Abundance and distribution of diazotrophs determined via qPCR from R/V Kilo Moana KM0703, R/V Seward Johnson SJ0609 in the tropical and subtropical SW Pacific and tropical N Atlantic from 2006-2007 (DIAZOTROPHS project)

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Project
» Biology and Ecology of Newly Discovered Diazotrophs in the Open Ocean (DIAZOTROPHS)

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Dataset Description

Abundance and distribution of diazotrophs determined via qPCR

Methods & Sampling

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Data Processing Description

BCO-DMO Processing Notes
Generated from original spreadsheet contributed by Kendra Turk: "Diazo_Distribution_Zehr_forBCO-DMO.xlsx"

BCO-DMO Edits
- CRUISE (cruise ids) inserted
- Parameter names modified to conform to BCO-DMO convention
- "nd" (BCO-DMO flag for no data) inserted into blank fields
- data submitted as seperate cruises combined into one dataset

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Data Files

File
Diazo_Distr.csv (Comma Separated Values (.csv), 40.70 KB) MD5:a85f95ad719bc67136955a4cf1778843 Primary data file for dataset ID 3376

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Parameters

Parameter	Description	Units
Cruise	Cruise Id	text
Sample_ID	Unique sample identifier	integer
Station	Station identifier	integer
Cast	CTD cast	integer
Bottle	Niskin bottle sampled	integer
Depth	Depth of water sample	meters
lon	longitude; negative denotes West	decimal degrees
lat	latitude; negative denotes South	decimal degrees
Filter_Size	size fraction associated with sample in microns (um); if 10 then data refers to the size fraction >10 microns; if 0.2 then the data refers to the size fraction between 0.2 and 10 microns	um
UCYN_A_nifH_copies	number of nifH genes per liter of seawater from uncultivated unicellular cyanobacteria group A (UCYN-A) as determined using qPCR; undetected (UD) or detected not quantified (DNQ)	L-1
UCYN_B_nifH_copies	number of nifH genes per liter of seawater from unicellular cyanobacteria group B (UCYN-B); Crocosphaera; as determined using qPCR	L-1
Trichodesmium_nifH_copies	number of nifH genes per liter of seawater from filamentous; non-heterocystous cyanobacteria Trichodesmium as determined using qPCR; undetected (UD) or detected not quantified (DNQ)	L-1
RR_het_1_nifH_copies	number of nifH genes per liter of seawater from heterocystous cyanobacteria Richelia associated with Rhizosolenia as determined using qPCR; undetected (UD) or detected not quantified (DNQ)	L-1
HR_het_2_nifH_copies	number of nifH genes per liter of seawater from heterocystous cyanobacteria Richelia associated with Hemiaulus as determined using qPCR; undetected (UD) or detected not quantified (DNQ)	L-1
CC_het_3_nifH_copies	number of nifH genes per liter of seawater from heterocystous cyanobacteria Calothrix associated with Chaetoceros as determined using qPCR; undetected (UD) or detected not quantified (DNQ)	L-1

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Instruments

Dataset-specific Instrument Name	CTD Sea-Bird SBE 911plus
Generic Instrument Name	CTD Sea-Bird SBE 911plus
Generic Instrument Description	The Sea-Bird SBE 911 plus is a type of CTD instrument package for continuous measurement of conductivity, temperature and pressure. The SBE 911 plus includes the SBE 9plus Underwater Unit and the SBE 11plus Deck Unit (for real-time readout using conductive wire) for deployment from a vessel. The combination of the SBE 9 plus and SBE 11 plus is called a SBE 911 plus. The SBE 9 plus uses Sea-Bird's standard modular temperature and conductivity sensors (SBE 3 plus and SBE 4). The SBE 9 plus CTD can be configured with up to eight auxiliary sensors to measure other parameters including dissolved oxygen, pH, turbidity, fluorescence, light (PAR), light transmission, etc.). more information from Sea-Bird Electronics

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

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Deployments

KM0703

Website	https://www.bco-dmo.org/deployment/58016
Platform	R/V Kilo Moana
Report	http://www.rvdata.us/catalog/KM0703
Start Date	2007-03-14
End Date	2007-04-18
	The cruise began in Townsville, Australia and sampled the Coral Sea, a transect southward toward the Tasman Sea, and a transect northward toward New Caledonia, with twelve hydrostations (001-012). It then made a run eastward to 170 deg W, a northward run to 15 deg S, then a transect to the east before ending in Suva, Fiji after carrying out fourteen stations (013-026). Cruise information and original data are available from the NSF R2R data catalog. **Methods & Sampling** Materials and Methods Water samples were collected from the R/V Kilo Moana (cruise KM0703) in 18 March - 14 April, 2007. Quantification of diazotrophs. Diazotroph abundances were determined using quantitative PCR (qPCR) targeting the nifH gene. Samples for DNA were collected with Niskin bottles from 8 depths at each station. Four to five liters of water was filtered first through 10 um (Osmonics) and next through 0.2 um Supor (Pall-Gelman) filters using a peristaltic pump. Filters were placed in bead beater tubes with 0.1 g sterile glass beads and immediately frozen in liquid N. Nucleic acid samples were kept in liquid N or liquid N fumes during the cruise and transportation to the University of California Santa Cruz where they were

Description	stored at –80ºC. DNA was extracted using a modified Qiagen Plant Minikit protocol (S1). A new qPCR primer-probe set (pps) was designed, tested and applied targeting Crocosphaera watsonii. The pps was designed using Primer Express software (Applied Biosystems). The pps has 100% nucleotide identity with C. watsonii WH8501 draft genome and an environmental clone DQ118216 (clone HT3312A10) from station ALOHA in the North Pacific Ocean. Specificity of the new primer probe set was tested against plasmid dilution series of 1-107nifH copies from other diazotroph groups including UCYN-A, Trichodesmium, symbiotic heterocystous cyanobacteria Het-1, Het-2, and Het-3. None of these diazotrophs were detected by the Crocosphaera primer probe set and all no template controls (blanks) were negative. QPCR TaqMan primer-probe sets from previous studies were used to target UCYN-A, the filamentous cyanobacterium Trichodesmium spp., heterocystous filamentous cyanobacterium Richelia associated with the diatom Rhizosolenia (Het-1) (S2-3), Richelia symbiont associated with the diatom Hemiaulus (Het-2) (S4-5), and a γ-Proteobacterial diazotroph for clone 24774A11 (GenBank accession number EU052413) (probe γ-Prot 24774A11) (S1). The probes were 5'FAM and 3'TAMRA labeled (Sigma Genosys). For qPCR template, DNA from 0.2-10 um size fraction was either diluted 1:10 (vol:vol) or used undiluted, and DNA extracted from the >10 um size fraction was used undiluted as the template. The 0-10 um size fraction samples were processed for UCYN-A, Crocosphaera, and y-Prot 24774A11, and all other targets except UCYN-A and γ-Prot 24774A11 were detected in >10 um size fraction samples. Gene copy abundances of Crocosphaera in the two size fractions were pooled to obtain total abundance. Three surface samples (5, 15, and 30 m) from station 25 were omitted as outliers in the Crocosphaera dataset (total n=98); low density surface water lens influenced vertical distributions at this station. Two uL of template DNA was added into the reaction mix at a 25 uL final volume that consisted of 12.5 uL ABI TaqMan Gene Expression mix, forward and reverse primers at 0.4 uM final concentration, and 0.2 uM final concentration of the TaqMan probe, and the volume was adjusted to 25 uL with water. Standards were included in duplicate with each set of samples run on the qPCR instrument, and composed of a dilution series with a range of 1 to 107nifH gene copies of linearized plasmid (pGEM-T, Promega) with the target insert. Four no template controls were run with each set of samples. Inhibition tests were carried out for each DNA sample by adding to the reaction mix two ul sample and two µl of standard containing 104 or 105 gene copies. QPCR was carried out using an ABI 7500 Real time PCR instrument (Applied Biosystems). The qPCR run conditions were 2 min at 50°C, then 45 cycles of 15 s at 95°C and 1 min at 60°C. The number of gene copies per sample was calculated as described in Short and Zehr (S6). One gene copy per reaction was determined as the limit of detection and 8 gene copies per reaction was determined as a limit of quantification (DNQ, "detected but not quantifiable"). In data analysis, DNQ was replaced with 1 gene copy L-1. In regression analyses, values from the upper mixed layer (UML) were included. Determination of the bottom of the UML is approximate, therefore values below UML were also included if UCYN-A or Crocosphaera abundance was in the same order of magnitude or higher than that in the UML. **Processing Description** BCO-DMO Processing Notes Generated from original spreadsheet contributed by Kendra Turk "Diazo_Distribution_Zehr_forBCO-DMO.xlsx", tab: KM0703 nifH qPCR DNA BCO-DMO Edits - CRUISE (cruise id) inserted - Parameter names modified to conform to BCO-DMO convention - "nd" (BCO-DMO flag for no data) inserted into blank fields

SJ0609

Website	https://www.bco-dmo.org/deployment/58017
Platform	R/V Seward Johnson
Start Date	2006-06-18
End Date	2006-07-31
	Leg 1 of the cruise began in Ft. Pierce FL with a rapid transit to Bridgetown, Barbados and two hydrostations (001-002) en route. Leg 2 extended from Barbados to Mindelo, Cape Verde, with nine hydrostations (003-010, 012). Leg 3 included a run south to the equator, then northwestward to Barbados with eleven hydrostations (013-023). **Methods & Sampling**

Description	Samples were collected during a cruise of the R/V Seward Johnson from 18 June to 25 July 2006. The cruise originated in Fort Pierce, Florida (27.44°N, 80.34°W), transited southeast to Barbados (13.05°N, 59.30°W), then east across the Atlantic Ocean to the Cape Verde Islands (15.02°N, 23.34°W), southwest to sampling station 17 at the equator (0.08°N, 34.99°W), and northwest to Barbados (see figure). Over 150 seawater samples were collected at stations 1 through 23 along the cruise track using Niskin bottles mounted on a conductivity-temperature-depth (CTD) rosette sampler. At each station, seawater samples were collected at 8 depths between 5 and 200 m to determine concentrations of chl a, inorganic nutrients and specific cyanobacterial nifH phylotypes. N2 fixation rates of small diazotrophs were measured at four depths at all stations but 6, 7 and 22. Fewer depths were sampled at stations 2, 22 and 23, and no samples were collected at station 11. **Processing Description** DNA extraction The Niskin bottles were drained into acid washed polycarbonate bottles, and then the seawater was filtered through sterile polypropylene filter holders using a peristaltic pump. Two litres of seawater from each depth were serially filtered through a 25 mm diameter, 10 um pore-size polyester filter (GE Osmonics, Minnetonka, MN) and a 25 mm, 0.2 um poresize Supor filter (Pall Corporation, Port Washington, NY, USA). Each filter was then stored in a polypropylene microcentrifuge tube, which contained 500 ul of Tris EDTA buffer (Ambion, Foster City, CA, USA) plus an approximate 0.2 g mixture of 0.1 mm and 0.5 mm diameter autoclaved glass beads (BioSpec Products, Bartlesville, OK, USA). The samples were immediately flash frozen in liquid nitrogen and subsequently stored frozen at -80°C until processed for nucleic acid extraction. DNA was extracted from the samples using the modified DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) protocol detailed in Moisander and colleagues (2008). However, during the final elution step, the samples were eluted twice with 25 ul of Buffer AE instead of 100 ul. The extracted DNA was stored at -20°C. Quantitative PCR A qPCR method using a TaqMan 5'-fluorogenic exonuclease assay was used to investigate the abundance and distribution of several nifH cyanobacterial sequence types, called nifH phylotypes. Probes were 5' labelled with the fluorescent reporter FAM (6-carboxyfluorescein) and 3' labelled with the quenching dye TAMRA (6-carboxytetramethylrhodamine). TaqMan oligonucleotide primers and specific fluorogenic probes were used to target the nifH gene of three unicellular cyanobacteria (groups A, B and C, also known as UCYN-A, UCYN-B and UCYN-C) and Trichodesmium spp., and the host-specific nifH sequences of three diatom-cyanobiont symbioses (H-R, R-R and C-C) (Table 4) (Church et al., 2005a; Church et al., 2005b; Foster et al., 2007). The cyanobionts associated with each type of diatom have been designated, based of nifH sequences, as het-1 (H-R), het-2 (R-R) and het-3 (C-C). The primer and probe sets for the diatom-cyanobiont symbioses were used to analyse extracts from the > 10 um size fraction of each sample, whereas the primer and probe sets for unicellular cyanobacteria were used to analyse extracts from the < 10 um size fraction (0.2 um to 10 um). The Trichodesmium primer and probe sets were run on extracts from both size fractions of each sample. The qPCR reactions were prepared in 96-well optical reaction plates with optical caps (Applied Biosystems, Foster City, CA, USA) and run on a ABI 7500 Real-time PCR System (Applied Biosystems) with the following thermocycling settings: 50°C for 2 min, 95°C for 10 min, and then 45 cycles of 95°C for 15 s followed by 60°C for 1 min. The sample reactions (25 ul) were run in triplicate and contained 12.5 ul of 2 x TaqMan Mastermix (Applied Biosystems), 8 ul of 5 kD filtered nuclease-free water (Ambion), 1 ull each of the forward and reverse primers (0.4 uM final concentration), 0.5 ul of fluorogenic TaqMan probe (0.2 uM final concentration) and 2 ul of template DNA (ranging from 0.05 to 161.27 ng DNA). Controls without any DNA target (no template controls) contained 2 ul of 5 kD filtered nuclease-free water (Ambion) instead of the template DNA, and were run on each plate to check for contamination. Linearized recombinant plasmids containing the nifH gene targets were used as standards for qPCR by making a dilution series covering 8 orders of magnitude (100–107 nifH gene copies per reaction), and the full series was run on each plate. The nifH gene copies l-1 were calculated for each nifH target set using linear regression parameters fit to a plot of cycle threshold (Ct) versus log gene copy for the standards run on each plate. The average efficiencies of the qPCR reactions for each of the assays were 102 ± 2% for UCYN-A, 102 ± 3% for UCYN-B, 98 ± 3% for Trichodesmium sp., 93 ± 3% for H-R and 101 ± 3% for R-R. Each sample was tested for inhibition using one primer/ probe set by spiking the qPCR reaction with a standard of 104 nifH gene copies per reaction, and determining the per cent inhibition using the following formula 1 - [(Ct,sample - Ct,standard)/ Ct,standard] x 100. In the few cases (less than 2% of the samples) where inhibition was observed, the sample was serially diluted until the addition of template DNA did not cause inhibition. In a majority of these cases, the extent of inhibition was minor, as non-inhibited amplification was observed after 10-fold

	dilutions. The LOD and limit of quantification (LOQ) have been determined empirically to be 1 and 8 nifH gene copies per reaction, respectively (data not shown). Taking into consideration the qPCR reaction volume, volume of nucleic acid extractions, and the volume of seawater filtered, this translates to a LOD of 10 nifH gene copies l-1 seawater and LOQ of 80 nifH gene copies l-1 seawater for a majority of the samples. LODs and LOQs are considerably higher for samples that needed to be diluted. Samples where amplification was observed, but the detected signal fell below the LOQ, were designated as 'detected not quantified' (DNQ). In order to calculate the depth-integrated nifH gene copies m-2, a sample that was below the LOD was considered to have zero nifH gene copies l-1, and a DNQ sample was assumed to have one nifH gene copy l-1. Table 4. Oligonucleotide TaqMan primers and probes used to detect the nifH phylotypes, and the corresponding target base region. Target Forward primer (5'-3') Probe Reverse primer (5'-3') UCYN-Aa AGCTATAACAACGTTTTATGCGTTGA TCTGGTGGTCCTGAGCCTGGA ACCACGACCAGCACATCCA 106–131 133–153 1 56–174 UCYN-Ba TGGTCCTGAGCCTGGAGTTG TGTGCTGGTCGTGGTAT TCTTCTAGGAAGTTGATGGAGGTGAT 138–157 160–176 178–203 UCYN-Cb ATACCAAGGAATCAAGTGTGTTGAGT CGGTGGTCCCGAGCCTGGAG ACCACGACCAGCACATCCA 106–124 133–153 156–174 H-Rb TGGTTACCGTGATGTACGTT TCTGGTGGTCCTGAGCCTGGTGT AATGCCGCGACCAGCACAAC 106–124 133–155 158–177 R-Ra CGGTTTCCGTGGTGTACGTT TCCGGTGGTCCTGAGCCTGGTGT AATACCACGACCCGCACAAC 105–124 133–155 158–177 C-Cb CGGTTTCCGTGGCGTACGTT TCTGGTGGTCCAGAACCTGGTGT AATACCACGACCAGCACAAC 106–124 133–155 133–155Trichodesmiuma GACGAAGTATTGAAGCCAGGTTTC CATTAAGTGTGTTGAATCTGGTGG CGGCCAGCGCAACCTA TCCTGAGC 217–241 246–278 284–300 The probes were 5' labelled with the fluorescent reporter FAM and 3' labelled with the quenching dye TAMRA. a. Primer and probe designed by Church et al., 2005a. b. Primer and probe designed by Foster et al., 2007. BCO-DMO Processing Notes Generated from original spreadsheet contributed by Kendra Turk "Diazo_Distribution_Zehr_forBCO-DMO.xlsx", tab: SJ0609 nifH qPCR DNA BCO-DMO Edits - CRUISE (cruise id) inserted - Parameter names modified to conform to BCO-DMO convention - "nd" (BCO-DMO flag for no data) inserted into blank fields

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Project Information

Biology and Ecology of Newly Discovered Diazotrophs in the Open Ocean (DIAZOTROPHS)

Coverage: Tropical and Subtropical Southwest Pacific and tropical North Atlantic

Biology and Ecology of Newly Discovered Diazotrophs in the Open Ocean

The productivity of the oceans is limited by the availability of nutrients,

which has implications for the fluxes of carbon between the atmosphere and

oceans. In a previous award the PIs found that previously unrecognized

N2-fixing unicellular cyanobacteria are active and abundant in oligotrophic

oceans. This finding has important implications for nitrogen cycling in the

oceans and for the role of "new" nitrogen in carbon fixation.

The PIs will address three major issues:

First, there are at least two distinct groups of cyanobacteria that appear

to be separated in space and time, due to unknown ecological variables.

Second, the geographic distribution and factors controlling the distribution are unknown, so it is not clear how these organisms should be included in biogeochemical models.

Finally, one of the groups of cyanobacteria appears to fix N2 during the day, which revives the enigma of simultaneous nitrogen fixation and photosynthesis that was previously limited to discussions of Trichodesmium.

PUBLICATIONS PRODUCED AS A RESULT OF THIS RESEARCH

Burns, J.A., Zehr, J.P., Montoya, J P, Kustka, A.B., and Capone, D. G.. "Effect of EDTA addtiions on natural Trichodesmium spp. (CYANOPHYTA) populations," Journal of Phycology, v.42, 2006, p. 900.

Campbell, L, E.J. Carpenter, J.P. Montoya, A.B. Kustka, D.G. Capone. "Picoplankton community structure within and outside a Trichodesmium bloom in the southwestern Pacific Ocean," Vie et Milieu, v.55, 2005, p. 185.

Capone, D.G., J.A. Burns, J.P. Montoya, A. Subramaniam, C. Mahaffey, T. Gunderson, A.F. Michaels, and E.J. Carpenter. "Nitrogen fixation by Trichodesmium spp.: An important source of new nitrogen to the tropica and subtropical North Atlantic Ocean," Global Biogeochemical Cycles, v.19, 2005, p. doi:10.10.

Holl, C.M. & J.P. Montoya. "Interactions between nitrate uptake and nitrogen fixation continuous cultures of the marine diazotroph Trichodesmium (Cyanophyta)," Journal of Phycology, v.41, 2005, p. 1178.

Holl, C.M., T.A. Villareal, C.D. Payne, T.D. Clayton, C. Hart, J.P. Montoya. "Trichodesmium in the western Gulf of Mexico: 15N2-fixation and natural abundance stable isotope evidence," Limnology and Oceanography, v.52, 2007, p. 2249.

Holl, C.M., Waite, A.M., Pesant, S., Thompson, P, Montoya, J P. "Unicellular diazotrophy as a source of nitrogen to Leeuwin Current coastal eddies," Deep-Sea Research I, v.54, 2007, p. 1045.

Krauk, J.M, T.A. Villareal, J.A. Sohm, J.P. Montoya, and D.G. Capone. "Plasticity of N:P ratios in laboratory and field populations of Trichodesmium spp.," Aquatic Microbial Ecology, v.72, 2006, p. 243.

Montoya, J P, Holl, C.M., Zehr, J.P., Hansen, A., Villareal, T.A., Capone, D.G.. "High rates of N2-fixation by unicellular diazotrophs in the oligotrophic Pacific," Nature, v.430, 2004, p. 1027.

Montoya, J.P., M. Voss, and D.G. Capone. "Spatial variation in N2-fixation rate and diazotroph activity in the Tropical Atlantic," Biogeosciences, v.4, 2007, p. 396.

Subramaniam, A, P.L. Yager, E.J. Carpenter, C. Mahaffey, K. Bjorkman, S. Cooley, A. Kustka, J.P. Montoya, A. Sañudo-Wilhelmy, R. Shipe, and D.G. Capone. "Amazon River enhances diazotrophy and carbon sequestration in the tropical North Atlantic Ocean," Proc. Natl. Acad. Sci, v.105, 2008, p. 10460.

Waite, AM; Muhling, BA; Holl, CM; Beckley, LE; Montoya, JP; Strzelecki, J; Thompson, PA; Pesant, S. "Food web structure in two counter-rotating eddies based on delta N-15 and delta C-13 isotopic analyses," DEEP-SEA RESEARCH PART II-TOPICAL STUDIES IN OCEANOGRAPHY, v.54, 2007, p. 1055-1075. View record at Web of Science

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Funding

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