Results from a study of physiological responses of Ulva lactuca to ocean acidification and nutrient enrichment

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Project
» Ocean Acidification: Scope for Resilience to Ocean Acidification in Macroalgae (Seaweed OA Resilience)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
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Abstract
This dataset results from a study of physiological responses of Ulva lactuca to ocean acidification and nutrient enrichment. It includes measurements of growth, carbohydrates, protein, nitrate reductase activity, ammonium pool, nitrate pool, ammonium and nitrate uptake rates, Pmax, alpha, and Ik.

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Coverage

Spatial Extent: **Lat**:34.0413889 **Lon**:-118.567556
Temporal Extent: 2016-05-26 - 2016-07-27

Methods & Sampling

The experiment took place in the laboratory at the Department of Biology, California State University, Northridge. The specimens used in the experiment were collected in Malibu, CA (34° 02' 29.0" N, 118° 34' 03.2" W) on May 26, 2016 for trial 1 and July 5, 2016 for trial 2. Each trial lasted 22 days.

Carbonate Chemistry
Carbonate chemistry parameters were calculated multiple times during each trial using measurements of pH and total alkalinity (AT). AT samples were collected in 50 mL Falcon tubes, stored wrapped in Parafilm at 4°C in the dark, and analyzed within two weeks by potentiometric titration coupled to a pH electrode (Mettler Toledo DGi-115-SC with T5 Rondolino) and thermometer. Most AT samples were measured on the day of sampling.

The performance of the machine was checked with each measurement using certified reference material (CRM) from the Dickson laboratory at the Scripps Oceanographic Institute and the pH electrode was calibrated using TRIS buffer (Dickson et al., 2007). A spectrophotometric technique using m-cresol as an indicator dye was used to determine pHT (pH total scale). AT was calculated using potentiometric titration data and pHT using spectrophotometric data in the R-package Seacarb V 3.0.14 (Lavigne et al., 2011).

Nitrate reductase activity
An in vivo assay of nitrate reductase activity (NRA) was done according to the methods of Thompson & Valiela (1999) resulting in a colorimetric reaction with NO2- produced via NRA under dark, anoxic conditions. Absorbance was measured at 540 nm using a spectrophotometer.

Nutrient Analysis
NH4+ concentrations in the culture tanks were measured with a fluorometric method using OPA (Holmes et al., 1999) with the suggested modifications of Taylor et al. (2007), which included using an improved method for measuring background fluorescence. The raw fluorescence measurement of a sample was calibrated to a standard curve of an NH4+ stock solution using the standard additions protocol I of Taylor et al. (2007) which accounts for matrix effects that can alter fluorescence measurements.

NO3- concentrations in the culture tanks were determined from samples sent to the University of California, Santa Barbara Marine Science Institute Analytical Lab and were analyzed using a Lachat Instruments flow injection analysis instrument (QuikChem 8000).

Nutrient uptake rates
Uptake rates of NH4+ and NO3- were measured in situ on day twenty of the trials 8 – 10 hours into the light cycle for a period of one hour. The formula for chemostat nutrient uptake by Carmona et al. (1996) was used to determine nutrient uptake rates.

CN analyses and carbon stable isotope ratios
Tissue samples were dried for 24 hours at 60 degrees Celsisu. Dried samples were prepared for analysis by homogenizing samples using a metal laboratory scoop, cleaned with ethanol between each sample, which resulted in a fine power. Then, approximately 3 mg of the Ulva lactuca tissue powder was measured using an analytical balance (Mettler Toledo XP205) into a tin capsule and carefully enclosed with clean forceps. The tin capsules were put into 96-well tray plates and sent to the University of California, Davis Stable Isotope Facility (UCD-SIF). The samples were analyzed for δ15N and δ13C using the elemental analysis – isotope ratio mass spectrometry technique, which also provides results for tissue C and N content.

Seawater carbon stable isotopes
Seawater samples for delta13C of dissolved inorganic carbon (DIC) were stored in 20 mL glass vials with cone lids to exclude air from sample. Samples were stored at room temperature in low light until prepared for analysis using the exetainer gas evolution technique for DIC (Li et al., 2007). Then, the samples were sent to the UCD-SIF for analysis using the GasBench – isotope ratio mass spectrometry technique.

Internal soluble nitrogen pools
Internal NH4+ and NO3- pools were measured using the boiling water method (Hurd et al., 1996). One piece (0.04 ± 0.02 g FW, mean ± SEM) from each treatment was rinsed with deionized water to remove salt and nutrients on the surface. The pieces were placed in test tubes with 15 mL of deionized water and placed in a boiling water bath for 40 minutes. The water was decanted and analyzed for NH4+ and NO3-. This process was repeated on the same algal piece three times and the concentrations of internal soluble NH4+ and NO3- pools were calculated using the sum of the NH4+ and NO3- concentrations of the three water samples of each algal piece.

Soluble protein and carbohydrates
Pieces of Ulva lactuca tissue (0.04 ± 0.004 g FW, mean ± SEM) were ground in a mortar and pestle in 2 mL of a β-mercaptoethanol buffer, pH 7.5 and stored at 4°C for up to 72 hours. The extract was centrifuged at 16,000 g for 5 minutes. Soluble proteins and carbohydrates were determined spectrophotometrically (Milton Roy Spectronic Genesys 5) using the supernatant fraction. Soluble proteins were determined according to Bradford (1976) and soluble carbohydrates were determined using the phenol-sulfuric acid method according to Kochert (1978).

Chlorophyll a
Chlorophyll a was extracted in dimethylsulfoxide (DMSO) and methanol according to the methods of Duncan and Harrison 1982. Pieces of Ulva lactuca tissue (0.5 g FW) were placed in 1.25 mL of 80% DMSO for 10 minutes, and then suspended in two sequential 3 mL solutions of methanol for 10 min each to complete the extraction. The absorbance of the DMSO and methanol were measured using a spectrophotometer at the

wavelengths indicated in the formulas below. The absorbance at each wavelength, volume of solvent, and the fresh weight of a fragment were used to calculate the concentration of Chl a from each solvent using the following formulas:

DMSO Solution Chl a (mg g^{-1}) = [(A_{665}/(72.8)) * 1000] / g FW

Methanol solution Chl a (mg g^{-1}) = (13.8A_{668} - 1.3A_{635}) / g FW

The Chl a concentration was the sum of the concentrations of the DMSO and methanol extracts.

Photosynthetic rates
Photosynthetic O2 evolution rates were measured using the Qubit systems O2 electrode in a water-jacketed cuvette connected to a laptop using a LabProTM interface. Small pieces of Ulva lactuca (1 – 2 cm2) were cut from thalli at least one hour prior to measurements. The pieces were placed in 20 mL of culture water at 16°C in a 2 cm2 mesh bag which held the pieces at a 90° angle to the Qubit LED light source. A photosynthesis-irradiance (P-I) curve was generated using various photon flux densities from 0 – 700 µM photons m-2 s-1 for 200 seconds each, following a 200 second dark period to measure dark respiration rate. The maximum photosynthetic rate (Pmax), light saturation point (Ik), and photosynthetic efficiency (the initial slope of the P-I curve) (α) were determined from the P-I curves.

Data Processing Description

Data Processing
General linear mixed models (GLMM) were used to evaluate the physiological responses of Ulva lactuca to a range of pCO2 and NH4+ concentrations. Data analysis was done in R using the lmer4 and AICcmodavg packages (Mazerolle 2017).

BCO-DMO Processing
- Renamed fields to conform with BCO-DMO naming conventions.

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Data Files

File
ulva_response.csv (Comma Separated Values (.csv), 3.77 KB) MD5:6fe2fd57f8eed67b5044b46c25e388b9
Primary data file for dataset ID 861111

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Related Publications

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248–254. doi:10.1016/0003-2697(76)90527-3
Methods

Carmona, R., Vergara, J. J., Perez-Llorens, J. L., Figueroa, F. L., & Niell, F. X. (1996). Photosynthetic acclimation and biochemical responses of Gelidium sesquipedale cultured in chemostats under different qualities of light. Marine Biology, 127(1), 25–34. doi:10.1007/bf00993640
Methods

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to Best Practices for Ocean CO2 Measurements. PICES Special Publication 3, 191 pp https://isbnsearch.org/isbn/1-897176-07-4
Methods

Duncan, M. J., & Harrison, P. J. (1982). Comparison of Solvents for Extracting Chlorophylls from Marine Macrophytes. Botanica Marina, 25(9). doi:10.1515/botm.1982.25.9.445

Methods

Hurd, C. L., Harrison, P. J., & Druehl, L. D. (1996). Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of Macrocystis integrifolia from wave-sheltered and exposed sites. Marine Biology, 126(2), 205–214. doi:10.1007/bf00347445
Methods

Kochert, G. (1978). Carbohydrate determination by the phenol sulfuric acid method. In J. A. Hellbust & J. S. Craigie (Eds.), Handbook of phycological methods: Physiological and biochemical methods (pp. 95–97). Cambridge: Cambridge University Press.
Methods

Lavigne H, Epitalon, JM, Gattuso JP, 2011. Seacarb: seawater carbonate chemistry with R. https://cran.r-project.org/web/packages/seacarb/index.html
Software

Li, Z.-P., Tao, M.-X., Li, L.-W., Wang, Z.-D., Du, L., & Zhang, M.-F. (2007). Determination of Isotope Composition of Dissolved Inorganic Carbon by Gas Chromatography-Conventional Isotope-ratio Mass Spectrometry. Chinese Journal of Analytical Chemistry, 35(10), 1455–1458. doi:10.1016/s1872-2040(07)60089-9
Methods

Mazerolle, M. J. (2017). Model selection and multimodel inference based on (Q)AIC(c). R Package, Version 2.1-1. http://CRAN.R-project.org/package=AICcmodavg
Methods

Taylor,B. D., Keep,C. F., Hall,R. O., Koch, B. J., Tronstad, L. M., Flecker,A. S. & Ulseth, A. J. (2007). Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. Journal of the North American Benthological Society 26(2), 167-177. doi:10.1899/0887-3593(2007)26[167:ITFAMM]2.0.CO;2
Methods

Thompson, S. M., & Valiela, I. (1999). Effect of Nitrogen Loading on Enzyme Activity of Macroalgae in Estuaries in Waquoit Bay. Botanica Marina, 42(6). doi:10.1515/bot.1999.059
Methods

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Parameters

Parameter	Description	Units
Experiement	Trial 1 or Trial 2	unitless
Pot	Pot number assigned to each experimental unit	unitless
Nutrient	Experimental unit received enriched (20 umol [NH4+]) or unenriched (no NH4+)	micromoles per liter (umol/L)
NH4	The average difference between header tank NH4+ concentration and culture tank NH4+ concentration	micromoles per liter (umol/L)
pCO2_avg_Day_5	Average pCO2 calculated from measurements of pH and TA sampled periodically throughout experiments up to day 5	parts per million (ppm)
pCO2_avg_Day_10	Average pCO2 calculated from measurements of pH and TA sampled periodically throughout experiments on days 5 -10	parts per million (ppm)

pCO2_avg_Day_20	Average pCO2 calculated from measurements of pH and total alkalinity sampled periodically throughout experiments on days 10 - 20	parts per million (ppm)
Growth_Day_5	Growth rate days 0 - 5 using fresh weight	perecent per day (% day-1)
Growth_Day_10	Growth rate days 5 - 10 using fresh weight	perecent per day (% day-1)
Growth_Day_20	Growth rate days 10 - 20 using fresh weight	perecent per day (% day-1)
Carbohydrates	Carbohydrate content of Ulva at end of experiment	milligrams per millilter per gram fresh weight (mg/ml g-1 FW)
Protein	Protein content of Ulva at end of experiment	milligrams per millilter per gram fresh weight (mg/ml g-1 FW)
NRA	An in vivo assay of nitrate reductase activity	micrometers NO2- per gram fresh weight (um NO2- g-1 FW)
Ammonium_Pool	Internal NH4+ and NO3- pools were measured using the boiling water method	milligrams NH4+ per gram fresh weight (mg NH4+ g-1 FW)
Nitrate_Pool	Internal NH4+ and NO3- pools were measured using the boiling water method	milligrams NO2- per gram fresh weight (mg NO2- g-1 FW)
Ammonium_Uptake_Rate	Measured in situ on day twenty of the trials 8 - 10 hours into the light cycle for a period of one hour.	microMolar per gram fresh weight per hour (uM g-1 FW h-1)
Nitrate_Uptake_Rate	Measured in situ on day twenty of the trials 8 - 10 hours into the light cycle for a period of one hour.	micromoles per gram fresh weight per hour (uM g-1 FW h-1)

Pmax	Pmax = maximum photosynthetic rate. A photosynthesis-irradiance (P-I) curve was generated using various photon flux densities from 0 – 700 uM photons m-2 s-1 for 200 seconds each, following a 200 second dark period to measure dark respiration rate. The maximum photosynthetic rate (Pmax), light saturation point (Ik), and photosynthetic efficiency (the initial slope of the P-I curve) (alpha) were determined from the P-I curves.	micromoles O2 per gram fresh water per minute (uM O2 g FW-1 min-1)
Alpha	Alpha = photosynthetic efficiency. A photosynthesis-irradiance (P-I) curve was generated using various photon flux densities from 0 – 700 uM photons m-2 s-1 for 200 seconds each, following a 200 second dark period to measure dark respiration rate. The maximum photosynthetic rate (Pmax), light saturation point (Ik), and photosynthetic efficiency (the initial slope of the P-I curve) (alpha) were determined from the P-I curves.	unitless
Ik	Ik = light saturation point. A photosynthesis-irradiance (P-I) curve was generated using various photon flux densities from 0 – 700 uM photons m-2 s-1 for 200 seconds each, following a 200 second dark period to measure dark respiration rate. The maximum photosynthetic rate (Pmax), light saturation point (Ik), and photosynthetic efficiency (the initial slope of the P-I curve) (alpha) were determined from the P-I curves.	micromoles photons per square meter per second (uM photon m-2 s-1)
Chl_a	Concentration of chlorophyll a + b at the end of the experiment	milligrams per gram fresh weight (mg g-1 FW)
Tissue_C	Average amount of carbon in algal tissue	micrograms C per milligram dry weight (ug C mg-1 DW)
Tissue_N	Average amount of nitrogen in algal tissue	micrograms N per milligram dry weight (ug N mg-1 DW)
C_N_ratio	Ratio of carbon to nitrogen in algal tissue	unitless
Respiration	Respiration rate	micromoles O2 per gram fresh weight per minute (uM O2 g FW-1 min-1)
DeltaC	Discrimination of carbon stable isotopes = (d13Csource - d13Cplant) / (1 + d13Cplant)	unitless
dN	Ratio of nitrogen stable isotopes 15N:14N	unitless

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Instruments

Dataset-specific Instrument Name	chemostat
Generic Instrument Name	Chemostat
Generic Instrument Description	Devices in which controlled conditions are maintained for a chemical process to be carried out by organisms or biochemically active substances derived from such organisms.

Dataset-specific Instrument Name	Lachat Instruments flow injection analysis instrument (QuikChem 8000)
Generic Instrument Name	Flow Injection Analyzer
Dataset-specific Description	NO3- concentrations in the culture tanks were determined from samples sent to the University of California, Santa Barbara Marine Science Institute Analytical Lab and were analyzed using a Lachat Instruments flow injection analysis instrument (QuikChem 8000)
Generic Instrument Description	An instrument that performs flow injection analysis. Flow injection analysis (FIA) is an approach to chemical analysis that is accomplished by injecting a plug of sample into a flowing carrier stream. FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector. Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

Dataset-specific Instrument Name	elemental analysis – isotope ratio mass spectrometry
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Samples were analyzed for δ15N and δ13C using the elemental analysis – isotope ratio mass spectrometry technique, which also provides results for tissue C and N content
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Qubit LED light
Generic Instrument Name	LED light
Generic Instrument Description	A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons.

Dataset-specific Instrument Name	Mass flow controlled (MFC) gas mixing system
Generic Instrument Name	Mass Flow Controller
Dataset-specific Description	Mass flow controlled (MFC) gas mixing system (Qubit Systems, Ontario, Canada)
Generic Instrument Description	Mass Flow Controller (MFC) - A device used to measure and control the flow of fluids and gases

Dataset-specific Instrument Name	OrionStar A329
Generic Instrument Name	Multi Parameter Portable Meter
Dataset-specific Description	pHT was calculated from daily measurements of conductivity, salinity, and temperature (Thermo scientific, OrionStar A329)
Generic Instrument Description	An analytical instrument that can measure multiple parameters, such as pH, EC, TDS, DO and temperature with one device and is portable or hand-held.

Dataset-specific Instrument Name	Qubit systems O2 electrode
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	Photosynthetic O2 evolution rates were measured using the Qubit systems O2 electrode in a water-jacketed cuvette connected to a laptop using a LabProTM interface
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	Mettler Toledo DGi-115-SC
Generic Instrument Name	pH Sensor
Dataset-specific Description	pH electrode for total alkalinity (Mettler Toledo DGi-115-SC with T5 Rondolino). The performance of the machine was checked with each measurement using certified reference material (CRM) from the Dickson laboratory at the Scripps Oceanographic Institute and the pH electrode was calibrated using TRIS buffer.
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

Dataset-specific Instrument Name	Milton Roy Spectronic Genesys 5
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Soluble proteins, carbohydrates, and chl a were determined spectrophotometrically (Milton Roy Spectronic Genesys 5)
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.

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Project Information

Ocean Acidification: Scope for Resilience to Ocean Acidification in Macroalgae (Seaweed OA Resilience)

Coverage: Temperate coastal waters of the USA (30 - 45 N latitude, -66 to -88 W and -117 to -125 W longitude)

Benthic macroalgae contribute to intensely productive near shore ecosystems and little is known about the potential effects of ocean acidification on non-calcifying macroalgae. Kübler and Dudgeon will test hypotheses about two macroalgae, *Ulva* spp. and *Plocamium cartilagineum*, which, for different reasons, are hypothesized to be more productive and undergo ecological expansions under predicted changes in ocean chemistry. They have designed laboratory culture-based experiments to quantify the scope for response to ocean acidification in *Plocamium*, which relies solely on diffusive uptake of CO2, and populations of *Ulva* spp., which have an inducible concentrating mechanism (CCM). The investigators will culture these algae in media equilibrated at 8 different pCO2 levels ranging from 380 to 940 ppm to address three key hypotheses. The first is that macroalgae (such as Plocamium cartilagineum) that are not able to acquire inorganic carbon in changed form will benefit, in terms of photosynthetic and growth rates, from ocean acidification. There is little existing data to support this common assumption. The second hypothesis is that enhanced growth of Ulva sp. under OA will result from the energetic savings from down regulating the CCM, rather than from enhanced photosynthesis per se. Their approach will detect existing genetic variation for adaptive plasticity. The third key hypothesis to be addressed in short-term culture experiments is that there will be a significant interaction between ocean acidification and nitrogen limited growth of *Ulva* spp., which are indicator species of eutrophication. Kübler and Dudgeon will be able to quantify the individual effects of ocean acidification and nitrogenous nutrient addition on *Ulva* spp. and also, the synergistic effects, which will inevitably apply in many highly productive, shallow coastal areas. The three hypotheses being addressed have been broadly identified as urgent needs in our growing understanding of the impacts of ocean acidification.

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Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:
NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1316198

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