The density (mg CaCO3/cm^3) of the skeleton of Clathromorphum nereostratum, when assessed as function of increasing seawater temperature and pCO2 concentration

Website: https://www.bco-dmo.org/dataset/755809
Data Type: experimental
Version: 1
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Project
» Ocean Acidification: Century Scale Impacts to Ecosystem Structure and Function of Aleutian Kelp Forests (OA Kelp Forest Function)

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
Skeletal density (mg CaCO3/cm^3) of Clathromorphum nereostratum, evaluated as a function of seawater temperature and pCO2 level that it was cultured in for 4 months in mesocosm. Density measurements were made using micro-computed tomography.

Table of Contents

Coverage

Temporal Extent: 2015 - 2016

Dataset Description

Skeletal density (mg CaCO3/cm^3) of *Clathromorphum nereostratum*, evaluated as a function of seawater temperature and pCO2 level that it was cultured in for 4 months in mesocosm. Density measurements were made using micro-computed tomography.

Methods & Sampling

To evaluate whether rates of calcification within *C. nereostratum* have changed or will change with ocean warming and acidification, we cultured *C. nereostratum* under experimental conditions mimicking past, present, and predicted future levels of ocean temperature and pCO2 in the region, then followed this four-month incubation period with measurements of skeletal density using micro-computed tomography (microCT). Small *C. nereostratum* colonies (~4-5 cm diameter) were live collected from Adak in 2015 and immediately transported to the Northeastern University Marine Science Center in Nahant, Massachusetts. There, all specimens were acclimated to laboratory conditions at 8.5 degrees C for two weeks, after which individual *C. nereostratum* colonies were attached to the underside of plastic Petri dishes using cyanoacrylate glue and then allowed to acclimate for an additional two weeks before being moved to experimental aquaria. Conditions were then incrementally modified to achieve target temperature and pCO2 levels (see below) over a one-week period. After reaching target conditions, each 42-L aquarium was dosed with 213 mL of calcein fluorescent dye (Western Chemicals Inc.), which was recirculated in the aquaria for three days and then flushed from the system. Coralline algae incorporate the dye into their skeleton, thus creating a distinct line that can be viewed via fluorescent microscopy to demark the region of new growth within each individual.

We employed four pCO2 conditions and three temperatures that, while factorially crossed, spanned pre-industrial, present-day, and projected year 2100 conditions (assuming an IPCC "business as usual" carbon emissions scenario; Pachauri and Meyer 2014). More extreme temperature (12.5 degrees C) and pCO2 (2800 micro-atm) conditions were also employed in the broader experiment but were not included in our study because they are not predicted to occur until year 2500, or later. For each treatment, we set values to average summertime conditions, the time when ~75% of *C. nereostratum* growth occurs (Adey et al. 2013).

All treatments (4 pCO2 concentrations x 3 temperatures, fully factorial) were housed on individual shelves and consisted of three 42-liter acrylic aquaria and one 65-liter sump (n = 3 tanks/treatment). The aquaria were connected to a sump via a common overflow and return line but were each independently and continuously replenished with new seawater—thereby establishing them as true experimental replicates. The sump contained a filter box with a nylon mesh particle filter and activated carbon, a protein skimmer (Eshopps PSK-75), and a return pump, all of which was connected to a water chiller (Coralife 1/4HP). Filtered natural seawater was added via Darhor manual flow controllers at a rate of 50 mL/min/tank, resulting in full replacement of treatment water every ~21 hours - sufficiently fast to prevent material depletion of the dissolved constituents of the seawater yet slow enough to allow the mixed gases being sparged into the experimental treatments to approach equilibrium with the seawater. Mixed gases were sparged into each tank with 91 cm long flexible bubblers at the rate of ~1 L/min via Darhor needle-valve gas flow controllers. Two 12,000K LED light arrays (Ecoxotic Panorama, Pro 24V) were mounted above each tank and set to an irradiance that mirrored average summer daylight irradiance at 10 m depth in the Aleutian Islands (~258 micro-E m-2 s-1; 12 hr light:12 hr dark cycle).

Over the course of the four-month experiment, we measured pH (Accumet AB15 pH meter with Accufet solid state probe), salinity (YSI3200 meter with K=10 conductivity electrode and temperature probe), and temperature (NIST traceable red spirit glass thermometer) in each tank every Monday, Wednesday, and Friday. The pCO2 of the gas mixtures was measured with a Qubit S151 infrared CO2 analyzer and calibrated with certified mixed CO2 from Airgas Incorporated. Every 10 days, we characterized the full carbonate system chemistry of the experimental treatments from measured total alkalinity, dissolved inorganic carbon, temperature, and salinity. For this, seawater samples were obtained in 250 mL borosilicate ground-glass-stoppered bottles and immediately poisoned with 100 micro-L of saturated HgCl2 solution to halt biological activity (Dickson et al. 2007). Total alkalinity was measured via closed-cell potentiometric Gran titration and dissolved inorganic carbon was measured with a UIC 5400 Coulometer on a VINDTA 3C (Marianda Incorporated) using Dickson certified seawater reference material. Seawater pCO2, pH, carbonate ion concentration ([CO32-]) bicarbonate ion concentration ([HCO3-]), aqueous CO2, and calcite saturation state were calculated with the program CO2SYS (Lewis and Wallace 1998), using Roy and colleague's (1993) values for the K1 and K2 carbonic acid constants, the Mucci (1983) value for the stoichiometric calcite solubility product, the seawater pH scale, and an atmospheric pressure of 1.015 atm.

At the beginning of the experiment we measured the buoyant weight of each specimen. We then scrubbed each specimen with a toothbrush and reweighed it every month and at the end of the experiment. With each weighing, we also photographed the specimen with a ruler and Reef Watch coral bleaching card in the field of view. We then measured the 2-d surface area of the photographed specimens (Image J, NIH). At the end of the experiment, all coralline algae were sectioned with a diamond lapidary saw (Inland Craft SwapTop 6.5” Diamond Trim Saw) and either frozen for genetic analysis or sectioned into 6 mm slices, rinsed in a series of two 90% Ethanol baths, and allowed to air dry for further examination of growth and skeletal density.

We measured the density of the calcified skeleton deposited by *C. nereostratum* during the four-month laboratory experiment via micro-computed tomography (microCT); see Chan et al. (2017) for methods

development and analytical setup. In brief, samples were scanned in a GE Locus RS-9 (General Electric Health Care, London, Ontario) x-ray microCT at an energy of 90kVp and tube current of 450 micro-A. Two frames, each 4500 ms in duration, were averaged at 900 projection angles over a 360-degree rotation of the gantry to produce data that was processed into a 3D image with 20 micron isotropic voxel spacing. Only specimens raised in the experimental temperature and pCO2 treatments employed in the feeding assay (6 treatments, n = 3 specimens/treatment) were studied. For each specimen, three cuboid regions of interest (ROI) were then selected, focusing on the region of new growth as indicated by the calcein mark. ROI size was similar for all measurements (1445-1575 voxels); however, dimensions were adjusted depending on the amount of accretion incurred and to avoid overlap with the epithallus or tissues deposited prior to the experiment. Grayscale thresholding to eliminate non-calcified tissue was unnecessary, given that conceptacles were not present in the newly deposited tissue and intracellular pore spaces (6 microns) are smaller than the microCT voxel size (20 microns) and were therefore not resolved. However, an analysis employing thresholding (Chan et al. 2017) produced virtually identical results. We quantified the skeletal density within each ROI by calculating the fractional mineral content of the ROI (i.e., fractional composition of each voxel that is CaCO3), converting each value to units of pure crystal calcite (physical density: 2.71 g/cm^3), then averaging over all voxels in the ROI.

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

[table of contents | back to top]

Data Files

File	
lab_cca_skeletal_density_fx_temp_pCO2.csv	(Comma Separated Values (.csv), 1.96 KB) MD5:1d0d4e90d967b141bbffd3d577a03c8f
Primary data file for dataset ID 755809	

[table of contents | back to top]

Related Publications

Adey, W. H., Halfar, J., & Williams, B. (2013). The coralline genus Clathromorphum Foslie emend. Adey: biological, physiological, and ecological factors controlling carbonate production in an arctic-subarctic climate archive. Smithsonian Contributions to Marine Science 40, 1-41 https://hdl.handle.net/10088/21560
Methods

Chan, P., Halfar, J., Norley, C. J. D., Pollmann, S. I., Adey, W., & Holdsworth, D. W. (2017). Micro-computed tomography: Applications for high-resolution skeletal density determinations: An example using annually banded crustose coralline algae. Geochemistry, Geophysics, Geosystems, 18(9), 3542-3553. doi:10.1002/2017gc006966 https://doi.org/10.1002/2017GC006966
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. ISBN: 1-897176-07-4. URL: https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html https://hdl.handle.net/11329/249
Methods

Lewis, E., Wallace, D., & Allison, L. J. (1998). Program developed for CO2 system calculations (No. ORNL/CDIAC-105). Brookhaven National Lab., Dept. of Applied Science, Upton, NY (United States); Oak Ridge National Lab., Carbon Dioxide Information Analysis Center, TN (United States). doi: 10.2172/639712
Methods

Mucci, A. (1983). The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. American Journal of Science, 283(7), 780-799. doi:10.2475/ajs.283.7.780

Methods

Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., ... & Dubash, N. K. (2014). Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change (p. 151). IPCC.
https://hdl.handle.net/10013/epic.45156
Methods

Roy, R. N., Roy, L. N., Vogel, K. M., Porter-Moore, C., Pearson, T., Good, C. E., Millero, F. J., Campbell, D. M. (1993). The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. Marine Chemistry, 44(2-4), 249-267. doi:10.1016/0304-4203(93)90207-5
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
treatment_temp	target temperature	degrees Celsius
treatment_pCO2	target pCO2 concentration	microatmospheres (uatm)
tank	replicate tank	unitless
tank_temp_ave	average tank temperature during study	degrees Celsius
tank_pCO2_ave	average tank pCO2 concentration during study	microatmospheres (uatm)
sample_ID	unique identifier for each algal sample	unitless
replicate	replicate coralline alga	unitless
ROI	region of interest scanned within each alga	unitless
mg_CaCO3_cm3	skeletal (CaCO3) density within the ROI	milligrams/cm^3

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Coralife 1/4HP
Generic Instrument Name	Aquarium chiller
Dataset-specific Description	To maintain temperature in tanks
Generic Instrument Description	Immersible or in-line liquid cooling device, usually with temperature control.

Dataset-specific Instrument Name	Accumet AB15 pH meter with Accufet solid state probe
Generic Instrument Name	Benchtop pH Meter
Dataset-specific Description	To measure pH of tanks
Generic Instrument Description	An instrument consisting of an electronic voltmeter and pH-responsive electrode that gives a direct conversion of voltage differences to differences of pH at the measurement temperature. (McGraw-Hill Dictionary of Scientific and Technical Terms) This instrument does not map to the NERC instrument vocabulary term for 'pH Sensor' which measures values in the water column. Benchtop models are typically employed for stationary lab applications.

Dataset-specific Instrument Name	Qubit S151 infrared CO2 analyzer
Generic Instrument Name	CO2 Analyzer
Dataset-specific Description	To measure pCO2 in tanks
Generic Instrument Description	Measures atmospheric carbon dioxide (CO2) concentration.

Dataset-specific Instrument Name	UIC 5400 Coulometer on a VINDTA 3C
Generic Instrument Name	CO2 Coulometer
Dataset-specific Description	To measure dissolved inorganic carbon
Generic Instrument Description	A CO2 coulometer semi-automatically controls the sample handling and extraction of CO2 from seawater samples. Samples are acidified and the CO2 gas is bubbled into a titration cell where CO2 is converted to hydroxyethylcarbonic acid which is then automatically titrated with a coulometrically-generated base to a colorimetric endpoint.

Dataset-specific Instrument Name	GE Locus RS-9 (General Electric Health Care, London, Ontario) x-ray microCT
Generic Instrument Name	Computerized Tomography (CT) Scanner
Dataset-specific Description	To produce 3D imagery of specimens
Generic Instrument Description	A CT scan makes use of computer-processed combinations of many X-ray measurements taken from different angles to produce cross-sectional (tomographic) images (virtual "slices") of specific areas of a scanned object.

Dataset-specific Instrument Name	
Generic Instrument Name	MARIANDA VINDTA 3C total inorganic carbon and titration alkalinity analyser
Generic Instrument Description	The Versatile INstrument for the Determination of Total inorganic carbon and titration Alkalinity (VINDTA) 3C is a laboratory alkalinity titration system combined with an extraction unit for coulometric titration, which simultaneously determines the alkalinity and dissolved inorganic carbon content of a sample. The sample transport is performed with peristaltic pumps and acid is added to the sample using a membrane pump. No pressurizing system is required and only one gas supply (nitrogen or dry and CO2-free air) is necessary. The system uses a Metrohm Titrino 719S, an ORION-Ross pH electrode and a Metrohm reference electrode. The burette, the pipette and the analysis cell have a water jacket around them. Precision is typically +/- 1 umol/kg for TA and/or DIC in open ocean water.

Dataset-specific Instrument Name	Darhor manual flow controllers
Generic Instrument Name	Mass Flow Controller
Generic Instrument Description	Mass Flow Controller (MFC) - A device used to measure and control the flow of fluids and gases

Dataset-specific Instrument Name	YSI3200 meter with K=10 conductivity electrode and temperature probe
Generic Instrument Name	Salinometer
Dataset-specific Description	To measure salinity and temperature of tanks
Generic Instrument Description	A salinometer is a device designed to measure the salinity, or dissolved salt content, of a solution.

Dataset-specific Instrument Name	NIST traceable red spirit glass thermometer
Generic Instrument Name	Thermometer
Dataset-specific Description	To measure temperature in the tanks
Generic Instrument Description	A device designed to measure temperature.

[table of contents | back to top]

Project Information

Ocean Acidification: Century Scale Impacts to Ecosystem Structure and Function of Aleutian Kelp Forests (OA Kelp Forest Function)

Extracted from the NSF award abstract:

Marine calcifying organisms are most at risk to rapid ocean acidification (OA) in cold-water ecosystems. The investigators propose to determine if a globally unique and widespread calcareous alga in Alaska's Aleutian archipelago, *Clathromorphum nereostratum*, is threatened with extinction due to the combined effects of OA and food web alterations. *C. nereostratum* is a slow growing coralline alga that can live to at least 2000 years. It accretes massive 'bioherms' that dominate the regions' rocky substrate both under kelp forests and deforested sea urchin barrens. It develops growth bands (similar to tree rings) in its calcareous skeleton, which effectively record its annual calcification rate over centuries. Pilot data suggest the skeletal density of *C. nereostratum* began to decline precipitously in the 1990's in some parts of the Aleutian archipelago. The

investigators now propose to use high-resolution microscopy and microCT imaging to examine how the growth and skeletal density of *C. nereostratum* has changed in the past 300 years (i.e., since the industrial revolution) across the western Aleutians. They will compare their records of algal skeletal densities and their variation through time with reconstructions of past climate to infer causes of change. In addition, the investigators will examine whether the alga's defense against grazing by sea urchins is compromised by ongoing ocean acidification. The investigators will survey the extent of *C. nereostratum* bioerosion occurring at 10 sites spanning the western Aleutians, both inside and outside of kelp forests. At each site they will compare these patterns to observed and monitored ecosystem trophic structure and recent *C. nereostratum* calcification rates. Field observations will be combined with laboratory experiments to determine if it is a decline in the alga's skeletal density (due to recent OA and warming), an increase in grazing intensity (due to recent trophic-level dysfunction), or their interactive effects that are likely responsible for bioerosion patterns inside vs. outside of forests. By sampling *C. nereostratum* inside and outside of forests, they will determine if kelp forests locally increase pH via photosynthesis, and thus buffer the effects of OA on coralline calcification. The combination of field observations with laboratory controlled experiments, manipulating CO2 and temperature, will help elucidate drivers of calcification and project how these species interactions will likely change in the near future. The project will provide the first in situ example of how ongoing ocean acidification is affecting the physiology of long-lived, carbonate producing organisms in the subarctic North Pacific. It will also be one of the first studies to document whether OA, ocean warming, and food web changes to ecological processes are interacting in complex ways to reshape the outcome of species interactions in nature.

[table of contents | back to top]

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)

[table of contents | back to top]

Funding

Funding Source	Award
NSF Arctic Sciences (NSF ARC)	PLR-1316141

[table of contents | back to top]