Size and chemical responses of two dinoflagellate species used in natural high light exposure experiments (Protist Signaling project)

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Project
» Environmental stress and signaling based on reactive oxygen species among planktonic protists (Protist signaling)

Contributors	Affiliation	Role
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Abstract
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Coverage

Temporal Extent: 2015-07-15 - 2015-09-10

Dataset Description

Size and chemical responses of two dinoflagellate species used in natural high light exposure experiments.

Methods & Sampling

Dinoflagellate culturing: *Alexandrium fundyense* (strain CCMP 1911) was obtained from the National Center for Marine Algae and Microbiota (NCMA). The strain was originally isolated from Sequim Bay, Washington, USA. *Heterocapsa rotundata* (strain K-0483), obtained from the Scandanavian Culture Collection of Algae and Protozoa, was isolated from the southern Kattegat Sea near Denmark. Dinoflagellate cultures were maintained in f/2 medium without added silicate at 15°C under a 12L:12D light cycle and transferred every two to three weeks into new media. Growth irradiance for *A. fundyense* was 53 µmol photons m-2 s-1; growth irradiance for *H. rotundata* was 12 µmol photons m-2 s-1.

Cell concentration and cell volume: Cell concentration (cells mL-1) and cell volume (µm3 cell-1) estimates for *H. rotundata* were obtained from live samples measured with a Beckman Coulter Z2 Particle Count and Size Analyzer with Z2 AccuComp software. For *A. fundyense*, samples were preserved in acid Lugol's solution (final concentration 2%). Cells were counted in a 1 ml Sedgewick Rafter chamber using light microscopy; cell volume was estimated from length and width of cells (n = 42 or 83 cells per sample) measured with Leica Application Suite X image analysis software and assuming cell shape was approximated by an oblate ellipsoid.

Chlorophyll-a: Chl-a concentrations were measured by filtering samples through 0.7 µm effective pore size 25 mm glass fiber filters. Pigments were then extracted in 90% acetone for 24 h (dark, -20°C) and fluorescence was measured on a Turner 10-AU fluorometer using the acidification method. Concentrations are reported per cell and per unit cell volume.

Dissolved and particulate dimethylsulfoniopropionate (DMSP): DMSP samples (4 ml) were gravity-filtered through precombusted 0.7 µm effective pore size 25 mm glass fiber filters so as not to rupture the cells. For measurement of particulate (intracellular) DMSP (DMSPp), filters were placed into sealed 20-ml glass vials containing 3 ml of 5 N NaOH. For measurement of DMSP in the dissolved (extracellular) phase (DMSPd), the first 4.5 mL of each sample's filtrate were caught in a 5 ml polystyrene culture tube, which was capped and stored at -80°C. Later, DMSPd samples were thawed and sparged with N2 gas for 1 min to remove any dimethyl sulfide (DMS) already present. Each sparged sample (4 ml) was then dispensed into a 20-ml vial containing 1 ml of 5 N NaOH, and sealed. Upon being sealed, prepared vials for either analysis were allowed to equilibrate for at least 24 h, allowing for the 1:1 conversion of DMSP to gaseous DMS, detectable via gas chromatography. Standards for DMSPp were prepared from pre-diluted DMSP solutions at the same time that samples were filtered and sealed into vials, while DMSPd standards were made at the same time that samples were sparged and sealed into vials. Samples and standards were analyzed using a Shimadzu Gas Chromatograph 14-A equipped with a flame photometric detector and a Supelco packed Chromosil 330 column. The chromatograph was operated isothermally at 90°C with flow rates of hydrogen, air, and helium (carrier gas) at 50, 60, and 150 kPa, respectively. DMSPp samples and standards were measured via direct injection while DMSPd samples and standards were measured with a headspace sweep (He flow rate 40 kPa). DMSPp concentrations are reported per cell and per unit cell volume. DMSPd concentrations are reported per cell and per unit seawater volume.

Data Processing Description

BCO-DMO Data Processing Notes:

- reformatted column names to comply with BCO-DMO standards
- replaced all blank cells with nd
- reformatted data into long format instead of wide
- date reformatted to yyyy/mm/dd
- spaces replaced with underscores

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Data Files

File
vol_chem.csv (Comma Separated Values (.csv), 14.06 KB) MD5:0ade28c2e6d899044896c32d897398cf Primary data file for dataset ID 723277

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Related Publications

Cooney, E. C. "The Effect of High-Intensity Visible Light on the Bloom Niches of the Phototrophic Dinoflagellates Alexandrium Fundyense and Heterocapsa Rotundata." WWU Masters Thesis Collection, Western Washington University, Western Washington University, 2016, cedar.wwu.edu/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=1534&context=wwuet.

https://cedar.wwu.edu/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=1534&context=wwuet
Related Research

Cooney, E. C., Fredrickson, K. A., Bright, K. J., & Strom, S. L. (2019). Contrasting effects of high-intensity photosynthetically active radiation on two bloom-forming dinoflagellates. Journal of Phycology, 55(5), 1082–1095. Portico. https://doi.org/10.1111/jpy.12890
Methods

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Parameters

Parameter	Description	Units
Experiment_ID	Experiment ID number	unitless
Experiment_Date	Experiment date; YYYY/MM/DD	unitless
Dinoflagellate_species	Species of sample	unitless
Total_PAR_during_exposure_treatment	Total dose of photosynthetically active radiation received during light exposure treatment	mol photons m^-2
Sampling_timepoint	Time sample was taken	unitless
Replicate_cell_counts	Cell concentration in seawater media	cells ml-1
Replicate_cell_volume	Volume of individual dinoflagellate cells	um^3
Replicate_determinations_chlorophyll_a	Chlorophyll-a content of cells	pg cell-1
Replicate_determinations_chlorophyll_a_per_unit_cell_volume	Intracellular concentration of chlorophyll-a	mg L-1
Replicate_determinations_particulate_DMSP_fmolcell1	Dimethylsulfoniopropionate content of cells	fmol cell-1
Replicate_determinations_particulate_DMSP_mmolL_cellvol1	Intracellular concentration of dimethylsulfoniopropionate	mmol L cell vol-1
Replicate_determinations_dissolved_DMSP_nmolL1	Concentration of dimethylsulfoniopropionate dissolved in seawater medium normalized to cell concentration in medium	nmol L-1
Replicate_determinations_dissolved_DMSP_fmolcell1	Concentration of dimethylsulfoniopropionate dissolved in seawater medium normalized to cell concentration in medium	fmol cell-1
Replicate_determinations_total_DMSP	Concentration of dissolved plus particulate dimethylsulfoniopropionate	umol L-1

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Instruments

Dataset-specific Instrument Name	Beckman Coulter Z2 Particle Count and Size Analyzer
Generic Instrument Name	Automated Cell Counter
Dataset-specific Description	Used to determine cell concentration
Generic Instrument Description	An instrument that determines the numbers, types or viability of cells present in a sample.

Dataset-specific Instrument Name	Shimadzu Gas Chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Used to analyze samples and standards
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

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Deployments

Strom_2014

Website	https://www.bco-dmo.org/deployment/684891
Platform	lab Strom
Start Date	2014-09-01
End Date	2017-08-01
Description	Five strains of coccolithophore Emiliania huxleyi were grown in the lab. Originally collected from the Salish Sea: 48.5, -122.75.

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Project Information

Environmental stress and signaling based on reactive oxygen species among planktonic protists (Protist signaling)

Coverage: Salish Sea: 48.5, -122.75

Description from NSF proposal:
This proposal arises from the central premise that the oxidative stress response is an emergent property of phototrophic cellular systems, with implications for nearly every aspect of a phytoplankton cell's life in the upper ocean. Oxidative stress (OS) arises from the uncompensated production of reactive oxygen species (ROS) within a cell, which can occur in response to a myriad of environmental stressors (e.g. nutrient limitation, temperature extremes, toxins, variable light exposure). In addition to the biochemical damage and physiological impairment that OS can cause, the phytoplankton OS response also includes increased net production and extracellular release of ROS, osmolytes, and other compounds that are known or suspected to be potent signals regulating protist behavior. We hypothesize that, through chemical signaling, oxidative stress acts to

govern relationships among environmental variability, phytoplankton condition, and protist predation. Our proposed study of these integrated signaling and response processes has three overarching objectives: 1) Create and characterize oxidatively stressed phytoplankton. We will use light stress (variable exposure to visible light and UV) to create oxidatively stressed phytoplankton in the laboratory. Common coastal taxa with contrasting stress responses will be characterized using an array of fluorescent probes, biochemical measurements, and physiological assays. In addition, intracellular production and extracellular release of ROS and the associated chemical signal DMSP will be quantified. Use of *Phaeodactylum tricornutum* light stress mutants will add an independent means of connecting OS to signal production and predation response. 2) Examine protist predator responses to oxidatively stressed phytoplankton and associated chemical signals. Responses will be investigated by means of manipulation experiments and thorough characterization of associated signal chemistry. Assessment of predator response will be via predation rate measurements and population aggregation/dispersal behaviors in structured columns. 3) Investigate the prevalence of OS, its environmental correlates, and the microzooplankton predation response in the natural waters of a well-characterized local embayment. Application of ROS probes and OS assays to the natural environment and the design of OS manipulation experiments will be informed by the laboratory experiments using local protist species.

Our work will help to elucidate some of the multiple ways in which the OS response can affect phytoplankton fitness, contributing information that can be used to characterize the position of key coastal species along an OS response spectrum. Ultimately such information could be used in trait-based conceptual and numerical models in a manner analogous to cell size and other 'master traits'. Our research will also inform the relatively new and exciting field of chemical signaling in planktonic communities, exploring DMSP- and ROS-based signaling between two of the most significant groups in the plankton, the eukaryotic phytoplankton and their protist predators. Finally, findings will help elucidate the links between environmental stress, phytoplankton response, and predation in planktonic ecosystems. These links relate to central issues in biological oceanography, including the predator-prey interactions that influence bloom demise, and the mechanisms by which protists feed selectively and thereby structure prey communities. The proposed research is a cross-cutting endeavor that unites subjects usually studied in isolation through a novel conceptual framework. Thus the findings have the potential to generate broadly applicable new insights into the ecological and evolutionary regulation of this key trophic link in planktonic food webs.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1434842

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