# qPCR data for Orbicella faveolata recruits throughout a symbiont acquisition laboratory experiment conducted in 2018 and 2019

Website: https://www.bco-dmo.org/dataset/920860

**Data Type**: experimental

Version: 1

Version Date: 2024-03-04

#### **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

qPCR data for Orbicella faveolata recruits throughout symbiont acquisition laboratory experiment. Includes proportion of different Symbiodinaceae genera hosted, and symbiont-to-host cell ratios (symbiont density). These data correspond to research presented in Williamson et al. (2021), published in Coral Reefs and funded in part by the NSF project "Symbiont Shifts on Reefs". They were used to test if Orbicella faveolata recruits could establish symbiosis with D. trenchii supplied by nearby "donor" colonies and examined the resulting ecological trade-offs to evaluate early Symbiodiniaceae manipulation as a scalable tool for reef restoration. We exposed aposymbiotic recruits to 29 °C or 31 °C and to fragments of Montastraea cavernosa (containing Cladocopium ITS2 type C3) or Siderastrea siderea (containing D. trenchii). Next, a subset of recruits were exposed to a 60-day heat stress. These data include survivorship and symbiont acquisition rates, symbiont identity and density data (derived using qPCR), polyp area measurements, and scoring of bleaching and survivorship during a heat stress experiment. Overall, proportion of D. trenchii hosted was negatively correlated with polyp size and symbiont density, indicating a trade-off between growth (of both host and symbiont) and heat tolerance. These findings suggest that, while donor colonies may be effective sources for seeding coral recruits with thermotolerant symbionts, practitioners will need to balance the likely benefits and costs of these approaches when designing restoration strategies.

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#### Coverage

**Location**: Parents of recruits used in this study were located on Horseshoe Reef in Key Largo, FL at 5 - 8 meters depth (25.1388°N, 80.2950°W).

**Spatial Extent: Lat:**25.1388 **Lon:**-80.295

**Temporal Extent**: 2018-08-01 - 2019-05-01

#### Methods & Sampling

This section includes methodology for several related treatments and experiments conducted in the course of research presented in Williamson et al. (2021). Each BCO-DMO page provides access to one of these datasets, see the "Related Datasets" section for access to all related datasets.

Parents of recruits used in this study were located on Horseshoe Reef in Key Largo, FL at 5 - 8 meters depth.

**Symbiont uptake experiment** (see related dataset "Recruit survivorship and symbiont acquisition" <a href="https://www.bco-dmo.org/dataset/920853">https://www.bco-dmo.org/dataset/920853</a>)

Throughout symbiont uptake period, a dissecting microscope was used to count the number of: (1) surviving recruits and (2) recruits visibly infected with symbionts. When infection was first observed in all aquaria (day 21) and again after 60 days in their treatments, three to five recruits from each aquarium were sacrificed using a razor blade. To standardize sampling, only solitary polyps not clumped with others were sacrificed. Sacrificed recruits were placed in individual 1.5-mL microcentrifuge tubes with 200  $\mu$ L of 1% SDS + DNAB and incubated at 65 °C for one hour. Genomic DNA was extracted from SDS archives following modified organic extraction methods (Rowan and Powers 1991; Baker and Cunning 2016).

**Growth and symbiont density** (see related dataset "Recruit area measurements" <a href="https://www.bco-dmo.org/dataset/920846">https://www.bco-dmo.org/dataset/920846</a>)

Five months (150 days) after settlement, a random sample of recruits from each of the four original treatments was photographed under a dissecting microscope with QCapture Suite Plus. Only solitary polyps were photographed to maximize accuracy of area measurements. ImageJ was used to calculate recruit skeletal area in mm2. A subset of the photographed recruits was then sacrificed to measure symbiont identity and density at the time of growth measurements.

**Heat stress experiment** (see related dataset "Heat stress experiment data" <a href="https://www.bco-dmo.org/dataset/920837">https://www.bco-dmo.org/dataset/920837</a>)

A subset of plugs with each symbiont type were placed into new aquaria, where temperature was increased from 22 to 28 °C over six days, and then to 32 °C over 48 h. At this point, all recruits (n = 66) were infected with symbionts. About half the recruits (n = 32) were pre-exposed to mild heat stress (reared at 31 °C during Experiment 1), while the other half (n = 34) were naïve to heat stress (reared at 29 °C). Temperature was maintained at 32 °C for ten days, then raised to 33 °C for ten days, then raised to 34 °C for 40 days. Every two to five days, recruits were scored as "healthy," "pale," "bleached," or "dead" using a fluorescence microscope. After 60 days, all remaining living recruits were sacrificed for symbiont community analysis.

#### **Recruit PCR (qPCR)** (data included in this dataset)

Quantitative PCR (qPCR) assays were used to identify Symbiodiniaceae to genus level and quantify symbiont-to-host (S:H) cell ratios for each recruit sampled. Since O. faveolata commonly hosts members of Symbiodinium, Breviolum, Cladocopium, and Durusdinium (Kemp et al. 2015), assays targeting specific actin loci for each genus were performed using a QuantStudio 3 Real-Time PCR Instrument (Applied Biosystems, USA). Assays for O. faveolata, Symbiodinium, and Breviolum followed reactions described in Cunning and Baker (2013), whereas Cladocopium and Durusdinium assays were multiplexed as described in Cunning et al. (2015).

Instruments: For qPCR data, assays targeting specific actin loci for each genus were performed using a QuantStudio 3 Real-Time PCR Instrument (Applied Biosystems, USA).

#### **Data Processing Description**

Quantitative PCR (qPCR) assays: The StepOneR software package in R was used to quality-filter assay results, calculate relative abundance of each symbiont, and compute S:H cell ratios.

- \* Submitted file "Ofav.gPCR.csv" was imported into the BCO-DMO data system for this dataset.
- \*\* Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.
- \* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]
- \* Date converted to ISO 8601 format
- \* Taxon ids for names used in metadata were matched on 2024-03-11 using the World Register of Marine Species (WoRMS) taxa match tool. Supplemental file taxon\_ids.csv added.

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#### **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

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

Kemp, D. W., Thornhill, D. J., Rotjan, R. D., Iglesias-Prieto, R., Fitt, W. K., & Schmidt, G. W. (2015). Spatially distinct and regionally endemic Symbiodinium assemblages in the threatened Caribbean reef-building coral Orbicella faveolata. Coral Reefs, 34(2), 535–547. https://doi.org/10.1007/s00338-015-1277-z Methods

Rowan, R., & Powers, D. (1991). Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Marine Ecology Progress Series, 71, 65–73. https://doi.org/10.3354/meps071065 <a href="https://doi.org/10.3354/MEPS071065">https://doi.org/10.3354/MEPS071065</a> Methods

Williamson, O. M., Allen, C. E., Williams, D. E., Johnson, M. W., Miller, M. W., & Baker, A. C. (2021). Neighboring colonies influence uptake of thermotolerant endosymbionts in threatened Caribbean coral recruits. Coral Reefs, 40(3), 867–879. https://doi.org/10.1007/s00338-021-02090-1

Results

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#### **Related Datasets**

#### IsRelatedTo

Williamson, O., Baker, A. (2024) **Area of Orbicella faveolata recruits hosting different proportions of various symbiont genera from a symbiont acquisition laboratory experiment conducted in 2018 and 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-04 http://lod.bco-dmo.org/id/dataset/920846 [view at BCO-DMO] Relationship Description: Datasets collected as part of symbiont acquisition and heat stress experiments reported in results publication Williamson et al. (2021).

Williamson, O., Baker, A. (2024) Proportions of healthy, pale, bleached, and dead Orbicella faveolata recruits over time during a heat stress laboratory experiment conducted in 2018 and 2019, corresponding to proportions of different symbiont genera hosted. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-04 http://lod.bco-dmo.org/id/dataset/920837 [view at BCO-DMO]

Relationship Description: Datasets collected as part of symbiont acquisition and heat stress experiments reported in results publication Williamson et al. (2021).

Williamson, O., Baker, A. (2024) **Survivorship and proportion of recruits infected with Symbiodiniaceae over time during a symbiont acquisition laboratory experiment conducted in 2018 and 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-04 http://lod.bco-dmo.org/id/dataset/920853 [view at BCO-DMO] Relationship Description: Datasets collected as part of symbiont acquisition and heat stress experiments reported in results publication Williamson et al. (2021).

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## **Parameters**

Parameter	Description	Units
Sample	Sample identification that indicates tank, then unique recruit identifier number within the tank, then time point when sample was taken (i.e. $7 = \text{time point } \#7$ )	
tank	Tank identifier	unitless
temp	Temperature at which recruits had been previously reared - same as temperature treatment during symbiont uptake in "Experiment 1" (first $\sim$ 60 days of life, either elevated [31C] or ambient [29C])	
adult	enus of symbiont that the O.faveolata adult donor colony in this tank hosted ither Cladocopium or Durusdinium)	
days	days after settlement	days
Coral_CT_mean	mean Ct value (threshold cycle) for O. faveolata target during qPCR	unitless
A_CT_mean	mean Ct value (threshold cycle) for Symbiodinium (formerly clade A) symbiont target during qPCR	unitless
B_CT_mean	mean Ct value (threshold cycle) for Breviolium (formerly clade B) symbiont target during qPCR	unitless
C_CT_mean	mean Ct value (threshold cycle) for Cladocopium (formerly clade C) symbiont target during qPCR	unitless
D_CT_mean	mean Ct value (threshold cycle) for Durusdinium (formerly clade D) symbiont target during qPCR	unitless
A_Coral	ratio of Symbiodinium cells to O. faveolata cells in sample	unitless
B_Coral	ratio of Breviolum cells to O. faveolata cells in sample	unitless
C_D	ratio of Cladocopium cells to Durusdinium cells in sample	unitless
C_Coral	ratio of Cladocopium cells to O. faveolata cells in sample	unitless
D_Coral	ratio of Durusdinium cells to O. faveolata cells in sample	unitless
total_sh	ratio of all symbiont cells to O. faveolata cells in sample	unitless
prop_D	proportion of symbionts in coral sample that belong to the genus Durusdinium	unitless
prop_B	proportion of symbionts in coral sample that belong to the genus Breviolum	unitless
prop_C	proportion of symbionts in coral sample that belong to the genus Cladocopium	unitless
prop_A	proportion of symbionts in coral sample that belong to the genus Symbiodinium	unitless
prop_nonD	proportion of symbionts in coral sample that do not belong to the genus Durusdinium	unitless
logsh	log of the ratio of all symbiont cells to O. faveolata cells in sample	unitless
species	coral species (Ofav = Orbicella faveolata)	unitless
dominant	the most abundant symbiont genus or genera in the sample (anything that makes up greater than 10% of all the symbionts). If multiple are listed, they are codominant (i.e. "A/D"). A = Symbiodinium, B = Breviolum, C = Cladocopium, D = Durusdinium	

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# Instruments

Dataset-specific Instrument Name	QuantStudio 3 Real-Time PCR Instrument (Applied Biosystems, USA)
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	For qPCR data, assays targeting specific actin loci for each genus were performed using a QuantStudio 3 Real-Time PCR Instrument (Applied Biosystems, USA).
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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# **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 heat-tolerant 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 ~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.

# Funding

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

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