Lesion frequencies and sizes after fish feces treatment on coral samples collected on the north shore of Mo'orea, French Polynesia, Oct 2020 to Jun 2021

Website: https://www.bco-dmo.org/dataset/933832
Data Type: experimental
Version: 1
Version Date: 2024-09-24

Project
» CAREER: Testing the effects of predator-derived feces on host symbiont acquisition and health (Fish transmit microbes)

Contributors	Affiliation	Role
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Abstract
This datafile contains frequencies and sizes (number of polyps killed) developed in coral tissues after the application and removal of fish feces to test whether fish from different guilds affect coral health in distinct ways.There were five treatments: fresh feces from a corallivorous butterflyfish (FC); fresh feces from a grazer/detritivore (FG); sterilized feces from a corallivorous butterflyfish (SC); sterilized feces from a grazer/detritivore (SG); no-feces control (C). For the fresh feces treatments (FC, FG), we applied 100 µl of fresh feces isolated from the hindgut of the butterflyfish Chaetodon ornatissimus (FC) or the grazer/detritivore Ctenochaetus striatus (FG) directly onto each coral fragment. For the sterilized feces treatments (SC, SG), fecal pellets were sterilized in a pressure cooker for 40 minutes at 120°C and then applied in the same manner as fresh feces. The experiment ran for ~22 hours. The experiment was conducted in three iterations in Mo'orea, French Polynesia, over two years (2020, 2021).

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Coverage

Location: The North shore of Mo'orea, French Polynesia
Spatial Extent: **N**:-17.474444 **E**:-149.827222 **S**:-17.483056 **W**:-149.849444
Temporal Extent: 2020-10 - 2021-06

Methods & Sampling

A feces addition experiment was conducted three times (Final N = 11 replicates) using fragments of multiple

species of *Pocillopora* in Moorea, French Polynesia. An initial experimental iteration was performed in October 2020 (four replicates); two additional iterations took place in June 2021 (5 replicates each). Coral colonies were collected from the fore reef on the north shore of Mo'orea on Scuba at 4-8 m depth and immediately returned to the research station and placed into flow-through seawater tables. After 24 h, all corals were cut into five ~10 cm long fragments and left to acclimate for 48 hours, and any macrosymbionts (e.g., Trapezia crabs, shrimp) were removed. All fragments were transferred to glass jars containing 500 ml of 0.2 µm-filtered (sterile) seawater with bubblers and air stones to promote aeration and water circulation, and photographed under a dissection microscope with 3.5-180X zoom (AmScope SM-1TSZZ-144S-10M).

We then blindly assigned each jar containing a coral fragment to one of the following five treatments (such that one fragment from each coral colony was assigned to each treatment, 4 replicate colonies x 5 treatments = 20 coral fragments in separate jars): fresh feces from a corallivorous butterflyfish (FC); fresh feces from a grazer/detritivore (FG); sterilized feces from a corallivorous butterflyfish (SC); sterilized feces from a grazer/detritivore (SG); no-feces control (C). For the fresh feces treatments (FC, FG), we applied 100 µl of fresh feces isolated from the hindgut of the butterflyfish Chaetodon ornatissimus (FC) or the grazer/detritivore Ctenochaetus striatus (FG) directly onto each coral fragment. Fish were collected on the north shore of Mo'orea using a Hawaiian sling and immediately transported on ice to the laboratory. Feces were isolated from the hindgut using sterile tools by making an incision from the anus to the pelvic fin and removing the intestinal tract; feces were then squeezed from the hindgut into sterile collection tubes using sterile tweezers and pipetted onto coral fragments using 1 ml filter tips that were modified to widen the tip opening using a sterilized razor blade. For the sterilized feces treatments (SC, SG), fecal pellets were sterilized in a pressure cooker for 40 minutes at 120 °C and then applied in the same manner as fresh feces. The fresh feces used in the experiment were subsampled for DNA extractions by preservation in DNA/RNA Shield (Zymo Research, CA). No manipulation was conducted on the no-feces control fragments. The experiment ran for ~22 hours; this treatment duration is reflective of time periods over which corals may sometimes be in direct contact with fish feces in situ (up to 48 hours; Ezzat et al., 2019; Ezzat et al., 2021).

Minor modifications were incorporated into the design of the second and third experimental iterations: for these iterations, five replicate colonies were used instead of four. Additionally, fecal treatments in these iterations were composed of feces from two individuals per fish species that were mixed prior to application (instead of using feces from a single fish individual per treatment as in the first experimental iteration).

To test how microbial communities in fish feces affected coral health, we quantified the frequencies and sizes of coral lesions caused by fecal treatments (in addition to measuring the photosynthetic efficiency of each fragment). In brief, fecal pellets were removed from each fragment and photographs were taken using a dissection microscope.

Data Processing Description

Dissection microscope photographs were analyzed in ImageJ (version 1.53f51; Abràmoff, 2004). Each fragment was binned to one of three categories: “apparently healthy” (no change in fragment compared to before fecal application), “lesion” (fragment contains a novel patch of bare calcium carbonate, where coral tissue died and sloughed off following fecal application), or “dead” (fragment no longer contains live tissue; all tissue sloughed off following fecal application). The size of each lesion (excluding fragments binned as “apparently healthy” or “dead”) was measured by determining the average polyp size on each fragment in ImageJ by counting the number of polyps in a polygon of standardized size in triplicate. Then, the size of the lesion was measured (in pixels), and the lesion size was expressed as the estimated number of polyps that had died.

BCO-DMO Curation Notes

- Imported original files "Lesion frequencies.CSV" and "Lesion sizes.CSV" into the BCO-DMO system.
- Concatenated "graco_iteration", "Treatment", and "Colony" columns into a sample id for each table to join on
- Joined tables into single dataset "933832_v1_coral_lesion_fish_feces.csv"
- Renamed fields to comply with system requirements
- Added month of collection column as indicated in methods section
- Added columns for lat and lon of reef as a general collection location
- Saved final file as "933832_v1_coral_lesion_fish_feces.csv"
- Checked all scientific names referenced in methods section and the methods section of related dataset using

World Register of Marine Species (WoRMS) Taxon Match. All scientific names referenced are valid and accepted names as of 2024-09-23. Included the complete list in taxon_identifiers.csv as a supplemental file
- Included "Identification_in_methods", "matched_AphiaID', "LSID", "AphiaID_accepted", and "ScientificName_accepted" in "taxon_identifiers.csv" supplemental file

Problem Description

The third iteration of the experiment ended after ~15 hours (instead of ~22 hours) because fragments (including control fragments) from three of five colonies were exhibiting signs of tissue loss consistent with stressors outside the scope of our experiment. Tissue loss in some other fragments (that were not selected for use in the experiment) of these coral colonies had started before treatments were applied to experimental fragments. Thus, all data for these three colonies (the entire set of samples—all treatments) were discarded and not included in subsequent analyses. Additionally, there are no data on lesion sizes for any of the treatments for replicate 1 in iteration 1 of the experiment.

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Data Files

File	
933832_v1_coral_lesion_fish_feces.csv	(Comma Separated Values (.csv), 2.50 KB) MD5:fd810f43a760a43bf7715980a1f77e59
Primary data file for dataset ID 933832, version 1	

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Supplemental Files

File	
taxon_identifiers.csv	(Comma Separated Values (.csv), 1.69 KB) MD5:191ca61e01017292ed5b26c2ce30036a
Taxon identifiers (AphiaID and LSID) for scientific names listed in methods section of this and related datasets. Generated using the World Register of Species taxa match tool performed 2024-09-24.	

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Related Publications

Abràmoff, M.D, Magalhães, P.J., Ram, S.J. 2004. Image processing with ImageJ. Biophotonics International 11(7): 36−42
Software

Ezzat, L., Lamy, T., Maher, R., Munsterman, K., Landfield, K., Schmeltzer, E., Gaulke, C., Burkepile, D., & Vega Thurber, R. (2019). Surgeonfish feces increase microbial opportunism in reef-building corals. Marine Ecology Progress Series, 631, 81–97. https://doi.org/10.3354/meps13119
Methods

Ezzat, L., Merolla, S., Clements, C. S., Munsterman, K. S., Landfield, K., Stensrud, C., Schmeltzer, E. R., Burkepile, D. E., & Vega Thurber, R. (2021). Thermal Stress Interacts With Surgeonfish Feces to Increase Coral Susceptibility to Dysbiosis and Reduce Tissue Regeneration. Frontiers in Microbiology, 12. https://doi.org/10.3389/fmicb.2021.620458
Methods

Grupstra, C. G. B., Howe-Kerr, L. I., van der Meulen, J. A., Veglia, A. J., Coy, S. R., & Correa, A. M. S. (2023). Consumer feces impact coral health in guild-specific ways. Frontiers in Marine Science, 10. https://doi.org/10.3389/fmars.2023.1110346
Results

Rasband, W. S. (n.d.). ImageJ. U.S. National Institutes of Health. https://imagej.net/ij/ *Software*

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Related Datasets

IsRelatedTo

Correa, A. M., Grupstra, C. (2024) **Bacterial communities and relative abundances of the pathogen Vibrio coralliilyticus in feces of coral reef fish collected on the north shore of Mo'orea, French Polynesia, Oct 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-11-13 doi:10.26008/1912/bco-dmo.935908.1 [view at BCO-DMO]
Relationship Description: Related dataset includes bacterial 16S rRNA gene metabarcoding on fecal, corals, algae, sediments, and seawater samples.

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Parameters

Parameter	Description	Units
Sample_ID	Unique identifer of samples and treatment.	unitless
Collection_Date	Date of sampling as year and month.	unitless
Experiment	Iteration of the experiment, graco1 (2020), graco2 (2021), or graco3 (2021).	unitless
Treatment	Treatment name; C= control, FC= fresh corallivore feces, FG= fresh grazer feces, SC= sterile corallivore feces, SG=sterile grazer feces.	unitless
Colony	Coral colony number; note that these are unique per year (graco1 = 2020, graco2 and graco3= 2021).	unitless
Replicate	Replicate; combination of treatment name and colony number.	unitless
Health	Health state of the fragment after removal of the fecal pellet; healthy (there was no lesion), lesion (there was one lesion, i.e., a patch where coral tissue had died), or dead (the entire fragment died).	unitless
Dead_Polyps	Number of polyps that died due to application of fecal treatment. If a replicate was "healthy" or "dead" in the above file, then it is not included in this file.	unitless

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Instruments

Dataset-specific Instrument Name	Imaging PAM maxi (k-6) (Walz, Germany)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Dissection scope (AmScope SM-1TSZZ-144S-10M)
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	To test how microbial communities in fish feces affected coral health, we quantified the frequencies and sizes of coral lesions caused by fecal treatments (in addition to measuring the photosynthetic efficiency of each fragment). In brief, fecal pellets were removed from each fragment and photographs were taken using a dissection microscope.
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

CAREER: Testing the effects of predator-derived feces on host symbiont acquisition and health (Fish transmit microbes)

NSF Award Abstract:
Climate change and local-scale anthropogenic stressors are degrading coral reefs across the globe. When conditions become too stressful on reefs, corals can lose beneficial microbial symbionts (e.g., dinoflagellates in the family Symbiodiniaceae) that live in their tissues via a process called "bleaching". Although Symbiodiniaceae play key roles in the health of coral colonies, we know little about the processes that make symbionts available in the environment to prospective host corals. This research tests the extent to which the feces of coral-eating fish, which contain live Symbiodiniaceae, facilitate symbiont acquisition by corals in their early life stages. It will generate seminal knowledge on how corallivore feces impact coral symbioses and health, and will assess the ecological importance of corallivorous fishes as drivers of coral symbiont assemblages. This research also test the extent to which corallivore feces are a source of food and nutrients that impact coral health; this has particular relevance to the survival and recovery of bleached adult corals. This research can ultimately inform intervention strategies to support reef resilience and mitigate reef degradation. Results from this project will be communicated widely in scientific arenas, in undergraduate education programs, and to the public via multimedia content and outreach. The Houston Independent School District (HISD, Houston, TX) is the nation's 7th largest public school system. This work will also support economically disadvantaged and first-generation undergraduate students in pursuing STEM majors and careers through multi-year research experiences.

Symbioses between foundation species (e.g., corals, sponges, trees) and microbiota (e.g., microeukaryotes, bacteria) underpin the biodiversity, productivity, and stability of ecosystems. Consumers, such as predators and herbivores, shape communities of these foundation species through trophic interactions. For instance, grazers contribute to the maintenance of coral dominance on reefs via consumption of macroalgal

competitors. However, the indirect effects of other consumers on foundation species are rarely examined. Few studies have tested how consumers affect microbiota assembly in corals, even though coral symbionts (e.g., dinoflagellates in the family Symbiodiniaceae) play key roles in reef function and persistence. Corallivorous (coral-eating) fishes were recently demonstrated to egest large quantities of live Symbiodiniaceae cells as they swim across reefs. This research is testing the hypothesis that corallivore feces promote coral dominance on reefs by supporting coral acquisition of key symbionts and nutrients. The following research objectives will be accomplished: (1) to quantify the contribution of corallivorous fish feces to coral symbiont acquisition; and (2) to test the extent to which corallivorous fish feces influence coral health and recovery from thermal stress. Reefs are being degraded globally due to climate-change induced bleaching and associated mortality. This project is teasing apart the extent to which nutrients and/or live symbionts associated with corallivore feces contribute to the resilience of bleached corals under ambient and heat stress conditions. The research is tightly integrated with two education objectives: (1) to organize a Research Experience for Teachers (RET) program in which rigorous learning modules that high school teachers can incorporate into their Environmental Systems course offerings are developed and tested; and (2) to provide undergraduate students with a multi-year research experience through a partnership with the Rice Emerging Scholars Program (RESP).

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-2145472

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