Continuous culture studies of possible climate change effects: Thalassiosira pseudonana CCMP1335 growth in nitrate-limited and nutrient-replete cultures

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

Contributors	Affiliation	Role
Laws, Edward	Louisiana State University College of the Coast and Environment (LSU-CC&E [formerly SC&E])	Principal Investigator
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Abstract
The marine diatom Thalassiosira pseudonana clone CCMP 1335 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 CO2, and temperatures ranging from 5 to 32 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.

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Coverage

Spatial Extent: **Lat**:30.4089 **Lon**:-91.18412
Temporal Extent: 2016-01-25 - 2019-10-01

Dataset Description

The marine diatom *Thalassiosira pseudonana* clone CCMP 1335 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 CO2, and temperatures ranging from 5 to 32 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 culture was grown in either a nitrate-limited or nutrient-replete continuous culture system on a 14:10 L:D cycle of illumination at temperatures of 5, 10, 15, 20, 25, 30, 31, and 32°C. The irradiance during the photoperiod was either 50 or 300 micro-mol photons m–2 s–1. 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. The CO2 concentration in the laboratory was monitored with a CO2METER model AZ-004 meter calibrated at 0 and 400 ppm CO2 with a standard gas mixture.

The system was judged to be in steady state when cell counts, measured with a Beckman Coulter model Z1 particle counter, had 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. Concentrations of particulate carbon (PC) and particulate nitrogen (PN) were determined by filtering replicate 50-mL samples from the growth chamber onto GF/F glass fiber filters followed by analysis with an Exeter Analytical model CE-440 elemental analyzer. pH was measured with a Thermo Spectronic Heios 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, 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 2365 meq per liter. Nutrient concentrations corresponded to f/2 medium, with the exception of trace metals, which were added at the concentrations specified by Sunda and Hardison (Limnology & Oceanography 52[6]: 2496–2506 [2007]). The nitrate concentration in the nitrate-limited experiments was 20 micromolar. The medium was sterile filtered (0.2 micron) 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 2183 mL. In the first few experiments, the cells in the growth chamber were uniformly labeled with C-14 by adding 20 microcuries of C-14 bicarbonate to the nutrient reservoir to facilitate monitoring the concentration of organic carbon in the growth chamber. In those first few experiments, five-milliliter samples for C-14 activity in the organic carbon were withdrawn in triplicate from the growth chamber at two-hour intervals during the photoperiod. 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. During those first few studies, we determined that addition of C-14 in this way was unnecessary because we could adequately monitor the concentrations of PC by withdrawing samples for CHN analysis. Subsequent experiments relied entirely on CHN analyses for determination of particulate carbon and nitrogen concentrations.

Short-term (5-minute) photosynthesis-versus-irradiance curves (P-E 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 5, 10, 20, 30, 55, 80, 120, 150, 200, 250, 300, and 350 micro-mol photons per square meter per second 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, et al 2007. DIC 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 in these short-term experiments were found to be best described by a hyperbolic tangent function of the form P = Pm*tanh(E*alpha/Pm), where E is the irradiance, alpha is the initial slope of the photosynthesis-irradiance curve, and Pm is the asymptotic light-saturated photosynthetic rate. The values of Pm with units of grams carbon per gram chlorophyll a per hour and the value of alpha with units of meters squared (carbons/photon) per gram chlorophyll a were determined by least squares.

Measurements of Fv/Fm ratios (the ratio of variable fluorescence to maximal fluorescence after dark adaptation) were made within 30 minutes of each P-E assay, using a Z985 AquaPen fluorometer (Qubit Systems). Briefly, a 4-mL aliquot of culture from the growth chamber was added to each of three plastic 1-cm cuvettes, and each cuvette was immediately wrapped in aluminum foil. The cuvettes were incubated at the

growth chamber temperature for 30–40 minutes, after which the foil was removed and a single Fv/Fm measurement was made on each cuvette in a darkened room. The background-corrected Fv/Fm ratio was automatically calculated by the AquaPen software. The light intensities of the saturating pulse and measurement pulse were 2100 and 0.03 micro-mol photons per square meter per second, respectively, both at a wavelength of 450 nm.

Data Processing Description

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

$$d(PC)/dt = P - D \times PC \quad (1)$$

where P is the rate of production of PC in the growth chamber, D is the dilution rate of the growth chamber and $d(PC)/dt$ is the rate of change of PC in the growth chamber. The solution of equation (1) between two points in time is

$$P = D(PC_t - PC_0 \exp(-Dt)) / (1 - \exp(-Dt)) \quad (2)$$

where PC_0 and PC_t are the concentrations of PC 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 averaged over the 14-h photoperiod.

Dark respiration rates were calculated from the natural logarithm of the ratio of the PC concentration at the end of the photoperiod and the beginning of the subsequent photoperiod. The natural logarithm of the ratio of the *PC concentrations* was equated to $(D + D_r)10/24$, where D_r is the dark respiration rate (with units of inverse days) and D is the dilution rate (with units of inverse days). Division by 24 converts these rates to hourly rates, and multiplication by 10 corrects for the fact that the duration of the dark period was 10 hours. Thus

$$D_r = (24/10)\ln (PC_e / PC_b) - D \quad (3)$$

where PC_e and PC_b are the PC 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 two rows that contained a mean and stdev for 'relative growth rate' (growth_relative)
- moved the stdev and n (number of values in mean) for 'growth rate per day' (growth_day) to another column and called it 'growth_stdev_n'
- reformatted date from yyyy.m.d to yyyy-mm-dd

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Data Files

File
Tpseudo_growth.csv (Comma Separated Values (.csv), 6.55 KB) MD5:1d4cfeec0feeb22d65f7e4cb32a8fdd4
Primary data file for dataset ID 779368

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Supplemental Files

File
Figure 1. Diagram of chemostat filename: Fig1.PNG (Portable Network Graphics (.png), 44.88 KB) MD5:86ee53227b38eb97fca8b4d151715b7d Figure 1. Diagram of chemostat with three-way valve for bubbling air and collecting samples.
Figure S2. Estimated photosynthetic rate filename: FigS2.PNG (Portable Network Graphics (.png), 40.77 KB) MD5:0db32f12fba86e280c0de0c3924e0384 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.PNG (Portable Network Graphics (.png), 31.43 KB) MD5:92770433c52e42eabf118f2df8589de7 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

Sunda, W. G., & Ransom Hardison, D. (2007). Ammonium uptake and growth limitation in marine phytoplankton. Limnology and Oceanography, 52(6), 2496–2506. doi:10.4319/lo.2007.52.6.2496
Methods

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Related Datasets

Replaces Old Versions

Laws, E., Passow, U. (2017) **[Deprecated] Laboratory growth, photosynthetic, and growth rates of Thalassiosira pseudonana clone 3H in nitrate replete culture (Stressors on Marine Phytoplankton project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). Version Date 2017-09-14 http://lod.bco-dmo.org/id/dataset/715193 [view at BCO-DMO]

Laws, E., Passow, U. (2017) **[Deprecated] Laboratory growth, photosynthetic, and respiration rates of Thalassiosira pseudonana clone 3H in nitrate limited culture (Stressors on Marine Phytoplankton project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). Version Date 2017-09-14 http://lod.bco-dmo.org/id/dataset/714846 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
date	sampling date formatted as yyyy-mm-dd	unitless

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
irrad_CO2	Irradiance-CO2 combination: H = high; L = low	unitless
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
dark_resp_growth	dark respiration/growth rate	unitless
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
PM_stderr	standard error of 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
alpha_stderr	standard error of 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)
N_to_chl	Nitrogen:chlorophyll ratio	unitless (grams/grams)
P_PM	Ratio of mean photosynthesis to maximum photosynthetic production (P/PM)	unitless
growth_stdev_n	This column contains both the standard deviation of the daily growth, plus the number of values used in the calculation.	per day

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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	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	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. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

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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