Experimental results of nitrate-limited or nitrate-replete continuous culture studies of Thalassiosira pseudonana at 5 temperatures with either high or low pCO2 and irradiance

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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
Thalassiosira pseudonanana were grown in nitrate limited culture at five temperatures, 400 umol photons m–2 s–1, and 1000 ppm CO2 and irradiance levels of 50 and 300 umol photons m-2 s-1. Growth rates, photosynthetic rates, respiration rates, C:N ratio, C:Chlorophyll-a ratio, maximum quantum yield are reported.

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Coverage

Temporal Extent: 2016-01-25 - 2018-07-22

Dataset Description

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Methods & Sampling

The Thalassiosira pseudonana culture was grown in a nitrate-limited or nitrate-replete continuous culture system on a 14:10 L:D cycle of illumination at temperatures of 10, 15, 20, 25, and 30°C. The irradiance during the photoperiod was either 50 or 300 mmol photons m–2 s–1. Continuous aeration of the culture was with 0.2 µm-filtered ambient air with a CO2 concentration of either 400 or 1000 ppm. 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 temperature-controlled water bath through the outer jacket of the reaction chamber. The dilution rate of the growth chamber was controlled with a peristaltic pump (Masterflex Model 77200-60) to within ± 0.002 per day. For 400 ppm CO2 treatments, the CO2 concentration in the laboratory was monitored with a nondispersive infrared absorption-based CO2 meter (AZ-0004, CO2meter.com, Ormond Beach, FL) calibrated at 0 and 400 ppm CO2 with standard gas mixture. For elevated CO2 treatments (1000 ppm), the growth chambers and media reservoirs were bubbled constantly (2–5 L/min each) with sterile-filtered ambient air amended with 100% Bone-Dry 3.0 grade CO2 (Airgas, Inc., Radnor, PA) using the system described in Fig. 1. The CO2 concentration of the amended air was monitored using a CO2 meter as for 400 ppm treatments.

See Figure 1 for Schematic of CO2 amendment and distribution. (See Supplemental Documents below)

The system was judged to be in steady state when cell counts, measured with a Beckman Coulter model Z1 particle counter, and been reproducible to within 2% for at least 4 doubling times. Chlorophyll a concentrations were determined from samples collected on glass fiber filters and extracted in methanol. The absorbances were measured at 664 and 750 nm with a Cary Model 50 spectrophotometer. Total-scale pH was measured with a Thermo Spectronic Helios spectrophotometer, as described in SOP 6B by 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. with minor modifications, and with a Hach SensION model PH31 pH meter calibrated with standards on the total pH scale, prepared as per Millero, F.J., et al. "The use of buffers to measure the pH of seawater." Marine Chemistry 44.2 (1993): 143-152. with minor modifications. The growth medium consisted of artificial seawater with a total alkalinity of 2.365 meq L–1. Nutrient concentrations corresponded to f/2 medium, with the exception of nitrate, which was added at a concentration of 40 mM. The medium was sterile filtered (0.2 mm) into a 40-liter glass carboy that had been previously autoclaved. The growth chamber was an autoclaved glass reaction flask with a working volume of 2143 mL. The cells in the growth chamber were uniformly labeled with C-14 by adding 20 microcuries of C-14 bicarbonate to the nutrient reservoir. Five-milliliter samples for C-14 activity in the organic carbon were withdrawn in triplicate from the growth chamber at two-hour intervals. The samples were acidified with 1 mL of 1 N HCl to drive off inorganic carbon. The activity of C-14 in the samples was then determined by counting on a Packard Tri-Carb model 3100 TR liquid scintillation counter. 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 0.85 microcuries of C-14 bicarbonate. The vials were incubated at irradiances of 0, 5, 10, 20, 30, 55, 80, 120, 150, 200, 250, 300, and 350 mmol photons m–2 s–1 for 5 minutes. Fixation was stopped by adding 0.5 mL of 1 N HCl to the vials. Total alkalinity was determined using the open cell titration method described as SOP 3B by 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. DIC and equilibrium CO2 concentrations were then calculated from temperature, salinity, total alkalinity, and pH using the equations in Zeebe and Wolf-Gladrow, CO2 in Seawater: Equilibrium, Kinetics, Isotopes. Photosynthetic rates as a function of irradiance were fit via least squares with the following functions: piecewise linear, simple hyperbola, hyperbolic tangent, and negative exponential. Each of these functions is described by two parameters, the light-saturated photosynthetic rate (Pmax) with units of grams carbon per gram chlorophyll a per hour and a parameter Ek with units of mmol photons m–2 s–1 that determines the characteristics of the function at irradiances sub-saturating irradiances. For example, for a simple hyperbola, Ek is the irradiance at which the photosynthetic rate equals one-half Pmax. Dark-adapted photosynthetic quantum yield (QY) was measured in triplicate for each continuous culture in steady state at mid-photoperiod. QY measurements were made with a PSI AquaPen C100 with 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 at the treatment temperature for 30 minutes, after which QY was measured in a darkened room.

See Figure 2 for plotted results of Thalassiosia pseudonana CO2 and light experiments. (See Supplemental Documents below)

Data Processing Description

The rate of change of the concentration of particulate organic carbon in the growth chamber is described by the differential equation

$d(POC)/dt = P – u \times POC$

Where POC is the concentration of particulate organic carbon, u is the dilution rate of the growth chamber,

and P is the photosynthetic rate. The solution of this equation for P over the time interval t_1 to t_2 is

P = (POC_2 e^(u(t_2-t_1)) – POC_1 / (e^(–u(t_2 - t_1))^-1)

Where POC_1 and POC_2 are the concentrations of POC at times t_1 and t_2, respectively. Photosynthetic rates were calculated on the basis of POC concentrations measured at two-hour intervals throughout the 14-h photoperiod. The photosynthetic rates so-calculated were divided by the corresponding chlorophyll a concentrations to determine the productivity indices (photosynthetic rates normalized to chlorophyll a) in units of g C g^{-1} chl a h^{-1} and then averaged over the photoperiod.

Short-term (5-minute) photosynthesis versus irradiance plots were described by the Hill equation with a Hill coefficient of 2:

P = (P_m * I^2) / ((E_K)^2 + I^2)

Where I is the irradiance, P is the photosynthetic rate, P_m is the light-saturated photosynthetic rate, and E_K is a constant. When I = E_K, P is equal to P_m/2. Based on this equation, the minimum number of photons required to fix one carbon atom is 2E_K/P_m.

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 blank rows
- added parameter name 'treatment' to a column with no header
- copied temp and limiting factor values to cells below within same treatment series (4 rows)
- converted date format from yyyy.m.d to yyyy-mm-dd

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Data Files

File
Tp_cyclostat.csv (Comma Separated Values (.csv), 5.17 KB) MD5:fddf1a5859093e7f41ff14030a1f85c8 Primary data file for dataset ID 746398

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Supplemental Files

File
Figure 1 filename: Laws_schematic_CO2.png (Portable Network Graphics (.png), 75.39 KB) MD5:3c5e6b729114af0e27c3de7ce01fe47c Schematic of CO2 amendment and distribution.
Figure 2 filename: Tpseudoresults_2018-08.jpg (JPEG Image (.jpg), 61.13 KB) MD5:cd1eb95b0d4eff1c0f5b4c715dac8c3a Plotted results of Thalassiosia pseudonana CO2 and light experiments

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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

Millero, F. J., Zhang, J.-Z., Fiol, S., Sotolongo, S., Roy, R. N., Lee, K., & Mane, S. (1993). The use of buffers to measure the pH of seawater. Marine Chemistry, 44(2-4), 143–152. doi:10.1016/0304-4203(93)90199-x https://doi.org/10.1016/0304-4203(93)90199-X
Methods

Zeebe, R. E., & Wolf-Gladrow, D. (2001). CO2 in seawater: equilibrium, kinetics, isotopes (No. 65). Gulf Professional Publishing. https://isbnsearch.org/isbn/978-0444509468
Methods

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Parameters

Parameter	Description	Units
date	measurement date formatted as yyyy-mm-dd	date
temp	target treatment temperature	degrees Celsius
Limiting_factor	Limiting factor for photosynthesis: either nitrate or temperature	unitless
irradiance	irradiance during photoperiod	micro-mol photons per square meter per second (mmol photons/m^2/s)
pCO2	pCO2	parts per million by volume (ppmv)
treatment	experimental treatment: high and low levels of irradiance and temperature (in that order): CO2 treatments: L = 400 ppm CO2; H = 1000 ppm CO2; irradiance tmt: L = 50 umol photons m-2 s-1; H = 300 umol photons m-2 s-1	unitless
growth	growth rate	per day
PI	mean Photosynthetic Irradiance	grams Carbon per gram chlorophpyll per hour (gC/g chl/hour)
dark_resp	dark respiration rate	per day
Fv_Fm	The normalized ratio of variable fluorescence to maximum fluorescence; Fv/Fm	dimensionless
PM_lights_on	Maximum photosynthesis (Pmax) when growth chamber lights first come on.	grams Carbon per gram chlorophpyll per hour (gC/g chl/hour)

PM_midday	Maximum photosynthesis (Pmax) at midday	grams Carbon per gram chlorophpyll per hour (gC/g chl/hour)
PM_lights_off	Maximum photosynthesis (Pmax) when the lights go off.	grams Carbon per gram chlorophpyll per hour (gC/g chl/hour)
PM_mean	mean maximum photosynthesis (Pmax); calculated for the 14-hour photoperiod.	grams Carbon per gram chlorophpyll per hour (gC/g chl/hour)
Ek_lights_on	the light saturation index or saturation irradiance (Ek) when the lights first come on.	micro-mol photons per square meter per second (umol photons/m^2/s)
Ek_midday	the light saturation index or saturation irradiance (Ek) at midday	micro-mol photons per square meter per second (umol photons/m^2/s)
Ek_lights_off	the light saturation index or saturation irradiance (Ek) at lights go off	micro-mol photons per square meter per second (umol photons/m^2/s)
Ek_mean	the mean light saturation index or saturation irradiance (Ek)	micro-mol photons per square meter per second (umol photons/m^2/s)
min_quanta_lights_on	minimum mol quanta per mol C at lights on	mol quanta per mol Carbon (mol quanta/mol C)
min_quanta_midday	minimum mol quanta per mol C at midday	mol quanta per mol Carbon (mol quanta/mol C)
min_quanta_lights_off	minimum mol quanta per mol C at lights off	mol quanta per mol Carbon (mol quanta/mol C)
min_quanta_mean	mean of minimum quanta per mol C	mol quanta per mol Carbon (mol quanta/mol C)
C_to_N	carbon to nitrogen content ratio	grams per grams
C_to_Chl	ratio of carbon to chlorophyl a	grams per grams
P_to_Pm	ratio of photosynthesis to maximum photosynthesis	dimensionless

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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	Varian 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	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	A nondispersive infrared absorption-based CO2 meter. 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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