[Deprecated] Laboratory growth, photosynthetic, and respiration rates of Thalassiosira pseudonana clone 3H in nitrate limited culture (Stressors on Marine Phytoplankton project)

Website: https://www.bco-dmo.org/dataset/714846
Data Type: experimental
Version: 0
Version Date: 2017-09-14

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

Contributors	Affiliation	Role
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Abstract
This dataset has been deprecated and replaced. Please see dataset https://www.bco-dmo.org/dataset/779368.

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Coverage

Spatial Extent: **Lat**:30.4089 **Lon**:-91.18412

Dataset Description

This dataset has been deprecated and replaced. Please see dataset dataset https://www.bco-dmo.org/dataset/779368.

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

Methods & Sampling

The culture was grown in a nitrate-limited continuous culture system on a 14:10 L:D cycle of illumination at temperatures of 10, 15, 20, 25, 30, and 32°C. The irradiance during the photoperiod was 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 CO_2 concentration in the laboratory was monitored with a CO2METER model AZ-004 meter calibrated at 0 and 400 ppm CO_2 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 organic carbon (POC) 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 illero, 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 L^{-1}. Nutrient concentrations corresponded to f/2 medium, with the exception of nitrate, which was added at a concentration of 40 micromolar, and trace metals, which were added at the concentrations specified by Sunda and Hardison (Limnology & Oceanography 52[6]: 2496–2506 [2007]). 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. 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 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. 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 5, 10, 20, 30, 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. 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, CO_2 in Seawater: Equilibrium, Kinetics, Isotopes.

Photosynthetic rates as a function of irradiance were found to be best described by Hill reaction kinetics with n = 2 (Hill, A. V., J. Physiol. 40, iv-vii [1910]). For n = 2, the Hill equation takes the form

$$P = P_{max} I^{\wedge 2} / (K_I{}^{\wedge 2} + I^{\wedge 2})$$

The light-saturated photosynthetic rate (P_{max}) with units of grams carbon per gram chlorophyll *a* per hour and the parameter K_I (Hill coefficient) with units of micro-mol photons m^{-2} s^{-1} were determined by least squares. 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 room temperature for 30 minutes, after which QY was measured in a darkened room.

References:

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 191 pp.

Hill, A. V. 1910. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J. Physiol.* 40: iv–vii.

Sunda, W. G., and D. R. Hardison. 2007. Ammonium uptake and growth limitation in marine phytoplankton. Limnol. Oceanogr. 52(6): 2496–2506.

Data Processing Description

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

$d(POC)/dt = P - m \times POC$ (1)

where P is the rate of production of *POC* in the growth chamber, m 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 (1) between two points in time is

$P = m(POC_t - POC_0\, e^{-mt})/(1 - e^{-mt})$ (2)

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 total organic carbon ^{14}C activity at the end of the photoperiod and the beginning of the subsequent photoperiod. All $TO^{14}C$ activities were corrected for blank counts, which were typically 24 counts per minute. The natural logarithm of the ratio of the $TO^{14}C$ counts was equated to $(m + m_r)10/24$, where m_r is the dark respiration rate (d^{-1}) and m is the dilution rate (d^{-1}). Division by 24 converts these rates to h^{-1}, and multiplication by 10 corrects for the fact that the duration of the dark period was 10 hours. Thus

$m_r = (24/10)\ln(TO^{14}\,C_e / TO^{14}\,C_b) - m$ (3)

where $TO^{14}\,C_e$ and $TO^{14}\,C_b$ are the ^{14}C counts in the TOC at the end of one photoperiod and the beginning of the next photoperiod, respectively.

The number of photons absorbed per carbon atom fixed was calculated for each irradiance of the five-minute photosynthesis-irradiance experiments by assuming a chlorophyll *a*-specific absorption coefficient of 14 $m^2\ g^{-1}$ chl *a* based on Atlas and Bannister (Limnology & Oceanography 25(1): 157–159 [1980]).

The minimum quantum requirement (i.e., smallest number of photons required to fix one carbon atom) was estimated at the beginning, midpoint, and end of the 14-h photoperiod based on the 5-minute uptake of inorganic carbon (gram C per gram chlorophyll per hour) versus irradiance. Hill kinetics with n = 2 was assumed (Hill, A. V., J. Physiol. 40, iv-vii [1910]). For n = 2, the Hill equation takes the form

$P = P_{max} I^2/(K_I^2 + I^2)$

For such an uptake curve, the minimum quantum requirement equals $2K_I A_{abs}/P_{max}$, where A_{abs} is the chlorophyll-specific absorption coefficient of light. Because our experiments were conducted with white light (daylight fluorescent lamps), we assumed an A_{abs} value of 14 m^2 per gram of chlorophyll based on Atlas and Bannister, Limnology & Oceanography 25(1): 157–159 (1980). If K_I has units of micro-mol photons $m^{-2}\ s^{-1}$ and P_{max} has units of g C g^{-1} chl *a* h^{-1}, the minimum quantum requirement based on the Hill equation with n = 2 is $1.2096K_I/P_{max}$. Minimum quantum requirements were calculated at the beginning, middle, and end of the 14-h photoperiod and are reported as the averages of those three estimates.

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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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

Hill, A. V. 1910. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J. Physiol. 40: iv–vii.
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

Replaced By New Versions

Laws, E., Passow, U. (2020) **Continuous culture studies of possible climate change effects: Thalassiosira pseudonana CCMP1335 growth in nitrate-limited and nutrient-replete cultures.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2020-05-07 doi:10.26008/1912/bco-dmo.779368.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
temperature	temperature of culture	degrees Celsius
growth_rate	growth rate	per day
C_N	carbon to nitrogen ratio	grams C/grams N
C_chla	carbon to chlorophyll-a ratio, or F ratio (Strickland, 1960)	grams C/grams chlorophyll-a
PI	Productivity Index, PI: the photosynthetic rate normalized to the chlorophyll concentrations	grams C/grams chlorophyll-a/hour
Pmax	maximum photosynthetic rate	grams C/grams chlorophyll-a/hour
K_I	Hill coefficient, KI	micromole photons/meter^2/second
quantum_min	minimum quantum requirement; derived from the inverse of the maximum quantum yield (Falkowski and Raven)	photons per carbon
dark_respiration_rate	dark respiration rate	per day
Fv_Fm	maximum quantum yield (QY=Fv/Fm)	unitless
time_interval	time interval of study	unitless

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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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