Experimental results: Exopolymer production by phytoplankton under oxidative stress; conducted at the Thornton lab, TAMU from 2007-2012 (Diatom EPS Production project)

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Project
» Effect of Temperature on Extracellular Polymeric Substance Production (EPS) by Diatoms (Diatom EPS Production)

Contributors	Affiliation	Role
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Abstract
Data from laboratory experiment on exopolymer production by the diatom Thalassiosira wessiflogii (CCMP 1051) and the cyanobacterium Synechococcus elongates_cf (CCMP 1379) under conditions of oxidative stress.

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Dataset Description

Data from laboratory experiment on exopolymer production by the diatom *Thalassiosira wessiflogii* (CCMP 1051) and the cyanobacterium *Synechococcus elongates_cf* (CCMP 1379) under conditions of oxidative stress.

Related references:
Chen, J. 2014. Factors affecting carbohydrate production and the formation of transparent exopolymer particles (TEP) by diatoms. Ph.D. dissertation, Texas A&M University, College Station, TX.

Acquisition Description

Growth of the phytoplankton
The diatom *Thalassiosira wessiflogii* (CCMP 1051) and the cyanobacterium *Synechococcus elongates_cf* (CCMP 1379) were obtained from the National Center for Culture of Marine Algae and Microbiota (NCMA). Replicated (n = 3) Batch cultures were grown in artificial seawater (Berges et al. 2001) containing nitrogen, phosphorus and silicon at 400 µM (as NaNO3), 25 µM (NaH2PO4), and 400 µM (Na2SiO3), respectively. Culture temperatures were maintained at 20 ± 1 °C. Photon flux density on the surface of the culture bottles was 40 to 45 µmol m-2 s-1 on a 14 hour light: 10 hour dark cycle. During exponential growth, each culture was split into three treatments in which oxidative stress was induced by the addition of hydrogen peroxide at final concentrations of 0 (control), 10 and 100 µM H2O2. The treatments were sampled once a day over the next three days.

Measures of phytoplankton abundance and biomass
Counts of 400 cells from each culture were made using hemocytometers (Guillard and Sieracki 2005) from samples preserved in Lugol's iodine (Parsons et al. 1984) using a light microscope (Axioplan 2, Carl Zeiss MicroImaging). Turbidity of the cultures, used as an indicator of growth, was measured by absorbance at 750 nm in a 1 cm path cuvette using a UV-Mini 1240 spectrophotometer (Shimadzu Corporation).

Chlorophyll *a* concentration 90% acetone extractions from biomass retained on GF/C (Whatman) were measured using a Turner Designs 700 fluorometer, which was calibrated using chlorophyll *a* standards (Sigma) (Arar and Collins 1997). The extract was diluted with 90% acetone if the chl *a* concentration were too high.

Cell permeability
Uptake and staining with the membrane-impermeable SYTOX Green (Invitrogen) was used to determine what proportion of the diatom population had permeable cell membranes (Veldhuis et al. 2001, Franklin et al. 2012). Four hundred cells were examined using an epifluorescence microscope (Axioplan 2, Carl Zeiss MicroImaging) and the number of cells that stained with SYTOX Green was enumerated.

TEP staining and analysis

Transparent exopolymer particles (TEP) were sampled according to Alldredge et al. (1993) and TEP abundance was enumerated by image analysis (Logan et al. 1994, Engel 2009). Ten photomicrographs were taken of each slide using a light microscope (Axioplan 2, Carl Zeiss MicroImaging). Images were analyzed using ImageJ software (National Institutes of Health) based on the method of Engel (2009). Thresholding during image processing was done using the triangle method (Zack et al. 1977).

CSP staining and analysis

Coomassie staining particles (CSP) were sampled according to Long and Azam et al. (1996) and CSP abundance was enumerated by image analysis (Logan et al. 1994, Engel 2009). Ten photomicrographs were taken of each slide using a light microscope (Axioplan 2, Carl Zeiss MicroImaging). Images were analyzed using ImageJ software (National Institutes of Health) based on the method of Engel (2009). Thresholding during image processing was done using the triangle method (Zack et al. 1977).

Quantum yield of photosystem II

The quantum yield of photosystem II was used as an indicator of phytoplankton health and measured using the saturating pulse method (Genty et al. 1989) using a pulse amplitude modulated fluorometer (PAM-210, Heinz Walz GmbH) folowing a protocol based on Marwood et al. (1999).

Caspase-like activity

Caspase-like activity was measured based on the method of Bouchard & Purdie (2011). Phytoplankton were collected by centrifugation, then lysed in a buffer, and the caspase-3 like activity was measured in the extracted proteins using a Enzcheck Caspase-3 Assay Kit #1 (Invitrogen inc.). The fluorescent product was measured by fluorescence using a microplate reader (SPECTRAmax GeminiEM, Molecular Devices).

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Processing Description

Limited processing was necessary with this dataset. As this was a laboratory experiment it was designed in such a way to ensure that the parameters measured were likely to be within a measurable range and therefore there were no measurements below detection limits. Chlorophyll concentrations were frequently too high; this was resolved by diluting the sample into the measurable range. Measured parameters were normalized to volume as most of the parameters were expressed as concentrations.

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Parameters

Parameter	Description	Units
species	Species name.	dimensionless
day	Day of the experiment.	dimensionless
H2O2	Hydrogen peroxide concentration.	micromolar (uM)
turbidity	Turbidity of the cultures measured by absorbance at 750 nm in a 1 cm path cuvette using a spectrophotometer.	NTU
cell_conc	Cell concentration. Counts of 400 cells were made by transmitted light microscopy using a hemacytometer (Fuchs-Rosenthal ruling Hauser Scientific) (Guillard & Sieracki 2005).	cells per milliliter (cells mL-1)
chla	Chlorophyll a measured by fluorescence (Arar & Collins 1997; Method 445.0. EPA).	micrograms per liter (ug L-1)
PSII	Quantum yield of photosystem II measured after Marwood et al. (1999) using pulse amplitude modulated chlorophyll fluorometer.	quantum yield of photosystem II

caspase_like_activity	Caspase-like activity was measured after Bouchard & Purdie (2011).	relative fluorescence units per milligrams protein per hour (RFU mg protein-1 h-1)
stained_cells_pcnt	% of SYTOX Green stained cells. Uptake and staining with the membrane-impermeable SYTOX Green (Invitrogen) was used to determine what proportion of the diatom population had permeable cell membranes (Veldhuis et al. 2001; Franklin et al. 2012). Four hundred cells were examined using an epifluorescence microscope and the number of cells that stained with SYTOX Green was enumerated.	percent (%)
TEP_conc	Transparent exopolymer particles (TEP) concentration. TEP retained on 0.4 polycarbonate filters and stained with Alcian blue (Alldredge et al. 1993).	micrometers TEP per milliliter (um2 mL-1)
TEP_abund	Transparent exopolymer particles (TEP) abundance. TEP retained on 0.4 polycarbonate filters and stained with Alcian blue (Alldredge et al. 1993).	TEP per milliliter (mL-1)
TEP_per_chla	Transparent exopolymer particles (TEP) per chlorophyll a.	square millimeters of TEP per nanogram of chla (mm2 (ng chl. a)-1)
CSP_conc	Coomassie staining particles (CSP) concentration. CSP retained on 0.4 polycarbonate filters and stained with Coomassie briliant blue blue (Long & Azam 1996).	micrometers CSP per milliliter (um2 mL-1)
CSP_abund	Coomassie staining particles (CSP) abundance. CSP retained on 0.4 polycarbonate filters and stained with Coomassie briliant blue blue (Long & Azam 1996).	CSP per milliliter (mL-1)

CSP_per_chla	Coomassie staining particles (CSP) per chlorophyll a.	square millimeters of CSP per nanogram of chlorophyll a (mm2 (ng chl. a)-1)

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Instruments

Dataset-specific Instrument Name	Pulse Amplitude Modulated Fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	The quantum yield of photosystem II was measured using the saturating pulse method (Genty et al. 1989) using a pulse amplitude modulated fluorometer (PAM-210, Heinz Walz GmbH).
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	UV-Mini 1240 Spectrophotometer
Generic Instrument Name	UV Spectrophotometer-Shimadzu
Dataset-specific Description	Turbidity of the cultures was measured by absorbance at 750 nm in a 1 cm path cuvette using a UV-Mini 1240 Spectrophotometer (Shimadzu Corporation).
Generic Instrument Description	The Shimadzu UV Spectrophotometer is manufactured by Shimadzu Scientific Instruments (ssi.shimadzu.com). Shimadzu manufacturers several models of spectrophotometer; refer to dataset for make/model information.

Dataset-specific Instrument Name	Turner Designs 700 Fluorometer
Generic Instrument Name	Turner Designs 700 Laboratory Fluorometer
Dataset-specific Description	Chlorophyll a concentration 90% acetone extractions from biomass retained on GF/C (Whatman) were measured using a Turner Designs 700 fluorometer, which was calibrated using chlorophyll a standards (Sigma) (Arar and Collins 1997).
Generic Instrument Description	The TD-700 Laboratory Fluorometer is a benchtop fluorometer designed to detect fluorescence over the UV to red range. The instrument can measure concentrations of a variety of compounds, including chlorophyll-a and fluorescent dyes, and is thus suitable for a range of applications, including chlorophyll, water quality monitoring and fluorescent tracer studies. Data can be output as concentrations or raw fluorescence measurements.

Dataset-specific Instrument Name	Epifluorescence Microscope
Generic Instrument Name	Microscope-Fluorescence
Dataset-specific Description	Cell permeability was determined using an epifluorescence microscope (Axioplan 2, Carl Zeiss MicroImaging).
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Hemocytometer
Generic Instrument Name	Hemocytometer
Dataset-specific Description	Counts of 400 cells from each culture were made using hemocytometers (Guillard and Sieracki 2005) from samples preserved in Lugol's iodine (Parsons et al. 1984) using a light microscope.
Generic Instrument Description	A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html.

Dataset-specific Instrument Name	Light Microscope
Generic Instrument Name	Microscope-Optical
Dataset-specific Description	Counts of 400 cells from each culture were made using hemocytometers (Guillard and Sieracki 2005) from samples preserved in Lugol's iodine (Parsons et al. 1984) using a light microscope (Axioplan 2, Carl Zeiss MicroImaging). A light microscope (Axioplan 2, Carl Zeiss MicroImaging) was also used to enumerate TEP and CSP by image analysis.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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Deployments

lab_Thornton

Website	https://www.bco-dmo.org/deployment/506141
Platform	TAMU
Start Date	2007-09-01
End Date	2012-08-01
Description	Experiments conducted in the lab of Daniel C.O. Thornton located at: Department of Oceanography Texas A&M University College Station, Texas, 77843 United States

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Project Information

Effect of Temperature on Extracellular Polymeric Substance Production (EPS) by

Diatoms (Diatom EPS Production)

Coverage: O&M Building, Texas A&M University, College Station, TX 77840

Description from NSF Propsoal: It is necessary to determine the fate of organic matter in the ocean to understand marine food webs, biogeochemical cycles, and climate change. Diatoms fix approximately a quarter of the net global primary production each year, and a significant proportion of this production is excreted as extracellular polymeric substances (EPS). EPS have a profound impact on pelagic ecosystems by affecting the formation of aggregates. Diatoms and other particulate organic carbon (POC) sink rapidly as aggregates, affecting the biological carbon pump, which plays a pivotal role in the sequestration of carbon in the ocean. The proposed research will test the central hypothesis: Temperature increase affects diatom release of EPS, which act as a glue, increasing aggregation. Previous work by the investigator showed that increased temperatures affected the aggregation of Skeletonema costatum. Four specific hypotheses will be tested: H1: Diatoms produce more EPS with increasing temperature. H2: Diatoms produce more transparent exopolymer particles (TEP) with increasing temperature. H3: The quantity or composition of cell-surface carbohydrates in diatoms changes with temperature. H4: Aggregation of diatom cultures and natural plankton increases with temperature. Laboratory experiments (years 1 - 2) will be conducted with three model diatom species grown at controlled growth rates and defined limitation (nitrogen or light) in continuous culture. Culture temperature will be stepped up or down in small increments to determine the effect of the temperature change on EPS production, aggregation, and partitioning of carbon in intra- and extracellular pools. Similar experiments in year 3 will be carried out using natural plankton populations from a coastal site where diatoms contribute a significant proportion to the biomass. The proposed research will increase our understanding of the ecology and physiology of one of the dominant groups of primary producers on Earth. EPS are a central aspect of diatom biology, though the physiology, function and broader ecosystem impacts of EPS production remain unknown. This research will determine how temperature, light limitation, and nutrient limitation affect the partitioning of production between dissolved, gel, and particulate phases in the ocean. Measurements of plankton stickiness (alpha) under different conditions will be important to model aggregation processes in the ocean as alpha is an important (and variable) term in coagulation models. Determining how carbon is cycled between the ocean, atmosphere and lithosphere is key to understanding climate change on both geological and human time scales. This is a major societal issue as atmospheric CO2 concentrations are steadily increasing, correlating with a 0.6 C rise in global average temperature during the last century. This research will address potential feedbacks between warming of the surface ocean, diatom ecophysiology and the biological carbon pump. Related Publications: Rzadkowolski, Charles E. and Thornton, Daniel C. O. (2012) Using laser scattering to identify diatoms and conduct aggregation experiments. Eur. J. Phycol., 47(1): 30-41. DOI: 10.1080/09670262.2011.646314 Thornton, Daniel C. O. (2009) Effect of Low pH on

Carbohydrate Production by a Marine Planktonic Diatom (Chaetoceros muelleri). Research Letters in Ecology, vol. 2009, Article ID 105901, 4 pages. DOI: 10.1155/2009/105901 Thornton, D.C.O. (2014) Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. European Journal of Phycology 49: 20-46. DOI: 10.1080/09670262.2013.875596 Thornton, D.C.O., Visser, L.A. (2009) Measurement of acid polysaccharides (APS) associated with microphytobenthos in salt marsh sediments. Aquat Microb Ecol 54:185-198. DOI: 10.3354/ame01265

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