Prey-only exposure results from light stress in phytoplankton and dinoflagellate grazing response experiments from July of 2015 to July of 2017

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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
Prey-only exposure results (single-factor) results from light stress in phytoplankton and dinoflagellate grazing response experiments from July of 2015 to July of 2017. These data were published in Strom et al. (2020).

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Coverage

Spatial Extent: **Lat**:48.5 **Lon**:-122.75
Temporal Extent: 2015-07-14 - 2017-07-18

Dataset Description

Prey-only exposure results (single-factor) results from light stress in phytoplankton and dinoflagellate grazing response experiments from July of 2015 to July of 2017. These data were published in Strom et al. (2020).

Related datasets also from light stress grazing experiments:
* Light stress grazing: photosynthetic efficiency https://www.bco-dmo.org/dataset/779033
* Light stress grazing: Prey and predator exposure https://www.bco-dmo.org/dataset/779050

Methods & Sampling

Phytoplankton light stress – dinoflagellate grazing experiments

General information

Emiliania huxleyi strains were grown in f/50 without added Si, except for CCMP1516 which was grown in f/2 for experiments D and I and in f/50 otherwise. All other phytoplankton were grown in f/2 medium without added Si. Most strains (designated CCMP) were obtained from the National Center for Marine Algae and Microbiota except Heterocapsa rotundata, which was from the Norwegian Culture Collection of Algae (NORCCA). Heterotrophic dinoflagellates Amphidinium longum and Oxyrrhis marina were isolated from marine waters of the Salish Sea, grown in ciliate medium (Gifford 1985), and maintained on a mixture of phytoflagellate species. All cultures of any type were grown at a salinity of 30 and a temperature of 15°C. Phytoplankton were grown at a range of low to moderate irradiances, depending on experiment on a 12L:12D cycle. Heterotrophic dinoflagellates were grown at 10-20 µmol photons m-2 s-1 on a 12L:12D cycle. Before use in experiments, dinoflagellate predators were fed only Rhodomonas sp. 755 (A. longum) or Dunaliella tertiolecta (O. marina) and allowed to consume these prey until they were nearly gone from the culture.

Cells were exposed to experimental light treatments outdoors in a shallow tank filled with flowing seawater supplied from nearby coastal waters. Temperature during experiments was monitored at regular intervals with a thermometer mounted in an unscreened incubation bottle, and ranged from 14-15°C except for Exp. A, where it averaged 17°C. Light (incident photosynthetically active radiation, or PAR) was measured with a Li-Cor 2π sensor, and logged at 5-min intervals so that total experiment light dose (mol photons m-2) could be computed for specific incubation periods. Control treatments were incubated in 60-ml polycarbonate bottles screened with sufficient neutral density screening to approximate growth irradiances. Higher light exposures were achieved using fewer (or no) layers of neutral density screening, depending on experiment. Except for Exp. E, which used polycarbonate bottles only, all high light treatments used 60-ml Teflon bottles, which are transparent to UV wavelengths. In some experiments high light treatments included both Teflon (UV-transparent) and polycarbonate (UV-opaque) bottles, to isolate the effects of UV on protist responses. Bottles were incubated at ~10 cm depth in the outdoor tank.

Experiments A-F exposed only the phytoplankton prey to the light stress treatments ('Single_factor_grazing (prey-only)' data set https://www.bco-dmo.org/dataset/779043). Cultures were divided into incubation bottles (n=3-5 depending on experiment) and placed in the outdoor tank for 60-120 min. Photosynthetic efficiency (Fv/Fm) was monitored before cells were taken outside (t=0) and, after gentle mixing, at 30-min intervals during the incubations ('FvFm' data set https://www.bco-dmo.org/dataset/779033). After outdoor exposure, phytoplankton were returned to the laboratory and a subsample from each replicate was added to a corresponding 30-ml polycarbonate bottle containing heterotrophic dinoflagellate predator A. longum to initiate predation experiments. The remainder of the phytoplankton culture volume was placed in an incubator at the culture growth irradiance level, and Fv/Fm monitored at regular intervals during this recovery period.

Prey concentrations for predation experiments ranged from 5.0 x 103 cells ml-1 for dinoflagellate Heterocapsa rotundata to 5.0 x 104 cells ml-1 for the various E. huxleyi strains. Prey biomass densities were equivalent for all prey types, at ~500 µg C liter-1. Carbon per cell for each phytoplankton species was estimated from measured cell volumes and published C:volume conversion factors (Menden-Deuer & Lessard 2000). A. longum concentrations were ~1-2 x 103 cells ml-1, and O. marina concentration (Exp. I only, see below) was 260 cells ml-1. For 'prey only exposure' experiments, predation tests were conducted for 50 min in a laboratory incubator at 15°C and ~50 µmol photons m-2 s-1. For 'prey and predator exposure' experiments, predation tests were conducted for 40-60 min under either control or high light outdoor illumination conditions. Predation tests were terminated by adding cells to cold 10% glutaraldehyde and DAPI stain (final concentrations 0.5% and 0.1 µg ml-1, respectively). After fixation overnight in 4°C and darkness, samples were filtered (3 or 5 µm pore-size polycarbonate filters), mounted on slides, and frozen for later examination by epifluorescence microscopy. UV excitation was used to locate and identify dinoflagellate predators from the DAPI-induced fluorescence of their nuclei. Ingested prey were detected using blue light excitation, from the orange (cryptophyte) or red (all other prey) autofluorescence of the prey pigments inside the predator food vacuoles Because A. longum uses a peduncle to feed on cryptophytes, rather than phagocytizing intact cells, the number of ingested prey per predator cannot be quantified for this predator – prey combination. Therefore for all predator and prey types, each micrograzer cell was scored as 'feeding' or 'not feeding'. At least 250 micrograzers per slide were scored; predation intensity was calculated as fraction of the population feeding (= # micrograzers with ingested prey / total # micrograzers scored).

Experiments G, H, and I used a matrix design in which predators and prey were exposed to experimental irradiances separately, then combined in various ways and predation measured in outdoor irradiance conditions ('Prey and predator exposure' data set https://www.bco-dmo.org/dataset/779050). Cultures of predators and prey were incubated in separate bottles for the first 1-1.2 h of exposure time. After that, appropriate volumes of prey with various exposure histories were introduced into predator bottles with various exposure histories, and those predation tests incubated for an additional 40-60 min at the original predator irradiance level. Fv/Fm was monitored throughout ('Photosynthetic efficiency' data set https://www.bco-dmo.org/dataset/779033), first in the original phytoplankton-only bottles and then in the remaining

phytoplankton volume after predation tests were initiated, and finally through a recovery period in the laboratory as described above. At the end of the predation test period, samples were fixed and slides prepared as described above.

For more information see Strom et al. (2020).

Data Processing Description

Data manager processing notes:
* Data exported from Excel to csv file in preparation for exporting into the BCO-DMO data system.
* Date format changed to ISO 8601 format yyyy-mm-dd
* Charcter # used in column names replaced with "num" to enable data reusability in various systems.

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Data Files

File
singlefactor.csv (Comma Separated Values (.csv), 7.46 KB) MD5:b6f2c516871d147354371e1c8d7e8d26 Primary data file for dataset ID 779043

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Supplemental Files

File
Species list for light stress experiments filename: light_stress-species_list.csv (Comma Separated Values (.csv), 849 bytes) MD5:f915fdf1048759f3dc2d0eda9fb70aea List of names used in the datasets, the scientific name, aphiaID taxanomic identifier and link to the World Register of Marine Species for that species. Note that the "names_in_data" uses a wildcard character * to denote multiple names used in the data (e.g. Ehux_* includes Ehux_1516, Ehux_3266, etc.)

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Related Publications

Gifford, DJ (1985) Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). Mar Ecol Prog Ser 23:257-267. doi: 10.3354/meps023257
General

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography, 45(3), 569–579. doi:10.4319/lo.2000.45.3.0569
General

Strom, S., Barberi, O., Mazur, C., Bright, K., & Fredrickson, K. (2020). High light stress reduces dinoflagellate predation on phytoplankton through both direct and indirect responses. Aquatic Microbial Ecology, 84, 43–57. doi:10.3354/ame01924
Methods

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Parameters

Parameter	Description	Units
Experiment_ID	Shows letter (A-I) corresponding to experiment ID system used in Strom et al. (submitted), followed by experiment ID used in Strom lab.	unitless
Experiment_Date	Calendar date on which experiment was conducted ISO 8601 Date format yyyy-mm-dd	unitless
Phytoplankton_Species	Shows species and strain number (CCMP), where available, for phytoplankton used in light stress experiments. See species list (supplemental document) for species codes and the corresponding species names.	unitless
Micrograzer_Species	Shows species and strain number (CCMP), where available, for micrograzers used in light stress experiments. See species list (supplemental document) for species codes and the corresponding species names.	unitless
Bottle_Type	Composition of bottles used for outdoor light exposure; PC = polycarbonate	unitless
Num_Screens	Number of neutral density screen layers used to wrap bottles during outdoor exposure period	unitless
Phytoplankton_PAR_Dose	Total dose of photosynthetically active radiation received by phytoplankton during outdoor 'light stress' incubation period, before phytoplankton prey were added to micrograzers under low light laboratory conditions.	mol photons m-2
Replicate_Number	Identifies an individual replicate bottle	unitless
Fraction_Feeding	Fraction of the total enumerated micrograzer population that contained ingested phytoplankton prey after the 50-min predation test period in the laboratory.	dimensionless

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Instruments

Dataset-specific Instrument Name	Pulse-Amplitude Modulated Fluorometer: Walz Water PAM
Generic Instrument Name	Fluorometer
Dataset-specific Description	Photosynthetic efficiency measurements: Pulse-Amplitude Modulated Fluorometer: Walz Water PAM
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	Li-Cor 1400 data logger with 2-pi (cosine) photosynthetically active radiation (PAR) sensor
Generic Instrument Name	LI-COR Biospherical PAR Sensor
Dataset-specific Description	Irradiance measurements: Li-Cor 1400 data logger with 2-pi (cosine) photosynthetically active radiation (PAR) sensor
Generic Instrument Description	The LI-COR Biospherical PAR Sensor is used to measure Photosynthetically Available Radiation (PAR) in the water column. This instrument designation is used when specific make and model are not known.

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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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