

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).

Laboratory analysis

We used 100 µL aliquots from the SDS lysates to extract and purify genomic DNA following established procedures (Baker and Cuning 2016). 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; Cuning and Baker 2013), was estimated using qPCR assays that target the actin gene in *Pocillopora* and its symbionts in the genus *Cladocopium* and *Durussdinium*. Primers, probes and PCR conditions followed Cuning and Baker (2013). All reactions were run in duplicate on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA), with a ΔR_n threshold = 0.01 to estimate the cycle threshold (CT) of each sample and target.

The StepOneR repository for R (Cuning 2018) was used to calculate the genus-specific symbiont to host (S/H) cell ratios (*Cladocopium* to Host [C/H], and *Durussdinium* to Host [D/H]). This repository uses the cycle threshold (CT) values obtained from the qPCR reactions to calculate the ratios using the formula $2^{(CT_{\text{host}} - CT_{\text{symbiont}})}$. The ratios are corrected using additional information about target ploidy, fluorophore intensity, and DNA extraction efficiency (Mieog et al. 2009; Cuning and Baker 2013). 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 *Durussdinium* 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

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