**Symbiont community monitoring in Gulf of Chiriquí and Gulf of Panama 2018**

**Methods**

***Field sampling***

During August 2018, 131 colonies of *Pocillopora* spp. were sampled to establish coral’s Symbiodiniaceae community composition. All samples were collected from the tops of colonies by clipping ~1 cm from a branch tip using bone cutters. Samples were kept in sealed plastic bags inside in a shaded bucket with seawater, until processed 5 to 6 h after the first sample collection. The samples were preserved by incubating for 90 min at 65°C in 400-800 μL of a solution of 1% SDS and DNA buffer[(Rowan and Powers 1991)](https://paperpile.com/c/uhkxKa/nkogW).

### ***Laboratory analysis***

We used 100 μL aliquots from the SDS lysates to extract and purify genomic DNA following established procedures[(Baker and Cunning 2016)](https://paperpile.com/c/uhkxKa/yLnxY). Total genomic DNA was used as template in TaqMan-MGB (Life Technologies) qPCR assays to assess the composition (identity and abundance) of algal symbiont communities. The symbiont to host (S/H) cell ratio, a metric of symbiont abundance [(Mieog et al. 2009; Cunning and Baker 2013)](https://paperpile.com/c/uhkxKa/LYKDK+ik1BX), was estimated using qPCR assays that target the actin gene in *Pocillopora* and its symbionts in the genus *Cladocopium* and *Durusdinium*. Primers, probes and PCR conditions followed Cunning and Baker (2013). All reactions were run in duplicate on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA), with a ΔRn threshold = 0.01 to estimate the cycle threshold (CT) of each sample and target.

The StepOneR repository for R[(Cunning 2018)](https://paperpile.com/c/uhkxKa/Cqp9U) was used to calculate the genus-specific symbiont to host (S/H) cell ratios (*Cladocopium* to Host [C/H], and *Durusdinium* to Host [D/H]). This repository uses the cycle threshold (CT) values obtained from the qPCR reactions to calculate the ratios using the formula 2^(CT host - CT symbiont). The ratios are corrected using additional information about target ploidy, fluorophore intensity, and DNA extraction efficiency[(Mieog et al. 2009; Cunning and Baker 2013)](https://paperpile.com/c/uhkxKa/LYKDK+ik1BX). Finally, we calculated the total S/H symbiont to host cell ratio as the sum of all algal genera ratios present in the sample (total S/H = C/H + D/H) and the proportion of *Durusdinium* in the total community [(D/H)/(S/H)]. Prior to data analysis, we applied quality filters to discard data from plates with amplification in no-template (negative) controls, targets in which one of the two technical replicates did not amplify, and samples in which the CT standard deviation between technical replicates was higher than 1.5.

**References**

[Baker, Andrew, and Ross Cunning. 2016. “Bulk gDNA Extraction from Coral Samples v1 (protocols.io.dyq7vv).” *Protocols.io*. https://doi.org/](http://paperpile.com/b/uhkxKa/yLnxY)[10.17504/protocols.io.dyq7vv](http://dx.doi.org/10.17504/protocols.io.dyq7vv)[.](http://paperpile.com/b/uhkxKa/yLnxY)

[Cunning, Ross. 2018. *SteponeR: R Package for Importing qPCR Data from StepOneTM Software* (version v0.1.0). https://doi.org/](http://paperpile.com/b/uhkxKa/Cqp9U)[10.5281/zenodo.1173321](http://dx.doi.org/10.5281/zenodo.1173321)[.](http://paperpile.com/b/uhkxKa/Cqp9U)

[Cunning, Ross, and Andrew C. Baker. 2013. “Excess Algal Symbionts Increase the Susceptibility of Reef Corals to Bleaching.” *Nature Climate Change* 3: 259.](http://paperpile.com/b/uhkxKa/ik1BX)

[Mieog, Jos C., Madeleine J. H. van Oppen, Ray Berkelmans, W. T. Stam, and Jeanine L. Olsen. 2009. “Quantification of Algal Endosymbionts (Symbiodinium) in Coral Tissue Using Real-Time PCR.” *Molecular Ecology Resources* 9 (1): 74–82.](http://paperpile.com/b/uhkxKa/LYKDK)

[Rowan, Rob, and Dennis A. Powers. 1991. “Molecular Genetic Identification of Symbiotic Dinoflagellates (zooxanthellae).” *Marine Ecology Progress Series* 71: 65–73.](http://paperpile.com/b/uhkxKa/nkogW)