# Results from qPCR assays to quantify the abundance and photochemical performance of symbionts relative to coral cells in three coral species collected from colonies in southeast Florida in April and October 2019 before, during, and after heat stress tests 

Website: https://www.bco-dmo.org/dataset/918220
Data Type: Other Field Results
Version: 1
Version Date: 2024-01-22

## Project

" Collaborative Research: Assessing the changing symbiotic milieu on Caribbean coral reefs under climate change: magnitude, tradeoffs, interventions, and implications (Symbiont Shifts on Reefs)

| Contributors | Affiliation | Role |
| :--- | :--- | :--- |
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#### Abstract

This dataset contains results from assays to quantify the abundance and photochemical performance of Breviolum, Cladocopium, and Durusdinium symbionts relative to coral cells in Montastraea cavernosa, Orbicella faveolata, and Siderastrea siderea corals collected from colonies in southeast Florida in April 2019 and in October 2019, before, during and after aquarium-based experimental heat stress tests. Bulk genomic DNA was extracted from tissue scrapings taken from 2.5 cm diameter cores of corals and was used as a template for symbiont genus-specific qPCR assays. The resulting CT values were used to calculate the relative abundance of each symbiont genus within each coral core over the course of the heat stress. The photochemical efficiency of each coral core was also measured periodically throughout heat stress tests using an imaging pulse amplitude modulated (I-PAM) fluorometer. The publication based on these data is Buzzoni, et al. (2023) (DOI: 10.1007/s00338-023-02428-x).


## Table of Contents

- Coverage
- Dataset Description
- Methods \& Sampling
- Data Processing Description
- BCO-DMO Processing Description
- Data Files
- Supplemental Files
- Related Publications
- Related Datasets
- Parameters
- Instruments
- Project Information
- Funding


## Coverage

Location: Emerald Reef off Key Biscayne, SE Florida (USA)
Spatial Extent: N:25.6788 E:-80.0974 S:25.6773 W:-80.1005
Temporal Extent: 2019-05-22-2020-03-05

## Methods \& Sampling

Coral cores of 2.5-centimeter (cm) diameter were collected from tagged colonies of three coral species in either April 2019 or October 2019 from Emerald Reef in Southeast Florida, USA. Cores were then maintained in 300-liter (L) indoor flow-through aquaria (in the Marine Technology and Life Science Seawater complex at the University of Miami's Rosenstiel School of Marine, Atmospheric, and Earth Science) and subjected to experimental heat stress. Before, during, and following the heat stress, 2 -millimeter ( mm ) tissue samples were collected from cores and genomic DNA was subsequently extracted from these samples as per Cunning \& Baker (2016). Quantitative PCR was performed using Taqman Environmental Master Mix and symbiont genusspecific and coral species-specific primers and probes targeting the actin gene (Cunning \& Baker, 2013; Cunning et al., 2015). In addition to collecting tissue samples, the photochemical efficiency of symbionts within coral cores was also assayed before, during, and after heat stress using an imaging-PAM fluorometer.

Full methodology described in Buzzoni, et al. (2023) (DOI: 10.1007/s00338-023-02428-x)

## Data Processing Description

All imaging-PAM and qPCR data were analysed in RStudio V4.2.1 (R Core Team, 2022). qPCR data were filtered to exclude samples lacking two technical replicates which amplified with Ct values under 40. Ct data were used to calculate the relative abundances of symbiont genera in tissue samples (relative to all Symbiodiniaceae present and relative to coral host cells) using the stepOneR package (Cunning, 2018). Ct data were also adjusted for differences between symbiont genera and coral species in target locus copy number, DNA extraction efficiency, and probe fluorescence intensity.

The Supplemental File "Ct_corrections_table.pdf" contains correction coefficients fed into StepOneR software along with qPCR Ct data. These correct for differences in host and symbiont DNA extraction efficiency and gene copy number, and for differences in fluorescence of target-specific probes.

## BCO-DMO Processing Description

- Imported original file "qPCR_IPAM.csv" into the BCO-DMO system.
- Flagged "NA" as a missing data identifier (missing data are empty/blank in the final CSV file).
- Converted latitude and longitude values to decimal degrees and rounded to 4 decimal places.
- Converted the date column to YYYY-MM-DD format.
- Replaced the abbreviated species name with the full genus + species name.
- Renamed fields/columns to comply with BCO-DMO naming conventions.
- Saved the final file as "918220_v1_qpcr_ipam.csv".
[ table of contents | back to top ]


## Data Files

## File

918220_v1_qpcr_ipam.csv(Comma Separated Values (.csv), 42.06 KB) MD5:533da1834438240361b966909f9c5ecc
Primary data file for dataset ID 918220, version 1
[ table of contents | back to top ]

## File

## Ct_corrections_table.pdf

(Portable Document Format (.pdf), 344.23 KB) MD5:7a2ac5c366f2f3ee6894890ea868a6c1

Supplemental File for dataset ID 918220. Contains correction coefficients fed into StepOneR software along with qPCR Ct data. These correct for differences in host and symbiont DNA extraction efficiency and gene copy number, and for differences in fluorescence of target-specific probes.

BCO-DMO converted the original file "Ct corrections table.docx" from .docx format to PDF.
[ table of contents | back to top ]

## Related Publications

Baker, A., \& Ross Cunning, not provided. (2015). Bulk gDNA extraction from coral samples v1. https://doi.org/10.17504/protocols.io.dyq7vv

## Methods

Buzzoni, D., Cunning, R., \& Baker, A. C. (2023). The role of background algal symbionts as drivers of shuffling to thermotolerant Symbiodiniaceae following bleaching in three Caribbean coral species. Coral Reefs, 42(6), 1285-1295. https://doi.org/10.1007/s00338-023-02428-x Results

Cunning, R. (2018). Steponer: R Package For Importing Qpcr Data From Stepone ${ }^{T M}$ Software. Zenodo. https://doi.org/10.5281/ZENODO. 1173322
Software
Cunning, R., \& Baker, A. C. (2013). Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nature Climate Change, 3(3), 259-262. doi:10.1038/nclimate1711
Methods
Cunning, R., Silverstein, R. N., \& Baker, A. C. (2015). Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proceedings of the Royal Society B: Biological Sciences, 282(1809), 20141725. https://doi.org/10.1098/rspb.2014.1725
Methods
RStudio Team (2022) RStudio: Integrated Development for R. Version 4.2.1,. RStudio, Inc., Boston, MA. http://www.rstudio.com/
Software
[ table of contents | back to top ]

## Related Datasets

## IsRelatedTo

Buzzoni, D., Cunning, R., Baker, A. (2024) In situ temperature data from August 2019 to May 2020 from one HOBO temperature logger deployed at Emerald Reef in Southeast Florida, USA. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-01-24 doi:10.26008/1912/bco-dmo.918364.1 [view at BCO-DMO]
[ table of contents | back to top ]

## Parameters

| Parameter | Description | Units |
| :---: | :---: | :---: |
| Species | Coral host species (M. cavernosa, O. faveolata or S. siderea) | unitless |
| Treatment | Experimental heat stress treatment that core was assigned to (Bleached or Control) | unitless |
| Core | Unique identifier of each 2.5 cm coral core ('coral species'-'colony tag'-'core tag') | unitless |
| Collection_Latitude | Latitude of parent collection from which cores were collected; positive values = North | decimal degrees |
| Collection_Longitude | Longitude of parent collection from which cores were collected; negative values $=$ West | decimal degrees |
| Timepoint | Context of the time tissue sample or IPAM measurement was taken with respect to the experimental heat stress (Pre-bleach, Post-bleach, Recovery, or Pre-outplant) | unitless |
| Sym_Host | In each tissue sample, the ratio of symbiont (the sum of Breviolum, Cladocopium and Durusdinium) cells to host coral cells, calculated using the StepOneR package (see methods for details) from technical replicates of qPCR target-specific Ct values (to 9 decimal places) | unitless |
| Y2 | Maximum quantum yield of symbionts' PSII of a coral core, as detected by IPAM fluorometer (to 3 decimal places) | unitless |
| PropD | In each tissue sample, the ratio of Durusdinium to total symbiont (the sum of Breviolum, Cladocopium and Durusdinium) cells, calculated using the StepOneR package (see methods for details) from technical replicates of qPCR target-specific Ct values (to 9 decimal places) | unitless |
| Batch | Denotes whether core was collected and heat stressed in either April 2019 (1) or October 2019 (2) | unitless |
| Date | Date tissue sample/ IPAM measurement was taken in the format YYYY-MM-DD (Eastern Standard Time) | unitless |
| Colony | Tag number of field colony from which cores were extracted ( $2,3,4,13,16$, $18,19,21,22,23,26,27,28,34,35,36,39,41,48,62,64,65,66,67,68$, $71,72,81,87,100)$ | unitless |

[ table of contents | back to top ]

## Instruments

| Dataset- <br> specific <br> Instrument <br> Name | submersible drill |
| :--- | :--- |
| Generic <br> Instrument <br> Name | Drill |
| Dataset- <br> specific <br> Description | Coral cores were extracted from reef colonies using a submersible drill (Nemo Power Tools Ltd.) <br> fitted with a diamond core drill bit (Montana Brand Tools). |
| Generic <br> Instrument <br> Description | A drill is a tool used for making round holes or driving fasteners. There are many types of drills: <br> some are powered manually, and others use electricity (electric drill) or compressed air as the <br> motive power. Drills with a percussive action (hammer drills) are mostly used in hard materials <br> such as masonry (brick, concrete, and stone) or rock. Some types of hand-held drills are also <br> used to drive screws and other fasteners. |


| Dataset- <br> specific <br> Instrument <br> Name | imaging pulse amplitude modulated (PAM) fluorometer |
| :--- | :--- |
| Generic <br> Instrument <br> Name | Fluorometer |
| Dataset- <br> specific <br> Description | Photochemical efficiency was calculated using measurements from an imaging pulse amplitude <br> modulated fluorometer (MAXI Version IMAGING-PAM M-Series, Walz, Effeltrich, Germany). |
| Generic <br> Instrument <br> Description | A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its <br> intensity and wavelength distribution of emission spectrum after excitation by a certain <br> spectrum of light. The instrument is designed to measure the amount of stimulated <br> electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water <br> sample or in situ. |


| Dataset-specific <br> Instrument Name | QuantStudio3 real-time PCR system |
| :--- | :--- |
| Generic Instrument <br> Name | qPCR Thermal Cycler |
| Dataset-specific <br> Description | qPCR was performed using a QuantStudio3 real-time PCR system (Applied <br> Biosystems). |
| Generic Instrument <br> Description | An instrument for quantitative polymerase chain reaction (qPCR), also known as <br> real-time polymerase chain reaction (Real-Time PCR). |

[ table of contents | back to top ]

## Project Information

## Collaborative Research: Assessing the changing symbiotic milieu on Caribbean coral reefs under climate change: magnitude, tradeoffs, interventions, and implications (Symbiont Shifts on Reefs)

Coverage: Coral reefs of the Caribbean and Western Atlantic

## NSF Award Abstract:

Climate change represents an existential threat to coral reef ecosystems worldwide, with coral bleaching driven by continued ocean warming presenting the most pressing challenge to the persistence of these ecosystems over the next few decades. Given the severity and urgency of this threat it is critical to investigate mechanisms by which some corals might survive warming, assess the degree to which this is happening on reefs, and apply these discoveries to inform conservation interventions that might improve survival trajectories wherever possible. This project aims to fulfill these objectives by testing whether reef corals in the Caribbean are undergoing shifts in their algal symbionts in favor of more heat-tolerant types, what the consequences of these shifts might be for coral reef ecosystems, and the way in which we might use this information to help conserve them. Scientific objectives will be leveraged to improve the effectiveness of reef restoration efforts in the Caribbean by applying findings to ongoing intervention trials which aim to seed outplanted corals (both adult fragments raised in nurseries, and sexually derived coral recruits) with heat tolerant algae that are climate-resistant. It also takes advantage of emerging opportunities at two major public aquariums to highlight the plight of coral reefs to engaged public audiences primed to receive this message and learn about the role of science in both understanding and mitigating the problem. Finally, numerous high school, undergraduate, and graduate students will receive mentorship during this project, helping to train the next generation of marine scientists.

This project tests whether continued climate warming is causing heat-tolerant algal symbionts (such as Durusdinium trenchii) to become increasingly common on coral reefs in the Caribbean. Understanding the changing symbiotic "milieu" in the region, the processes underlying the spread of $D$. trenchii, and the consequences of this spread, are very timely questions that have the potential to help us understand future
reef states. This project will: (1) Manipulate coral symbioses in the laboratory, including a number of Caribbean coral species never before attempted, to assess in a standardized way their relative ability to acquire heattolerant symbionts; (2) Outplant corals with manipulated symbiont communities to reefs to assess real-world ecophysiological tradeoffs to heat tolerance, such as reduced growth rate; (3) Introduce heat-tolerant symbionts to coral colonies in the field using tissue implants in order to understand environmental controls on the persistence or loss of introduced symbionts; (4) Evaluate transgenerational feedbacks in the symbiotic milieu by investigating the roles of temperature and $D$. trenchii availability on the acquisition and establishment of these symbionts in newly settled coral larvae; and (5) Quantify changes in the incidence and relative abundance of heat-tolerant symbionts in the Caribbean over the last $\sim 20$ years using unique archived samples dating back to 1995-2002 from Florida, Bahamas, Belize, and Bermuda.

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.
[ table of contents | back to top ]

## Funding

| Funding Source | Award |
| :--- | :--- |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1851392 |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1851305 |

[ table of contents | back to top ]

