Results from bulk cyclic AMP (cAMP) assays conducted to investigate the role of soluble adenylyl cyclase (sAC) in sperm from the gonochoric, broadcast spawning coral Astrangia poculata

Website: https://www.bco-dmo.org/dataset/920168
Data Type: experimental
Version: 1
Version Date: 2024-02-09

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
Most stony corals liberate their gametes into the water column via broadcast spawning, where fertilization hinges upon the activation of directional sperm motility. Sperm from gonochoric and hermaphroditic corals display distinct morphological and molecular phenotypes, yet it is unknown whether the signallng pathways controlling sperm motility are also distinct between these sexual systems. We addressed this knowledge gap using the gonochoric, broadcast spawning coral Astrangia poculata. This dataset is from bulk cyclic AMP (cAMP) assays conducted to investigate the role of soluble adenylyl cyclase (sAC) in sperm. Data are associated with Glass et al. (2023) Proceedings of the Royal Society B (10.1098/rspb.2023.0085). These data are also published in Dryad under DOI 10.5061/dryad.rn8pk0pg8.

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Coverage

Temporal Extent: 2021-08-01 - 2022-12-31

Methods & Sampling

Corals were collected from Narragansett Bay, Rhode Island, USA. Experiments were performed at the University of Rhode Island, and samples were processed and data collected at the University of Pennsylvania.

Adult *Astrangia poculata* (Ellis & Solander, 1786) colonies (figure 1a of Glass et al., 2023) were collected from Narragansett Bay, Rhode Island, USA (41.49231, -71.41883) in early August 2021 and 2022 and transported in seawater to the University of Rhode Island (URI). Corals were induced to spawn by increasing the temperature from approximately 22 degrees Celsius (°C) to 31°C over 30 minutes and by physical touch. Once spawning began (figure 1b) of Glass et al., 2023), males were moved to individual glass bowls containing 50 milliliters (mL) seawater and left unperturbed until spawning activity ceased (figure 1c of Glass et al., 2023). The seawater containing sperm (i.e. 'sperm water') was then passed through a 100 micrometer (µm) cell strainer. For *in vivo* assays, sperm from each male were concentrated separately by centrifugation at 1500 × g for 5 minutes. The supernatant was removed, additional sperm water from the same male was added atop the sperm pellet, and centrifugation was repeated to form a large sperm pellet. Following this process, the supernatant was removed and sperm were resuspended in approximately 15 mL sodium-free seawater (NaFSW), a seawater substitute with the same salinity as seawater but lacking sodium ions (Na+). The absence of Na+ in NaFSW prevents the outward flux of H^+ through an Na+/H+ exchanger and thus facilitates cytosolic alkalinization following the addition of NH_4Cl. Sperm in NaFSW were used for all *in vivo* assays within 2.5 hours after spawning. Since coral sperm capacity for motility can decline over time following spawning, each pool of sperm in NaFSW was tested for motility capacity immediately prior to use in each *in vivo* assay by stimulating a small aliquot of the pool with 20 millimolar (mM) NH_4Cl; all sperm pools had at least 50% motility as assessed via phase contrast microscopy (Nikon Eclipse Ni-U microscope and digital camera system DS-Ri1).

Assessment of *in vivo* sAC activity:
Within 0.5 to 1.5 hours post-spawning, pools of sperm in sodium-free seawater (NaFSW) (N = 3 pools; 1 pool per male) were divided in half and incubated with either dimethyl sulfoxide (DMSO; 0.5% v/v) as a carrier control or the sAC inhibitor KH7 (50 micromolar (µM) in DMSO; Tocris Bioscience) for 30 minutes, then aliquoted into a 96-well plate. For each sperm pool, half of the replicate wells of sperm from each treatment (DMSO or KH7) were amended with either 100 mM NH_4Cl to a final concentration of 20 mM NH_4Cl (stimulated) or an equivalent volume of NaFSW (unstimulated). Triplicate wells per treatment and stimulation condition were lysed at time = 0, 0.1, 0.5, 1, 2, and 5 minutes with the addition of HCl (0.167 M final concentration) with 0.01% (v/v) Triton-X. Cyclic adenosine monophosphate (cAMP) was quantified in each sample well using an enzyme-linked immunosorbent assay according to manufacturer's instructions (ArborAssays K019). Data were analyzed using a four-parameter logistic curve of cAMP standards to determine the nanomoles (nmol) of cAMP in each well, which was normalized to nanograms (ng) of protein in the well determined via Bradford assays.

Assessment of *in vivo* PKA activity:
Protein kinase A (PKA) activity was assessed *in vivo* to test the hypothesis that PKA is activated downstream of sAC following cytosolic alkalinization in sperm from *A. poculata*. Within 1.5 to 2 hours post-spawning, pools of sperm in NaFSW (N = 3 males; 1 pool per male) were divided into three aliquots and treated with either DMSO (0.5% v/v), the sAC inhibitor KH7 (50 µM in DMSO), or the PKA inhibitor H-89 (20 µM in DMSO; Cell Signaling Technologies) for 30 minutes. Sperm from each treatment were then aliquoted into each of six 1.5 mL tubes. One tube from each treatment was flash-frozen after the addition of NaFSW as an unstimulated control. Then, each of the remaining tubes was stimulated with the addition of a stock solution of 100 mM NH_4Cl (20 mM NH_4Cl final concentration). One tube per treatment was then flash-frozen in liquid nitrogen at either 0.1, 0.5, 1, 2, or 5 minutes post-stimulation. Later, proteins were extracted from each sample, quantified via Bradford assays, and run in gel electrophoresis. Following transfer, membranes were probed with a commercial primary antibody recognizing phosphorylated substrates of PKA (Abcam). All blots were developed with pico chemiluminescence reagents (Thermo Fisher Scientific) and imaged on an Amersham Imager 600 (General Electric) with automatic exposure. Total band intensity was quantified for each lane and normalized to the time = 0 lane for each blot in ImageJ.

Assessment of sperm motility:
Sperm motility was assessed within 0.5 to 2.5 hours of spawning under various conditions to determine the roles of sAC and PKA in motility. Videos of sperm in seawater or NaFSW were recorded before and immediately after stimulation with 20 mM NH_4Cl. For videos of sperm in seawater, sperm were never transferred to NaFSW and sat in seawater for up to 2.5 hours, so motility results of sperm in seawater should be interpreted with caution as motility may have declined over time. Next, sperm in NaFSW were amended with either DMSO (0.05% v/v), KH7 (50 µM in DMSO), or H-89 (20 µM in DMSO) and incubated for 30 minutes, and then stimulated with 20 mM NH_4Cl and immediately videoed under a phase contrast microscope (Nikon Eclipse Ni-U microscope and digital camera system DS-Ri1). This experiment was repeated using sperm from each of three males. In all movies, most non-motile sperm could be seen twitching, indicating viability. Each movie was renamed with a random string of characters and corresponding treatment information was recorded in a spreadsheet, then movies were scored by an observer blinded to the treatment as 0, 20, 40, 60, 80, or 100%

motile according to the percentage of sperm displaying progressive, directional motility. Motility scores for each treatment were averaged for each male (technical replicates) and then across the males (biological replicates).

BCO-DMO Processing Description

- Imported original file "cAMP ELISA data.xlsx" into the BCO-DMO system.
- Saved final file as "920168_v1_camp_elisa_data.csv".

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Data Files

File
920168_v1_camp_elisa_data.csv (Comma Separated Values (.csv), 13.44 KB) MD5:863837158ab41097f77527f9c614cdc2 Primary data file for dataset ID 920168, version 1

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Related Publications

Glass, B. H., Ashey, J., Okongwu, A. R., Putnam, H. M., & Barott, K. L. (2023). Characterization of a sperm motility signalling pathway in a gonochoric coral suggests conservation across cnidarian sexual systems. Proceedings of the Royal Society B: Biological Sciences, 290(2004). https://doi.org/10.1098/rspb.2023.0085 *Results*

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Related Datasets

IsRelatedTo

Barott, K., Putnam, H., Okongwu, A., Glass, B., Ashey, J. (2024) **Results from motility assays carried out as part of a study investigating the role of soluble adenylyl cyclase (sAC) in sperm from the gonochoric, broadcast spawning coral Astrangia poculata.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-02-23 doi:10.26008/1912/bco-dmo.920623.1 [view at BCO-DMO]

Barott, K., Putnam, H., Okongwu, A., Glass, B., Ashey, J. (2024) **Results from protein kinase A (PKA) substrate phosphorylation assays conducted to investigate the role of soluble adenylyl cyclase (sAC) in sperm from the gonochoric, broadcast spawning coral Astrangia poculata.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-02-13 doi:10.26008/1912/bco-dmo.920273.1 [view at BCO-DMO]

Different Version

Glass, B., Ashey, J., Okongwu, A., Putnam, H., & Barott, K. (2023). Data for: Characterization of a sperm motility signaling pathway in a gonochoric coral suggests conservation across sexual systems (Version 3) [Data set]. Dryad. https://doi.org/10.5061/dryad.rn8pk0pg8

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Parameters

Parameter	Description	Units
Colony	Identifies which male (M) coral colony from which sperm for the assay were collected	unitless
Time	Time (in seconds) after stimulation of sperm motility that the assay was stopped via cell lysis	seconds
Treatment	Modifications made to sperm before the assay, including DMSO (control), KH7 (sAC inhibitor), and H-89 (PKA inhibitor)	unitless
Stim	Denotes presence (yes) or absence (no) of stimulation of sperm	unitless
cAMP_pmol_mL	Concentration of cAMP (in pmol/mL) in well of 96-well plate in which assay was performed	picomoles per milliliter (pmol/mL)
Protein_ug_mL	Concentration of protein (in ug/mL) in well of 96-well plate in which assay was performed	micrograms per milliliter (ug/mL)
cAMP_nmol_mg	cAMP in well normalized to milligrams of protein in well (previous two columns divided)	nanomoles per milligram (nmol/mg)
cAMP_nmol_ng	cAMP in well normalized to nanograms of protein in well (previous two columns divided)	nanomoles per nanomgram (nmol/ng)

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Instruments

Dataset-specific Instrument Name	Amersham Imager 600
Generic Instrument Name	Amersham Imager 600
Dataset-specific Description	Amersham Imager 600 (General Electric) for imagining of Western blots
Generic Instrument Description	The Amersham Imager 600 series is an imaging instrument designed to identify and quantify proteins or DNA in gels or membranes. A cooled 3.2 megapixel CCD camera is used to capture high-resolution digital images of protein and DNA bands in gels and on membranes. The 600 series includes the Amersham Imager 600, 600 UV, 600 QC, 600 RGB. Three different modes are available from the 600 series: Chemiluminescence capture mode is available in all configurations; Fluorescence capture mode is available on the 600 UV, 600 QC, and 600 RGB; Colorimetric capture mode is available in all configurations.

Dataset-specific Instrument Name	digital camera system
Generic Instrument Name	Camera
Dataset-specific Description	Phase contrast microscope (Nikon Eclipse Ni-U microscope and digital camera system DS-Ri1) for recording sperm motility videos
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Phase contrast microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Phase contrast microscope (Nikon Eclipse Ni-U microscope and digital camera system DS-Ri1) for recording sperm motility videos
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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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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