Series 1B-1: Multiple stressor experiments on T. pseudonana (CCMP1335) – pH, Dissolved Inorganic carbon (DIC), and Macronutrient concentrations in experiments

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

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Abstract
Four follow-up experiments on the combined effect of light and temperature changes on the growth rate (μ) and photophysiology of Thalassiosira pseudonana CCMP 1335 were conducted to supplement / repeat series 1A experiments. This was necessary because doubt existed regarding the growth during 1A experiments. 1A experiments were conducted in artificial seawater. 1B experiments were conducted in artificial seawater supplemented with 5% sterilized seawater. This data set contains the carbonate system parameters.The experiments were designed to test the combined effects of four temperatures, and eight light intensities on the growth and photophysiology of the diatom T. pseudonana CCMP1335 in a multifactorial design. This dataset contains measurements of pH, DIC and macronutrient (N, P and Si) concentrations made over the course of the experiments

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Coverage

Temporal Extent: 2018-11-17 - 2019-04-16

Acquisition Description

Experimental setup:

The experiments were designed to test the combined effects of four temperatures, and eight light intensities on growth and photophysiology of the diatom T. pseudonana CCMP1335 in a multifactorial

design. Four temperatures were tested: 15°C, 18°C, 22°C, and 26°C. Within each temperature, eight light levels were tested: 30, 40, 70,90,105,125,140 and 265 µmol photons m-2 s-1. All lights were set at a 12 h day: 12 h dark cycle. For logistical reasons, experiments were partially conducted in series.

Experiments were conducted in Multicultivator MC-1000 OD units (Photon Systems Instruments, Drasov, Czech Republic). Each unit consists of eight 85 ml test-tubes immersed in a thermostated water bath, each independently illuminated by an array of cool white LEDs set at specific intensity and timing. A 0.2µm filtered ambient air was bubbled through sterile artificial seawater, and the humidified air was supplied to each tube. Each experiment was split into two phases: An acclimation phase spanning 3 days, was used to acclimate cultures to their new environment. Pre-acclimated, exponentially-growing cultures were then inoculated into fresh media and incubated through a 4-day experimental phase during which assessments of growth, photophysiology, and nutrient cycling were carried out daily. All sampling started 6 hours into the daily light cycle to minimize effects of diurnal cycles.

Experiments were conducted with artificial seawater (ASW) prepared using previously described methods (Kester et. al 1967), and enriched with 50mL per liter of UV sterilized natural seawater and nitrate (NO3), phosphate (PO4), silicic acid (Si[OH]4), at levels ensuring that the cultures would remain nutrient-replete over the course of the experiment. Trace metals and vitamins were added as in f/2 (Guillard 1975). The pH of the growth media was measured spectrophometrically using the m-cresol purple method (Dickson 1993), and adjusted using 0.1N HCl or 0.1M NaOH.

pH measurements:

Three ml samples were taken at the start and end of the experiment to assess pH. The pH was measured with a spectrophotometer (Genesys 10SVIS) using the indicator dye m-cresol purple (Sigma Aldrich) at 25°C. The absorbance was measured at 730 nm, 578 nm, and 434 nm before and after dye addition (Clayton & Byrne 1993, Fangue et al. 2010). A TRIS buffer solution in synthetic seawater with known pH, supplied by A. Dickson (Scripps Institution of Oceanography, USA) was used to calibrate the dye.

Dissolved Inorganic Carbon (DIC) measurements:

DIC was measured in freshly prepared media, and at the end of the experiment phase. 25 ml of the sample was siphoned into clean glass serum vials, fixed with HgCL2 (0.035 % final conc. v/v), and sealed with butyl rubber septa. Samples were stored at 4°C until analyzed. Prior experiments had confirmed that no gas exchange, and/or change in DIC occurred during sample storage for up to 30 days using this method. Total dissolved inorganic carbon (TCO2) samples were analyzed using an automated infrared inorganic carbon analyzer (AIRICA). The AIRICA-23 (MARIANDA, Kiel, Germany), is a high precision instrument used to measure total dissolved inorganic carbon in seawater. The system uses a high precision syringe and a mass flow controller to deliver a known volume of sample into a stripper where it is then acidified, converting the inorganic carbon species into CO2 and delivered under constant flow to nondispersive infrared detector. The CO2 is then carried using an inert reference gas (N2) into a LICOR-7000 that measures pCO2 using the difference in infrared absorbance between a sample and reference cell. The pCO2 is recorded over time and integrated by the AIRICA software. This integrated value is proportional to the amount of dissolved inorganic carbon evolved from the sample and converted to carbon units using a conversion factor (CT Factor). The CT Factor is determined by calibration of the system against a certified reference material of known value (Dickson et al. 2007. Guide to Best Practices for Ocean CO2 Measurements). The value is converted to gravimetric units (umol/kg) using the volume, temperature, and salinity of the sample. In order to check for analytical stability of the system throughout a run, a certified reference material is used in between every 5 samples. Replicate DIC measurements were averaged.

Macronutrient concentrations:

Media was filtered through 0.2 µm filters into clean (plastic) bottles and stored at -20°C until analyses for nutrients. Phosphate (PO4), Nitrate (NO3) + Nitrite (NO2), and Silicic Acid (Si(OH)4) were measured by Flow injection analysis (FIA) using a QuikChem 8500 Series 2 AutoAnalyzer (Lachat Instruments, Zellweger Analytics, Inc.).

Processing Description

BCO-DMO Processing Notes:
- data submitted in Excel file "BCODMO_Series 1B - 1_pH_DIC_Nuts_4Aug2020.xlsx" sheets "pH_DIC" and "Nutrients" extracted to csv
- the three sheets were joined into a single table
- added conventional header with dataset name, PI name, version date
- renamed columns to conform with BCO-DMO naming conventions (removed spaces and special characters

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Related Publications

Clayton, T. D., & Byrne, R. H. (1993). Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. Deep Sea Research Part I: Oceanographic Research Papers, 40(10), 2115–2129. doi:10.1016/0967-0637(93)90048-8
Methods

Dickson, A. G. (1993). The measurement of sea water pH. Marine Chemistry, 44(2-4), 131–142. doi:10.1016/0304-4203(93)90198-w https://doi.org/10.1016/0304-4203(93)90198-W
Methods

Dickson, A. G., & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers, 34(10), 1733–1743. doi:10.1016/0198-0149(87)90021-5
Methods

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 https://isbnsearch.org/isbn/1-897176-07-4
Methods

Fangue, N. A., O'Donnell, M. J., Sewell, M. A., Matson, P. G., MacPherson, A. C., & Hofmann, G. E. (2010). A laboratory-based, experimental system for the study of ocean acidification effects on marine invertebrate larvae. Limnology and Oceanography: Methods, 8(8), 441–452. doi:10.4319/lom.2010.8.441
Methods

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. Culture of Marine Invertebrate Animals, 29–60. doi:10.1007/978-1-4615-8714-9_3
Methods

Kester, D. R., Duedall, I. W., Connors, D. N., & Pytkowicz, R. M. (1967). Preparation of Artificial Seawater 1. Limnology and Oceanography, 12(1), 176–179. doi:10.4319/lo.1967.12.1.0176
Methods

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). MEASUREMENT OF THE APPARENT DISSOCIATION CONSTANTS OF CARBONIC ACID IN SEAWATER AT ATMOSPHERIC PRESSURE1. Limnology and Oceanography, 18(6), 897–907. doi:10.4319/lo.1973.18.6.0897
Methods

Pierrot, D. E. Lewis,and D. W. R. Wallace. 2006. MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a.
Methods

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Parameters

Parameter	Description	Units
Phase	Indicates whether the sample was collected during the acclimation phase or the experiment phase of the experiment	unitless
Temp	Indicates the temperature at which the samples were incubated.	degrees Celsius
Irradiance	Indicated light level at which the samples were incubated units of µmol photons m-2 s-1	micromol photons/meter^2/second
Day	Indicates the timepoint (day) of sampling. D0 = day 0; D1 = day 1; etc.	unitless
Replicate	Indicates replication within a treatment if applicable (not applicable).	unitless
Phosphate_uM	Phosphate concentrations	micromol/liter
Silicate_uM	Silicate concentrations	micromol/liter
NO3_NO2_uM	Nitrate + Nitrite concentrations	micromol/liter
pH_25C	pH measurements at 25°C	pH units
DIC_umol_kg	DIC measurements (in umol/kg)	micromol/kilogram
Comment	Comments on values below instrument detection limits. (Note that some concentrations in some treatments were below detection limits)	unitless

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Instruments

Dataset-specific Instrument Name	• QuikChem 8500 Series 2 AutoAnalyzer (Lachat Instruments, Zellweger Analytics, Inc.)
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Used for analysis of nutrient (N, P, Si) concentrations.
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

Dataset-specific Instrument Name	Genesys 10SVIS
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Used for measurement of 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.

Dataset-specific Instrument Name	Multicultivator MC-1000 OD (Photon Systems Instruments, Drasov, Czech Republic)
Generic Instrument Name	Cell Cultivator
Dataset-specific Description	Used for incubation of TP1014 cultures.
Generic Instrument Description	An instrument used for the purpose of culturing small cells such as algae or bacteria. May provide temperature and light control and bubbled gas introduction.

Dataset-specific Instrument Name	AIRICA-23 (MARIANDA, Kiel, Germany)
Generic Instrument Name	Inorganic Carbon Analyzers
Dataset-specific Description	An Automated infrared inorganic carbon analyzer (AIRICA) for analysis of dissolved inorganic carbon.
Generic Instrument Description	Instruments measuring carbonate in sediments and inorganic carbon (including DIC) in the water column.

Dataset-specific Instrument Name	LICOR-7000
Generic Instrument Name	Inorganic Carbon Analyzers
Dataset-specific Description	A CO2/H2O Analyzer for analysis of dissolved inorganic carbon.
Generic Instrument Description	Instruments measuring carbonate in sediments and inorganic carbon (including DIC) in the water column.

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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-1538602

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