Session 16

Larval recruitment on coral reefs facing global change

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


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 cm² 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.

Keywords: coral recruitment, coral reefs, settlement tiles, 16S rRNA Illumina sequencing, water quality
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-Laе (“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 coral 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 $0.73 \pm 1.75$ per 100 cm$^2$ at BL and $0.90 \pm 1.97$ per 100 cm$^2$ at BD (mean $\pm$ SD). There was no significant difference in recruitment on natural substrate between both sites. 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, $p = 7.675 \times 10^{-9}$ and multiple post-hoc comparisons, see Fig. 1). Most coral larvae settled on downward-facing tiles with $6.45 \pm 9.03$ recruits per 100 cm$^2$ on the lower tiles and $2.31 \pm 2.45$ per cm$^2$ on the upper tiles. No larvae settled on the exposed surface of upper tiles and only $0.17 \pm 0.30$ 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 $\sim 80\%$ 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 Oscillatoria, Desulfovibrio and Phormidium were related to the water quality as well.
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.

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).

**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.

![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).](image)

**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.

![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 (NO₃), dissolved organic carbon (DOC), suspended particulate matter (SPM), salinity (Sal), Chlorophyll a (ChlA) and phosphate (PO₄).](image)
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 cm² 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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References


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