TITLE: Effects of the endocrine disruptor 17α-ethinylestradiol on bacterial community associated with the scleractinean coral *Mussismilia harttii*

AUTHORS: VILELA, C.L.S.[1,2]; VILLELA, H.D.M. [1,2]; DUARTE, G.A.S.[1,2]; SANTORO, E.P.[1,2]; RACHID, C.T.C.C.[1], PEIXOTO, R.P.[1,2]

INSTITUTION: [1] University of Rio de Janeiro, Rio de Janeiro, Brazil;

[2] Rio de Janeiro Marine Aquarium Research Center, Brazil.

ABSTRACT:

Water pollution is affecting the wildlife in all ecosystem, mainly the marine environment. 17α-ethinylestradiol (EE2) is an estrogenic endocrine disrupting compound (EDC) related with alterations in the reproductive cycles of several animals in aquatic environments, cancers, feminization and others health disorders. Some studies have focused on revealing estrogen impacts on physiology of different marine organisms, however no data has been published about the diversity and changes in their associated microbial communities. Therefore, the aim of this work was to investigate potential EE2 effects on *Mussismilia hartti* health and their associated bacteria. Thus, a closed microcosm system with circulating water was set up. In this system,3 different treatments were tested in quadruplicates (4 controls without EE2, 4 aquaria containing 100ng/L of EE2 and 4 with 100µg/L), to simulate EE2 pollution in a coral reef, at real concentrations found in aquatic environments. The experiment was carried out for 17 days and sample collections were performed at days 0, 3, 9 and 17. Water parameters (such as pH, temperature and nutrients) were constantly monitored and coral samples were taken for DNA analyses in the same sampling days. Maximum photosynthetic efficiency (Fv/Fm) of corals' zooxanthellae was measured throughout the experiment as

a proxy for coral health. No difference on the values was detected and the average ranged around 550 of Fv/Fm. Non-metric multidimensional scaling (NMDS) demonstrated that the collection time is statistically significant on the microbiome structure, which does not happen with the presence of EE2 in the treatments, that did not present significant difference. Taxonomic analysis showed that there were no significant microbial community changes among conditions with or without estrogen. Nonetheless, bioindicator analysis presented significantly divergent bacterial groups in different treatments, suggesting that the presence of EE2 could affect specific OTUs associated with corals. Therefore, although the preliminary results indicated that the Fv/Fm proxy and the coral associated community were not impacted by the endocrine disruptor using the tested concentrations. Deeper analyses should be performed to corroborate these findings, such as the use of metagenomics, transcriptomics and longer periods of exposure to EE2.

Keywords: *Mussismilia harttii*, 17α-ethynilestradiol (EE2), seawater pollution, micropollutants, coral microbiome, endocrine disruptor, synthetic estrogen, next generation sequencing

Development agency: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior)