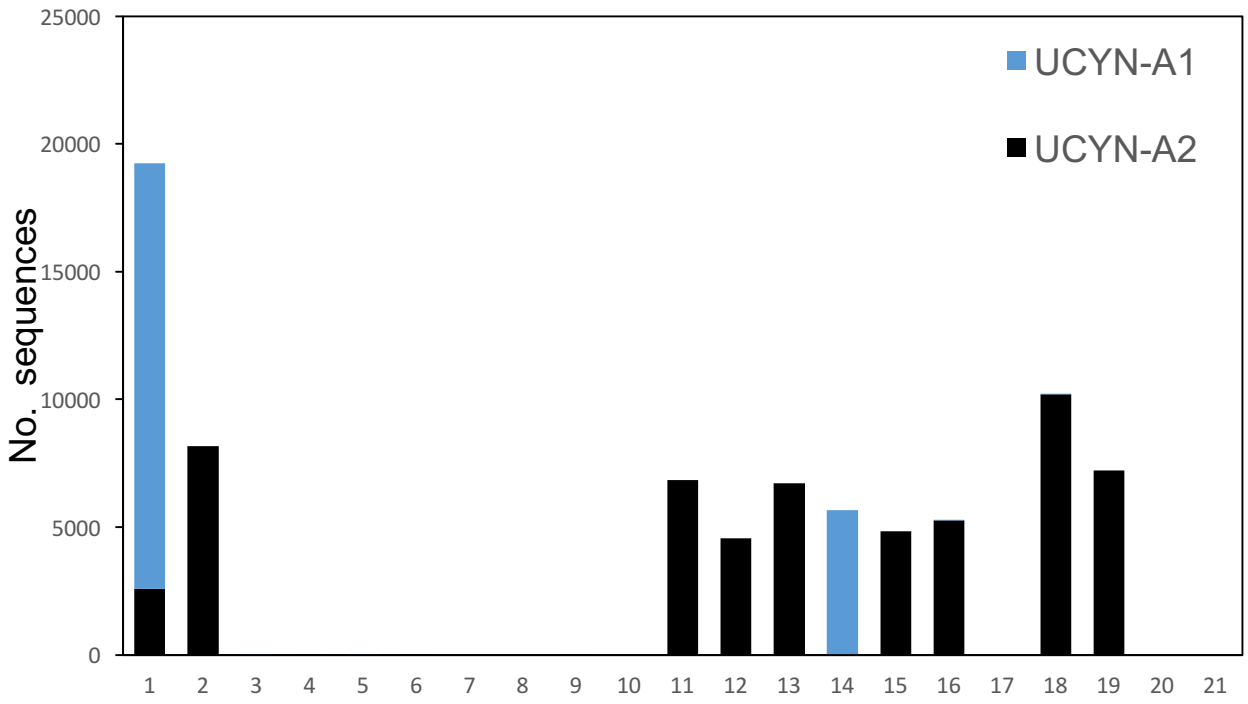


Figure S3. UCYN-A qPCR data. UCYN-A strain abundances and distribution in surface samples by qPCR. UCYN-A1 (blue) and UCYN-A2 (black) are present in the Bering Sea, Chukchi Sea, on the Chukchi Shelf and the Beaufort Sea. Error bars represent standard deviation of technical replicates. Bars below dashed line are sample below the limit of quantification, given the minimum value for visualization purposes.

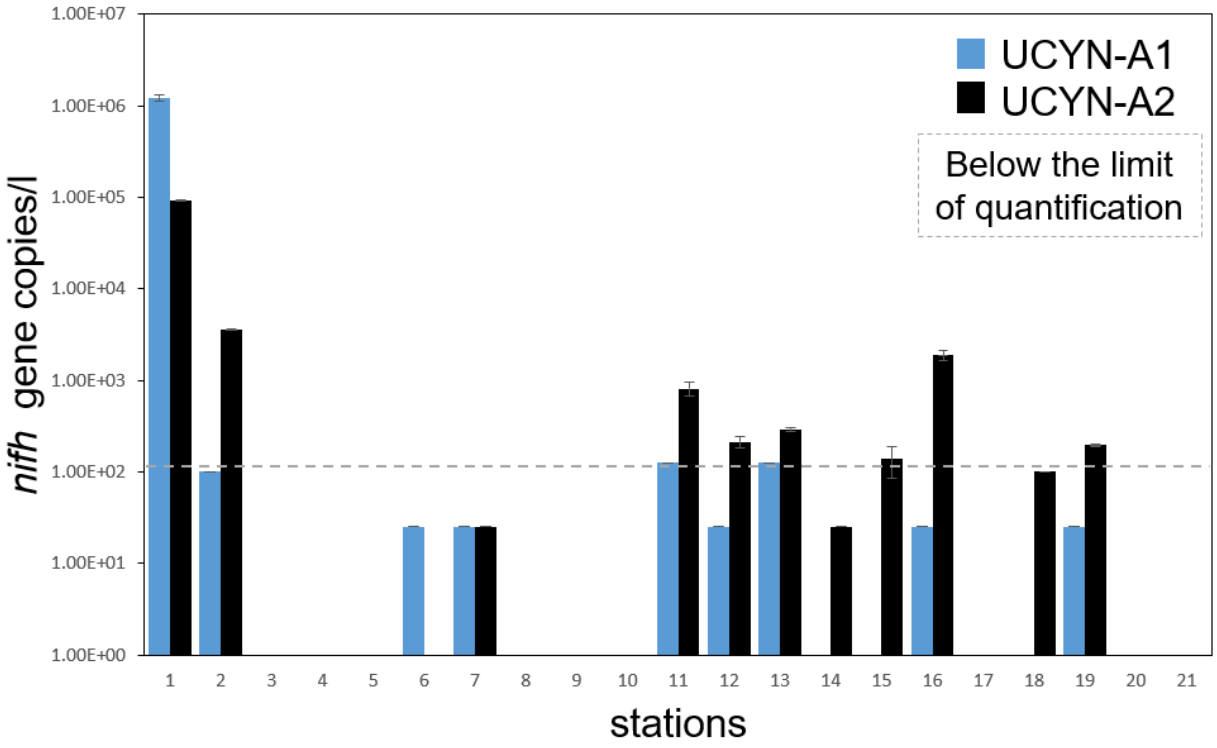
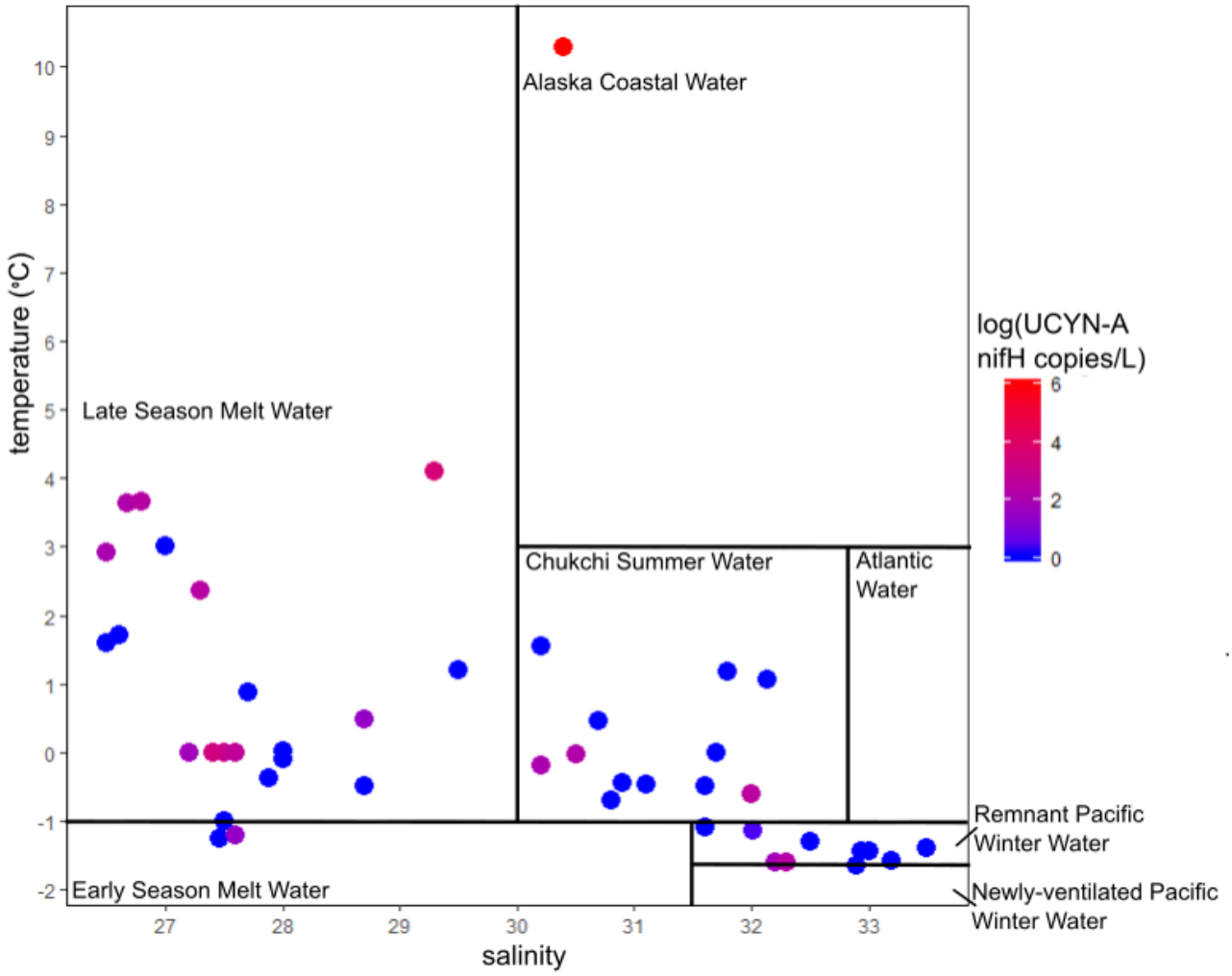


Figure S4. UCYN-A relationship to T/S. Water masses are defined as in (27). Water samples from all depths categorized by temperature and salinity. Color gradient of points indicates total UCYN-A (UCYN-A1 + UCYN-A2) abundances determined by qPCR.



Supplemental Tables S1 – S3

Table S1. Double CARD-FISH probes and oligonucleotide competitors and helpers used to visualize morphologies and target for nanoSIMS analysis. The last column indicates the

presence (Y) or absence (N) of Horseradish-peroxidases (HRP) conjugated to the CARD-FISH probes.

Probe	**Target**	**Sequence (5' to 3')**	**Reference**	**HRP?**
UCYN–A1 732	Unicellular cyanobacteria UCYN-A1	GTTACGGTCCAGTAGCAC	(29)	Y
UCYN–A2 732	Unicellular cyanobacteria UCYN-A2, used as competitor	GTT**G**CGGTCCAGTAGCAC	(18)	Y
UPRYM-69	UCYN-A1 host	CACATAGGAACATCCTCC	(18)	Y
UBRADO-69	UCYN-A2 host	CACATTGGAACATCCTCC	(18)	Y
UCYN-A1 competitor	UCYN-A2 probe used as A2 competitor	GTT**G**CGGTCCAGTAGCAC	(18)	N
UCYN-A2 competitor	UCYN-A1 probe used as A2 competitor	GTTACGGTCCAGTAGCAC	(18)	N
Helper A–732	Unicellular cyanobacteria UCYN-A	GCCTTCGCCACCGATGTTCTT	(29)	N
Helper B–732	Unicellular cyanobacteria UCYN-A	AGCTTTCGTCCCTGAGTGTCA	(29)	N
UPRYM-69 competitor	UCYN-A2 host, used as competitor	CACAT**T**GGAACATCCTCC	(18)	N
UBRADO-69 competitor	UCYN-A1 host, used as competitor	CACAT**A**GGAACATCCTCC	(18)	N
Helper A-PRYM	*Haptophyta*	GAAAGGTGCTGAAGGAGT	(18)	N
Helper B-PRYM	*Haptophyta*	AATCCCTAGTCGGCATGG	(18)	N

Table S2. Table summarizing stations sampled. Station numbers according to station map (Fig. S1) repeating station numbers are different depths sampled. Depths between 17 to 38 m correspond to the location of the chlorophyll maximum at that station. Location corresponds to the sea or hydrographical location of the sample, shelf corresponds to stations above the Chukchi Shelf but not in the Chukchi Sea proper. UCYN-A abundances were determined by qPCR. Relative % UCYN-A *nifH* sequence, is the total UCYN-A (UCYN-A1 + UCYN-A2) *nifH* sequences as a percent of the total number of *nifH* sequences. Bulk N_2 fixation rates, standard deviation of the rate, limit of detection (LOD) and minimum quantifiable rate (MQR) were calculated as described in methods as the sum of values 3 μm and 0.3 μm size fractions. In the

$^{15}N_2$ fixation column, ‘x’ indicates no bulk measurement made at station, ND (not detected) shows rate was below the limit of detection. If the rate was above detection but below minimum quantifiable rate, rate is included but marked with DNQ, (detected, not quantified). Enrichment of $^{15}N_2$ in incubations, as determined by MIMS, is shown as the average of triplicate incubations for each station.

Station #	Latitude	Longitude	sample depth (m)	location	salinity	temp (°C)	UCYN-A1 nifH copies/L	UCYN-A1 standard dev.	rel. % UCYN-A *nifH* gene seqs.	15N2 fixation (nmol N/l day)	15N2 fix St.Dev.	MQR	LOD	15N2 At% enrichment
1	64.9	-167.2	2	Bering	30.4	10.3	1.2E+06	8.4E+04	34	6.85	3.84	4.36	0.32	3.14
2	72.0	-164.6	2	Chukchi	29.3	4.1	1.0E+02	0.0E+00	45	0.17 (DNQ)	0.19	0.23	0.09	7.23
3	73.7	-164.0	2	Chukchi	28.5	-0.1	0.0E+00	0.0E+00	0	0.14	0.08	0.07	0.13	7.37
4	71.8	-164.8	2	shelf	27.7	0.9	0.0E+00	0.0E+00	0	0.23 (DNQ)	0.21	0.24	0.13	4.73
5	73.2	-161.0	2	Chukchi	27.5	-1.3	0.0E+00	0.0E+00	0	ND	-	0.09	0.18	3.83
6	72.2	-162.7	2	shelf	28.7	0.5	2.5E+01	0.0E+00	0	0.53	0.34	0.46	0.13	4.47
7	71.7	-158.4	2	shelf	27.2	0.0	2.5E+01	0.0E+00	0	0.25 (DNQ)	0.35	0.49	0.11	4.17
8	72.2	-158.9	2	shelf	27.5	-1.0	0.0E+00	0.0E+00	0	0.14 (DNQ)	0.14	0.22	0.06	6.77
9	73.0	-161.4	2	shelf	28.0	0.0	0.0E+00	0.0E+00	0	0.37 (DNQ)	0.24	0.59	0.16	4.13
10	74.5	-163.0	2	shelf	27.9	-0.4	0.0E+00	0.0E+00	0	ND	-	0.11	0.05	10.93
11	71.8	-155.4	2	shelf	27.5	0.0	1.3E+02	0.0E+00	0	0.42	0.14	0.19	0.07	7.47
12	72.3	-156.4	2	shelf	27.3	2.4	2.5E+01	0.0E+00	31	0.27	0.08	0.12	0.05	6.70
13	73.0	-157.9	2	Beaufort	27.6	0.0	1.3E+02	0.0E+00	0	1.19	1.38	1.15	0.09	5.93
14	74.3	-160.0	2	Beaufort	27.6	-1.2	0.0E+00	0.0E+00	0	0.70 (DNQ)	0.62	0.98	0.17	3.40
15	72.0	-151.8	2	Beaufort	26.7	3.6	0.0E+00	0.0E+00	0	0.14 (DNQ)	0.11	0.16	0.05	6.20
16	73.3	-154.1	2	Beaufort	27.4	0.0	2.5E+01	0.0E+00	0	0.37	0.19	0.17	0.08	8.13
17	74.5	-155.7	2	Beaufort	26.7	3.0	0.0E+00	0.0E+00	0	0.53	0.22	0.28	0.07	4.97
18	74.9	-156.4	2	Beaufort	26.5	2.9	0.0E+00	0.0E+00	0	0.39	0.23	0.22	0.04	5.27
19	74.0	-152.3	2	Beaufort	26.8	3.7	2.5E+01	0.0E+00	0	0.40	0.17	0.14	0.06	5.20
20	74.9	-154.6	2	Beaufort	26.5	1.6	0.0E+00	0.0E+00	0	0.28	0.15	0.19	0.04	4.00
21	75.4	-152.0	2	Beaufort	26.6	1.7	0.0E+00	0.0E+00	0	0.20	0.09	0.17	0.03	5.13
2	72.0	-164.6	27	Chukchi	31.80	1.2	0.0E+00	0.0E+00	0	0.42 (DNQ)	0.42	0.45	0.09	5.70
3	73.7	-164.0	25	Chukchi	32.00	-1.3	0.0E+00	0.0E+00	0	0.21	0.16	0.20	0.20	7.47
4	71.8	-164.8	24	shelf	32.10	1.1	0.0E+00	0.0E+00	0	0.49	0.28	0.36	0.14	5.87
5	71.4	-160.7	20	Chukchi	32.00	-1.1	0.0E+00	0.0E+00	0	x	x	x	x	x
6	72.2	-162.7	22	shelf	28.70	-0.5	0.0E+00	0.0E+00	0	0.57	0.35	0.46	0.21	4.20
7	71.7	-158.4	27	shelf	30.80	-0.7	0.0E+00	0.0E+00	0	0.24 (DNQ)	0.41	0.73	0.13	4.70
8	72.2	-158.9	26	shelf	31.70	0.0	0.0E+00	0.0E+00	0	0.14 (DNQ)	0.18	0.18	0.07	6.83
9	73.0	-161.4	29	shelf	31.60	-0.5	0.0E+00	0.0E+00	0	ND	-	0.41	0.12	5.77
10	74.5	-163.0	17	shelf	29.50	1.2	0.0E+00	0.0E+00	0	0.23	0.13	0.14	0.05	10.97
11	71.8	-155.4	23	shelf	30.50	0.0	0.0E+00	1.5E+02	44	0.41	0.19	0.22	0.04	6.20
12	72.3	-156.4	38	shelf	31.60	-1.1	0.0E+00	0.0E+00	0	0.17	0.06	0.07	0.04	6.13
13	73.0	-157.9	34	Beaufort	32.20	-1.6	0.0E+00	5.0E+01	0	0.39	0.42	0.39	0.05	4.97
14	74.3	-160.0	33	Beaufort	32.30	-1.6	2.0E+02	0.0E+00	17	1.39	0.64	0.57	0.05	2.37
15	72.0	-151.8	35	Beaufort	30.90	-0.5	0.0E+00	0.0E+00	0	0.13	0.08	0.12	0.03	6.80
16	73.3	-154.1	25	Beaufort	30.20	-0.2	0.0E+00	1.0E+02	3	0.25	0.13	0.12	0.20	7.17
17	74.5	-155.7	28	Beaufort	30.20	0.5	0.0E+00	0.0E+00	0	0.87	0.31	0.34	0.14	5.20
18	74.9	-156.4	35	Beaufort	30.20	1.5	0.0E+00	0.0E+00	0	0.33 (DNQ)	0.17	0.70	0.05	5.60
19	74.0	-152.3	35	Beaufort	30.70	0.5	0.0E+00	0.0E+00	0	0.33	0.28	0.29	0.05	6.30

Station 1 $^{15}N_2$-fixation rate represents replicate samples, not triplicate. Only the 0.3 µm size fraction was measured for $^{15}N_2$-fixation rates at Station 3 at both depths listed.

Table S3. Volumetric calculations of UCYN-A derived N_2 fixation rates. Summary table comparing nanoSIM measured cell-specific N_2 fixation rates, calculated volumetric rates (from cell-specific N_2 fix rates and qPCR abundances) and bulk particulate N_2 fixation rates.

Sample	UCYN-A specific N_2 fix (fmol N cell^{-1}day^{-1})	*nifH* gene copies l^{-1}	UCYN-A total N_2 fix rate (nmol N l^{-1} d^{-1})	measured bulk N_2 fix rate (nmol N l^{-1} d^{-1})
UCYN-A1 Bering	7.6	$1.2x10^6$	9.3 ± 17.7	6.9 ± 3.8
UCYN-A2 Bering	13.0	$9.2x10^4$	1.2 ± 0.7	
UCYN-A2 Chukchi	1.1	$3.6x10^3$	0.004 ± 0.006	0.17 ± 0.19

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