Discrete raw measurements from upwelling experiments conducted on two phytoplankton species isolated from the California Upwelling Zone

Website: https://www.bco-dmo.org/dataset/826448
Data Type: experimental
Version: 1
Version Date: 2020-10-13

Project
» CAREER: An integrated molecular and physiological approach to examining the dynamics of upwelled phytoplankton in current and changing oceans (Upwelled Phytoplankton Dynamics)

Contributors	Affiliation	Role
Marchetti, Adrian	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Two phytoplankton species, Chaetoceros decipiens (UNC1416) and Emiliania huxleyi (UNC1419), were cultured at 12 °C in artificial Aquil* medium using trace metal clean (TMC) techniques. Macronutrients were supplied such that nitrate would become limiting for growth (50 µmol L^{-1} NO3, 10 µmol L^{-1} PO4, 200 µmol L^{-1} H4SiO4) and two iron treatments were used (1370 nmol L^{-1} and 3.1 nmol L^{-1} total Fe). Upwelling conveyor belt cycle (UCBC) simulations were performed by transitioning the cultures to different nutrient and light regimes. They were grown by first growing the cultures with ample light (115 µmol photons m^{-2} s^{-1}) until nitrate depletion and stationary growth. Cultures were then moved to no light for 10 days to simulate the sinking out of the euphotic zone. After this dark period, the cultures were subsequently transferred back to fresh medium under ambient light levels, and grown until stationary phase again. Samples were collected for six different time points associated with the different phases throughout the simulated upwelling cycle. This dataset includes the treatment information, replicate IDs, and the discrete raw measurements made for the upwelling experiments.

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Coverage

Temporal Extent: 2017-01 - 2017-09

Methods & Sampling

Methodology:
Two phytoplankton species, *Chaetoceros decipiens* (UNC1416) and *Emiliania huxleyi* (UNC1419), were cultured at 12 °C in artificial Aquil* medium using trace metal clean (TMC) techniques. Macronutrients were supplied such that nitrate would become limiting for growth (50 µmol L^{-1} NO_3, 10 µmol L^{-1} PO_4, 200 µmol L^{-1} H_4SiO_4) and two iron treatments were used (1370 nmol L^{-1} and 3.1 nmol L^{-1} total Fe). Upwelling conveyor belt cycle (UCBC) simulations were performed by transitioning the cultures to different nutrient and light regimes. They were grown by first growing the cultures with ample light (115 µmol photons $m^{-2}s^{-1}$) until nitrate depletion and

stationary phase growth. Cultures were then moved to no light for 10 days to simulate the sinking out of the euphotic zone. After this dark period, the cultures were subsequently transferred back to fresh medium under ambient light levels, and grown until stationary phase again. Samples were collected for six different time points associated with the different phases throughout the simulated upwelling cycle.

Sampling:
Cell counts – 5 mL samples was preserved in 2% Lugol's solution in glass vials. Cells were then enumerated on an Olympus CKX41 inverted microscope using a 1 mL Sedgwick-Rafter counting chamber after allowing the cells to settle for five minutes and counting a minimum of 300 cells within 10-30 fields of view.

Chlorophyll – 50 mL of sample was filtered through 0.45 µm mixed cellulose ester filters under gentle vacuum pressure (< 100 mm Hg) and frozen at -80 °C immediately. Extraction was performed using 90% acetone at -20 °C for 24 hours and measured using a Turner 10-AU fluorometer (Parsons et al., 1984)

F_v:F_m – The maximum photochemical yield of photosystem II (F_v:F_m) was measured using the Satlantic FIRe (Gorbunov & Falkowski, 2005; Kolber et al., 1998). Samples were acclimated to low light for 20 minutes prior to the measurements. Data were blank corrected using microwave-sterilized Aquil medium. The resulting F_v:F_m was derived from the induction profile using a saturating pulse (Single Turnover Flash; 20,000 µmol photons $m^{-2}s^{-1}$) for a duration of 100 µs. The gain was optimized for each sample (400, 600, or 800), and the average of 50 iterations was obtained.

Particulate carbon and nitrogen – 50 mL of sample was vacuum-filtered onto pre-combusted GF/F filters. Filters were then immediately stored in Petri dishes at -20 °C, and dried at 65 °C for 24 hours before being pelletized. Total nitrogen and carbon were quantified with a Costech 4010 CHNOS Elemental Combustion system according to U.S. Environmental Protection Agency Method 440.0 (Zimmermann et al., 1997). Three blanks were run alongside the samples and were all below the detection limits (0.005 mg N and 0.071 mg C). Carbon and nitrogen per cell were calculated by dividing particulate carbon and nitrogen concentration by cell concentration.

Dissolved nitrate and nitrite – Filtrate from the 0.45 µm filters used for RNA was transferred to polypropylene tubes and immediately frozen at -80 °C. Dissolved nitrate + nitrite (NO_3 + NO_2) concentrations were quantified with an OI Analytical Flow Solutions IV auto analyzer according to EPA method 353.4 (Zhang et al., 1997). The detection limit for NO_3 + NO_2 was 0.2 µmol L^{-1}.

RNA Extraction – 300 mL of sample was filtered onto 0.45 µm Pall Supor polyethersulfone filters (47 mm) filters using gentle vacuum pressure and immediately frozen and stored at -80 °C. Total RNA was extracted using RNAqueous-4PCR Total RNA Isolation Kit for

Chaetoceros cultures, and TRIzol reagent for *E. huxleyi* cultures. RNA was then purified and examined for quality and quantity using a Nanodrop spectrophotometer.

Analysis: (see related dataset for the NCBI project accession and library information)
RNA libraries were constructed using a custom protocol for 3' poly-A-directed mRNA-seq (also known as TagSeq) based on Meyer et al. (2011) and adapted for Illumina HiSeq based on Lohman et al. (2016) and Strader et al. (2016). For most samples 1 µg but as low as 250 ng of total RNA were fragmented by incubating at 95 °C for 15 min.

First strand cDNA was synthesized with SMARTScribe Reverse Transcriptase (Takara Bio, Mountain View, CA, USA), an oligo-dT primer, and template switching to attach known sequences to each end of the poly-A mRNA fragments. cDNA was then amplified with a PCR reaction consisting of 32 µL sterile H_2O, 5 µL dNTPs (2.5 mM each), 5 µL 10X Titanium Taq Buffer (Takara Bio), 1 µL of each primer (10 µM) designed amplify the sequences attached during cDNA synthesis, and 1 µL of Titanium Taq DNA Polymerase (Takara Bio). The PCR was run with an initial denaturing step at 95 °C for 5 min, then 17 cycles consisting of 95 °C for 1 min, 63 °C for 2 min, and 72 °C for 2 min. cDNA amplification was then verified on a 1% agarose gel and purified with the QiaQuick PCR Purification Kit (Qiagen). cDNA was quantified with the Quant-iT dsDNA High-Sensitivity Assay (Invitrogen) and cDNA concentrations were then normalized to the same volume.

Samples were then barcoded with a PCR reaction consisting of 11 µL sterile H_2O, 3 µL dNTPs (2.5 mM each), 3 µL 10X Titanium Taq Buffer (Takara Bio), 0.6 µL TruSeq Universal Primer (10 µM), 6 µL barcoding primer (1 µM), Titanium Taq Polymerase (Takara Bio), and 6 µL of purified cDNA with an initial denaturing step at 95 °C for 5 min, then 5 cycles consisting of 95 °C for 40 s, 63 °C for 2 min, and 72 °C for 1 min. Samples were barcoded from both ends using unique combinations of 6 bp sequences on both the TruSeq universal primer and barcoding primers. Products were then again confirmed on a 2% agarose gel and combined into small pools of 6-8 samples. The 400-500 bp region of these pools was extracted with the QIAquick Gel Extraction Kit

(Qiagen), quantified with the Quant-iT dsDNA High-Sensitive Assay, and then mixed in equal proportions. The library was sequenced at the University of Texas at Austin Genomic Sequencing and Analysis Facility on Illumina HiSeq 2500 (three lanes, single-end 50bp reads) with a 15% PhiX spike-in to target approximately 8 million reads per sample.

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Data Files

File
discrete_data.csv (Comma Separated Values (.csv), 3.48 KB) MD5:e6f30c4c544e592df1dbd849429da612 Primary data file for dataset ID 826448

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Related Publications

Barwell-Clarke, J. & Whitney, Frank. (1996). IOS nutrient methods and analysis. Can Tech Rep Hydrog Ocean Sci. 182. 1-43.
Methods

Bertrand, E. M., McCrow, J. P., Moustafa, A., Zheng, H., McQuaid, J. B., Delmont, T. O., … Allen, A. E. (2015). Phytoplankton–bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. Proceedings of the National Academy of Sciences, 112(32), 9938–9943. doi:10.1073/pnas.1501615112
Methods

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. doi:10.1093/bioinformatics/btu170
Methods

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Methods

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Methods

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Methods

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Methods

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Software

Kanehisa, M. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Research, 28(1), 27–30. doi:10.1093/nar/28.1.27
Methods

Kolber, Z. S., Prášil, O., & Falkowski, P. G. (1998). Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1367(1-3), 88–106. doi:10.1016/s0005-2728(98)00135-2
General

Lampe, R. H., Hernandez, G., Lin, Y. Y., & Marchetti, A. (2020). Representative diatom and coccolithophore

species exhibit divergent responses throughout simulated upwelling cycles. doi:10.1101/2020.04.30.071480
Results

Lohman, B. K., Weber, J. N., & Bolnick, D. I. (2016). Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. Molecular Ecology Resources, 16(6), 1315–1321. doi:10.1111/1755-0998.12529
Methods

Meyer, E., Aglyamova, G. V., & Matz, M. V. (2011). Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Molecular Ecology. doi:10.1111/j.1365-294x.2011.05205.x https://doi.org/10.1111/j.1365-294X.2011.05205.x
Methods

Parsons, T. R., Y. Maita, and C. M. Lalli. "A Manual of Chemical and Biological Methods of Seawater Analysis", Pergamon Press (1984). ISBN: 9780080302874
Methods

Strader, M. E., Aglyamova, G. V., & Matz, M. V. (2016). Red fluorescence in coral larvae is associated with a diapause-like state. Molecular Ecology, 25(2), 559–569. doi:10.1111/mec.13488
Methods

Tang, S., Lomsadze, A., & Borodovsky, M. (2015). Identification of protein coding regions in RNA transcripts. Nucleic Acids Research, 43(12), e78–e78. doi:10.1093/nar/gkv227
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The UniProt Consortium (2016). UniProt: the universal protein knowledgebase. Nucleic Acids Research, 45(D1), D158–D169. doi:10.1093/nar/gkw1099
Methods

Zhang J., Ortner P. B., Fischer C. J. (1997). Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis. National Exposure Research Laboratory Office of Research and Development US Environmental Protection Agency Cincinatti. Method 353.4. URL: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100PDOG.txt
Methods

Zimmerman, C. F., C. W. Keefe, & J. Bashe. (1997). Method 440.0 Determination of Carbon and Nitrogen in Sediments and Particulatesof Estuarine/Coastal Waters Using Elemental Analysis. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-15/009.
Methods

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Related Datasets

IsSupplementedBy

Marchetti, A. (2021) **NCBI project accession and library information on each sample analyzed in upwelling experiments conducted on two phytoplankton species isolated from the California Upwelling Zone.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-03-17 doi:10.26008/1912/bco-dmo.826494.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Treatment	Description of treatment (species + conditions): Chae = Chaetoceros deicipiens; Ehux = Emiliania Huxleyi; HighFe = high iron; LowFe = low iron.	unitless
Timepoint	Measurement time point; samples were collected for six different time points associated with the different phases throughout the simulated upwelling cycle.	unitless
Replicate	Replicate number	unitless
Cells	Cell count	cells per milliliter (cells/mL)
Chl	Chlorophyll a	micrograms per liter (ug/L)
PN	Particulate nitrogen concentration	micromoles per liter (umol/L)
PC	Particulate carbon concentration	micromoles per liter (umol/L)
Fv_Fm	Maximum photosystem II photochemical efficiency as measured by the Satlantic FIRe (Fluorescence Induction and Relaxation System).	unitless

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Instruments

Dataset-specific Instrument Name	Costech 4010
Generic Instrument Name	Costech International Elemental Combustion System (ECS) 4010
Dataset-specific Description	Total nitrogen and carbon were quantified with a Costech 4010 CHNOS Elemental Combustion system according to U.S. Environmental Protection Agency Method 440.0 (Zimmermann et al., 1997).
Generic Instrument Description	The ECS 4010 Nitrogen / Protein Analyzer is an elemental combustion analyser for CHNSO elemental analysis and Nitrogen / Protein determination. The GC oven and separation column have a temperature range of 30-110 degC, with control of +/- 0.1 degC.

Dataset-specific Instrument Name	Olympus CKX41 inverted microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Cells were enumerated using a Sedgwick-Rafter counting chamber on an Olympus CKX41 inverted microscope.
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

Dataset-specific Instrument Name	Astoria Analyzer
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Dissolved nitrate and nitrite was quantified with an Astoria Analyzer.
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

Dataset-specific Instrument Name	Satlantic FIRe
Generic Instrument Name	Satlantic Fluorescence Induction and Relaxation of Emission Spectrometer
Dataset-specific Description	Fv:Fm was measured by the Satlantic FIRe (Fluorescence Induction and Relaxation System).
Generic Instrument Description	The Satlantic FIRe (Fluorescence Induction and Relaxation) System is a bio-optical technology used to measure variable chlorophyll fluorescence in photosynthetic organisms. Based on the Fast Repetition Rate Fluorometry (FRRF) technique, the FIRe was developed in collaboration with Dr. Maxim Gorbunov and Dr. Paul Falkowski from Rutgers University. More information on FIRe (PDF).

Dataset-specific Instrument Name	Nanodrop 2000
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	RNA was purified and examined for quality and quantity using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	Turner 10-AU fluorometer
Generic Instrument Name	Turner Designs Fluorometer 10-AU
Dataset-specific Description	Chlorophyll was measured using a Turner 10-AU fluorometer.
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

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Project Information

CAREER: An integrated molecular and physiological approach to examining the dynamics of upwelled phytoplankton in current and changing oceans (Upwelled Phytoplankton Dynamics)

Coverage: California Upwelling Zone

NSF Award Abstract:
Upwelling zones are hotspots of photosynthesis that are very dynamic in space and time. Microsocopic algae, known as phytoplankton, bloom when deep, nutrient-rich waters are upwelled into sunlit surface layers of the ocean, providing nourishment that supports productive food webs and draws down carbon dioxide (CO2) from the atmosphere to the deep ocean. Photosynthetic microbes in these regions must constantly adapt to changes in their chemical and physical environments. For example, subsurface populations respond to changes in light as they approach the surface. When upwelled waters move offshore, cells sink out of the illuminated zone, establishing seed populations that remain inactive until the next upwelling event. This process is called the upwelling conveyor belt cycle (UCBC). How phytoplankton respond to these changes in environmental conditions and how they may influence their nutrient requirements remains unknown. With future ocean changes predicted to alter seawater chemistry, including ocean acidification and decreased iron availability, some phytoplankton groups may be more vulnerable than others. Accompanying educational activities provide learning experiences to enhance understanding and awareness of marine microbes. The development of a research hub at UNC aims to provide infrastructure and support for scientists and students conducting research on environmental genomics. A laboratory component for an upper-level undergraduate course focused on marine phytoplankton is being developed. Educational outreach activities to broader communities include creation of a lesson plan on phytoplankton in upwelling zones and a virtual research cruise experience for middle-school students, as well as a hands-on lab activity for a local museum focused on marine phytoplankton and the important roles they play in shaping our planet.

The project examines how phytoplankton respond at the molecular and physiological level to the different UCBC stages, which seed populations (i.e., surface versus subsurface) contribute most to phytoplankton blooms during upwelling events of varying intensity, how phytoplankton elemental compositions are altered throughout UCBC stages, and how future predicted ocean conditions will affect the phytoplankton responses to UCBC conditions. This project contains both laboratory and fieldwork. In the laboratory, phytoplankton isolates recently obtained from upwelling regions are exposed to simulated UCBC conditions to examine changes in gene expression, growth and photosynthetic characteristics and elemental composition. Cultures are subjected to both current and future ocean conditions, including reduced iron availability and higher CO2. In the field, research cruises within upwelling regions study the dynamics of natural phytoplankton communities (both surface and subsurface) experiencing upwelling and relaxation and within simulated upwelling incubation experiments. Knowledge of how phytoplankton are affected by UCBC conditions at an integrated molecular, physiological and elemental level under both current and future scenarios is imperative for the proper conservation and management of these critically important ecosystems.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1751805

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