Symbiodinium sequence abundance in St. John, US Virgin Islands from 1994-2010 (RUI-LTREB project)

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Data Type: Other Field Results
Version: 1
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Project
» RUI-LTREB Renewal: Three decades of coral reef community dynamics in St. John, USVI: 2014-2019 (RUI-LTREB)

Contributors	Affiliation	Role
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Table of Contents

Coverage

Spatial Extent: **Lat**:18.32 **Lon**:-64.723
Temporal Extent: 1994 - 2010

Dataset Description

Study site and ecology of Orbicella annularis:
The time-series analysis on the Tektite reef is based on three parallel 10-m transects along which 10 contiguous photoquadrats (1 x 1 m) are recorded annually (n = 30 photoquadrats y-1). Transects cross the flat upper surface of a single coral buttress and do not depart appreciably from 14-m depth across their length. Images were acquired on 35 mm film (Kodachrome 64) from 1987-2000, and digitally from 2001-2004 (3.34 megapixel), and 2005-2010 (6.1 megapixels). Images are archived (http://mcr.lternet.edu/vinp/overview/), and have been analyzed for percentage cover of the benthic community using CPCe software. In addition to photoquadrats, seawater temperature has been recorded in Great Lameshur Bay since 1989, initially at 9-m depth at Yawzi Point (~1 km away from the Tektite reef) from 1989 to 2011, and at the 14 m Tektite site since 2004. The temperature records used here include values from Yawzi Point from 1994-2003, and from Tektite from 2004-2009. Temperatures were recorded using a Ryan Industries Tempmentor (± 0.3°C [Ryan Industries, Redmond, WA]) from January 1992 to April 1997 and November 1997 to August 1999, an Optic Stowaway logger (± 0.2°C accuracy [Onset Industries, Bourne, MA]) at 9-m depth from May 1997 to October 1997, and from August 1999 to August 2001, and an Aquapro Logger (± 0.2°C accuracy [Onset Industries, Bourne, MA]) at 9-m depth from August 2001 to August 2010. Loggers recorded temperature every 15-30 mins and these data were collapsed by day, and described using the mean and interquartile ranges by year.

Colonies of Orbicella annularis were first sampled August 12th and 16th 1994 for DNA fingerprinting. In 1994, biopsies of tissue and skeleton were collected in two photoquadrats using a 13-mm diameter steel punch. To prevent cross-contamination, a sterile punch was used for each colony and each biopsy bagged individually underwater. Care was taken to sample biopsies from the horizontal upper surfaces of colonies, and the sampled holes were filled with non-toxic modeling clay. Photoquadrats were then photographed to allow

sampled colonies to be located again and to evaluate the effects of sampling on these colonies. Biopsies were transported to the lab on ice (~30 min), and crushed in 2 ml of ice-cold guanadinium hydrochloride (GHCl) buffer (8 M GHCl, 0.1M sodium acetate (pH 5.2), 5 mM dithiothreitol, 0.5% N-lauryl sarcosine. After grinding, the slurry was stored at 4°C and transported at room temperature to California State University, Northridge (CSUN). Some samples were processed, while others were stored at 4°C until 2010.

Between August 5th and 8th 2010 (i.e., virtually the same time of year as in 1994) the same colonies of Orbicella annularis biopsied in 1994 were sampled with the objectives of genotyping the coral host and their Symbiodinium. To ensure identical colonies were sampled, the annual photoquadrats were used to track over time the shape, color, and orientation of the colonies first sampled in 1994. Based on the capacity to relocate colonies for which archived DNA from 1994 was available, and the constraints of permits issued by the Virgin Island National Park, 12 colonies were tracked from 1994 to 2009. In 2010, laminated prints of the photoquadrats were used underwater to locate the same colonies for a second sampling, and to ensure that the sampling orientations were the same as employed 16 years earlier. Sampling in the same position on each colony was important to reduce the likelihood that variation in Symbiodinium genotypes was caused by sampling different microhabitats across the colony surface. Our analysis therefore assumes that differences in Symbiodinium genotypes between the two samplings reflected the passage of time and not differences in sampling position within each colony.

In 2010, the 12 colonies of O. annularis were sampled using 6-mm diameter punches, and the holes filled with non-toxic modeling clay. A sterile punch was used for each colony to prevent cross-contamination, and biopsies were bagged individually underwater, stored on ice, and returned to the lab for processing. Biopsies were stored in DNA extraction buffer (50% (w/v) guanidinium isothiocyanate, 50 mM Tris pH 7.6, 10 □M ethylenediaminetetraacetic acid (EDTA), 4.2% (w/v) sarkosyl, and 2.1 % (v/v) □-mercaptoethanol) and shipped at room temperature for processing at the University of Hawaii (Symbiodinium) or Florida State University (O. annularis host).

To evaluate growth, each of the 12 colonies was measured in the annual photoquadrats. Most colonies were found every year, although occasionally it was not possible to measure their size if they were obscured by the camera framer or gorgonians. Colonies were measured using ImageJ 1.42q software to outline coral tissue and calculate planar area (cm2). Each colony was tracked over 16 y and the trajectory of changing size used as a measure of success. Success of the 12 colonies was placed in a broader context by evaluating overall coral cover using the 30 photoquadrats sampled annually. Coral cover was measured using CPCe software and a grid of 200 randomly located points on each image.

DNA extraction:
Genomic DNAs (coral host and Symbiodinium combined) were extracted following Pochon et al. (2001). The 1994 samples were vortexed and 200 microliters of supernatant placed in tubes with 400 microliters of guanidinium buffer. These tubes, as well as the crushed coral biopsies from 2010, were incubated at 72 degrees C for 20 min, centrifuged at 16,000 g for 5 min, and 300 microliters of the supernatant mixed with an equal volume of isopropanol and incubated at -20 degrees C overnight. DNA was precipitated by centrifugation at 16,000 g for 15 min, and the DNA pellet washed in 70% ethanol, resuspended, and stored in Tris Buffer (0.1 M pH 8).

Symbiodinium analysis:
Two genes from different cellular compartments (nuclear and chloroplastic), and two analytical approaches (cloning and pyrosequencing) were used to genotype the Symbiodinium in the 24 coral biopsies (12 each from 1994 and 2010). These approaches added the support of multiple markers and methodologies to tests of our hypotheses. Moreover, pyrosequencing provided more resolution to the analysis of temporal variation in Symbiodinium assemblages and its effects on holobiont performance in comparison to studies employing fingerprinting gel-based analysis. Previous studies have focused on banding patterns generated by using gel electrophoresis to separate DNA from Symbiodinium based on denaturing gradients (i.e., DGGE fingerprints), and while such approaches have good resolution for identifying dominant patterns, they have limited capacity to more finely resolve genetic variation or to detect rare genotypes. While interpreting the biological significance of rare Symbiodinium genotypes remains controversial in light of the presence of intragenomic variation associated with ITS2, the increasing number of examples of Symbiodinium-host symbioses in which rare Symbiodinium genotypes have been found argue strongly for using techniques with the resolution to detect such forms. This argument was compelling to us in the decision to expand our analytical approach to tag pyrosequencing of nrITS2 amplicons, as was the success of this approach in advancing other fields requiring fine-grained genetic resolution of single-celled organisms (Sogin et al. 2006). An important limitation of this early-adoption of new techniques is we assume that the debate over the meaning of rare Symbiodinium genotypes will resolve in favor of their biological significance.

We PCR amplified the nuclear Internal Transcribed Spacer 2 (nrITS2) region and the chloroplastic 23S domain V

(cp23S) region of the ribosomal arrays using the nrITS2 primers 'its-dino' and 'its2rev2' and the cp23S primers '23S4F' and '23S7R' (after Stat et al. 2009, Pochon & Gates 2010). Each 50 microliters PCR reaction contained 1 microliters of DNA template, 5 microliters of 10x ImmoBuffer (Bioline, MA), 0.2 microliters IMMOLASE™ Hot-Start DNA Polymerase (Bioline, MA), 2 microliters of 50 mM MgCl2, 1 microliters of 10 mM total dNTPs (10 mM each), 1 microliters of each primer (10 pmol each), and 38.8 microliters of deionized sterile water. A touchdown PCR protocol was performed on a BioRad iCycler™ using the following conditions: 7 min at 95 degrees C, 20 cycles of 94 degrees C for 30 s, 62 degrees C for 30 s (decreased by 0.5 degrees C at each cycle), 72 degrees C for 1 min, and followed by 12 additional cycles with an annealing temperature set at 52□C, and a final extension of 72□C for 7 min. PCR products were purified using the QIAquick™ PCR Purification Kit (Qiagen), and ligated into the pGEM-T Easy vector™ (Promega). A minimum of 10 positive inserts per clone library were amplified using plasmid-specific (M13) primers, and sequenced in both directions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit and an ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems). Bi-directional sequences were inspected and assembled using Sequencher v4.7 (Gene Codes Corporation, Ann Arbor, MI, USA), aligned using BioEdit v7.0.5.3, and Symbiodinium were identified to clade and subclade level using the Basic Local Alignment SearchTool (BLAST) in nrITS2 and cp23S databases generated from sequences archived in GenBank. This database is Supplementary Material as a fasta file.

The patterns of dominant-cloned Symbiodinium sequence types (i.e., the most abundant sequences in each sample) obtained using nrITS2 and cp23S were similar, but there were a few differences between the abundances in the cloned sequence numbers. This suggested that too few clones had been sequenced to fully capture the diversity of rare Symbiodinium sequence types. To address this issue, all DNAs were re-analyzed using multiplexed tag pyrosequencing of the nrITS2 amplicons (described as pyrosequencing hereafter). This method, pioneered in the field of microbial ecology, is replacing cloning and DGGE as the technique of choice for addressing diversity (Huse et al. 2008), and has been successfully employed for characterizing cryptic Symbiodinium diversity within O. faveolata and O. franksi in the Gulf of Mexico. We used pyrosequencing to increase the mean number of Symbiodinium sequences acquired per coral sample from ~10 to >1300. For pyrosequencing, 24 nrITS2 Symbiodinium amplicon libraries were submitted to Research and Testing Laboratory, LLC (Texas, USA) for analysis. Pyrosequencing involved ligating sample-specific tags for each of the 24 samples during the ITS2 amplification (cycling conditions described above, amplified separately from clone libraries), followed by amplicon purification, emulsion PCR, and high-throughput sequencing on a Roche GS FLX pyrosequencing system.

As described above, we acknowledge that ITS2 does not provide the correct characteristics for use as an alpha diversity marker. Prior work has recognized the inability to link an individual biological entity to an individual sequences due to duplication in the ribosomal array resulting in the production of paralogs that preclude species assignment. ITS2 can however, be utilized in a comparative approach to determine patterns in the assemblage of sequences within a given sample. Our approach is primarily focused on identifying genetic variation over time and not to assign taxonomy to the sequences identified. Reconciling both ecological patterns and taxonomic identify for Symbiodinium must be a high priority if the full biological significance of Symbiodinium diversity is to appreciated for coral holobionts.

Bioinformatic analysis of pyrosequencing results

The 454 sequencing reads were first quality trimmed to a T threshold of 25, clustered using USEARCH (Edgar 2010), chimera-checked using UCHIIME, and de-noised based on a Quality score of 30 using a standard data analysis pipeline at the Research and Testing Laboratory, LLC. The full fasta file was demultiplexed and adapter-trimmed and subsequently filtered to remove any terminal regions with a PHRED quality score below 20. Sequences were filtered for length > 180 bp and maximum number of errors of 1 in forward primer, and trimmed of barcode, adapters, and forward and reverse primers with Integroomer (http://courge.ics.hawaii.edu/inte/groomer/). A Symbiodinium-specific bioinformatic pipeline (symTyper; M Belcaid unpublished method, https://github.com/bingo11/symTyper) was then employed to assign each Symbiodinium sequence an identity. Briefly, using HMMER v3 (http://hmmer.org/), the resulting sequences were compared to a database of Symbiodinium clade HMM profiles, which were generated from the ITS2 database. The sequencing reads were subsequently compared to each clade-specific profile and a read was assigned to clade based on two rules: 1) the alignment was significant (e-value ≤ 1e-20 and sequence alignment over 95% of the read) and 2) the sequences was unlikely to have originated in another clade (e-value for the first hit was at least 5 orders of magnitude smaller than that of the second hit). Sequences failing the first rule were classified as unknown, whereas the sequences failing the second rule were considered ambiguous.

Reads that were successfully assigned to a clade were subsequently compared using the Basic Local Alignment SearchTool (BLAST) in an nrITS2 database generated from sequences archived in GenBank and assigned the subtype of the reference with which they were most similar (97% similarity over 97% of their

length). Similar to the clade assignment stage, the non-ambiguity requirement was enforced by requiring that the first hit have a higher raw bit score than that of the second hit. The sequencing reads lacking pairwise similarity with database entries were classified as putatively new and were manually investigated for known systematic biases. Short sequences were dropped from the analysis, while ambiguous sequences aligning with similar quality to two or more subtypes were assigned to the lowest common ancestor node in the phylogenetic tree of its clade and reported to clade level only.

Each of the three steps described above: 1) clade assignment, 2) sequence subtyping, and 3) tree placement of ambiguous reads, were implemented in a python program, available for download from the following github repository: https://github.com/bingo11/symTyper.

Host genetics:
Six microsatellite loci developed by Severance et al. (2004) were used to genotype the Orbicella annularis host tissue. The PCR cocktail consisted of 2.4 microliters 5X PCR buffer (Promega), 1.2 microliters 1mM dNTPs, 0.15 microliters GoTaq, 1.0 microliters 10 M bovine serum albumin, 1.25-3.5 microliters 1.5mM MgCl2 (depending on primer), 0.5 microliters of fluorescently labeled forward primer, 0.5 microliters reverse primer, 2.0 microliters DNA (5 ng/microliters), and double-distilled water to bring to a total volume of 12 microliters. PCR amplification was run as follows: 95 degrees C for 3 min, then 30 cycles of 95 degrees C for 1 min, 50 degrees C (primers maMS11, maMS2-4) or 55 degrees C (primers maMS8, maMS12, maMS2-5, and maMS2-8), 72 degrees C for 2 min, then a final extension time of 30 min at 72degrees C. PCR product from three loci with different fluorescent labels was multiplexed (mulitplexI: maMS8, 2-4, 2-5 and multiplexII: maMS11, 12, 2-8) using HiDI Formamide (1:12) and 0.5 microliters analyzed with Applied Biosystems 3130xl Genetic Analyzer with Capillary Electrophoresis. Genemapper software (Applied Biosystems, version 4) was used to check for misidentified peaks and stutter bands. All alleles were binned into di- or tri-nucleotide sizes dependent on locus. Colonies with ambiguous genotypes were re-run to confirm binning. Loci were analyzed with Micro-checker software to calculate the observed and expected heterozygosity, the likelihood of large allele drop-out and null alleles. The probability of colonies having identical genotypes via sexual reproduction (as opposed to being clone-mates through asexual reproduction) was estimated using Gimlet.

Data 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

[table of contents | back to top]

Data Files

File
pyroseq_clade.csv (Comma Separated Values (.csv), 905 bytes) MD5:0e23f8405c8bb0c103c1dd80252beb74 Primary data file for dataset ID 750636

[table of contents | back to top]

Related Publications

Edmunds, P., Pochon, X., Levitan, D., Yost, D., Belcaid, M., Putnam, H., & Gates, R. (2014). Long-term changes in Symbiodinium communities in Orbicella annularis in St. John, US Virgin Islands. Marine Ecology Progress Series, 506, 129–144. doi:10.3354/meps10808
Results

[table of contents | back to top]

Parameters

Parameter	Description	Units
Sample_ID	The DNA collection identifier	unitless
Colony_no	The number of the colony in the quadrat	unitless
Photoquadrat	The physical location of the photographic quadrat	unitless
Date_sampled_yr	The year the sample was collected in YYYY format	unitless
Clade_A	The abundance of Clade A Symbiodinium	count
Clade_B	The abundance of Clade B Symbiodinium	count
Clade_C	The abundance of Clade C Symbiodinium	count
Clade_F	The abundance of Clade F Symbiodinium	count
Clade_G	The abundance of Clade G Symbiodinium	count
Clade_HIT_Sequence_Totals	The total number of sequences identified to a Symbiodinium clade	count

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	ABI 3100 Genetic Analyzer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems)
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.

[table of contents | back to top]

Project Information

RUI-LTREB Renewal: Three decades of coral reef community dynamics in St. John, USVI: 2014-2019 (RUI-LTREB)

Website: http://coralreefs.csun.edu/

Coverage: USVI

Describing how ecosystems like coral reefs are changing is at the forefront of efforts to evaluate the biological consequences of global climate change and ocean acidification. Coral reefs have become the poster child of these efforts. Amid concern that they could become ecologically extinct within a century, describing what has

been lost, what is left, and what is at risk, is of paramount importance. This project exploits an unrivalled legacy of information beginning in 1987 to evaluate the form in which reefs will persist, and the extent to which they will be able to resist further onslaughts of environmental challenges. This long-term project continues a 27-year study of Caribbean coral reefs. The diverse data collected will allow the investigators to determine the roles of local and global disturbances in reef degradation. The data will also reveal the structure and function of reefs in a future with more human disturbances, when corals may no longer dominate tropical reefs.

The broad societal impacts of this project include advancing understanding of an ecosystem that has long been held emblematic of the beauty, diversity, and delicacy of the biological world. Proposed research will expose new generations of undergraduate and graduate students to natural history and the quantitative assessment of the ways in which our planet is changing. This training will lead to a more profound understanding of contemporary ecology at the same time that it promotes excellence in STEM careers and supports technology infrastructure in the United States. Partnerships will be established between universities and high schools to bring university faculty and students in contact with k-12 educators and their students, allow teachers to carry out research in inspiring coral reef locations, and motivate children to pursue STEM careers. Open access to decades of legacy data will stimulate further research and teaching.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Environmental Biology (NSF DEB)	DEB-0841441
NSF Division of Environmental Biology (NSF DEB)	DEB-1350146

[table of contents | back to top]