Flow cytometry data from a controlled multi-stressor incubation experiment conducted in July 2017 using phytoplankton communities from Narragansett Bay, RI

Website: https://www.bco-dmo.org/dataset/968692
Data Type: Other Field Results
Version: 1
Version Date: 2025-07-16

Project
» Dimensions: Collaborative Research: Genetic, functional and phylogenetic diversity determines marine phytoplankton community responses to changing temperature and nutrients (Phytoplankton Community Responses)
» Quantifying Temperature Dependence In Growth & Grazing Rates of Planktonic Herbivores (Planktonic Herbivore Temp Dependence)
» RII Track-1: Rhode Island Consortium for Coastal Ecology Assessment, Innovation, and Modeling (C-AIM)

Program
» Dimensions of Biodiversity (Dimensions of Biodiversity)

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Abstract
These data include measurements of phytoplankton physiology and composition from a controlled multi-stressor incubation experiment conducted in July 2017. Whole phytoplankton communities were collected from the Narragansett Bay Long-Term Plankton Time Series site and incubated at the in-situ temperature (22 degrees Celsius) and at deviations from that temperature (±4 degrees Celsius) with both macronutrient amendments (N, P, Si addition) and unamended controls. Phytoplankton growth, abundance, size structure, and elemental composition, as well as microzooplankton grazing rates were measured throughout the incubations. These data improve our understanding of the phytoplankton community response to short-term changes in temperature or nutrients, providing valuable insight into the drivers of marine ecosystems.

Table of Contents

Coverage

Location: Narragansett Bay Long-term Plankton Time Series site
Spatial Extent: **Lat**:41.57 **Lon**:-71.39
Temporal Extent: 2017-07 - 2017-07

Methods & Sampling

Incubation Experiments:
Seawater was collected from surface seawater on July 17, 2017, from the Narragansett Bay (NBay) Long-term Plankton Time Series site (https://web.uri.edu/gso/research/plankton/; 41.57ºN, 71.39ºW). Collection and incubation procedures were as in Anderson et al. (2022) and Kling et al. (2023). Briefly, seawater was filtered through a 200-micrometer (µm) mesh to remove macro-zooplankton grazers and used to set up six incubations at three temperature treatments and two nutrient concentrations. Incubations were carried out in 2 liter (L) polypropylene bottles at the *in-situ* temperature (22 degrees Celsius (ºC)) and salinity (28), or at positive or negative deviations from 22ºC (ca. ±4ºC: 18ºC and 26ºC). Incubations were set up in triplicate at each temperature by performing 1:1 dilutions of NBay whole surface seawater with either nutrient amended or unamended (controls) 0.2 µm filtered seawater. Nutrient amended seawater contained final macronutrient concentrations as follows: 32 micromolar (µM) nitrate, 32 µM silicate, 2 µM phosphate. Controls contained vitamins and trace metals, but no macronutrients. Incubations were maintained on a 15:9 light:dark cycle at 150 micromoles photons per square meter per second (µmol photons m-2 s-1) for four days to mimic summer *in-situ* conditions.

Grazing rates and intrinsic phytoplankton growth rates (growth in the absence of predation) were assessed with a separate set of incubations run in tandem, but following the same methodology. Grazing and intrinsic phytoplankton growth were determined using the two-point dilution method (Morison and Menden-Deuer 2017; Franzè et al. 2023). Briefly, incubation water from each temperature and nutrient treatment was used to prepare two samples: an undiluted sample (100% whole sea water from each incubation) representing full grazing conditions and a 10% dilution (diluting with either amended or unamended filtered seawater, depending on treatment), where the density of grazers is lowered with negligible impacts to phytoplankton growth. The 10% dilution is assumed to have minimal predator-prey interactions, allowing for the calculation of phytoplankton intrinsic growth rates.

Phytoplankton Size Composition:
On days 0, 2, and 4 of the incubation experiment, phytoplankton size composition was discerned with size-fractionated chlorophyll *a* (chl-*a*) and flow cytometry. For size-fractionated chl-*a*, phytoplankton biomass was divided into 0.7-5 µm, 5-20 µm, and >20 µm fractions by either filtering whole seawater in triplicate directly onto 25-millimeter (mm) GF/F filters (Whatman), pre-filtering whole seawater through a 20 µm mesh and then filtering onto 25 mm GF/F filters, or filtering onto 5 µm polyester filters (Sterlitech) following the methods in Anderson et al. (2022). For flow cytometry analyses (see related dataset), samples from each incubation were fixed in triplicate with 0.1% glutaraldehyde and 2% paraformaldehyde, final concentrations. Samples were then prefiltered through 20 µm mesh to avoid clogging the flow cell, and counted using a Guava easyCyte flow cytometer (Luminex). Particles were identified as either eukaryotic or from the genus *Synechococcus* using red (692/40 nanometer (nm)) or orange fluorescence (580/30 nm), respectively. Plankton size structure was analyzed using density distributions of forward scatter (FSC), binned log-linearly, while taxonomic composition used counts of eukaryotic cells and *Synechococcus*, discerned via fluorescence, as well as density distributions of side-scatter (SSC), binned log-linearly.

Elemental Composition:
To discern changes in phytoplankton community elemental composition, Particulate Organic Carbon (POC), Particulate Organic Nitrogen (PON), and Biogenic Silica (BSi) content were assessed on days 0 and 4 following the methods in Anderson et al. (2022). For POC and PON, cells were harvested in triplicate onto pre-

combusted 25-mm GF/F filters and analyzed on an Elemental Combustion System (Costech Analytical Technologies Inc.). Samples for BSi were filtered in triplicate onto 2 µm polycarbonate filters and evaluated on a SP-830 spectrophotometer (Barnstead Turner Inc), following the methods of Strickland and Parsons (1972). Additionally, *in situ* nutrient concentrations (dissolved ammonium, phosphate, silicate, and total inorganic nitrogen) were assessed using a Lachat Quikchem 8500 analyzer (Hach).

Data Processing Description

Size-fractionated Chlorophyll:
Phytoplankton biomass was calculated for the following size fractions: 0.7-5 µm, 5-20 µm, and >20 µm. First, whole seawater was filtered in triplicate either directly onto 25 mm GF/F filters (> 0.7 µm), pre-filtered through a 20 µm mesh and then filtered onto 25 mm GF/F filters (0.7-20 µm), or filtered onto 5 µm polyester filters (> 5 µm). Then, desired size fractions were calculated from the results of each filtration. Total chlorophyll is reported as the mean and standard deviation of biological triplicates at each treatment, while size fractions are given in percent biomass.

Flow cytometry:
Phytoplankton samples were prefiltered through 20 µm mesh and then counted using a Guava easyCyte flow cytometer (Luminex). Particles were identified as either eukaryotic or from the genus *Synechococcus* using red (692/40 nm) or orange fluorescence (580/30 nm), respectively. Plankton size and taxonomic structure were then analyzed using density distributions of forward scatter (FSC) and side scatter (SSC), binned log-linearly. Flow cytometry data were processed in Python version 3.9.7 with FlowCal (Castillo-Hair et al. 2016).

Growth and Grazing Rates:
Phytoplankton intrinsic growth and microzooplankton grazing rates were determined using the two-point dilution method (Morison and Menden-Deuer 2017; Franzè et al. 2023). Briefly, incubation water from each temperature and nutrient treatment was used to prepare two samples: an undiluted sample (100% whole sea water from each incubation) representing full grazing conditions and a 10% dilution (diluting with either amended or unamended filtered seawater, depending on treatment), where the density of grazers is lowered with negligible impacts to phytoplankton growth. The 10% dilution is assumed to have minimal predator-prey interactions, allowing for the calculation of phytoplankton intrinsic growth rates. The difference in phytoplankton growth rates during a 24-hour incubation period between dilutions then provided grazing rate estimates [10% dilution (intrinsic growth) – 100% whole seawater (growth with grazing)]. Physiological data are reported as the mean and standard deviation of biological triplicates at each treatment.

BCO-DMO Processing Description

- Imported original file "Dimensions_July_FCM_master.csv" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved the final file as "968692_v1_july_2017_fcm.csv".

Problem Description

Because of the time-consuming nature of dilution experiments, incubations for intrinsic growth and grazing measurements were only conducted over two 24-hr periods (beginning days 2 and 5), while other phytoplankton measurements were conducted three times on days 0, 2 and 4.

[table of contents | back to top]

Data Files

File
968692_v1_july_2017_fcm.csv (Comma Separated Values (.csv), 9.08 KB) MD5:2422250a3ee30b397bdf9cc355cfdd4d Primary data file for dataset ID 968692, version 1

File

[table of contents | back to top]

Supplemental Files

File
Dimensions_July_FCM_T4.zip (ZIP Archive (ZIP), 68.37 MB) MD5:781714dbb30bc766c3b1a6b92963a46b Supplemental file for dataset ID 968692, version 1. These flow cytometry data include measurements of forward scatter (FSC) and side scatter (SSC) from day 4 of a controlled multi-stressor incubation experiment conducted in July 2017. Natural plankton communities from Narragansett Bay, RI were exposed to altered temperature and nutrient conditions during a short-term incubation while the community composition was characterized. Each file is named with a plate and well number. A 'well_key.csv' file then lists which plate and well number are associated with each treatment.

[table of contents | back to top]

Related Publications

Anderson, S. I., Franzè, G., Kling, J. D., Wilburn, P., Kremer, C. T., Menden-Deuer, S., Litchman, E., Hutchins, D. A., & Rynearson, T. A. (2022). The interactive effects of temperature and nutrients on a spring phytoplankton community. Limnology and Oceanography, 67(3), 634–645. Portico. https://doi.org/10.1002/lno.12023
Methods

Castillo-Hair, S. M., Sexton, J. T., Landry, B. P., Olson, E. J., Igoshin, O. A., & Tabor, J. J. (2016). FlowCal: A User-Friendly, Open Source Software Tool for Automatically Converting Flow Cytometry Data from Arbitrary to Calibrated Units. ACS Synthetic Biology, 5(7), 774–780. https://doi.org/10.1021/acssynbio.5b00284
Software

Franzè, G., Anderson, S. I., Kling, J. D., Wilburn, P., Hutchins, D. A., Litchman, E., Rynearson, T. A., & Menden-Deuer, S. (2022). Interactive effects of nutrients and temperature on herbivorous predation in a coastal plankton community. Limnology and Oceanography. Portico. https://doi.org/10.1002/lno.12289
Results

Kling, J. D., Lee, M. D., Walworth, N. G., Webb, E. A., Coelho, J. T., Wilburn, P., Anderson, S. I., Zhou, Q., Wang, C., Phan, M. D., Fu, F., Kremer, C. T., Litchman, E., Rynearson, T. A., & Hutchins, D. A. (2023). Dual thermal ecotypes coexist within a nearly genetically identical population of the unicellular marine cyanobacteriumSynechococcus. Proceedings of the National Academy of Sciences, 120(47). https://doi.org/10.1073/pnas.2315701120
Methods

Morison, F., & Menden-Deuer, S. (2017). Doing more with less? Balancing sampling resolution and effort in measurements of protistan growth and grazing-rates. Limnology and Oceanography: Methods, 15(9), 794–809. doi:10.1002/lom3.10200
Methods

Strickland, J. D. H., & Parsons, T. R. (1972). A Practical Handbook of Seawater Analysis, 2nd edition. Fisheries Research Board of Canada. https://doi.org/10.25607/OBP-1791
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

Anderson, S. I., Franzè, G., Kling, J. D., Wilburn, P., Kremer, C. T., Menden-Deuer, S., Litchman, E., Hutchins, D. A., Rynearson, T. A. (2025) **Phytoplankton physiology and composition under temperature-nutrient multi-stressor incubation from July 2017 in Narragansett Bay, RI.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-06-27 doi:10.26008/1912/bco-dmo.966570.1 [view at BCO-DMO]

[table of contents | back to top]

Parameters

Parameter	Description	Units
time_d	Time	days
temp_C	Temperature	degrees Celsius
nutr	Nutrient treatment, either nutrient amended or control.	unitless
bin	Bin number from 1-50.	unitless
limit	Upper bound of log-linearly spaced bins from 0 to 10000.	FSC or SSC
fsc_per	Percent of cells within each forward scatter (fsc) bin.	percent
ssc_per	Percent of cells within each side scatter (ssc) bin.	percent

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Elemental Combustion System
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Particulate carbon and nitrogen was evaluated with an Elemental Combustion System (Costech Analytical Technologies Inc.).
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Guava easyCyte flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Phytoplankton community analyses were carried out with a Guava easyCyte flow cytometer (Luminex).
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Lachat Quikchem 8500 analyzer
Generic Instrument Name	Lachat QuikChem 8500 flow injection analysis system
Dataset-specific Description	Nutrients were analyzed on a Lachat Quikchem 8500 analyzer (Hach).
Generic Instrument Description	The Lachat QuikChem 8500 Series 2 Flow Injection Analysis System features high sample throughput and simple, but rapid, method changeover. The QuikChem 8500 Series 2 system maximises productivity in determining ionic species in a variety of sample types, from sub-ppb to percent concentrations. Analysis takes 20 to 60 seconds, with a sample throughput of 60 to 120 samples per hour.

Dataset-specific Instrument Name	SP-830 spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Biogenic silica (BSi) was assessed with a SP-830 spectrophotometer (Barnstead Turner Inc).
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	10-AU fluorometer
Generic Instrument Name	Turner Designs Fluorometer-10
Dataset-specific Description	Chlorophyll content was assessed with a 10-AU fluorometer (Turner).
Generic Instrument Description	The Turner Designs Model 10 fluorometer (manufactured by Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA) is used to measure Chlorophyll fluorescence. No information could be found for this specific model.

[table of contents | back to top]

Project Information

Dimensions: Collaborative Research: Genetic, functional and phylogenetic diversity determines marine phytoplankton community responses to changing temperature and nutrients (Phytoplankton Community Responses)

Coverage: Narragansett Bay, RI and Bermuda, Bermuda Atlantic Time-series Study (BATS)

NSF Award Abstract:
Photosynthetic marine microbes, phytoplankton, contribute half of global primary production, form the base of most aquatic food webs and are major players in global biogeochemical cycles. Understanding their community composition is important because it affects higher trophic levels, the cycling of energy and elements and is sensitive to global environmental change. This project will investigate how phytoplankton communities respond to two major global change stressors in aquatic systems: warming and changes in nutrient availability. The researchers will work in two marine systems with a long history of environmental monitoring, the temperate Narragansett Bay estuary in Rhode Island and a subtropical North Atlantic site near Bermuda. They will use field sampling and laboratory experiments with multiple species and varieties of phytoplankton to assess the diversity in their responses to different temperatures under high and low nutrient concentrations. If the diversity of responses is high within species, then that species may have a better chance to adapt to rising temperatures and persist in the future. Some species may already be able to grow at high temperatures; consequently, they may become more abundant as the ocean warms. The researchers will incorporate this response information in mathematical models to predict how phytoplankton assemblages would reorganize under future climate scenarios. Graduate students and postdoctoral associates will be trained in diverse scientific approaches and techniques such as shipboard sampling, laboratory experiments, genomic analyses and mathematical modeling. The results of the project will be incorporated into K-12 teaching, including an advanced placement environmental science class for underrepresented minorities in Los Angeles, data exercises for rural schools in Michigan and disseminated to the public through an environmental journalism institute based in Rhode Island.

Predicting how ecological communities will respond to a changing environment requires knowledge of genetic, phylogenetic and functional diversity within and across species. This project will investigate how the interaction of phylogenetic, genetic and functional diversity in thermal traits within and across a broad range of species determines the responses of marine phytoplankton communities to rising temperature and changing nutrient regimes. High genetic and functional diversity within a species may allow evolutionary adaptation of that species to warming. If the phylogenetic and functional diversity is higher across species, species sorting and ecological community reorganization is likely. Different marine sites may have a different balance of genetic and functional diversity within and across species and, thus, different contribution of evolutionary and ecological responses to changing climate. The research will be conducted at two long-term time series sites in the Atlantic Ocean, the Narragansett Bay Long-Term Plankton Time Series and the Bermuda Atlantic Time Series (BATS) station. The goal is to assess intra- and inter-specific genetic and functional diversity in thermal responses at contrasting nutrient concentrations for a representative range of species in communities at the two sites in

different seasons, and use this information to parameterize eco-evolutionary models embedded into biogeochemical ocean models to predict responses of phytoplankton communities to projected rising temperatures under realistic nutrient conditions. Model predictions will be informed by and tested with field data, including the long-term data series available for both sites and in community temperature manipulation experiments. This project will provide novel information on existing intraspecific genetic and functional thermal diversity for many ecologically and biogeochemically important phytoplankton species, estimate generation of new genetic and functional diversity in evolution experiments, and develop and parameterize novel eco-evolutionary models interfaced with ocean biogeochemical models to predict future phytoplankton community structure. The project will also characterize the interaction of two major global change stressors, warming and changing nutrient concentrations, as they affect phytoplankton diversity at functional, genetic, and phylogenetic levels. In addition, the project will develop novel modeling methodology that will be broadly applicable to understanding how other types of complex ecological communities may adapt to a rapidly warming world.

Quantifying Temperature Dependence In Growth & Grazing Rates of Planktonic Herbivores (Planktonic Herbivore Temp Dependence)

Coverage: Narragansett Bay

NSF Award Abstract:
Plankton, single-celled organisms that inhabit the world's oceans are responsible for the generation of oxygen, cycling energy and matter between the atmosphere and the deep ocean and are the basis for virtually all seafood harvested. These life-giving functions critically depend on the relative rates at which plankton grow and get eaten. How temperature influences those rates is essential to understand plankton responses to environmental changes and ocean dynamics. It is well established that plankton grow faster when temperatures are higher however, whether feeding has a similar temperature dependence is unknown. That means oceanographers are missing key data required to build global predictive models. This project will fill essential knowledge gaps and measure physiological rates of singled celled zooplankton across temperature gradients representing the global ocean, from polar to tropical regions and throughout the seasonal cycle. Researchers will combine laboratory experiments with specimens taken from the coastal ocean (Narragansett Bay), which is exemplary in its strong seasonal temperature variations. These data will provide a clear picture of the production capacity and activity of plankton in a global and dynamic ocean. The project supports an early career scientist, as well as graduate and undergraduate students. Scientists will continue communicating their research to the public through large-scale outreach events, education at the high-school level, and engagement through online and other media. Moreover, researchers will continue collaborating with the Metcalf Institute for Marine & Environmental Reporting to support their Annual Science Immersion Workshop for Journalists and their ongoing work to disseminate research findings through web-based seminars.

Grazing is the single largest loss factor of marine primary production and thus affects a key transfer rate between global organic and inorganic matter pools. Remarkably, data for herbivorous protist growth and grazing rates at temperatures representative of the vast polar regions and during winter and spring periods are extremely sparse. By combining laboratory experiments with ground truthing fieldwork, this project alleviates a central knowledge gap in oceanography and delivers the empirical measurements necessary to derive algorithms to incorporate temperature dependence of heterotrophic protist growth and grazing rates into biogeochemical models. The extraordinary seasonal temperature fluctuations in a temperate coastal estuary (Narragansett Bay) are exploited to measure rates of heterotrophic protists isolated from different temperatures and seasons and to quantify the temperature and acclimation responses of these ecotypes. This project delivers data urgently needed to solve the conundrum of whether herbivorous growth and predation is depressed at low temperatures, implying low trophic transfer rates and high carbon export, or if predation proceeds at rates comparable to temperate systems with primary production largely lost to predation. Large temperature gradients in the global ocean mean that cross-biome and biogeochemical models are particularly sensitive to assumptions about the temperature dependence in modeled rate processes. Establishment of the dependence of heterotrophic plankton physiological rates (growth and grazing) to gradients of temperature, mimicking realistic conditions experienced by plankton in a changing ocean, is a key step towards integrating much needed biological information in biogeochemical modeling efforts. This project makes a significant contribution to linking ecological research with ecosystem models by providing empirically rooted algorithms of the temperature dependence of protistan herbivory and growth rates, key processes in the transformation of organic matter in global biogeochemical cycles and tools critically missing in ecosystem models.

RII Track-1: Rhode Island Consortium for Coastal Ecology Assessment, Innovation, and Modeling (C-AIM)

Coverage: Narragansett Bay, Rhode Island

NSF Award Abstract:

Non-technical Description
The University of Rhode Island (URI) will establish the Consortium for Coastal Ecology Assessment, Innovation, and Modeling (C-AIM) to coordinate research, education, and workforce development across Rhode Island (RI) in coastal marine science and ecology. C-AIM addresses fundamental research questions using observations, computational methods, and technology development applied to Narraganset Bay (NB), the largest estuary in New England and home to important ecosystem services including fisheries, recreation, and tourism. The research will improve understanding of the microorganisms in NB, develop new models to predict pollution and harmful algal bloom events in NB, build new sensors for nutrients and pollutants, and provide data and tools for stakeholders in the state. Observational capabilities will be coordinated in an open platform for researchers across RI; it will provide real-time physical, chemical, and biological observations ? including live streaming to mobile devices. C-AIM will also establish the RI STEAM (STEM + Art) Imaging Consortium to foster collaboration between artists, designers, engineers, and scientists. Research internships will be offered to undergraduate students throughout the state and seed funding for research projects will be competitively awarded to Primarily Undergraduate Institution partners.

Technical Description
C-AIM will employ observations and modeling to assess interactions between organisms and ecosystem function in NB and investigate ecological responses to environmental events, such as hypoxia and algal blooms. Observations of the circulation, biogeochemistry, and ecosystem will be made using existing and new instrument platforms. The Bay Observatory ? a network of observational platforms around NB - will be networked to trigger enhanced water sampling and sensing during specific environmental events, such as hypoxic conditions or phytoplankton blooms. Biogeochemical, ecological, and coastal circulation models will be integrated and coupled to focus on eutrophication and pollutant loading. Data and models will be integrated on multiple scales, from individual organisms and trophic interactions to food-web responses, and from turbulence to the regional ocean circulation. New sensing technologies for nutrients and pollutants will be developed, including affordable, micro-fluidic (Lab-on-a-Chip) devices with antifouling capabilities. The results will be synthesized and communicated to stakeholders.

[table of contents | back to top]

Program Information

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503446

Coverage: global

(adapted from the NSF Synopsis of Program)
Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to

understand the roles of biodiversity in critical ecological and evolutionary processes.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638958
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638804
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638834
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736635
NSF Office of Integrative Activities (NSF OIA)	OIA-1655221

[table of contents | back to top]