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Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea

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ABSTRACT

Coral-associated bacteria play an increasingly recognized part in coral health. We investigated the effect of local anthropogenic impacts on coral microbial communities on reefs near Jeddah, the largest city on the Saudi Arabian coast of the central Red Sea. We analyzed the bacterial community structure of water and corals (*Pocillopora verrucosa* and *Acropora hemprichii*) at sites that were relatively unimpacted, exposed to sedimentation & local sewage, or in the discharge area of municipal wastewaters. Coral microbial communities were significantly different at impacted sites: in both corals the main symbiotic taxon decreased in abundance. In contrast, opportunistic bacterial families, such as e.g. Vibrionaceae and Rhodobacteraceae, were more abundant in corals at impacted sites. In conclusion, microbial community response revealed a measurable footprint of anthropogenic impacts to coral ecosystems close to Jeddah, even though the corals appeared visually healthy.

1. Introduction

Reef-building corals are metaorganisms living in symbiosis with dinoflagellates from the genus *Symbiodinium* and a plethora of other microbes, such as bacteria, archaea, fungi, and also viruses (Rosenberg et al., 2007). While *Symbiodinium* spp. are largely responsible for photoautotrophic energy production, other coral-associated microbes are shown to provide important contributions to coral holobiont functioning. They can facilitate the fixation of nitrogen in oligotrophic waters (Lema et al., 2012; Lesser et al., 2004) and play a role in many metabolic processes (Wegley et al., 2007), such as the cycling of sulfur compounds (González et al., 2003). Furthermore, it has been suggested that microbial communities may facilitate acclimatization of the coral holobiont to changes in the environment through rapid restructuring of the microbial community composition (Reshef et al., 2006), and studies indicate that an intact coral microbiome is essential to coral immunity and health (Krediet et al., 2013; Mao-Jones et al., 2010; Rosenberg et al., 2007).

Coral microbiomes are assumed to be host species specific (Sunagawa et al., 2010). However, this specificity is dependent on the health state of the coral; in diseased tissues different coral hosts display bacterial community compositions that are more similar in comparison to their healthy counterparts. This pattern has mostly been explored for visible coral disease states: in *Diploria strigosa*, *Montastraea cavernosa*, and *Orbicella annularis* from Curacao (Frias-Lopez et al., 2002) and in *Pavona duerdeni* and *Porites lutea* from Thailand (Roder et al., 2014a). Moreover, when compared across oceans, the disease microbiome of corals was found to be conserved across large geographic scales independent of host species (Roder et al., 2014b). Commonly, the development and progression of coral disease is associated with an increase in microbial diversity and the occurrence of opportunistic microbial taxa, such as e.g. Vibrionaceae and Rhodobacteraceae (Rosenberg and Ben-Haim, 2002), that can lead to changes in the function of the microbiome and the development of disease (Vega Thurber et al., 2009) and bleaching (Rosenberg and Falkovitz, 2004). On a global scale, outbreaks of coral bleaching and coral disease are amongst the most pervasive threats to coral reefs. In the Caribbean, for instance, White Plague Disease has driven Acroporid coral species to the brink of extinction within two decades (Miller et al., 2002) and in the Persian/Arabian Gulf, coral communities are projected to be on a trajectory of decline due to the combined impacts of bleaching and coral disease (Riegl et al., 2013).

Besides disease, local anthropogenic influences are also suspected to be drivers affecting the coral microbial community and coral health. For example, the experimental enrichment with inorganic nutrients of a

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coral reef habitat induced shifts in coral microbial communities of *Acropora hemprichii* (Jessen et al., 2013) and caused outbreaks of coral disease (Bruno et al., 2003; Vega Thurber et al., 2014). Further, damage from fishing gear causes wounds on coral colonies that can become infected and lead to higher rates of coral disease in fished reefs compared to protected areas (Lamb et al., 2015). Increased nutrients coupled with overfishing also has indirect effects on coral microbial communities, by promoting algal growth that may mediate microbe-induced coral mortality (Smith et al., 2006). Driven by burgeoning coastal populations, increasing amounts of sewage enter coastal ecosystems introducing high loads of inorganic nutrients, sediments, and organic compounds, which can have deleterious effects on coral reefs (Fabricius, 2005; Lamb et al., 2015; Wear and Thurber, 2015). Moreover, sewage also introduces many microbial taxa and recently a strong link between sewage-associated human pathogens and the development of coral disease in Caribbean corals has been made (Closek et al., 2014; Sutherland et al., 2010, 2011). Even in the absence of potential stressors or threats, prevailing environmental conditions are shown to align with coral-associated microbial communities. Roder et al. (2015) showed that highly structured microbiomes of *Ctenactis echinata* were encountered at sites where *C. echinata* was most common; these were sites characterized by open rocky substrates and clear water, indicating that microbiome composition reflects habitat adequacy.

Red Sea reefs are for the most part relatively unimpacted, but they border on coastal areas experiencing rapid population growth and increasing local anthropogenic influences. Evidence is accumulating that Red Sea reefs are changing as a result of these factors (Riegl et al., 2012), but to our knowledge, data on microbial infections of corals from the Red Sea is lacking and research exploring these coral reef habitats in their transition is urgently needed. The aim of this study was to investigate the effect of chronic anthropogenic pollution (i.e. sedimentation, sewage, nutrients) on microbial communities associated with seemingly healthy coral colonies of *Acropora hemprichii* and *Pocillopora verrucosa*, close to the metropolitan area of Jeddah, Saudi Arabia, in order to better understand the nature of anthropogenic impacts to Red Sea coral reefs.

2. Materials and methods

2.1. Sampling sites and anthropogenic impacts

The study sites were located in direct vicinity of the major city of Jeddah (current population 4 mio, annual 3% growth rate), Saudi Arabia, situated on the coast of the central Red Sea. Unlike many other parts of the Saudi Arabian Red Sea, the coastline around Jeddah has been extensively affected by development. For instance, there has been widespread infilling over the nearshore fringing reefs extending from the center of the city to 50 km northwards. Besides causing direct loss of coral communities and associated lagoonal habitat, at many points this has resulted in increased turbidity and sedimentation. Existing facilities for the treatment of wastewater often work over their recommended capacities and are insufficient. As a consequence there is extensive discharge of untreated or only partially-treated sewage into the oligotrophic coastal waters (Mudarris and Turki, 2006). Three large municipal wastewater outfalls release high volumes of effluent onto the coast and adjacent reefs; the outfalls at Al Shabab and Al Arbaeen respectively release approximately 35,000 $m^3\ d^{-1}$ and 68,000 $m^3\ d^{-1}$ of effluent within Jeddah Bay (El-Rayis and Moammar, 1998). At the third outfall at Al Kumrah, further to the south, approximately 300,000 $m^3\ d^{-1}$ of effluent are released (Basaham et al., 2009). In addition, numerous unapproved sewage outlets exist that discharge an estimated 99,000 $m^3\ d^{-1}$ of sewage near the shore over ca. 30 km of coastline extending north from the city center (Al-Farawati, 2010; Peña-García et al., 2014; Risk et al., 2009). In general, a series of previous studies have found a significant elevation in nutrient levels in the vicinity of these wastewater outfalls (e.g. Al-Farawati, 2010; El Sayed, 2002; Peña-García et al., 2014). Moreover, the local reefs are heavily fished, thus likely reducing the grazing pressure on benthic algae, which compete with corals for space and may affect their bacterial assemblages (Morrow et al., 2013). The combination of these impacts has resulted in declining hard coral cover on reefs in the area (DeVantier and Pilcher, 2000; Kotb, 2010; Pilcher and Alsuhaibany, 2000).

Given the above, six study sites were chosen to investigate influences of these prevalent anthropogenic impacts on coral-associated microbial communities (Fig. S1). The six sites had a similar geomorphology and provided paired replicates of three conditions. Two relatively unimpacted sites (henceforth referred to as sites A and B), one located at a patch Reef south of Ras Dha'l Lama at the entrance to Sharm Suleiman (site A: N 21°52′22.83″ E 38°58′01.61″) and the other at a fringing reef at Bohairat, off Al Zummrad to the northern end of the municipal area (site B: N 21°47′08.23″, E 39°02′28.56″) served as control sites. The second set of sites was located adjacent to the heavily-developed Jeddah Corniche, a major road running north along the shore from the city center, and the focus of ongoing coastal construction. These sites, which experience turbidity, sedimentation, and local sewage outfall were on the fringing reef south of Green Island (site C: N 21°36′54.53″, E 39°06′17.92″) and between Al Nawras and the Fakieh Aquarium (site D: N 21°34′34.21″, E 39°06′27.27″). The last two sites were located on reefs not far from Jeddah Port and the associated industrial zones at the southern end of the city (henceforth referred to as sites E and F). Site E was located on a patch reef off the center of Jeddah Bay close to the municipal wastewater outfalls of Al Shabab and Al Arbaeen (N 21°26′21.41″, E 39°06′28.49″) and site F was located south of Al Khumrah outfall (N 21°15′33.92″, E 39°06′42.37″). Both sites were within about 5 km of the three main discharge points from the city's main sewerage systems and treatment works, but proved sufficiently distant from the municipal wastewater discharge points to be only occasionally subject to elevated turbidity.

2.2. Benthic surveys

The line intercept transect (LIT) method (English et al., 1997; Loya, 1978) was used to assess differences between coral assemblages at the different sites, linked to differences in anthropogenic impacts (see above). At each site, a 30 m-long leaded line was laid along the reef following the 5 m depth contour. The substrate underlying the transect line was recorded cm by cm.

2.3. Coral and water sampling

At each of the six sites (i.e., A, B, C, D, E, F), three visually-healthy appearing colonies of the scleractinian corals *P. verrucosa* and *A. hemprichii* were sampled at 5 m depth by removing a branch. Sampled branches were placed in zip-lock bags under water and upon return to the boat were rinsed with filtered seawater (0.22 μm) and subsequently transferred into a N_2 dry shipper. Water samples were taken at the same depth as the coral samples, transported back to the shore in the dark in cooled boxes and 1 l of seawater was immediately filtered through 0.22 μm Durapore PVDF filters (Millipore, Billerica, MA, USA). The filters were subsequently frozen with the coral samples until transported to KAUST.

2.4. DNA extraction and sequencing

Coral fragments and PVDF filters were stored at −80°C. Prior to DNA extraction 1.5 ml AP1 extraction buffer (DNeasy Plant Mini Kit, Qiagen, Hilden, Germany) were applied on each coral sample while the frozen fragment was thawing. For each sample, coral tissue was blasted off the skeleton using airflow from a sterile pipet tip (1000 μl filter barrier tips, Neptune, USA) connected via a rubber hose to a bench top air pressure valve. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For DNA

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