Reproductive histology and energetics in Acropora hyacinthus in response to the 2019 Moorea bleaching event.

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Project
» RAPID: Collaborative Research: Studies of recovery from bleaching in Acropora hyacinthus: epigenetic shifts, impacts on reproductive biology and carry-over effects (Moorea coral bleaching)

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Abstract
The French Polynesian island of Mo'orea experienced a severe mass bleaching event in 2019 accompanied by widespread coral mortality. At the most heavily impacted sites, Acropora hyacinthus individuals that were resistant to bleaching were observed alongside colonies that bleached but showed signs of symbiont recovery shortly after the bleaching event. Fragments of healthy A. hyacinthus colonies five months post-bleaching were sampled for energetic assays and histological measurements. Despite healthy appearances in both resistant and recovered corals, recovered colonies had significantly reduced energy reserves compared to resistant colonies. In addition, compound effects of stress on reproduction were observed: recovered colonies displayed both a lower probability of containing gametes and lower fecundity per polyp.

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Coverage

Spatial Extent: **Lat**:-14.4751 **Lon**:-149.817
Temporal Extent: 2019-10-06 - 2019-10-15

Methods & Sampling

Methodology and analytical procedures documented in Leinbach et al., 2021, but are included here as well:
In tagged coral colonies that were resistant to or recovered from bleaching, the energetic condition of the host was assessed (Nresistant = 12, Nrecovered = 20). One small branch (~ 2–4 centimeters in length) was sampled from each colony and airbrushed in filtered seawater to remove coral tissue and algal cells (blastate)

from the skeleton. The blastate was homogenized and 200 µL was collected and preserved in Z-fix (10% zinc formalin) for algal symbiont counts. The remaining blastate was centrifuged at 2000×g for 2 minutes to separate the host tissue from endosymbiont cells. Host tissue slurry was preserved at − 20 °C until further processing. Microalgal endosymbiont density was quantified using a hemocytometer (Hausser Scientific, Horsham, PA) under an Olympus BH-2 microscope. Total host protein content was quantified using a Bradford assay with bovine-serum albumin (BSA) as a standard (Pierce Coomassie Plus Assay Kit, Thermo Fisher Scientific). Total host carbohydrate content was quantified using a modified phenol–sulfuric acid method. All physiological metrics were standardized to coral skeleton surface area following the paraffin wax-dipping technique.

Small fragments from tagged colonies were sampled by hand via SCUBA (Nresistant = 26, Nrecovered = 21) in October 2019. For each colony, the selected fragment was sampled 5–10 centimeters (cm) from the colony edge, and branch tips and colony edges were avoided. Samples were immediately preserved in Z-fix for 24 hours and then stored in 100% ethanol until histological processing. Samples were decalcified with a 1% EDTA decalcifier solution for 48–72 h and stored in 70% ethanol until processing on a Leica ASP6025 tissue processer. Paraffinized tissue was embedded in wax blocks (Leica EG1150H embedding machine) and then allowed to cool in a freezer 24 hours prior to sectioning. Blocks were serially sectioned at 5 micrometer (µm) thickness on a Leica RM2125RTS microtome every 300 micrometer (µm), which corresponds to the average oocyte diameter. Sections were arranged on microscope slides and stained using a modified Heidenhain's aniline blue stain on a Leica ST5020 multistainer.

Histological sections were analyzed for measurements of reproductive effort: (1) presence/absence of male and female gametes, (2) diameter of oocytes, and (3) relative fecundity, detailed below. Gametes (oocytes and spermatocytes) were staged from I-V following the classification of Szmant-Froelich et al. Slides were examined using an Olympus BX41 microscope with an Olympus SC180 camera attachment. Measurements were made using ImageJ. Oocyte diameter was determined by averaging the longest and shortest axis of each oocyte. A total of 25 oocytes were measured from each colony. In fragments containing fewer than 25 oocytes, the maximum number of oocytes observed was used (Supplementary Table S1, Leinbach et al., 2021). Only oocytes with a visible nucleus were measured to ensure no oocytes were counted more than once and that the maximum diameter was measured.

Due to the small size of the fragments and polyps, as a proxy for fecundity, three polyps were randomly selected on the middle slide from each individual. When there was an even number of slides, the first of the two middle slides were used. Because only one slide from each individual was examined, there was no risk of double-counting oocytes, so the number of both nucleated and non-nucleated oocytes was counted in each of the randomly selected polyps. These counts were averaged to produce the average number of oocytes per polyp for each individual as a measure of relative fecundity. It should be noted that this relative estimate is lower than true fecundity.

Data Processing Description

BCO-DMO Processing description:
- Adjusted field/parameter names to comply with BCO-DMO naming conventions
- Missing data identifier 'NA' replaced with 'nd' (BCO-DMO's default missing data identifier)
- Added a conventional header with dataset name, PI names, version date
- Rounded columns to 3 decimal places

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Data Files

File	
energetics_fecundity.csv	(Comma Separated Values (.csv), 5.77 KB) MD5:fff274b1e788c2bd1385da4e8adb3401
Primary data file for dataset ID 876072	

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Related Publications

Leinbach, S. E., Speare, K. E., Rossin, A. M., Holstein, D. M., & Strader, M. E. (2021). Energetic and reproductive costs of coral recovery in divergent bleaching responses. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-02807-w
Results

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Parameters

Parameter	Description	Units
coral_ID	coral identification number	unitless
depth	depth of coral sample	meters (m)
response	response (resistant, bleached, recovered)	unitless
tagged_month	month of sample tagging	month
May_status	status in May	unitless
August_status	status in August	unitless
October_status	status in October	unitless
histology_sampled_date	histology sample date in format YYYY-MM-DD	unitless
energetics_sampled_date	energetics sample date in format YYYY-MM-DD	unitless
oocytes_measured	number of oocytes measured	unitless
average_relative_fecundity	average relative fecundity	units
surface_area	surface area	centimeters squared
symbiont_normSA	microalgal endosymbiont density	symbionts per centimeters squared
protein_normSA	total host protein content	micrograms per centimeters squared
carb_normSA	total host carbohydrate content	micrograms per centimeters squared
colony_area	area of coral colony	centimeters squared

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Instruments

Dataset-specific Instrument Name	Olympus SC180
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	
Generic Instrument Name	Hemocytometer
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.

Dataset-specific Instrument Name	Olympus BH-2
Generic Instrument Name	Microscope - Optical
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".

Dataset-specific Instrument Name	Olympus BX41
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	used with Olympus SC180 camera attachment
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".

Dataset-specific Instrument Name	Eica ASP6025
Generic Instrument Name	Tissue processor
Generic Instrument Description	An automated system used to process tissue specimens for examination through fixation, dehydration, and infiltration.

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Project Information

RAPID: Collaborative Research: Studies of recovery from bleaching in Acropora hyacinthus: epigenetic shifts, impacts on reproductive biology and carry-over effects (Moorea coral bleaching)

Coverage: Moorea, French Polynesia; Auburn University; University of California, Santa Barbara

NSF Award Abstract:

Coral reefs provide strong economic and ecological benefits, yet they are declining worldwide largely due to extreme heat events that cause bleaching, a disturbance of the essential relationship between the algae that live inside the coral and the coral. There is currently a mass coral bleaching event in Moorea, French Polynesia where up to 90% of corals show some level of bleaching in response to heat stress. This location is ideal to study adaptation and acclimation thanks to the facilities and sampling of the Moorea Coral Reef (MCR) Long Term Ecological Research (LTER) site. This project explores how strong natural disaster events shape genetic differences in populations through time. By using historical environmental data it may be possible to identify modifications of the genome linked to past bleaching events. This knowledge will help establish models to predict reef recovery after disturbance and will be useful for choosing colonies with the best chance of survival in restoration efforts. This project also investigates how the bleaching history of the parents impacts characteristics of the next generations, such as reproductive output, larval, survival and heat tolerance. This project will provide training and involvement in research for three senior PhD students and at least five undergraduates. Coral restoration efforts rely on understanding how corals might adapt to environmental stress.

The mass coral bleaching event currently occurring in French Polynesia (April 2019) offers an opportunity to test hypotheses regarding mechanisms of rapid response to large scale disturbances. This project investigates potential epigenetic and genetic mechanisms involved in either resisting stress or recovering from bleaching. The research leverages the Moorea Coral Reef (MCR) LTER, which integrates the high resolution oceanographic metrics and data on long-term community dynamics into the study of rapid adaptation of Acropora hyacinthus. Genetic and epigenetic signatures of a natural selection event (bleaching) are tracked in the field to test the impact of bleaching history on reproductive and carry-over effects in larval and juvenile corals. Both physiological and molecular methods, such as 2bRAD genotyping and reduced representation bisulfite sequencing, are employed to investigate correlations between phenotypes and genetic and epigenetic differences in the genome. This work explores associations between selection on genetic variation and epigenetic variation as well as the potential role of DNA methylation in phenotypic change across a generation in association with coral bleaching. In this era of global change, there is mounting evidence that rapid evolutionary processes are occurring at time scales relevant to ecological processes. Therefore, capitalizing on a system with rich long-term ecological data, such as that associated with the MCR LTER, is ideal to investigate mechanisms of rapid adaptation.

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-1935308
NSF Division of Ocean Sciences (NSF OCE)	OCE-1935305

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