Mitochondrial membrane potential (MMP) of male gametes following parental ocean acidification during lab experiments conducted in spring 2022.

Website: https://www.bco-dmo.org/dataset/920827
Data Type: experimental
Version: 1
Version Date: 2024-03-24

Project
» Influence of environmental pH variability and thermal sensitivity on the resilience of reef-building corals to acidification stress (Coral Resilience)

Contributors	Affiliation	Role
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Abstract
Ocean acidification (OA) resulting from anthropogenic CO2 emissions is impairing the reproduction of marine organisms. While parental exposure to OA can protect offspring via carryover effects, this phenomenon is poorly understood in many marine invertebrate taxa. We examined how parental exposure to acidified (pH 7.40) versus ambient (pH 7.72) seawater influenced reproduction and offspring performance across six gametogenic cycles (13 weeks) in the estuarine sea anemone Nematostella vectensis. This dataset pertains to the physiology of gametes released by adults in the OA treatment, specifically the mitochondrial membrane potential (MMP) of sperm.

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Coverage

Location: Laboratory at the University of Pennsylvania
Temporal Extent: 2022-01-26 - 2022-05-02

Dataset Description

Data generated as part of a Nematostella ocean acidfication experiment published in Glass et al., 2023. (see Related Publications). Related Zenodo datasets provides further analysis and plotting of the BCO-DMO dataset

here. (see Related Dataset).

Methods & Sampling

Nematostella vectensis (Stephenson, 1935) anemones were collected from a salt marsh in Brigantine, New Jersey in the fall of 2020. Females were identified by inducing spawning, and 14 individuals that released eggs were chosen as the genotype pool for this experiment. Each female was then horizontally bisected through the body column using a razor blade, resulting in two genotypically identical individuals that were divided between the two experimental groups (ambient and acidic).

A clonal male population, also originating from the United States Atlantic coast, was obtained from the laboratory of Dr. Katerina Ragkousi (Amherst College) in the spring of 2021. The male population size was increased via bisection, resulting in a total of 20 genetically identical males for the experiment (N=10 per treatment).

All anemones were kept in 12 parts per thousand (ppt) artificial seawater (ASW; Instant Ocean Reef Crystals reef salt, Spectrum Brands, Blacksburg, VA, USA) at pH 7.7–8.1 and 18°C. The animals were maintained in a dark incubator (Boekel Scientific, Feasterville-Trevose, PA, USA) and fed approximately every other day with Artemia nauplii. The experiment was performed approximately 1–1.5 years after animal collection.

Data Processing Description

Sperm mitochondrial membrane potential (MMP) was measured using the fluorescent dye JC-1 (Thermo Fisher Scientific, Waltham, MA, USA) in weeks 11 and 13. Sperm were pooled by treatment and 1 ml subsamples were incubated with 20 µM JC-1 for 15 min in the dark. A separate aliquot was treated with carbonyl cyanide m-chlorophenyl hydrazone (CCCP; Cell Signaling Technology, Danvers, MA, USA) at a final concentration of 50 µM for 15 min followed by JC-1 as a negative control. Sperm were then centrifuged at 1500×***g*** for 5 min to remove excess dye, resuspended 12 ppt ASW at a concentration of 5×10^5 sperm ml^{-1}, and distributed in triplicate into a 96-well plate. The plate was kept dark and loaded into a Guava easyCyte™ HT flow cytometer (MilleporeSigma, St. Louis, MO, USA). Samples were excited at 488 nm, and fluorescence was detected at two wavelengths: GRN-B (525/30 nm) and YEL-B (583/26 nm). Each well was read for at least 60 s, resulting in more than 1.5×10^4 cells quantified per well. Using in Guava InCyte, plots of green versus yellow fluorescence produced by samples treated with both CCCP and JC-1 were used for gating, then gates were used on all other sample plots to quantify percentages of sperm with high MMP.

BCO-DMO Curation Notes

* Adjusted parameter names to comply with database requirements

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Data Files

File	
920827_v1_spermmmp.csv	(Comma Separated Values (.csv), 319 bytes) MD5:738f3616a86c4d1217b0a0420855b63b
Primary data file for dataset ID 920827, version 1	

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Related Publications

Glass, B. H., Schmitt, A. H., Brown, K. T., Speer, K. F., & Barott, K. L. (2023). Parental exposure to ocean

acidification impacts gamete production and physiology but not offspring performance in Nematostella vectensis. Biology Open, 12(3). https://doi.org/10.1242/bio.059746
Results

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Related Datasets

IsRelatedTo

Glass, B. H., Schmitt, A. H., Speer, K. F., & Barott, K. L. (2022). *Nematostella OA* [Data set]. Zenodo. https://doi.org/10.5281/ZENODO.6941530 https://doi.org/10.5281/zenodo.6941530

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Parameters

Parameter	Description	Units
Treatment	Experimental treatment into which anemones were placed (ambient or acidic seawater pH)	unitless
Date	Sampling week (week 1 start = 2022-01-26)	unitless
Percent_High_Mitochondrial_Membrane_Potential	Percentage of sperm cells with high mitochondrial membrane potential as determined via flow cytometry	percentage (%)

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Instruments

Dataset-specific Instrument Name	PreSens SensorDish Reader
Generic Instrument Name	PreSens OXY-10 Mini oxygen meter
Dataset-specific Description	PreSens SensorDish Reader (Precision Sensing, Regensburg, Germany) for respiration measurements
Generic Instrument Description	The OXY-10 mini is a precise multi-channel oxygen meter for up to 10 'in-house' sensors, simultaneously controlling and reading them. The meter is used with oxygen sensors based on a 2mm optical fibre. The meter is compatible with sensors that are type PSt3 which has a detection limit 15 ppb, 0 - 100% oxygen.

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Project Information

Influence of environmental pH variability and thermal sensitivity on the resilience of reef-building corals to acidification stress (Coral Resilience)

Coverage: Kaneohe Bay, Oahu, HI; Heron Island, Queensland, Australia

NSF Award Abstract:
Coral reefs are incredibly diverse ecosystems that provide food, tourism revenue, and shoreline protection for coastal communities. The ability of coral reefs to continue providing these services to society is currently threatened by climate change, which has led to increasing ocean temperatures and acidity that can lead to the death of corals, the animals that build the reef framework upon which so many species depend. This project examines how temperature and acidification stress work together to influence the future health and survival of corals. The scientists are carrying out the project in Hawaii where they have found individual corals with different sensitivities to temperature stress that are living on reefs with different environmental pH conditions. This project improves understanding of how an individual coral's history influences its response to multiple stressors and helps identify the conditions that are most likely to support resilient coral communities. The project will generate extensive biological and physicochemical data that will be made freely available. Furthermore, this project supports the education and training of undergraduate and high school students and one postdoctoral researcher in marine science and coral reef ecology. Hands-on activities for high school students are being developed into a free online educational resource.

This project compares coral responses to acidification stress in populations experiencing distinct pH dynamics (high diel variability vs. low diel variability) and with distinct thermal tolerances (historically bleaching sensitive vs. tolerant) to learn about how coral responses to these two factors differ between coral species and within populations. Experiments focus on the two dominant reef builders found at these stable and variable pH reefs: Montipora capitata and Porites compressa. Individuals of each species exhibiting different thermal sensitivities (i.e., bleached vs. pigmented) were tagged during the 2015 global coral bleaching event. This system tests the hypotheses that 1) corals living on reefs with larger diel pH fluctuations have greater resilience to acidification stress, 2) coral resilience to acidification is a plastic trait that can be promoted via acclimatization, and 3) thermally sensitive corals have reduced capacity to cope with pH stress, which is exacerbated at elevated temperatures. Coral cells isolated from colonies from each environmental and bleaching history are exposed to acute pH stress and examined for their ability to recover intracellular pH in vivo using confocal microscopy, and the expression level of proteins predicted to be involved in this recovery (e.g., proton transporters) is examined via Western blot and immunolocalization. Corals from each pH history are exposed to stable and variable seawater pH in a controlled aquarium setting to determine the level of plasticity of acidification resilience and to test for pH acclimatization in this system. Finally, corals with different levels of thermal sensitivity are exposed to thermal stress and recovery, and their ability to regulate pH is examined over time. The results of these experiments help identify reef conditions that promote coral resilience to ocean acidification against the background of increasingly common thermal stress events, while advancing mechanistic understanding of coral physiology and symbiosis.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923743

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