Experimental results: Exopolymer and carbohydrate production by diatoms with growth; conducted at the Thornton lab, TAMU from 2007-2012 (Diatom EPS Production project)

Website: https://www.bco-dmo.org/dataset/511526
Data Type: experimental
Version: 1
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Project
» Effect of Temperature on Extracellular Polymeric Substance Production (EPS) by Diatoms (Diatom EPS Production)

Contributors	Affiliation	Role
Thornton, Daniel C.O.	Texas A&M University (TAMU)	Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Data from laboratory experiment on exopolymer and carbohydrate production by the diatoms *Thalassiosira weissflogii* (CCMP 1051), *Skeletonema marinoi* (CCMP 1332), and *Cylindrotheca closterium* (CCMP 339) during the growth to death phases of the cultures.

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.

Methods & Sampling

Growth of the diatoms
The diatoms *Thalassiosira weissflogii* (CCMP 1051), *Skeletonema marinoi* (CCMP 1332), and *Cylindrotheca closterium* (CCMP 339) were grown in artificial seawater (Berges et al. 2001) in batch culture at 20 °C with 100 µM NaNO3, 200 µM of NaH2PO4·H2O, and 200 µM of Na2SiO3·9H2O. Illumination was on a 14 h:10 h light:dark cycle at a photon flux density of 160 µmol m-2 s-1. There were three replicate cultures. Cultures were sampled during both the growth and death of the cultures over several weeks.

Measures of diatom abundance and biomass
Counts of 400 cells from each culture were made using a hemacytometer (Fuchs-Rosenthal ruling, Hauser Scientific) (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).

Cell volume was determined using live cells (Menden-Deuer and Lessard 2000). The volume of 25 diatoms from

each replicate culture was determined by measuring cell length (pervalver length) and width (valver length) at 400x magnification using a light microscope (Axioplan 2, Carl Zeiss MicroImaging). Cell volume was calculated based on the assumption that both *T. wessiflogii* and *S. marinoi* were cylinders. The volume of *Cylindrotheca closterium* was estimated assuming that its shape was equivalent to two cones.

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.

Bacteria abundance
Bacteria (400 cells) were counted using an epifluorescence microscope (Axioplan 2, Carl Zeiss MicroImaging) after staining with 4'6-diamidino-2-phenylindole dihydrochloride (DAPI) (Porter and Feig 1980) at a final concentration of 0.25 µg ml-1.

Carbohydrate analysis
Two spectrophotometric methods were used to measure carbohydrates, the phenol sulfuric acid (PSA) method (Dubois et al. 1956) and the 2, 4, 6-tripyridyl-s-triazine (TPTZ) method (Myklestad et al. 1997). The color produced by both methods was measured in 1 cm path length cuvette using UV-Mini 1240 spectrophotometer (Shimadzu Corporation). Both methods were calibrated using D-glucose and the results are expressed as D-glucose equivalents. Different fractions of carbohydrate were extracted from the cultures using methods described in Underwood et al. (1995) and Underwood et al. (2004): total, colloidal, exopolymers (EPS), intracellular carbohydrate (hot water (HW) extraction), cell-wall associated carbohydrates (hot bicarbonate (HB) extraction), and residual. These carbohydrate fractions were measured using the PSA method. The TPTZ method was used to measure the intracellular and extracellular monosaccharide pools and the intracellular and extracellular polysaccharide pools after acid hydrolysis of the sample.

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).

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Data 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 we 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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Data Files

File
growth_phase_exopolymers.csv (Comma Separated Values (.csv), 18.94 KB) MD5:013dac5262adb9dd8c9b3a97328819ee Primary data file for dataset ID 511526

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Parameters

Parameter	Description	Units
species	Species name.	dimensionless
growth_phase	Growth phase of the diatom (exponential, stationary, declining, death).	dimensionless
day	Day of the experiment.	dimensionless
culture	Identifier of the culture replicate.	dimensionless
cell_abundance	Cell count. Counts of 400 cells were made by transmitted light microscopy using a hemacytometer (Fuchs-Rosenthal ruling Hauser Scientific) (Guillard & Sieracki 2005).	cells per milliliter (mL-1)
cell_vol_mean	Mean cell volume calculated assuming that both T. wessiflogii and S. marinoi were cylinders. The volume of Cylindrotheca closterium was estimated assuming that its shape was equivalent to two cones.	cubic micrometers (um^3)
chla	Concentration of chlorophyll a measured by fluorescence (Arar & Collins 1997; Method 445.0. EPA).	micrograms per liter (ug L-1)
chla_per_cell	Concentration of chlorophyll a per cell.	picograms per cell (pg cell-1)
chla_per_cell_vol	Concentration of chlorophyll a per cell volume.	femtograms per cubic micrometer (fg um-3)
tot_carb	Total carbohydrate concentration measured using the PSA method (Dubois et al. 1956).	micrograms per milliliter (ug mL-1)
tot_carb_per_cell	Total carbohydrate concentration per cell.	picograms per cell (pg cell-1)
tot_carb_per_cell_vol	Total carbohydrate concentration per cell volume.	femtograms per cubic micrometer (fg um-3)
colloidal_carb	Colloidal carbohydrate concentration. Different fractions of carbohydrate were extracted from the cultures using methods described in Underwood et al. (1995) and Underwood et al. (2004). The colloidal carbohydrate fractions were measured using the PSA method (Dubois et al. 1956).	micrograms per milliliter (ug mL-1)
collodial_per_cell	Colloidal carbohydrate concentration per cell.	picograms per cell (pg cell-1)
colloidal_per_cell_vol	Colloidal carbohydrate concentration per cell volume.	femtograms per cubic micrometer (fg um-3)
EPS_carb	Exopolymer (EPS) carbohydrate concentration. Different fractions of carbohydrate were extracted from the cultures using methods described in Underwood et al. (1995) and Underwood et al. (2004). The EPS carbohydrate fractions were measured using the PSA method (Dubois et al. 1956).	micrograms per milliliter (ug mL-1)

EPS_carb_per_cell	Exopolymer (EPS) carbohydrate concentration per cell.	picograms per cell (pg cell-1)
EPS_carb_per_cell_vol	Exopolymer (EPS) carbohydrate concentration per cell volume.	femtograms per cubic micrometer (fg um-3)
HW_carb	Intracellular carbohydrate (hot water (HW) extraction) concentration. Different fractions of carbohydrate were extracted from the cultures using methods described in Underwood et al. (1995) and Underwood et al. (2004). The HW carbohydrate fractions were measured using the PSA method (Dubois et al. 1956).	micrograms per milliliter (ug mL-1)
HW_carb_per_cell	Intracellular carbohydrate (hot water (HW) extraction) concentration per cell.	picograms per cell (pg cell-1)
HW_carb_per_cell_vol	Intracellular carbohydrate (hot water (HW) extraction) concentration per cell volume.	femtograms per cubic micrometer (fg um-3)
HB_carb	Cell-wall associated carbohydrate (hot bicarbonate (HB) extraction) concentration. Different fractions of carbohydrate were extracted from the cultures using methods described in Underwood et al. (1995) and Underwood et al. (2004). The HB carbohydrate fractions were measured using the PSA method (Dubois et al. 1956).	micrograms per milliliter (ug mL-1)
HB_carb_per_cell	Cell-wall associated carbohydrate (hot bicarbonate (HB) extraction) concentration per cell.	picograms per cell (pg cell-1)
HB_carb_per_cell_vol	Cell-wall associated carbohydrate (hot bicarbonate (HB) extraction) concentration per cell volume.	femtograms per cubic micrometer (fg um-3)
residual_carb	Residual carbohydrate concentration. Different fractions of carbohydrate were extracted from the cultures using methods described in Underwood et al. (1995) and Underwood et al. (2004). The residual carbohydrate fractions were measured using the PSA method (Dubois et al. 1956).	micrograms per milliliter (ug mL-1)
residual_carb_per_cell	Residual carbohydrate concentration per cell.	picograms per cell (pg cell-1)
residual_carb_per_cell_vol	Residual carbohydrate concentration per cell volume.	femtograms per cubic micrometer (fg um-3)
TPTZ_intracell_mono	Intracellular monosaccharide concentration determined using the TPTZ method (Myklestad et al. 1997).	micrograms per milliliter (ug mL-1)
TPTZ_extracell_mono	Extracellular monosaccharide concentration determined using the TPTZ method (Myklestad et al. 1997).	micrograms per milliliter (ug mL-1)
TPTZ_intracell_polysacc	Intracellular polysaccharide concentration determined using the TPTZ method (Myklestad et al. 1997).	micrograms per milliliter (ug mL-1)

TPTZ_extracell_polysacc	Extracellular polysaccharide concentration determined using the TPTZ method (Myklestad et al. 1997).	micrograms per milliliter (ug mL-1)
TEP_conc_mean	Mean transparent exopolymer particle (TEP) concentration. TEP retained on 0.4 polycarbonate filters and stained with Alcian blue (Alldredge et al. 1993).	TEP per milliliter (TEP mL-1)
TEP_mean_size	Mean size of Transparent exopolymer particles (TEP).	square micrometers (um^2)
tot_TEP_area	Total TEP area.	square millimeters per milliliter (mm^2 mL-1)
CSP_conc_mean	Mean coomassie staining particle (CSP) concentration. CSP retained on 0.4 polycarbonate filters and stained with Coomassie briliant blue blue (Long & Azam 1996).	CSP per milliliter (mL-1)
CSP_mean_size	Mean size of coomassie staining particle (CSP).	square micrometers (um^2)
tot_CSP_area	Total CSP area.	square millimeters per milliliter (mm^2 mL-1)
stained_cells_pcnt	% of SYTOX Green stained cells. Cell permeability was determined by SYTOX Green staining (Veldhuis et al. 1997). Four hundred cells were examined using an epifluorescence microscope and the number of cells that stained with SYTOX Green was enumerated.	percent (%)
bacteria	Bacteria abundance determined by DAPI staining and counts using an epifluorescence microscope (Porter & Feig 1980).	cells per milliliter (mL-1)
bact_per_diatom	Bacteria abundance per diatom.	dimensionless

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Instruments

Dataset-specific Instrument Name	Epifluorescence Microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Bacteria were counted and 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 a hemocytometer (Fuchs-Rosenthal ruling, Hauser Scientific) (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 a hemacytometer (Fuchs-Rosenthal ruling, Hauser Scientific) (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 was also used to determine cell volume and 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".

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	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). Two spectrophotometric methods were used to measure carbohydrates, the phenol sulfuric acid (PSA) method (Dubois et al. 1956) and the 2, 4, 6-tripyridyl-s-triazine (TPTZ) method (Myklestad et al. 1997). The color produced by both methods was measured in 1 cm path length cuvette using 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.

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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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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0726369

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