Continuous culture studies of Synechococcus elongatus CCMP1629 in nitrate-limited and nutrient-replete cultures plus DNA results

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Project
» Collaborative Research: Effects of multiple stressors on Marine Phytoplankton (Stressors on Marine Phytoplankton)

Contributors	Affiliation	Role
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Abstract
This dataset summarizes the results of continuous culture studies of the marine cyanobacterium Synechococcus CCMP 1629 grown under controlled conditions at temperatures from 20 to 45 degrees Celsius, nutrient-replete and nitrate-limited conditions, high and low CO2 partial pressures (1000 and 400 ppm CO2), and high and low irradiance (300 and 50 micro mol quanta per square meter per second). The dataset covers a total of 48 steady states (6 temperatures x 2 CO2 partial pressures x 2 irradiances x nitrate-limited or nutrient-replete). In all cases the cells were grown on a 14:10 light:dark cycle of illumination. Nitrate-limited cells were grown at approximately 50% of the nutrient-replete growth rate at the same irradiance, temperature, and CO2 partial pressure. The data include growth rates, C/N ratios, C/chlorophyll ratios, productivity indices, light respiration rates, and the initial slopes and asymptotic values of short-term CO2 uptake experiments (photosynthesis versus irradiance).

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Coverage

Temporal Extent: 2017-01 - 2021-01

Dataset Description

The marine cyanobacterium *Synechococcus elongatus* clone CCMP1629 was grown in a continuous culture system on a 14:10 light-dark cycle under either nitrate-limited or nutrient-replete conditions, a photoperiod irradiance of either 50 or 300 micro-mol photons per square meter per second, partial pressures of either 400

or 1000 ppm CO_2, and temperatures ranging from 20 to 45 degrees Celsius. Growth rates, photosynthetic rates, respiration rates, C:N ratios, C:Chlorophyll-a ratios, productivity indices, Fv/Fm ratios, and the initial slope and light-saturated asymptote of short-term photosynthesis-irradiance curves are reported.

Methods & Sampling

The marine cyanobacterium *Synechococcus elongatus* clone CCMP1629 was grown in continuous culture systems to study the interactive effects of temperature, irradiance, nutrient limitation, and the partial pressure of CO_2 (pCO_2) on its growth and physiological characteristics. The culture was grown in either a nitrate-limited or nutrient-replete continuous culture system on a 14:10 light:dark cycle of illumination with light provided by a bank of fluorescent lamps. The irradiance on the growth chamber during the photoperiod was either 300 or 50 micro mol quanta per square meter per second. Photosynthetically active radiation (400–700 nm) was measured with a Biospherical Instruments model QSL 2100 quantum sensor. Temperature was controlled to within 0.1°C by circulating water from a Haake model DC10 thermoregulated water bath through the outer jacket of the polycarbonate reaction/growth chamber. Experiments were carried out in 5°C increments from 20°C to 45°C. The dilution rate of the growth chamber was controlled with a peristaltic pump (Masterflex Model 77200-60) to within ± 0.002 per day. The partial pressure of CO_2 was regulated by bubbling air with the desired partial pressure of CO_2 (either 400 or 1000 ppm) into the growth chamber. The pCO_2 in the growth medium was calculated from measurements of pH and total alkalinity. The CO_2 concentration in the laboratory was monitored with an infrared absorption-based CO_2 meter (CO2METER.com, model AZ-004) calibrated at 0 and 400 ppm CO_2 with a standard gas mixture.

Initial experiments were carried out beginning January 2016 through December 2017 under nutrient-replete conditions to determine what the nutrient-saturated growth rates were at the given irradiance, temperature, and pCO_2. Once that growth rate had been determined, the nitrate-limited experiments were initiated with the same factors. The growth chambers were autoclaved glass reaction flasks. The nitrate-limited experiments were run in a growth chamber with a working volume of 2143 ±5 mL. The nutrient-replete experiments were run in an identical growth chamber but with a working volume of 1306 ±4 mL that was reduced by lowering the level of the overflow tube (see Figure 1, Supplemental Files section). At a fixed medium pumping rate, the growth rates in the nutrient-replete system were therefore 2143/1306 = 1.64 times the growth rates in the nitrate-limited system. High growth rates could therefore be maintained in the nutrient-replete system without sterile filtering new medium reservoirs at frequent intervals.

When samples were not being collected from the growth chamber, air containing either 400 or 1000 ppm CO_2 was bubbled into the growth chamber through a sterile air filter and a three-way valve, as shown in Figure 1. When the chemostat was not being sampled, the three-way valve was turned so that the sterile air bubbled into the bottom of the growth chamber. Bubbling with sterile air created a pressure in the headspace that was sufficient to force medium through the overflow line. Sterile medium from the nutrient reservoir was continuously dripped into the growth chamber at a controlled rate by the peristaltic pump. When it was time to sample the chemostat, the overflow line was clamped, and pressure rapidly built up in the headspace. Within about 5 seconds, the three-way valve was then rotated 90° so that culture could flow up the aeration tube to the three-way valve and then out the collection tube. In this way samples could be collected from the growth chamber within a matter of seconds and without introducing non-sterile air into the headspace. After collection of a sample, the three-way valve was rotated back to its normal position and the clamp on the overflow line was removed.

Cell counts were measured every day at the same time during the photoperiod with a Beckman Coulter model Z1 particle counter. The system was judged to be in steady state when cell counts had been reproducible to within ± 2% for at least 4 doubling times. Apart from measurements of cell counts, all sampling was done after the steady state condition had been reached.

Chlorophyll a concentrations were measured every 2 hours during the photoperiod. Triplicate 50-mL samples from the growth chamber were filtered onto glass fiber (GF/F) filters and extracted in methanol. The absorbances were measured at 664 and 750 nm with a Cary Model 50 spectrophotometer. Triplicate samples were likewise collected at 2-hour intervals during the photoperiod for particulate carbon (PC) and particulate nitrogen (PN) by filtering 50-mL samples from the growth chamber onto GF/F glass fiber filters, which were then stored frozen. The filters were dried at 60°C for 24 hours prior to analysis with an Exeter Analytical model CE-440 elemental analyzer. The sampling for chlorophyll and CHN samples extended over as many as 12 days because we required that the amount of sample withdrawn from the growth chamber never exceed the

amount of fresh medium that would be added to the growth chamber during the subsequent two-hour interval. In some cases, this required taking one 50-mL sample every 4 hours, in which case collecting 3 chlorophyll samples and 3 CHN samples every two hours required sampling for a total of 12 days. On odd days one 50-mL sample was collected at times 0, 4, 8, and 12 hours after the start of the photoperiod and on even days at times 2, 6, 10, and 14 hours after the start of the photoperiod.

The growth medium consisted of artificial seawater with a total alkalinity of 2365microequivalents per liter (µeq L–1). For the nitrate-limited chemostats, nutrient concentrations corresponded to f/2 medium, with the exception of nitrate, which was added at a concentration of 20 micromolar, and trace metals, which were added at the concentrations specified by Sunda and Hardison (2007). We found that the biomass produced with 20 micromolar nitrate in the nutrient reservoir was sufficiently low that the pCO_2 in the growth chamber did not deviate by more than ±10% from the targeted partial pressures. For the nutrient-replete chemostats, the medium was identical, except that nitrate concentrations were 883 micromolar, the concentration specified for f/2 medium. The medium was sterile filtered (0.2 micron) into a 40-liter glass carboy that had been previously autoclaved. Cell concentrations in the nitrate-limited and nutrient-replete growth chambers were similar at the same irradiance, temperature, and pCO_2. Therefore, nitrate concentrations in the growth chamber under nutrient-replete conditions were in excess of 800 micromolar.

When operation of the chemostat had consumed most of the contents of one 40-liter reservoir, another carboy was autoclaved, and 40 liters of fresh medium were sterile filtered into it. The reservoir-carboy was then replaced with the new carboy using sterile techniques to make the necessary connection to the growth chamber. To ensure that there was no perturbation to the steady state, carboys were only (and always) changed between steady states, e.g. sampling at a given steady state was never initiated unless there was sufficient medium in the carboy to complete all sampling for that steady state.

After the sampling for CHN and chlorophyll a had been completed at a given steady-state growth rate, additional samples were collected during the next three days so that short-term (5-minute) photosynthesis-versus-irradiance curves could be measured at the start, middle, and end of the photoperiod. For these experiments, triplicate 5-mL aliquots from the growth chamber were added to liquid scintillation vials pre-inoculated with 0.5 microcuries of C-14 bicarbonate. A platform was set up in front of a bank of fluorescent lamps, and 20-mL glass vials were positioned on the platform at varying distances from the lamps so as to achieve irradiances of 55, 80, 120, 150, 200, 250, 300, and 350micromole photons per square meter per sec (µmol photons m−2 s−1), as measured with a quantum scalar light meter (Biospherical Instruments model QSL 2100). Three replicate vials were placed at each irradiance position. To account for background activity and any dark uptake, an additional three vials were wrapped in aluminum foil but otherwise treated in the same manner as the exposed vials. To each vial, a fixed volume (80–100 µL) of a 4.5-mL solution containing approximately 20 µCi of ^{14}C (40–60 µCi/µmol $NaH^{14}CO_3$; Perkin Elmer) at pH 12 was added. At the beginning of each assay, a 5.0-mL aliquot of culture was added to each vial in series, 5 seconds apart. The timing was controlled using a stopwatch, and each vial was incubated for a total of 5 minutes. At the conclusion of the 5-minute incubation, 0.5 mL of 1 N HCl was added to each vial to halt photosynthesis and convert any non-assimilated ^{14}C to CO_2. The uncapped vials were then allowed to vent for a further 48 hours, after which 10 mL of scintillation cocktail (Ultima Gold XR, Perkin Elmer) was added. Additionally, to determine the total ^{14}C activity that was added to each vial, the same volume of ^{14}C solution was combined with 5.0 mL of filtered artificial seawater and 0.5 mL of 0.1 N NaOH, and the resulting solution was immediately capped. After addition of scintillation cocktail, the activity in each vial was measured for 5 minutes on a scintillation counter (Tri-Carb 3100TR, Perkin Elmer). Background activity was determined based on duplicate solutions containing 5 mL of filtered artificial seawater and 10 mL scintillation cocktail.

The pH was measured with a Thermo Spectronic Helios spectrophotometer, as described in SOP 6b by Dickson, et al (2007) with minor modifications, and with a Hach SensION model PH31 pH meter calibrated with standards on the total pH scale, prepared as per Millero, et al. (1993), with minor modifications.

[See the notepad doc for details and revisions]

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After the sampling for CHN and chlorophyll a had been completed, short-term (5-minute) photosynthesis

versus irradiance curves were measured at the start, middle, and end of the photoperiod. For these experiments, triplicate 5-mL aliquots from the growth chamber were added to liquid scintillation vials pre-inoculated with 0.5 microcuries of C-14 bicarbonate. The vials were incubated at irradiances of 55, 80, 120, 150, 200, 250, 300, and 350 micro-mol photons m–2 s–1 for 5 minutes. Fixation was stopped by adding 0.5 mL of 1 N HCl to the vials, and samples were allowed to vent for 48 hours. The activity of C-14 in the samples was then determined by counting on a Packard Tri-Carb model 3100 TR liquid scintillation counter.

Total alkalinity was determined using the open-cell titration method described in SOP 3b by Dickson et al. (2007), using a pH electrode and meter (Hach SensION model PH31). Briefly, 100 mL of culture was brought to 25°C and initially titrated to a pH of 3.50 with standardized HCl (Fisher) diluted to 0.10 N in 0.6 M NaCl. The solution was then stirred vigorously for 5–10 minutes. Afterwards, the solution was further titrated stepwise with 50-µL additions of the HCl solution to ultimately achieve a pH of 3.00. The total alkalinity was then calculated based on the relative potential of the electrode after each acid addition and on the cumulative volume of acid added at each step. DIC concentrations were then calculated from temperature, salinity, total alkalinity, and pH using the equations in Zeebe and Wolf-Gladrow, CO_2 in Seawater: Equilibrium, Kinetics, Isotopes.

Three functions, a simple hyperbola, a negative exponential, and a hyperbolic tangent were assessed to determine which one best described the relationship between irradiance and photosynthetic rate. Based on a least squares criterion, the hyperbolic tangent (tanh) consistently gave the best description of the data.

P = Pmax*tanh(Ea/ Pmax) (1)

Here, E is the irradiance with units of umol photons m–2 s–1, Pmax is the light-saturated photosynthetic rate with units of grams carbon per gram chlorophyll a per hour, and a is the initial slope of the photosynthesis-irradiance curve (i.e., the slope in the limit as E ® 0). Values of Pmax and a were determined by least squares (Figure S2).

Fig. 2, see Supplemental Files.

The calculated values of a were converted to units of m2 (carbons/photon) g−1 chl a

by multiplying by 106 to convert mmoles to moles, dividing by 3600 to convert hours to seconds, and dividing by 12 to convert grams of carbon to moles of carbon.

The dark-adapted Fv/Fm ratios were measured in triplicate for each continuous culture in steady state at the start, middle, and end of the photoperiod. Fv/Fm measurements were made with a PSI AquaPen C100 with the manufacturer’s supplied plastic cuvettes containing 4 mL of culture each. Dark-adaptation of the culture samples was achieved by wrapping each of three cuvettes in aluminum foil and incubating them for 30–40 minutes in the same thermoregulated water bath used to control the temperature of the growth chamber. Afterwards, Fv/Fm was measured in a darkened room.

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The cells were grown in either a nutrient-replete mode or nitrate-limited. For the nitrate-limited studies, the concentration of nitrate in the nutrient reservoir was 40 micro molar. The growth medium was artificial seawater enriched with f/2 nutrients with the exception of nitrate for the nitrate-limited studies. The medium was sterile filtered into a 40-liter nutrient reservoir and pumped into the growth chamber at a controlled rate with a peristaltic pump.

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The pH was measured with a Thermo Spectronic Helios spectrophotometer, as described in SOP 6b by Dickson, et al 2007 with minor modifications, and with a Hach SensION model PH31 pH meter calibrated with standards on the total pH scale, prepared as per Millero, et al. (Marine Chemistry 44[2]: 143–152 [1993]), with minor modifications.

Table 1, see Supplemental Files.

Samples for analysis of particulate carbon, particulate nitrogen (i.e., CHN), and chlorophyll a were collected in triplicate every two hours from the start to the end of the 14-h photoperiod (a total of 7 time points). Because

this sampling would have exceeded the dilution rate of the chemostat in all cases, sampling had to take place over a period as long as 12 days. The protocol required that the volume of culture withdrawn for a sample be replaced by fresh medium before another sample could be taken. At low growth rates, this criterion required that 50-mL samples be collected at 4-hour intervals. The sampling was therefore staggered on alternate days. For example, on day one, single samples were collected at times 0, 4, 8, and 12 hours, and on day two samples were collected at times 2, 6, 10, and 14 hours. Collecting triplicate samples for both CHN and chlorophyll a analysis in such cases required that sampling extend over a period of 12 days.

Data Processing Description

Photosynthetic rates during two-hour time intervals during the photoperiod were calculated by solving the differential equation

$$d(POC)/dt = P - u \acute{} POC \quad (2)$$

where P is the rate of production of *POC* in the growth chamber, u is the dilution rate of the growth chamber and d(*POC*)/dt is the rate of change of *POC* in the growth chamber. The solution of equation (2) between two points in time is

$$P = u\,(POC_t - POC_0\, e^{-ut})/(1 - e^{-ut}) \quad (3)$$

where POC_0 and POC_t are the concentrations of *POC* at the beginning and end of the time interval, respectively, and t is the duration of the time interval, which in this experiment was 2 hours. Values of P were calculated for each two-hour time interval during the photoperiod, normalized to the chlorophyll *a* concentration during each time interval, and then averaged to determine the photosynthetic rate per unit chlorophyll (productivity index or PI) during the photoperiod. Results are reported as grams of carbon per gram of chlorophyll *a* per hour.

Dark respiration rates were calculated from the natural logarithm of the ratio of the particulate carbon concentrations at the end of the photoperiod and the beginning of the subsequent photoperiod. The natural logarithm of the ratio of the particulate carbon concentrations was equated to $(u + u_r)10/24$, where u_r is the dark respiration rate (d^{-1}) and u is the dilution rate (d^{-1}). Multiplication by 10/24 corrects for the fact that the duration of the dark period was 10 hours. Thus

$$u_r = (24/10)\ln(PC_e/PC_b) - u \quad (4)$$

where PC_e and PC_b are the particulate carbon concentrations at the end of one photoperiod and the beginning of the next photoperiod, respectively.

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- removed column 'ratio of alpha nutrient-replete to nutrient-limited'

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Supplemental Files

File	
Figure 1. Diagram of chemostat filename: Fig1_chemostat_diagram.pdf	(Portable Document Format (.pdf), 239.11 KB) MD5:be4aae6bbe320cca4038b5fccb56f41c
Diagram of chemostat with three-way valve for bubbling air and collecting samples.	
Figure S2. Estimated photosynthetic rate filename: FigS2_photosynthetic_rate.pdf	(Portable Document Format (.pdf), 340.56 KB) MD5:7f044ce895703ee1d71857353beaf483
Figure S2. Estimated photosynthetic rate from 5-minute uptake of 14C at 10□C and 50 □mol photons m–2 s–1. The smooth curve is a hyperbolic tangent fit to the data by least squares.	
Table 1. Characteristics of seawater filename: Table1_seawater_characteristics.pdf	(Portable Document Format (.pdf), 243.05 KB) MD5:d6d21579a771a820154c407ee396303a
Table 1. Characteristics of seawater with a total alkalinity of 2365 meq L–1 as a function of pCO2 and temperature based on equations in Zeebe and Wolf-Gladrow, CO2 in Seawater: Equilibrium, Kinetics, Isotopes (2001)	

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Related Publications

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO2 measurements. PICES Special Publication 3, 191 pp. ISBN: 1-897176-07-4. URL: https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html https://hdl.handle.net/11329/249
Methods

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Parameters

Parameter	Description	Units
temp	temperature of culture	degrees Celsius
limiting_factor	limiting factor	unitless
irradiance	irradiance during photoperiod	micromole photons/meter^2/second
pCO2	partial pressure of carbon dioxide	parts per million by volume
growth_day	growth rate	per day
growth_relative	relative growth rate: the ratio of the nutrient-limited growth rate to the nutrient-replete growth rate under otherwise identical conditions. Therefore the relative growth rates of nutrient-replete cultures are automatically 1.	per day
PI_mean	mean of the Productivity-Irradiance curve	grams Carbon/gram chl/hour
dark_resp_day	dark respiration rate	per day
Fv_FM	maximum quantum yield (QY=Fv/Fm)	unitless
PM_lights_on	maximum photosynthetic rate at lights on	grams Carbon/gram chl/hour
PM_midday	maximum photosynthetic rate at midday	grams Carbon/gram chl/hour
PM_lights_off	maximum photosynthetic rate at lights off	grams Carbon/gram chl/hour
PM_mean	mean maximum photosynthetic rate	grams Carbon/gram chl/hour
alpha_lights_on	alpha at lights on	meters^2(moles Carbon/moles photons)/gram chla
alpha_midday	alpha at midday	meters^2(moles Carbon/moles photons)/gram chla
alpha_lights_off	alpha at lights off	meters^2(moles Carbon/moles photons)/gram chla
alpha_mean	mean alpha	meters^2(moles Carbon/moles photons)/gram chla
C_to_N	Carbon:Nitrogen ratio	unitless (grams/grams)
C_to_chl	Carbon:chlorophyll ratio	unitless (grams/grams)

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Instruments

Dataset-specific Instrument Name	Hach SensION model PH31 pH meter
Generic Instrument Name	Benchtop pH Meter
Generic Instrument Description	An instrument consisting of an electronic voltmeter and pH-responsive electrode that gives a direct conversion of voltage differences to differences of pH at the measurement temperature. (McGraw-Hill Dictionary of Scientific and Technical Terms) This instrument does not map to the NERC instrument vocabulary term for 'pH Sensor' which measures values in the water column. Benchtop models are typically employed for stationary lab applications.

Dataset-specific Instrument Name	Cary Model 50 spectrophotometer
Generic Instrument Name	Cary 50 spectrophotometer
Dataset-specific Description	Used to measure absorbances were measured at 664 and 750 nm
Generic Instrument Description	A Cary 50 spectrophotometer measures absorbance (200-800 nm).

Dataset-specific Instrument Name	
Generic Instrument Name	Chemostat
Generic Instrument Description	Devices in which controlled conditions are maintained for a chemical process to be carried out by organisms or biochemically active substances derived from such organisms.

Dataset-specific Instrument Name	an Exeter Analytical model CE-440 elemental analyzer
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	Used to measure concentrations of particulate organic carbon (POC) and particulate nitrogen (PN)
Generic Instrument Description	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

Dataset-specific Instrument Name	Beckman Coulter model Z1 particle counter
Generic Instrument Name	Coulter Counter
Dataset-specific Description	Use to make cell counts
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	PSI AquaPen C100
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used to measure the maximum quantum yield, QY (Fv/Fm) with the manufacturer's supplied plastic cuvettes containing 4 mL of culture each.
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Z985 Cuvette Aquapen (Qubit Systems)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used to measure instantaneous chlorophyll fluorescence (F0). AquaPen settings: f = 30, F=71, A = 50.
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Packard Tri-Carb model 3100 TR liquid scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Used to measure the activity of C-14 in the samples
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

Dataset-specific Instrument Name	CO2METER model AZ-004
Generic Instrument Name	pCO2 Sensor
Dataset-specific Description	Used to monitor CO2 concentration in the laboratory. Calibrated at 0 and 400 ppm CO2 with a standard gas mixture
Generic Instrument Description	A sensor that measures the partial pressure of CO2 in water (pCO2)

Dataset-specific Instrument Name	Masterflex Model 77200-60 peristaltic pump
Generic Instrument Name	Pump
Dataset-specific Description	Used to control the dilution rate of the growth chamber
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	Biospherical Instruments model QSL 2100 quantum sensor
Generic Instrument Name	Radiometer
Dataset-specific Description	Used to measure photosynthetically active radiation (400–700 nm)
Generic Instrument Description	Radiometer is a generic term for a range of instruments used to measure electromagnetic radiation (radiance and irradiance) in the atmosphere or the water column. For example, this instrument category includes free-fall spectral radiometer (SPMR/SMSR System, Satlantic, Inc), profiling or deck cosine PAR units (PUV-500 and 510, Biospherical Instruments, Inc). This is a generic term used when specific type, make and model were not specified.

Dataset-specific Instrument Name	Thermo Spectronic Heios spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Used to measure pH
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.

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Project Information

Collaborative Research: Effects of multiple stressors on Marine Phytoplankton (Stressors on Marine Phytoplankton)

The overarching goal of this project is to develop a framework for understanding the response of phytoplankton to multiple environmental stresses. Marine phytoplankton, which are tiny algae, produce as much oxygen as terrestrial plants and provide food, directly or indirectly, to all marine animals. Their productivity is thus important both for global elemental cycles of oxygen and carbon, as well as for the productivity of the ocean. Globally the productivity of marine phytoplankton appears to be changing, but while we have some understanding of the response of phytoplankton to shifts in one environmental parameter at a time, like temperature, there is very little knowledge of their response to simultaneous changes in several parameters. Increased atmospheric carbon dioxide concentrations result in both ocean acidification and increased surface water temperatures. The latter in turn leads to greater ocean stratification and associated changes in light exposure and nutrient availability for the plankton. Recently it has become apparent that the response of phytoplankton to simultaneous changes in these growth parameters is not additive. For example, the effect of ocean acidification may be severe at one temperature-light combination and negligible at another. The researchers of this project will carry out experiments that will provide a theoretical understanding of the relevant interactions so that the impact of climate change on marine phytoplankton can be predicted in an informed way. This project will engage high schools students through training of a teacher and the development of a teaching unit. Undergraduate and graduate students will work directly on the research. A cartoon journalist will create a cartoon story on the research results to translate the findings to a broader general public audience.

Each phytoplankton species has the capability to acclimatize to changes in temperature, light, pCO2, and nutrient availability - at least within a finite range. However, the response of phytoplankton to multiple simultaneous stressors is frequently complex, because the effects on physiological responses are interactive. To date, no datasets exist for even a single species that could fully test the assumptions and implications of existing models of phytoplankton acclimation to multiple environmental stressors. The investigators will combine modeling analysis with laboratory experiments to investigate the combined influences of changes in pCO2, temperature, light, and nitrate availability on phytoplankton growth using cultures of open ocean and coastal diatom strains (Thalassiosira pseudonana) and an open ocean cyanobacteria species (Synechococcus sp.). The planned experiments represent ideal case studies of the complex and interactive effects of environmental conditions on organisms, and results will provide the basis for predictive modeling of the response of phytoplankton taxa to multiple environmental stresses.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536581

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