Data and code from an examination of growth rates of cyanobacteria co-cultured with a heterotrophic bacterium, Alteromonas, under either present-day or predicted future pCO2 conditions

Website: https://www.bco-dmo.org/dataset/925841
Data Type: experimental
Version: 1
Version Date: 2024-04-24

Project
» Impacts of Evolution on the Response of Phytoplankton Populations to Rising CO2 (P-ExpEv)
» Collaborative Research: Ecology and Evolution of Microbial Interactions in a Changing Ocean (LTPE)

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
The CO2 content of Earth's atmosphere is rapidly increasing due to human consumption of fossil fuels. Models based on short-term culture experiments predict that major changes will occur in marine phytoplankton communities in the future ocean, but these models rarely consider how the evolutionary potential of phytoplankton or interactions within marine microbial communities may influence these changes. Here we experimentally evolved representatives of four phytoplankton functional types (silicifiers, calcifiers, coastal cyanobacteria, and oligotrophic cyanobacteria) in co-culture with a heterotrophic bacterium, Alteromonas, under either present-day or predicted future pCO2 conditions. The data and analysis code in this dataset show that the growth rates of cyanobacteria generally increased under both conditions, and the growth defects observed in ancestral Prochlorococcus cultures at elevated pCO2 and in axenic culture were diminished after evolution. Evolved Alteromonas were also poorer "helpers" for Prochlorococcus, supporting the assertion that the interaction between Prochlorococcus and heterotrophic bacteria is not a true mutualism but rather a competitive interaction stabilized by Black Queen processes. This work provides new insights on how phytoplankton will respond to anthropogenic change and on the evolutionary mechanisms governing the structure and function of marine microbial communities.

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Coverage

Location: Laboratories at the University of Alabama at Birmingham
Temporal Extent: 2013-08-01 - 2024-01-31

Methods & Sampling

Detailed methods can be found in the manuscript "Marine phytoplankton and heterotrophic bacteria rapidly adapt to future pCO2 conditions in experimental co-cultures". A summary of major methods are provided here.

The phytoplankton used in this study as well as the media in which they were grown are *Prochlorococcus* MIT9312 (PEv medium), *Synechococcus* CC9311 (SEv medium), *Synechocystis* PCC6803 (SEv medium), *Thalassiosira oceanica* CCMP1005 (FEv medium), and *Emiliania huxleyi* CCMP371 (FEv medium). All media types were derived from media commonly used to cultivate each organism detailed in "Algal Culturing Techniques" edited by Andersen. Prior to use in experiments, phytoplankton cultures were rendered clonal and axenic, then mixed with pure cultures of the heterotrophic bacterium A*lteromonas* EZ55, obtained by streaking for isolation on YTSS agar.

Cultures were experimentally evolved for approximately 500 generations at 22 degrees Celisius (°C) under approximately 75 micromoles photons per square meter per second (µmol photons m-2 s-1) in acid-washed conical-bottom glass tubes with airtight caps where the carbonate system was manipulated by the addition of acid or base to achieve present-day (400 parts per million (ppm)) or year 2100 (800 ppm) pCO2 conditions. Phytoplankton growth was measured every 48 hours using a Guava HT1 flow cytometer equipped with a 488 nanometer (nm) laser. When phytoplankton cell densities crossed a cutoff value, cultures were diluted 26-fold into fresh media, representing $\log_2 26$ or 4.7 generations per transfer. Samples from each lineage were cryopreserved every 25 generations and again at the end of the experiment.

At the end of the evolution period, we subcultured clonal evolved *Alteromonas* strains by spread-plating evolved cultures on YTSS agar and selecting single, isolated colonies for growth in YTSS broth. *Prochlorococcus* was rendered axenic by the addition of 100 micrograms per milliliter (µg mL-1) streptomycin. All growth experiments were initiated by mixing axenic *Prochlorococcus* with a specific *Alteromonas* clone (or else remaining axenic) and acclimating the co-culture for 3 transfer cycles (approximately 14 generations) at the target pCO2 concentration. Growth was then monitored by flow cytometry as described above for at least 3 subsequent transfers under constant conditions.

Data Processing Description

The impact of experimental treatments on growth parameters was statistically analyzed using linear models in R with post-hoc statistical testing using extended marginal means with the *emmeans* package. Malthusian and exponential growth rates were calculated as described in our previous work (Hennon et al. 2017, ISME Journal). Because these experiments involved thousands of measurements collected over several years, a variety of clearly erroneous data points were recorded that led to several outlier growth rates that had a disproportionate impact on model output; rather than attempt to manually curate all growth rates, we simply removed either the most extreme 5% high and low Malthusian growth rates or eliminated exponential growth rates with r2 values lower than 0.95 for each strain in each experiment before conducting statistical tests.

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Data Files

File

FinalGR.csv

(Comma Separated Values (.csv), 86.07 KB)
MD5:3722d33f5be206e3ecb1875464a3ffe6

Contains growth rates for experiments done at the end of the experiment comparing the ancestral and evolve phytoplankton organisms. Each row represents a single transfer cycle as in the above description. These data were used to generate main text Figure 2 Lu, et al. (2024).

Column headings are:

Strain: which phytoplankton strain was measured -- Prochlorococcus MIT9312, Synechococcus CC9311, Thalassiosira oceanica CCMP1005, or Emiliania huxleyi CCMP371.

EvolTreat: Whether the culture represents an ancestor (Anc), 400 ppm pCO2 evolved population (CO2-), or 800 ppm pCO2 evolved population (CO2+)

AssayTreat: Whether the culture was assessed at 400 ppm (CO2-) or 800 ppm (CO2+) pCO2

t: the number of days between culture inoculation and final reading

N0: initial cell density

Nt: final cell density

EGR: exponential growth rate, calculated as described in the Methods

rsq: r-squared value of the regression used to calculate the EGR; cultures with r-squared values less than 0.95 were removed from the analysis

MGR: Malthusian growth rate, calculated as described in the METHODS

LTPE_RCode_Cultures.R

(R Script, 20.39 KB)
MD5:452bf22e737ea02e4bc246607a339ec2

Contains the code necessary to run the statistical analyses described in the text (Lu, et al., 2024).

File

LTPE_Transfers.csv

(Comma Separated Values (.csv), 424.63 KB)

MD5:6a1f34b9c3019fedcb8e09a66bef937c

Contains the day-by-day flow cytometry data collected during the evolution phase of the Long Term Phytoplankton Evolution experiment that forms the basis of the manuscript. Each row represents the results of a single transfer cycle of a single evolving lineage; i.e., the beginning and ending cell densities, dates, and so forth. These data were used to generate main text Figure 1 Lu, et al. (2024).

Column heading explanations are:

Strain: which phytoplankton strain was measured -- Prochlorococcus MIT9312, Synechococcus CC9311, Synechocystis PCC6803, Thalassiosira oceanica CCMP1005, or Emiliania huxleyi CCMP371.

Replicate: which replicate lineage each measured sample was taken from. Each lineage has a unique designation.

Treatment: whether the lineage was grown at 400 ppm (CO2-) or 800 ppm (CO2+) pCO2.

Method: how the phytoplankton biomass was measured. Most samples were assessed for cell density using a Guava flow cytometer, but some early samples were assessed by in vivo chlorophyll-a fluorescence with a Turner Designs Trilogy fluorometer.

Restart: Rarely, a lineage would crash or become contaminated and would have to be restarted. Measurements applying to the first culture transfer after a restart and indicated here. Cultures that would eventually crash are not depicted in main text Figure 1.

Transfer: the sequential number of each transfer, representing the evolutionary distance between the culture and its ancestor. Each transfer represents log(2) 26 = 4.7 generations.

T0: date of culture inoculation

Tend: date of culture transfer

deltaT: days elapsed between T0 and Tend

InitDens: initial cell density (or chl-a fluorescence)

FinalDens: final cell density (or chl-a fluorescence)

MGR: Malthusian growth rate, calculated as described in the Methods

Notes: special notes pertaining to certain transfers corresponding to reasons for breaks in transfer number or dates (e.g., crash, contamination, cryopreservation prior to moving labs)

File

MixnMatch.csv (Comma Separated Values (.csv), 53.83 KB)
MD5:70be38910633d2f667f28ed4b2982a0d

Contains the data from experiments where ancestral and evolved Prochlorococcus were grown with different strains of Alteromonas EZ55 (or axenically). These data were used to generate main text Figure 4B of Lu, et al. (2024).

Column headings are as follows:

LTPE: LTPE strain designation of the Prochlorococcus strain in the experiment.

Pro: Whether the Prochlorococcus strain was ancestral (Anc), evolved at 400 ppm pCO2 (Evo400), or evolved at 800 ppm pCO2 (Evo800)

Het: Whether the culture was axenic (X), grown in co-culture with ancestral Alteromonas EZ55 (A), or with an Alteromonas strain evolved at the pCO2 condition the culture was grown at in co-culture with Prochlorococcus (P), Thalassiosira oceanica (TO), or Emiliania huxleyi (EH)

t: Elapsed time between culture inoculation and final measurement

IniDens: Initial cell density of the culture

FinDens: Final cell density of the culture

EGR: Exponential growth rate as calculated in the Methods

R^2: r-squared value of the regression used to calculate the EGR; cultures with r-squared values less than 0.95 were removed from the analysis

MGR: Malthusian growth rate as calculated in the Methods

Viability.csv (Comma Separated Values (.csv), 40.41 KB)
MD5:65590822089f426801c26281aeea48be

Contains the data from experiments measuring the impact of different EZ55 strains or axenic culture on mortality of Prochlorococcus strains. These data were used to make Figure 4A Lu, et al. (2024).

Column headings are as follows:

LTPE: LTPE Strain designation of the Prochlorococcus strain in the experiment.

Pro: Whether the Prochlorococcus strain was ancestral (Anc), evolved at 400 ppm pCO2 (400ppm), or evolved at 800 ppm pCO2 (800ppm)

Het: Whether the culture was axenic (X), grown in co-culture with ancestral Alteromonas EZ55 (A), or with an Alteromonas strain evolved at the pCO2 condition the culture was grown at (E)

CO2: The pCO2 condition under which the culture was grown

ViaRes: Whether or not the culture survived (s) or failed (f). Failure was considered to occur if the culture did not attain the cutoff cell density for transfer of 2,600,000 cells per milliliter within 30 days.

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Supplemental Files

File

ReadMe.cultures.txt (Plain Text, 5.61 KB)
MD5:b9399712fe6524cca81b140a60ced517

Explanation of the files in this dataset, how the data files are organized, and how to run the code to replicate the analyses we used in our manuscript (Lu, et al., 2024).

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Related Publications

Hennon, G. M., Morris, J. J., Haley, S. T., Zinser, E. R., Durrant, A. R., Entwistle, E., … Dyhrman, S. T. (2017). The impact of elevated CO2 on Prochlorococcus and microbial interactions with “helper” bacterium Alteromonas. The ISME Journal, 12(2), 520–531. doi:10.1038/ismej.2017.189.
Methods

Lu, Z., Entwistle, E., Kuhl, M. D., Durrant, A. R., Filho, M. M. B., Goswami, A., & Morris, J. J. (2024). Marine phytoplankton and heterotrophic bacteria rapidly adapt to future pCO2conditions in experimental co-cultures. https://doi.org/10.1101/2024.02.07.579367
Results

R. Andersen, Ed., Algal Culturing Techniques, (Academic Press, Burlington, MA, 2005), pp. 596. https://isbnsearch.org/isbn/9780120884261
Methods

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Related Datasets

IsRelatedTo

Morris, J. J., Entwistle, E., Lu, Z. (2024) **Data and analysis code used to experimentally evolve representatives of four phytoplankton functional types in co-culture with a heterotrophic bacterium under either present-day or predicted future pCO2 conditions.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-04-25 doi:10.26008/1912/bco-dmo.925872.1 [view at BCO-DMO]

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

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

Dataset-specific Instrument Name	Guava HT1 flow cytometer with 488nm laser
Generic Instrument Name	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)

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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.

Collaborative Research: Ecology and Evolution of Microbial Interactions in a Changing Ocean (LTPE)

Coverage: Lab work: Birmingham, Alabama and New York, New York. Field Work: Bermuda Atlantic Time Series.

NSF Award Abstract:
Carbon dioxide released from fossil fuels is causing the ocean to become more acidic. Much attention has been given to how this will affect shelled animals like corals, but acidification also affects the algae that form the base of the ocean food chain. It is possible that future algal communities will look very different than they do today, with potentially negative consequences for fisheries, recreation, and climate. Alternatively, it is possible that these algae will be able to adapt rapidly enough to avoid the worst of it. This study looks at algae adapting to acidification in real time in the lab, focusing on "marketplace" interactions between the algae and the bacteria they live alongside. The researchers also go to sea to learn whether adaptations from the lab experiments are beneficial under real-world conditions. Ultimately, this project is helping scientists better understand how the ocean's most important and most overlooked organisms will respond to the changes humans are causing in their habitat. The researchers also use their scientific work to create fun educational opportunities from grade school to college, including agar art classes where students learn about microbial ecology by "painting" with freshly-isolated ocean bacteria.

The effect of ocean acidification on calcifying organisms has been well-studied, but less is known about how changing pH will affect phytoplankton. Previous work showed that the mutualistic interaction between the globally abundant cyanobacterium Prochlorococcus and its "helper" bacterium Alteromonas broke down under projected future CO2 conditions, leading to a strong decrease in the fitness of Prochlorococcus. It is possible that such interspecies interactions between microbes are important for many ecological processes, but a lack of understanding of how these interactions evolve makes it difficult to predict how important they are. This project is using laboratory evolution experiments to discover how evolution shapes the interactions between bacteria and algae like Prochlorococcus, and how these co-evolutionary dynamics might influence the biogeochemical processes that shape Earth's climate. Four research cruises to the Bermuda Atlantic Time Series are also planned to study how natural algal/bacterial communities respond to acidification, and whether evolved microbes from laboratory experiments have a competitive advantage in complex, natural communities exposed to elevated CO2. The ultimate goal of this project is to gain a mechanistic understanding of microbial interactions that can be used to inform models of Earth's oceans and biological feedbacks on global climate.

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Program Information

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

Website: https://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 (https://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-1316101
NSF Division of Ocean Sciences (NSF OCE)	OCE-1540158
NSF Division of Ocean Sciences (NSF OCE)	OCE-1851085

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