# OTU molecular abundances for coral Symbiodinium, Belize Mesoamerican Barrier Reef System (MBRS), 2014-2015

**Website**: https://www.bco-dmo.org/dataset/734674
**Data Type**: Other Field Results
**Version**: 1
**Version Date**: 2018-04-16

**Project**
» Investigating the influence of thermal history on coral growth response to recent and predicted end-of-century ocean warming across a cascade of ecological scales (Thermal History and Coral Growth)

| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
This dataset contains relative abundance (counts?) of operational taxonomic units (OTUs) from Sumbiodinium samples collected from three coral species (S. siderea, S. radians, and P. strigosa) at nine sites across four latitudes along the Belize MBRS in 2014 and 2015. These sites were previously characterized into three thermally distinct regimes (lowTP, modTP, highTP) and exhibited variations in coral species diversity and richness.

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## Coverage

**Spatial Extent**: **N**:17.64363 **E**:-88.12 **S**:16.13013 **W**:-88.62943
**Temporal Extent**: 2014-11 - 2015-10

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## Dataset Description

This dataset contains relative abundance (counts?) of operational taxonomic units (OTUs) from Sumbiodinium samples collected from three coral species (S. siderea, S. radians, and P. strigosa) at nine sites across four latitudes along the Belize MBRS in 2014 and 2015. These sites were previously characterized into three thermally distinct regimes (lowTP, modTP, highTP) and exhibited variations in coral species diversity and richness.

### Acquisition Description

From Baumann et al (2017):

DNA Extraction

Coral holobiont (coral, algae, and microbiome) DNA was isolated from each sample following a modified phenol-chloroform [86,87,88] method described in detail by Davies et al. [87]. Briefly, DNA was isolated by immersing the tissue in digest buffer (100 mM NaCL, 10 mM Tris-Cl pH 8.0, 25 mM EDTA pH 9.0, 0.5% SDS, 0.1 mg ml-1 Proteinase K, and 1 µg ml-1 RNaseA) for 1 h at 42 °C followed by a standard phenol-chloroform extraction. Extracted DNA was confirmed on an agarose gel and quantified using a Nanodrop 2000 Spectrophotometer (Thermo Scientific).

PCR Amplification and Metabarcoding

The ITS-2 region (350 bp) was targeted and amplified in each sample using custom primers that incorporated Symbiodinium specific ITS-2-dino-forward and its2rev2-reverse regions [65, 73, 89]. Each primer was constructed with a universal linker, which allowed for the downstream incorporation of Illumina specific adapters and barcodes during the second PCR as well as four degenerative bases whose function was to increase the complexity of library composition. The forward primer was 5'-GTCTCGTCGGCTCGG + AGATGTGTATAAGAGACAG+ NNNN + CCTCCGCTTACTTATATGCTT-3', where the underlined bases are the 5'-universal linker,

italicized bases indicate spacer sequences, Ns denote degenerative bases, and the bold bases are the ITS-2-dino. The reverse primer was 5'-TCGTCGGCAGCGTCA + AGATGTGTATAAGAGACAG + NNNN + GTGAATTGCAGAACTCGTG-3'.

Each 20 µL PCR reaction contained 5-100 ng DNA template, 12.4 µL Milli-Q H2O, 0.2 µM dNTPs, 1 µM forward and 1 µM reverse primers, 1× Extaq buffer, and 0.5 U (units) Extaqpolymerase (Takara Biotechnology). PCR cycles were run for all samples using the following PCR profile: 95 °C for 5 min, 95 °C for 40 s, 59 °C for 2 min, 72 °C for 1 min per cycle and a final elongation step of 72 °C for 7 min. The optimal number of PCR cycles for each sample was determined from visualization of a faint band on a 2% agarose gel (usually between 22 and 28 cycles) as per Quigley et al. [65]. PCR products were cleaned using GeneJET PCR purification kits (Fermentas Life Sciences), and then a second PCR reaction was performed to incorporate custom barcode-primer sequences [65] modified for Illumina Miseq as in Klepac et al. [90]. Custom barcode primer sequences included 5'-Illumina adaptor + 6 bp barcode sequence + one of two universal linkers-3' (e.g., 5'-CAAGCAGAAGACGGCATACGAGAT + GTATAG + GTCTCGTGGGCTCGG-3', or 5'-AATGATACGGCGACCACCGAGATCTACAC + AGTCAA + TCGTCGGCAGCGTC-3'). Following barcoding, PCR samples were visualized on a 2% agarose gel and pooled based on band intensity (to ensure equal contributions of each sample in the pool). The resulting pool was run on a 1% SYBR Green (Invitrogen) stained gel for 60 min at 90 V and 120 mA. The target band was excised, soaked in 30 µL of Milli-Q water overnight at 4 °C, and the supernatant was submitted for sequencing to the University of North Carolina at Chapel Hill High Throughput Sequencing Facility across two lanes of Illumina MiSeq (one 2 × 250, one 2 × 300). The two lanes produced similar mapping efficiencies (73 and 73%, respectively; Table S3).

Bioinformatic Pipeline

The bioinformatic pipeline used here builds upon previous work by Quigley et al. [65] and Green et al. [73]. Raw sequences were renamed to retain sample information, and then all forward (R1) and reverse (R2) sequences were concatenated into two files, which were processed using CD-HIT-OTU [91]. CD-HIT-OTU clusters concatenated reads into identical groups at 100% similarity for identification of operational taxonomic units (OTUs). Each sample was then mapped back to the resulting reference OTUs, and an abundance count for each sample across all OTUs was produced. A BLASTn search of each reference OTU was then run against the GenBank (NCBI) nucleotide reference collection using the representative sequence from each OTU to identify which Symbiodinium lineage was represented by each OTU (Table S2).

The phylogeny of representative sequences of each distinct Symbiodinium OTU was constructed using the PhyML tool [92, 93] within Geneious version 10.0.5 (http://geneious.com) [94]. PhyML was run using the GTR+I model (chosen based on delta AIC values produced from

jModelTest [92, 95]) to determine the maximum likelihood tree. The TreeDyn tool in Phylogeny.fr was used to view the tree (Fig. 2) [96, 97, 98]. The reference sequences included in the phylogeny were accessed from GenBank (Table S6).

**These data are reported in:**

Baumann, J.H., Davies, S.W., Aichelman, H.E. and Castillo, K. D. (2017) Coral Symbiodinium Community Composition Across the Belize Mesoamerican Barrier Reef System is Influenced by Host Species and Thermal Variability. Microb Ecol. https://doi.org/10.1007/s00248-017-1096-6.

**Methodology References:**

65. Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz MV, Bay LK (2014) Deep-sequencing method for quantifying background abundances of Symbiodinium types: exploring the rare Symbiodinium biosphere in reef-building corals. PLoS One 9:e94297

73. Green EA, Davies SW, Matz MV, Medina M (2014) Quantifying cryptic Symbiodinium diversity within Orbicella faveolata and Orbicella franksi at the Flower Garden Banks, Gulf of Mexico. PeerJ 2:e386

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87. Davies SW, Rahman M, Meyer E, Green EA, Buschiazzo E, Medina M, Matz MV (2013) Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, Montastraea faveolata. Mar Biodivers 43:167-172

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95. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772-772

96. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard J-F, Guindon S, Lefort V, Lescot M (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36:W465-W469

97. Dereeper A, Audic S, Claverie J-M, Blanc G (2010) BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol. Biol. 10:8

98. Chevenet F, Brun C, Bañuls A-L, Jacq B, Christen R (2006) TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC bioinformatics 7:439

**Processing Description**

**BCO-DMO Processing Notes:**
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- added species names column

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## Related Publications

Baumann, J. H., Davies, S. W., Aichelman, H. E., & Castillo, K. D. (2017). Coral Symbiodinium Community Composition Across the Belize Mesoamerican Barrier Reef System is Influenced by Host Species and Thermal Variability. Microbial Ecology, 75(4), 903–915. doi:10.1007/s00248-017-1096-6

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Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nature Protocols, 1(2), 581–585. doi:10.1038/nprot.2006.83

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9(8), 772–772. doi:10.1038/nmeth.2109

Davies, S. W., Rahman, M., Meyer, E., Green, E. A., Buschiazzo, E., Medina, M., & Matz, M. V. (2012). Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, Montastraea faveolata. Marine Biodiversity, 43(2), 167–172. doi:10.1007/s12526-012-0133-4

Dereeper, A., Audic, S., Claverie, J.-M., & Blanc, G. (2010). BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evolutionary Biology, 10(1), 8. doi:10.1186/1471-2148-10-8

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Green, E. A., Davies, S. W., Matz, M. V., & Medina, M. (2014). Quantifying crypticSymbiodiniumdiversity withinOrbicella faveolataandOrbicella franksiat the Flower Garden Banks, Gulf of Mexico. PeerJ, 2, e386. doi:10.7717/peerj.386

Guindon, S., & Gascuel, O. (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. Systematic Biology, 52(5), 696–704. doi:10.1080/10635150390235520

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3), 307–321. doi:10.1093/sysbio/syq010

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649. doi:10.1093/bioinformatics/bts199

Klepac, C., Beal, J., Kenkel, C., Sproles, A., Polinski, J., Williams, M., … Voss, J. (2015). Seasonal stability of coral-Symbiodinium associations in the subtropical coral habitat of St. Lucie Reef, Florida. Marine Ecology Progress Series, 532, 137–151. doi:10.3354/meps11369

Li, W., Fu, L., Niu, B., Wu, S., & Wooley, J. (2012). Ultrafast clustering algorithms for metagenomic sequence analysis. Briefings in Bioinformatics, 13(6), 656–668. doi:10.1093/bib/bbs035

Quigley, K. M., Davies, S. W., Kenkel, C. D., Willis, B. L., Matz, M. V., & Bay, L. K. (2014). Deep-Sequencing Method for Quantifying Background Abundances of Symbiodinium Types:

Exploring the Rare Symbiodinium Biosphere in Reef-Building Corals. PLoS ONE, 9(4), e94297. doi:10.1371/journal.pone.0094297

R., A., W., P., M., T., & K., K. (2002). The 1998 bleaching event and its aftermath on a coral reef in Belize. Marine Biology, 141(3), 435–447. doi:10.1007/s00227-002-0842-5

Stat, M., Loh, W. K. W., LaJeunesse, T. C., Hoegh-Guldberg, O., & Carter, D. A. (2009). Stability of coral–endosymbiont associations during and after a thermal stress event in the southern Great Barrier Reef. Coral Reefs, 28(3), 709–713. doi:10.1007/s00338-009-0509-5

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| species | taxonomic species name | unitless |
| species_code | species code | unitless |
| Sample | coral sample identifier | unitless |
| site | site identifier: nearby city and the thermally distinct regime code: 1=low; 2=moderate; 3=high | unitless |
| thermal_type | thermal regime code: 1=lowTP; 2=modTP; 3=highTP. These 3 categories are based on low; moderate; and high temperature parameters (see Baumann et al 2016 for details) | unitless |
| lat_location | site location and code number | unitless |
| illumina_run | Illumina run number | unitless |
| otu_diversity | total number of operational taxonomic units (OTU) in sample | OTU's |
| C1_I | relative abundance of OTU C1.I | unitless |
| B1_I | relative abundance of OTU B1.I | unitless |
| C1_II | relative abundance of OTU C1.II | unitless |
| C1_III | relative abundance of OTU C1.III | unitless |
| D1a | relative abundance of OTU D1a | unitless |
| B1_II | relative abundance of OTU B1.II | unitless |
| G3 | relative abundance of OTU G3 | unitless |
| A4a | relative abundance of OTU A4a | unitless |
| B_BG | relative abundance of OTU B.BG | unitless |
| C3 | relative abundance of OTU C3 | unitless |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Illumina Mi-seq |
| **Generic Instrument Name** | Automated DNA Sequencer |
| **Generic Instrument Description** | General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Nanodrop 2000 Spectrophotometer (Thermo Scientific) |
| **Generic Instrument Name** | Spectrophotometer |
| **Dataset-specific Description** | Used to confirm presence of extracted DNA on agarose gel. |
| **Generic Instrument Description** | An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | PCR Thermal Cycler |
| **Generic Instrument Description** | General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html) |

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## Project Information

**Investigating the influence of thermal history on coral growth response to recent and predicted end-of-century ocean warming across a cascade of ecological scales (Thermal History and Coral Growth)**

**Website**: http://www.unc.edu/~kdcastil/research.html

**Coverage**: Western Caribbean

Description from NSF award abstract: Rising global ocean surface temperatures have reduced coral growth rates, thereby negatively impacting the health of coral reef ecosystems worldwide. Recent studies on tropical reef building corals reveal that corals' growth in response to ocean warming may be influenced by their previous seawater temperature exposure - their thermal history. Although these recent findings highlight significant variability in coral growth in response to climate change, uncertainty remains as to the spatial scale at which corals' thermal history influences how they have responded to ocean warming and how they will likely respond to predicted future increases in ocean temperature. This study investigates the influence of thermal history on coral growth in response to recent and predicted seawater temperatures increases across four ecologically relevant spatial scales ranging from reef ecosystems, to reef communities, to reef populations, to an individual coral colony. By

understanding how corals have responded in the past across a range of ecological scales, the Principal Investigator will be able to improve the ability to predict their susceptibility and resilience, which could then be applied to coral reef conservation in the face of climate change. This research project will broaden the participation of undergraduates from underrepresented groups and educate public radio listeners using minority voices and narratives. The scientist will leverage current and new partnerships to recruit and train minority undergraduates, thus allowing them to engage high school students near field sites in Florida, Belize, and Panama. Through peer advising, undergraduates will document this research on a digital news site for dissemination to the public. The voice of the undergraduates and scientist will ground the production of a public radio feature exploring the topic of acclimatization and resilience - a capacity for stress tolerance within coral reef ecosystems. This project will provide a postdoctoral researcher and several graduate students with opportunities for field and laboratory research training, teaching and mentoring, and professional development. The results will allow policy makers from Florida, the Mesoamerican Barrier Reef System countries, and several Central American countries to benefit from Caribbean-scale inferences that incorporate corals' physiological abilities, thereby improving coral reef management for the region. Coral reefs are at significant risk due to a variety of local and global scale anthropogenic stressors. Although various stressors contribute to the observed decline in coral reef health, recent studies highlight rising seawater temperatures due to increasing atmospheric carbon dioxide concentration as one of the most significant stressors influencing coral growth rates. However, there is increasing recognition of problems of scale since a coral's growth response to an environmental stressor may be conditional on the scale of description. This research will investigate the following research questions: (1) How has seawater temperature on reef ecosystems (Florida Keys Reef Tract, USA; Belize Barrier Reef System, Belize; and Bocas Del Toro Reef Complex, Panama), reef communities (inshore and offshore reefs), reef populations (individual reefs), and near reef colonies (individual colonies), varied in the past? (2) How has seawater temperature influenced rates of coral growth and how does the seawater temperature-coral growth relationship vary across these four ecological spatial scales? (3) Does the seawater temperature-coral growth relationship forecast rates of coral growth under predicted end-of-century ocean warming at the four ecological spatial scales? Long term sea surface temperature records and small-scale high-resolution in situ seawater temperature measurements will be compared with growth chronologies for the reef building corals Siderastrea siderea and Orbicella faveolata, two keystone species ubiquitously distributed throughout the Caribbean Sea. Nutrients and irradiance will be quantified via satellite-derived observations, in situ measurements, and established colorimetric protocols. Field and laboratory experiments will be combined to examine seawater temperature-coral growth relationships under recent and predicted end-of-century ocean warming at four ecologically relevant spatial scales. The findings of this study will help us bridge the temperature-coral growth response gap across ecologically relevant spatial scales and thus improve our understanding of how corals have responded to recent warming. This will lead to

more meaningful predictions about future coral growth response to climate change.

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## Funding


| Funding Source | Award |
| --- | --- |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1459522 |


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