Gradients of anthropogenic stress in Indonesian coral reefs

Implications for bacterial community composition and aggregate formation

A dissertation by

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To my family, old and new

Summary

The Indonesian Spermonde Archipelago, like other coral reefs located in close vicinity to urban areas, is confronted with a multitude of local anthropogenic stressors. With a growing coastal population those stressors, including eutrophication, pollution and overfishing, increase. Coastal eutrophication, the increased availability of inorganic nutrients and organic matter, likely leads to an increase in phytoplankton biomass, often measured as chlorophyll *a* concentrations. This abundant phytoplankton community, stimulated by heterotrophic bacteria, releases copious amounts of transparent exopolymer particles (TEP). The result is often a severalfold increase of the suspended material, aggregate formation and higher sedimentation rates. A pronounced water quality gradient in relation to the distance from mainland Sulawesi has been proposed for several decades and decreasing water quality was often suggested as one of the main reasons for the decline of the fringing reefs of the Archipelago. But so far only few studies investigated bacteria, which fulfill important functions in organic matte cycling and disease ecology in coral reef ecosystems, in their response to water quality parameters in the Spermonde Archipelago. Therefore the main questions were: (1) *How does the availability of organic and inorganic nutrients influence the abundance and community structure of bacteria in the water column, sediments and on benthic marine biota of the Spermonde Archipelago?* (2) *What role does water quality have in aggregate formation and bacterial community composition on settling aggregates along large scale gradients in tropical reef waters? (3) Do the small inhabited islands of the archipelago have a significant impact on water quality parameters of the surrounding water masses?*

A combination of experimental and observational studies was conducted at several fringing reefs and in the water column, crossing the entire shelf from 1 to 55 km distance from mainland Sulawesi during two sampling campaigns in February 2013 (after the rainy season) and in June 2014 (dry season). Surveys of bacterial communities (from reef sediments, water column, aggregates and coral mucus) and simultaneous water quality measurements, including inorganic nutrients, chlorophyll *a*, dissolved organic carbon (DOC), TEP and suspended particulate matter (SPM), were conducted along this regional gradient. Additionally comparative small-scale gradients were investigated at the back-reef areas of a populated and an uninhabited island to see whether sewage and waste water seepage from inhabited islands of the Archipelago could have a measurable influence of the water quality of the surrounding water masses.

In the first part of this thesis, the spatial impact of eutrophication on bacterial community composition at the regional scale was investigated. In contrast to previous studies water quality parameters were not gradually decreasing with increasing distance from Makassar. Instead, there were two contrasting regions, one inshore zone of highly elevated concentrations of chlorophyll *a*, SPM, TEP, aggregate formation potential and sedimentation rates, and one oligotrophic outer shelf zone with much lower concentrations of the measured parameters. Bacterial community compositions were very distinct between those two described zones. Copiotrophic bacteria dominated communities in the inshore zone, especially those attached to aggregates and inhabiting the sediments (e.g. the relative abundance of *Gammaproteobacteria* in sediments dropped from 46.6 % in the eutrophic inshore zone to 23.8 % in the outer shelf zone). The prevalence of bacterial groups containing potentially pathogenic strains was also higher at the chronically impacted inshore zone. The measured water quality parameters, including chlorophyll *a*, TEP and SPM were significantly elevated at the inshore zone and had a significant impact on the bacterial community composition. But in contrast to many previous studies conducted in the Spermonde Archipelago, the influence of untreated sewage and riverine input is restricted to a narrow band of eutrophic water very close to Makassar.

The second part investigated how the contrasting conditions of the two zones, eutrophic inshore vs. oligotrophic outer shelf zone, affect aggregate formation and sedimentation rates, as well as the bacterial community composition on settling aggregates. While the zonation from the first part was confirmed again, additionally, significantly higher total aggregated volume (21.80 ± 5.46 cm^3) and sedimentation rates (20.92 ± 5.15 mg cm^{-2} d^{-1}) were observed in the eutrophic zone compared sampling within the oligotrophic outer shelf zone (0.29 ± 0. 26 cm^3 and 0.87 ± 0.29 mg cm^{-2} d^{-1}). As a consequence bacterial community composition on aggregates sampled after the end of the rolling tank experiment were shifted almost completely from *Gammaproteobacteria* (83.44 %) at the inshore zone to *Alphaproteobacteria* (72.39 %) in the outer shelf zone. Results from the second part implied that enhanced nutrient uptake, phytoplankton abundance, aggregate formation and sedimentation in the inshore zone seem to prevent an increased nutrient and organic matter loading to oligotrophic sites on the outer shelf.

In the third part of this thesis the impact of dense island population on water quality and bacterial communities was investigated. This comparative study revealed significantly elevated concentrations of water quality parameters, especially chlorophyll *a* (0.61 ± 0.02 µg L^{-1}) and TEP (127.12 ± 33.03 µg Xeq L^{-1}), in back-reef waters of the inhabited island compared to the uninhabited one (0.02 ± 0.02 μg L^{-1} and 85.05 ± 22.97 μg Xeq L^{-1}). Redundancy analysis revealed that the significant differences in bacterial community composition between both islands can be related to the inhabitation status of the island. Among the dominant classes *Gammaproteobacteria* of the particle-attached fraction of the water column more than doubled in relative abundance (34.65 % compared to 15.23 %). Overall this part of the study shows indications for an impact of dense islands populations in the Spermonde Archipelago on the water quality and bacterial community composition. In combination with results from the first part this implies that management efforts to mitigate causes for eutrophication can be focused on nearshore waters and the inhabited islands, e.g. with the installation of septic tanks or basic sewage treatment facilities.

In conclusion, data presented in this thesis conclusively showed that dense human populations on the mainland and on the individual islands exerted a clear and measurable influence on the investigated water quality parameters, but that influence was restricted to a narrow zone close to the coast. There were significant repercussions in the bacterial communities, especially in the sediments and associated to aggregates, and differences in water quality between the eutrophic inshore/oligotrophic outer shelf sites and inhabited/uninhabited island were identified as significant drivers of those shifts. And bacterial community shifts towards copiotrophic and potentially pathogen-containing groups mainly occurred at this eutrophic inshore zone. The findings made in this thesis can prove valuable to focus water quality management efforts on the areas most affected by untreated sewage and nutrient input.

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Zusammenfassung

Ebenso wie andere Korallenriffe in der Nähe von größeren Stadtgebieten, wird das indonesische Spermonde Archipel mit einer Vielzahl von lokalen, anthropogenen Stressfaktoren konfrontiert. Mit zunehmender Bevölkerungsdichte an der Küste nehmen auch diese Stressfaktoren, wie Eutrophierung, Verschmutzung und Überfischung, zu. Eutrophierung der Küstengewässer, d.h. die erhöhte Verfügbarkeit von anorganischen Nährstoffen und organischem Material, führt zu einem Anstieg der Phytoplanktonbiomasse, die häufig als Chlorophyll *a* Konzentration gemessen wird. Diese reichhaltige Phytoplanktongemeinschaft wird durch heterotrophe Bakterien dazu stimuliert, erhebliche Mengen an transparenten Exopolymerpartikeln (TEP) abzusondern. Daraus folgt häufig eine Vervielfachung des gelösten Materials in der Wassersäule, die Bildung von Aggregaten sowie erhöhte Sedimentationsraten. Während der vergangenen Jahrzehnte wurde ein ausgeprägter Gradient in der Wasserqualität, abhängig von der Entfernung zur Küste Sulawesis, beschrieben und die Abnahme der Wasserqualität wurde mehrfach als Hauptgrund für die Zustandsverschlechterung der Saumriffe im Archipel benannt. Bisher untersuchten jedoch nur wenige Studien im Spermonde Archipel die Auswirkung von Wasserqualität auf Bakterien, die wichtige Funktionen im Kreislauf organischen Materials sowie Krankheitsökologie in Korallenriffökosystemen erfüllen. Die Hauptfragestellungen waren *(1) Wie beeinflusst die Verfügbarkeit von organischem und anorganischen Nährstoffen im Spermonde Archipel die Abundanz und Struktur von Bakteriengemeinschaften in der Wassersäule, in Sedimenten und auf benthischen Meeresorganismen? (2) Welche Rolle spielt die Wasserqualität in tropischen Gewässern entlang eines regionalen Gradienten bei der Bildung von Aggregaten und der Zusammensetzung der Bakteriengemeinschaften auf diesen? 3) Haben die kleinen bewohnten Inseln des Archipels einen messbaren Einfluss auf die Wasserqualitätsparamter in umgebenden Wassermassen?*

Eine Kombination aus experimentellen und untersuchenden Studien wurde während zwei Feldkampagnen im Februar 2013 (nach der Regenzeit) und im Juni 2014 (während der Trockenzeit) durchgeführt, die mehrere Saumriffe und die Wassersäule entlang des gesamten Schelfs von 1 bis 55 km Entfernung zum Festland Sulawesis umfasste. Entlang des regionalen Gradienten wurden Analysen der Bakteriengemeinschaften (in Riffsedimenten, der Wassersäule, auf Aggregaten sowie in Korallenschleim) durchgeführt, bei gleichzeitiger Untersuchung der Wasserqualitätsparameter durch Bestimmung von anorganischen Nährstoffen, Chlorophyll *a*, gelösten organischen

Kohlenstoffs (DOC), TEP sowie partikuläre Schwebstoffe (SPM). Zusätzlich wurden auf vergleichbare Weise lokale Gradienten entlang der Rifflagune einer bewohnten, sowie einer unbewohnten Insel untersucht, um festzustellen, ob das Einsickern von ungeklärten Abwässern der bewohnten Inseln des Archipels in die umgebenden Wassermassen einen messbaren Einfluss auf die Wasserqualität hat.

Im ersten Teil dieser Arbeit wurde der räumliche Einfluss von Eutrophierung auf Bakteriengemeinschaften entlang des regionalen Gradienten untersucht. Im Gegensatz zu vorherigen Studien nahmen die Wasserqualitätsparameter nicht gleichmäßig mit zunehmender Entfernung zu Makassar ab. Stattdessen konnten zwei unterschiedliche Zonen bestimmt werden, eine schmale, küstennahe Zone mit stark erhöhten Konzentrationen von Chlorophyll *a*, SPM, TEP, erhöhtem Potenzial zur Aggregatbildung und höheren Sedimentationsraten, sowie eine breite nährstoffarme Zone weiter draußen auf dem Schelf, in welcher deutlich niedrigere Konzentrationen der untersuchten Parameter gemessen wurden. In beiden Zonen wurden verschiedenartige Zusammensetzungen der Bakteriengemeinschaft ermittelt. Heterotrophe Bakterien dominierten die Gemeinschaften in der küstennahen Zone, besonders die auf Aggregaten sowie in Sedimenten (die relative Häufigkeit von *Gammaproteobakteria* in Sedimenten fiel beispielweise von 46.6 % in der eutrophierten küstennahen Zone auf 23.8 % in der äußeren Zone). Das Vorherrschen von Bakteriengruppen, welche potenziell pathogene Stämme enthalten können, war erhöht in der chronisch beeinflussten küstennahen Zone. Die gemessenen Wasserqualitätsparameter, einschließlich Chlorophyll a, TEP und SPM waren signifikant höher in der küstennahen Zone, und hatten einen signifikanten Einfluss auf die Zusammensetzung der Bakteriengemeinschaft. Im Gegensatz zu vielen bisherigen Studien die im Spermonde Archipel durchgeführt wurden, fanden wir jedoch heraus, dass der Einfluss der ungeklärten Abwässer sowie Einträgen aus Flüssen auf eine schmale Zone eutrophierten Wassers in der Nähe von Makassar begrenzt ist.

Der zweite Teil untersuchte die Auswirkung der unterschiedlichen Bedingungen der eutrophierten küstennahen Zone im Vergleich zur nährstoffarmen äußeren Zone auf Aggregatbildung und Sedimentationsraten, sowie auf die Zusammensetzung der Bakterien auf Aggregaten. Hierbei wurde die Zonierung aus dem ersten Teil dieser Arbeit bestätigt, und zusätzlich wurden in der eutrophierten küstennahemn Zone signifikant erhöhte Aggregat-Volumina (21.80 ± 5.46 cm^3) und Sedimentationsraten (20.92 ± 5.15 mg cm^{-2} d^{-1}) im Vergleich zu Messungen in der nährstoffarmen äußeren Zone gemessen (0.29 ± 0.26 cm^3 und 0.87 ± 0.29 mg cm^{-2} d^{-1}). Als Konsequenz daraus verlagerte sich die

Zusammensetzung der Bakteriengemeinschaften auf Aggregaten am Ende der Experimente in 'rolling tanks' fast komplett von *Gammaproteobacteria* (83.44 %) in der küstennahen Zone, zu *Alphaproteobacteria* (72.39 %) in der äußeren Zone. Die Ergebnisse des zweiten Teiles implizieren, dass erhöhte Nährstoffaufnahmen, Überfluss an Phytoplankton, Aggregatbildung und Sedimentation in der küstennahen Zone eine Erhöhung der Nährstoffwerte und Belastung durch organisches Material in der äußeren Zone verhindern kann.

Im dritten Teil dieser Arbeit wurde der Einfluss von starker Inselbesiedlung auf die Wasserqualität und die Bakteriengemeinschaften untersucht. Diese vergleichende Studie offenbarte signifikant erhöhte Konzentrationen von Wasserqualitätsparameter, inbesonders Chlorophyll *a* (0.61 ± 0.02 µg L^{-1}) und TEP (127.12 ± 33.03 µg Xeq L^{-1}), im Wasser der Rifflagune der bewohnten Insel im Vergleich zur unbewohnten (0.02 ± 0.02 µg L^{-1} und 85.05 ± 22.97 µg Xeq L^{-1}). Eine Redundanzanalyse zeigte, dass die signifikanten Unterschiede in der Zusammensetzung der Bakteriengemeinschaft zwischen beiden Inseln direkt auf den Besiedlungsstatus der Inseln zurückzuführen ist. Unter den häufigsten Bakterienklassen nahmen besonders die Häufigkeit der *Gammaproteobacteria* in der an Partikel gebundenen Fraktion der Wassersäule um mehr als das Doppelte zu (34.65 % im Vergleich zu 15.23 %). Insgesamt deuten die Ergebnisse dieses Teils darauf hin, dass hohe Bevölkerungsdichten auf den Inseln im Spermonde Archipel die Wasserqualität sowie die Zusammensetzung der Bakteriengemeinschaften beeinflussen. In Kombination mit den Ergebnissen des ersten Teils dieser Arbeit lässt sich daraus schließen, dass Managementstrategien zur Verminderung von Eutrophierungseinflüssen auf küstennahe Gebiete und bewohnte Inseln konzentriert werden sollten, beispielsweise durch den Bau von Klärbehältern und einfachen Abwasserbehandlungsanlagen.

Zusammenfassend zeigen die Daten dieser Arbeit schlüssig, dass dichte Besiedlung durch Menschen auf dem Festland und auf den einzelnen Inseln einen klaren und messbaren Einfluss auf die untersuchten Wasserqualitätsparameter ausüben, aber dass dieser Einfluss auf eine schmale, küstennahe Zone beschränkt ist. Es gab signifikante Einflüsse auf die Bakteriengemeinschaften, besonders in Sedimenten und gebunden an Aggregate. Unterschiede in der Wasserqualität zwischen der eutrophierten küstennahen und der nährstoffarmen äußeren Zone sowie zwischen bewohnten und unbewohnten Inseln konnten als Ursachen für diese Veränderungen identifiziert werden. Eine Verschiebung der Zusammensetzung von Bakteriengemeinschaften zu heterotrophen und potenziell Pathogen-enthaltenden Gruppen fand in dieser eutrophierten küstennahen Zone

statt. Diese Arbeit liefert wertvolle Erkenntnisse um das Management von Wasserqualität auf solche Zonen zu fokussieren, welche besonders von ungeklärten Abwässern und Nährstoffeinträgen betroffen sind.

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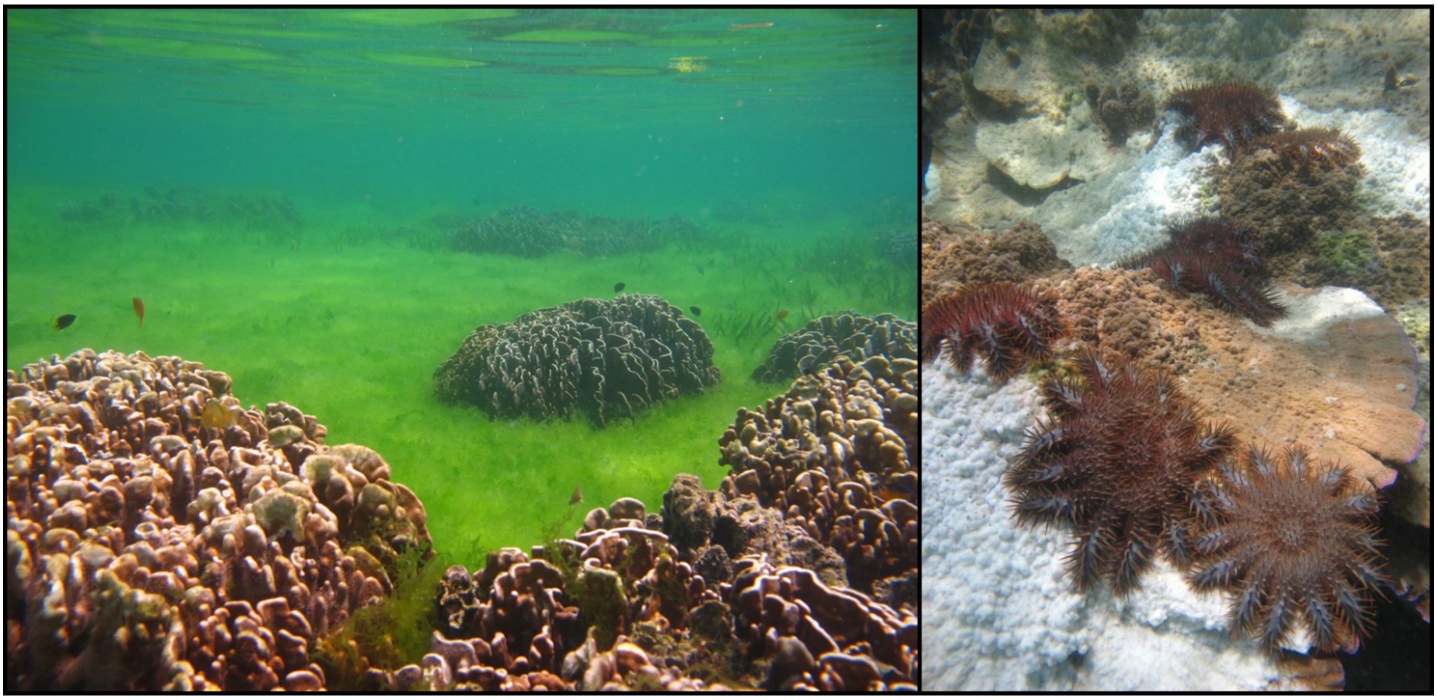
Table of Contents

Chapter 1

General introduction



THE SPERMONDE ARCHIPELAGO AS MODEL ECOSYSTEM

Indonesia is the largest archipelago on this planet, comprising of more than 17,000 islands stretching approximately 5,000 km from the eastern Indian Ocean to the western tropical Pacific. Its productive and highly diverse waters are located in the center for tropical marine biodiversity (Veron et al., 2009) and support one of the largest fisheries industries of the world (Hopley and Suharsono, 2000). The Spermonde Archipelago is located in southern Sulawesi, in the eastern part of Indonesia. More than 100 islands with surrounding fringing reefs and submerged reefs are spread across the 60 km wide continental shelf off metropolitan Makassar (Tomascik et al., 1997). All of the islands which support an autochthonous water supply are inhabited, in many cases very densely (Schwerdtner Máñez et al., 2012). Sewage input, pollution and sedimentation from the harbor city of Makassar (population 1.4 million) and the Jene Berang river are both impacting the fringing reefs and related organisms in the archipelago (Becking et al., 2006; Edinger et al., 1998, 2000; Moll, 1983; Plass-Johnson et al., 2015). Nutrient levels have been found highest close to Makassar and gradually decreasing towards the outer-shelf islands (Moll, 1983; Renema and Troelstra, 2001). Despite the ongoing water quality deterioration and reef degradation, fishing is still a major source of income (Ferse et al., 2012; Pet-Soede et al., 2001). Both for food consumption and the ornamental fish trade, fishing is often conducted in illegal and unsustainable ways, using bombs or cyanide (Burke et al., 2011; Pet-Soede and Erdmann, 1998). Further, the islands of the archipelago itself are also of particular scientific interest, because they are close to each other, but vary in human population density. As there is often no local wastewater treatment, there is a continuous diffuse influx of water rich in inorganic and organic nutrients entering the fringing reefs surrounding those islands. Thus, the Spermonde Archipelago is an ideal natural laboratory to investigate anthropogenic stressors to coral reefs at various spatial scales, ranging from local impact of the individual island populations to large scale changes across the entire shelf gradient.

TROPICAL CORAL REEFS AND ASSOCIATED BACTERIAL COMMUNITIES

Warm water scleractinian corals thrive in the oligotrophic waters of the tropics and subtropics (Odum and Odum, 1955), with a diversity and productivity rivaling those of tropical rainforests (Connell, 1978; Sebens, 1994). This paradoxon has already been observed and described by Charles Darwin during his early voyages on the *HMS Beagle* (Darwin, 1842) and continued to puzzle marine scientists for more than a century. A highly efficient internal recycling and exchange of organic matter and inorganic nutrients between the coral host and mutualistic dinoflagellates from the genus *Symbiodinium* (often referred to *as* zooxanthellae) are the cornerstones of the high productivity of reef-building corals (Muscatine and Porter, 1977).

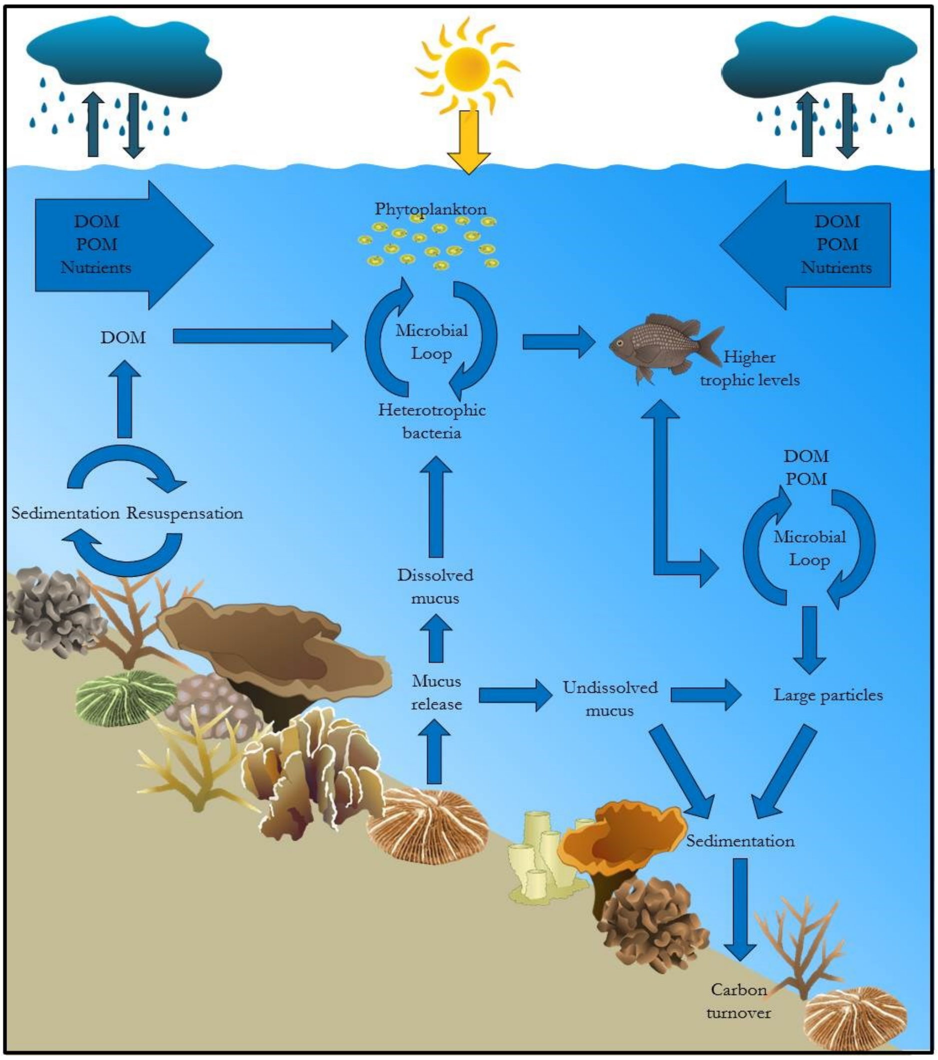


Fig.1-1 Key biogeochemical processes with bacterial contribution occurring in tropical coral reef ecosystems. Modified from Bourne and Webster (2013).

Bacterial communities in coral reefs inhabit very different but intricately linked habitats (Fig. 1-1), from the overlying water column to the reef sediments, including a range of benthic organisms, such as corals and sponges (Bourne and Webster, 2013). The crucial role of bacteria as key players in coral reef ecosystems is now well accepted (Azam and Malfatti, 2007; Sorokin, 1973). They regulate important elemental cycles (Azam and

Malfatti, 2007; Carlson et al., 2007; Kopylov et al., 2007; Pomeroy, 1974), promote or inhibit the aggregation of particles in the water column (Grossart and Ploug, 2001; Heissenberger and Herndl, 1994), and play a major role in coral health and disease (Bourne et al., 2009; Harvell et al., 2007; Rosenberg et al., 2007; Willis et al., 2004). By measuring the metabolic activity of the smallest size fraction of planktonic organisms scientists showed already several decades ago that microbes play an important trophic role in transforming organic matter and energy in the water column (Biddanda and Pomeroy, 1988; Pomeroy, 1974; Sorokin, 1973, 1991). The transfer of dissolved organic matter (DOM), as such often unavailable to other organisms, through bacterial incorporation into higher trophic levels of planktonic food webs was described and termed the 'microbial loop' (Azam et al., 1983; Fenchel, 2008). Available nutrients in oligotrophic reef waters are rapidly assimilated by the most abundant photoautotrophic organisms, *Cyanobacteria* (picophytoplankton) of the genera *Prochlorococcus* and *Synechococcus* (Agawin et al., 2000; Mouriño-Carballido et al., 2016; Partensky et al., 1999). Living phytoplankton releases significant amounts of accumulated photosynthates as DOM into the water column (Bertilsson et al., 2007; Thornton, 2014; Zlotnik and Dubinsky, 1989). Ineffective feeding by larger eukaryotic phytoplankton (Pinhassi et al., 2004), sloppy feeding, senescence cell death and cell lysis also increase the availability of organic matter (Agustí and Duarte, 2013; Stocker, 2012; Thornton, 2014). Benthic organisms such as corals (Naumann et al., 2010; Sorokin, 1991; Wild et al., 2008, 2010) and macroalgae (Haas et al., 2010, 2011, 2013; Wild et al., 2008) also release significant amounts of DOM into the water column. Not all of this organic matter is readily available for bacterial degradation (Nelson et al., 2011), but the bulk mass supports a highly diverse and active free-living heterotrophic bacterial community in the water column (Van Duyl and Gast, 2001; Karl et al., 1998; Nelson et al., 2013; Sakka et al., 2002) with abundances in the order of $1 - 2 \times 10^6$ cells cm^{-3} (Weinbauer et al., 2010). DOM is often patchily distributed within the water column (Stocker, 2012) and chemotaxis is widespread among free-living bacteria to effectively exploit those patches (Kiørboe and Jackson, 2001; Stocker, 2012; Stocker et al., 2008) and track motile algae or their respective organic matter plumes (Barbara and Mitchell, 2003). Different sources of phytoplankton-derived organic matter also promote different bacterial lineages, indicating different nutritional quality of the exudates (Sarmento and Gasol, 2012), likewise to different benthic sources of organic matter (Nelson et al., 2013). In return bacteria can be beneficial for phytoplankton they surround by enhancing nutrient regeneration in immediate vicinity to the phytoplankton cells by creating remineralization hot-spots of increased inorganic nutrient availability (Azam and Malfatti, 2007). Several studies revealed that the water residence time plays an essential role in the retention of organic matter, the cycling of nutrients and the shaping of bacterial communities (Delesalle and Sournia, 1992; Mari et al., 2007; Nelson et al., 2011; Torréton and Dufour, 1996; Weinbauer et al., 2010). Additionally, different metabolic pathways between bacterial life strategies can mitigate the detrimental effects of an increased labile organic matter availability of degraded reefs by channeling energy fluxes through non-pathogenic, autothrophic picoplankton such as *Synechococcus* and *Prochlorococcus* (McDole Somera et al., 2016).

During the abiotic formation biotically produced precursor material coagulates to form submicron particles (Kepkay, 1994) and gels (Chin et al., 1998; Verdugo and Santschi, 2010; Verdugo et al., 2004). Eventually those particles and gels will coagulate further to TEP and larger aggregates, in the μm to mm size range within the DOM-particulate organic matter (POM) continuum (Passow, 2000, 2002; Verdugo et al., 2004). Biological formation of TEP mostly occurs during the decline of phytoplankton blooms. Some diatoms and cyanobacteria produce mucous substance which forms free TEP when sloughing off during senescence of the cell (Passow, 2000). With its high stickiness TEP is essential in the formation of larger particles and marine snow (Simon et al., 2002). Coral mucus released into the water column also contributes to the POM pool (Coles and Strathmann, 1973; Ducklow and Mitchell, 1979; Wild et al., 2004a) Bacteria can either enhance or inhibit the formation of larger aggregates via the excretion of mucus (Simon et al., 2002; Wolanski et al., 2003) or hydrolytic enzymes (Heissenberger and Herndl, 1994; Smith et al., 1992), thus actively shaping organic matter fluxes. Several studies experimentally showed that the formation of macroaggregates often ceased after removal of bacteria, providing evidence that the presence of specific bacteria can lead to profound changes in phytoplankton aggregation behavior (Biddanda, 1985; Gärdes et al., 2011; Van Oostende et al., 2013). Thus, varying roles of heterotrophic bacteria in phytoplankton aggregation can be explained by differences in algal exopolymer release, cell stickiness, and bacterial community composition (Grossart et al., 2005).

TEP and aggregates themselves are hot-spots for microbial colonization and activity (Fig. 1-2) (Kiorboe et al., 2002, 2003; Passow, 2002; Simon et al., 2002). Bacteria inhabiting aggregates are several orders of magnitude more abundant per unit of volume compared to the surrounding seawater (Simon et al., 2002). Community composition is also very distinct from the free-living counterparts (Delong et al., 1993; Michotey et al., 2012; Weinbauer et al., 2010; Zhang et al., 2007). Colonizing bacteria express a large variety of ectohydrolases to digest the organic matter of the aggregates (Azam and Malfatti, 2007; Smith et al., 1992). Only little of the lysate is used by the attached bacteria (Smith et al., 1992), the remainder is trailing the sinking aggregates as a plume of DOM readily available to free-living bacteria (Kiørboe and Jackson, 2001). Those plumes might help to retain important nutrients, such as nitrogen, phosphorus and iron in the water column, and therefore stimulate a continuous primary production (Azam and Malfatti, 2007).

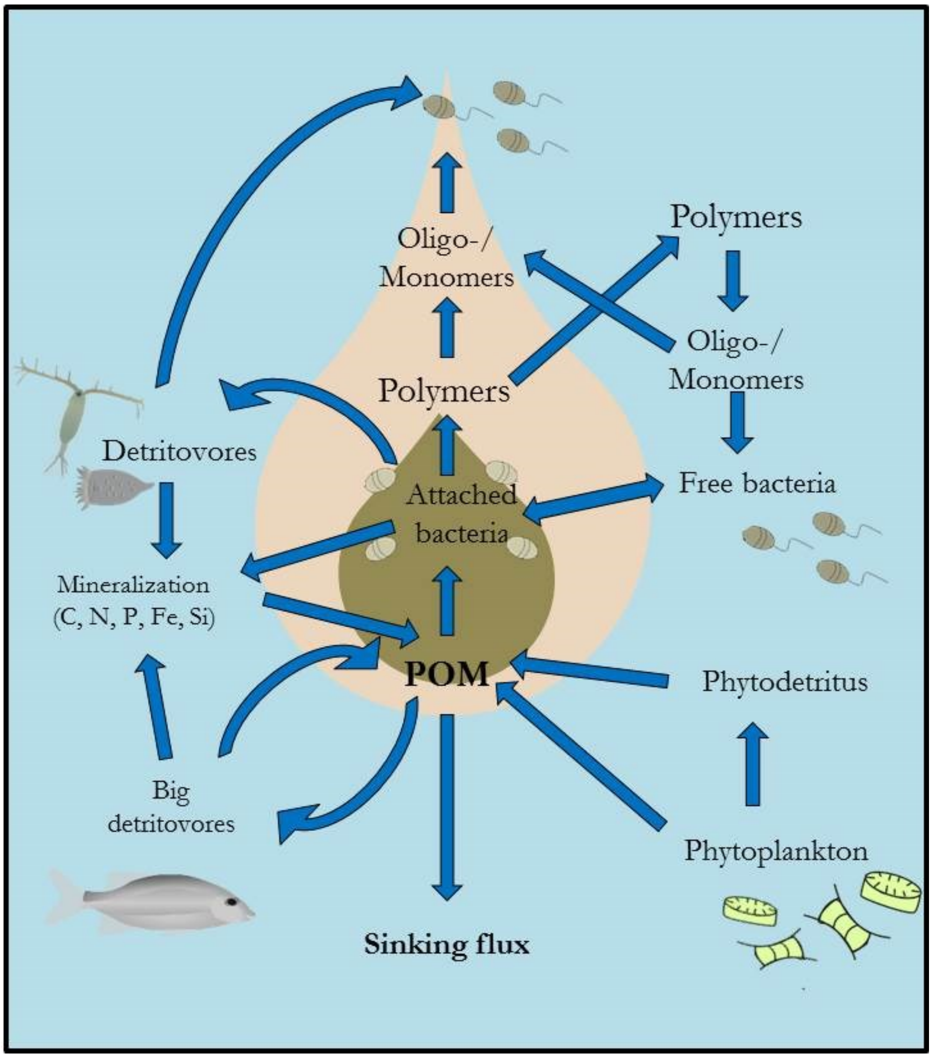


Fig. 1-2 Interaction processes of bacteria with sinking aggregates and particulate organic matter (POM) and relevant decomposition pathways. Modified from Simon et al. (2002).

Tropical, shallow water coral reef sediments, due to their biogenic origin, consist of highly porous calcareous sands (Rasheed et al., 2003). They are often densely colonized by bacteria, reaching abundances of up to 1–2 × 10^9 cells cm^{-2} (Schöttner et al., 2011; Wild et al., 2006). Bacterial communities, especially in the well-oxygenated surface layers of the sediment, are also very diverse, as reported e.g. from the Great Barrier Reef, Australia and Hawaii, USA (Hewson and Fuhrman, 2006; Hewson et al., 2003; Rusch et al., 2009;

Uthicke and McGuire, 2007). The most abundant taxa are *Proteobacteria*, *Planctomycetaceae*, members of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) consortium and *Cyanobacteria* (Rusch et al., 2009; Uthicke and McGuire, 2007). Sediment bacterial communities fulfill several key functions in the coral reef environment. They metabolize and remineralize large proportions of organic matter released in other reef habitats and therefore facilitate the efficient recycling in the reef by retaining nutrients and organic matter (Werner et al., 2006; Wild et al., 2004a, 2004b). But despite the high rates of recycling occurring in the reef environment, additional nutrients are required for net growth of the system (Szmant-Froelich, 1983). Recent findings indicate that bacteria capable of fixing atmospheric dinitrogen play a key role in sustaining the high productivity encountered in coral reefs (Capone et al., 2008; Lesser et al., 2004; Neil and Capone, 2008), especially in highly oligotrophic conditions or at reefs subject to a strong seasonality where nitrogen is often the limiting nutrient (Cardini et al., 2015; Falkowski et al., 1993; Howarth et al., 1988; Thomas, 1970). Thus, nitrogen fixation by diazotrophs in reef sediments is an essential process to introduce new nutrients to the system to maintain primary productivity (Bednarz et al., 2015; Charpy-Roubaud et al., 2001; Werner et al., 2008).

It is now an established paradigm that corals do not only form a mutualistic relationship with zooxanthellae, but they also closely interact with bacteria to form the ‘coral holobiont’ (Bourne et al., 2009; Rohwer et al., 2001, 2002). Whether bacterial communities inhabiting corals are species specific and stable over large geographic areas is yet to be resolved (Barott et al., 2011; Ceh et al., 2011; Klaus et al., 2005; Rohwer et al., 2002). But it is now widely accepted that those bacterial communities forming the ‘coral holobiont’ are not only distinct from the surrounding seawater (Frias-Lopez et al., 2002), but also within niches in the mucus, coral tissue and skeleton of individual coral species (Bourne and Munn, 2005; Koren and Rosenberg, 2006). The number of prokaryotic cells derived from direct in situ cell counts of coral mucus and coral tissue was in the range of abundances encountered in the water column with 1×10^6 cells mL^{-1} (Garren and Azam, 2010). In accordance with the overall high biodiversity encountered in coral reefs, a study by Rohwer et al. (2002) revealed that bacterial species richness was also very high, with a high number of novel genera and species.

In the different niches within the coral holobiont bacteria carry out a large variety of functions (Fig. 1-3) essential in providing the coral host and its symbionts with carbon, nitrogen and other nutrients (Cardini et al., 2015; Knowlton and Rohwer, 2003; Lesser et al., 2004; Rohwer et al., 2002). Kimes et al. (2010) found a high number of bacterial genes involved in the degradation of cellulose, chitin and other polysaccharides in the coral *Montastrea faveolata*. The bacterial community of that coral was also capable of degrading a wide array of xenobiotic compounds such as hydrocarbons or heterocyclic and chlorinated aromatic molecules. In healthy corals bacteria associated with the coral mucus can also fend of opportunistic pathogens (Krediet et al., 2013; Ritchie, 2006; Sweet et al., 2011).

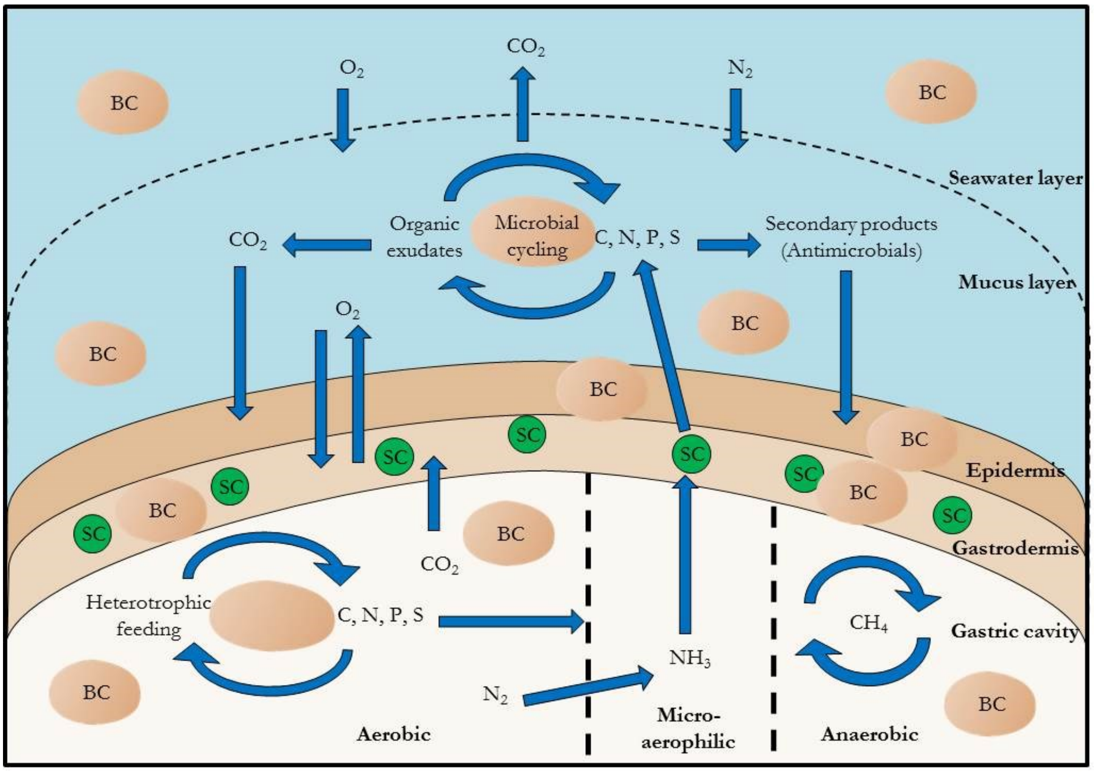


Fig. 1-3 Graphical representation of important processes involving coral-associated bacterial communities. Green circles represent *Symbiodinium* cells, brown ovals represent bacterial communities. Modified from Bourne and Webster (2013).

EUTROPHICATION EFFECTS ON BACTERIAL COMMUNITIES IN TROPICAL CORAL REEF ECOSYSTEMS

Eutrophication is defined as a process of an increasing load of nutrients and organic matter to an ecosystem (Fabricius, 2011). As one of the most common local anthropogenic threats, it can have many detrimental effects in coastal coral reef environments (Bell, 1992; Burke et al., 2011), including the decrease of water clarity through enhanced productivity, increasing sedimentation, enriching sediments with organic matter or changing biogeochemical pathways (Fabricius, 2011). As outlined above, bacteria are key players in many of those habitats subject to eutrophication. And, due to their high surface to volume ratio and very short generation times they will respond much faster to changes in organic and inorganic nutrient availability (Eilers et al., 2000; Meyer-Reil and Köster, 2000).

Although the role of bacteria in important processes in the water column was known for much longer (Azam et al., 1983), investigations of the response of tropical microbial communities in the water column to eutrophication are rare and did not start until the early 1990ies (Gast et al., 1999). Bacterial cell abundance often increases with an increased primary production (Billen et al., 1990; Gasol and Duarte, 2000; Horner-Devine et al., 2003). Bacterial communities of lagoons in the Great Barrier reef shifted towards indicator species for sewage discharge with increased riverine input of particulate matter, nitrogen, phosphorus and particulate organic carbon (POC) (Angly et al., 2016). As primary production and the accumulation of phytoplankton biomass in coastal waters are strongly coupled to the inorganic nutrient input, eutrophication will therefore also enhance the release of organic matter in dissolved and particulate form (Garren et al., 2008). Bacterial communities showed distinct differences in community composition and diversity of the free-living and particle-attached size fractions along gradients from very high inorganic and organic nutrient concentrations at fish ponds to the sites furthest away from the eutrophication source (Garren et al., 2008). A recent study conducted at another, non-tropical reef sites indicated that with higher nutrient concentration the number of prokaryotic cells in seawater and sediments was higher (Zhang et al., 2009). The bacterioplankton in the investigated harbor in Hong Kong was distinctive in the dominance of 16S rRNA sequences of often copiotrophic *Gammaproteobacteria* and *Cytophaga* over those of *Alphaproteobacteria* and the lack of otherwise ubiquitous coastal marine microorganisms such as the *SAR11* group (Zhang et al., 2009). These findings were confirmed in another study of bacterioplankton in the Venice lagoon (Simonato et al., 2010), where copiotrophic bacteria dominated the eutrophic lagoon station. Aggregates formed in those conditions of high productivity with a larger contribution to copiotrophic and pathogenic bacterial groups, so they can act as densely inhabited refugees for pathogenic bacteria and subsequently act as vectors for disease transfer to the benthos (Lyons et al., 2007, 2010).

Bacterial community composition and the distribution in relation to the predominant environmental conditions and water quality parameters in shelf sediments is still understudied compared to other habitats (Huettel et al., 2014). But there is already ample evidence that bacterial activity and oxygen demand can increases several fold after the addition of organic matter, through sedimentation or mucus deposition (Alongi et al., 2007; Bayraktarov and Wild, 2014; Wild et al., 2004b). Carbonate sediments of the Red Sea showed strong seasonal variability in bacterial community composition between the highly stratified, oligotrophic summer well-mixed and productive winter periods (Schöttner et al., 2011). Eutrophication was also identified as the main driver of shifts in sediment bacterial assemblages in Hong Kong, China, where nitrogen, carbon and chlorophyll *a* were the main drivers of differences in community composition (Thiyagarajan et al., 2010). Variation in bacterial community composition of coral reef sands along an inshore to offshore gradient in Indonesia was also primarily driven by differences in satellite-derived colored DOM concentrations (Polónia et al., 2015).

Eutrophication further has the potential to induce shifts in coral-associated microbial community composition and / or activity. Inorganic nutrient and DOC availability can directly enhance the number of bacterial disease-related genes in the coral holobiont and the abundance of disease-associated bacteria inhabiting the coral (Vega Thurber et al., 2009). It also increases the severity of diseases caused by bacteria, such as yellow band disease (Bruno et al., 2003; Furby et al., 2014). But in most cases the effects of increased nutrient loadings are more indirect and related to the stimulation of phytoplankton and macroalgae growth. Increased concentrations of DOC can disrupt the delicate balance in the coral-associated microbial community in the surface mucus layer through the stimulation of bacterial growth, with negative consequences for the health of the coral host (Kline et al., 2006; Mitchell and Chet, 1975). For example, the release of DOM by algae stimulates microbial activity, leading to coral stress in close proximity to the algae (Smith et al., 2006). On larger scales, such as island ecosystems in the Pacific, increased nutrient availability at human-impacted sites also led to an increased macroalgae and phytoplankton abundance and subsequently to higher availability of labile DOM (Dinsdale et al., 2008). The heavily impacted sites were in turn characterized by higher potential pathogen abundance, disease prevalence and overall lowest coral cover. Fish farm effluent treatments, characterized by elevated levels of DOC and chlorophyll *a*, also induced rapid and profound shifts in the coral-associated bacterial community, which included an increase in the prevalence of human and coral pathogens (Garren et al., 2009). It is noteworthy though, that corals often exhibited a strong recovery potential after short term exposure to organic matter stress (Garren et al., 2009; Kuntz et al., 2005), and bacterial communities often returned to the original composition (Garren et al., 2009).

GAPS OF KNOWLEDGE/JUSTIFICATION OF STUDY

Indonesia is home to the highest biological diversity of scleractinian corals and coral reef fishes (Veron et al., 2009). Over 80 % of its more than 240 million inhabitants live within 50 km to the coast (Ferse et al., 2012). And it is also home to one of the largest marine fisheries of the world (Hopley and Suharsono, 2000). The fisheries mainly rely on small-scale artisanal fisherman, which make up 95 % of the total catch landed annually (Ferse et al., 2012). But, despite increasing coastal populations and warnings of a fisheries collapse already more than two decades ago, intensive fishing, often using destructive, indiscriminate and illegal techniques, continues unabated (Erdmann, 1995; Pet-Soede and Erdmann, 1998; Pet-Soede et al., 2001). In addition to a deteriorating water quality that is threatening coral reefs of the archipelago, they also have to cope with frequent mechanical damage from bombings, resulting in habitat destruction and loss of functionally critical fish communities (Plass-Johnson et al., 2016).

Nevertheless, the fishermen of the Spermonde Archipelago continue to rely on reefs to sustain the high yields required to provide adequate food and income. And healthy reefs, in turn, are dependent on favorable conditions concerning water quality to recover, survive and proliferate (Bell et al., 2014; Fabricius, 2005; Fabricius et al., 2003). Given the key role bacteria play in a functioning reef, from organic matter recycling to coral health; it is noteworthy that very little effort has been made to understand how bacterial communities from different habitats are affected by both changing environmental conditions and water quality in the Spermonde Archipelago. This response has been tested extensively over the past decades for coral cover (Becking et al., 2006; Cleary et al., 2005; Edinger et al., 1998; Moll, 1983), sponges (Becking et al., 2006; de Voogd et al., 2006), reef fish functional variability and herbivory (Plass-Johnson et al., 2015, 2016), as well as foraminifera (Becking et al., 2006; Cleary et al., 2005; Renema and Troelstra, 2001). To date, there are only two studies of the Spermonde Archipelago that have focused on bacterial diversity, one on settlement tile biofilms (Sawall et al., 2012) and another on bacterial communities from different reef habitats, specifically within sponges and the functional role of the associated bacteria (Cleary et al. 2015). Our study further examined the relationship between bacterial communities, habitats, and the gradients from inshore to the outer shelf in the Spermonde Archipelago. In addition to previous studies we also included mucus of the common hard coral genus *Fungia* as an important bacterial habitat. This will allow us to investigate whether the coral host will be able to maintain control over its bacterial symbiont community composition at different amplitudes of anthropogenic stress induced by eutrophication. So far, investigations of bacterial communities have only been conducted along larger scale gradients. Our aim was to elucidate whether larger scale processes driven by terrestrial input or localized stressors from untreated sewage of the individual islands have a greater potential to shift bacterial community composition into undesirable states dominated by copiotrophic and potentially pathogenic taxa. Therefore we designed that study to be able to discriminate between those two spatial scales by comparing an uninhabited to a densely populated at a very similar distance from riverine and untreated sewage sources. This will close an important knowledge gap and enable coastal managers to better align the applied methods with identified local or regional eutrophication hot-spots.

We also included TEP analyses, to the best of our knowledge, as the first study in the Spermonde Archipelago. TEP is an important, but frequently overlooked, biogeochemical water quality parameter that is pivotal for the organic matter composition and transition from the dissolved to the particulate fraction. Due to its high stickiness it also greatly enhances the aggregation potential of smaller particles, therefore playing a key role in the flux of large aggregates rich in organic matter to the benthos. As increased sedimentation can have many detrimental effects to benthic communities, including scleractinian corals, TEP should be an integral parameter in standard water quality assessments. But if increased sedimentation occurs in localized and confined areas close to the source, and away from coral reefs, it might even be helpful in containing the extent of the negative impact of eutrophic conditions. Through TEP it can channel the inorganic nutrients through phytoplankton biomass and aggregates directly to the sediment. Additionally, its formation is tightly linked to the interaction of phytoplankton with bacteria (Gärdes et al., 2011). With those properties, TEP may also significantly alter bacterial community composition and their provided ecosystem functions in the water column as well as in the sediments receiving the formed aggregates (Buchan et al., 2014; Passow, 2002; Taylor et al., 2014).

Within this framework three main questions were addressed in this thesis:

1) *How does the availability of organic and inorganic nutrients influence the abundance and community structure of bacteria in the water column, sediments and on benthic marine biota of the Spermonde Archipelago?*
2) *What role does water quality have in aggregate formation and bacterial community composition on settling aggregates along large scale gradients in tropical reef waters?*
3) *Do the small inhabited islands of the archipelago have a significant impact on water quality parameters of the surrounding water masses?*

This thesis is based on two field studies to the Spermonde Archipelago, Indonesia in 2013 and 2014 and one research stay in Misool, Indonesia in 2013. The different research questions were addressed in three main chapters (*Chapters 2, 3 and 4*), following this general introduction and identification of major research gaps for the Spermonde Archipelago (*Chapter 1*). *Chapter 5* lists additional publications also conducted in the framework of this thesis which report further investigations and observations. The general discussion (*Chapter 6*) synthesizes the main research chapters and discusses the findings in the broader context of the Spermonde Archipelago.

PUBLICATION OUTLINE

Chapter 2 – Bacterial communities along a cross-shelf eutrophication gradient

Coastal eutrophication controls the bacterial community composition in different reef habitats of the Spermonde Archipelago, Indonesia

H F Kegler, M Lukman, M Teichberg, J G Plass-Johnson, C Wild, A Gärdes

Contributions: This project was initiated by H. F. Kegler, A. Gärdes, C. Wild, M. Teichberg and M. Lukman. The experimental design for this study was developed by H. F. Kegler, A. Gärdes and C. Wild. Sampling was conducted by H. Kegler, J. G. Plass-Johnson, A. Gärdes. Data analysis was conducted by H. F. Kegler and the manuscript was written by H. F. Kegler with improvements from all contributing authors.

Chapter 3 – Aggregate formation and associated bacterial communities along gradients of eutrophication

Intensive nutrient uptake and particle aggregation mitigate eutrophication at more distant reefs at a tropical coastal shelf ecosystem

H F Kegler, T C Jennerjahn, P Kegler, J Jompa, A Gärdes

Contributions: This project was initiated by H. F. Kegler, A. Gärdes, T. C. Jennerjahn and J. Jompa. The experimental design for this study was developed by H. F. Kegler, A. Gärdes and T. C. Jennerjahn. Sampling was conducted by H. F. Kegler, P. Kegler and A. Gärdes. Data analysis was conducted by H. F. Kegler and the manuscript was written by H. F. Kegler with improvements from all contributing authors.

Chapter 4 – Small scale gradients of eutrophication and bacterial community composition

Small tropical islands with dense human populations: Decreased water quality but little effects on bacterial community composition

H F Kegler, P Kegler, T C Jennerjahn, M Lukman, C Hassenrueck, J Jompa, A Gärdes

Contributions: This project was initiated by H. F. Kegler, A. Gärdes, T. C. Jennerjahn, M. Lukman and J. Jompa. The experimental design for this study was developed by H. F. Kegler, A. Gärdes and T. C. Jennerjahn. Sampling was conducted by H. F. Kegler, P. Kegler and A. Gärdes. Data analysis was conducted by H. F. Kegler, P. Kegler and C. Hassenrück and the manuscript was written by H. F. Kegler with improvements from all contributing authors.

Chapter 5 – Related publication to the main work carried out in the course of this thesis

The formation of aggregates in coral reef waters under elevated concentrations of dissolved inorganic and organic carbon: A mesocosm approach

A Cárdenas, F W Meyer, **H F Schwieder***, C Wild, A Gärdes

Contributions: This project was initiated by H. F. Schwieder, A. Cárdenas and A. Gärdes. The experimental design for this study was developed by A. Cárdenas, F. Meyer, H. F. Schwieder and A. Gärdes. Sampling and data analysis were conducted by A. Cárdenas and F. Meyer and the manuscript was written by A. Cárdenas with improvements from all contributing authors.

Persist or desist: revealing (the lack of) patterns in benthic-fish association on coral reefs of the Spermonde Archipelago, Indonesia

J G Plass-Johnson, V N Bednarz, **H F Kegler**, C Wild, A Gärdes, M Lukman, H Reuter, S C A Ferse, M Teichberg

Contributions: This project was initiated by J. G. Plass-Johnson, M. Teichberg, C. Wild and S. C. A. Ferse. The experimental design for this study was developed by J.G. Plass-Johnson, M. Teichberg and S. C. A. Ferse. Sampling was conducted by J. G. Plass-Johnson, H. F. Kegler, V. N. Bednarz, A. Gärdes, H. Reuter and S. C. A. Ferse. Data analysis was conducted by J.G. Plass-Johnson and the manuscript was written by J. G. Plass-Johnson with improvements from all contributing authors.

Different levels of anthropogenic impact influence coral larvae settlement and bacterial biofilm communities in the Spermonde Archipelago, Indonesia

P Kegler, **H F Kegler**, A Kunzmann, A Gärdes, Y R Alfiansah, M Lukman, C Hassenrück, S C A Ferse

Contributions: This project was initiated by P. Kegler, H. F. Kegler, A. Kunzmann and A. Gärdes. Experimental design was developed by P. Kegler, H. F. Kegler, A. Kunzmann, A. Gärdes and S. C. A. Ferse. Sampling was conducted by P. Kegler, H. F. Kegler and A. Gärdes, aided by M. Lukman. Data analysis was performed by P. Kegler and C. Hassenrück. Data discussion and writing of the manuscript was done mainly by P. Kegler with contributions from all authors.

A recent *Acanthaster planci* outbreak in the Spermonde Archipelago, Indonesia

J G Plass-Johnson, **H F Schwieder***, J P Heiden, L Weiand, C Wild , S C A Ferse, J Jompa, M Teichberg

Contributions: This project was initiated by J. G. Plass-Johnson, M. Teichberg, and S. C. A. Ferse. The experimental design for this study was developed by J. G. Plass-Johnson, M. Teichberg and S. C. A. Ferse. Sampling was conducted by J. G. Plass-Johnson, J. P. Heiden, H. F. Schwieder, L. Weiand, and S. C. A. Ferse. Data analysis was conducted by J. G. Plass-Johnson and the manuscript was written by J. G. Plass-Johnson with improvements from all contributing authors.

First assessment of bacterial community composition in relation to water quality and human sewage input in the epicenter of coral biodiversity: Misool, Raja Ampat, Indonesia

A Gärdes, **H F Kegler,** Purwanto, J T Jeske, J Jompa

Contributions: This project was initiated by A. Gärdes, H. F. Kegler and J. Jompa. Experimental design was developed by A. Gärdes, H. F. Kegler and Purwanto. Sampling was conducted by A. Gärdes, H. F. Kegler and Purwanto. Data analysis was performed by J. T. Jeske, Purwanto and H. F. Kegler. Writing of the manuscript was done by A. Gärdes, J. T. Jeske, H. F. Kegler and Purwanto with improvements from J. Jompa.

*** these manuscripts were submitted under the authors name given at birth (Hauke Fabian Schwieder)**

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Chapter 2

Coastal eutrophication controls the bacterial community composition in different reef habitats of the Spermonde Archipelago, Indonesia.

Coastal eutrophication controls the bacterial community composition in different reef habitats of the Spermonde Archipelago, Indonesia.

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ABSTRACT

Coastal eutrophication is a key driver of shifts in bacterial communities, but related knowledge is very scarce. With fringing and patch reefs at varying distances from the coast the Spermonde Archipelago in southern Sulawesi, Indonesia offers ideal conditions to study the effects of coastal eutrophication along a spatially defined gradient. The present study investigated bacterial community composition of three coral reef habitats: the water column, sediments and mucus of the hard coral genus *Fungia*, along that cross-shelf environmental and water quality gradient. The main research questions were: (1) How do bacterial communities respond to changes in water quality along a spatial gradient? (2) Which water quality parameters influence the bacterial community composition? (3) Are there bacteria community differences between the different investigated microbial habitats? For this purpose a range of key water parameters were measured at eight stations in distances from 2 to 55 km from urban Makassar. This was supplemented by sampling of bacterial communities of important microbial habitats using 454 pyrosequencing. Findings revealed that the population center Makassar had a strong effect on the concentrations of chlorophyll *a*, suspended particulate matter (SPM) and transparent exopolymer particles (TEP), which were all significantly elevated at the inshore compared the other seven sites. Shifts in the bacterial communities were specific to each sampled habitat. In the water column, the relative abundance of *Gammaproteobacteria* increased with distance from Makassar, while that of *Alphaproteobacteria* decreased. In the sediments, there was a pronounced dominance of *Gammaproteobacteria* at the inshore site, which decreased along the gradient. There was no gradual shift in bacterial classes for samples obtained from the *Fungia* mucus. We observed a strong positive correlation between *Bacteroidia*, *Chlamydiia* and *Acidobacteria_Gp6* and chlorophyll *a*, TEP and SPM. *Deinocooci*, *Verrumicrobiae* and *Alphaproteobacteria* were, on the other hand, positively correlated to DOC concentrations. In addition, we observed very distinct communities between the investigated habitats. Our data shows strong changes in the bacterial community composition at the inshore site for water column and sediment samples. Alarmingly, this led to a higher prevalence of potentially pathogenic bacteria at the chronically impacted site closest to Makassar.

INTRODUCTION

Coastal coral reef systems in close vicinity to highly populated urban areas are often impacted by land-based activities. The Spermonde Archipelago, including its approximately 150 small islands, is located on a narrow, 60 km wide carbonate shelf platform in southern Sulawesi, Indonesia. The coral reefs fringing the islands are essential to sustain the livelihoods of thousands of fishermen in the archipelago as a source of income and building material for local construction (Pet-Soede et al., 2001). The Archipelago is characterized by an eutrophication gradient from nutrient-rich coastal waters and oligotrophic offshore waters (Edinger et al., 1998). Untreated sewage and pollutants from Makassar enter the system directly or via the river Jene Berang, which additionally discharges sediments and inorganic nutrients from the hinterland (Renema and Troelstra, 2001). This leads to eutrophication, one of the primary local threats to coastal marine ecosystems (Burke et al., 2011; Paerl et al., 2014). The first response to eutrophication is often an increase in phytoplankton biomass (Fabricius, 2011). The result is an increased availability of organic matter such as dissolved organic carbon (DOC) and subsequently transparent exopolymer particles TEP (Passow, 2000; Verdugo and Santschi, 2010; Verdugo et al., 2004). High concentrations of TEP in the water column will in turn intensify aggregation and sedimentation processes due to their high stickiness (Azam and Malfatti, 2007; Passow, 2002). The sinking particles and TEP itself are rich sources of organic matter for both free-living and particle-attached bacteria in the water column (Kiørboe and Tang, 2003; Kramer et al., 2013; Passow and Alldredge, 1994).

Several studies found significant shifts in the bacterial community composition in eutrophic and organic matter rich conditions of reef waters (Meyer-Reil and Köster, 2000; de Voogd et al., 2015; Weinbauer et al., 2010), microbial biofilms (Sawall et al., 2012; Witt et al., 2012) and sediments (Uthicke and McGuire, 2007) often alongside an increase in total bacterial cell counts (Dinsdale et al., 2008; Zhang et al., 2007, 2009). The changes are often related to a transition from autotrophic to heterotrophic bacterial communities (Meyer-Reil and Köster, 2000; Witt et al., 2012). There are often two important groups of bacteria, free-living and particle-attached, which are both influenced differently by changes in water quality and use different carbon sources (Becquevort et al., 1998; Zhang et al., 2007). Understanding the response of microbial communities in different coral reef habitats to spatial gradients in eutrophication is of great importance in the context of increasing anthropogenic perturbations to coastal water quality in the Spermonde

Archipelago. As they play such an important role in biogeochemical cycling and coral reef health, small shifts induced by increased anthropogenic eutrophication can further alter nutrient cycling, sedimentation and organic matter export as well as promoting coral pathogens (Bruno et al., 2003; Fabricius, 2005; Lyons et al., 2010).

To date, there are only two studies of the Spermonde Archipelago that have focused on bacterial diversity of settlement tile biofilms (Sawall et al., 2012) and bacterial communities from different reef habitats, specifically within sponges and the functional role of the associated bacteria (Cleary et al. 2015). Our study now further examines the relationship between bacterial communities, habitats and water quality gradients in the Spermonde Archipelago, and additionally includes mucus of the common hard corals genus *Fungia* as another important bacterial habitat. We also included TEP, to the best of our knowledge, as the first study in the Spermonde Archipelago. TEP is an important, but frequently overlooked, biogeochemical water quality parameter that is pivotal for the organic matter composition and transition from the dissolved to the particulate fraction. It's formation is tightly linked to the interaction of phytoplankton with bacteria (Gärdes et al., 2011). With those properties, TEP may significantly alter bacterial communities and their provided ecosystem functions (Buchan et al., 2014; Passow, 2002; Taylor et al., 2014). Through this multifaceted approach, including the eutrophication-related parameters and bacterial communities from different habitats, we wanted to elucidate if the relative abundance of different bacterial phylogenetic groups shift in response to changes in environmental and water quality parameters including: pH, salinity, inorganic nutrient availability, chlorophyll *a*, DOC, TEP and SPM along the eutrophication gradient. The main research questions were: a) How do microbial (bacterial) communities respond to changes in water quality along a spatial gradient? b) Which water quality parameters influence the bacterial community composition? c) What are the bacteria community differences between different microbial habitats (water column, reef sediments, coral mucus)?

MATERIALS AND METHODS

Study sites

Sampling was carried out at eight islands across the continental shelf of the Spermonde Archipelago in South Sulawesi, Indonesia after the wet monsoon season in February 2013 (Fig. 2-1). Due to environmental and ecological variability across the shelf, the archipelago has been divided into several ecological zones running parallel to the coast line (Moll, 1983;

Renema and Troelstra, 2001). The chosen sites represent varying exposure to eutrophication from an inshore station in close proximity to metropolitan Makassar (1 km) to the outer shelf break at 55 km, and were therefore classified into four zones (modified from Moll, (1983); Fig. 2-1). The inshore site is characterized by greatly reduced water clarity and is frequently exposed to discharge from rivers and effluents from the city of Makassar (Moll, 1983; Renema and Troelstra, 2001; Sawall et al., 2011). Near-shore and midshelf sites only receive additional effluent loads during times of increased riverine inputs in monsoon seasons (Cleary et al., 2005). All other sites are only affected while experiencing extreme rain events. All islands with one exception are inhabited. Only the outer shelf site at 27 km is a submerged reef platform. We standardized the sampling by always sampling during high tide in the morning hours and by choosing a site in the northwestern area of each island, the area of highest reef accretion.

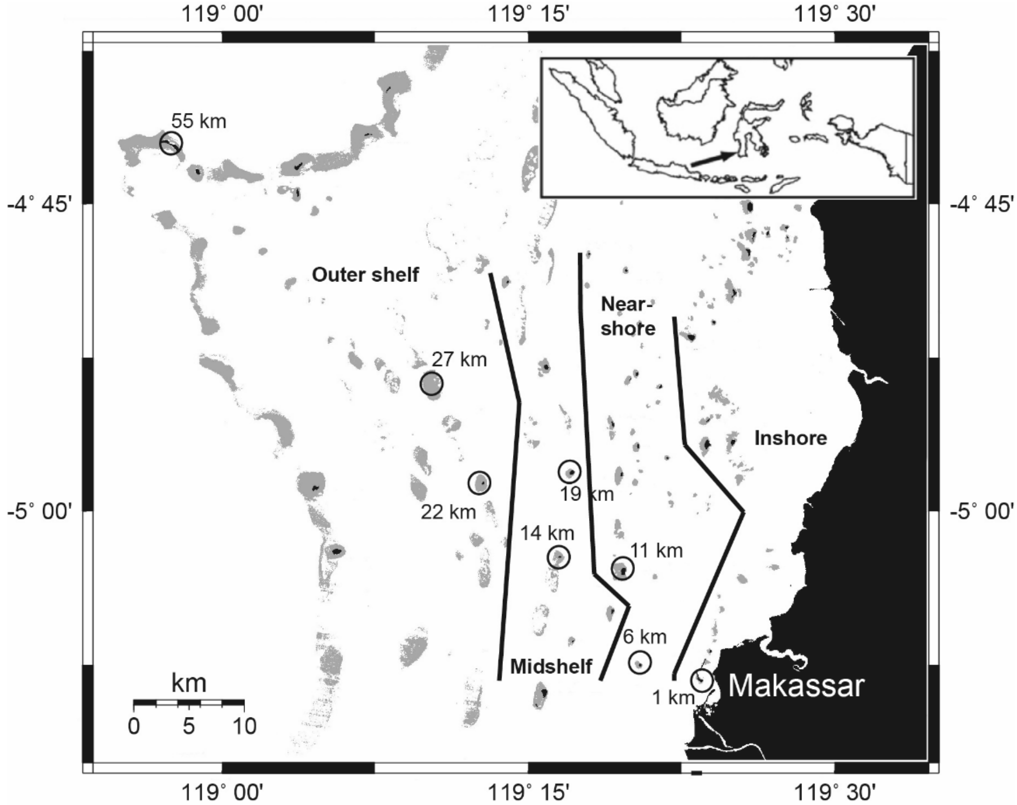

Fig. 2-1 Map of the Spermonde Archipelago in southern Sulawesi. Sampling stations are circled with the according distance to Makassar. Map modified from Glaser et al. (2010), zonation modified from Renema and Toelstra (2001).

Environmental and water quality parameters

Five replicate water samples were taken from 5 m water depth (which was approximately 1 m above the substrate) with a 5 L Niskin bottle (*HydroBios, Kiel, Germany*). From each replicate subsamples were taken for the water quality parameters (inorganic nutrients, DOC, TEP and SPM), while the environmental parameters and chlorophyll *a* were measured *in-vivo* with a Eureka Manta 2 multiprobe (*eureka water probes, Austin, USA*) equipped with an optical sensor. Samples were stored at -20 °C in the dark until analysis immediately after returning to the field station. The longest travel time was no more than three hours. Inorganic nutrients (nitrite, nitrate, phosphate and silicate) where measured spectro-photometrically with a Flowsys continuous flow analyzer (*Unity scientific, Brookfield, USA*). For dissolved organic carbon (DOC), samples were filtered through 0.45 µm pore Whatman GF/F filters *(Whatman, GE Healthcare, Pittsburgh, USA)* and acidified with HCl (pH below 2). The measurement was completed via high-temperature oxic combustion (HTOC) using a TOC-VCPH TOC analyzer (Shimadzu, Mandel, Canada). Hansell artificial seawater standards (Hansell laboratory RSMAS, University of Miami) and ultrapure water blanks were used for calibration and quality control. To determine SPM mass, pre-combusted GF/F filter were weighed on a ME 36S (Sartorius, Göttingen, Germany) before and after filtration of known volume of sample water. Difference in weight was determined after filters were dried for 24h at 40 °C. TEP was quantified with an updated protocol (Engel, 2009) of the spectrophotometric method first introduced by Passow and Alldredge (1995). In short this method relates the adsorption of a dye to the weight of polysaccharides filtered on 0.4 µm polycarbonate filters. To relate the absorbance measured on the filters to a reference polysaccharide we prepared a calibration curve by filtering and staining different volumes of Gum Xanthan from *Xanthomonas campestris* cultures.

DNA extraction and 454 sequencing

From each site, a 1 L subsample was filtered sequentially using 3 µm and 0.2 µm Whatman Nuclepore polycarbonate filters *(Whatman, GE Healthcare, Pittsburgh, USA)*, to separate bacterioplankton in two selected size fractions representing 'particle-attached' and 'free-living' bacteria, respectively. DNA extraction followed for water column samples the protocol established by Boström et al. (2004) without modification. Surface sediment was collected from the uppermost 1 cm of sediment at each site. Sediment samples were allocated to 2.0 mL tubes (*Eppendorf, Germany*) and stored at -20 °C until extraction. Coral mucus samples were obtained from individual corals of the genus *Fungia* collected at the same depth by exposing them to air for about 1 min. DNA extraction for sediments and mucus were conducted using the PowerSoil^{TM} DNA Isolation Kit (*MO BIO Laboratories, Carlsbad, USA*) with modification of two steps of the protocol: 1) we did not incubate for 5 min at 4 °C but went straight for the centrifugation and 2) we used 50 µL of elution buffer instead of 100 µL. Extracted DNA samples were sequenced, after 16S rRNA amplification (PCR), Roche/454-tagging and preparation of the Pico Titer Plate, on a Genome Sequencer FLX System + Titanium (*Roche, Basel, Switzerland*) by LGC Genomics (Berlin, Germany). 16S rRNA primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 1061R (5'-CRRCACGAGCTGACGAC-3') were used, targeting the V3 – V6 hypervariable region (Ong et al., 2013). We carried out all subsequent analyses using the RDP sequencing pipeline online tool package provided by the Center for Microbial Ecology at Michigan State University, USA (Cole et al., 2009). During the first initial step we removed the primers and barcodes from the sequences. Sequences < 500 bp were excluded, as well as samples with quality values lower than 20. Additionally, sequences were removed when containing more than two sequencing mismatches or ambiguous bases. All sequences that passed the quality control were checked for chimeric sequences using USEARCH 6.0 (Uchime denovo mode, Edgar et al., 2011). Processed and trimmed sequences where then aligned with the secondary-structure aware Infernal aligner version 1.1rc4 (Nawrocki et al., 2009) before a complete linkage clustering was conducted. The number of OTUs, OTU richness, Chao1 and Shannon-Weaver diversity indices were determined at 3% dissimilarity. Using the RDP tools naïve Bayesian classifier we also did the classification of the present OTUs at a 80% confidence cutoff.

Statistical analysis

Differences among sites in environmental parameters and water quality were analyzed with SigmaPlot 13.0 software (*Systat Software, Inc., San Jose, California, USA*). Concentrations are given in arithmetic mean ± standard deviation. All parameters failed the Shapiro-Wilk test for normal distribution, so alternative non-parametric Kruskal-Wallis test on ranks was performed. Whenever significant differences were detected, pair-wise comparisons using Tukey’s HSD post-hoc tests were carried out. Principle component analysis (PCA), non-metrical multi-dimensional scaling (NMDS) plotting and hierarchical clustering of changes between the stations including all environmental and water quality parameters was conducted using the PRIMER 6.16 software (Clarke and Gorley, 2006).

Statistical analysis of the bacterial communities to compare diversity, changes in abundance, and to relate bacterial communities to differences in environmental and water quality parameters were carried out with the PRIMER 6.16 (Clarke and Gorley, 2006) and Calypso 3.4 software (Queensland Institute of Medical Research, http://bioinfo.qimr.edu.au/calypso/).

RESULTS

Spatial variation of environmental and water quality parameters

There was no observable trend in environmental parameters (Salinity, temperature, pH) or inorganic nutrients (PO_4, NO_x) across the surveyed gradient from the inshore site to the outer shelf break (Table 2-1). In contrast to inorganic nutrients, all measured organic nutrient parameters showed changes associated with distance from shore, confirming previous studies of a land-derived water quality gradient (Fig. 2-2; Edinger et al., 1998; Renema and Troelstra, 2001; Sawall et al., 2012). Chlorophyll *a* exhibited significantly higher concentrations inshore (1.50 ± 0.21 µg L^{-1}) compared to the furthest outer shelf site (0.14 ± 0.05 µg L^{-1}). No significant differences were found in SPM concentrations although there was a general decreasing trend from inshore to the outer shelf. There were significant differences between the highest concentrations of DOC (97.44 ± 17.76 µmol L^{-1}) at the closest near-shore station and lowest concentrations, 78.46 ± 18.04 µmol L^{-1}, at the inshore station. Differences in DOC concentrations between these two stations were significantly different. But although cross-shelf concentrations of DOC were significantly different ($P < 0.01$), there was only a very low correlation to the distance from Makassar ($r^2 = 0.04$). Overall chlorophyll *a*, TEP and SPM showed a very similar pattern with steeply declining concentrations between the inshore and the first near-shore site. Near-shore concentrations of TEP were significantly ($P < 0.02$) higher (301.38 ± 11.98 µg Xeq L^{-1}) than the most distant outer shelf site (26.34 ± 1.43 µg Xeq L^{-1}) The two near-shore sites showed intermediate concentrations while all following stations were in a comparable range to the outermost site.

The first two principal components of the PCA (Fig. 2-3) accounted for 66.2 % of the variation in environmental and water quality parameters among sites. The inshore site separates clearly from near-shore and mid-shelf sites by the first principal component. A second group of offshore sites separated on the second PC largely driven by lower salinity and pH values. This was confirmed by the hierarchical clustering (Suppl. 2-1) where there was low similarity between the inshore island and the remaining sites. Within those remaining islands two additional larger clusters formed. Samalona and Barrang Lompo, both categorized as near-shore islands showed a very high similarity. Additionally all mid-shelf islands formed a distinct group significantly different from the other groups.

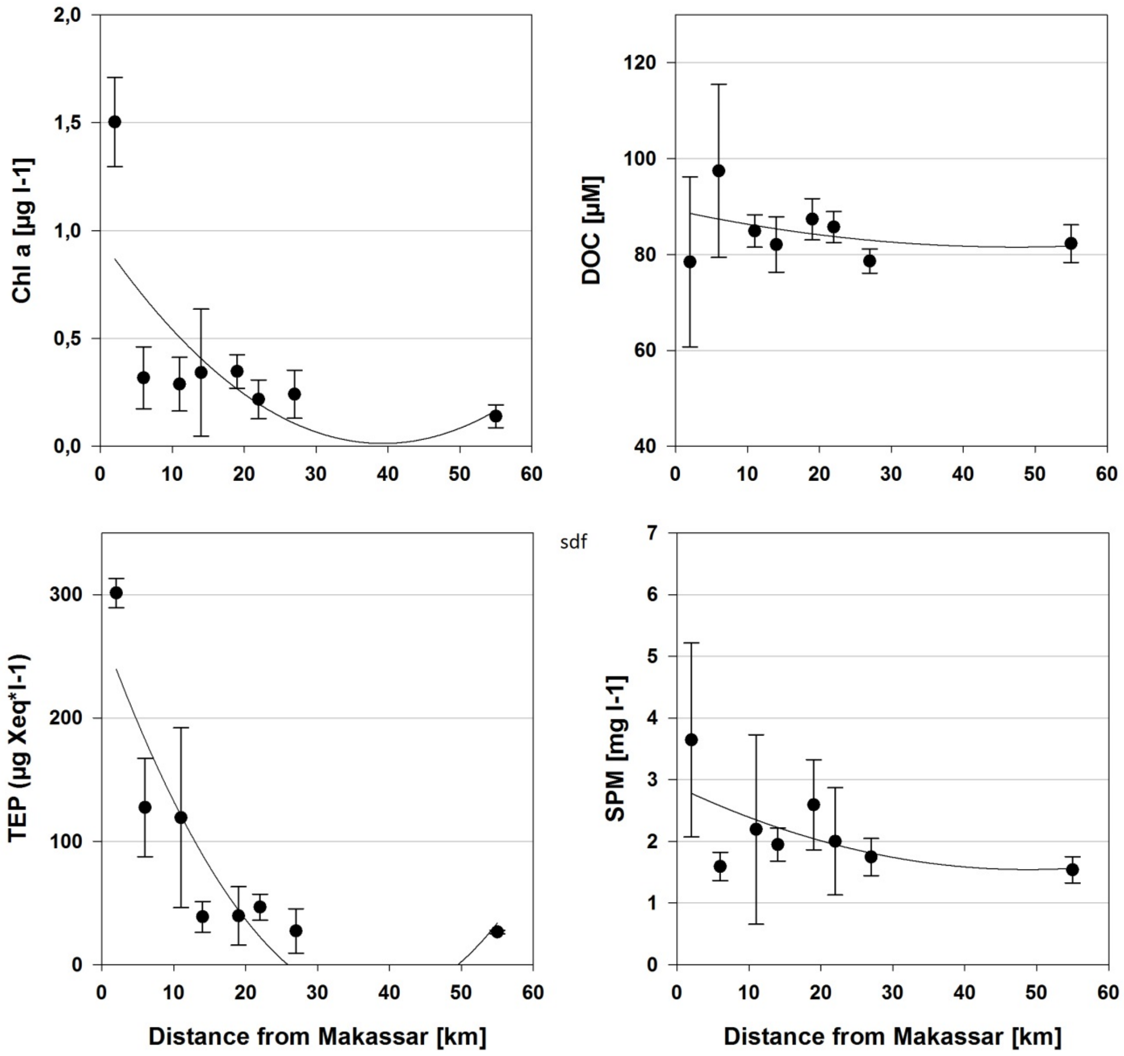

Fig. 2-2 Mean (±SD) concentrations of the selected water quality parameters that were sampled along the hypothesized cross-shelf gradient from the island closest to Makassar (2 km) to the most distant island on the outer shelf (55 km). Best-fitting regressions were included. Correlation to distance was strongest for TEP (r2 = 0.75), followed by chlorophyll a (r2 = 0.41), SPM (r2 = 0.13) and DOC (r2 = 0.04).

Spatial variation of bacterial communities

There was an average of 572 and 804 sequences per station for the 0.2 µm and 3.0 µm size fractions of the water column, respectively. For the investigated reef sediments we identified an average of 351 sequences at each station, while there was an average of 665 sequences per station in the *Fungia* mucus. Shannon-Diversity (H' = 1.92) and Evenness (J' = 0.69) of the mucus were only slightly lower compared to the water column. Water column Shannon-Diversity and Evenness were H' = 2.01 and J' = 0.70, respectively.

The observed bacterial communities of the sediments were generally more evenly distributed (J’ = 0.90) and diverse (H’ = 3.20) compared to index values from the water column and the coral mucus.

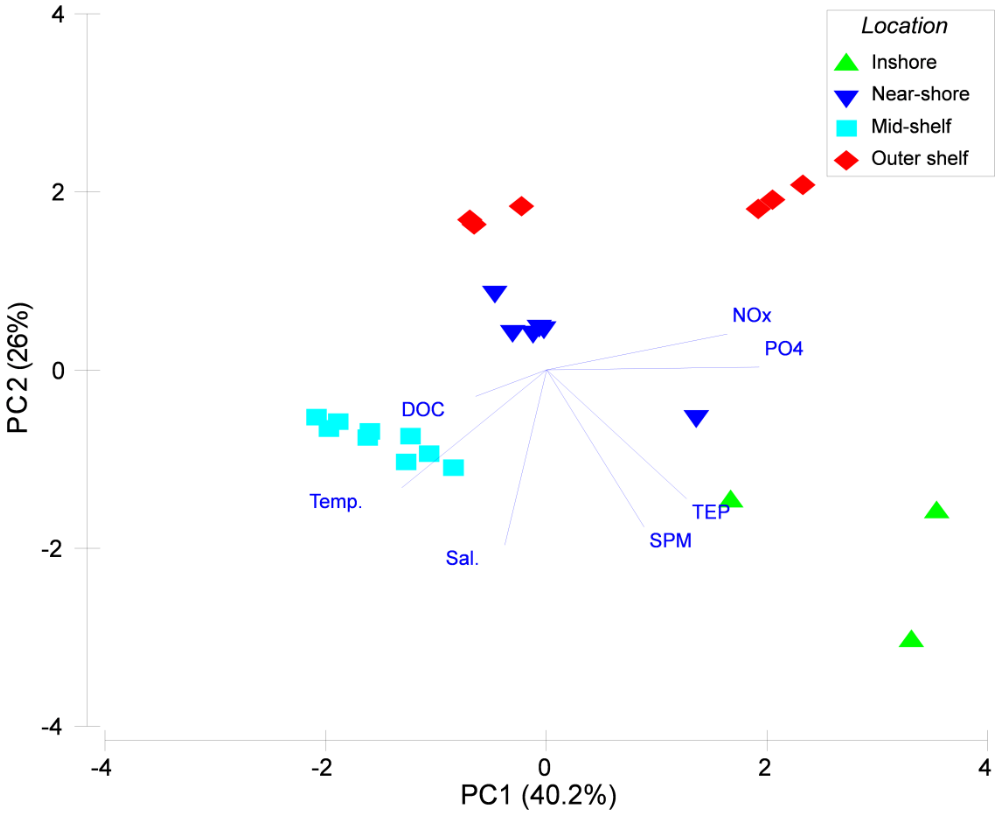

Fig. 2-3 Principal component (PC) analysis of environmental and water quality parameters for the islands included in the cross-shelf gradient. Green triangles show samples from the inshore site, blue inverted triangles samples from the inshore area, turquoise squares mid-shelf sites and red diamonds indicates samples from the outer shelf sampling stations.

No DAPI counts were conducted for the for the water column of the furthest outer shelf station and the sediments. DAPI cell counts of the combined water column bacterial abundance ranged from 7.94 x 10^6 ± 1.08 x 10^6 cells per mL at the inshore site to 4.39 x 10^6 ± 4.70 x 10^6 cells per mL in the outer shelf area. 5.87 x 10^6 ± 5.45 x 10^5 to 3.23 x 10^6 ± 2.66 x 10^5 cells were counted in the free-living size fraction at the inshore and midshelf sites, respectively. DAPI cell counts for the particle-attached fraction ranged from 2.07 x 10^6 ± 5.22 x 10^5 at the inshore site to 1.02 x 10^6 ± 7.44 x 10^5 cells within the midshelf sites. Bacterial abundance in the *Fungia* mucus ranged from 9.61 x 10^7 ± 7.88 x 10^6 cells at one of the midshelf sites to 3.34 x 10^7 ± 2.58 x 10^6 cells found within the near-shore area. Biological oxygen demand of the water column was highest closer to shore, while that of sediments was more variable and was more related to local conditions.

Water column

The "free-living" fraction of the water column bacterial communities showed variation among stations across the spatial gradient. Four classes constituted the majority of all identified bacteria of the "free-living" fraction of the water column: the *Actinobacteria, Alpha-*, *Beta-* and *Gammaproteobacteria* (Fig. 2-4). The relative dominance of *Gammaproteobacteria* increased from 26.7 % at the inshore sampling station to 48.5 % at the stations on the outer shelf break (Fig. 2-4). In contrast, *Alphaproteobacteria* showed highest concentrations at the inshore site (19.7 %) and decreased towards the mid-shelf sites (average 8.3 %). *Actinobacteria* and *Betaproteobacteria* showed no obvious trends in changing abundances across the gradient, and their relative contribution to the total community varied from 10.7 – 20.4 % and 22.2 – 37.9 %, respectively. All mentioned classes contain genera that are potential human pathogens.

Among all the bacterial classes that were retained on a 3.0 µm filter, and thus being defined as "particle-attached", the dominant classes where again *Actinobacteria*, *Alpha-*, *Beta-* and *Gammaproteobacteria*. *Betaproteobacteria*, on average the most abundant class across the whole gradient, were generally found to be less abundant at the inshore station (21.4 %, Fig. 2-4b). The relative abundance was highest at the mid-shelf stations (34.1 %). At all stations, members of the genus *Ralstonia* dominated the *Betaproteobacteria* class. Abundance of *Alphaproteobacteria* decreased from 27.8 % at the inshore site to 17.2 % at the outer shelf. The *Gammaproteobacteria* were mainly made up of *Escherichia/Shigella* and *Stenotrophomonas* groups and both of them contain potentially pathogenic bacteria.

Reef sediments

Overall the diverse sediments showed little variation across the shelf (Fig. 2-5). Only two bacterial groups exhibited stronger changes in relative abundance across the gradient. *Gammaproteobacteria*, and within this class the genera *Escherichia/Shigella* and *Steroidobacter*, showed much higher OTU counts at the inshore station, which is continuously affected by effluents from urban Makassar (46.6 %) compared to further off-shore (mean of 23.8 % across all other stations). The other one, *Planctomycetatia*, showed an increase from inshore to midshelf.

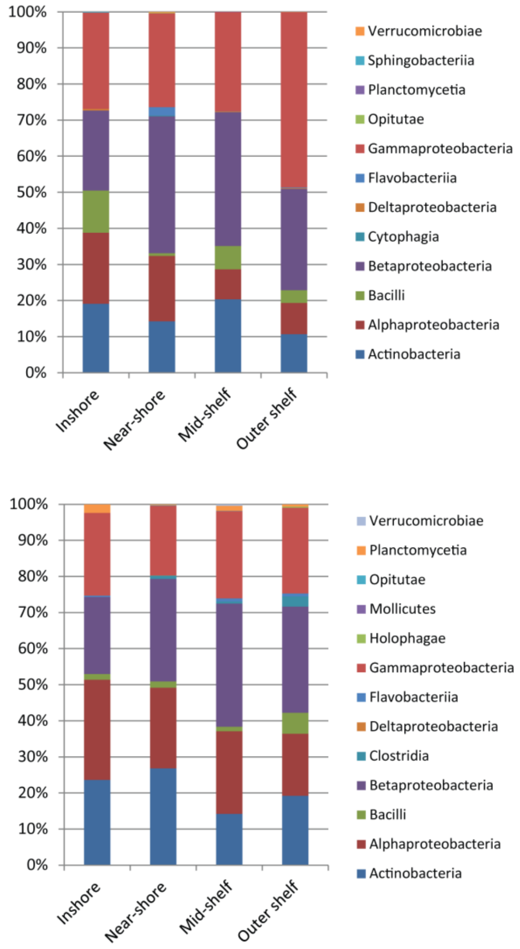

Fig. 2-4 Relative abundance of bacterial classes in the top: free-living, bottom: particle-attached fraction of the water column from inshore to outer shelf reef sites.

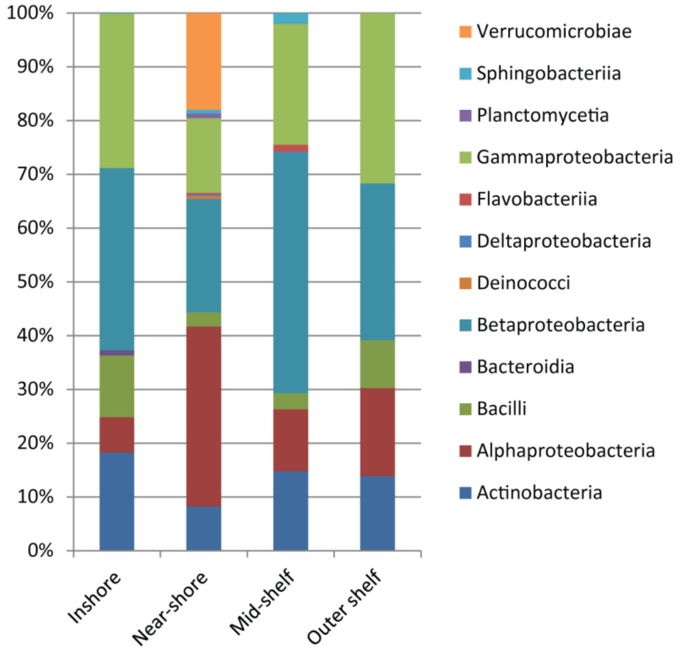

Fig. 2-5 Relative abundance of bacterial classes inhabiting the *Fungia* mucus from inshore to outershelf reef sites.

Fungia mucus

Patterns of relative dominance among the different bacterial groups were similar across the sites. *Betaproteobacteria* were the most abundant group in *Fungia* mucus, ranging from 21.1 % at near-shore sites to 44.6 % at the mid-shelf (Fig. 2-6). As in the water column, *Ralstonia* was the single most dominant genus. The *Gammaproteobacteria* contributed to 13.9 % - 31.7 % of the total bacterial community, *Alphaproteobacteria* 6.6 % - 33.5 % and *Actinobacteria* ranged from 8.2 % - 18.3 %. Within the *Actinobacteria*, two genera showed a strong decrease from inshore to offshore. Members of the pathogen-containing genera *Propionibacterium* and *Mycobacterium* were abundant at near-shore stations but only observed in much lower numbers at mid-shelf and outer shelf sampling stations. This also accounts for the genus *Escherichia/Shigella* of the class *Gammaproteobacteria* which were also found in high abundances at the inshore site, which is continuously subjected to effluents from Makassar, but decreased towards the mid-shelf sites.

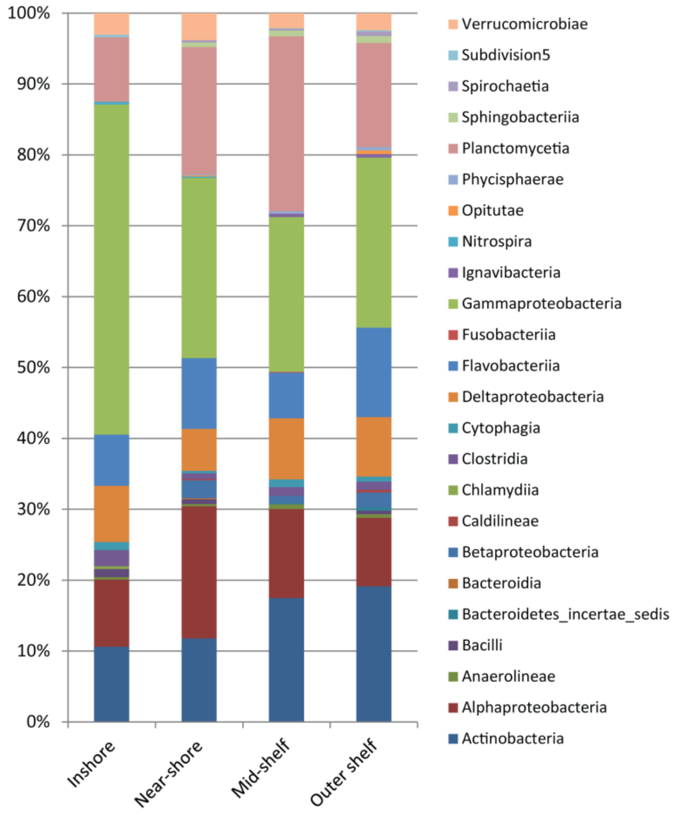

Fig. 2-6 Relative abundance of bacterial classes in the coral reef sediments.

Environmental influence on bacterial community composition

In the heatmap two clusters of bacterial orders with positive correlations to the measured water quality parameters become obvious (Fig. 2-7). A strong positive Pearson correlation to DOC was detected for *Cyanobacteria, Alphaproteobacteria, Acidobacteria_Gp9, Deinococci, Verrucomicrobiae* and *Opitutae*. Another cluster of bacterial classes, including *Acidobacteria_Gp6, Chlamydia* and *Bacteroidia*, is strongly correlated to chlorophyll *a*, TEP and SPM.

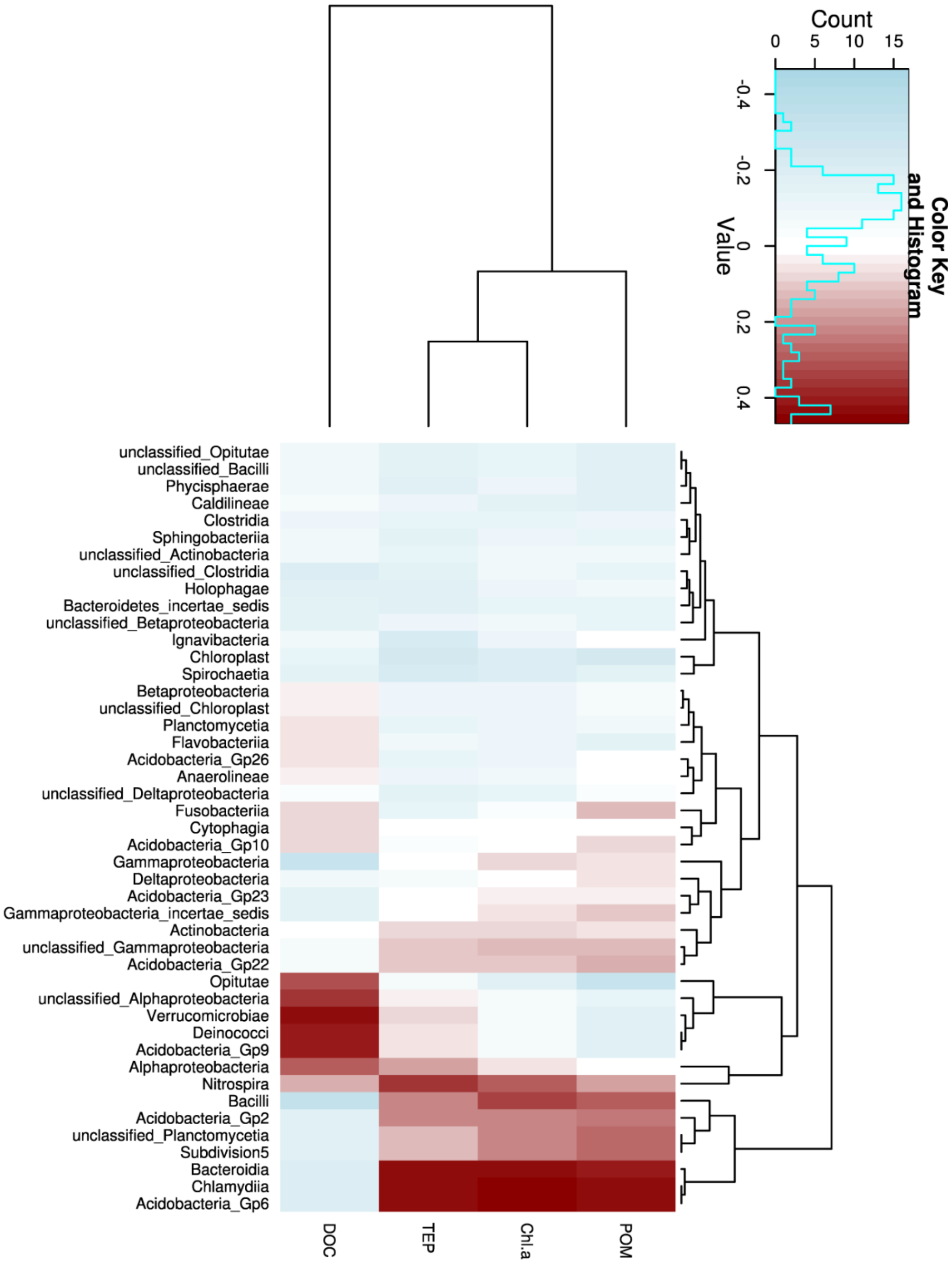

Fig. 2-7 Pearson correlations of bacterial classes from all habitats to measured water quality parameters. Red colors indicate a positive correlation, blue colors a negative correlation.

Habitat comparison

Although bacterial communities in the reef sediments were generally more diverse and the taxa were more evenly distributed compared to the coral mucus and water column, overall more sequences were obtained from the other habitats. Overall the clustering displays a clear and significant separation of sediment communities to the other habitats (Fig. 2-8). This was confirmed by an analysis of similarity (ANOSIM, p = 0.001) which revealed significantly different communities. Subsequent pairwise tests showed that all habitats, except the *Fungia* mucus and the free-living fraction of the water column where no differences were detected, were significantly different from each other (ANOSIM, P ≤ 0.006).

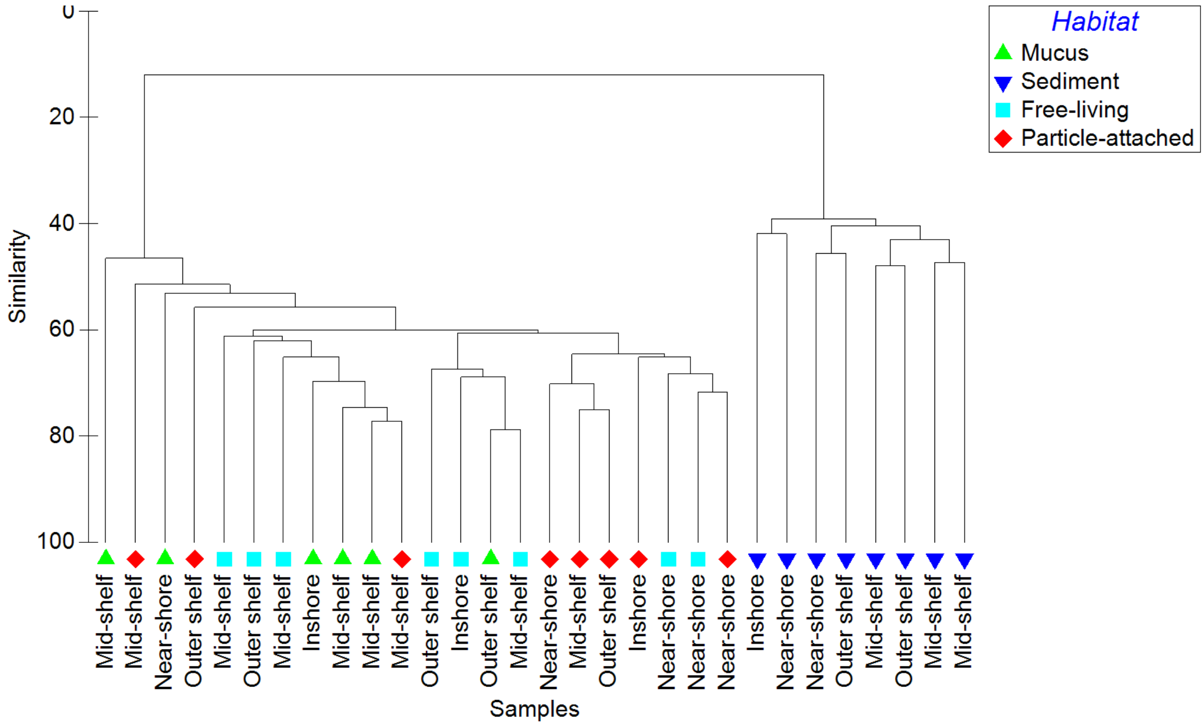

Fig. 2-8 Hierarchical clustering, based on Bray-Curtis dissimilarities, of the investigated habitats according to their location on the shelf. Green triangles show samples from coral mucus, blue inverted triangles samples from the sediments, turquoise squares indicate the free-living and red diamonds particle-attached samples from the water column, respectively.

We found a high dominance of few taxa in coral mucus or the water column, whereas within the sediments, the overall contribution of individual classes or families is much lower. There are classes, which are almost exclusively found in the sediment, including *Planctomycetia, Flavobacteria* and *Deltaproteobacteria.* At the class and family level there are distinct differences in bacterial community composition between the habitats. *Betaproteobacteria* is the most abundant class in the water column and coral mucus, but almost absent from the sediment samples (Fig. 2-9). *Bacili* are also significantly more abundant in those habitats. On the family level the picture is very similar. *Burkholderiaceae*, the predominant group within the *Betaproteobacteria*, and *Enterobacteriacea*, members of *Gammaproteobacteria*, constitute a large proportion of bacterial mucus and water column communities and are again found in the sediments in only very low numbers.

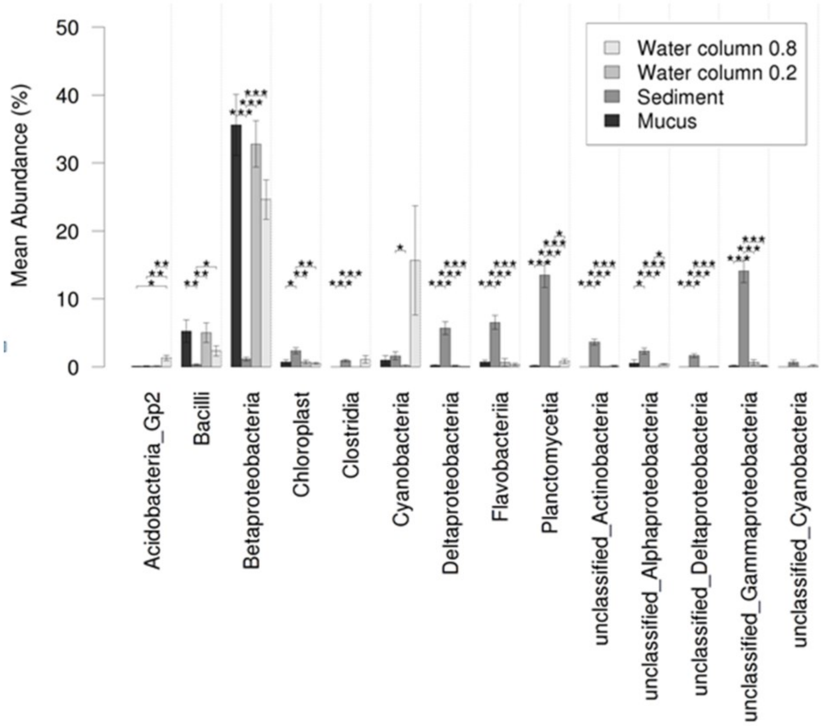

Fig. 2-9 Mean abundances of bacterial classes showing significant differences among the sampled habitats. Asterisks indicate significant differences between habitats within the classes.

DISCUSSION

With this study we can shed new light on shifts in bacterial communities of the water column, reef sediments and coral mucus in response to coastal eutrophication. Similar to other previous investigations we found strong changes in water quality parameters along the gradient (Sawall et al., 2011). But effects could only be detected for the inshore site and were limited to a few parameters, namely chlorophyll *a* and TEP. Although SPM showed a very similar pattern, the differences were not significant. The clearest change in relative bacterial class abundance was noticeable in the reef sediments. *Gammaproteobacteria* were significantly more abundant at the site with highest organic matter availability. Interestingly the relative abundance of free-living *Gammaproteobacteria* in the water-column decreased with decreasing chlorophyll *a* and SPM concentrations along the surveyed gradient. Chlorophyll *a*, TEP and SPM were the main drivers of changes in the bacterial communities. There are distinct differences between the habitats. Overall, the sediment bacterial communities were more diverse and heterogeneous (Hewson et al., 2003). Moreover, while *Betaproteobacteria* was the most dominant class in the water column and *Fungia* mucus, *Gammaproteobacteria* were the most abundant class in the reef sediments.

Environmental parameters

Excluding the highly eutrophic inshore site, concentrations of chlorophyll *a*, TEP and SPM in the Spermonde Archipelago were within the range of other near-shore reef ecosystems of the western Pacific, e.g. the Great Barrier Reef, Australia (Alongi et al., 2015), New Caledonia (Fichez et al., 2010) or, for chlorophyll *a*, previous studies of the same area (Sawall et al., 2011). Interestingly, the effect of riverine and urban sewage is limited only to the inshore site. One possible reason is a dilution effect by a strong longshore current in the Spermonde Archipelago. A large proportion of the Indonesian through-flow, connecting the Pacific with the Indian Ocean, is channeled through the Makassar Strait, leading to a constant southward flow of water (Gordon et al., 2003). As concentrations of chlorophyll *a* and the severity of eutrophication-related processes were highly correlated to water residence time (Delesalle and Sournia, 1992; Howarth et al., 2011), the rapid flushing can have a dilution effect on the measured parameters. This is also the case during the rainy season, when increased riverine input also delivers more nutrients to the inshore and near-shore sites. But since the predominant current through the Makassar Strait is southward, the river plume of the Jene Berang river is also deflected south and only reaches out to the closest near-shore station (Renema and Troelstra, 2001). In both cases excess nutrients will not be available for phytoplankton growth and bacterial production in areas north of the deflected plume, e.g. the mid-shelf and outer shelf sites.

TEP concentrations can be used as a good integrative indicator for differences in the water quality among the sites as well as for a strong interaction between phytoplankton and the bacterial communities, of which TEP is a product (Gärdes et al., 2011; Smith et al., 1995). TEP was strongly correlated to chlorophyll *a*, SPM concentrations and DAPI cell counts in the water column. Additionally TEP may also serve as a food source and habitat for bacteria (Passow, 2002), increases particle aggregation and subsequent sedimentation (Cárdenas et al., 2015; Gärdes et al., 2011).

Combining these important ecological and biogeochemical attributes and the pivotal role TEP plays in organic matter turnover and export, this suggests TEP may be an ideal candidate for an integrative eutrophication indicator in situations where shifts in aggregate formation rates or bacterial community composition are also of interest.

Concentrations of DOC in the Spermonde Archipelago are at the higher end described for tropical reef ecosystems (Dinsdale et al., 2008; Nelson et al., 2011), but they do not follow the general decreasing trend observed for chlorophyll *a*, TEP and SPM. This could be explained through a combination of two processes: DOC is (1) rapidly taken up by heterotrophic bacteria in the water column and (2) aggregates into larger particles (Nelson et al., 2011; Passow and Alldredge, 1994; Passow, 2000).

Bacterial community composition

In the present study, significantly higher bacterial cell counts in the water column were observed. This can result in an increased uptake of DOC at the inshore and near-shore stations closer to Makassar (Ferrier-Pagès et al., 1998). Compared to the number of obtained sequences in the water column and *Fungia* mucus, the number of sequences extracted from the sediments was much lower. Rarefaction curves, contrary to water column and mucus samples, still increased almost linearly. This indicates that some degree of diversity was not uncovered due to the comparatively low number of sequences obtained from the sediments, although already much higher diversity indices for the reef sediments compared to the other habitats were calculated.

Spatial variation of bacterial communities

On the community level there were no corresponding shifts along the measured water quality gradients. With an increased availability of nutrients and organic matter, the expected shift would be towards a community dominated by heterotrophic bacteria specialized in assimilating the available organic matter, such as *Gammaproteobacteria*. Interestingly, this pattern was neither reflected in the free-living and particle-attached fractions of the water column. Nevertheless we found strong differences in the abundance of some groups. And likely there is a time lag between the additional availability of food sources and the response in the bacterial community (Wietz et al., 2015). Together with the low water residence time in the Spermonde Archipelago, this can lead to a shifted pattern in community composition. In support of that, there was a much higher relative *Gammaproteobacteria* abundance at the outer shelf sites compared to the eutrophic inshore station. To the contrary, *Alphaproteobacteria*, which were most abundant in the water column of the chlorophyll-rich inshore waters of the Spermonde Archipelago, are known to correlate well to waters rich in inorganic nutrients and phytoplankton biomass (Allers et al., 2007; Teira et al., 2008). There was no observable gradual change in the *Betaproteobacteria* occurrence across the shelf in the Spermonde Archipelago, likely due to overriding local effects at the individual study sites. The islands of the Archipelago are densely populated, and all islands lack waste water treatment (Ferse et al., 2012; Williams et al., 2014). This indicates a local source of organic matter for *Betaproteobacteria* from island effluents. This is further corroborated at the only uninhabited site, a submerged reef platform in the mid-shelf area, which showed the lowest relative abundance in *Betaproteobacteria* in free-living and particle-attached habitats.

The highly diverse reef sediment community was dominated by *Gammaproteobacteria*, particularly at the inshore site closest to Makassar. *Gammaproteobacteria* have a large array of extracellular hydrolytic enzymes, making them an ecologically and biogeochemically important group in the rapid assimilation of organic matter (Azam and Malfatti, 2007; Dang et al., 2009; Edwards et al., 2010). This supports our water quality data, with highest concentrations of chlorophyll *a*, TEP and SPM at near-shore sites, indicating the highest productivity and anthropogenic impact at the site closest to shore. One result from high chlorophyll *a* and SPM concentrations is also a constant supply of particles rich in organic matter to the sediments, which may favor growth of *Gammaproteobacteria*.

Observations made on the community composition of the mucus samples were similar to those of the water column. Again *Betaproteobacteria* were, on average the most dominant class. Findings from the present study are supported by comparisons of bacterial communities in mucus of *Fungia* sp. from the Red Sea and aquaria by Kooperman et al. (2007). They also found increased occurrences of *Betaproteobacteria* in the aquaria samples with altered water quality. They are also known to correlate strongly to the availability of organic matter in the marine environment (Tada et al., 2011).

Influence of water quality parameters

In general, some bacterial genera can benefit greatly from changes in environmental parameters or water quality. In the present study there are two clusters of correlations to the measured water quality parameters. We detected positive correlations of groups such as *Bacteroidia, Chlamydiia*, and to a lesser extent *Gammaproteobacteria*, to chlorophyll *a*, TEP and SPM. Alternatively, bacterial groups such as *Alphaproteobacteria, Deinococci* and *Verrumicrobiae* were positively correlated to DOC concentrations. This supports other field and experimental studies where *Alphaproteobacteria* were often associated to nutrient-depleted environments or phytoplankton blooms (Pinhassi et al., 2004; Riemann et al., 2000). At the anthropogenically impacted inshore site three genera, *Escherichia/Shigella, Propionibacterium* and *Mycobacterium*, were much more abundant compared to the sites further out on the shelf. The two latter ones were almost exclusively found at the station closest to Makassar. In the present study this was especially evident in the *Fungia* mucus.

Those above mentioned genera, members of the *Actinobacteria, Betaproteobacteria* and *Gammaproteobacteria*, which were more abundant at the inshore site, influenced by constant sewage input, contain pathogens of corals and humans (Allers et al., 2008; Dinsdale et al., 2008; Kaper et al., 2004; Lyons et al., 2010). Alarmingly, in the present study, high abundances of known pathogenic genera, such as *Escherichia/Shigella, Propionibacterium* and *Mycobacterium*, were found in all habitats except the free-living fraction of the water column at the inshore site.

Inter-habitat variability

Our results suggest that much of the observed variability in bacterial community composition in the Spermonde Archipelago is a result of differences between the sampled habitats. Previous studies already showed that microbiota associated to a host are often species specific and to a large extent stable across environmental gradients. Barott et al. (2011) showed clear difference in bacterial community composition of different types of algae and corals. Another recent study from the Spermonde Archipelago confirmed this for a variety of reef sponges (Cleary et al., 2015). The production of specific carbohydrates and antibiotics very likely plays an important role in shaping those stable host-specific microbial communities (Ritchie, 2006; Rosenberg et al., 2007). All three habitats in the present study were also statistically different in their bacterial community composition. *Betaproteobacteria* exhibited the highest average relative abundance across all sampled habitats except the reef sediments. They are a dominant group of coastal and shelf bacterioplankton (Giovannoni and Stingl, 2005; Rappé et al., 2000) and may be used as indicators for freshwater and/or sewage input as they occur often more prevalent in polluted waters (Newton et al., 2013; Patel et al., 2014).

CONCLUSIONS

This study contributes the first conclusive overview of changes in bacterial communities from the water column, sediments and coral mucus in relation to changes in the water quality of the Spermonde Archipelago. Bacterial communities were very distinct among the three microbial habitats water column, sediments, and coral mucus. While *Betaproteobacteria* was the most abundant taxon in the water column and on the coral mucus, *Gammaproteobacteria* dominated the sediments, especially at the inshore site. There were strong differences in the bacterial communities between the near shore site at 1 km and all other sites located further on the shelf. Alarmingly, the prevalence of potentially pathogenic bacteria was much higher at this chronically impacted sampling site. If coastal development and waste water management remains unchanged this may have unpredictable consequences for the coastal and island populations that strongly depend on the natural resources taken from the Archipelago.

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Chapter 3

Intensive nutrient uptake and particle aggregation mitigate eutrophication at more distant reefs at a tropical coastal shelf ecosystem

Intensive nutrient uptake and particle aggregation mitigate eutrophication at more distant reefs at an Indonesian coastal shelf ecosystem

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ABSTRACT

Coastal eutrophication in the Spermonde Archipelago, Indonesia, has long been assumed to be a key driver of detrimental community shifts in the fringing and patch reefs at varying distances from the coast. But detailed information on the relevant water quality parameters was often lacking. The present study investigated important water quality parameters across a regional gradient from an inshore site close to a metropolitan area, to sites further out on the shelf. This data was related to sedimentation rates and bacterial community composition from the water column, the reef sediments and aggregates from incubation experiments. The aim was to elucidate how bacterial community in those habitats change in response to eutrophication and role aggregate formation and the associated bacterial communities might have in mitigating detrimental effects of eutrophic waters to the reefs on the shelf. Findings revealed that significantly elevated concentrations of chlorophyll *a*, and transparent exopolymer particles (TEP), suspended particulate matter (SPM) and sedimentation rates could only be observed at the very inshore site. Bacterial community composition on the incubated aggregates and in the free-living fraction of the water column was significantly different between the inshore station and the ones further on the shelf. Interestingly, *Cyanobacteria* of the genus *Synechococcus* dominated the particle-attached communities. This study implicates that in the immediate vicinity to the shore the high nutrient and organic matter assimilation activity of phytoplankton, *Cyanobacteria* and heterotrophic bacteria, in connection with the high on-site sedimentation rates, can act as a biological filter and prevents eutrophic waters from the reefs further out on the shelf. In conclusion this biological filtration could be an important mechanism to maintain oligotrophic shelf waters and healthy coral reefs in a system under constant anthropogenic pressure.

INTRODUCTION

The riverine input of nitrogen and phosphorus into the ocean has more than doubled in the past decades, especially from the 1960s to the 1990s, leading to the worldwide observed eutrophication of coastal waters (Galloway et al., 2004; Vitousek et al., 1997). And although nitrogen limitation of coastal seas is common because of an unfavorable N/P ratio below the Redfield ratio of 16:1 (Goldman et al., 1979; Malone et al., 1996), the sheer amount of nutrients available rendered the unfavorable N/P ratio less important. This often fostered excessive phytoplankton blooms with often potentially toxic cyanobacterial growth in many estuarine and coastal areas (reviewed by Heisler et al., 2008 and O'Neil et al., 2012). In oligotrophic conditions overall inorganic nutrient concentrations and the N/P ratio are much more important controls of phytoplankton growth and primary production (Gruber and Galloway, 2008; Moore et al., 2013; Smith, 1984) and single-celled *Cyanobacteria* such as *Synechococcus* and other picophytoplankton gain an competition advantage due to their advantageous surface to volume ratio and capabilities to access other energy sources (Moisan et al., 2010; Revelante and Gilmartin, 1988).

An elevated abundance of phytoplankton frequently results in an increased availability of organic matter, such as dissolved organic carbon (DOC) and subsequently transparent exopolymer particles (TEP) (Passow, 2000; Verdugo and Santschi, 2010; Verdugo et al., 2004). Due to the high stickiness TEP will in turn intensify aggregation of suspended materials, detritus or faeces into larger aggregates and sedimentation to the benthos. (Azam and Malfatti, 2007; Passow, 2002). If sedimentation occurs localized and in high rates close to the source of the eutrophication it can restrict the extent of the eutrophic conditions across the shelf (Herbeck et al., 2013). It has been assumed so far that the proportion of the primary production produced by single-celled photoautotrophic *Cyanobacteria* is retained in the upper layers of the water column through the 'microbial loop' (Azam et al., 1983; Pomeroy, 1974). Thus, their contribution to the carbon export, in relation to their relative abundance, was therefore hypothesized to be much lower compared to that of larger phytoplankton species (Michaels and Silver, 1988). The sinking particles and TEP itself are also a rich source of organic matter for both free-living and particle-attached bacteria in the water column, as well as those inhabiting reef sediments (Kiørboe and Tang, 2003; Kramer et al., 2013; Passow and Alldredge, 1994). Several studies found shifts in the bacterial community composition, often towards heterotrophic communities, in eutrophic and organic matter rich conditions of reef waters (Meyer-Reil and Köster, 2000; de Voogd et al., 2015; Weinbauer et al., 2010) and sediments (Uthicke and McGuire, 2007).

TEP and larger marine particles themselves are hot-spots for microbial colonization and activity (Kiorboe et al., 2002, 2003; Passow, 2002; Simon et al., 2002b). Bacteria inhabiting those aggregates are several orders of magnitude more abundant per unit of volume compared to communities from the surrounding seawater (Simon et al., 2002b). Community composition is also very distinct at different environmental conditions (Zhang et al., 2007) and differs from the free-living counterparts (Delong et al., 1993; Michotey et al., 2012; Weinbauer et al., 2010). Colonizing bacteria express a large variety of ectohydrolases to digest the organic matter of the aggregates (Azam and Malfatti, 2007; Smith et al., 1992). Only little of the lysate is actually used by the attached bacteria (Smith et al., 1992), the remainder is trailing the sinking aggregates as a plume of dissolved organic matter (DOM) readily available to free-living bacteria (Kiørboe and Jackson, 2001). Those plumes might help to retain important nutrients, such as nitrogen, phosphorus and iron in the water column, and therefore stimulate a continuous primary production (Azam and Malfatti, 2007; Azam et al., 1983).

With this study we aim to show for the first time that highly elevated primary production and localized organic matter export in the very inshore areas, in immediate vicinity to metropolitan Makassar and the river plumes, mitigates detrimental eutrophication effects at reefs further offshore in the Indonesian Spermonde Archipelago. A cross-shelf water quality gradient has been previously assumed in several publications (Edinger et al., 1998; Renema and Troelstra, 2001). But so far the mentioning of this gradient often remained anecdotal and assumptions were based on only few water quality parameters that were actually measured. The present study now conducted parallel sampling of a larger array of relevant water quality parameters and bacterial communities from the water column and sediments. In additionally conducted 48 h rolling tank experiments, during dark incubations, we investigated how the aggregate formation potential and bacterial colonization changed along sampling sites with increasing distance to the mainland. To our knowledge it is the first study of the Spermonde Archipelago to investigate bacterial community composition on single aggregates. The main research questions addressed in this study were: (1) How do bacterial communities respond to anthropogenic nutrient and organic matter availability? (2) What processes restrict eutrophic conditions from expanding to outer shelf reefs? (3) Do the formation of macroscopic aggregates, along with changing bacterial communities, contribute to those process?

METHODS

Study sites

Sampling was carried out at the continental shelf off Makassar in the Spermonde Archipelago in South Sulawesi, Indonesia during the dry season in May and June 2014 (Fig. 3-1). Due to environmental and ecological variability across the shelf, the archipelago has been divided into several ecological zones running parallel to the coast line, assumingly representing varying degrees of exposure to eutrophication from riverine input and sewage from metropolitan Makassar (Edinger et al., 1998; Moll, 1983; Renema and Troelstra, 2001). Three islands and two open water sites with increasing distance from Makassar were investigated. Lae Lae was the island closest to Makassar at 1 km distance to Makassar. It is characterized by constant riverine and sewage input as well as low water clarity (< 5 m) and high sediment load. Barrang Lompo (11 km distance) has higher water clarity and receives only very little riverine input from the mainland, mostly during rainy season. Badi, at 19 km distance to Makassar, is not impacted by city effluents and features the highest water clarity. The two open water sampling sites are located at 6 and 15 km, between Lae Lae and Barrang Lompo and between Barrang Lompo and Badi, repectively.

Environmental and water quality parameters

The environmental parameters and chlorophyll *a* were measured in the reef, using a Eureka Manta 2 multiprobe (*eureka water probes, Austin, USA*). Water quality parameters (inorganic nutrients, DOC, SPM and TEP) were measured from water samples taken from 5 m water depth (n=6) with a 5 L Niskin bottle (*HydroBios, Kiel, Germany*). Samples were stored at -20 °C in the dark until analysis within 3 h of sampling. For each parameter replicate subsamples were taken. For DOC, samples were filtered through 0.45 µm pore Whatman GF/F filters *(Whatman, GE Healthcare, Pittsburgh, USA)* and acidified with HCl (pH below 2). The filters were analyzed using a TOC-VCPH TOC analyzer (*Shimadzu, Mandel, Canada*) with high-temperature oxic combustion (HTOC). For calibration and quality control Hansell artificial seawater standards (*Hansell laboratory RSMAS, University of Miami*) and ultrapure water blanks were included. To determine SPM mass, a known volume of sample water was filtered using pre-combusted GF/F filters. After filtration these filters were rinsed with aqua dest. and dried for 24 h at 40 °C before determination of the weight difference before and after filtration (using a ME 36S, *Sartorius, Göttingen, Germany)*. The filters were then cut into quarters and analyzed for total CN content and inorganic/organic carbon ratio using a Eurovector EA 3000 elemental analyzer (*Eurovector SPA, Redavalle, Italy*) with Organic Analytical Standard, Certificate No. *207835*, as reference standard. Δ15N stable isotope analysis was carried out using Thermo Finnigan Delta Plus (*Thermo Fisher Scientific, Waltham, USA*) gas isotope ratio mass spectrometer (MS) after high temperature combustion (1000 °C) in a Flash 1112 EA elemental analyzer (*Thermo Fisher Scientific, Waltham, USA*). Δ15N is given as the ‰ deviation from atmospheric air. Inorganic nutrients (nitrite, nitrate, phosphate and silicate) where measured spectrophotometrically with a Flowsys continuous flow analyzer (*Unity scientific, Brookfield, USA*). TEP was quantified with an updated protocol (Engel, 2009) of the spectrophotometric method first introduced by Passow and Alldredge (1995). The adsorption of a dye is related to the weight of polysaccharides filtered on 0.4 μm polycarbonate filters. A calibration curve was prepared by filtering and staining different volumes of Gum Xanthan from *Xanthomonas campestris* cultures to relate the absorbance measured on the sample filters to this reference polysaccharide.

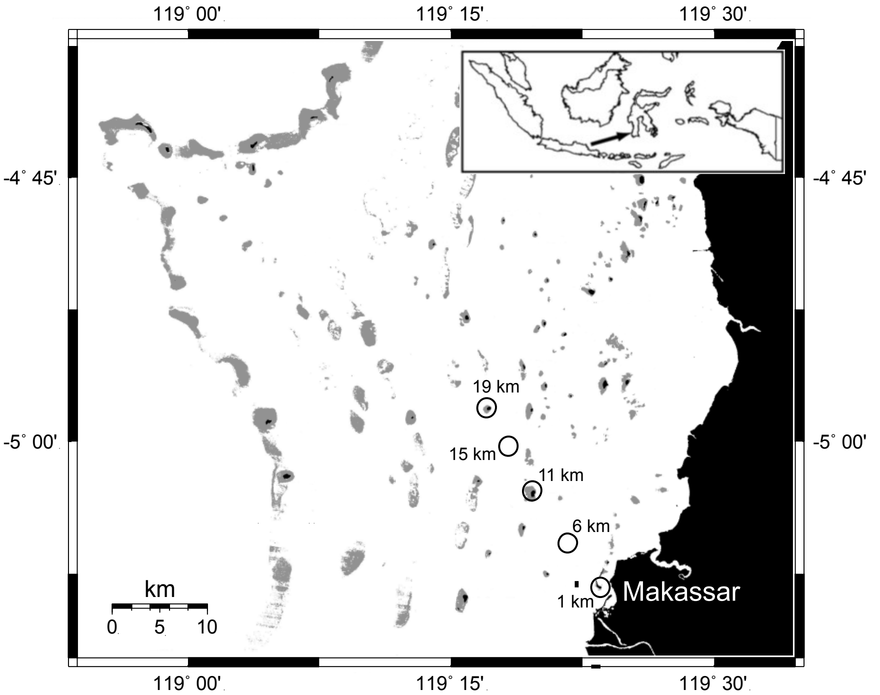

Fig. 3-1 Map of the Spermonde Archipelago, Indonesia, including the selected sampling stations (black circles).

Rolling tank experiments

From each site, additional water was collected for five replicate rolling tanks of 1.25 L volume each. All stations except one, which was stopped after 10 h as an additional time point, were rolled at 3 rpm for 48 h in the dark. Oxygen concentrations were measured *in situ* with a Firesting O_2 optical sensor (*PyroScience GmbH, Aachen, Germany*). Particles within the rolling tanks were counted at 15, 30 and 48h. Particles were visually categorized into five size classes: < 1, 1 – 3, 3 – 5, 5 – 10 and > 10 mm. Total aggregated volume was calculated assuming an ellipsoid form of the aggregates. At the end of the rolling period, individual aggregates were collected from the tanks and stored in RNAlater solution (*Thermo Fisher Scientific, Waltham, USA*) at -20 °C until DNA extraction.

DNA extraction and Illumina sequencing

DNA was extracted from three different habitats; sediments and two selected size fractions from the water column. To separate the 'free-living' and 'particle-attached' size fractions, a 1 L subsample from each site was filtered sequentially using 3 µm and 0.2 µm Whatman Nuclepore polycarbonate filters *(Whatman, GE Healthcare, Pittsburgh, USA)*. DNA extraction for these water column samples followed the protocol established by Boström et al. (2004) without modification. At each site surface sediment was collected from the uppermost 1 cm of sediment and stored in 2.0 mL tubes (*Eppendorf, Germany*) at -20 °C until extraction. At the end of the rolling tank experiments after 10 h or 48 h individual aggregates were collected and stored in RNAlater in 2 mL tubes (*Eppendorf, Germany*). The PowerSoil$^{\text{TM}}$ DNA Isolation Kit (*MO BIO Laboratories, Carlsbad, USA*) was used for DNA extraction from sediments and aggregates. Sequencing of the DNA samples was carried out through an Illumina MiSeq V3 pipeline (*Illumina Inc., San Diego, USA*) by LGC Genomics (*Berlin, Germany*). To target the V3-V4 hypervariable region, 16S rRNA primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-CTACCAGGGTATCTAATCC-3') were used. Quality trimming of the sequences was conducted with Trimmomatic (Bolger et al., 2014), followed by paired-end read merging with PEAR (Zhang et al., 2014). The Swarm algorithm (Mahé et al., 2014) was used to cluster the remaining OTUs (97 % cutoff) *de novo.* In a last step the clustered OTUs were aligned and taxonomically classified with the SILVA Incremental Aligner (SINA, Pruesse et al., 2012), using the SILVA Version 1.19 ribosomal RNA gene database (Quast et al., 2013).

Statistical analysis

Environmental and water quality parameters were analyzed for differences between sites using SigmaPlot 13.0 (*Systat Software, Inc., San Jose, California, USA*). All concentrations are reported as arithmetic mean ± standard deviation. Principle component analysis (PCA), non-metrical multi-dimensional scaling (NMDS) plotting and hierarchical clustering of changes between the stations including all environmental and water quality parameters was conducted using the PRIMER 6.16 software (Clarke and Gorley, 2006). This program was also used for analysis of the bacterial communities. Diversity of the communities and changes in abundance of bacteria classes were determined and bacterial communities related to differences in environmental and water quality parameters. Enrichment of phenotypic functions were predicted using the METAGENassist online tool, which maps taxonomic information associated to phenotypes (Arndt et al., 2012).

RESULTS

Field sampling

Combined concentrations of nitrite and nitrate (NO_x) ranged from 0.21 ± 0.02 µM to 0.71 ± 0.10 µM, with highest concentrations 19 km off the coast (Fig. 3-2 a). The differences were significantly different (ANOVA, $p \leq 0.001$, with subsequent Holm-Sidak post-hoc test) for all stations except between the 1 and 6 km stations. Concentrations of inorganic phosphate (PO_4) were highest at 1 km distance (0.18 ± 0.01 µM) and lowest at 15 km (0.10 ± 0.01 µM) (Fig. 3-2 a, Supp. 4-1). A Kruskal-Wallis test on ranks revealed significant differences between stations ($p \leq 0.001$, Tukey's post-hoc pairwise comparisons). There was no general decreasing or increasing trend in silicate (Si) concentrations, although the concentrations were significantly different (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons). Measured concentrations ranged from 2.76 ± 0.29 µM to 6.69 ± 0.31 µM (Fig 3-2 a). Observed chlorophyll *a* values (Fig. 3-2 b) were more than 40 times higher at the most inshore station at 1 km distance (0.80 ± 0.58 µg L^{-1}) compared to the one furthest out on the shelf (0.02 ± 0.01 µg L^{-1}). A similar trend was observed for TEP (Fig 3-2d), with significantly decreasing concentrations from 1 km (326.57 ± 97.26 µg Xeq L^{-1}) to 19 km (105.12 ± 35.63 µg Xeq L^{-1}). The strongest decline in concentrations for both chlorophyll *a* and TEP was observed between the inshore stations at 1 km and the next one at 6 km. DOC (Fig. 3-2 c) ranged from 69.31 ± 8.43 µM to 90.94 ± 30.76 µM while SPM (Fig. 3-2 e) concentrations ranged from

2.85 ± 1.23 mg L^{-1} to 7.76 ± 0.87 mg L^{-1}. Differences in concentrations of DOC and SPM were statistically significant (Kruskal-Wallis test and ANOVA, $p \leq 0.001$, respectively). Sedimentation rates in the eutrophic inshore zone (20.92 ± 5.15 mg cm^{-2} d^{-1}) were significantly higher (Kruskal-Wallis test, $p = 0.009$, Tukey's post-hoc pairwise comparisons) compared to lowest rates measured in the oligotrophic outer shelf zone (0.87 ± 0.29 mg cm^{-2} d^{-1}). Ratios of NO_x/PO_4 increased significantly (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons) from 1 km (1.16 ± 0.18) to 19 km (6.09 ± 0.82). The carbon/nitrogen ratio of the SPM was significantly higher at the 6 km station (12.07 ± 3.14) compared to the ratios of 6.24 ± 0.48 and 6.90 ± 0.70 at the 1 and 11 km sampling sites, respectively (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons). Sedimentation rates were severalfold higher at the 1 km site (Fig. 3-2 f) than those further offshore (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons). Enrichment in δ15N stable isotopes in samples from the water column (Fig. 3-2 g) was highest at 11 km (6.98 ± 0.49 ‰) and lowest at 15 km (3.88 ± 0.62 ‰). Differences were statistically significant (ANOVA, $p \leq 0.001$, with subsequent Holm-Sidak post-hoc test), especially between the three sampling sites closer to shore and the two further out on the shelf at 15 and 19 km. Material collected from the sediment traps also showed a higher δ15N signature (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons) in inshore samples at 1 km (5.53 ± 0.52 ‰) than those from the 19 km site (3.85 ± 0.19 ‰).

Results of the principal component analysis were plotted to visualize variability in environmental and water quality parameters in a two-dimensional space. The two first components of the PCA explained 40.5 % and 21.9 % of the observed variation between the sampled stations (Fig. 3-3). There is a clear separation of the most inshore station at 1 km from the other stations further on the shelf along principal component 1, which is explained by inorganic phosphate, chlorophyll *a*, and TEP. The other stations were mainly separated by the second principal component.

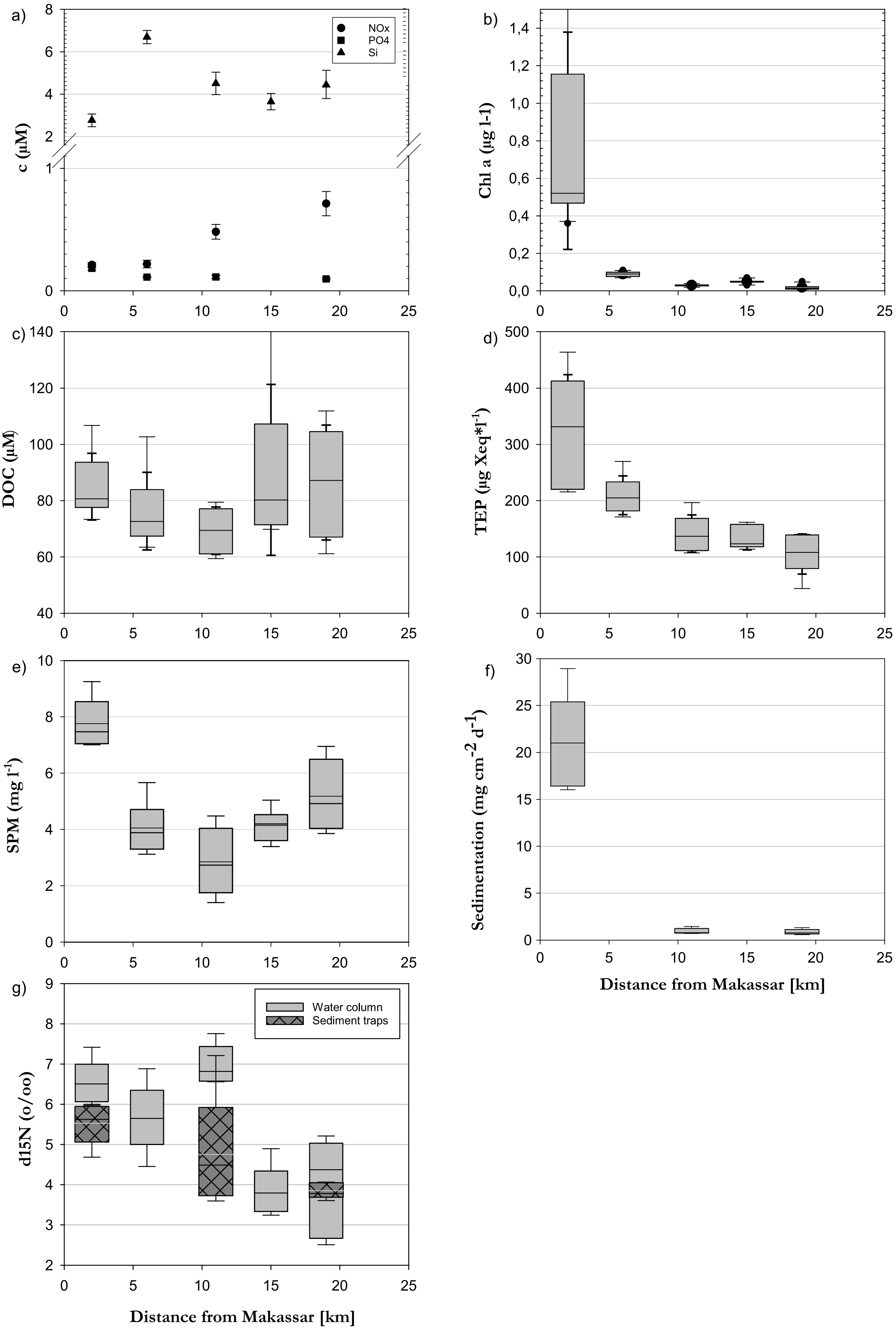

Fig. 3-2 Water quality parameters along the sampled cross-shelf gradient. a) inorganic nutrients, b) Chlorophyll *a*, c) DOC, d) TEP, e) SPM, f) sedimentation rates and g) δ15N stable isotope enrichment. Error bars show standard deviation.

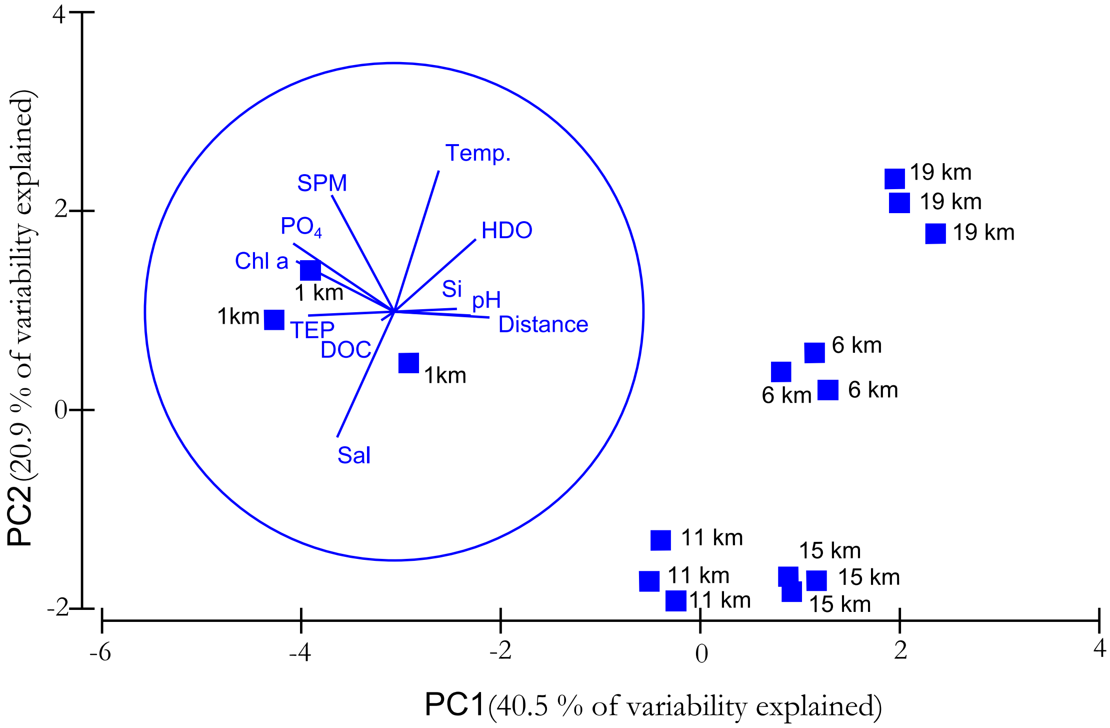

Fig. 3-3 PCA ordination of the stations across the shelf according to normalized environmental and water quality parameters. PC1 and PC2 explained 40.5 % and 20.9 % of total variation, respectively. Eigenvectors of the assessed parameters along the two principal components are plotted in blue.

Bacterial community composition

Total DAPI cell counts also decreased significantly by more than 50 % (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons) from $1.29 \times 10^7 \pm 1.45 \times 10^6$ cells mL^{-1} the 1 km site to $5.59 \times 10^6 \pm 8.04 \times 10^5$cells mL^{-1} at the site furthest away from Makassar (19 km). Among other indices for alpha-diversity, the Shannon-Weaver H' diversity index was calculated (Supplementary material Tab. S 3-1). Within-habitat variation of H' was low along the gradient. H' ranged from 4.09 at the 19 km site to 4.99 at the 11 km site for the free-living fraction of the water column. Diversity in the article-attached fraction was lowest at the 19 km site (H' = 2.92) and highest at the 6 km site (H' = 3.07). Within the investigated sediments diversity was very similar (from 6.97 at the 19 km site to 7.29 at the 1 km site). Between the habitats significantly higher H' was observed in the sediments compared to the two water column size fractions (ANOVA, $p \leq 0.001$, with subsequent Holm-Sidak post-hoc test). Highest OTU numbers were found in sediment samples (1394 OTU at the 19 km site to 1600 OTU at the 1 km site), followed by the 0.2 µm (594 OTU at 11 km to 623 OTU at 19 km) and 3.0 µm (460 OTU at the 1 km site to 560 OTU at the 11 km site) size fractions of the water column, respectively (Supplementary material Tab. S 3-1). In the free-living fraction of the water column *Gammaproteobacteria,* *Alphaproteobacteria, Deltaproteobacteria* and *Acidomicrobiia* are the most dominant bacterial classes (Fig. 3-4). The relative abundances of the 1 km and 11 km stations are quite similar, but the relative abundance of the *Gammaproteobacteria* more than doubles to 69.54 % at the 19 km sampling site.

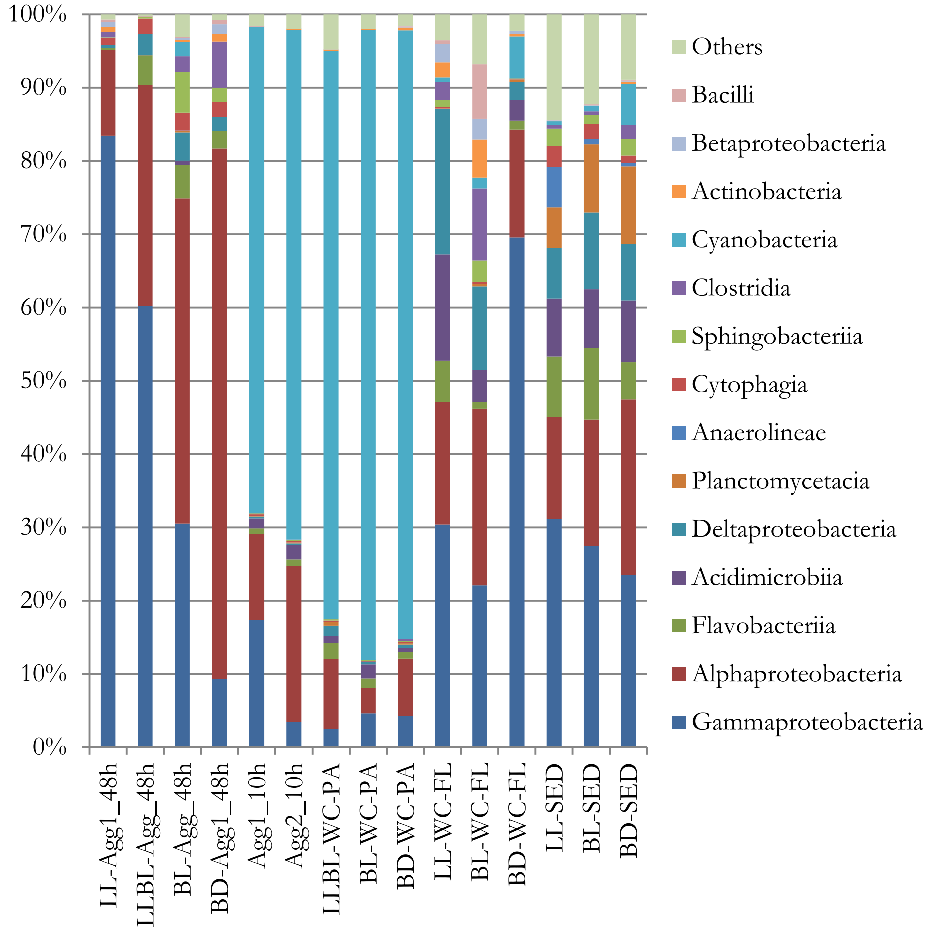

Fig. 3-4 Relative abundance of the bacterial classes with more than 5 % contribution in at least one sample for aggregates incubated for longer than 48 h and those stopped after 10 h, Agg) aggregate samples, FL) bacteria of the free-living fraction of the water column(> 0.2 µm), PA) "particle-attached" fraction (> 3 µm) and SED) from sediment samples.

The particle-attached communities of the water column were at all sampling stations, at 6, 11 and 19 km distance to Makassar, dominated by *Cyanobacteria*, which always constituted more than 80 % relative abundance. Otherwise important groups, such as *Alpha-* or *Gammaproteobacteria*, contributed much less to the community composition compared to the free-living fraction or sediments. In the sediment, *Gammaproteobacteria, Alphaproteobacteria, Flavobacteria* and *Acidomibrobiia* were the main constituents of the bacterial communities.

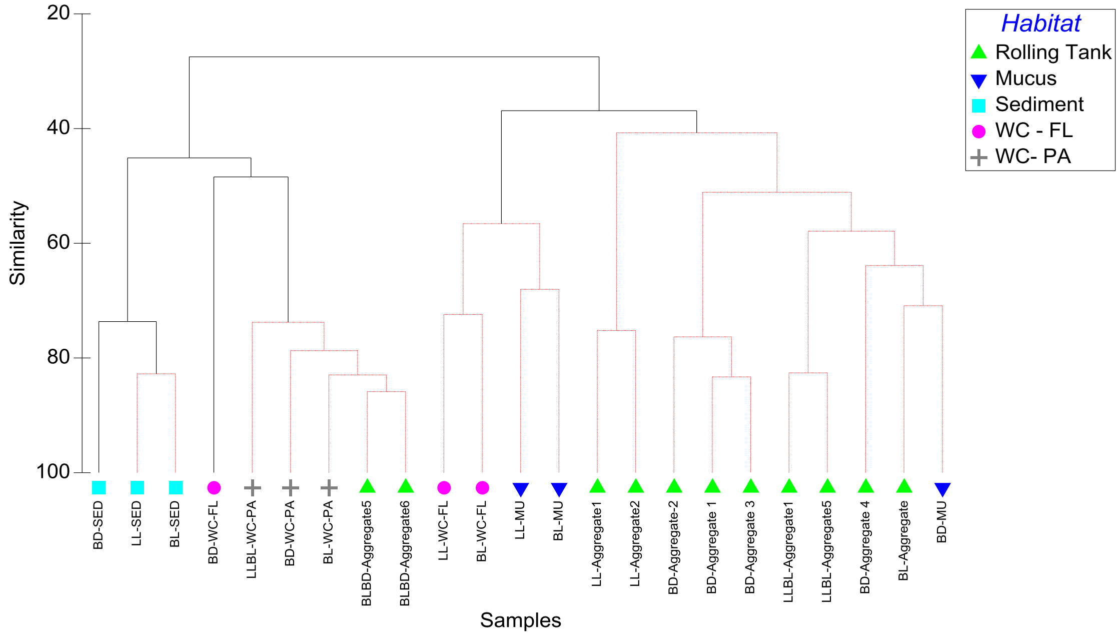

Fig. 3-5 Hierarchical clustering of the sampled habitats: the water column, particle attached (grey cross), free-living (pink circle), *Fungia* mucus (blue inverse triangle), reef sediments (turquoise square) and rolling tank aggregates (green triangle). Black lines indicate significant differences between clusters and samples, whereas red dotted lines show no differences.

Contrary to the free-living fraction of the water column the relative abundance of *Gammaproteobacteria* decreases from 31.12 % to 23.47 %, while that of *Alphaproteobacteria* increases from 13.91 % to 23.99 % along the gradient from 1 to 19 km. Hierarchical clustering (Fig. 3-5) revealed significant differences between the free-living and particle-attached fraction of the water column (Simprof test, p = 0.001) as well as between the free-living bacterial community at 19 km and the other two stations closer to Makassar (Simprof test, $p = 0.015$). With METAGENassist we predicted the abundance of dinitrogen-fixing bacteria in the sampled habitats (Fig. 3-6). There were significant differences between the habitats (Kruskal-Wallis test on ranks, $p < 0.05$), with highest per habitat-normalized abundance found in the particle-attached fraction of the water column and the reef sediments.

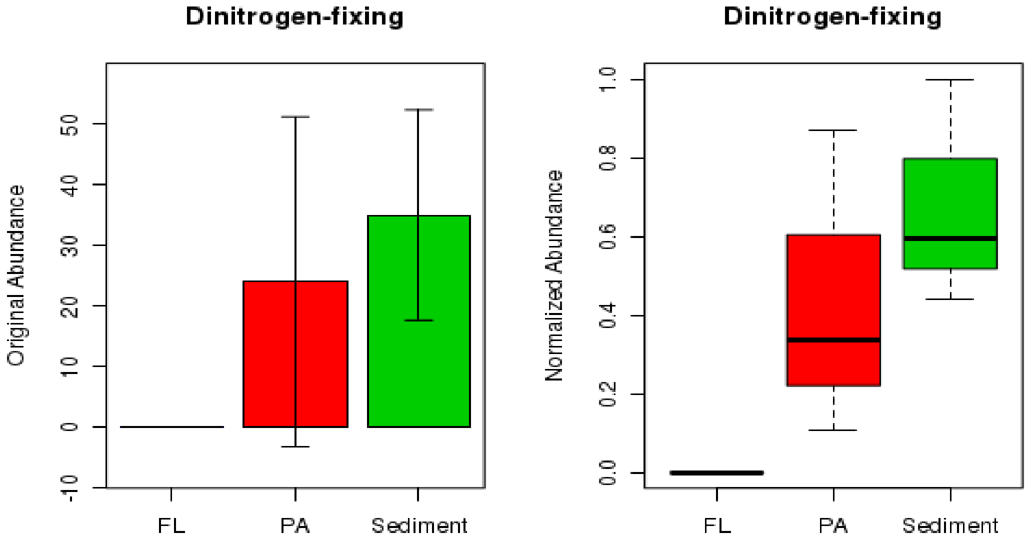

Fig. 3-6 Original (left) and normalized (right) abundance of predicted dinitrogen-fixing metabolism in the free-living (FL, 0.2µm) and, particle-attached (PA, 3.0µm) size fractions of the water column, as well as the sediments. Error bars depict standard deviation.

Rolling tank experiment

Total aggregated volume of the rolled aggregates (Fig. 3-7) was also significantly higher at the inshore site at 1 km (21.80 ± 5.46 cm^3) compared to 6 km (10.64 ± 0.51 cm^3), 11 km (4.16 ± 2.21 cm^3) and 19 km (0.29 ± 0. 26 cm^3). Most particles and aggregates counted after 15 h were found in the size classes of 3-5 mm and 5-10 mm, respectively. Only aggregates at the 6 km station were mostly found in the largest size fraction of >10 mm. After 30 h and 48 h of incubation in the rolling tanks, the total aggregated volume for the 1 km and 6 km stations was almost exclusively found in the >10 mm size fraction of the aggregates. This was also the case for the 11 km station after 48 h of rolling. There were also significant differences in oxygen consumption rates measured from the rolling tanks incubated with water from the field sampling stations (Fig. 3-8, Kruskal-Wallis test, $p = 0.002$). Consumption rates at the stations closer to Makassar (0.115 ± 0.003 mg O_2 L^{-1} h^{-1} and 0.105 ± 0.031 mg O_2 L^{-1} h^{-1}, respectively) were significantly higher compared to the ones at 11 km (0.021 ± 0.003 mg O_2 L^{-1} h^{-1}) and 19 km (0.032 ± 0.009 mg O_2 L^{-1} h^{-1}).

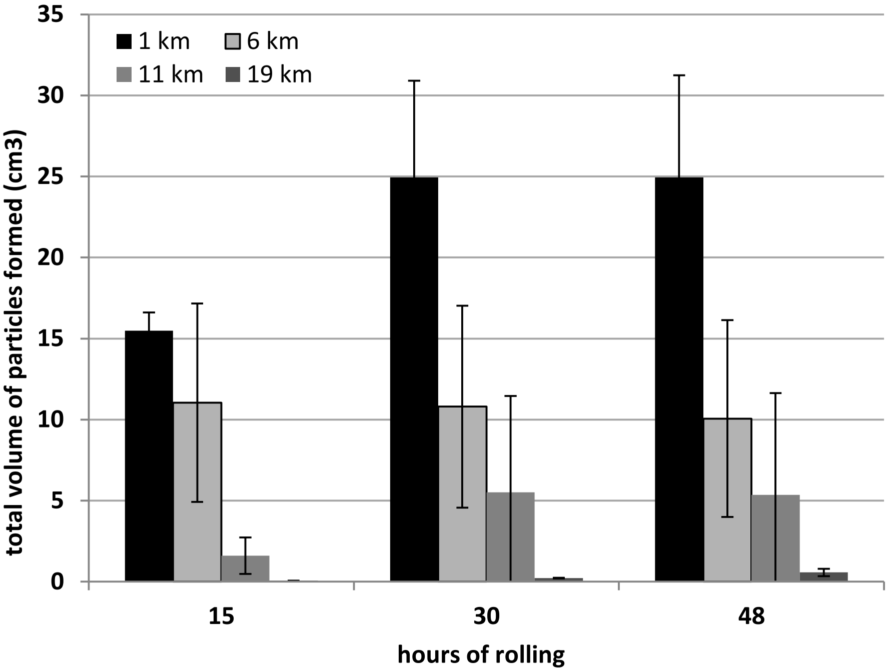

Fig. 3-7 Mean total aggregated volume (± standard deviation) of particles formed in varying distance from shore.

The number average number of OTUs extracted from aggregate samples (707 ± 101) was significantly higher compared to those from the particle-attached size fraction (513 ± 50) of the water column (ANOVA, $p \leq 0.001$, with subsequent Holm-Sidak post-hoc test). Calculated Shannon-Weaver H' diversity was also significantly higher in the aggregates collected from the rolling tank experiment (4.88 ± 0.56 compared to 2.99 ± 0.08 in the particle-attached size fraction of the water column (ANOVA, $p \leq 0.001$, with subsequent Holm-Sidak post-hoc test). Spatial shifts in aggregate-associated bacterial community composition along the cross-shelf gradient after 48 h of rolling were distinct (Fig 3-4). The sample from the 1 km site, closest to Makassar, consisted almost exclusively of *Gammaproteobacteria* (84.32 %). There was only one other bacterial classes with relative abundance higher than 1 %, *Alphaproteobacteria* (11.43 %). The relative abundance of *Gammaproteobacteria* then decreases continuously along the cross-shelf gradient to 8.71 % at the outermost station at 19 km distance from Makassar while, conversely, the abundance of *Alphaproteobacteria* increases to 73.40 % at 19 km. The difference in community composition is also shown in the complete linkage hierarchical clustering, which revealed significant differences (SIMPROF, $p \leq 0.01$) in community composition between sampling sites (Fig. 3-5). The rolling tank experiment showed a rapid shift in bacterial community composition from an almost complete dominance of *Synechococcus* to Proteobacteria within

48 h of rolling in the dark. In rolling tanks which we stopped after 10 h time period we still observed high abundances of *Synechococcus* of 71.41 %, which reflected the data of the field sampling. 48 h later the genus was only found in very low abundances, if found at all. This was in contrast to particle-attached bacterial communities from the aggregates sampled after 48 h of incubation in the rolling tanks, were *Cyanobacteria* were almost absent from the communities.

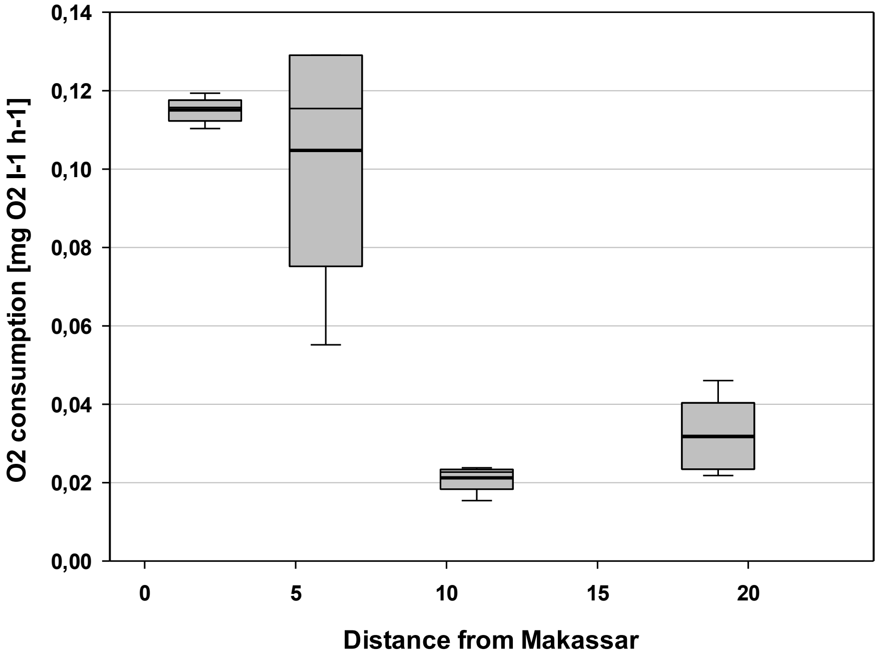

Fig. 3-8 Mean biological oxygen demand measured of the 48 h rolling tank experiment. The graph shows the average hourly oxygen consumption over the whole rolling period of 48 h.

DISCUSSION

The increased occurrence and severity of eutrophication of coastal waters related to agricultural nutrient and anthropogenic sewage input is a frequently observed phenomenon (reviewed by Howarth et al., 2011; Smith and Schindler, 2009; Smith et al., 2006). This observation is often accompanied by unfavorable shifts in phytoplankton and benthic communities, increased productivity, sedimentation or hypoxia (Fabricius, 2011; Fabricius et al., 2005; Meyer-Reil and Köster, 2000; Smith, 2006). However, the coastal shelf waters of the Spermonde Archipelago, outside the closest reef at 1 km distance, were consistently depleted in inorganic nutrients. As a result chlorophyll *a* concentrations, as a surrogate for phytoplankton biomass also decreased several-fold in the same distance range and were generally low compared to other tropical shelf areas, such as the Philippines (Agawin et al., 2003). This is quite a unique feature for a tropical coastal ecosystem which is under constant pressure from anthropogenic sewage by a city of more than 1.5 million and agricultural inputs from various smaller rivers along the coastline. Enrichment in δ15N, measured from SPM and sediment trap material collected at increasing distance from the mainland indicates a decreasing trend. This is in line with another study conducted on Sulawesi, which used δ15N as an indicator of sewage load and found decreasing enrichment at increasing distance from a metropolitan area (Risk et al., 2009). The data presented in this study also indicates that high inorganic nutrient and organic matter assimilation by eukaryotic phytoplankton, *Cyanobacteria* and heterotrophic bacteria in the inshore areas close to Makassar function as a biological filter for untreated sewage and riverine input discharged into the coastal Spermonde Archipelago. Additional inorganic nutrients are immediately transferred into phytoplankton and bacterial biomass and deposited to the sediments in immediate vicinity to their source, preventing eutrophic waters to reach reefs further out on the shelf. The drawdown of inorganic silicate at the station with highest chlorophyll *a* concentrations is a strong indication for a diatom-dominated community, which is commonly found at silicate concentrations > 2 µM (Egge and Aksnes, 1992). Aggregates consisting of diatoms are known to exhibit higher sinking rates, especially during senescence and silicate depletion, which could also explain higher sedimentation rates and fast export at the near-shore site (Bienfang et al., 1982; Eppley et al., 1967).

In oligotrophic conditions, such as those found in the more offshore reefs of the Spermonde Archipelago, *Cyanobacteria* of the genus *Synechococcus* or closely related *Prochlorococcus* are often dominant members of the phytoplankton community (Irwin et al., 2006; Tremblay and Legendre, 1994). *Synechococcus* were ubiquitous and dominant in the larger size fraction of the Spermonde Archipelago. This diverse and commonly found phototrophic bacterium has a fundamental role in global marine primary productivity and biogeochemistry (Agawin et al., 2000; Li, 1994; Richardson and Jackson, 2007; Smith and Kemp, 2001). Due to their advantageous surface to volume ratio most *Synechococcus* are not capable of fixing nitrogen, but are well adapted to rapidly assimilate low concentrations of available inorganic nutrients in oligotrophic conditions (Moisan et al., 2010). In the nutrient-depleted conditions of the Spermonde Archipelago they are able to maintain high photosynthetic activity by assimilating the available low concentrations of inorganic nutrients and organic nitrogen (Berman and Chava, 1999; Tremblay and Legendre, 1994;

Wawrik et al., 2009). Therefore they contribute significantly to the production of new organic matter (Fuks et al., 2005; Phlips et al., 1989). This channeling of large amounts of nutrients and energy through autotrophic, non-pathogenic bacteria into higher trophic levels can help mitigate detrimental effects of fleshy macroalgae growth and subsequent organic matter release on coral reefs (McDole Somera et al., 2016). In reefs under threat by multiple stressors, such as those of the Spermonde Archipelago, this autotrophic microbialization can help to slow down the phase-shift to algae dominance and support a recovery to a less-degraded reef state (Haas et al., 2016; McDole Somera et al., 2016).

Bacterial diversity is generally assumed to be lower with increasing perturbation to the system (Atlas, 1984; Zhang et al., 2009, 2013). But intermediate, non-catastrophic disturbances also create new niches, therefore in fact increasing observed diversity (Connell, 1978). In the Spermonde Archipelago this would imply a greatly reduced bacterial diversity at the inshore station at 1 km. Exposure to increased organic matter loading and inorganic nutrient availability is chronic, so a non-desirable steady state dominated by copiotrophic bacteria adapted to the often anaerobic degradation of organic matter and overall lower diversity would be expected (Huettel et al., 2014). Interestingly diversity within the individual habitats did not vary significantly between the eutrophic inshore and oligotrophic outer shelf zone. Bacterial communities within the water column are exposed to a highly dynamic environment, with constantly changing food availability (Stocker, 2012). As in other contrasting tropical marine environments, diversity of water-column bacteria does not necessarily change due to differences in environmental conditions (Michotey et al., 2012). There is also a constant exchange between the free-living bacteria and those inhabiting aggregates via successional colonization and detachment processes, which could also maintain a high diversity under varying environmental conditions (Grossart et al., 2002; Kiorboe et al., 2003; Rath et al., 1998; Simon et al., 2002).

Alpha- and *Gammaproteobacteria* are often found to be the dominating classes in the free-living fraction of the water column (Crespo et al., 2013; Crump et al., 1999; Zhang et al., 2009). *Alphaproteobacteria*, which were more abundant in the water column of stations closer to shore, are known to correlate well to waters with higher inorganic nutrient concentrations and phytoplankton biomass (Allers et al., 2007; Teira et al., 2008). *Gammaproteobacteria* feature a wide arrangement of extracellular hydrolytic enzymes which enable them to rapidly assimilate organic matter, even at low concentrations (Azam and Malfatti, 2007; Edwards et al., 2010). Sediments, in contrast to the overlying water masses with short residence times, can integrate sedimentation processes of the water column over time and act as an archive to investigate long-term differences between the two contrasting environments of the inshore and outer shelf zone. This is also reflected in the bacterial community composition of the sediments, again with a dominance of *Alpha-* and *Gammaproteobacteria*. Given the constant supply of organic matter from the water column heterotrophic bacteria play a key role in remineralizing the deposited material. This change in composition has been observed at other coastal sampling sites (Zhang et al., 2007).

One additional peculiarity of the Makassar Strait are the large water masses moving from the Pacific through the Strait into the Indian Ocean at a relatively high speed (Gordon et al., 2008). Water residence time is an important indicator of productivity (Delesalle and Sournia, 1992). Thus the predominantly southerly flow of the water can lead to a lateral movement of sewage plumes and agricultural input to the south, which mitigates the nutrient loading to the shelf waters. Overall with our data we can show that the influences of untreated sewage from Makassar and riverine agricultural nutrient input on the water quality across the shelf are low. Both factors, physical dilution by lateral water movement as well as inshore biological uptake by photoautotrophic organisms and subsequent on-site sedimentation considerably reduce the extent to which eutrophication affects the more distant reefs on the shelf. The differences in the bacterial communities sampled from contrasting environments of the two zones after 48 h of rolling give a valuable insight into the quality of the aggregated material from the different sites across the gradient. With decreasing chlorophyll *a*, TEP and DOC concentrations from inshore to offshore stations the bacterial communities sampled from the aggregates changed from *Gammaproteobacteria* to *Alphaproteobacteria* dominance. This was corroborated by a decreasing microbial bacterial activity, measured as biological oxygen demand, from inshore to offshore stations. These experimental observations support our hypothesis about a biological filter protecting the reefs of the Spermonde Archipelago from excessive nutrient and organic matter input. Enrichment of the sinking aggregates with *Gammaproteobacteria* at the inshore site indicates that remineralization already starts on the particles. When those aggregates settle on the sediment they already contain a bacterial community well adapted for the degradation of organic matter and therefore also contribute to remineralization processes in the top layers of the sediments.

The insights gained from this study also emphasize the need to include the analysis of bacterial community composition into routine assessments of water quality (Garren and Azam, 2012). Bacterial communities often respond much faster to changes in environmental conditions and water quality, therefore specific indicator groups could function as an early warning system before detrimental effects can be detected in corals and other reef organisms. Samples can be stored for long times in regular fridges or freezers, and the costs for next-generation sequencing analysis have decreases dramatically in the past year, together with the advent of more advanced –omic tools to offer even more in-depth analyses, e.g. the expression of pathogenicity- or virulence-related genes (Garren and Azam, 2012; Tan et al., 2015).

CONCLUSIONS

In conclusion we propose that the high nutrient and organic matter assimilation activity of phytoplankton, *Cyanobacteria* and heterotrophic bacteria, in connection with the high on-site sedimentation rates, in the immediate vicinity to Makassar act as a biological filter that prevents eutrophic waters from harming coral reefs further out on the shelf. Inorganic nutrients are directly metabolized and deposited to inshore sediments. The calm waters further out on the shelf, low N/P ratio and overall low nutrient concentrations, together with stable DOC concentrations, provide favorable conditions for the growth of *Cyanobacteria* in the water column, which maintain a stable primary productivity. The biological filtration could be an important mechanism to maintain oligotrophic shelf waters and healthy coral reefs in a system under constant anthropogenic pressure.

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Chapter 4

Small tropical islands with dense human populations: Decreased water quality results in distinct bacterial communities

Small tropical islands with dense human populations: Decreased water quality results in distinct bacterial communities

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ABSTRACT

Anthropogenic stress, such as enrichment in inorganic and organic matter, is one of the major local threats to coral reefs in Indonesia. So far emphasis has often been put on processes along larger scale impacts of those unfavorable changes across whole estuaries or entire shelfs. The present study investigated, for the first time in the Spermonde Archipelago, differences in water quality and bacterial community composition in different coral reef habitats, including the water column and back-reef sediments, between a densely populated and an uninhabited island of comparable distance to shore. The main aim was to elucidate if a) water quality related parameters, including inorganic nutrients, chlorophyll *a*, DOC and TEP differ between an uninhabited and a densely populated island of the archipelago and b) whether there are related difference in bacterial community composition in back-reef sediments, and the water column, which can be attributed to differences in water quality. Several key water quality parameters, including, PO_4, chlorophyll *a*, DOC and TEP were significantly elevated at the inhabited island. *Flavobacteriaceae* and various groups within the *Gammaproteobacteria*, bacterial taxa well adapted to organic matter rich environments, were significantly enriched at the inhabited island. Within the habitats changes of bacterial community composition between the islands were significantly correlated to differences in water quality. With this study we could show that not only large, urban areas can have a severe impact on the water quality and bacterial community. Dense island populations also have the ability to shape bacterial of the surrounding reef flats by altering the water quality of the surrounding waters. From a management perspective they could be priority targets to implement improved sewage treatment facilities, as shift in bacterial community composition are not as pronounced in the highly impacted inshore zone of the Spermonde Archipelago.

INTRODUCTION

Coastal coral reef systems in close vicinity to highly populated urban areas are often impacted by land-based activities (Reopanichkul et al., 2010; Wolanski et al., 2009). The Spermonde Archipelago in southern Sulawesi, Indonesia, including its approximately 150 small islands, is an excellent example for that anthropogenic impact, as several previous studies revealed (Plass-Johnson et al., 2015; Renema and Troelstra, 2001; Sawall et al., 2012). The Archipelago has been described to be characterized by a cross-shelf gradient from chlorophyll *a*-rich inshore to oligotrophic offshore waters (Becking et al., 2006; Renema and Troelstra, 2001). But although this gradient has been related to sewage input from metropolitan Makassar (population: 1.4 million), no extensive assessment of water quality parameters related to eutrophication, e.g. inorganic nutrients, chlorophyll *a*, dissolved organic carbon (DOC) or transparent exopolymer particles (TEP), have been carried out so far. Untreated sewage and pollutants from Makassar enter the system directly or via the river Jene Berang, which additionally discharges sediments and inorganic nutrients from the hinterland (Renema and Troelstra, 2001). In addition to anthropogenic disturbances on the larger-scale, driven by sewage and riverine input from the mainland and Makassar, the individual islands are also densely populated and lack proper sewage and waste water treatment facilities. Thus, they are also potential sources of inorganic and organic nutrients to the fringing reefs, introduced via groundwater seepage (Laws et al., 2004; Paytan et al., 2006). Those nutrients are quickly assimilated and converted into phytoplankton biomass (Koop et al., 2001). This leads to eutrophication, one of the primary local threats to coastal marine ecosystems (Burke et al., 2011; Paerl et al., 2014). Consequences are an increased release of DOC and TEP (Karl et al., 1998; Passow, 2002) and subsequent aggregation and sedimentation of large particles rich in organic matter (Cárdenas et al., 2015; Kiørboe and Hansen, 1993).

Bacterial community composition in reef waters can shift significantly in organic matter-rich conditions (Meyer-Reil and Köster, 2000; de Voogd et al., 2015; Weinbauer et al., 2010), microbial biofilms (Sawall et al., 2012; Witt et al., 2012) and sediments (Uthicke and McGuire, 2007). This shift often occurs alongside an increase in total bacterial cell counts (Dinsdale et al., 2008; Zhang et al., 2007, 2009) are related to a transition from autotrophic to heterotrophic bacterial communities (Meyer-Reil and Köster, 2000; Witt et al., 2012). High bacterial abundance and remineralization activities increase the oxygen consumption and lead to hypoxia, with potentially fatal consequences for benthic reef organism (Kline et al., 2006). Bacteria also play an important role in biogeochemical cycling and coral reef health, therefore, small shifts induced by increased anthropogenic eutrophication can further alter nutrient cycling, sedimentation and organic matter export, as well as promoting coral pathogens (Bruno et al., 2003; Fabricius, 2005; Lyons et al., 2010).

There is only few studies on bacterial community composition in the Spermonde Archipelago, which have focused on diversity of settlement tile biofilms (Sawall et al., 2012) and bacterial communities from different reef habitats, specifically within sponges and the functional role of the associated bacteria (Cleary et al. 2015). The present study now aims to elucidate how the inhabitation status (uninhabited and densely populated) of two islands of similar distance to mainland Sulawesi is reflected in differences in water quality parameters, including inorganic nutrients, chlorophyll *a*, DOC and TEP. Bacterial community composition was investigated simultaneously to find out if those changes in water quality could lead to differences in bacterial community composition within the reef sediments and two free-living as well as particle-attached fractions of the water column. Previous studies have shown that enrichment with organic matter can lead to an increased prevalence of pathogenic bacteria in scleractinian corals (Garren et al., 2009). And deteriorating coral reefs of the fringing islands could put the livelihoods of thousands of fishermen in the Spermonde Archipelago at risk (Pet-Soede et al., 2001).

The main research questions of this study were: a) Do water quality related parameters, including inorganic nutrients, chlorophyll *a*, DOC and TEP differ between an uninhabited and a densely populated island of the archipelago? b) Are there related difference in bacterial community composition in back-reef sediments, and the water column, which can be attributed to differences in water quality?

MATERIAL AND METHODS

Two transects each at two islands of similar distance to Makassar, a city of approx. 1.4 million inhabitants, were sampled during the dry south-east monsoon in May and June 2014 (Fig. 4-1). One island, Pulau Barrang Lompo, is densely populated, with more than 4,000 people inhabiting the 20 ha island, while Pulau Kodinggareng Keke was chosen as an uninhabited reference island of similar distance to shore. Two replicate transects were always laid out from 25 m to 300 m across the northern and southern part of the back-reef area to see if different parts of the back reef received different nutrient input. All sampling was conducted while snorkeling during high tide in the morning hours. Temperature (30.65 ± 0.40 °C to 31.33 ± 0.44 °C), pH (8.02 ± 0.06 to 8.14 ± 0.03) and salinity (32.47 ± 0.08 to 33.10 ± 0.04) were all within a very narrow range across both islands.

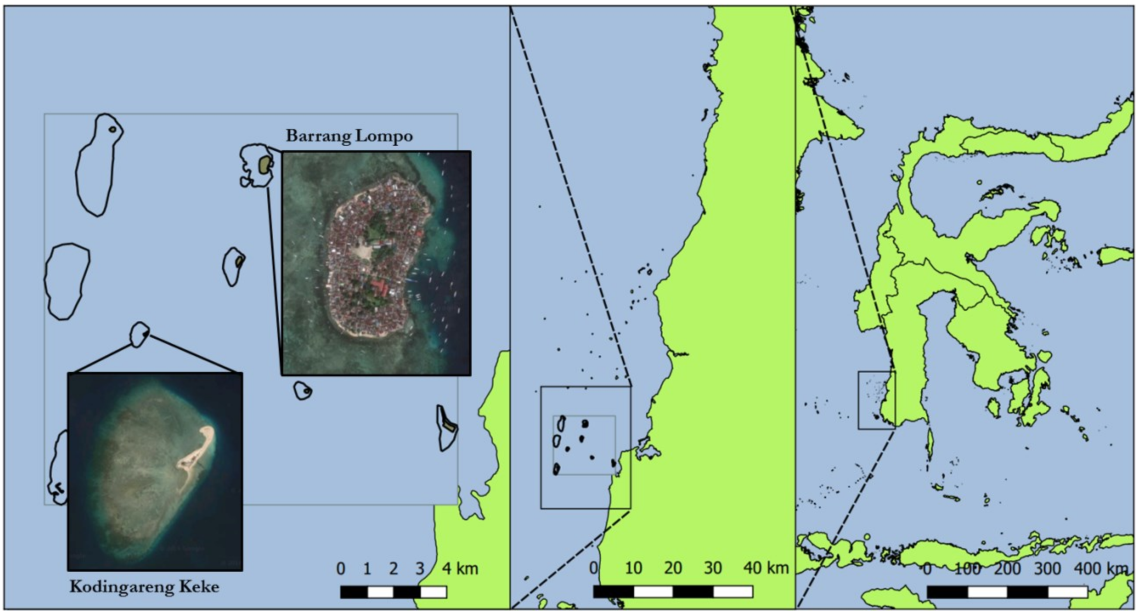

Fig. 4-1 Map of the two sampled islands (left) Barrang Lompo (inhabited) and Kodingareng Keke (uninhabited) in the Spermonde Archipelago (middle), Sulawesi (right), Indonesia.

Environmental and water quality parameters

Measured water quality parameters included inorganic nutrients, dissolved organic carbon (DOC), transparent exopolymer particles (TEP) as well as environmental parameters and chlorophyll *a*. While the environmental parameters and chlorophyll *a* were measured *in-vivo* with a Eureka Manta 2 multiprobe (*eureka water probes, Austin, USA*), surface water was collected at all sampling points using three replicate 5 L canisters for determination of inorganic nutrients, DOC, TEP and SPM. From each replicate subsamples were taken for these water quality parameters . Samples were stored at -20 °C in the dark until analysis immediately after returning to the field station, which never exceeded 3 h after sampling. After filtration through a 0.7 µm GF/F filter (*GE Healthcare Bio-Sciences, Pittsburgh, USA)* and subsequent fixation with mercury chloride, inorganic nutrients (nitrite, nitrate, phosphate and silicate) where measured spectro-photometrically with a Flowsys continuous flow analyser (*Unity scientific, Brookfield, USA*). To determine DOC, subsamples were filtered through 0.45 µm pore Whatman GF/F filters *(Whatman, GE Healthcare, Pittsburgh, USA)* and acidified with HCl (pH below 2). High-temperature oxic combustion (HTOC) using a TOC-VCPH TOC analyzer (*Shimadzu, Mandel, Canada*) completed the measurement. Calibration and as quality control Hansell artificial seawater standards (*Hansell laboratory RSMAS, University of Miami*) and ultrapure water blanks were used. SPM mass was analyzed by filtering a known volume of sample water through pre-combusted GF/F filters that were weighed on a ME 36S (*Sartorius, Göttingen, Germany*) before and after filtration. SPM was determined as the difference in weight after filters were dried for 24 h at 40 °C. For TEP analysis the spectrophotometric method first introduced by Passow and Alldredge (1995) was used with an updated protocol as described by Engel (2009). Briefly described, this method relates the adsorption of a dye to the weight of polysaccharides filtered on 0.4 μm polycarbonate filters. A calibration curve was prepared using the reference polysaccharide Gum Xanthan from *Xanthomonas campestris* cultures.

DNA extraction and Illumina sequencing

To separate bacterioplankton in two selected size fractions representing 'particle-attached' and 'free-living' bacteria, a 1 L subsample from each site was filtered sequentially using 3 μm and 0.2 μm Whatman Nuclepore polycarbonate filters *(Whatman, GE Healthcare, Pittsburgh, USA)*. The protocol established by Boström et al. (2004) for DNA extraction from water column samples was applied without modification. Surface sediment was collected from the uppermost 1 cm of sediment at each transect point from. Sediment samples were allocated to 2.0 mL tubes *in-situ* (*Eppendorf, Germany*) and stored at -20 °C until extraction using the PowerSoil™ DNA Isolation Kit (*MO BIO Laboratories, Carlsbad, USA*) with modification of two steps of the protocol: 1) we did not incubate for 5 min at 4 °C but went straight for the centrifugation and 2) we used 50 μL of elution buffer instead of 100 μL. All extracted DNA samples were sequenced by LGC Genomics (*Berlin, Germany*) through an Illumina MiSeq V3 pipeline (*Illumina Inc., San Diego, USA*). The 16S rRNA primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-CTACCAGGGTATCTAATCC-3') were used, targeting the V3-V4 hypervariable region. Quality trimming of the sequences was done by applying the Trimmomatic tool (Bolger et al., 2014), before paired-end read merging with PEAR (Zhang et al., 2014). Remaining OTUs where then *de novo* clustered with the Swarm algorithm, a novel method which tries to avoid the pitfalls of globally set clustering thresholds and input-order dependencies (Mahé et al., 2014). Afterwards the clustered OTUs were aligned and taxonomically classified with the SILVA Incremental Aligner (SINA, Pruesse et al., 2012), using the SILVA Version 1.19 ribosomal RNA gene database (Quast et al., 2013).

Statistical analysis

Concentrations of environmental parameters and water quality are given in arithmetic mean ± standard deviation. To determine differences among sites in these parameters statistical analysis was conducted using the SigmaPlot 13.0 software (*Systat Software, Inc., San Jose, California, USA*). Whenever parameters failed the Shapiro-Wilk test for normal distribution an alternative non-parametric Kruskal-Wallis test on ranks was performed. Whenever significant differences were detected, pair-wise comparisons using Holm-Sidak (parametric) or Tukey's HSD (non-parametric) post-hoc tests were carried out. Principle component analysis (PCA), non-metrical multi-dimensional scaling (NMDS) plotting and hierarchical clustering of changes between the stations including all environmental and water quality parameters was conducted using the R software package, version 3.2.2 (R Core Team, 2015) and R-Studio, version 0.99.482 (RStudio Team, 2015). NMDs plotting, based on Bray-Curtis dissimilarities, was also conducted to visualize patterns in the bacterial community composition. Analyses of similarity (ANOSIM) were used to test for significant differences of bacterial communities from different habitats and within the sampled habitats between the two islands. Redundancy analysis (RDA) was used to test the contributions of 'habitat' and 'island' within the individual habitats to the explanation of observed variability in the different bacterial communities. The Aldex function (Fernandes et al., 2014) was applied to detect differentially abundant OTUs based on BH-adjusted *p*-value of 0.1 or an absolute effect size of more than 2. The statistical analysis of the bacterial communities to compare diversity, changes in abundance, and to relate changes in bacterial community composition to variability in water quality parameters, were carried out with the R software package and R-Studio. Additional packages used within R were 'vegan' (Oksanen et al., 2016) and 'ALDEx2' (Fernandes et al., 2014).

RESULTS

Water quality parameters

All measured water quality parameters, except NO_x and silicate, were significantly elevated at the inhabited island, and often decreased from the station closest to the island to the reef crest (Fig. 4-2, Supplementary material Fig. S 4-1). Highest Nitrite/Nitrate (Fig. 4-2 a) and phosphate (Fig. 4-2 b) concentrations (0.59 ± 0.01 µM and 0.18 ± 0.01 µM, respectively) were measured on the southern transect of the inhabited island, compared to highest concentrations of 0.16 ± 0.01 and 0.14 ± 0.01 µM at the uninhabited island.

Differences between the islands were statistically significant for inorganic phosphate (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons), At both transects, north and south, chlorophyll *a* concentrations were always higher at the inhabited island (Fig. 4-2 d). Again, differences between the two islands were statistically significant (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons). Concentrations of different size spectra of organic matter, DOC (Fig. 4-2 e) and TEP (Fig. 4-2 f), were also elevated at the inhabited island. Lowest DOC concentrations (67.40 ± 1.00 µM) were measured on the southern transect of the uninhabited island while highest concentration (95.76 ± 9.06 µM) was measured at the southern transect of the inhabited island. TEP concentrations were also almost threefold higher at the inhabited island (184.36 ± 42.59 compared to 67.78 ± 6.59 µg Xeq × L^{-1}). Both parameters were significantly elevated at the inhabited island (Kruskal-Wallis test, $p \leq 0.001$, Tukey's post-hoc pairwise comparisons). The first two principal components of the PCA (Fig. 4-3, Tab. 4-1) accounted for 70.8 % of the variation in the water quality parameters among islands. Based on the eigenvectors of the individual water quality parameters the sampling sites from the inhabited island separated clearly from the southern transect of the uninhabited site, and to a lesser degree from the northern transect of the uninhabited site, by the first principal component. Those differences along the first main principal component were mainly driven by TEP, PO_4, NO_x and DOC (Tab. 4-1). Differences along the second principal component were driven by chlorophyll *a* and silicate, separating the northern transect of the inhabited from the southern transect of the uninhabited island.

Tab. 4-1 List of eigenvectors of the individual water quality parameters contributing to the principal components of Fig. 4-3.

Variable	PC1	PC2	PC3	PC4	PC5
NO_x	-0,437	-0,373	-0,321	0,282	0,209
PO_4	-0,444	0,312	0,180	-0,564	-0,476
Si	-0,280	-0,676	0,471	-0,356	0,225
Chlorophyll *a*	-0,369	0,540	0,407	0,197	0,606
DOC	-0,417	0,102	-0,678	-0,299	0,194
TEP	-0,473	-0,060	0,130	0,589	-0,524

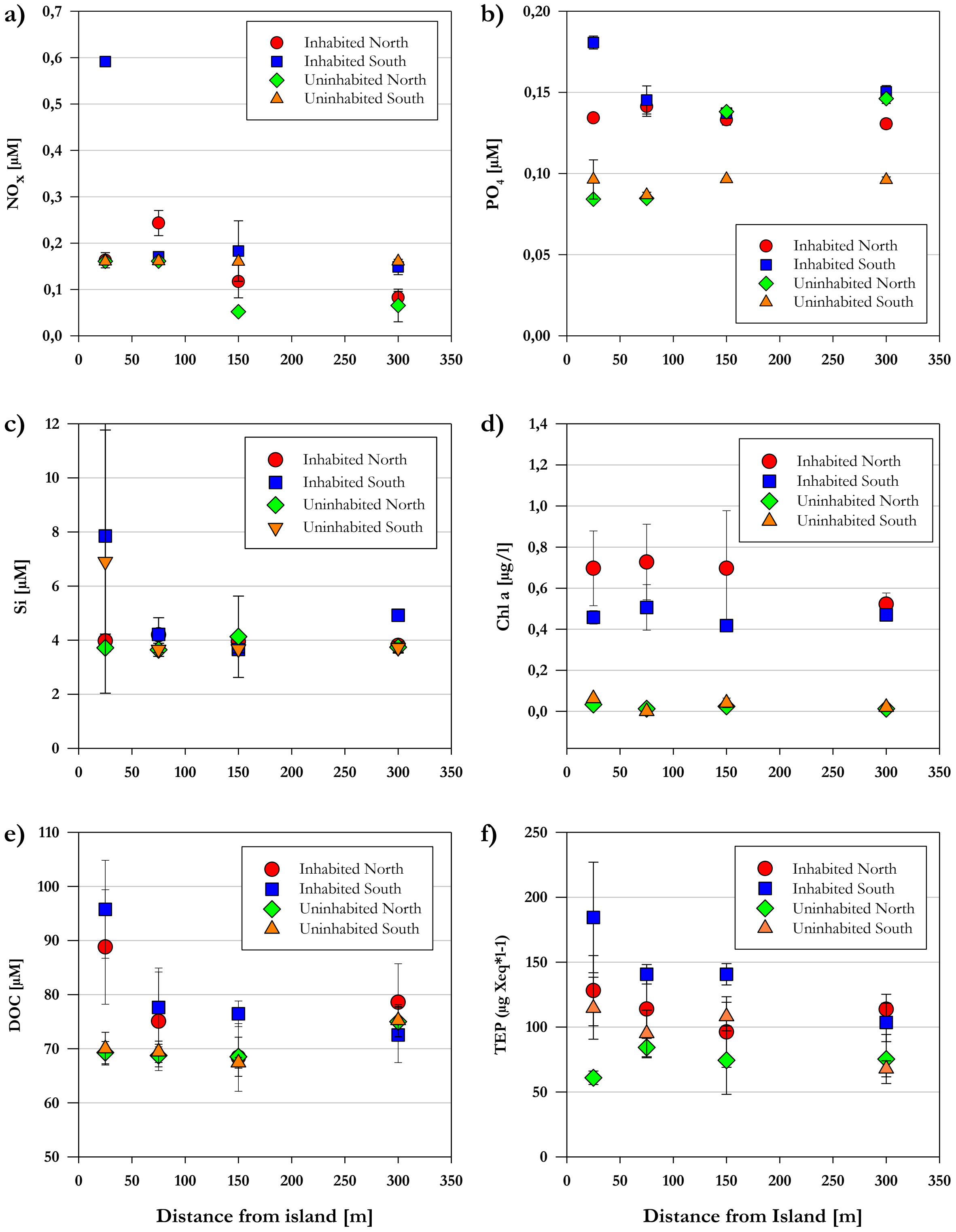

Fig. 4-2 Water quality parameters of the inhabited (red and blue symbols) and uninhabited (green and orange symbols) island. a) NO$_x$, b) PO$_4$, c) Si, d) Chlorophyll *a*, e) DOC and f) TEP. Error bars depict standard deviation.

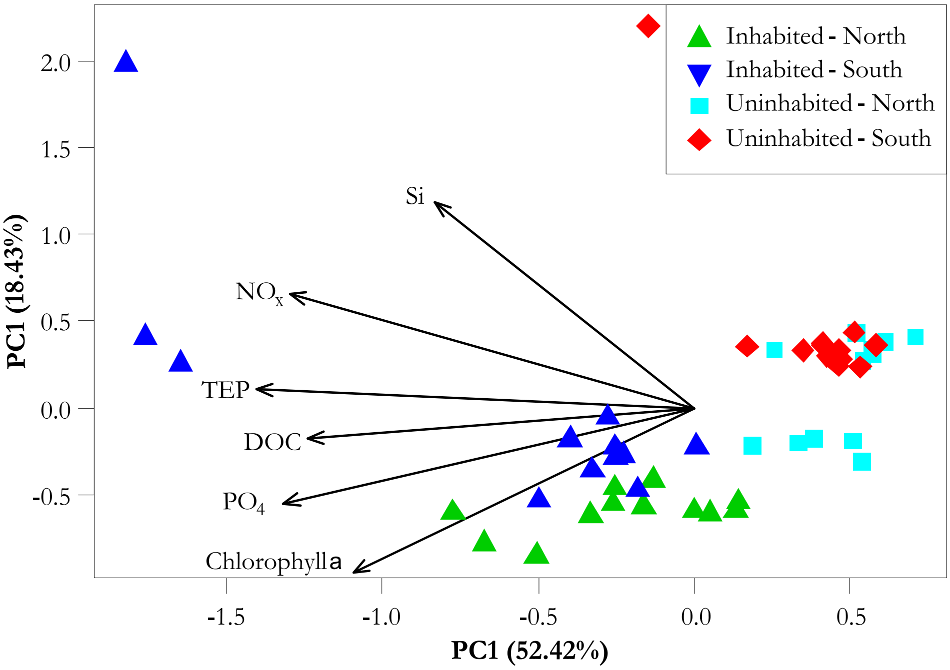

Fig. 4-3 PCA ordination of samples of the inhabited (green and blue symbols) and uninhabited (turquoise and red symbols) according to normalized water quality parameters. PC1 and PC2 explained 52.42 % and 18.43 % of total variation, respectively.

Overall and alpha diversity

After quality control, and removing of singletons and OTUs which were not detected at least three times in two samples, a total of 554,436 Sequences remained, distributed among 44 samples. On average there were 12,601 sequences per sample (ranging from 831 to 55,682 reads). The number of OTUs per sample ranged from 384 to 1,575. Highest average OTU counts (1,421) were obtained from the sediments, compared to 707 and 540 from the particle-attached and free-living fraction of the water column, respectively. Across both islands and all three habitats *Gammaproteobacteria* was the most abundant class (23.67 % of all counts). *Alphaproteobacteria* and *Cyanobacteria* both contributed to 18.53 % of the bacterial community. Additional main contributors, with more than 1 % contribution, to total observed bacterial were *Flavobacteriia* (8.88 %), *Deltaproteobacteria* (6.36 %), *Acidimicrobiia* (6.03 %), *Planctomycetacia* (4.03 %), *Sphingobacteriia* (1.40 %), *Clostridia* (1.35 %), *Phycisphaerae* (1.29 %) and *Cytophagia* (1.16 %). Average Shannon diversity was also highest in samples obtained from the sediment, followed by the free-living and particle-attached fraction of the water column (Supplementary material, Tab. S 4-1).

Bacterial community composition differences between habitats and islands

The NMDS plot revealed distinct differences between the communities originating from the three sampled habitats, the free-living and particle-associated fraction of the water column as well as the sediments (Fig. 4-4). Within the habitats there is also a clear separation between the inhabited and the uninhabited islands in the NMDS bi-plot. *Gamma-* and *Alphaproteobacteria* were the most dominant fraction (17.04 % and 15.55 %) of the sediments at the inhabited island (Fig. 4-5). That order was reversed for the uninhabited island, with 14.54 % and 18.22 % contribution to the bacterial community composition, respectively. *Flavobacteriia* and *Planctomycetacia* were the third most common classes (10.73 % and 15.13 %) at the inhabited and uninhabited island, respectively. The free-living fraction of the water column was also dominated by members of the *Gamma-* and *Alphaproteobacteria*, which, together, contributed to more than 50 % of the community at both islands. *Flavobacteriia* (15.29 %) was the third most abundant class at the inhabited island while this was *Acidomicrobiia* (13.78 %) for the uninhabited island. The particle-attached fraction of the water column bacterial community was dominated by *Cyanobacteria* (34.77 %), closely followed by *Gammaproteobacteria* (34.65 %) and *Alphaproteobacteria* (16.12 %). Cyanobacterial dominance (59.54 %) was even more pronounced at the uninhabited island. *Gamma-* and *Alphaproteobacteria* (15.23 % and 12.43 %) followed as second and third highest contributors to of the particle-attached bacterial community composition.

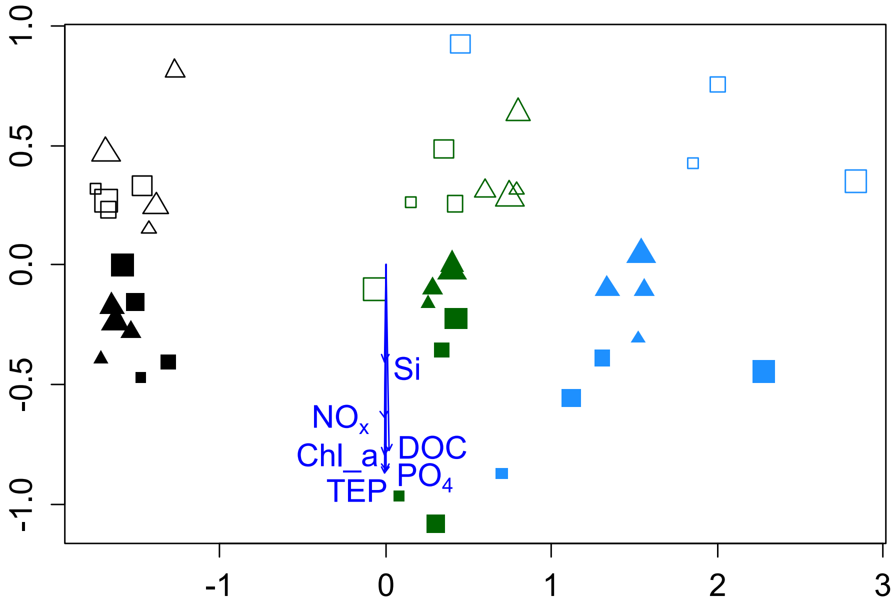

Fig. 4-4 NMDS plot of the sampled habitats at the inhabited island (filled symbols) and uninhabited island (empty symbols): free-living (blue) and particle-associated (green) fraction of the water column as well as reef sediment communities (black). Triangles and squares indicate samples from northern and southern transects, respectively. Arrows of the water quality parameters indicate envfit explanation of variability between the two islands.

Those differences between communities of different habitats were highly significant (ANOSIM, $p \leq 0.001$). This was also corroborated by the hierarchical clustering (Supplementary material Fig. S 4-1). For the sediment (ANOSIM, $p = 0.001$) and particle-attached (ANOSIM, $p = 0.004$) bacterial communities there were also significant differences in community composition between the inhabited and uninhabited island. A redundancy analysis (RDA) showed a strong and highly significant (adjusted $R^2 = 0.34$, *post-hoc* ANOVA, $F = 12.71$, $p \leq 0.001$) explanation of the variability within the bacterial community by the factor 'habitat'. To a lesser degree the factor 'island' could additionally explain differences of the overall community composition. The RDA revealed a lower variability explained (adjusted $R^2 = 0.04$), which was nonetheless highly significant (*post-hoc* ANOVA, $F = 3.95$, $p \leq 0.001$). Within the three habitats the factor 'island' was always significant in explaining variability between the two communities, although adjusted R^2 for sediment (adjusted $R^2 = 0.18$, *post-hoc* ANOVA, $F = 4.39$, $p \leq 0.001$), free-living (adjusted $R^2 = 0.10$, *post-hoc* ANOVA, $F = 2.28$, $p \leq 0.004$) and particle-associated (adjusted $R^2 = 0.10$, *post-hoc* ANOVA, $F = 2.72$, $p \leq 0.002$) communities was never as high as for the overarching factor 'habitat'.

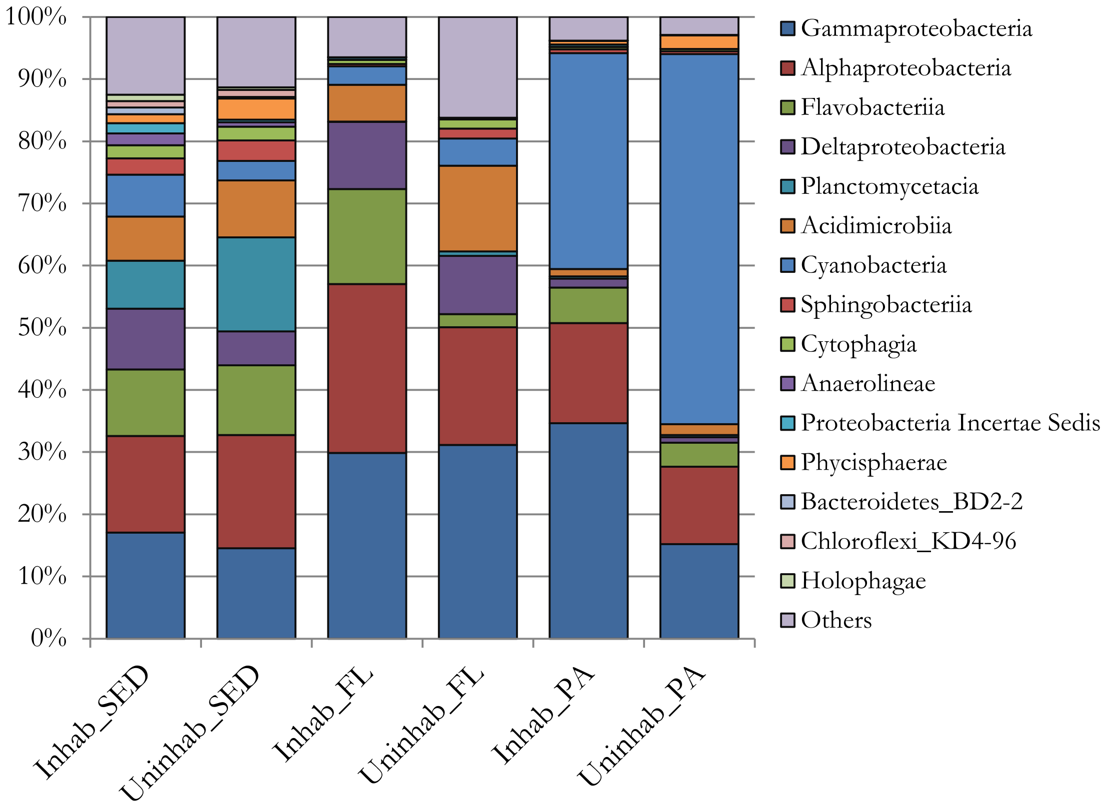

Fig. 4-5 Relative abundance of the bacterial classes from the samples of the inhabited and uninhabited island: SED) reef sediments, FL) bacteria of the free-living fraction of the water column (> 0.2 µm), and PA) "particle-attached" fraction (> 3 µm) of the water column.

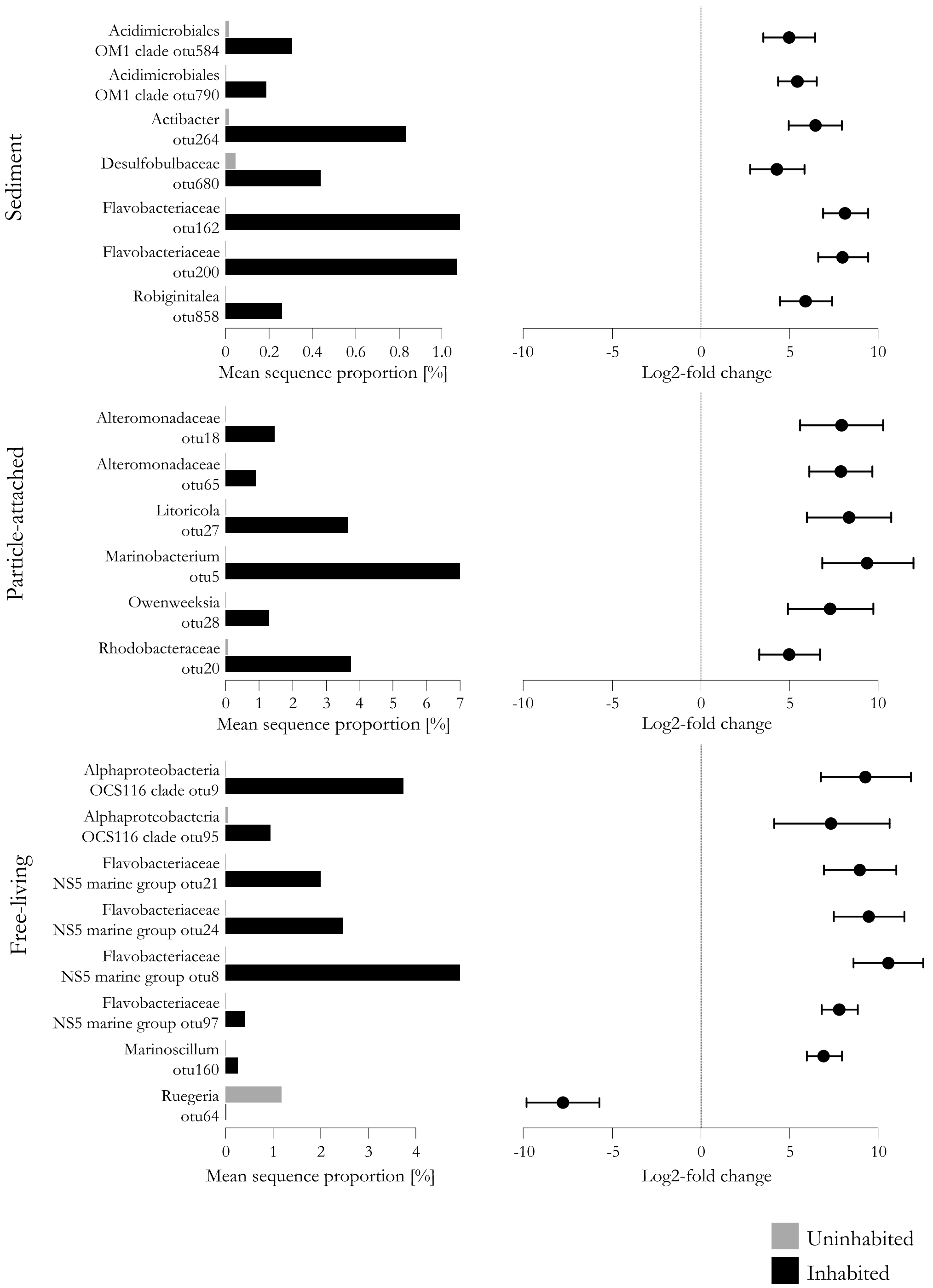

Fig. 4-6 Mean relative abundance and log2-fold change of bacterial communities from the sediments (top panel), particle-attached (particle-attached (middle panel) and free-living fraction (bottom panel) of the water column from the uninhabited (gray bars) and inhabited island (black bars).

To determine which OTU were most contributing to the differences between the inhabited from the uninhabited island within the individual habitats we tested for differences between the relative abundance of the two islands. Within the sediments OTU from the families *Acidimicrobiales OM1 clade*, *Flavobacteriaceae*, *Desulfobulbaceae* and the genera

Actibacter and *Robiginitalea* were significantly enriched at the inhabited island (Fig 4-6, top panel). Particle-attached bacterial communities (Fig. 4-6, middle panel) of the inhabited island were several-fold more abundant in two OTU of the families *Alteromonadaceae* and *Rhodobacteraceae*, as well as the genera *Litoricola*, *Marinobacterium* and *Owenweeksia*. Free-living bacteria from the *Alphaproteobacteria OCS 116* clade, *Flavobacteriaceae NS5* marine group and *Marinoscillum* were significantly more abundant in the water column surrounding the inhabited island (Fig. 4-6, bottom panel). There was one OTU, *Ruegeria*, in the free-living fraction of the uninhabited island which was enriched compared to the inhabited one. Differences in community composition between the two islands could be attributed to changes in the measured water quality parameters (NMDS, Fig. 4-4). All observed parameters showed an overall increase in concentration from the uninhabited to the inhabited island.

DISCUSSION

It is well established that dense human populations inhabiting the small islands of the Spermonde Archipelago can have profound effects on the physical environment of those islands. There is now a well-documented imbalance of available freshwater und demand with increasing population density and changed water retention capability through construction activities (Schwerdtner Máñez et al., 2012). Scleractinian corals are also still being mined for construction activities or farmed for the international aquarium trade, deteriorating the reefs supporting the vital fisheries of the archipelago (Ferse et al., 2012). Blast fishing also continues unabated, with a high destructive potential for the affected reefs (Nurdin and Grydehøj, 2014; Pet-Soede et al., 2001). But even though a lot of attention in the past decades has been focused on investigations of the larger scale effects of changes in water quality in the Spermonde Archipelago (Moll, 1983; Polónia et al., 2015; Renema and Troelstra, 2001; Sawall et al., 2012), no studies were conducted to investigate localized changes in water quality among the islands yet.

Water quality parameters

Results from measurements of the water quality parameters indicate that the inhabited island is significantly affected by anthropogenic inorganic nutrient input. Sewage outflow enters the reefs waters through diffusive groundwater seepage. This can lead to elevated concentrations of inorganic nutrients in the near-shore areas, as was observed in this study as well as at other, larger islands (Kim, 2003; Street et al., 2008; Umezawa et al., 2002). Interestingly, most of the introduced inorganic and organic nutrients were likely assimilated, into phytoplankton, benthic algae or seagrass, and transformed into the particulate fraction or metabolized at the inhabited island, as measured concentrations decreased across the back-reef gradients. Those observations of steeply declining concentrations (with the exception of chlorophyll *a*) from sampling sites very close to the island to the ones further out on the reef flat, resemble those of observations made at several other tropical and subtropical reef locations in the Hawaiian Archipelago, USA (Laws et al., 2004) and in Moorea, French Polynesia (Nelson et al., 2011). Those declining concentrations of water quality parameters are often related to biological and physical removal processes within the back-reef. Inorganic nutrients are rapidly assimilated by benthic macroalgae (Koop et al., 2001; Lapointe et al., 2004) and phytoplankton (Hallock and Schlager, 1986). DOM, released by phytoplankton, often aggregates into larger molecules, such as TEP and ultimately POM (Passow and Alldredge, 1994; Verdugo et al., 2004) and subsequently settles to the sediments. There it is metabolized by heterotrophic bacteria (Wild et al., 2004). The changes in concentrations of water quality parameters at the small scale gradients are also mirrored by observations along the larger scale gradient from Makassar to the shelf break investigated in previous studies, where a steep decline especially in organic nutrients was observed from the eutrophic zone close to Makassar compared to the oligotrophic zone further out on the shelf (Kegler, chapter 2 and 3, Becking et al., 2006; Edinger et al., 1998; Sawall et al., 2012). However, that enrichment caused by the human island population is small in amplitude compared to eutrophication caused by sewage outflow and riverine nutrient input to the inshore zone off Makassar.

Bacterial community composition differences between habitats and islands

Taking all samples into account, differences in community composition between habitats, the sediments, free-living and particle-attached fraction of the water column, were the overriding factor determining community composition along the sampled gradient. This result is supported by many studies from tropical coral reef ecosystems, including the Spermonde Archipelago, which showed very distinct bacterial communities between various habitats within the reefs (Cleary et al., 2015; Polónia et al., 2015; Rohwer et al., 2002; Tout et al., 2014). Different habitats vary considerably in substrate heterogeneity, colonizable surfaces and food availability. Some habitats are stable over longer periods of time, such as the pelagic water column, while others, such as rapidly flushed coastal ecosystems, are disturbed more frequently. And both habitats will favor different bacterial life strategies (Lauro et al., 2009). Thus, investigations trying to disentangle the driving forces of community variability along gradients of water quality or contrasting environmental parameters should focus on the within-habitat differences of bacterial community composition.

In the present case study the differences between the densely populated and the uninhabited island were always highly significant within the individual habitats, and differences in water quality could explain significant parts of the community variability. This is a novel finding for the Spermonde Archipelago, as local drivers of bacterial community variability were neglected so far. It is well known that changes in the availability of water quality parameters such as inorganic nutrients, chlorophyll *a*, DOM or TEP can lead to shifts in bacterial community composition (Allers et al., 2007; Horner-Devine et al., 2003; Weinbauer et al., 2010). Often the changes favor opportunistic, copiotrophic and potentially pathogenic bacteria (Dinsdale et al., 2008; Eilers et al., 2000). In this study several OTUs from the *Flavobacteriaceae* family were significantly enriched in sediments and the free-living fraction of the water column at the inhabited island. Members of *Flavobacteria*, the largest group within the *Bacteroidetes*, are key players in the initial degradation of high molecular mass (HMW) fraction of organic matter derived from algae production and detritus (Kirchman, 2002; Pinhassi et al., 2004). In turn more labile DOM is available for bacterial groups specialized in the uptake in smaller polysaccharides, such as *Alphaproteobacteria* and *Gammaproteobacteria* (Williams et al., 2012). Thus, they can be considered the start of the chain of DOM degradation fueling the microbial loop (Azam et al., 1983; Fenchel, 2008). In the back-reef area of the inhabited island chlorophyll *a* was consistently elevated compared to the uninhabited island as well as sampling off the reef crest (Kegler, chapter 4 of this thesis). This can explain the increased relative abundance of *Flavobacteriaceae* and is consistent with observations of other studies (Pinhassi et al., 2004; Williams et al., 2012).

Another interesting family, which is significantly enriched in the sediments at the anthropogenically impacted island, are *Desulfobulbaceae*. These are long, filamentous bacteria which have been reported recently in the coupling of biogeochemical processes between the oxic and anoxic zone within sediments, often crossing several cm (Pfeffer et al., 2012; Reguera, 2012). In the upper, well-aerated layer of the sediment oxygen is used as the electron acceptor during the metabolism of organic matter. This quickly depletes oxygen concentrations and alternative acceptors, such as sulfate, are used by bacteria of the deeper, anoxic sediment layers to metabolize organic matter. This releases hydrogen sulfide into the sediments, which is toxic to oxygen-consuming organisms. If oxygen consumption increases in surface sediment, e.g. due to stimulated bacterial activity through increased organic matter input, the sulfide regions expands (Nielsen et al., 2010). *Desulfobulbaceae*, as reported by Pfeffer et al. (2012), play an important role in sediments of high organic matter input, as they can prevent this expansion through a coupling of sulfide oxidation in anaerobic sediment layers to oxygen reduction to the aerobic part, spanning up to 1.5 cm.

Particle-associated bacterial communities were mainly enriched in OTUs belonging to the *Gammaproteobacteria*. They feature a diverse repertoire of extracellular, hydrolytic enzymes which enable them to access and assimilate various forms of organic matter on the aggregates in the water column or in the sediments (Azam and Malfatti, 2007; Edwards et al., 2010). *Gammaproteobacteria* is also a class which contains many strains of potentially pathogenic bacteria, such as *Vibrio* sp., and aggregates are known to be a hot-spot for pathogens (Dinsdale et al., 2008; Garren et al., 2009; Lyons et al., 2010). As *Gammaproteobacteria* were significantly enriched at the inhabited island with higher organic matter availability this could have potential detrimental consequences for benthic organisms in the back-reef area.

Overall, with this study, we can conclusively show that there is a significant effect of a dense island population on the measured water quality parameters in the Spermonde Archipelago. The measured parameters are also elevated compared to sampling from off the reef crest, but the effect is very limited compared to the impact of riverine and sewage input on the near-shore zone. Those differences in water quality, reflected in the higher availability of inorganic and organic nutrients, also caused shifts in the bacterial community composition, mainly favoring heterotrophic *Flavobacteria* and *Gammaproteobacteria*. Those classes contain potentially pathogenic taxa, therefore those shifts could also imply negative consequences for human or reef organism health. But again, in comparison to changes in community composition of the highly eutrophic near-shore zone the changes are not as severe. This indicates that the functional composition of the communities has also not shifted as dramatically. In that context a detailed metatranscriptomic analysis can add to the current knowledge and help to understand whether those differences in bacterial community composition also entail a shift in functionality.

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Chapter 5

Supporting publications

The formation of aggregates in coral reef waters under elevated concentrations of dissolved inorganic and organic carbon: A mesocosm approach

A Cárdenas, F Meyer, H Schwieder, C Wild, A Gärdes

ABSTRACT

The transformation of dissolved organic carbon (DOC) to particulate organic carbon is the major mechanism through which large sinking organic particles are formed in aquatic systems. Global stressors, such as high concentrations of dissolved inorganic carbon (DIC) due to ocean acidification, as well as local stressors, such as high DOC concentrations due to coastal eutrophication, can significantly affect the formation and settling of aggregates and thereby the marine biogeochemical carbon cycle. Increasing aggregate formation rates can contribute to the mortality of benthic organisms in coral reef ecosystems, but relevant knowledge is scarce. Therefore, the present study addresses this issue and studies the individual and combined effects of high DIC (900 µatm) and DOC (150 µM glucose) on organic matter dynamics as well as the formation of organic aggregates in a manipulative study over 42 days using 24 mesocosms dominated by either benthic calcifying algae or by hard corals. Organic aggregates in terms of transparent exopolymer particle (TEP) concentrations and total aggregated volume were measured. Results showed lower TEP concentrations and aggregated volume under high DIC concentrations. By contrast, under DOC enrichment higher rates of aggregate formation and microbial oxygen uptake were observed. Surprisingly, the highest aggregate formation rates were observed under the combined DIC and DOC enrichment. Furthermore, benthic organisms influenced the availability of DOC resulting in higher aggregate formation in coral compared to calcifying algae mesocosms. These experiments simulate future ocean conditions in coastal ecosystems where elevated DOC concentrations could aggravate the effect of high DIC on aggregate formation. In coral reef ecosystems, this may have important consequences on benthic organisms.

Persist or desist: revealing (the lack of) patterns in benthic-fish association on coral reefs of the Spermonde Archipelago, Indonesia

J G Plass-Johnson, V N Bednarz, H F Schwieder, C Wild, A Gärdes, M Lukman, H Reuter, S C A Ferse, M Teichberg

ABSTRACT

The Spermonde Archipelago is a complex of ~100 populated islands off Southwest Sulawesi, Indonesia in the middle of the Coral Triangle. The reefs in this area are exposed to a high level of disturbance due to intense fishing and land-based effluents. Previous studies have shown that variation in water quality is strongly linked to the benthos where coral cover is positively related to distance from the mainland, and turf algae is negatively linked. In this study we use seven islands of the archipelago, varying in distance from 1-55 km from the mainland, and three years of surveys, to examine the influence of benthic composition on the structure of the fish assemblage. Cluster analysis indicated that distinct groups of fish were associated with distance and a limited few species were present across the entire range of sites. Relating fish groupings to principle components of a principle component analysis performed on benthic composition, via multivariate generalized linear model confirmed that fish groups differ in their dependence on benthic structure. From this relationship we can identify sets of fish species that may be lost, or that might persist, given continued degradation of the Spermonde reefs. Lastly, incorporating water quality, benthic and fish indices revealed that the reefs of this system can respond positively after acute disturbances (*Acanthaster planci* outbreak). This study contributes an important, missing component (fish community structure), to the ever growing literature of the Spermonde Archipelago; a system that reflects similar environmental pressures to the greater Southeast Asian region.

Different levels of anthropogenic impact influence coral larvae settlement and bacterial biofilm communities in the Spermonde Archipelago, Indonesia

P Kegler, H F Kegler, A Kunzmann, A Gärdes, Y R Alfiansah, M Lukman, C Hassenrück, S C A Ferse

ABSTRACT

Recruitment of coral larvae is one of the key factors for coral reef recovery and is determined by larval behavior in the water column as well as settlement and post-settlement survival. Larval settlement and metamorphosis rely strongly on settlement cues emitted from bacterial biofilms and their bacterial community composition (BCC). These BCC can change drastically with altered environmental conditions and in turn may affect larval settlement behavior. BCC and coral larvae settlement were investigated at three sites with increasing distance from shore, and thus different levels of human impact and water quality, in the Spermonde Archipelago, Indonesia. Coral recruitment and BCC were analyzed on natural reef substrate and artificial ceramic tiles. Bacterial communities were comprised largely of *Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria* and *Flavobacteria* and were strongly correlated with water quality. No coral recruits were found at the inshore site where the highest anthropogenic impact was observed. Recruitment at the other two sites was 0.73 ± 1.75 and 0.90 ± 1.97 recruits per 100 cm^2 at BL and BD respectively, with no significant difference between them (ANOVA; $p>0.05$). Differences in both BCC and coral recruitment were detected between natural and artificial substrates at two of the three sites, underlining that the use of settlement tiles may yield different patterns than recruitment on natural substrates, depending on each specific location and sampling time. The results demonstrate that negative anthropogenic influences on water quality affect bacterial community composition, which in turn can affect recruitment of coral larvae. This highlights the importance of taking these often neglected factors into account when evaluating the recovery potential of coral reefs.

A recent outbreak of crown-of-thorns starfish (Acanthaster planci) in the Spermonde Archipelago, Indonesia

J G Plass-Johnson, H F Schwieder, J P Heiden, L Weiand, C Wild , S C A Ferse, J Jompa, M Teichberg

ABSTRACT

A recent review of crown-of-thorns starfish (*Acanthaster planci*; COTS) in Indonesia has suggested that their impacts have gone under-reported. In 2012–2013, we surveyed COTS at permanent transects within 12 sites of the Spermonde Archipelago, Indonesia, a coastal region close to two rivers and the heavily urbanized city of Makassar. Evidence of COTS was apparent at 8 of 12 sites surveyed with highest densities (37 starfish per 250 m^{-2} at Barrang Lompo) comparable to those reported in the Indonesian historical literature. At Barrang Lompo and Bonetambung, the COTS outbreak resulted in the loss of half the live coral. Terrestrial effluents have reduced water quality in the Spermonde Archipelago, which further supports recent work linking water quality and COTS outbreaks, thus providing a warning of future outbreaks to Indonesian coastal managers given the country's increased urbanization.

First assessment of bacterial community composition in relation to water quality and human sewage input in the epicenter of coral biodiversity: Misool, Raja Ampat, Indonesia.

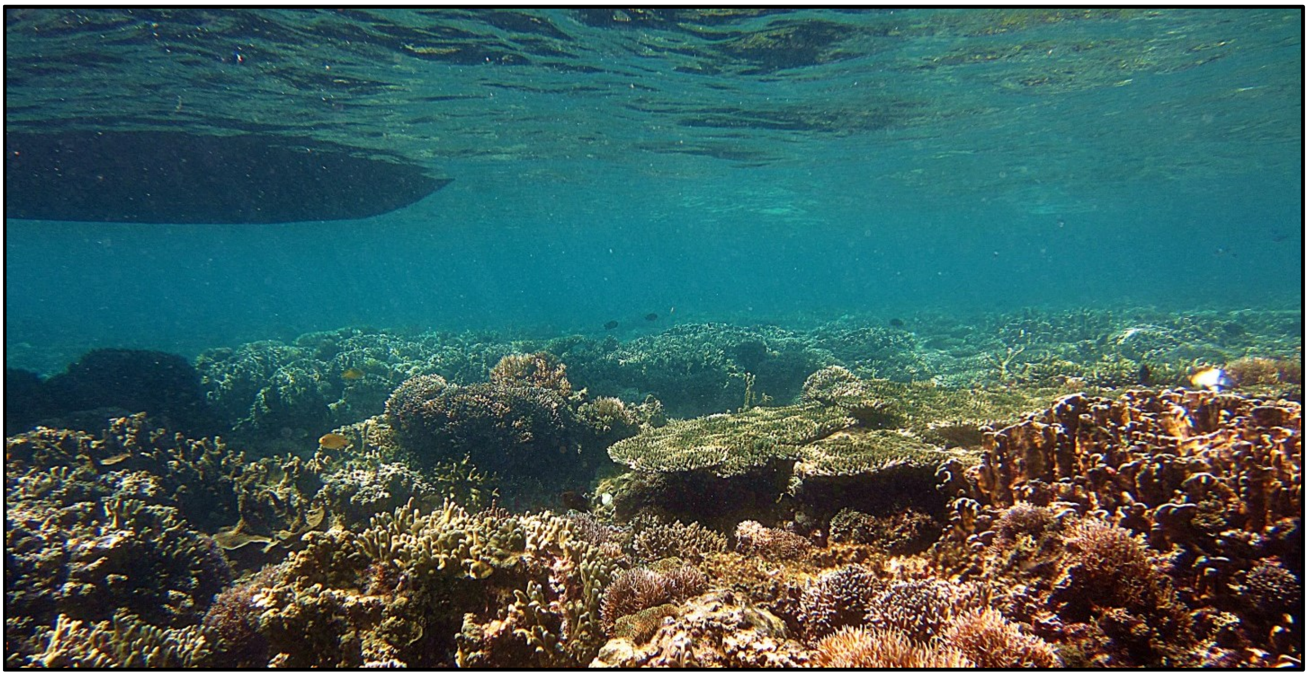
A Gärdes, H F Kegler, Purwanto, J T Jeske, J Jompa

ABSTRACT

The island of Misool is located in the center of coral reef biodiversity in eastern Indonesia. Although located in a very remote area, the number of human settlements and the extent of habitat modification through fishing and mining for natural resources are steadily increasing. As fast responding organisms bacteria are ideal candidates for a warning system for undesirable ecosystem changes. Here we present the first study assessing bacterial community composition (BCC) in the free-living and particle-attached fraction of reef waters throughout the southeastern Misool marine protected area (MPA). The simultaneous assessment of water quality parameters, including inorganic nutrients, chlorophyll *a*, DOC, TEP and SPM presents important baseline data for future management efforts. We supplemented the fieldwork with laboratory microcosm experiments to investigate community changes related to sewage water inflow from settlements within the MPA. We found that sampling sites differed significantly ($p < 0.05$) in terms of NO_x, PO_4, DOC and chlorophyll *a* concentrations. However, none of these were found to significantly change the observed BCC. Bacterial communities throughout the MPA were dominated by *Gammaproteobacteria* in the particle-attached, and *Cyanobacteria* in the free-living size fraction. In the microcosm experiment we observed DOC to have a statistically significant effect ($p<0.05$) on changes in BCC. From the initial BCC we observed increasing relative abundances of especially *Gammaproteo-*, and *Flavobacteria* in response to sewage water addition. Most strikingly though, we observed a clear decrease in general bacterial diversity as a result of the nutrient addition. Our data shows that naturally occurring bacterial communities in waters of the MPA are to some degree resilient to the observed and already present variability in water quality parameters. But the experimental nutrient addition to those natural communities also reveals realistic scenarios of shifts in BCC towards heterotrophic bacteria, potentially containing pathogenic strains, and an apparent loss of diversity, and very likely functionality. Management efforts in the MPA should include BCC from the very beginning as an early warning system for shifts in water quality and the potential detrimental consequences for the diverse coral reefs, associated organisms and, ultimately humans.

Chapter 6

General discussion



KEY FINDINGS

The main aim of this thesis was to provide a thorough study of the bacterial communities inhabiting different reef-associated habitats (water column, aggregates, sediments and *Fungia* mucus) of the anthropogenically impacted Spermonde Archipelago, Indonesia, and relate community variability to water quality parameters. There was a striking difference in anthropogenic disturbance, reflected in water quality, between a narrow inshore zone of highly eutrophic water masses and the remainder of the shelf, which remained mostly oligotrophic. Furthermore, in all habitats, water quality parameters were significant drivers of differences in bacterial community composition. As hypothesized the bacterial communities were very distinct between the investigated habitats, indicating distinct functionalities and biogeochemical roles of the communities between them. Aggregate formation rates, as an indicator for primary productivity, phytoplankton biomass and for organic matter fluxes from the water column to the sediments, were also significantly enhanced in the inshore zone. This was accompanied by a significant enrichments of aggregates with bacteria specialized in the degradation of more labile organic matter. Aggregates are known hot-spots of bacterial groups known to contain pathogenic strains, and can act as vectors of those pathogens to benthic habitats. This was, in fact, supported by observations in the eutrophic inshore zone close to Makassar, where bacterial groups known to contain potential pathogens were enriched at the site of highest sedimentation.

Dense inhabitation of the small islands on the shelf also had a significant impact on several key water quality parameters such as chlorophyll *a*, dissolved organic carbon (DOC) and transparent exopolymer particles (TEP), leading to elevated concentrations and shifts in bacterial community composition in the back-reef area of the inhabited island. However, among the many threats that the reefs of the Spermonde Archipelago are facing due to the increasing human coastal and island populations, from destructive and unregulated fishing to *Acanthaster planci* outbreaks, deteriorating water quality at the islands further out on the reef is very likely not one of the main concerns when it comes to devising proper management plans.

This chapter will refer back to the original research questions, summarize the key findings and put it in context of the Spermonde Archipelago.

HOW DOES THE AVAILABILITY OF ORGANIC AND INORGANIC NUTRIENTS INFLUENCE THE ABUNDANCE AND COMMUNITY STRUCTURE OF BACTERIA IN THE WATER COLUMN, SEDIMENTS AND ON BENTHIC MARINE BIOTA OF THE SPERMONDE ARCHIPELAGO?

Nearshore tropical shelf waters are under increasing pressure from a growing coastal human population and its detrimental impacts, such as eutrophication, pollution or overfishing (Burke et al., 2011). This anthropogenic stress is exerted at a multitude of geographical scales from large regional scales down to small, patchy packets of water. For the Spermonde Archipelago there is a long history of large-scale investigations of anthropogenic impacts on the water quality and subsequently on coral reef associated organisms (Becking et al., 2006; Cleary et al., 2015; Moll, 1983; Plass-Johnson et al., 2015a; Sawall et al., 2011, 2012). But until the start of this thesis there were no studies simultaneously investigating bacterial communities from different habitats in the Spermonde Archipelago. Results from it conclusively show that, at different spatial scales and during different sampling periods, enrichment in inorganic nutrients and organic matter can lead to profound shifts in bacterial community composition (**chapter 2, 3 and 4**). Those observations were consistent across both sampling campaigns conducted directly after the rainy season 2013 and during dry season in 2014. In some respects results from this thesis contradict previous studies in the finding that the large scale impacts of untreated sewage and riverine nutrient input, such as chlorophyll *a*, TEP and SPM concentrations, were limited to the immediate vicinity of metropolitan Makassar (**chapter 2 and 3**). In this narrow strip of water most introduced inorganic nutrients are immediately assimilated by phytoplankton, including the abundant picophytoplankton *Synechococcus*. This abundant phytoplankton community, stimulated by heterotrophic bacteria (Gärdes et al., 2011), released copious amounts of TEP (Passow, 2002). This resulted in a severalfold increase of the suspended material, aggregate formation and sedimentation rates observed at the inshore zone. At all other sites further out on the shelf, water quality parameters were corresponding to oligotrophic conditions typical for many coral reef environments. Therefore, instead of a gradient of declining concentrations of water quality parameters related to eutrophication, as proposed in many previous studies (Becking et al., 2006; Sawall et al., 2011, 2012), results from this thesis strongly suggest that the shelf is separated into two discrete zones: a very narrow band of highly eutrophic waters close to the coastline and a large shelf area of oligotrophic waters. Distinct shifts in the bacterial community composition reflected this zonation, especially for the sediments, aggregates and free-living fraction of the water column (**chapter 2 and 3**). As the aggregate formation and sedimentation rates show, increased water column organic matter production through phytoplankton and bacteria was tightly linked to higher on-site aggregate formation and sedimentation rates in the inshore zone (**chapter 3**). During both sampling campaigns aggregates and sediments sampled from the eutrophic inner zone were enriched in opportunistic, copiotrophic bacteria (e.g. *Gammaproteobacteria*), which efficiently capitalize on the organic matter enrichment (Allers et al., 2007; Dinasquet et al., 2013; Grossart and Ploug, 2001). The findings were corroborated by higher DAPI cell counts and sediment biological oxygen demand (as a measure for microbial activity in the water column) in the inshore zone (**chapter 2 and 3**). For the free-living faction that shift in community composition occurred further offshore, likely concurrent with the transport of water masses out of the highly eutrophic inshore zone in vicinity to Makassar. This proposed time lag is in accordance with lab experiments, during which hours to days passed between the enrichment and the bacterial response (Allers et al., 2008; Wietz et al., 2015). The bacterial communities inhabiting the *Fungia* mucus were not subject to shifts between the two zones (**chapter 2**). But those communities are known to be more stable, as there is some control of the metazoan host over its bacterial symbionts (Lee et al., 2010; Reveillaud et al., 2014; Ritchie, 2006).

However, the shifts in bacterial community composition, driven by increased organic matter availability can have detrimental effects for the health of humans and coral reef organisms. The abundance of potentially pathogenic bacterial genera, such as *Escherichia/Shigella, Propionibacterium* and *Mycobacterium*, was much higher in the inshore zone close to Makassar (**chapter 2**). And under the current regime of chronic eutrophication in the inshore zone the bacterial communities are not likely to return to a community composition with less detrimental effects on human and coral reef organisms (e.g. increased BOD, increased pathogen abundance, higher turbidity due to intensive aggregation in the water column). Bacterial communities are very resilient and have the proven ability to quickly return to more desirable and ‘natural’ community compositions (Garren et al., 2009). Therefore, with progress in sewage treatment capabilities and the reduction of riverine nutrient input, even the highly eutrophic inshore belt has the potential to return to bacterial communities within the different habitats that are characteristic of less impacted state with decreased phytoplankton biomass and sedimentation of organic matter.

WHAT ROLE DOES WATER QUALITY HAVE IN AGGREGATE FORMATION AND BACTERIAL COMMUNITY COMPOSITION ON SETTLING AGGREGATES ALONG LARGE SCALE GRADIENTS IN TROPICAL REEF WATERS?

Initial laboratory experiments showed that increased availability of DOC will lead to elevated concentrations of TEP and significantly higher total aggregate volume (Cárdenas et al., 2015, **chapter 5**). Between the two observed zones in the Spermonde Archipelago, aggregate formation and sedimentation rates were significantly higher in the eutrophic inshore site with highest chlorophyll and TEP concentrations (**chapter 3**). This is a commonly observed phenomenon, as phytoplankton abundance, aggregate formation and subsequent sedimentation are tightly linked (Legendre and Le Fevre, 1995; Richardson and Jackson, 2007). In accordance with other studies reporting distinct particle-attached bacterial communities from different environments (Crespo et al., 2013; Crump et al., 1999; Zhang et al., 2007) bacterial communities colonizing the sampled aggregates also showed a very strong variability between the eutrophic inshore zone and the more oligotrophic sites from the outer shelf zone (**chapter 3**). Classes adapted to readily available organic, especially *Gammaproteobacteria*, were notably enriched with decreasing distance to Makassar, accompanied by a significantly elevated biological oxygen demand during the rolling tank incubations. Excessive sedimentation is very detrimental to coral reefs (reviewed by Fabricius, 2005 and Rogers, 1990). Even short term exposure of coral recruits to increased sedimentation can lead to high coral recruit mortality, thus hampering the ability for reefs to repopulate and recover (Fabricius et al., 2003). Moreover, in addition to direct detrimental effects, in contrast to the surrounding seawater organic aggregates can harbor high abundances of pathogenic bacteria. Especially at the inshore zone that receives significantly higher sediment input, this could be potentially harmful to humans using resources from that area. In those contexts the site receiving the most sediment input in the Spermonde Archipelago was of particular interest concerning recovery potential. During an adjunct study investigating the relationship between water quality, bacterial biofilm communities and coral recruitment, no coral recruits were found at the inshore site (Kegler et al., 2016, **chapter 5**).

In the case of the already strained Spermonde Archipelago (Ferse et al., 2012; Pet-Soede and Erdmann, 1998; Plass-Johnson et al., 2015a, 2015b, **chapter 5**), enhanced aggregate formation and sedimentation at the inshore site seems to mitigate effects of increased nutrient and organic matter loading to sites further out on the shelf. Sedimentation rates (**chapter 3**) and chlorophyll *a* and TEP (**chapter 2 and 3**) measured in the oligotrophic outer shelf zone were significantly lower compared to the inshore site closest to Makassar, thus negative impacts implied by increased sedimentation rates (Dubinsky and Stambler, 1996; Fabricius, 2005; Fabricius et al., 2005; Hunte and Wittenberg, 1992; Kegler et al., 2016, **chapter 5**) are likely restricted to inshore areas.

DO THE SMALL INHABITED ISLANDS OF THE ARCHIPELAGO HAVE A MEASURABLE IMPACT ON WATER QUALITY PARAMETERS OF THE SURROUNDING WATER MASSES?

Aim of this thesis was to elucidate how small island populations affect water quality parameters (e.g. PO4, NOx, chlorophyll *a*, DOC and TEP) and bacterial community composition in comparison to uninhabited islands and larger scale inputs from mainland rivers or sewage from Makassar (**chapter 2 and 3**). This comparison was possible because both islands, uninhabited and inhabited, were located at very similar distances to the mainland. Results of this thesis revealed that the human inhabitation of the small, sandy islands can significantly increase the availability of inorganic nutrients, chlorophyll *a*, DOC and TEP along gradients across the back-reef area (**chapter 4**). Sewage and waste water are not treated on the islands and enter the back-reef water through diffusive groundwater discharge. This is a common occurrence on islands with coarse and porous sediments, which also leads to the increased availability of inorganic nutrients and DOC (Laws et al., 2004; Nelson et al., 2015; Street et al., 2008; Umezawa et al., 2002).

Differences in bacterial community composition between the densely populated and the uninhabited island were always highly significant within the individual habitats (free-living, particle-attached and back-reef sediment), and differences in water quality could explain significant parts of the community variability (**chapter 4**). With both islands being located in the oligotrophic outer shelf zone this is a clear indication of a human population effect on bacterial communities in the surrounding back-reef area. Many of the bacterial taxa with significant contribution to differences between the two islands were opportunistic copiotrophs. Previous work of this thesis (**chapter 2**) showed an increased prevalence of potentially pathogenic bacteria in the highly eutrophic inshore zone. This could now also be the case on smaller scales, in close vicinity to the inhabited islands of the Archipelago, as the enriched classes are known to contain many pathogenic groups (Dinsdale et al., 2008; Garren et al., 2009; Lyons et al., 2010). What is lacking so far is the information on the abundance and expression of pathogenicity-related genes at sites where potentially pathogenic bacteria were enriched. Therefore, further in-depth metagenomic and metatranscriptomic analyses could help fill those knowledge gaps (Sorek and Cossart, 2010; Tan et al., 2015).

Bacterial community composition of the free-living fraction of the back-reef water column was similar to those observed from the oligotrophic outer shelf zone (**chapter 3 and 4**). There is a steady water exchange due to currents and tides, which can explain the strong similarities. Sediment bacterial communities also showed a strong resemblance and shared many classes at similar relative abundances, such as *Alphaproteobacteria*, *Flavobacteria*, *Deltaproteobacteria*, *Planctomycetacia* and *Acidimicrobiia* (**chapter 2 and 4**). Two important classes showed larger differences. The relative abundance of *Gammaproteobacteria* was much higher in sediments of the outer shelf zone, while *Cyanobacteria* were severalfold enriched in sediments of the back-reef area at the inhabited island. It is very likely that they can benefit from the continuous supply of inorganic nutrients seeping from the islands through the porous sediments, as was also hypothesized by Brocke et al. (2015). Strong differences could be observed for the particle-attached fraction of the water column. While the particle-attached fraction of the oligotrophic waters in the outer shelf zone consisted to > 80 % of *Cyanobacteria*, communities sampled from the back-reef area were more evenly distributed, with *Gamma-* and *Alphaproteobacteria* constituting > 50 % of the relative abundance.

Mechanisms of aggregate formation in shallow, turbid systems differ significantly from that in deeper waters. Aggregates in the back-reef area do not only originate from phytoplankton and their excretion products but also receive material from resuspended sediments and biofilms (Simon et al., 2002). The process of constant resuspension and remineralization of small aggregates, rich in organic matter, in the back-reef area of the inhabited island can reinforce the sustained and elevated abundance of phytoplankton compared to the oligotrophic outer shelf waters. Overall, chapter 3 of this study could establish strong indications for an impact of dense islands populations in the Spermonde Archipelago on the biogeochemistry and bacterial community composition. Most measured water quality parameters were significantly elevated at the inhabited island. This higher availability of inorganic nutrients and organic matter then resulted in a higher abundance of phytoplankton (measured as chlorophyll *a*) and shifts in the bacterial community, most prominent in the particle-attached fraction, favoring heterotrophic bacteria.

OVERALL CONCLUSIONS AND FUTURE PERSPECTIVES

Data presented in this thesis conclusively showed that dense human populations on the mainland and on the individual islands exerted a clear and measurable influence on the investigated water quality parameters. There were significant repercussions in the bacterial communities, especially in the sediments and associated to aggregates, and differences in water quality between the eutrophic inshore/oligotrophic outer shelf sites and inhabited/uninhabited island were identified as significant drivers of those shifts. In contrast to previous publications of water quality in the Spermonde Archipelago the eutrophic conditions were only encountered in a very narrow belt of water in immediate vicinity to the coast. And bacterial community shifts towards copiotrophic and potentially pathogen-containing groups mainly occurred at this eutrophic inshore zone. Therefore measures aimed at mitigating causes of eutrophication can focus more on the waste water management of the individual islands and try to reduce the localized impact on the surrounding reefs located in otherwise oligotrophic waters.

For future studies it will be essential to exactly delimitate the extents of the eutrophic conditions along the shoreline and in a higher temporal resolution. The coast along the archipelago extends more 50 km north of Makassar, with several additional rivers discharging into the shelf area. This raises the question whether findings made in the present thesis are representative only for large metropolitan areas? Or can riverine input fed by rural and agricultural input result in similar eutrophic conditions? Moreover, a more thorough investigation of those bacterial groups containing potentially pathogenic strains by applying advanced next-generation sequencing methods unavailable during this thesis, such as metagenomics and metatranscriptomics, could help to prove our assumption of increased pathogen abundance and expression of pathogenicity-related genes in the inshore site (Tan et al., 2015). Those techniques could also reveal more about the ecological roles of the dominant taxa and their function in organic matter aggregation and remineralization within and between the two contrasting zones as well as the inhabited islands.

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Supplementary material

CHAPTER 2: COASTAL EUTROPHICATION CONTROLS THE BACTERIAL COMMUNITY COMPOSITION IN DIFFERENT REEF HABITATS OF THE SPERMONDE ARCHIPELAGO, INDONESIA

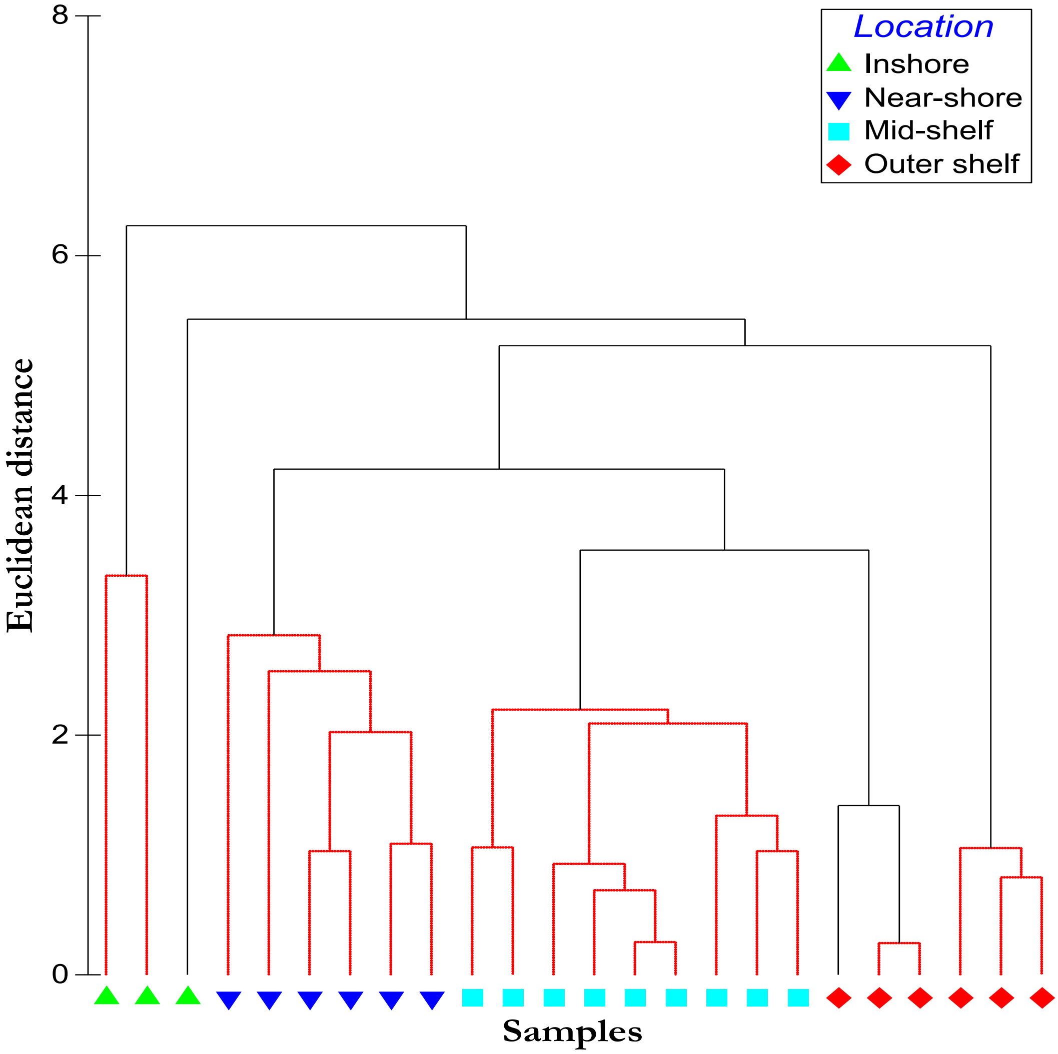

Fig. S 2-1 Hierachical clustering of the different sampling stations based on euclidean distance. Black lines indicate significant differences between stations, red lines indicate no differences.

CHAPTER 3: INTENSIVE NUTRIENT UPTAKE AND PARTICLE AGGREGATION MITIGATE EUTROPHICATION AT MORE DISTANT REEFS AT A TROPICAL COASTAL SHELF ECOSYSTEM

Tab. S 3-1 List of diversity indicators calculated for the different habitats and sampling stations. ace = abundance-based coverage estimator, invS = inverse Shannon-Weaver index, shannon = Shannon-weaver diversity index.

	nOTU	chao1	ace	invS	shannon
LL-WC-FL	621	4533	4593	22.54	4.73
BL-WC-FL	594	6602	5299	42.92	4.99
BD-WC-FL	623	5341	5630	6.27	4.09
LLBL-WC-PA	460	3652	3674	2.86	2.98
BL-WC-PA	560	6009	6646	2.73	3.07
BD-WC-PA	518	5936	7044	2.6	2.92
LL-SED	1600	25928	29059	1140.11	7.29
BL-SED	1455	12848	15219	713.15	7.1
BD-SED	1394	11168	12231	419.22	6.97
LL-AGG-1	692	6185	5759	17.72	4.76
LL-AGG-2	743	6246	6067	19.79	5
LLBL-AGG-1	713	6282	6218	34.97	5.13
LLBL-AGG-2	869	8922	10134	32.33	5.44
BL-AGG-1	888	5160	4875	169.12	6.09
BLBD-AGG-1	758	8631	9067	6.41	4.41
BLBD-AGG-2	630	7289	7744	5.59	4.02
BD-AGG-1	574	2950	2942	14.07	4.58
BD-AGG-2	634	329	3478	9.49	4.48
BD-AGG-3	632	3628	3682	18.98	4.78
BD-AGG-4	644	5589	5312	23.87	4.94

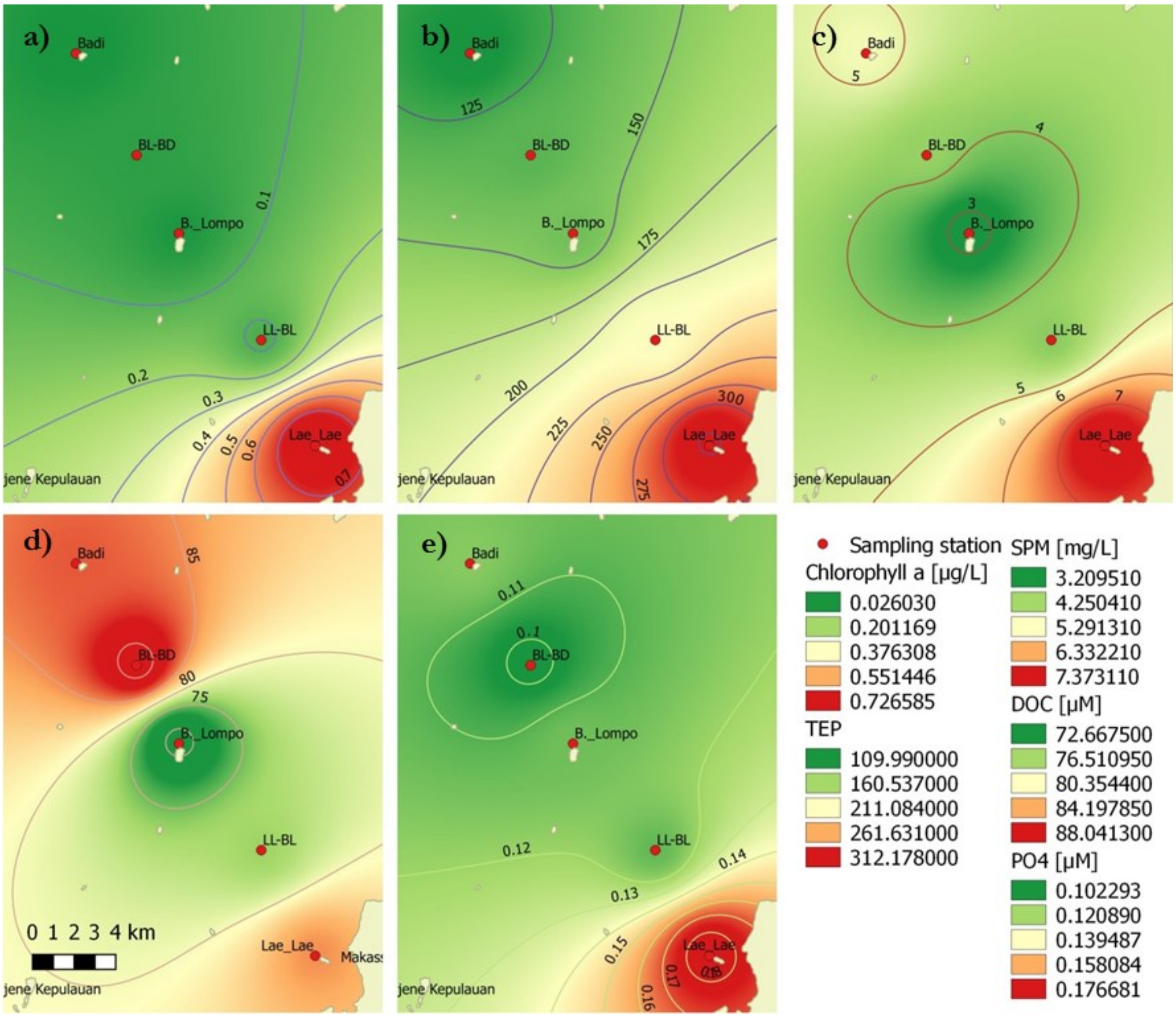

Fig. S 3-1 Integration of the measured water quality parameters a) chlorophyll *a*, b) TEP, c) SPM, d) DOC and e) PO_4 along the investigated sites on the shelf.

Chapter 4: Small tropical islands with dense human populations: Decreased water quality results in distinct bacterial communities

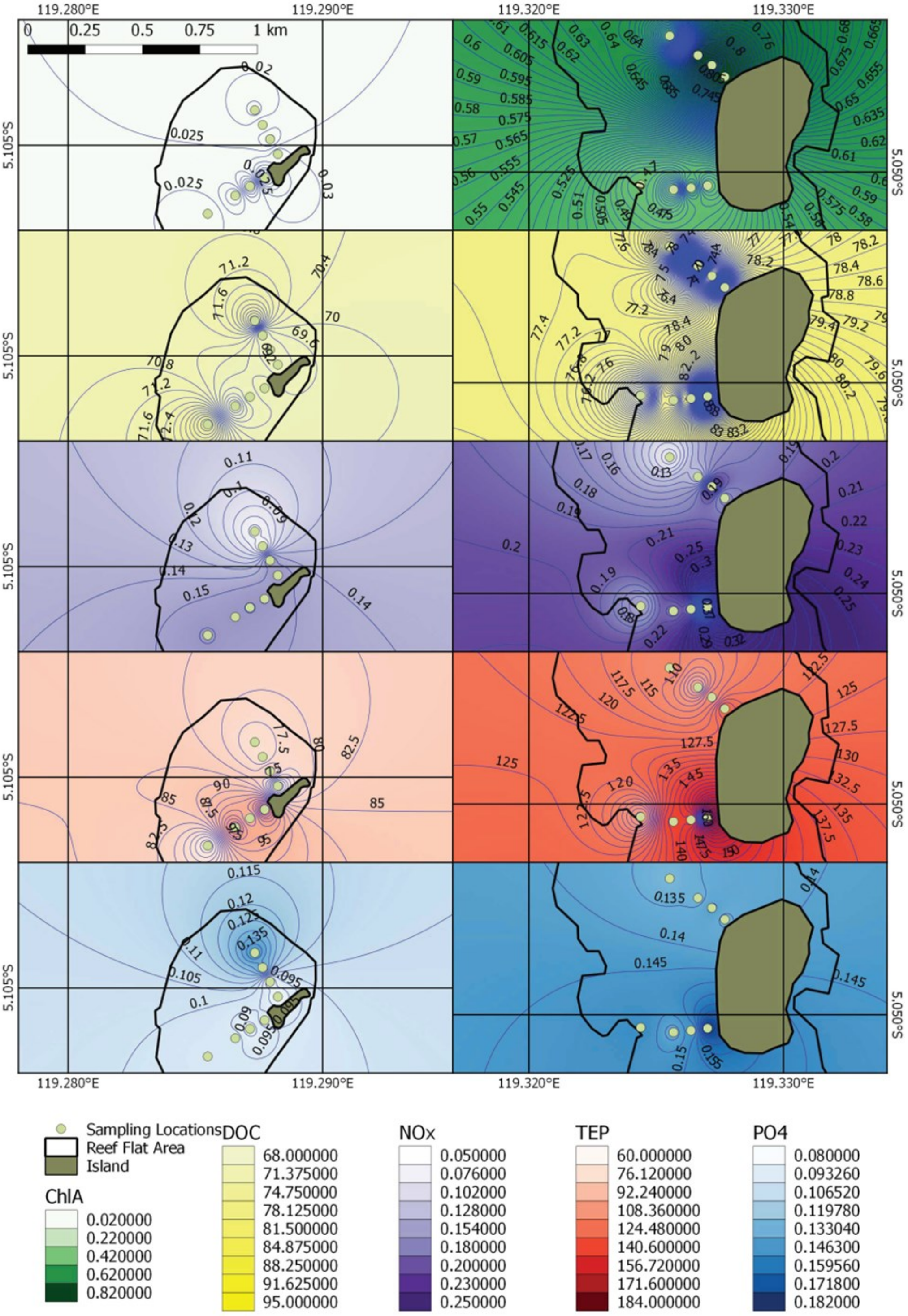

Fig. S 4-2 Interpolated contour lines of water quality parameters sampled at the uninhabited islands Kodingareng Keke (left column) and inhabited Pulau Barrang Lompo (right column).

Tab. S 4-1 List of diversity indicators calculated for the different habitats and sampling stations. ace = abundance-based coverage estimator, invS = inverse Shannon-Weaver index, shannon = Shannon-weaver diversity index.

	nOTU	chao1	ace	invS	shannon
Inhabited Sediment	1457.26	12715.1	14633.18	856.35	7.08
Uninhabited Sediment	1384.63	9199.27	10493.51	682.53	7.03
Inhabited Free-living	493.95	3774.34	3631.22	26.52	4.43
Uninhabited Free-living	631.49	4080.46	4487.17	30.45	4.92
Inhabited Particle-attached	747.83	7500.6	8064.83	12.3	4.65
Uninhabited Particle-attached	665.6	6533.45	7232.62	5.92	4.08

CHAPTER 5: SUPPORTING CO-AUTHORSHIP PUBLICATIONS

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The formation of aggregates in coral reef waters under elevated concentrations of dissolved inorganic and organic carbon: A mesocosm approach

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ABSTRACT

The transformation of dissolved organic carbon (DOC) to particulate organic carbon is the major mechanism through which large sinking organic particles are formed in aquatic systems. Global stressors, such as high concentrations of dissolved inorganic carbon (DIC) due to ocean acidification, as well as local stressors, such as high DOC concentrations due to coastal eutrophication, can significantly affect the formation and settling of aggregates and thereby the marine biogeochemical carbon cycle. Increasing aggregate formation rates can contribute to the mortality of benthic organisms in coral reef ecosystems, but relevant knowledge is scarce. Therefore, the present study addresses this issue and studies the individual and combined effects of high DIC (900 μatm) and DOC (150 μM glucose) on organic matter dynamics as well as the formation of organic aggregates in a manipulative study over 42 days using 24 mesocosms dominated by either benthic calcifying algae or by hard corals. Organic aggregates in terms of transparent exopolymer particle (TEP) concentrations and total aggregated volume were measured. Results showed lower TEP concentrations and aggregated volume under high DIC concentrations. By contrast, under DOC enrichment higher rates of aggregate formation and microbial oxygen uptake were observed. Surprisingly, the highest aggregate formation rates were observed under the combined DIC and DOC enrichment. Furthermore, benthic organisms influenced the availability of DOC resulting in higher aggregate formation in coral compared to calcifying algae mesocosms. These experiments simulate future ocean conditions in coastal ecosystems where elevated DOC concentrations could aggravate the effect of high DIC on aggregate formation. In coral reef ecosystems, this may have important consequences on benthic organisms.

1. Introduction

Coral reef decline occurs as a direct response to a single stressor or as a consequence of different global and local stressors acting simultaneously (Baker et al., 2008; Knowlton and Jackson, 2008; Szmant, 2002). Ocean acidification (OA) represents one of the most significant long-term threats to coral reefs. Experimental evidence suggests that a doubling of pre-industrial pCO_2 has reduced coral growth and calcification by up to 40% due to the reduction of aragonite formation (Hoegh-Guldberg et al., 2007). Local and regional threats generally include overfishing and land-based pollution (Moore and Best, 2001; Smith et al., 2003). These threats are generally linked to some of the main causes of coral reef degradation, such as algal overgrowth, increased disease prevalence (Bruno et al., 2003; Rosenberg et al., 2007; Voss and Richardson, 2006), bleaching (Glynn, 1993; Wiedenmann et al., 2013), and sedimentation (Devlin and Brodie, 2005; Fabricius, 2005). Elevated DOC may cause different pathologies and increase the rate of coral mortality (Kline et al., 2006; Kuntz et al., 2005). Negative effects of high DOC concentrations on coral reef health have been linked to enhanced bacterial growth rates and activities that cause coral death by oxygen depletion and accumulation of toxic substances (Gregg et al., 2013; Smith et al., 2006; Wild et al., 2010). Exogenous inputs of DOC in coral reefs are mainly derived from sewage, terrestrial run-off and marine fish farms (Fabricius, 2005; Garren et al., 2008, 2009). For instance, the global flux of riverine DOC has been calculated as high as 7.8×10^{14} g C yr^{-1} (Mantoura and Woodward, 1983). However, DOC inputs have spatial and temporal variations, being higher in areas exposed to river discharges and during the wet season when precipitation is higher and storm events are more frequent (Alongi and McKinnon, 2005; Joo et al., 2012). High concentrations of DOC can also enter the coral reef system in the form of exudates released by the benthic community, mainly from fleshy macroalgae and predominantly in the form of dissolved carbohydrates (Haas and Wild, 2010; Nelson et al., 2013; Wild et al., 2010). For instance, macroalgal blooms represent large amounts of continuous organic matter loads which can increase DOC

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concentrations up to 1000 μmol L^{-1} (Kline et al., 2006). Coral reefs are thus profoundly impacted by any activity linked to macroalgal overgrowth, as is the case of inorganic nutrient enrichment and overfishing (Smith et al., 2006).

Although DOC is the most dominant form of organic carbon in most marine ecosystems, large sinking particles constitute a biologically important, though often small, percentage of the total organic carbon (Alldredge, 2000; Verdugo et al., 2004). In coral reef waters, the particulate fraction may be trapped by coral mucus and is mainly remineralized by benthic bacterial communities in order to fuel new production (Huettel et al., 2006; Wild et al., 2004, 2005). However, high levels of suspended particulate matter directly affects light penetration and sedimentation rates and may subsequently promote coral mortality (Fabricius, 2005; Fabricius et al., 2003; Fichez et al., 2005; Garren and Azam, 2012).

The formation of large sinking particles from DOC generally involves acidic polysaccharide gel-like particles termed transparent exopolymer particles (TEPs) (Azetsu-Scott and Passow, 2004; Engel et al., 2004b; Passow, 2002). Abiotic TEP formation requires TEP-precursors present in some DOC pools, and depends on turbulence, ion density and concentration of inorganic colloids (Alldredge et al., 1993; Logan et al., 1995; Passow, 2002). However, TEP can also be directly produced from cell exudates of numerous organisms, particularly from phytoplankton cells as well as macroalgae, coral and bacteria (Gärdes et al., 2012; Passow, 2000; Ramaiah et al., 2001; Wotton, 2004).

Local and global stressors may significantly alter biogeochemical carbon cycling. For instance, declines in pH due to DIC enrichment cause changes of TEP properties and abundance, presumably because of alterations in total alkalinity (TA). This can lead to higher downward carbon exportation (Mari, 2008; Passow, 2012). Mesocosm experiments examining phytoplankton blooms under elevated DIC show an increased CO_2 uptake and subsequent TEP exudation of phytoplankton cells (Egge et al., 2009; Engel, 2002; Engel et al., 2004a), which can stimulate particle aggregation and acceleration of sedimentation (Gärdes et al., 2011; Logan et al., 1995; Mari, 2008) However, there are no studies focused on understanding particle aggregation under high DIC in coral reef environments.

DOC drives the microbial loop and further transformations into large aggregates. For example, anthropogenic activities can result in high loads of organic matter in coastal ecosystems, and these are often related to large quantities of particulate organic matter in the form of large suspended particles (Garren et al., 2008, 2009; Sarà et al., 2004). Experimental evidence also suggests increases in aggregate formation in eutrophic coastal waters, most likely due to the increased phytoplankton growth and subsequent DOC release (Degobbis, 1989; Kiorboe et al., 1998; Linley and Field, 1982). However, there is no evidence supporting the effects of high DOC on aggregate formation in coral reef systems, despite its importance in coral mortality related to increased sedimentation (Anthony, 1999; Anthony and Fabricius, 2000; Fabricius, 2005).

This study explores the individual and combined effects of elevated DIC and DOC concentrations on aggregate formation processes in the overlying water column in coral reef mesocosms. Furthermore, it intends to compare the effect of these stressors working on two groups of key players in coral reefs specifically, benthic calcifying algae and hard coral communities. Since aggregates and TEP always reach high concentrations in elevated nutrient scenarios of eutrophication, such as after phytoplankton blooms or upwelling events (Kiorboe et al., 1998; Passow and Alldredge, 1995b), higher concentrations of TEP and higher aggregate formation in DIC and DOC treatments were expected.

For this study we used replicated mesocosms deployed with calcifying algae or hard coral communities. The following future ocean scenarios were manipulated over 42 days: Elevated DIC concentrations (DIC), elevated DOC concentrations (DOC), the combined effect of elevated DIC and DOC (combined), and a control treatment. During the course of the experiment, the following parameters were measured: DOC, TEP and suspended particulate matter (SPM) concentrations, total aggregated volume, bacterioplankton cell density and oxygen uptake.

2. Methods

2.1. Origin and preparation of organisms

The experiments were carried out at the MARine Experimental Ecology facility (MAREE) of the Leibniz Center for Tropical Marine Ecology (ZMT). Coral and algal fragments were taken from existing colonies at the MAREE. The fragmentation was done 45 days prior to the starting point of the experiments and fragments were acclimatized for 15 days. The algal species used in the experiments were *Halimeda cuneata*, *Halimeda opuntia*, *Halimeda macroloba*, *Halimeda copiosa* and *Amphiroa foliacea*. Coral species used were *Acropora millepora*, *Pocillopora damicornis*, *Seriatopora hystrix*, *Stylophora pistillata* and *Acropora muricata*.

2.2. Setup of mesocosms

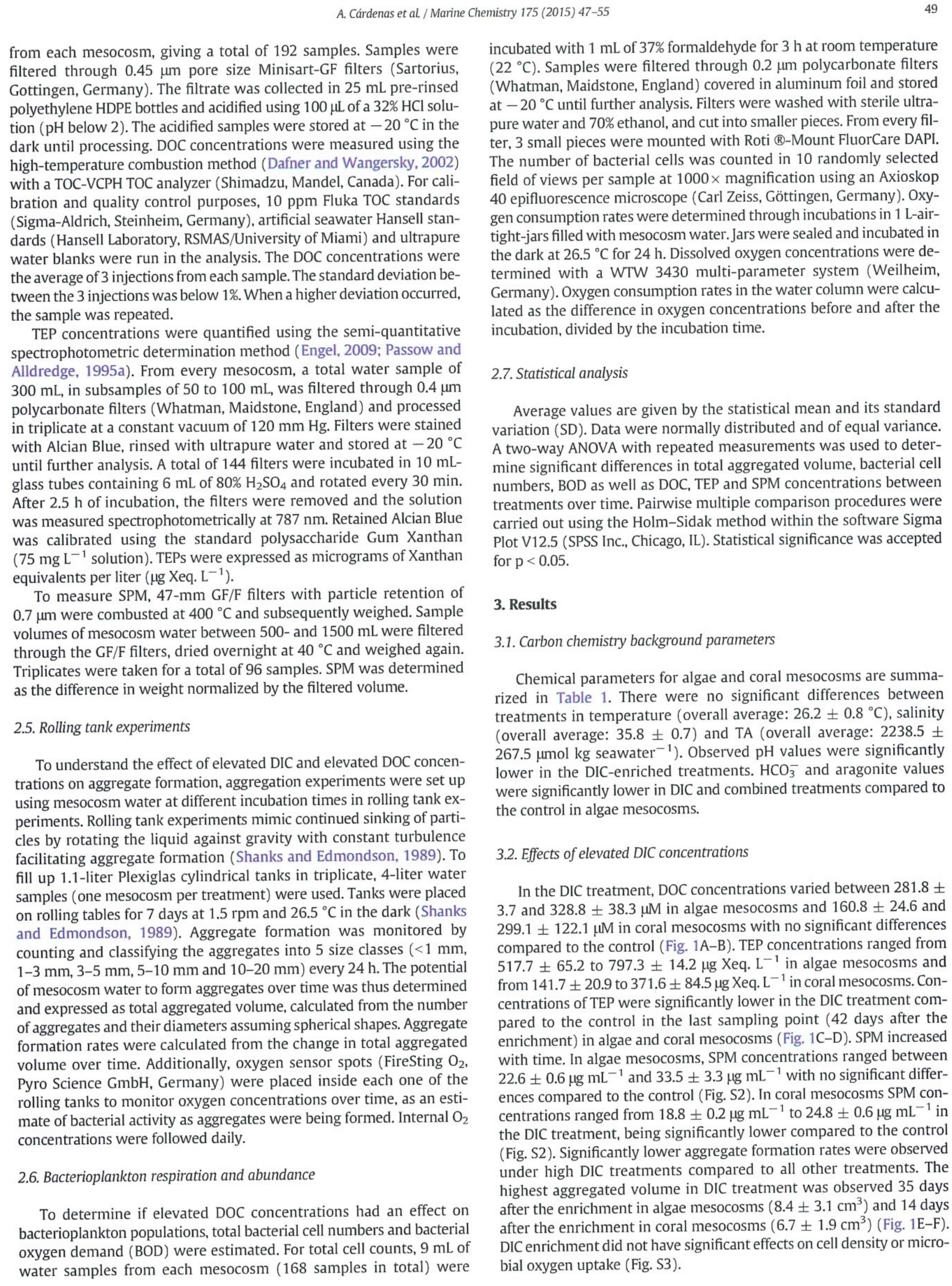
Mesocosms were assembled as an open system using two-compartment tanks with a total volume of 264 L and a constant flow between compartments (Fig. S1). Mesocosms were filled with reverse osmosis and ion exchange resin (Dowex™) prepared water with the addition of artificial sea salts. Every tank contained 20 L of oolite live sand (Ocean Direct™) with a fraction size ranging from 200 μm to 1000 μm as a substrate. Of the 24 tanks, twelve were set up with algae and twelve with coral fragments, with the following conditions: 3 tanks for DIC enrichments, 3 tanks for DOC enrichment, 3 tanks for the combined treatment and 3 tanks to perform the control treatment. Every mesocosm contained between 48 and 52 fragments comprising 5 species of algae or coral. Every tank contained a protein skimmer with 10% of the water being exchanged every week to ensure complete homogenization while minimizing material accumulation. Water was sampled from the lower compartment, directly at the inflow point of the upper compartment using polypropylene beakers (sulfuric acid-cleaned and artificial seawater-leached).

2.3. Background parameters and DIC/DOC enrichment

To obtain elevated DIC conditions, CO_2 was supplied constantly via aeration with 1000 μatm CO_2 pre-mixed gas (gas mixing system, HTK Hamburg, Germany) at a flow rate of 25 L min^{-1} in the DIC and combined treatments. Control and DOC tanks were also aerated at the same flow rates in order to avoid differences in microbial and chemical activity due to bubbling although using 400 μatm CO_2. In DOC and combined treatments, DOC enrichment was achieved by adding 1.5 μM h^{-1} glucose using peristaltic pumps and a glucose stock solution of 30 mmol L^{-1} exchanged once a week. Total alkalinity was maintained constant using 40 g L^{-1} $CaHCO_3$ stock solution by peristaltic pumps. The carbonate chemistry was monitored by measuring in situ pH_{NBS}, temperature, oxygen saturation and salinity using a multi-probe, (WTW 3430, Germany). TA was measured by end-point titration using the TitroLine alpha plus Titrator (SI Analytics, Germany) using 0.5 M HCl and certified reference material (Batch 111, CRM Andrew Dickson, Scripps Institution of Oceanography). The complete carbonate system was calculated from pH_{NBS} and TA using the CO_2 Sys Excel Macro (Lewis and Wallace, 1998) with the KSO_4 constants of Dickson (Dickson, 1990) and K1 and K2 from Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

2.4. Carbon pool analyses

Changes in concentration of DOC, TEP, and SPM were measured over time to follow changes in organic carbon pools during the various future ocean scenarios. For DOC analysis 20 mL of water were collected weekly



from each mesocosm, giving a total of 192 samples. Samples were filtered through 0.45 μm pore size Minisart-GF filters (Sartorius, Gottingen, Germany). The filtrate was collected in 25 mL pre-rinsed polyethylene HDPE bottles and acidified using 100 μL of a 32% HCl solution (pH below 2). The acidified samples were stored at −20 °C in the dark until processing. DOC concentrations were measured using the high-temperature combustion method (Dafner and Wangersky, 2002) with a TOC-VCPH TOC analyzer (Shimadzu, Mandel, Canada). For calibration and quality control purposes, 10 ppm Fluka TOC standards (Sigma-Aldrich, Steinheim, Germany), artificial seawater Hansell standards (Hansell Laboratory, RSMAS/University of Miami) and ultrapure water blanks were run in the analysis. The DOC concentrations were the average of 3 injections from each sample. The standard deviation between the 3 injections was below 1%. When a higher deviation occurred, the sample was repeated.

TEP concentrations were quantified using the semi-quantitative spectrophotometric determination method (Engel, 2009; Passow and Alldredge, 1995a). From every mesocosm, a total water sample of 300 mL, in subsamples of 50 to 100 mL, was filtered through 0.4 μm polycarbonate filters (Whatman, Maidstone, England) and processed in triplicate at a constant vacuum of 120 mm Hg. Filters were stained with Alcian Blue, rinsed with ultrapure water and stored at −20 °C until further analysis. A total of 144 filters were incubated in 10 mL-glass tubes containing 6 mL of 80% H_2SO_4 and rotated every 30 min. After 2.5 h of incubation, the filters were removed and the solution was measured spectrophotometrically at 787 nm. Retained Alcian Blue was calibrated using the standard polysaccharide Gum Xanthan (75 mg L^{-1} solution). TEPs were expressed as micrograms of Xanthan equivalents per liter (μg Xeq. L^{-1}).

To measure SPM, 47-mm GF/F filters with particle retention of 0.7 μm were combusted at 400 °C and subsequently weighed. Sample volumes of mesocosm water between 500- and 1500 mL were filtered through the GF/F filters, dried overnight at 40 °C and weighed again. Triplicates were taken for a total of 96 samples. SPM was determined as the difference in weight normalized by the filtered volume.

2.5. Rolling tank experiments

To understand the effect of elevated DIC and elevated DOC concentrations on aggregate formation, aggregation experiments were set up using mesocosm water at different incubation times in rolling tank experiments. Rolling tank experiments mimic continued sinking of particles by rotating the liquid against gravity with constant turbulence facilitating aggregate formation (Shanks and Edmondson, 1989). To fill up 1.1-liter Plexiglas cylindrical tanks in triplicate, 4-liter water samples (one mesocosm per treatment) were used. Tanks were placed on rolling tables for 7 days at 1.5 rpm and 26.5 °C in the dark (Shanks and Edmondson, 1989). Aggregate formation was monitored by counting and classifying the aggregates into 5 size classes (<1 mm, 1–3 mm, 3–5 mm, 5–10 mm and 10–20 mm) every 24 h. The potential of mesocosm water to form aggregates over time was thus determined and expressed as total aggregated volume, calculated from the number of aggregates and their diameters assuming spherical shapes. Aggregate formation rates were calculated from the change in total aggregated volume over time. Additionally, oxygen sensor spots (FireSting O_2, Pyro Science GmbH, Germany) were placed inside each one of the rolling tanks to monitor oxygen concentrations over time, as an estimate of bacterial activity as aggregates were being formed. Internal O_2 concentrations were followed daily.

2.6. Bacterioplankton respiration and abundance

To determine if elevated DOC concentrations had an effect on bacterioplankton populations, total bacterial cell numbers and bacterial oxygen demand (BOD) were estimated. For total cell counts, 9 mL of water samples from each mesocosm (168 samples in total) were incubated with 1 mL of 37% formaldehyde for 3 h at room temperature (22 °C). Samples were filtered through 0.2 μm polycarbonate filters (Whatman, Maidstone, England) covered in aluminum foil and stored at −20 °C until further analysis. Filters were washed with sterile ultrapure water and 70% ethanol, and cut into smaller pieces. From every filter, 3 small pieces were mounted with Roti ®-Mount FluorCare DAPI. The number of bacterial cells was counted in 10 randomly selected field of views per sample at 1000× magnification using an Axioskop 40 epifluorescence microscope (Carl Zeiss, Göttingen, Germany). Oxygen consumption rates were determined through incubations in 1 L-air-tight-jars filled with mesocosm water. Jars were sealed and incubated in the dark at 26.5 °C for 24 h. Dissolved oxygen concentrations were determined with a WTW 3430 multi-parameter system (Weilheim, Germany). Oxygen consumption rates in the water column were calculated as the difference in oxygen concentrations before and after the incubation, divided by the incubation time.

2.7. Statistical analysis

Average values are given by the statistical mean and its standard variation (SD). Data were normally distributed and of equal variance. A two-way ANOVA with repeated measurements was used to determine significant differences in total aggregated volume, bacterial cell numbers, BOD as well as DOC, TEP and SPM concentrations between treatments over time. Pairwise multiple comparison procedures were carried out using the Holm–Sidak method within the software Sigma Plot V12.5 (SPSS Inc., Chicago, IL). Statistical significance was accepted for $p < 0.05$.

3. Results

3.1. Carbon chemistry background parameters

Chemical parameters for algae and coral mesocosms are summarized in Table 1. There were no significant differences between treatments in temperature (overall average: 26.2 ± 0.8 °C), salinity (overall average: 35.8 ± 0.7) and TA (overall average: 2238.5 ± 267.5 μmol kg $seawater^{-1}$). Observed pH values were significantly lower in the DIC-enriched treatments. HCO_3^- and aragonite values were significantly lower in DIC and combined treatments compared to the control in algae mesocosms.

3.2. Effects of elevated DIC concentrations

In the DIC treatment, DOC concentrations varied between 281.8 ± 3.7 and 328.8 ± 38.3 μM in algae mesocosms and 160.8 ± 24.6 and 299.1 ± 122.1 μM in coral mesocosms with no significant differences compared to the control (Fig. 1A–B). TEP concentrations ranged from 517.7 ± 65.2 to 797.3 ± 14.2 μg Xeq. L^{-1} in algae mesocosms and from 141.7 ± 20.9 to 371.6 ± 84.5 μg Xeq. L^{-1} in coral mesocosms. Concentrations of TEP were significantly lower in the DIC treatment compared to the control in the last sampling point (42 days after the enrichment) in algae and coral mesocosms (Fig. 1C–D). SPM increased with time. In algae mesocosms, SPM concentrations ranged between 22.6 ± 0.6 μg mL^{-1} and 33.5 ± 3.3 μg mL^{-1} with no significant differences compared to the control (Fig. S2). In coral mesocosms SPM concentrations ranged from 18.8 ± 0.2 μg mL^{-1} to 24.8 ± 0.6 μg mL^{-1} in the DIC treatment, being significantly lower compared to the control (Fig. S2). Significantly lower aggregate formation rates were observed under high DIC treatments compared to all other treatments. The highest aggregated volume in DIC treatment was observed 35 days after the enrichment in algae mesocosms (8.4 ± 3.1 cm^3) and 14 days after the enrichment in coral mesocosms (6.7 ± 1.9 cm^3) (Fig. 1E–F). DIC enrichment did not have significant effects on cell density or microbial oxygen uptake (Fig. S3).

Table 1
Mesocosm water chemistry after 42 days of incubation. Average values (±SD) are given for each treatment.

	Treatment	Temperature (°C)	Salinity	pH (NBS)	TA (μmol kg^{-1})	DIC (μatm)	HCO_3^- (μmol kg^{-1})	Ω aragonite
Algae mesocosms	Control	25.8 ± 0.4	35.6 ± 0.3	8.130 ± 0.024	2143 ± 72	456 ± 56	1732 ± 140	2.75 ± 0.02
	DIC	26.0 ± 0.1	35.7 ± 0.3	7.991 ± 0.062	2206 ± 114	854 ± 115	2348 ± 41	2.19 ± 0.26
	DOC	25.9 ± 0.2	35.6 ± 0.5	8.097 ± 0.046	2320 ± 86	604 ± 82	2130 ± 196	2.82 ± 0.26
	Combined	26.6 ± 0.3	35.8 ± 0.6	7.933 ± 0.035	2195 ± 158	1170 ± 120	2823 ± 156	2.01 ± 0.14
Coral mesocosms	Control	25.6 ± 0.1	36.2 ± 0.4	8.183 ± 0.016	2401 ± 89	423 ± 33	1833 ± 107	3.66 ± 0.24
	DIC	25.2 ± 0.1	36.1 ± 0.3	8.085 ± 0.022	2333 ± 47	581 ± 45	1896 ± 55	2.79 ± 0.11
	DOC	25.5 ± 0.4	36.1 ± 0.5	8.114 ± 0.029	2430 ± 111	520 ± 18	1924 ± 65	3.27 ± 0.33
	Combined	25.3 ± 0.6	36.2 ± 0.2	8.07 ± 0.048	2310 ± 87	553 ± 64	1861 ± 61	2.87 ± 0.29

3.3. *Effects of elevated DOC concentrations*

Initial DOC concentrations in algae mesocosms were 688.3 ± 145.9 μM and reached 1448.0 ± 89.2 μM 21 days after the enrichment (Fig. 2A). In coral mesocosms initial concentrations were 201.8 ± 10.8 μM with a maximum of 365.5 ± 90.6 μM after 21 days of incubation (Fig. 2B). TEP concentrations were always significantly higher than the control mesocosms ($p < 0.01$). Initial TEP concentrations in algae

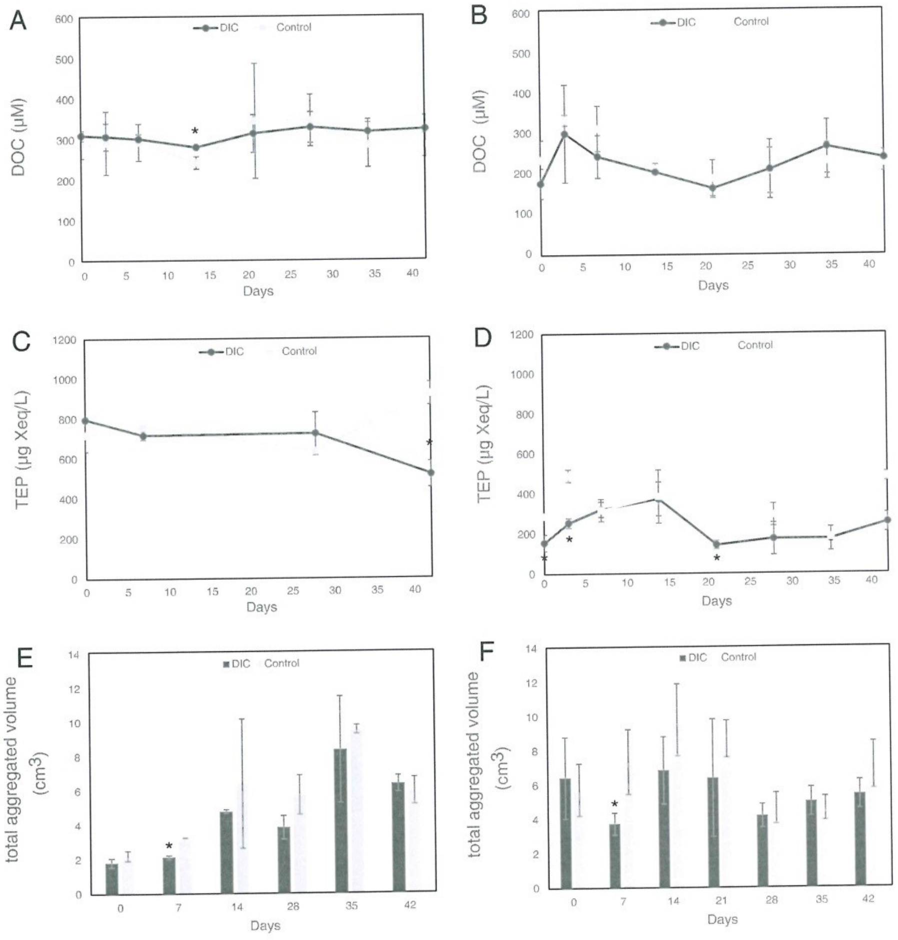


Fig. 1. Effects of elevated DIC enrichment on DOC and TEP concentrations as well as total aggregated volume. DOC concentrations in (A) algae and (B) coral mesocosms. TEP concentrations in (C) algae and (D) coral mesocosms. Total aggregated volumes in rolling tank experiments from (E) algae and (F) coral mesocosms. Aggregate volume per tank was calculated every 24 h during a total period of 72 h from particle numbers and their corresponding size assuming spherical shapes. The value shown on the graph represents the maximum value over the 72 h. Error bars denote ±SD. Asterisks indicate significantly different means compared to control at $p < 0.05$ (Holm–Sidak method).

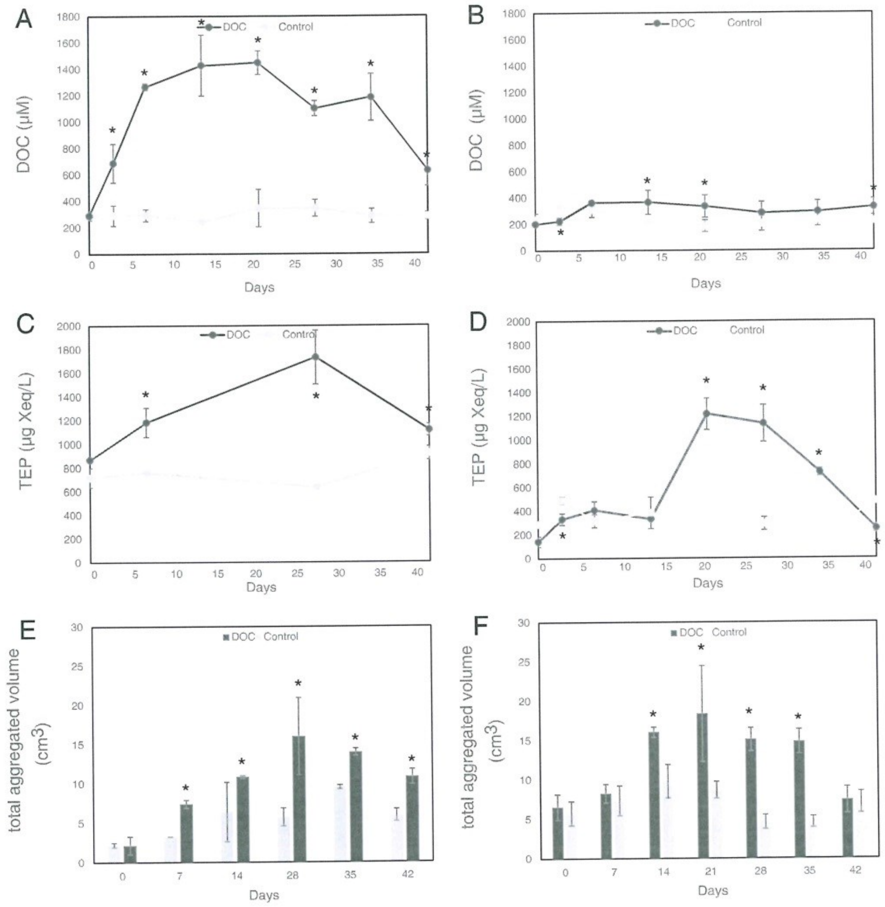


Fig. 2. Effects of elevated DOC enrichment on DOC and TEP concentrations as well as total aggregated volume. DOC concentrations in (A) algae and (B) coral mesocosms. TEP concentrations in (C) algae and (D) coral mesocosms. Total aggregated volumes in rolling tank experiments from (E) algae and (F) coral mesocosms. Aggregate volume per tank was calculated every 24 h during a total period of 72 h from particle numbers and their corresponding size assuming spherical shapes. The value shown on the graph represents the maximum value over the 72 h. Error bars denote ±SD. Asterisks indicate significantly different means compared to control at $p < 0.05$ (Holm–Sidak method).

Table 2
Significance values for pairwise comparisons between variables. Pairwise multiple comparison procedures were done by the pairwise comparison Holm–Sidak method using the software Sigma Plot V12.5 (SPSS Inc., Chicago, IL). Underlined values represent statistically significant differences compared to control treatments ($p < 0.05$).

	Algae mesocosms			Coral mesocosms		
	DIC	DOC	Combined	DIC	DOC	Combined
DOC concentrations	0.584	<0.001	<0.001	0.421	0.002	0.002
TEP concentrations	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
SPM concentrations	0.148	<0.001	<0.001	0.023	<0.001	<0.001
Aggregate formation	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cell density	0.196	0.236	0.146	0.052	0.177	0.084
Oxygen uptake	0.663	0.020	<0.001	0.682	0.050	0.104
Temperature	0.781	0.667	0.052	0.112	0.578	0.439
Salinity	0.173	0.289	0.346	0.833	0.828	0.878
TA	0.464	0.052	0.631	0.085	0.406	0.723
pH	0.022	0.332	<0.001	0.010	0.066	0.042
CO_2	0.006	0.061	<0.001	0.002	0.052	0.026
HCO_3	0.002	0.046	0.010	0.419	0.285	0.829
Aragonite	0.020	0.666	<0.001	0.072	0.039	0.165

mesocosms were 719.6 ± 82.8 μg Xeq. L^{-1} and increased to maximum values of 1733.1 ± 226.8 μg Xeq. L^{-1} by day 28 (Fig. 2C). In coral mesocosms, initial TEP concentrations were significantly lower than in algae mesocosms, (142.6 ± 43.9 μg Xeq. L^{-1}). Highest TEP concentrations in coral mesocosms were observed 21 days after the enrichment (1216.9 ± 132.4 μg Xeq. L^{-1}) (Fig. 2D). In algae mesocosms, SPM increased over time, reaching a peak value of 42.7 ± 4.3 μg mL^{-1} by day 42 (Fig. S2A). In coral mesocosms, a significant increase in SPM was observed from 14 to 35 days after the enrichment with maximum concentrations of 82.9 ± 3.5 μg mL^{-1} (Fig. S2B). Aggregate formation was significantly higher (Table 2) in DOC treatments compared to controls in both algae and coral mesocosms. Aggregate volume and formation rates increased especially during high TEP concentrations in the mesocosms (days 14 to 35) (Fig. 2E–F). In rolling tank experiments from algae mesocosms, the highest aggregated volume formed was 16.0 ± 4.9 cm^3 at high TEP concentrations (Fig. 2E). In rolling tank experiments from coral mesocosms, the highest aggregated volume formed was 35.0 ± 7.0 cm^3 in the DOC treatment (Fig. 2F). Cell numbers in the DOC treatment were not significantly higher in coral and algae mesocosms compared to the controls (Table 2, Fig. S3C–D). However, microbial oxygen uptake was significantly higher in the DOC treatment compared to the control treatments in both algae and coral mesocosms (Fig. S3A–B).

3.4. Combined effects of elevated DIC and DOC concentrations

DOC concentrations in algae mesocosms in the combined treatment were significantly lower than in the DOC treatment alone (Table 2). However, in coral mesocosms the combined treatment had no significant differences compared to the DOC treatment. The highest DOC concentrations observed were 1351.2 ± 47.8 μM by day 21 in algae mesocosms and 390.8 ± 25.1 μM in coral mesocosms by day 14 (Fig. 3A–B). Initial TEP concentrations in algae mesocosms were 760.3 ± 39.6 μg Xeq. L^{-1} and increased to maximum values of 1265.0 ± 82.2 μg Xeq. L^{-1} by day 28 (Fig. 3C). In coral mesocosms, initial TEP concentrations were significantly lower than in algae mesocosms (392.1 ± 79.7 μg Xeq. L^{-1}), but reached the highest observed TEP concentration (2750.7 ± 299.9 μM) 21 days after the enrichment (Fig. 3D). In algae mesocosms, the highest values of SPM observed were 50.8 ± 1.6 μg mL^{-1} after 35 days of enrichment (Fig. S2A). In coral mesocosms the SPM increased significantly from 14 to 35 days after the enrichment with maximum concentrations of 108.5 ± 7.7 μg mL^{-1} by day 28 (Fig. S2B). In both algae and coral mesocosms the highest aggregate formation was observed in the combined treatments especially from 14 to 35 days after the enrichment (Fig. 3E–F), corresponding to high TEP abundance. The highest aggregated volume observed was 20.7 ± 2.6 and 25.0 ± 4.2 cm^3 for algae and coral mesocosm rolling tank experiments, respectively. Bacterial oxygen uptake was only significantly higher in algae mesocosms compared to the control (Fig. S3A). Cell density values did not differ significantly from the control (Table 2).

3.5. Comparison between algae and coral mesocosms

The most remarkable difference between algae and coral mesocosms was the DOC concentration. In algae mesocosms, DOC concentrations were more than three times as high as for coral mesocosms (Figs. 2A–

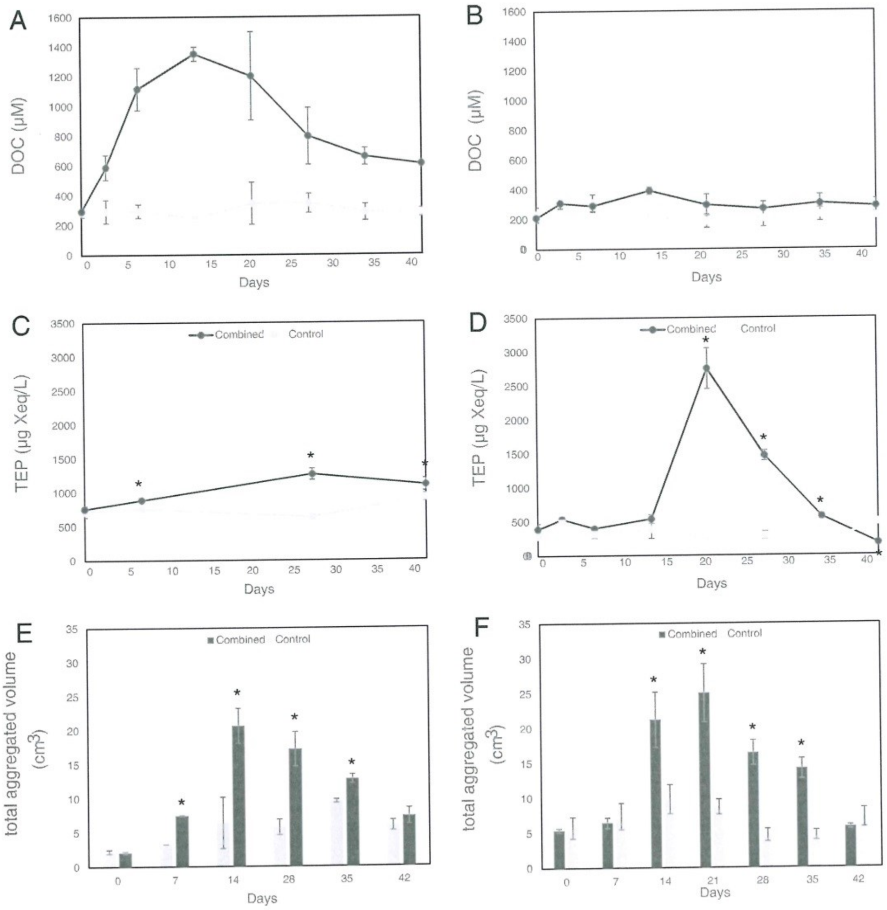


Fig. 3. Combined effects of elevated DOC and DIC enrichment on DOC and TEP concentrations as well as total aggregated volume. DOC concentrations in (A) algae and (B) coral mesocosms. TEP concentrations in (C) algae and (D) coral mesocosms. Total aggregated volumes in rolling tank experiments from (E) algae and (F) coral mesocosms. Aggregate volume per tank was calculated every 24 h during a total period of 72 h from particle numbers and their corresponding size assuming spherical shapes. The value shown on the graph represents the maximum value over the 72 h. Error bars denote ±SD. Asterisks indicate significantly different means compared to control at $p < 0.05$ (Holm–Sidak method).

B and 3A–B). TEP concentrations were higher in the DOC treatments in algae, and in the combined treatment in coral mesocosms (Figs. 2C–F and 3C–F). Furthermore, there were no significant differences in bacterial numbers and oxygen uptake between algae and coral mesocosms.

4. Discussion

4.1. Effects of high DIC on in-vitro particle aggregation

DIC enrichment is commonly associated with increased photosynthesis, exudation rates and TEP concentrations (Engel, 2002; Riebesell, 2004). It has previously been suggested that changes in both TA and pH may have an effect on TEP properties (Passow, 2012). In the present study, TEP concentrations were affected by DIC enrichment, remaining always significantly lower compared to the control mesocosms, even when TA remained constant. In the DIC treatment, even if the photosynthesis was increased and resulted in a larger transitory organic carbon pool, these photosynthates seemed to be mainly used to fuel benthic bacterial biomass rather than contribute to the TEP pool in the water column. Therefore, contrary to what was expected, exogenous glucose seemed to be the main contributor to TEP formation rather than increases in DIC.

Mari (2008) showed that a low pH reduces TEP stickiness, decreasing aggregate formation and therefore causing a decrease in the downward flux of carbon. Similarly, the lowest aggregate volumes were observed in the DIC treatment in this study, suggesting that TEP stickiness could have been affected by changes in pH. Nonetheless, other changes in TEP properties cannot be excluded and would require further study.

4.2. Effects of high DOC on in-vitro particle aggregation

Glucose, which was used in the present study to enrich DOC concentrations, not only represents one of the most abundant monosaccharides in coastal waters with anthropogenic influences (Cowie and Hedges, 1984; Huang et al., 2010) but also is a frequently dominant monosaccharide in natural exudates of several phytoplankton and macroalgae species (Barabanova et al., 2010; Urbani et al., 2005). Our results suggest that high DOC concentrations may trigger aggregate formation through the formation of TEP. Since a large fraction of algae and coral-derived DOC is composed of carbohydrates, which in turn are the main components of TEP, it may be that cell exudates may contribute to the DOC pool and therefore TEP precursors.

TEP concentrations did not increase steadily as has been observed in other mesocosm experiments (Engel et al., 2004a; Mari et al., 2005). A relationship exists between bacterial cell abundance and TEP concentrations, indicating that production and/or consumption of TEP in our experiments were coupled with microbial cell density. Due to the small differences in bacterial cell numbers and oxygen uptake over time, we assume that only a small amount of DOC was taken up by the bacterioplankton community and a larger proportion of DOC was either taken up by benthic bacterial communities or spontaneously assembled into TEP and SPM as has been shown in other studies (Alldredge et al., 1993; Passow, 2000). However, oxygen consumption was significantly higher in the DOC-enriched treatments (Table 2), suggesting that the addition of a carbon source favored heterotrophic activity in the system. Peaks in respiration coincided with points in time of higher aggregate formation. This indicates that suspended particle matter represents an important source of nutrients for the plankton community.

4.3. Combined compared to independent effects

Our results, in agreement with previous studies (Fabricius et al., 2003; Gärdes et al., 2011; Passow and Alldredge, 1995b), suggest TEP as one of the main factors driving aggregate formation. High concentrations of TEP in the mesocosms were always consistent with high aggregation rates in the rolling tank experiments. However, the largest aggregate volumes were observed in the combined treatment, even when the highest concentrations of TEP were measured in the DOC treatments. One possible explanation is that the slightly lower pH in the combined treatment compared to the DOC treatment, is enhancing aggregate formation in an unknown way. For instance, Mari (2008) showed that a low pH increased TEP buoyancy, causing flotation rather than sinking of aggregates. As a consequence, TEP with longer residence time in the water column may further enhance aggregate formation. In our study, aggregate formation in the combined treatment was five times higher than in the individual treatments. Nevertheless, it is still not possible to accurately relate how the combined effect of elevated DIC and elevated DOC concentrations are enhancing aggregate formation, and should be addressed in future studies.

4.4. The influence of reef communities on particle and aggregate formation

Previous results have shown either slight or no significant differences in DOC concentrations released by benthic calcifying algae (*H. opuntia*) compared to corals (*Porites lobata*) (Haas et al., 2011, 2013). However, present results showed severe differences in DOC concentrations between algae and coral mesocosms (Figs. 2A–B and 3A–B). Higher DOC concentrations in algae mesocosms are suggesting that in coral mesocosms DOC was either rapidly transformed into particulate organic matter (TEP and particle aggregates occurred earlier in coral mesocosms) or taken up directly by corals, as has been shown before (Gori et al., 2014; Grover et al., 2008; Holcomb et al., 2014). However, the activity of sediment microbial communities and communities hosted in the mucus layer of corals cannot be excluded, since they can significantly contribute to DOC uptake.

It is hypothesized that bacterial growth is triggered by the algae-derived DOC rather than coral-derived DOC (Haas et al., 2011, 2013). However, previous research demonstrates that calcifying algae release lower amounts and different compositions of exudates compared to their non-calcifying counterparts (Haas and Wild, 2010; Nakajima et al., 2010). We observed an increase in bacterial respiration in glucose-enriched treatments, but were unable to detect any significant differences in bacterial cell numbers and respiration rates between algae and coral mesocosms. This might suggest that calcifying algae release higher amounts of less labile DOC than corals, which in the end might yield the same oxygen uptake in the bacterial community. However, it is still necessary to consider DOC composition to understand all mechanisms involved in the transformations of organic carbon.

4.5. Ecological perspective

Elevated DOC concentrations are already one of the main concerns of coral reef health (Haas et al., 2011; Kline et al., 2006; Kuntz et al., 2005). Several studies reported that enhanced bacterial growth results in oxygen depletion and accumulation of toxic substances which ultimately leads to an increased coral mortality (Castro and Huber, 2003; Gregg et al., 2013; Kline et al., 2006; Smith et al., 2006). Unfortunately global and local stressors are not often considered simultaneously, thus neglecting the threats coral reefs face if exposed to multiple stressors. The combined effects of OA and increased DOC concentrations represent a more realistic scenario for many coastal ecosystems that are constantly under the effect of organic nutrient loads derived from anthropogenic activities. If proven in-situ, this could have profound biological implications in coral reef ecosystems as a consequence of higher aggregate formation and sinking rates, which in turn may enhance coral mortality caused by sedimentation. This could have a stronger impact in reefs that are located in poorly flushed locations or those with high exposure to pollutants. Furthermore, our results indicate that coral and algae release DOC in different quantities and compositions, which in turn has an impact on the aggregate formation. In a

community dominated by corals rather than calcifying algae, sedimentation could be faster. It is also important to include the effect of aggregate formation in coral reefs dominated by non-calcifying macroalgae in future studies, while considering that many coral reefs have already undergone phase-shifts from coral to non-calcifying macroalgae dominance (Hughes et al., 2007; McManus and Polsenberg, 2004). This algal overgrowth is known to elevate DOC levels and enhance microbial activity (Haas et al., 2013; Kline et al., 2006; Kuntz et al., 2005). In the same way, this might trigger coral mortality associated with suspended particle matter and increased sedimentation rates.

5. Conclusions

Experiments reported here were designed to shed light on the effects of local and global stressors on different organic carbon pools, ranging from the dissolved to the particulate state in coral reefs. The findings in this work demonstrate that combined effects of OA, in terms of elevated DIC concentrations and elevated DOC concentrations on aggregate formation, are more severe than each stressor individually. Combined effects of DIC and DOC enrichments increased TEP concentrations and formation of aggregates. These experiments could represent possible scenarios for future conditions, especially in coastal ecosystems where elevated DOC concentrations exert greater impact that could increase the susceptibility to ocean acidification. An increase in aggregate formation will likely result in higher export of carbon to the deep sea. However, in coral reef ecosystems this may lead to negative consequences due to an increase in sedimentation of labile organic carbon that could result in harmful effects for reef organisms. Nonetheless, the impact of increased sedimentation rates due to coral and algal-derived organic matter is not understood and needs to be addressed in further studies.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marchem.2015.04.002.

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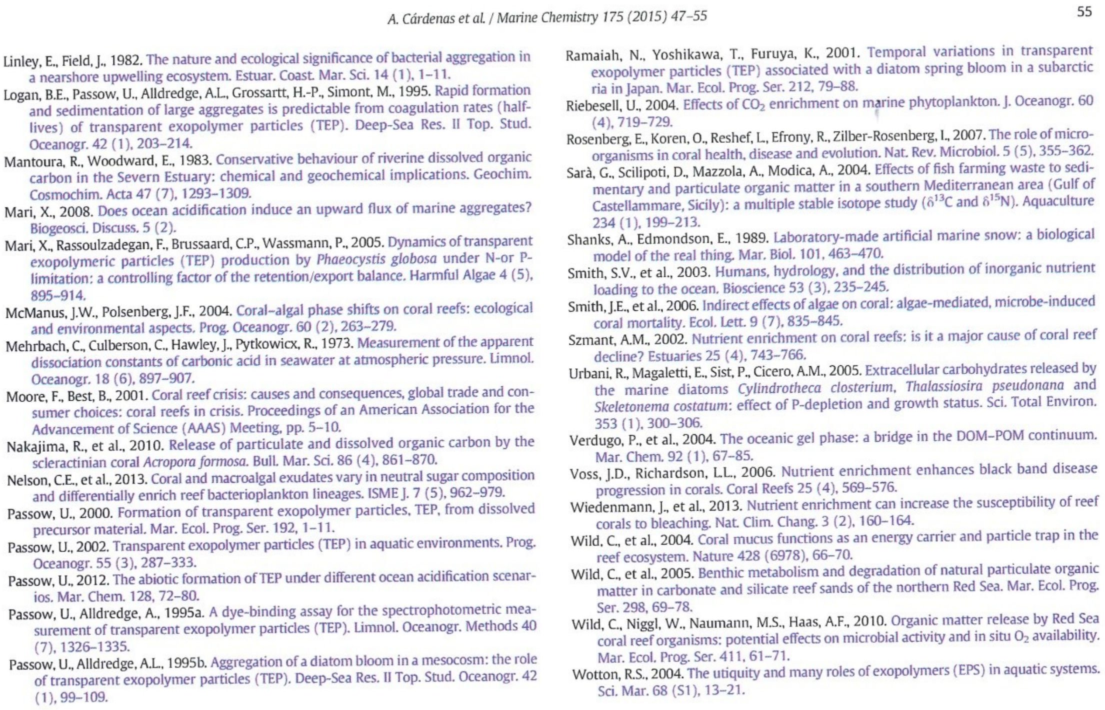

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RAPID COMMUNICATION

A recent outbreak of crown-of-thorns starfish (*Acanthaster planci*) in the Spermonde Archipelago, Indonesia

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Abstract A recent review of crown-of-thorns starfish (*Acanthaster planci*; COTS) in Indonesia has suggested that their impacts have gone under-reported. In 2012–2013, we surveyed COTS at permanent transects within 12 sites of the Spermonde Archipelago, Indonesia, a coastal region close to two rivers and the heavily urbanised city of Makassar. Evidence of COTS was apparent at 8 of 12 sites surveyed with highest densities (37 starfish per 250 m^{-2} at Barrang Lompo) comparable to those reported in the Indonesian historical literature. At Barrang Lompo and Bonetambung, the COTS outbreak resulted in the loss of half the live coral. Terrestrial effluents have reduced water quality in the Spermonde Archipelago, which further supports recent work linking water quality and COTS outbreaks, thus providing a warning of future outbreaks to Indonesian coastal managers given the country's increased urbanisation.

Keywords Coral reef · Crown-of-thorns starfish · Coral triangle · Makassar · Disturbance

Introduction

Acanthaster planci, or the crown-of-thorns starfish (COTS), is a significant threat to coral reefs in the Indian and Pacific oceans. Generally, COTS occur naturally in low abundances (<1 starfish ha^{-1}); however, population outbreaks of COTS can occur rapidly and be devastating, possibly resulting in up to 80 % mortality of corals (Pratchet et al. 2014). Damage by COTS can result in the loss of structural complexity, biodiversity, and productivity of corals and the greater reef community (Kayal et al. 2012). Despite the destructive potential of COTS, a lack of observations on the development and progression of population outbreaks impede our understanding surrounding the ecological processes of these events.

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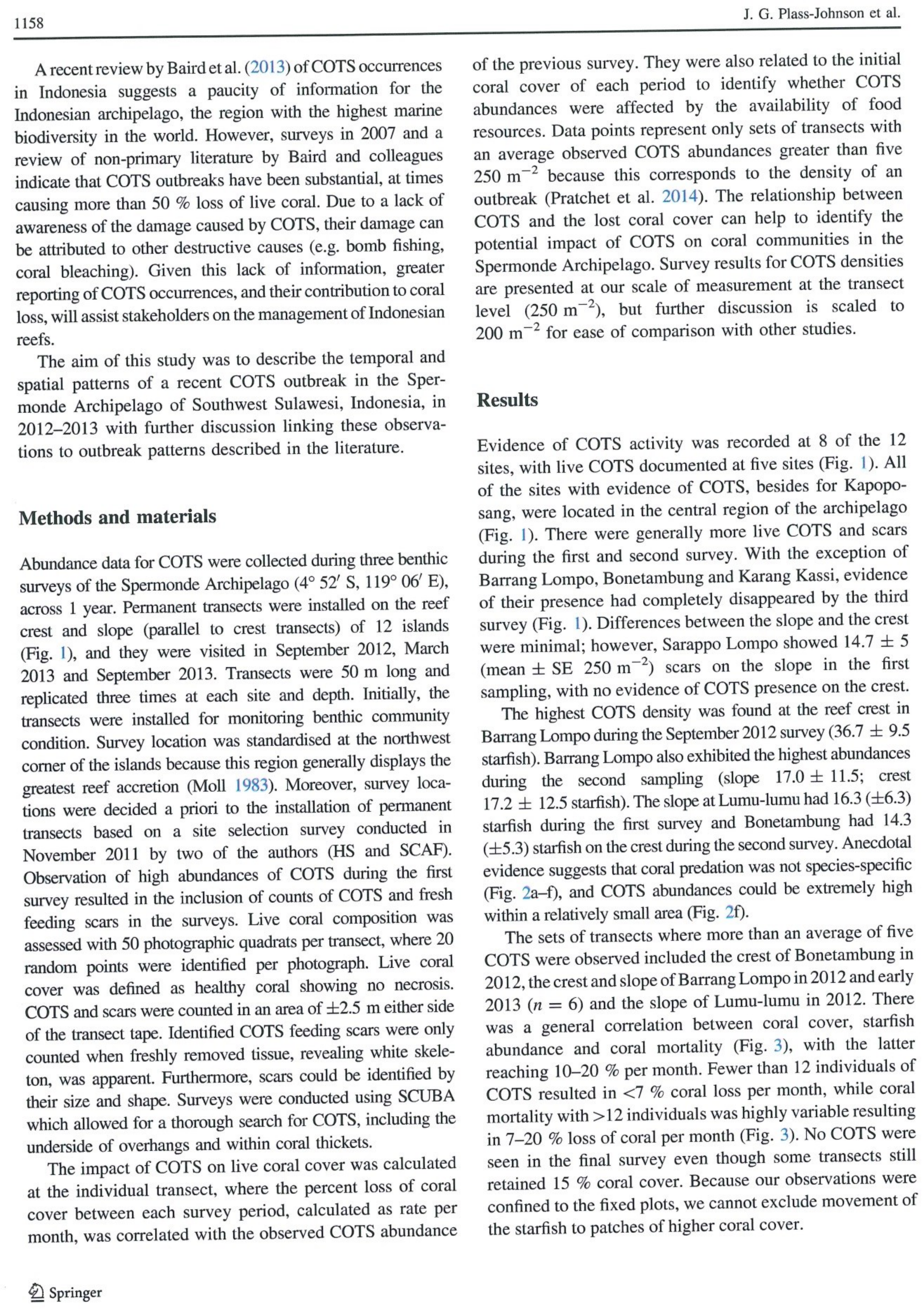

A recent review by Baird et al. (2013) of COTS occurrences in Indonesia suggests a paucity of information for the Indonesian archipelago, the region with the highest marine biodiversity in the world. However, surveys in 2007 and a review of non-primary literature by Baird and colleagues indicate that COTS outbreaks have been substantial, at times causing more than 50 % loss of live coral. Due to a lack of awareness of the damage caused by COTS, their damage can be attributed to other destructive causes (e.g. bomb fishing, coral bleaching). Given this lack of information, greater reporting of COTS occurrences, and their contribution to coral loss, will assist stakeholders on the management of Indonesian reefs.

The aim of this study was to describe the temporal and spatial patterns of a recent COTS outbreak in the Spermonde Archipelago of Southwest Sulawesi, Indonesia, in 2012–2013 with further discussion linking these observations to outbreak patterns described in the literature.

Methods and materials

Abundance data for COTS were collected during three benthic surveys of the Spermonde Archipelago (4° 52′ S, 119° 06′ E), across 1 year. Permanent transects were installed on the reef crest and slope (parallel to crest transects) of 12 islands (Fig. 1), and they were visited in September 2012, March 2013 and September 2013. Transects were 50 m long and replicated three times at each site and depth. Initially, the transects were installed for monitoring benthic community condition. Survey location was standardised at the northwest corner of the islands because this region generally displays the greatest reef accretion (Moll 1983). Moreover, survey locations were decided a priori to the installation of permanent transects based on a site selection survey conducted in November 2011 by two of the authors (HS and SCAF). Observation of high abundances of COTS during the first survey resulted in the inclusion of counts of COTS and fresh feeding scars in the surveys. Live coral composition was assessed with 50 photographic quadrats per transect, where 20 random points were identified per photograph. Live coral cover was defined as healthy coral showing no necrosis. COTS and scars were counted in an area of ±2.5 m either side of the transect tape. Identified COTS feeding scars were only counted when freshly removed tissue, revealing white skeleton, was apparent. Furthermore, scars could be identified by their size and shape. Surveys were conducted using SCUBA which allowed for a thorough search for COTS, including the underside of overhangs and within coral thickets.

The impact of COTS on live coral cover was calculated at the individual transect, where the percent loss of coral cover between each survey period, calculated as rate per month, was correlated with the observed COTS abundance of the previous survey. They were also related to the initial coral cover of each period to identify whether COTS abundances were affected by the availability of food resources. Data points represent only sets of transects with an average observed COTS abundances greater than five 250 m^{-2} because this corresponds to the density of an outbreak (Pratchet et al. 2014). The relationship between COTS and the lost coral cover can help to identify the potential impact of COTS on coral communities in the Spermonde Archipelago. Survey results for COTS densities are presented at our scale of measurement at the transect level (250 m^{-2}), but further discussion is scaled to 200 m^{-2} for ease of comparison with other studies.

Results

Evidence of COTS activity was recorded at 8 of the 12 sites, with live COTS documented at five sites (Fig. 1). All of the sites with evidence of COTS, besides for Kapoposang, were located in the central region of the archipelago (Fig. 1). There were generally more live COTS and scars during the first and second survey. With the exception of Barrang Lompo, Bonetambung and Karang Kassi, evidence of their presence had completely disappeared by the third survey (Fig. 1). Differences between the slope and the crest were minimal; however, Sarappo Lompo showed 14.7 ± 5 (mean ± SE 250 m^{-2}) scars on the slope in the first sampling, with no evidence of COTS presence on the crest.

The highest COTS density was found at the reef crest in Barrang Lompo during the September 2012 survey (36.7 ± 9.5 starfish). Barrang Lompo also exhibited the highest abundances during the second sampling (slope 17.0 ± 11.5; crest 17.2 ± 12.5 starfish). The slope at Lumu-lumu had 16.3 (±6.3) starfish during the first survey and Bonetambung had 14.3 (±5.3) starfish on the crest during the second survey. Anecdotal evidence suggests that coral predation was not species-specific (Fig. 2a–f), and COTS abundances could be extremely high within a relatively small area (Fig. 2f).

The sets of transects where more than an average of five COTS were observed included the crest of Bonetambung in 2012, the crest and slope of Barrang Lompo in 2012 and early 2013 ($n = 6$) and the slope of Lumu-lumu in 2012. There was a general correlation between coral cover, starfish abundance and coral mortality (Fig. 3), with the latter reaching 10–20 % per month. Fewer than 12 individuals of COTS resulted in <7 % coral loss per month, while coral mortality with >12 individuals was highly variable resulting in 7–20 % loss of coral per month (Fig. 3). No COTS were seen in the final survey even though some transects still retained 15 % coral cover. Because our observations were confined to the fixed plots, we cannot exclude movement of the starfish to patches of higher coral cover.

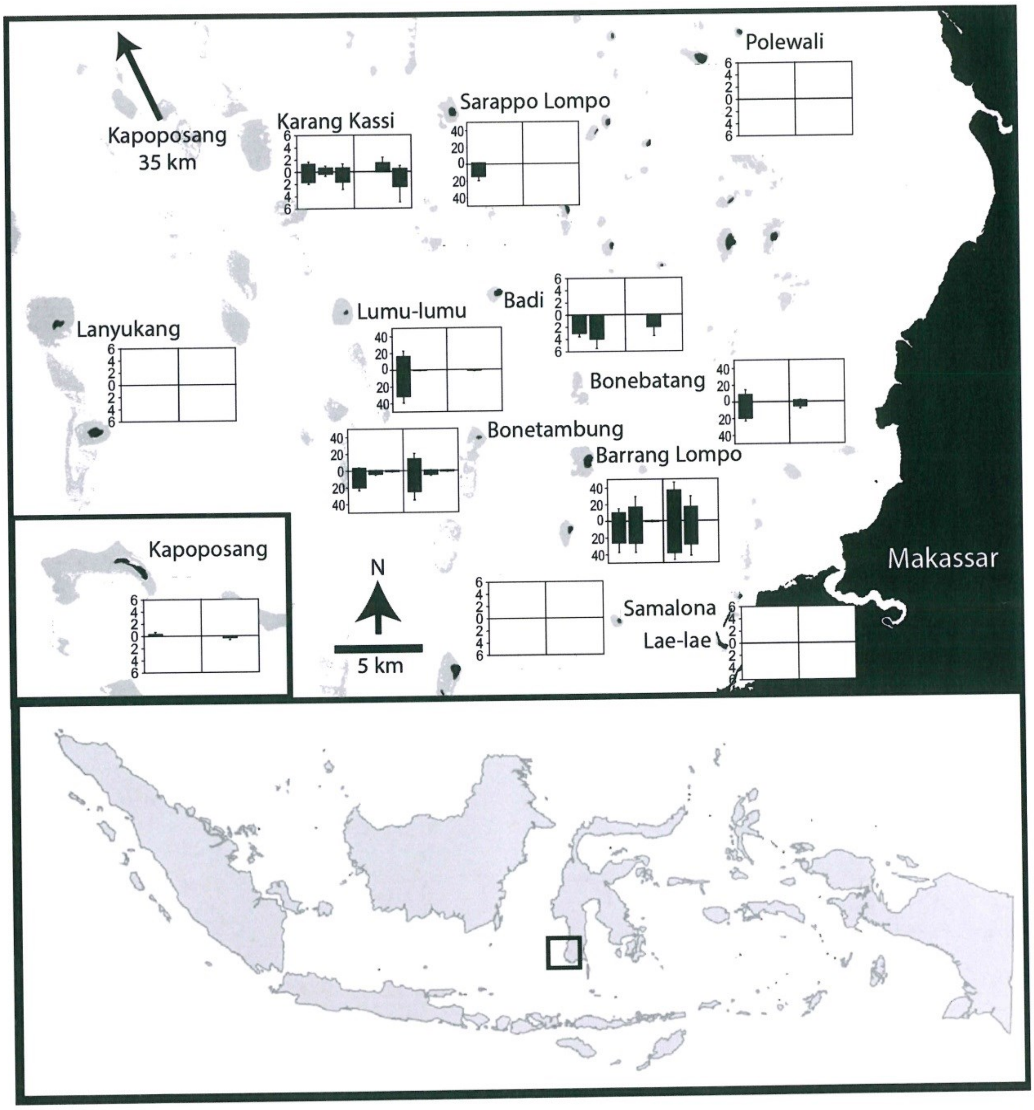

Fig. 1 Map of the Spermonde Archipelago (*top*) and its location within Indonesia (*bottom*). Data represent the mean starfish abundance (±SE) per survey (*top boxes*) and number of feeding scars (*bottom boxes*) on the slope (*left boxes*) and crest (*right boxes*). The *three bars* within a box indicate sampling time starting with the earliest on the *left* (September 2012) and latest on the *right* (September 2013). The absence of data for some sites of surveys indicates that there were no COTS present during the surveyed period. *Note* the different scales on the y-axis (mean starfish abundance)

Discussion

Baird et al. (2013) recently brought to attention the underreporting of *A. planci* outbreaks in Indonesia, despite the severe impact on live coral that they can have. The surveys of Baird and colleagues found abundances from one to 5.2 individuals 200 m^{-2}, and their supporting review of historical Indonesian literature revealed outbreaks of $\leq$26 individuals 200 m^{-2}. Direct comparison of the present data with that of Baird et al. (2013) is difficult due to differing

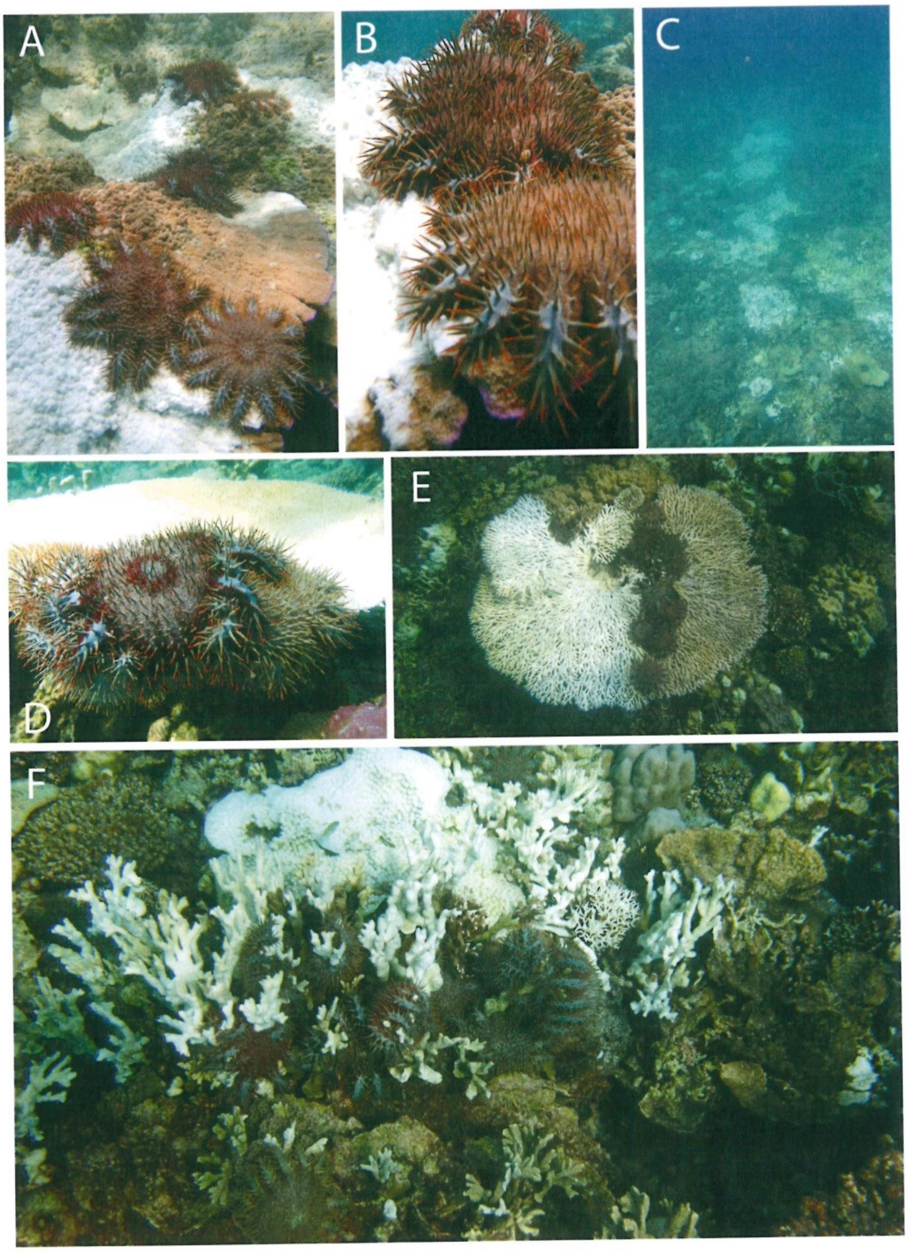

Fig. 2 Crown-of-thorns activity at Barrang Lompo (**a**, **d** and **f**), Bonetambung (**b** and **e**), and Lumu-lumu (**c**) recorded during the September 2012 survey. At Barrang Lompo, COTS were observed feeding on multiple genera of coral in very high densities. Eighteen starfish were observed feeding in roughly 2 m^{-2} at Barrang Lompo (**f**). **c** A track, approximately 20 m long, of fresh feeding scars left by COTS in September 2012

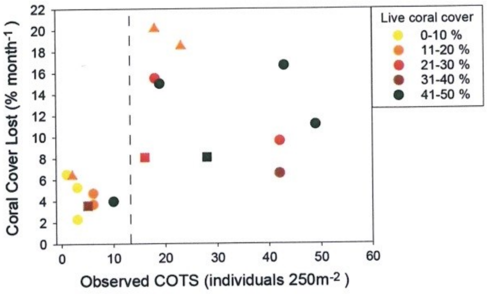

Fig. 3 Relationship between COTS abundances and the loss of live coral cover at Barrang Lompo (*circles*), Lumu-lumu (*squares*) and Bonetambung (*triangles*). The *dotted lines* denote abundances where COTS clearly increased their impact on live coral cover. *Colour* relates to the percentage of live coral cover observed when the COTS were counted (colour figure online)

survey methods; however, standardisation of data from Barrang Lompo during the peak observed outbreak (29.4 ± 5.1 starfish 200 m^{-2}) indicates COTS densities in the range of the worst historical outbreaks. Furthermore, these numbers are similar to those reported for Moorea, French Polynesia (30.3 ± 6.1 starfish 200 m^{-2}, Kayal et al. 2012) between 2004 and 2010, an outbreak recorded as the most intense disturbance of that reef in 40 years of monitoring, and one of the largest reported outbreaks in the last decade. Furthermore, this outbreak resulted in >90 % live coral loss.

It is important to note that the outbreak in our study had all but completely disappeared within 1 year. During the first survey, Barrang Lompo, Bonetambung, Lumu-lumu, Karang Kassi and Bonebatang all had COTS present, but by the second survey this was reduced to only two sites. By the third survey, all indications of COTS, including scars, were completely absent with the exception of a few starfish and scars at Karang Kassi. Previous observations have identified outbreak durations of roughly 1 year (Pratchett et al. 2009), while at other locations outbreaks have lasted for years (Kayal et al. 2012), even occurring cyclically (Pratchett 2005). In a survey of 180 m^2 of reef at Barrang Lompo in late 2011 (Muller et al. 2012) and a coarse but extensive (over 1 km length) survey by two of the authors (HS and SCAF) at the same time, no observations of COTS were reported, suggesting that our surveys may have identified the beginning of the outbreak, although individual animals in other sections of the reef may have remained undetected by the earlier surveys. Anecdotal observation by the lead author (JPJ) of a considerable number of starfish at Barrang Caddi, an island 4 km from Barrang Lompo, during the last survey (September 2013), suggests that COTS continued to persist in the archipelago.

The damage caused to live coral by the starfish was considerable, and this was apparent at both Barrang Lompo and Bonetambung. Individual transects on both islands lost up to half their coral cover between the first and last survey. Unfortunately, our surveys were spatially confined to the permanent transect so we could not track further movements of COTS to areas retaining high levels of coral cover. Although there are many potential drivers affecting coral reef degradation in Indonesian, careful observation indicates that the damage observed in this study was most likely caused by COTS. Similarly, Baird et al. (2013) found >50 % mortality of acroporids in seven of their 16 sites where COTS were present. Two of their sites, Samalona and Lae-lae (referred to as P. Semolina and P. Lele in Baird et al. 2013), were included in our study. Baird et al. (2013) observed COTS activity at both islands, and they report that more than half of Samalona was affected. During our study and an earlier visit in late 2011, COTS were never present, nor was there evidence of their feeding at either site. However, both of these sites have the lowest live coral cover of the surveyed archipelago (JPJ unpublished data), suggesting unsuitable conditions for the starfish. It is not clear whether the current status of Samalona and Lae-lae is a product of the outbreak observed by Baird and colleagues or due to other confounding factors such as high sediment loads and nutrients coming from nearby rivers and the city (Edinger et al. 2000) impeding coral growth. Nonetheless, our study provides support for this phenomenon and information about the spatial and temporal patterns and impact of COTS in the Spermonde Archipelago.

Lastly, previous work has shown links between COTS outbreaks and water quality (Fabricius et al. 2010; Pratchet et al. 2014) where increased phytoplankton greatly increases the probability of COTS larvae completing development (Fabricius et al. 2010). Anthropogenic and riverine matter export from the Makassar area contribute to high particulate organic matter and chlorophyll-*a* concentrations of coastal waters of the Spermonde Archipelago (Fabricius 2005; Sawall et al. 2011), suggesting possible causation for previous and current COTS observations. Although the exact cause of COTS outbreaks remains undetermined (Pratchet et al. 2014), deteriorating water quality caused by urbanisation should forewarn managers of Indonesian coral reefs that the potential for more COTS outbreaks will be high. The effects of the COTS outbreak in Spermonde show that, within 1 year, damage to live coral cover can be severe with up to 20 % loss $month^{-1}$, and therefore future outbreaks should be monitored closely.

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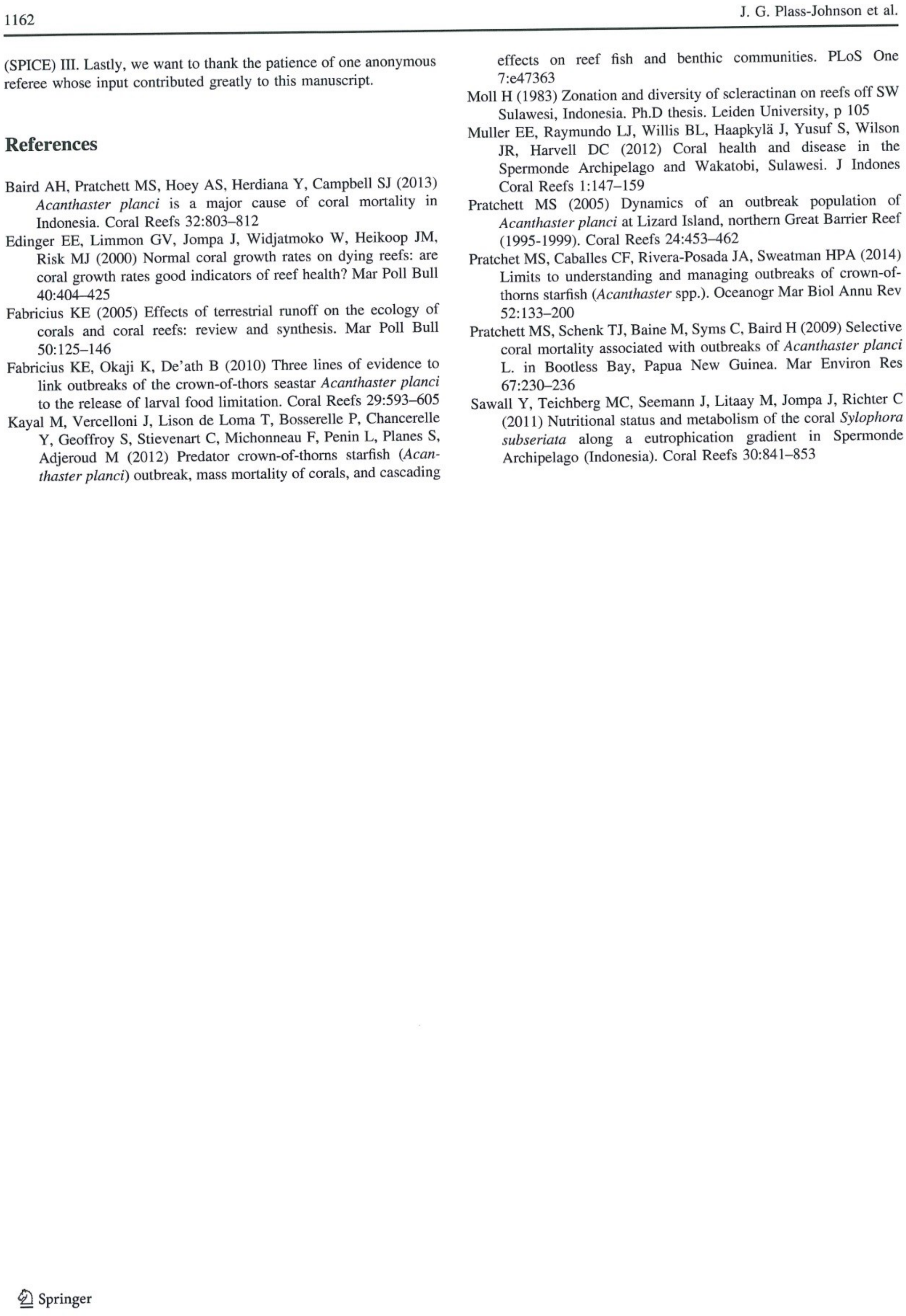

(SPICE) III. Lastly, we want to thank the patience of one anonymous referee whose input contributed greatly to this manuscript.

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DIFFERENT LEVELS OF ANTHROPOGENIC IMPACT INFLUENCE CORAL LARVAE SETTLEMENT AND BACTERIAL BIOFILM COMMUNITIES IN THE SPERMONDE ARCHIPELAGO, INDONESIA

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Abstract

Recruitment of coral larvae is one of the key factors for coral reef recovery and is determined by larval behavior in the water column as well as settlement and post-settlement survival. Larval settlement and metamorphosis rely strongly on settlement cues emitted from bacterial biofilms and their bacterial community composition (BCC). These BCC can change drastically with altered environmental conditions and in turn may affect larval settlement behavior. BCC and coral larvae settlement were investigated at three sites with increasing distance from shore, and thus different levels of human impact and water quality, in the Spermonde Archipelago, Indonesia. Coral recruitment and BCC were analyzed on natural reef substrate and artificial ceramic tiles. Bacterial communities were comprised largely of *Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria* and *Flavobacteria* and were strongly correlated with water quality. No coral recruits were found at the inshore site where the highest anthropogenic impact was observed. Recruitment at the other two sites was 0.73 ± 1.75 and 0.90 ± 1.97 recruits per 100 cm^2 at BL and BD respectively, with no significant difference between them (ANOVA; $p>0.05$). Differences in both BCC and coral recruitment were detected between natural and artificial substrates at two of the three sites, underlining that the use of settlement tiles may yield different patterns than recruitment on natural substrates, depending on each specific location and sampling time. The results demonstrate that negative anthropogenic influences on water quality affect bacterial community composition, which in turn can affect recruitment of coral larvae. This highlights the importance of taking these often neglected factors into account when evaluating the recovery potential of coral reefs.

Introduction

Scleractinian corals play an essential role in coral reef ecosystems as they provide the foundation and three-dimensional structure of the reef (Veron 2000) which is essential for reef associated species and ecosystem services (Graham and Nash 2013, Munday 2004). Coral reefs worldwide face multiple stressors (Hoegh-Guldberg et al. 2007, Burke et al. 2012), ranging from long term chronic stressors, such as increasing water temperatures, to specific destructive events, such as blast-fishing (Pet-Soede and Erdmann 1998) or severe storm damage (Harmelin-Vivien 1994). One of the key factors in the recovery of coral reefs is the sexual recruitment via coral larvae (Harrison 2011, Sawall et al. 2013).

Recruitment of coral larvae is determined by their behavior during the planktonic larval stage, as well as their settlement and post-settlement survival to the juvenile stage after building their skeleton (Keough and Downes 1982). Settlement of coral larvae depends on environmental stimuli including chemical cues emanating from biological sources that relay information about the respective habitat. For a large range of coral species crustose coralline algae (CCA) and their associated bacteria have been observed to induce settlement and metamorphosis of larvae (Heyward and Negri 1999, Price 2010, Webster et al. 2011). Several members of the genus *Pseudoalteromonas* have been shown to induce larval settlement and metamorphosis (Negri et al. 2001, Hadfield 2011, Tran and Hadfield 2011). CCA with high abundance of bacteria inhibiting coral pathogens, such as the genus *Roseobacter* (Nissimov et al. 2009), facilitated the settlement of coral larvae (Sneed et al. 2015). Due to their short generation times, bacterial community compositions in biofilms can shift rapidly with environmental conditions, subsequently affecting their ability to induce settlement of coral larvae (Bourne and Webster 2013, Webster et al. 2011).

Very little information exists on how anthropogenically altered environmental conditions affect bacterial biofilm communities in tropical marine environments (Qian et al. 2009) and on how changes will affect larval settlement. Previous authors were able to identify changing OTU numbers (Sawall et al. 2012) and altered community structures using T-RFLP fingerprinting (Qian et al. 2003). To determine the bacteria most affected by environmental changes would help greatly to further understand the interactions between BCC and larvae settlement with growing anthropogenic influence on marine ecosystems.

The current study investigated composition of bacterial biofilm communities (BCC) and settlement of coral larvae in reefs exposed to different levels of anthropogenic influence. Additionally coral recruitment and BCC were compared on natural reef substrate as well as on artificial ceramic tiles that are commonly used in settlement studies. The hypotheses were I.) that BCC and settlement of coral larvae would be different among sites, II.) that they would differ depending on set-up and orientation of artificial ceramic tiles and III.) that there would be no difference between natural and artificial substrates. This study is among the first to determine settlement of coral larvae under various environmental influences and to simultaneously investigate bacterial communities under the same conditions, using molecular sequencing methods.

Materials and Methods

Study area

The study was conducted in the Spermonde Archipelago in southern Sulawesi, Indonesia between April and June 2014. Three inhabited islands with varying distance from the city of Makassar were chosen for comparisons; Lae-Lae ("inshore", LL, approx. 1 km dist. from Makassar), Barrang Lompo ("near-shore", BL, 11 km dist) and Badi ("mid-shelf", BD, 19 km dist.). In this area water quality at the inshore site differs strongly from the other two sites, with water quality at LL being characterized by higher suspended particulate matter (SPM), Chlorophyll a concentration and phosphate levels and lower silicate and nitrite/nitrate(see also Plass-Johnson et al. 2016). Benthic community differs among the sites, with high levels of turf and macroalgae inshore and increasing coral cover and higher diversity of coral species at greater distances from the shore (see also Plass-Johnson et al. 2016). Water quality and benthic community composition determined during the present study confirmed this using the same methods as Plass-Johnson et al. (data not shown).

Natural surface analysis

To describe the natural reef substrate at each site, three 50 m transects in 3.5- 5.5 m water depth were analyzed per site. Coral recruits were counted along those transects during night dives (starting at 18:00), using fluorescence census techniques (Baird et al. 2006). All visible corals recruits (diameter 0.3 - 3 cm, presumed to be past the critical mortality phase) were counted inside a 20 x 20 cm quadrat placed within a 2 m belt from the transect (n=10 for each transect), wherever the substrate was suitable for settlement (i.e. excluding live corals or sand patches). From each transect small rocks of similar size and covered with crustose coralline algae were taken to assess the BCC by scraping the surface of each rock with a scalpel.

Artificial settlement tile analysis

At each site three metal frames containing ceramic tiles were positioned for analysis of coral larvae settlement and BCC on artificial substrates. Frames were placed at an angle of ~ 30 ° to reduce covering by sediments (English et al. 1997) and tiles were mounted in pairs, leaving a small gap of 0.5 cm (Maida et al. 1994). This resulted in four surfaces of tiles; an upper and a lower tile either facing up or down, to be analyzed after 8 weeks in the reef. Skeletons of coral recruits (>0.3 cm) were counted on all surfaces of four tile pairs from each frame after bleaching and drying and identified to the family level following Babcock et al. (2003). BCC was analyzed on all four surfaces of one tile pair from each frame by scraping a 1 cm wide patch with a scalpel.

Bacterial community analysis

Immediately after sampling organic material scraped from natural and artificial surfaces was stored in ~1.5 mL of "RNA later" (following Ambion, Texas, USA) at -20 °C until further analysis. DNA was extracted from these samples using the PowerSoil® isolation Kit from MoBio (www.mobio.com). Sequences of the V3-V4 hypervariable region of the 16S rRNA gene were obtained from paired-end Illumina MiSeq amplicon sequencing at LGC Genomics (Berlin, Germany).

Data analysis

Processing of Illumina sequences and statistical analysis of all data was performed in R (R v.3.0.2 using R Studio v.0.98.1056). Depending on the data, different statistical tests were performed: ANOVA was used to assess differences in recruit numbers on natural substrates, generalized linear mixed model analysis with the AD Model Builder platform (R package "glmmADMB") for differences in recruit numbers on artificial tiles, followed by a Kruskal Wallis test with multiple pairwise comparisons (functions kruskal.test and kruskalmc from R package "pgirmess"), and PERMANOVA (via adonis function from R package "vegan") was used for BCC comparisons.

Results

Coral recruitment

No coral recruits were found at the inshore site (LL). At the other two sites counts of coral recruits on natural substrate were 0.73 ± 1.75 per 100 cm^2 at BL and 0.90 ± 1.97 per 100 cm^2 at BD (mean ± SD). There was no significant difference in recruitment on natural substrate between both sites.

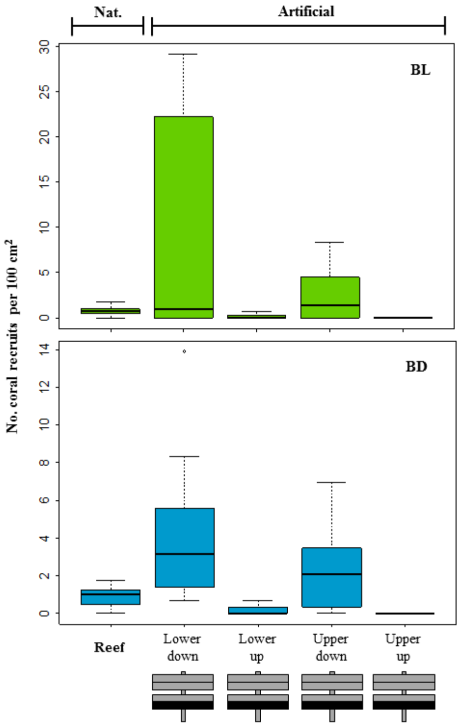

Fig. 1 – Coral recruitment on natural and artificial substrates. Upper panel shows recruit numbers at mid-shore Barrang Lompo (BL), lower panel recruit numbers at mid-shelf Badi (BD). The first bar in each graph is the number of recruits on natural reef substrate, the other four show the different surfaces of artificial ceramic tiles with an upper and lower tile either facing down or up. Letters indicate significance results from Kruskal Wallis multiple pairwise comparisons performed for each site separately.

Similarly, there was no significant difference in recruitment on settlement tiles between the near-shore (BL) and the mid-shelf site (BD), but among the tile surfaces (glmm, *p* <0.001 for surface effects). Significant differences in coral recruit counts were found among all surfaces except in the two surfaces facing down and the two surfaces facing up (Kruskal Wallis test, *p*= 7.675 e-09 and multiple post-hoc comparisons, see Fig. 1). Most coral larvae settled on downward-facing tiles with 6.45 ± 9.03 recruits per 100 cm^2 on the lower tiles and 2.31 ± 2.45 per cm^2 on the upper tiles. No larvae settled on the exposed surface of upper tiles and only 0.17 ± 0.30 per 100 cm^2 on upward-facing surface of the lower tiles. Within sites there was a high variation in recruit numbers among tile surfaces frames.

Differences in coral recruitment between substrates

There was no difference in recruitment between the two substrate types at the near-shore site (BL), while a significant difference was found at the mid-shelf site (BD, linear mixed effect model analysis). At this site higher numbers of recruits were found on the artificial tiles than in the reef, with significant differences between the transects and the surfaces on the lower tile (downward-facing *p*=0.030 and upward-facing *p*=0.001).

BCC

There were significant differences in BCC at the class level on natural substrate among the three sites (PERMANOVA, R^2= 0.298, *p*= 0.005). The most abundant bacteria classes on natural substrate were *Alphaproteobacteria, Cyanobacteria, Gammaproteobacteria* and *Flavobacteria* (together ~80 % abundance, Fig. 2). While at the inshore site (LL) a very high abundance of *Cyanobacteria* was found, the relative abundance of *Alphaproteobacteria* was increasing with distance from shore and was highest at mid-shelf (BD). BCC on natural substrate was strongly correlated to the water quality in terms of the measured water parameters (Fig. 3), which differed among the sites (mainly between the inshore and the other two sites). Bacteria genera relevant for corals, such as *Pseudoalteromonas* and *Roseobacter*, and potentially harmful bacteria like *Oscillaoria, Desulfovibrio* and *Phormidium* were related to the water quality as well.

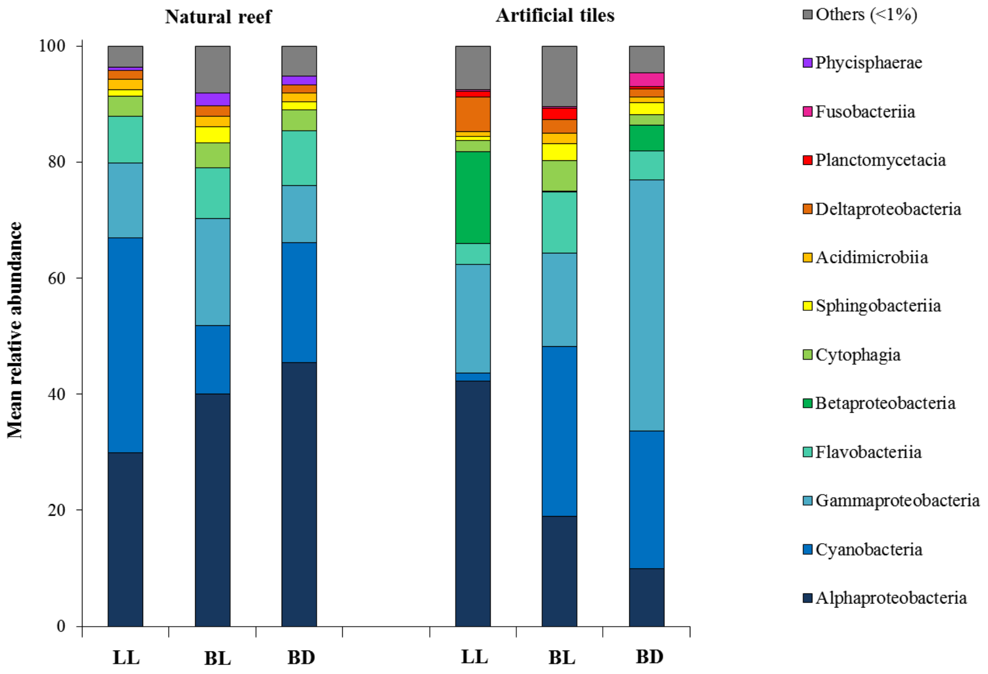

Fig. 2 - Mean relative abundance of classes of bacterial communities on natural reef substrate (left) and artificial ceramic tiles (right). The three sites are presented with increasing distance from shore from left to right; Lae-Lae (LL), Barrang Lompo (BL) and Badi (BD).

On artificial ceramic tiles, the same groups were found to be most abundant as on natural substrate, i.e. *Alphaproteobacteria, Cyanobacteria, Gammaproteobacteria* and *Flavobacteria* (Fig. 2). At the inshore site (LL) there was a very low abundance of *Cyanobacteria* and a high abundance of *Alphaproteobacteria*, as well as of *Betaproteobacteria*. The highest number of *Gammaproteobacteria* on artificial surfaces was detected mid-shelf (BD).

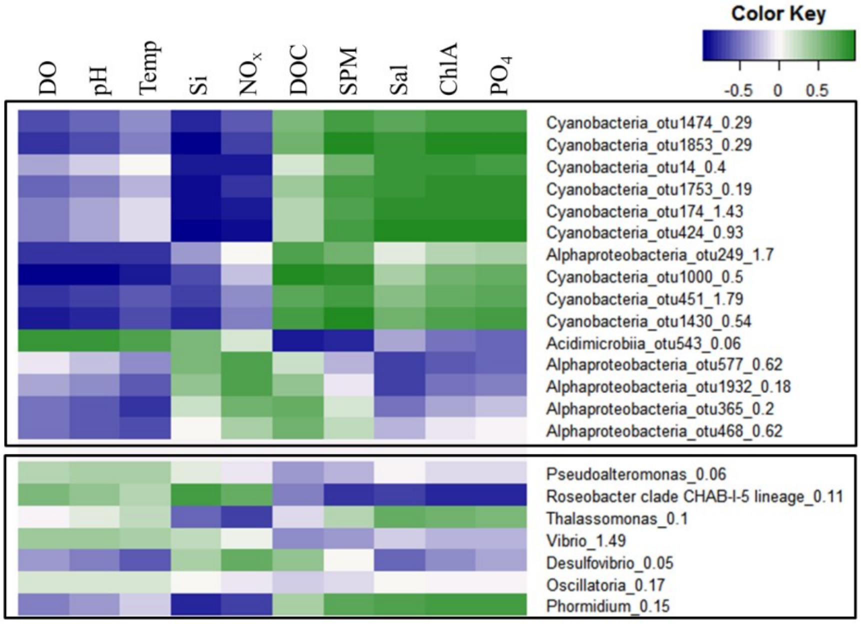

Fig. 3 – Heatmap correlating bacteria OTUs on natural substrate to the water parameters. The top panel shows the 15 most abundant OTUs labeled with their class level, OTU number and mean relative abundance in all samples. The lower panel shows seven bacteria genera relevant for coral recruits (*Pseudoalteromonas*: settlement inducing, *Roseobacter*: pathogen- inhibiting, *Thalassomonas* and *Vibrio*: some settlement inducing and some pathogenic strains, *Desulfovibrio*, *Oscillatoria* and *Phormidium*: coral pathogens) labeled with the mean relative abundance in all samples. Water parameters are dissolved oxygen (DO), pH, temperature (Temp), silicate (Si), nitrite/nitrate (NO_x), dissolved organic carbon (DOC), suspended particulate matter (SPM), salinity (Sal), Chlorophyll *a* (ChlA) and phosphate (PO_4).

Differences in BCC between substrates

BCC differed significantly between artificial and natural substrates at two of the three sites (PERMANOVA, BD: R^2=0.418, *p*=0.001, LL: R^2=0.553, *p*=0.002), while at BL there was no significant difference. The largest differences between the substrates at the inshore and mid-shelf sites were due to different abundances of the most abundant groups (*Alphaproteobacteria, Cyanobacteria* and *Gammaproteobacteria*), as well as the appearance of *Betaproteobacteria* on the artificial substrate, while it had less than 1 % abundance on natural substrate.

Discussion

This study showed differences in BCC and coral recruitment only between the inshore site and those further away, which was mirroring differences in the water quality at those sites. BCC and coral recruitment depended on the set-up and orientation of the artificial settlement tiles, with the largest differences being between the tiles facing up and those facing down. At two of the sites the analysis of coral recruitment and BCC revealed differences between natural and artificial substrates.

Recruitment and BCC

No recruitment was observed on either substrate at the site with the highest anthropogenic impact (LL). Although recruitment of larvae was recorded there five years previously on settlement tiles (Sawall et al. 2013) and live coral colonies were found in this area (~6.3 % live coral cover, data not shown), recruitment seems to either have ceased since then, or to be sporadic, indicating threatened viability of these reefs in the future.

Recruitment on natural substrate at BL and BD was slightly lower than recruitment recorded previously in the area with 1.46 ± 0.50 spat per 100 cm^2 over a 3 month period (Sawall et al. 2013) and was similar to other regions (see Glassom et al. 2004 for an overview).

There were significant differences between the BCC at the sampling sites, but in general the communities were similar to those found in coral reef sediments at the Great Barrier Reef, where many Proteobacteria were found in addition to *Cyanobacteria* and several other classes (Uthicke and McGuire 2007). While the bacterial communities at the near-shore (BL) and mid-shelf (BD) islands were similar, the community at the inshore site (LL) differed strongly from them. This was mainly due to a high abundance of *Cyanobacteria* and lower abundance of *Alphaproteobacteria* at the inshore site (LL). While *Cyanobacteria* are often an indicator for bad water quality and eutrophic conditions (Agawina et al. 2003, Paerl et al. 2011) and may inhibit coral recruitment (Carpy et al. 2011), *Alphaproteobacteria* are often associated with more oligotrophic systems (Yin et al. 2013) and were found to be dominant on CCA surfaces (Webster et al. 2011). The difference in BCC between the inshore site and the others was similar to the trend in water quality parameters, which were also mainly different between inshore and sites further out. BCC was correlated with site-specific characteristics of water quality, similar to findings on BCC in sediments in the same area (Polónia et al. 2015). Detrimental terrestrial influences on water quality in the Spermonde Archipelago are more marked during the wet season (Sawall et al. 2012), thus even stronger effects of water quality on BCC would be expected at other sampling times.

The bacterial genus most often recorded to induce settlement of coral larvae, *Pseudoalteromonas* (Negri et al. 2001, Hadfield 2011, Tran and Hadfield 2011), was detected most on settlement tiles at the inshore site (LL). In contrast, bacteria of the genus *Roseobacter*, which have inhibitory properties against several coral pathogens (Nissimov et al. 2009), were found only at the near-shore (BL) and mid-shelf (BD) reefs. To further determine the role of these bacteria in the settlement of coral larvae, an even closer look at these groups would be necessary.

The numbers of coral recruitment on artificial settlement tiles is comparable to those in other studies from Sulawesi (Ferse et al. 2013, Sawall et al. 2013). A clear spatial settlement pattern on the artificial substrate was observed, where most recruits settled on the downward-facing tile surfaces. This has been observed in other studies as well (Maida et al. 1994, Sawall et al. 2013) and is most likely caused by higher light intensities and sedimentation rates on the exposed upper sides.

On the artificial substrates the same bacteria groups were found to be most abundant as on the natural substrate. However, there was a surprisingly high abundance of *Alphaproteobacteria* inshore and a high abundance of *Gammaproteobacteria* mid-shelf, which was in contrast to the abundances of these groups on natural substrate.

Difference in recruitment and BCC between substrates

Previous studies found no differences in coral recruitment between natural reef substrate and artificial settlement substrates (Salinas-de-León et al. 2011). During this study at least at one of the sites with observed recruitment (at BD) a significant difference between the two substrates was detected, with a higher number of recruits counted on the natural reef surface. Although there are no clear reports for coral spawning times in Spermonde (Sawall et al. 2013), strong indications are that it occurs between February and April (Salinas de-León et al. 2013, Yusuf et al. 2013), i.e. slightly before our sampling period. Thus larvae might have already settled onto the reef while there was a lower abundance of larvae in the surrounding waters during the time of tile deployment. However that does not fully explain why differences were only found at one of the sites. Differences in larvae recruitment depending on substrate type have been reported for several artificial substrates (Baird et al. 2003, Burt et al. 2009) and the microstructure of substrates can increase spat survival (Nozawa 2008). Such factors should be taken into account to determine the suitability of settlement tiles used in providing similar conditions for recruitment as natural reef substrate, but neither fully explain the observed differences between the substrates. Another possibility remain differences in BCC, since at BD, where differences in recruitment were observed, there were also significant differences in BCC between artificial and natural substrate (similar at LL, however as at LL no coral recruitment was observed on either substrate, the implications of these differences in BCC for coral recruitment cannot be assessed). At the near-shore site (BL) no difference in BCC between the natural and artificial substrate was found, which is similar to the finding that at this site there was also no difference in coral recruitment on both substrates.

This study showed that at sites with the combined impact of unfavorable water quality and low coral cover, settlement of coral larvae can be significantly impaired. At sites with differences in BCC, differences in coral recruitment were found at the same time. As BCC are known to change quickly in response to changing environmental conditions, in particular water quality, this could be an indicator for a potential knock-on effect on coral larvae settlement. This study thus supports the conclusion from previous studies that BCC should be further investigated in connection with coral recruitment. However, while the present study provided an indication of a potential link between water quality, BCC and coral recruitment on different substrates, the study design does not permit conclusive evidence for such links. Further studies are needed that replicate sites of similar water quality in addition to sampling along a gradient in environmental conditions, and experimental manipulation of BCC in relation to coral settlement is needed to further assess the link between the two.

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