Data on laboratory cultures and statistical analysis code associated with the paper "Co-culture with Synechococcus facilitates the growth of Prochlorococcus under ocean acidification conditions" published in Environmental Microbiology

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Version Date: 2021-02-05

Project
» Impacts of Evolution on the Response of Phytoplankton Populations to Rising CO2 (P-ExpEv)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
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Abstract
Data on laboratory cultures and statistical analysis code pertaining to the paper "Co-culture with Synechococcus facilitates the growth of Prochlorococcus under ocean acidification conditions" published in Environmental Microbiology (doi:10.1111/1462-2920.15277). The data and code in this package allow one to re-run all the analyses performed in the paper.

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Dataset Description

The data and code in this package allow one to re-run all the analyses performed in the paper "Co-culture with Synechococcus facilitates Prochlorococcus growth under ocean acidification conditions", published in Environmental Microbiology (doi:10.1111/1462-2920.15277). Each file is provided separately and all files are packaged into the file Syn-Pro_Co-cultures.zip.

To re-run the analyses, place all the files in one directory, set that directory to the working directory in R, and copy-and-paste the entire contents of the file KnightDataAnalysis.txt into the R window and hit

"enter". You may need to install the plyr, lme4, and emmeans packages beforehand. A description of each file is in the ReadMe.txt file.

Acquisition Description

All data from this study are based on growth rate measurements from laboratory cultures of cyanobacteria acclimated to either 400 ppm or 800 ppm pCO2. Full details are included in Knight & Morris (2020). Brief summaries of the two experiments and their analyses are reported below.

1. Survey of *Prochlorococcus* and *Synechococcus* culture responses to year 2100 pCO2. We tested the growth rate response of several different strains of each genus, representing the most globally abundant ecotype varieties, to 800 ppm pCO2, similar to what is expected to exist by the end of the century. Cultures were acclimated for 3 cycles of semi-continuous culture at the target pCO2, and then growth curves were collected by flow cytometry for 3 further transfer cycles. Cultures were diluted into fresh media while still in exponential growth phase. Both exponential growth rates (slope of cell density vs. time during logarithmic growth) and Malthusian growth rates (based only on starting and ending cell densities, and the elapsed time of the culture) were calculated. Growth rate responses were expressed as the ratio of growth rate at 800 ppm over 400 ppm, and were compared to values reported previously in the literature for other marine cyanobacteria.

2. Head-to-head *Prochlorococcus* vs. *Synechococcus* experiments. We chose one strain of each genus from Experiment 1 to compete in co-culture with each other. Strains were chosen to have opposite growth rate responses, such that *Prochlorococcus* was predicted to be outcompeted at 800 ppm pCO2. Growth of each competitor was measured by flow cytometry, where the two genera have easily distinguishable fluorescence signatures. Relative fitness of *Prochlorococcus* was expressed as the difference in growth rate between *Prochlorococcus* and *Synechococcus*. Linear models were used to analyze the effects of cell density and relative frequency on competition outcomes.

Media preparation: All experiments were conducted in media with an artificial seawater base. Carbonate parameters were carefully measured using titration to determine alkalinity and either potentiometric or colorimetric assays to determine pH. pCO2 was manipulated by addition of calibrated doses of HCl or NaOH, along with NaHCO3 to preserve alkalinity if necessary, in hermetically sealed test tubes with approximately no headspace. Culture growth was assessed every two days by removing a 100 uL aliquot for flow cytometric analysis.

Processing Description

Data Processing: All data were analyzed in R v 3.6.1. Linear models were used for most analyses, with post-hoc comparisons conducted using the emmeans package Full code necessary to re-run our analyses is contained in the file KnightDataAnalysis.txt included with this package.

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Data Files

File	Version
CompDataProcessed.csv (Comma Separated Values (.csv), 11.50 KB) MD5:53b2154909a12fdc7e275597971f3d8e *CompDataProcessed.csv and CompDataProcess.outlierremoved.csv* *These are the co-culture competition experiments summarized in manuscript Figures 3 and 4.* *Experiment: Two experimental blocks were performed, one by author Knight (Maggie) and one by author Morris (Jeff).* *Culture: Each competition paired one of 6 Prochlorococcus clones, designated as LTPE 26-31, with one of 6 Synechococcus clones, designated LTPE 45-50.* *CO2: Either 400 ppm (0) or 800 ppm (1) pCO2.* *Replicate: In some cases multiple replicates of a particular clone pairing were performed, this column indicates which replicate a given row corresponds to.* *Transfer: Each culture was transferred into fresh media at least 2 times, giving at least 3 separate cultures in sequence. This column indicates which sequential transfer each row represents.* *Time: Time (days) between inoculation and transfer.* *InitPro, InitSyn, FinalPro, FinalSyn: densities in cells/mL of Prochlorococcus or Synechococcus at the beginning and end of the transfer.* *ProGR, SynGR: malthusian growth rate of Prochlorococcus and Synechococcus respectively, calculated as described in the manuscript.* *InitProFreq: Initial frequency (0-1) of Prochlorococcus.* *Wpro: Fitness calculated as the ratio of the Prochlorococcus malthusian growth rate vs. the Synechococcus malthusian growth rate. Dimensionless.* *Spro: Fitness calculated as the difference of the Prochlorococcus and Synechococcus malthusian growth rates. Units d^-1.* *InitDens, LogInitDens: Total cyanobacterial cell density at the beginning of the experiment.* *ProExGR, SynEXGR: as ProGR/SynGR, but using the exponential growth rate.* *WproEX, SproEx: as Wpro and Spro, but calculated using the exponential growth rates.* *LagPro, LagSyn: lag time in days, calculated as described in the manuscript.*	1
CompDataProcessed.outlierremoved.csv (Comma Separated Values (.csv), 23.93 KB) MD5:deadf46e9bc4a963c2dfde0d1cce7549 *CompDataProcessed.csv and CompDataProcess.outlierremoved.csv* *These are the co-culture competition experiments summarized in manuscript Figures 3 and 4.* *Experiment: Two experimental blocks were performed, one by author Knight (Maggie) and one by author Morris (Jeff).* *Culture: Each competition paired one of 6 Prochlorococcus clones, designated as LTPE 26-31, with one of 6 Synechococcus clones, designated LTPE 45-50.* *CO2: Either 400 ppm (0) or 800 ppm (1) pCO2.* *Replicate: In some cases multiple replicates of a particular clone pairing were performed, this column indicates which replicate a given row corresponds to.* *Transfer: Each culture was transferred into fresh media at least 2 times, giving at least 3 separate cultures in sequence. This column indicates which sequential transfer each row represents.* *Time: Time (days) between inoculation and transfer.* *InitPro, InitSyn, FinalPro, FinalSyn: densities in cells/mL of Prochlorococcus or Synechococcus at the beginning and end of the transfer.* *ProGR, SynGR: malthusian growth rate of Prochlorococcus and Synechococcus respectively, calculated as described in the manuscript.* *InitProFreq: Initial frequency (0-1) of Prochlorococcus.* *Wpro: Fitness calculated as the ratio of the Prochlorococcus malthusian growth rate vs. the Synechococcus malthusian growth rate. Dimensionless.* *Spro: Fitness calculated as the difference of the Prochlorococcus and Synechococcus malthusian growth rates. Units d^-1.* *InitDens, LogInitDens: Total cyanobacterial cell density at the beginning of the experiment.* *ProExGR, SynEXGR: as ProGR/SynGR, but using the exponential growth rate.* *WproEX, SproEx: as Wpro and Spro, but calculated using the exponential growth rates.* *LagPro, LagSyn: lag time in days, calculated as described in the manuscript.*	1

File	Version
GrowthRates.csv (Comma Separated Values (.csv), 28.03 KB) MD5:dd9a96af6cd1501a5509be2d1f1951dc *GrowthRates.csv:* *These represent the data summarized in manuscript Figure 1.* *Strain: Cyanobacterial strain.* *Genus: either Prochlorococcus or Synechococcus.* *CO2: Either 400 ppm (CO2-) or 800 ppm (CO2+) pCO2.* *Time: The number of days between culture inoculation and transfer into fresh media.* *DensInit: Culture density at inoculation, in cells/mL.* *DensFinal: Culture density at transfer, in cells/mL.* *RealizedGR, ExponentialGR: malthusian and exponential growth rates in d^-1, calculated as described in the text.* *Lag: lag time in days, calculated as described in the text.*	1
GRR.csv (Comma Separated Values (.csv), 2.82 KB) MD5:7ba9d168a3412d4b3c91f0bf2e8f2e8b *GRR.csv* *These represent the data summarized in manuscript Figure 2.* *Strain: Cyanobacterial strain.* *Dataset: Either this study (Morris) or our previous meta-analysis (Dutkiewicz).* *Genus: Either Prochlorococcus, Synechococcus, or "Diazotroph".* *Flag: Either Prochlorococcus (Pro) or not (NotPro).* *CO2-r, CO2+r: malthusian growth rate at 400ppm or 800ppm pCO2 respectively. Only available for experiments from this study; previous reports summarized in Dutkiewicz et al 2015 only included exponential growth rates.* *CO2-x, CO2+x: as above, but exponential growth rates.* *rgrr, xgrr: "growth rate response" for malthusian and exponential growth rates, calculated as described in the manuscript.*	1
KnightDataAnalysis.txt (Octet Stream, 54.49 KB) MD5:430c5412eada34f56aafa946a4870393 *To re-run the analyses, place all the files in one directory, set that directory to the working directory in R, and copy-and-paste the entire contents of the file KnightDataAnalysis.txt into the R window and hit "enter". You may need to install the plyr, lme4, and emmeans packages beforehand.*	1
ReadMe.txt (Octet Stream, 4.25 KB) MD5:8e292e82fd8e4f63f6ea72ea1bcabdd9 *ReadMe file describing all the other files and how to use them.*	1
Syn-Pro_Co-cultures.zip (ZIP Archive (ZIP), 42.19 KB) MD5:90ad67d8f6207b485578c60fcf624d3e *.zip package containing all the files needed to re-run the analyses performed in the paper "Co-culture with Synechococcus facilitates Prochlorococcus growth under ocean acidification conditions".*	1
Together.csv (Comma Separated Values (.csv), 24.26 KB) MD5:28c83887dfd3bc1e80c7b23ae94a3d4b *Together.csv* *This is the data summarized in manuscript Figure 5. It includes all the cultures from CompDataProcessed.outlierremoved.csv as well as comparison cultures with only one of the two cyanobacterial strains.* *Strain: Either Prochlorococcus or Synechococcus.* *Together: Row either corresponds to data collected from unialgal cultures (No) or co-cultures of both strains (Yes).* *Time: The number of days between culture inoculation and transfer into fresh media.* *DensInit: Culture density at inoculation, in cells/mL.* *DensFinal: Culture density at transfer, in cells/mL.* *RealizedGR, ExponentialGR: malthusian and exponential growth rates in d^-1, calculated as described in the text.* *Lag: lag time in days, calculated as described in the text.*	1

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Related Publications

Knight, M. A., & Morris, J. J. (2020). Co-culture with Synechococcus facilitates growth of Prochlorococcus under ocean acidification conditions. Environmental Microbiology, 22(11), 4876–4889. doi:10.1111/1462-2920.15277
Results

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Luminex Guava HT-1 flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Most data in this study were collected using a Luminex Guava HT-1 flow cytometer.
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	custom-calibrated pH probe
Generic Instrument Name	pH Sensor
Dataset-specific Description	pH was measured using either a custom-calibrated pH probe or colorimetrically using m-cresol purple administered by sequential injection in a BioTek Synergy H1 spectrophotometer plate reader.
Generic Instrument Description	General term for an instrument that measures the pH or how acidic or basic a solution is.

Dataset-specific Instrument Name	Mettler-Toledo titrator
Generic Instrument Name	Automatic titrator
Dataset-specific Description	Alkalinity was measured using a Mettler-Toledo titrator.
Generic Instrument Description	Instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

Dataset-specific Instrument Name	BioTek Synergy H1 spectrophotometer plate reader
Generic Instrument Name	plate reader
Dataset-specific Description	pH was measured using either a custom-calibrated pH probe or colorimetrically using m-cresol purple administered by sequential injection in a BioTek Synergy H1 spectrophotometer plate reader.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Percival algal growth chambers
Generic Instrument Name	Algal Growth Chamber
Dataset-specific Description	Cultures were incubated in Percival algal growth chambers.
Generic Instrument Description	A chamber specifically designed for the growth of algae in flasks. The chamber typically provides controlled temperature, humidity, and light conditions.

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Project Information

Impacts of Evolution on the Response of Phytoplankton Populations to Rising CO2 (P-ExpEv)

Coverage: Experiment housed in laboratories at Michigan State University

Note: This project is also affiliated with the NSF BEACON Center for the Study of Evolution in Action.

Project Description from NSF Award:
Human activities are driving up atmospheric carbon dioxide concentrations at an unprecedented rate, perturbing the ocean's carbonate buffering system, lowering oceanic pH, and changing the concentration and composition of dissolved inorganic carbon. Recent studies have shown that this ocean acidification has many short-term effects on phytoplankton, including changes in carbon fixation among others. These physiological changes could have profound effects on phytoplankton metabolism and community structure, with concomitant effects on Earth's carbon cycle and, hence, global climate. However, extrapolation of present understanding to the field are complicated by the possibility that natural populations might evolve in response to their changing environments, leading to different outcomes than those predicted from short-term studies. Indeed, evolution experiments demonstrate that microbes are often able to rapidly adapt to changes in the environment, and that beneficial mutations are capable of sweeping large populations on time scales relevant to predictions of environmental dynamics in the coming decades. This project addresses two major areas of uncertainty for phytoplankton populations with the following questions:
1) What adaptive mutations to elevated CO2 are easily accessible to extant species, how often do they arise, and how large are their effects on fitness?
2) How will physical and ecological interactions affect the expansion of those mutations into standing populations?

This study will address these questions by coupling experimental evolution with computational modeling of ocean biogeochemical cycles. First, cultured unicellular phytoplankton, representative of major functional groups (e.g. cyanobacteria, diatoms, coccolithophores), will be evolved under simulated year 2100 CO2 concentrations. From these experiments, estimates will be made of a) the rate of beneficial mutations, b) the magnitude of fitness gains conferred by these mutations, and c) secondary phenotypes (i.e., trade-offs) associated with these mutations, assayed using both physiological and genetic approaches. Second, an existing numerical model of the global ocean system will be modified to a) simulate the effects of changing atmospheric CO2 concentrations on ocean chemistry, and b) allow the introduction of CO2-specific adaptive mutants into the extant populations of virtual phytoplankton. The model will be used to explore the ecological and biogeochemical impacts of beneficial mutations in realistic environmental situations (e.g. resource availability, predation, etc.). Initially, the model will be applied to idealized sensitivity studies; then, as experimental results become available, the implications of the specific beneficial mutations observed in our experiments will be explored.

This interdisciplinary study will provide novel, transformative understanding of the extent to which evolutionary processes influence phytoplankton diversity, physiological ecology, and carbon cycling in the near-future ocean. One of many important outcomes will be the development and testing of nearly-neutral genetic markers useful for competition studies in major phytoplankton functional groups, which has applications well beyond the current proposal.

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Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:
NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1540158

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