# Results from growth rate experiment with the diatom Thalassiosira wessiflogii in semi-continuous culture; conducted at the Thornton lab, TAMU from 2007-2012 (Diatom EPS Production project)

**Website**: https://www.bco-dmo.org/dataset/506135
**Data Type**: experimental
**Version**: 1
**Version Date**: 2014-04-07

**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 growth rate and transparent exopolymer particles (TEP) in the diatom Thalassiosira wessiflogii (CCMP 1051) in a semi-continuous culture (four replicate cultures).

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## Dataset Description

Data from laboratory experiment on growth rate and transparent exopolymer particles (TEP) in the diatom *Thalassiosira wessiflogii* (CCMP 1051) in a semi-continuous culture (four replicate 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.

Chen, J., Thornton, D.C.O. (in revision). Effect of growth rate on TEP production and aggregation of *Thalassiosira weissflogii. Journal of Phycology.*

### Methods & Sampling

**Growth of the diatom**
*Thalassiosira wessiflogii* (CCMP 1051) was obtained from the National Center for Culture of Marine Algae and Microbiota (NCMA). The diatom was grown in artificial seawater (Berges et al. 2001) in nitrogen-limited 1000 ml semi-continuous cultures at a sequence of dilution rates. The macronutrient concentrations in the artificial seawater recipe were modified from Berges et al. (2001) to affect nitrogen limitation; concentrations of nitrogen, phosphorus and silicon were 60 µM (as NaNO3), 100 µM (NaH2PO4), and 100 µM (Na2SiO3), respectively. Culture temperature was maintained at 20 ± 0.1 °C throughout the experiment. Photon flux

density on the surface of the culture bottles was 150 µmol m-2 s-1. The cultures were stirred with 2.5 cm long stir bars using magnetic stirrers at 120 revolutions per minute. The cultures were grown at a sequence of dilution rates (0.3, 0.5, 0.7, 0.9 and 0.3 day-1) affected by daily dilution at 10:00 am every day. To induce a dilution rate of 0.3 day-1, 0.3 of the culture volume (300 ml) was removed and replaced with 300 ml of fresh medium to maintain a constant total culture volume (1000 ml).

**Measures of phytoplankton abundance and biomass**
Counts of 400 cells from each replicate culture were made by light microscopy using a hemocytometer (Fuchs-Rosenthal ruling, Hauser Scientific) (Guillard and Sieracki 2005) from samples preserved in Lugol's iodine (Parsons et al. 1984). Cell volume was determined using live cells (Menden-Deuer and Lessard 2000). The volume of 100 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 *T. wessiflogii* is a cylinder.

Chlorophyll *a* concentrations in the cultures was determined by fluorescence (Arar and Collins 1997). 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.

The carbon and nitrogen content of particulate organic matter in the cultures was determined by elemental analysis using a Carlo Erba NA1500 Elemental Analyzer. Standards were acetanilide, methionine, graphite (USGS 24, USGS 40, and USGS 41) (Verardo et al. 1990).

**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.

**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 and the number of cells that stained with SYTOX Green was enumerated.

**Total carbohydrate**
Total carbohydrate concentrations were determined in unfiltered liquid samples from the cultures using the phenol-sulfuric acid (PSA) method (Dubois et al. 1956) calibrated with d-glucose. The concentration of total carbohydrate was expressed as glucose equivalents.

**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 and the area of 100 TEP particles from each replicate culture was determined after manually drawing around each particle using Axio Vision 4.8 (Carl Zeiss MicroImaging ) image analysis software.

**Particle size distribution and aggregation**
The particle size distribution (PSD) and volume concentration of particles in the *T. weissiflogii* cultures was measured using laser scattering following the method of Rzadkowolski and Thornton (2012) using a Laser *In Situ* Scattering and Transmissometry instrument (LISST-100X, Type C; Sequoia Scientific). Sample (150 ml) from each replicate culture was placed into a chamber attached to the LISST and the PSD was measured 100 times at a rate of 1 Hz. The PSD of the culture was blank corrected by subtracting the PSD of 0.2 µm filtered artificial seawater.

**References cited**
Alldredge, A. L., Passow, U. & Logan B. E. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res. Oceanogr., I.* 40: 1131-1140. doi:10.1016/0967-0637(93)90129-Q

Arar, E. J. & Collins, G. B. 1997. Method 445.0. In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence U.S. Environmental Protection Agency, Cincinnati, Ohio.

Berges, J. A., Franklin D. J. & Harrison, P. J. 2001. Evolution of an artificial seawater medium: Improvements in enriched seawater, artificial water over the last two decades. *J. Phycol.* 37:1138-1145. doi:10.1046/j.1529-8817.2001.01052.x

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Logan, B. E., Grossart, H. P. & Simon, M. 1994. Direct observation of phytoplankton, TEP and aggregates on polycarbonate filters using brightfield microscopy. *J. Plankton Res.*16: 1811-1815.doi:10.1093/plankt/16.12.1811

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Porter, K. G. & Feig, Y. S. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25:943–948. doi:10.4319/lo.1980.25.5.0943

Rzadkowlski, C. E. & Thornton, D. C. O. 2012. Using laser scattering to identify diatoms and conduct aggregation experiments. *Eur. J. Phycol.*47:30-41. doi:10.1080/09670262.2011.646314

Veldhuis, M. J. W., Kraay, G. W. & Timmermans, K. R. 2001. Cell death in phytoplankton: correlation between changes in membrane permeability, photosynthetic activity, pigmentation and growth. *Eur. J. Phycol.* 36: 167–177. doi:10.1080/09670260110001735318

Verardo, D. J., Froelich, P. N. & McIntyre, A. 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. *Deep-Sea Res.A* 37:157-165. doi:10.1016/0198-0149(90)90034-S

### 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 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_rate_TEP.csv** (Comma Separated Values (.csv), 7.79 KB)<br>MD5:397732374bb0fce3fd14cc930d9ca2e7<br>Primary data file for dataset ID 506135 |

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| dilution_rate | Dilution rate. | per day (day-1) |
| culture | Identifier of the culture replicate. | dimensionless |
| day | Day of the experiment. | 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 |
| cell_vol_mean | Mean cell volume estimated assuming T. weissflogii (CCMP 1051) was a cyclinder using the method of Menden-Deuer & Lessard (2000). | cubic micrometers (um^3) |
| cell_vol_sd | Standard deviation of cell_vol_mean. | cubic micrometers (um^3) |
| cell_vol_n | n (number of cells) used in determination of cell_vol_mean. | dimensionless |
| 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) |
| TEP | Transparent exopolymer particles (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) |
| TEP_sd | Standard deviation of TEP_mean_size. | square micrometers (um^2) |
| TEP_n | n used in determination of TEP_mean_size. | dimensionless |
| tot_TEP_area | Total TEP area. | square millimeters per milliliter (mm^2 mL-1) |
| TEP_prod_rate | TEP production rate. | square millimeters per milliliter per day (mm^2 mL-1 day-1) |

| | | |
|---|---|---|
| vol_conc | Particulate volume concentration. Volume concentration and aggegation were measured using Laser in situ sacattering and transmissometry (LISST) (Rzadkowolski & Thornton 2012). | microliters per liter (uL L-1) |
| vol_conc_sd | Standard deviation of vol_conc. | microliters per liter (uL L-1) |
| vol_conc_n | n used in determination of vol_conc. | dimensionless |
| agg_vol_conc | Aggregated volume concentration (particles > 63 um ESD). Particulate volume concentration and aggegation were measured using Laser in situ sacattering and transmissometry (LISST) (Rzadkowolski & Thornton 2012). | microliters per liter (uL L-1) |
| agg_vol_conc_sd | Standard deviation of agg_vol_conc. | microliters per liter (uL L-1) |
| agg_vol_conc_n | n used in determination of agg_vol_conc. | dimensionless |
| stained_cells | Number 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. | cells per milliliter (cells 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 (cells mL-1) |
| bact_per_diatom | Bacteria abundance per diatom. | dimensionless |
| C_to_N | Ratio of carbon to nitrogen. C:N ratio was measured using a Carlo Erba NA1500 Elemental Analyzer. Standards were acetanilide, methionine, graphite (USGS 24, USGS 40, and USGS 41) (Verardo, Froelich, & McIntyre 1990). | dimensionless |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Carlo Erba NA1500 Elemental Analyzer |
| **Generic Instrument Name** | Carlo-Erba NA-1500 Elemental Analyzer |
| **Dataset-specific Description** | The carbon and nitrogen content of particulate organic matter in the cultures was determined by elemental analysis using a Carlo Erba NA1500 Elemental Analyzer. |
| **Generic Instrument Description** | A laboratory instrument that simultaneously determines total nitrogen and total carbon from a wide range of organic and inorganic sediment samples. The sample is completely and instantaneously oxidised by flash combustion, which converts all organic and inorganic substances into combustion products. The resulting combustion gases pass through a reduction furnace and are swept into the chromatographic column by the carrier gas which is helium. The gases are separated in the column and detected by the thermal conductivity detector which gives an output signal proportional to the concentration of the individual components of the mixture. The instrument was originally manufactured by Carlo-Erba, which has since been replaced by Thermo Scientific (part of Thermo Fisher Scientific). This model is no longer in production. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Epifluorescence Microscope |
| **Generic Instrument Name** | Fluorescence Microscope |
| **Dataset-specific Description** | Bacterial abunance and cell permeability were 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 replicate culture were made by light microscopy using a hemocytometer (Fuchs-Rosenthal ruling, Hauser Scientific). |
| **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** | The volume of 100 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). |
| **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** | LISST-100X Type C Sequoia Scientific |
| **Generic Instrument Name** | Sequoia Scientific Laser In-Situ Sediment Size Transmissometer |
| **Dataset-specific Description** | The particle size distribution (PSD) and volume concentration of particles in the T. weissiflogii cultures was measured using laser scattering following the method of Rzadkowolski and Thornton (2012) using a Laser In Situ Scattering and Transmissometry instrument (LISST-100X, Type C; Sequoia Scientific). |
| **Generic Instrument Description** | A self-contained unit which measures the scattering of LASER light at a number of angles. This primary measurement is mathematically inverted to give a grain size distribution, and also scaled to obtain the volume scattering function. The size distribution is presented as concentration in each of the grain-size class bins. Optical transmission, water depth and temperature are recorded as supporting measurements. The Sequoia LISST 100-X series instruments are available in two range sizes: 1.25-250 microns (Type B) and 2.5-500 microns (Type C). |

| | |
|---|---|
| **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. |

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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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