

RHODOLITHS: BIODIVERSITY-RICH HOLOBIONTS?

RODOLITOS: ¿HOLOBIONTES RICOS EN BIODIVERSIDAD?

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Rhodoliths, free-living marine benthic nodules that are predominantly accreted by crustose coralline red algae, are the main hard substrata for the attachment of benthic phototrophs offshore Louisiana in the Northwestern Gulf of Mexico. Rhodolith beds at Ewing Bank are associated with salt domes, unique deep bank habitats at ~55-90m depth on the continental shelf. Sampling expeditions following the 2010 Deepwater Horizon (DWH) oil spill to Ewing Bank revealed that most rhodoliths appeared bleached and were fully or partially denuded of surface macroalgae, a situation that has persisted in the field as of September 2014, our last expedition to Ewing Bank. However, when bare rhodoliths were taken from the field and placed in a series of 75-liter laboratory microcosms mirroring the site conditions, a shift in algal community reverted back to pre-spill conditions.

With the advent of additional sequencing technologies, methodologies for biodiversity assessments are now rapidly shifting to DNA metabarcoding, *i.e.* High-Throughput Sequencing of environmental DNA mixtures with standardized molecular markers for rapid, cost-effective biodiversity measurement. Metabarcoding assays of plastid *tufA* and 16S V4 targeting phototrophs from endolithic portions of rhodolith substrata reveal a hidden (cryptic) algal diversity targeting spores, gametes, propagules, and unsuspected life history stages. Analyses of combined metabarcodes and Sanger sequences result in novel *tufA* and 16S phylogenies for red, green and brown seaweeds. SEM, TEM and Fluorescence microscopy documented previously unrecognized benthic stages of bloom-forming microalgae such as the dinoflagellate *Prorocentrum lima*, the haptophyte *Ochrosphaera verrucosa*, and the unicellular red algal genus *Rhodosorus*, all residing endolithically inside *Lithothamnion* (Hapalidiales) rhodoliths; the taxonomic identity of these stages was confirmed by whole genome amplification on single cells and subsequent sequencing.

We will discuss the critical importance rhodoliths play in the life cycle completion of both macro- and microalgae, and as holobionts (macro-organisms together with their associated microbial communities) for the establishment and maintenance of biodiversity.

Keywords: benthic, biodiversity, Gulf of Mexico, high-throughput sequencing (HTS), metabarcoding, macroalgae, microalgae, *Prorocentrum*, *Ochrosphaera*, oil spill, rhodoliths, systematics