Experimental results: coral calcification, chlorophyll-a content, algal density, energy reserves, and tissue biomass from samples of reef systems collected from northwest Fiji in 2011

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Data Type: experimental
Version: 23 Sept 2015
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Project
» Interactive Effects of Temperature, Nutrients, and Ocean Acidification on Coral Physiology and Calcification (OA_coral_physiology)

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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Dataset Description

Coral calcification, chlorophyll *a* content, algal density, energy reserves, and tissue biomass from experiments carried out on coral samples from the taxa: *Acropora millepora, Pocillopora damicornis, Montipora monasteriata*, and T*urbinaria reniformis*.

Methods & Sampling

Full details of the experimental design are in:
Schoepf V**,** Grottoli AG, Warner ME, Cai W-J, Melman TF, Pettay DT, Hoadley K, Matsui Y, Baumann JH, Wang Y, Xu H, Li Q, & Hu X. 2013. Coral energy reserves and calcification in a high-CO2 world at two temperatures. PLoS ONE, 8(10): e75049. doi:10.1371/journal.pone.0075049.

A brief description of the analytical methods follows.

Coral collection (April 2011): northwest Fiji (17°29'19"S, 177°23'39"E)
Experiment (July/August 2011): Reef Systems Coral Farm, New Albany, Ohio, USA (40°07'24"N, 82°46'55"W)

Calcification. Net calcification was determined using the buoyant weight technique (Jokiel et al. 1978). Each

coral fragment was buoyantly weighed at the beginning, middle (after 11 experimental days), and at the end of the experiment (after 23 experimental days). As such, it was possible to assess if calcification rates varied during the experiment. Daily calcification rates were calculated as the difference between initial, middle, and final weights, divided by the respective number of days elapsed, and standardized to surface area (see below).

For tissue analyses, corals were frozen at −80 degrees C and a total of three branch tips or growing edge pieces were saved from each fragment for lipid, protein/carbohydrate, and tissue biomass analyses, respectively. The remaining tissue was airbrushed for chlorophyll *a* and endosymbiont density measurements.

Chlorophyll *a* and endosymbiont density. Coral tissue was stripped off the coral skeleton with a waterpik containing 40 ml of synthetic seawater (Instant Ocean). The endosymbionts were isolated from the host tissue via centrifugation and then resuspended in 10 ml of synthetic seawater. For chlorophyll *a* concentrations, 1 ml of this algal suspension was pelleted and the cells lysed in 1 ml of 4 degree C methanol using a bead-beater for 60 seconds. Samples were then immediately placed on ice and allowed to extract for one hour in the dark. Samples were centrifuged to remove cellular debris and measured spectrophotometrically (λ = 652, 665, & 750) on a 96-well plate reader. The equations for chlorophyll *a* in methanol described by Porra et al. 1989, along with path length correction (Warren 2008), were used to calculate chlorophyll *a* concentrations (pg/cell), and were then standardized to surface area (see below). Another 1 ml subsample of the algal suspension was preserved with 10 ul of 1% glutaraldehyde solution for endosymbiont quantification, which was calculated using 6 independent replicate counts on a hemocytometer, using a Nikon microphot-FXA epifluorescent microscope at 100× magnification. Photographs were analyzed through Image J using the analyze particles function.

Energy reserves and tissue biomass. For all energy reserve and tissue biomass measurements, only branch tips or samples with a growing edge were used. While tissue composition may vary across the surface of a coral (Oku et al. 2002), this approach was used to allow for comparison with previously published studies (Grottoli et al. 2004, Rodrigues and Grottoli 2007, Levas et al. 2013). Soluble lipids (referred to hereafter simply as lipids) were extracted from a whole, ground coral sample (skeleton + animal tissue + algal endosymbiont) in a 2:1 chloroform:methanol solution for 1 hour (Grottoli et al. 2004, Rodrigues and Grottoli 2007), washed in 0.88% KCl followed by 100% chloroform and another wash with 0.88% KCl. The extract was dried to constant weight under a stream of pure nitrogen (UPH grade 5.0) and standardized to the ash-free dry weight.

Animal soluble protein and carbohydrate (referred to hereafter simply as protein and carbohydrate, respectively) were extracted from grinding a whole second branch tip of the same fragment (Rodrigues and Grottoli 2007). Briefly, Milli-Q water was added to the ground coral sample and the resulting slurry was sonicated (5 min) and then centrifuged twice (5000 rpm, 10 min) to separate the animal tissue from the skeleton and endosymbiotic algae. Protein and carbohydrate was extracted from the animal tissue only. One subsample of this animal tissue slurry was used for protein extraction using the bicinchoninic acid method (Smith et al. 1985) with bovine serum albumin as a standard (Pierce BCA Protein Assay Kit). A second subsample was used for carbohydrate quantification using the phenol-sulfuric acid method (Dubois et al. 1956) with glucose as a standard. Soluble animal protein and carbohydrate concentrations were standardized to the ash-free dry weight.

Tissue biomass was measured by drying a third branch tip of whole coral sample (skeleton + animal tissue + algal endosymbiont) to constant dry weight (24 hours, 60 degrees C) and burning it (6 hours, 450 degrees C). The difference between dry and burned weight was the ash free dry weight which was standardized to the surface area of this branch tip.

Surface area. Surface area of plating *M. monasteriata* and *T. reniformis* fragments was determined using the aluminum foil technique (Marsh 1970), whereas surface area of branching *A. millepora* and *P. damicornis* fragments was determined using the single wax dipping technique (Stimson and Kinzie 1990, Veal et al. 2010) after the tissue had been removed. Natural wooden blocks of varying sizes and shapes were used as calibration standards (Veal et al. 2010). Wax dipping was conducted using household paraffin wax (Gulf Wax, Royal Oak Enterprises) heated to 65 degrees C. Dried coral skeletons and wooden calibration standards were maintained at room temperature prior to weighing.

Data Processing Description

Details of the statistical analysis methods are in Schoepf et al. 2013.

BCO-DMO Processing Notes:
- Missing values indicated by 'nd' (no data)

- Parameter names were modified to conform with BCO-DMO naming conventions
- 31 July 2015: dataset re-served with values displaying 4 decimal places
- 24 Sept 2015: new (corrected) version of dataset served; some protein and carbohydrate values were previously incorrect.

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Data Files

File
coral_calc_and_energy.csv (Comma Separated Values (.csv), 13.76 KB) MD5:cad1c88e64036c711e3813a2f98004ec Primary data file for dataset ID 546223

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Parameters

Parameter	Description	Units
species	Taxonomic name of the coral species.	unitless
coral_ID	ID number of the coral sample.	unitless
temp	Water temperature.	degrees Celsius (C)
pCO2	Partial pressure of CO2.	microatmospheres (uatm)
genotype	Genotype (parent colony) identifier.	unitless
calcification_1st_half	Calcification 1st half: This is the daily calcification rate measured on the 11th day of the experiment using the buoyant weight method and standardized to the surface area of the coral fragment.	milligrams per day per square centimeter (mg/day/cm2)
calcification_2nd_half	Calcification 2nd half: This is the daily calcification rate measured on the 23rd day of the experiment using the buoyant weight method and standardized to the surface area of the coral fragment.	milligrams per day per square centimeter (mg/day/cm2)
chlorophyll_a	The chlorophyll a content of the endosymbiont fraction standardized to surface area of the fragment.	micrograms per square centimeter (ug/cm2)
cell_density	The number of endosymbiont (Symbiodinium spp.) cells x10^6 per surface area of the coral fragment.	x10^6 cells per square centimeter (cells/cm2)
lipid	The soluble lipid content of the combined animal host and endosymbiont fraction standardized to ash-free dry weight (i.e. gdw).	grams per grams dry weight (g/gdw)
protein	The soluble protein content of the animal host fraction standardized to ash-free dry weight (i.e. gdw).	grams per grams dry weight (g/gdw)
carbs	The soluble carbohydrate content of the animal host fraction standardized to ash-free dry weight (i.e. gdw).	grams per grams dry weight (g/gdw)
tissue_biomass	The ash-free dry weight of the whole tissue (animal host and endosymbiont) standardized to surface area.	milligrams per square centimeter (mg/cm2)

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Instruments

Dataset-specific Instrument Name	Nikon microphot-FXA epifluorescent microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Endosymbiont quantification was calculated using 6 independent replicate counts on a hemocytometer, using a Nikon microphot-FXA epifluorescent microscope at 100X magnification.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Hemocytometer
Generic Instrument Name	Hemocytometer
Dataset-specific Description	Endosymbiont quantification was calculated using 6 independent replicate counts on a hemocytometer, using a Nikon microphot-FXA epifluorescent microscope at 100X magnification.
Generic Instrument Description	A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html.

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Deployments

Fiji_2011_Grottoli

Website	https://www.bco-dmo.org/deployment/546250
Platform	Fiji
Start Date	2011-04-01
End Date	2011-04-30
Description	Description from Schoepf V, Grottoli AG, Warner ME, Cai W-J, Melman TF, Pettay DT, Hoadley K, Matsui Y, Baumann JH, Wang Y, Xu H, Li Q, & Hu X. 2013. Coral energy reserves and calcification in a high-CO2 world at two temperatures. PLoS ONE, 8(10): e75049. doi:10.1371/journal.pone.0075049. "Six parent colonies of Acropora millepora, Pocillopora damicornis, Montipora monasteriata, and Turbinaria reniformis were purchased from Reef Systems Coral Farm (New Albany, Ohio, USA) which is a CITES permit holder. The parent colonies were specifically collected for this experiment from 3–10 m in northwest Fiji (17 29 19 S, 177 23 39 E) in April 2011. Colonies of the same species were collected at least 10 m apart to increase the probability that different genotypes of the same species were selected. All colonies were shipped to Reef Systems Coral Farm."

RSCF_Grottoli_2011

Website	https://www.bco-dmo.org/deployment/546255
Platform	Reef Systems Coral Farm
Start Date	2011-07-01
End Date	2011-08-31
Description	Experiements were conducted on coral reefs as part of the project, "Interactive Effects of Temperature, Nutrients, and Ocean Acidification on Coral Physiology and Calcification"

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Project Information

Interactive Effects of Temperature, Nutrients, and Ocean Acidification on Coral Physiology and Calcification (OA_coral_physiology)

Coverage: Reef Systems Coral Farm, New Albany, Ohio, USA

Extracted from the NSF award abstract:

Atmospheric and sea surface CO2 concentrations are expected to continue to increase substantially over the coming decades resulting in warmer and more acidic oceans, which will greatly stress the health of coral reefs. In addition, ocean margins where most corals live will also see continued increases in human-produced nutrient inputs. While there has recently been a considerable focus on how ocean acidification (due to higher CO2 alone) could negatively impact the growth of reef-building corals due to the projected loss in calcification, the combined impacts of CO2, temperature, and nutrients on coral physiology and calcification are poorly understood. This project will investigate the possible synergistic and antagonistic effects of elevated temperature, CO2, and nutrients on the physiology and internal calcifying chemistry of several species of corals in a laboratory setting. Research tools will include the assessment of coral energy reserves and metabolic demand, symbiotic algal physiology and molecular diversity, coral calcification, and direct measurement of the internal coral pH and carbonate concentration via microprobes. The results from this project have the potential to supply broad scientific impacts regarding how (or if) reef-building corals will survive future climate change scenarios, and will help establish several parameter ranges that could be used to strengthen ocean acidification and coral reef growth models.

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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 Emerging Frontiers Division (NSF EF)	EF-1041124
NSF Emerging Frontiers Division (NSF EF)	EF-1040940
NSF Emerging Frontiers Division (NSF EF)	EF-1041070

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