RESEARCH PROPOSAL FOR CORAL REEF RESEARCH IN FRENCH POLYNESIA 2012
INSTITUTE FOR PACIFIC CORAL REEFS (IRCP) – ROBERT WAN TAHITI PERLES

# BACTERIAL DIVERSITY AND METABOLISM IN TROPICAL CORAL REEF BIOFILMS AND SPONGES

Applicant: Verena Witt, Ludwig-Maximilians University (LMU) Munich, Germany
Department of Earth and Environmental Sciences, Palaeontology and Geobiology,
Molecular Geo- and Palaeobiology Lab

## Project Summary

Water quality in coastal regions around the world is declining in response to global (i.e., ocean warming and acidification) and local (i.e., land-based activities) anthropogenic impacts. Terrestrial runoff leads to decreased salinity, increased nutrient and reduced light availability to nearshore coral reefs with adverse effects. Bacterial communities associated with biofilms and sponges shift rapidly in response to changes in water quality and hence may serve as bioindicators for reef health. However, the potential of microbial bioindicators in reef organisms and whether microbial community shifts also alter community functioning remain unexplored. This study will therefore aim to identify microbial target indicator species, investigate the recovery/adaption potential of biofilm and sponge-associated microbes and determine potential consequent changes in organism functioning. Biofilms will be established on artificial substrates for 30 d in aquaria and sponges will be collected in situ along a water quality gradient comprising of inshore and offshore sites. Biofilms and sponges will then be exposed to inshore coastal sites highly exposed to terrestrial runoff (low light, high nutrient availability) and more pristine sites (high light, low nutrient availability). Transplant experiments will be performed with biofilm and sponge replicates (transplanted from impacted to pristine and vice versa) and sampled after 3 and 10 d exposure to the new site. 454 Tag-pyrosequencing will provide in-depth insight into the bacterial community composition and diversity. A metagenomic approach and direct measurement of biogeochemical $O_2$ fluxes (dark/light incubations/luminescent optode) will reveal whether community shifts also alter the function of whole bacterial communities in biofilms and sponges. Findings

may contribute to understanding consequences of terrestrial runoff on ecosystem functioning and resilience, and to the application of microbial biofilms/sponges in long-term water quality monitoring programmes and future coastal management.

## Introduction

Water quality in coastal regions worldwide is declining in response to global (i.e., ocean warming and acidification) and local (i.e., land-based activities) anthropogenic impacts. In particular during the wet season, rivers import large amounts of nutrients, sediments and freshwater onto coastal tropical waters. Terrestrial runoff leading to eutrophication, reduced light availability and decreased salinity, consequently deteriorates water quality of coastal tropical coral reefs (Wilkinson, 1999). Coastal development and land-clearing to facilitate agriculture destroy natural barriers thus promote greater impacts of terrestrial runoff and flood events during the wet season on vulnerable coral reef communities in South-East Asia (Cruz*, et al.*, 2007), the Great Barrier Reef (Fabricius, 2005), Hawaii and French Polynesia (Chin*, et al.*, 2011). Recent reports document significant alterations in the composition of benthic communities in response to degrading water quality e.g., on the Great Barrier Reef (GBR) including corals (Fabricius 2005, Fabricius et al. 2007(Fabricius, 2005, Fabricius*, et al.*, 2007, Haapkyla*, et al.*, 2011, Weber*, et al.*, 2012), algae (Schaffelke, 2005), foraminifers (Uthicke & Altenrath, 2010), sediments (Uthicke & McGuire, 2007) and bacterial biofilms (Kriwy & Uthicke, 2011, Witt*, et al.*, 2011, Witt*, et al.*, 2012). Another region of concern is French Polynesia in the Pacific, where land use farming practices result in terrestrial runoff from fertilizer (pineapple farming) and livestock waste (piggeries) importing nutrients and pollutants onto coastal coral reefs thus altering benthic community structure (Adjeroud, 1997, Chin*, et al.*, 2011).

Terrestrial runoff has recently been discovered to alter microbial communities associated with corals with negative impacts on the host's health (Haapkyla*, et al.*, 2011, Weber*, et al.*, 2012), thus intensifying the research focus on marine microbes. Marine bacteria are the first line of defence in coastal ecosystems due to their potential to mitigate the detrimental effects of certain anthropogenic pollutants, through degradation and transformation of harmful compounds. Marine bacteria predominantly organize themselves in surface-attached biofilm communities that play

essential roles in invertebrate larval settlement (Wieczorek & Todd, 1998) and nutrient turnover (Battin*, et al.*, 2003). As these communities are highly responsive indicators of changing environmental conditions as a consequence of their rapid life cycle (Paerl & Pinckney, 1996) and ability to structurally self-organize (Tolker-Nielsen & Molin, 2000), bacterial biofilms have been tested for their potential as indicators for water quality conditions in riverine (Araya*, et al.*, 2003), estuarine (Snyder*, et al.*, 2005, Moss*, et al.*, 2006, Jones*, et al.*, 2007, Nocker*, et al.*, 2007), polar (Webster & Negri, 2006) and marine (Dang*, et al.*, 2008) systems. Despite the importance of biofilm communities regarding coral reef resilience and functioning, only few studies have dealt with microbial biofilm bioindicators in coral reef ecosystems. These report that bacterial community composition in biofilms shift in response to local anthropogenic nutrient and consequent light impacts (Meyer-Reil & Koster, 2000, Nocker*, et al.*, 2007, Kriwy & Uthicke, 2011, Witt*, et al.*, 2011, Witt*, et al.*, 2012), increased sea surface temperatures (Witt*, et al.*, 2011, Witt*, et al.*, 2012) and ocean acidification (Witt*, et al.*, 2011). However, in-depth studies on microbial target indicator species and effects of anthropogenic impacts on biofilm functioning, to date, have been ignored.

Apart from biofilm communities, microbes also occur as symbionts in invertebrate hosts such as sponges. Coupled with their high diversity, sponge microorganisms are involved in important biogeochemical processes including nitrification and denitrification (Schläppy*, et al.*, 2010). It has been demonstrated that environmental stressors such as increased temperature (Webster*, et al.*, 2008) and heavy metal exposure (Webster*, et al.*, 2001, Selvin*, et al.*, 2009) cause shifts in typically stable microbial communities with cascading effects on the sponge host health. Contrastingly, recent reports observed no shifts in sponge-associated microbial communities in response to changes in light availability (Thoms*, et al.*, 2003) or increased sedimentation (Luter*, et al.*, 2012). Sponge-associated bacteria are sensitive and hence may be suitable bioindicators of changing environmental conditions. Nevertheless, there is a conspicuous absence of knowledge on the effects of terrestrial runoff (nutrient input) on the symbiotic partnerships between microbes and sponges, and their potential as bacterial bioindicators for water quality.

*Previous own work*

Previous own work identified that increased water quality parameters (in particular elevated Chl *a* and DOC concentrations) during the wet season shift bacterial community composition in field-grown biofilms showing changes in relative abundances of bacterial functional groups (Witt*, et al.*, 2012). For example, bacterial biofilms displayed an increased abundance of *Flavobacteria* and decrease in *Roseobacter* clade members when exposed to nutrient- (Witt*, et al.*, 2012) (Witt*, et al.*, 2012), thermal- (Webster*, et al.*, 2011, Witt*, et al.*, 2012) and acidification (Witt*, et al.*, 2011) stress. Further, bacterial community shifts have lead to changes in $O_2$ fluxes in response to nitrate, temperature and light (Witt*, et al.*, 2012), but not to ocean acidification (Witt*, et al.*, 2011). Further, GBR *in situ* inshore and offshore scenarios during the wet and dry seasons were simulated in aquaria by manipulating combinations of the factors temperature (26, 29, 31°C) nitrate (0.5, 1.0, 1.4 µM) and light (40 and 200 µmol photons $m^{-2}s^{-1}$). Biofilms in simulated 'offshore' conditions became nutrient-limited, while 'inshore' biofilms became light-limited. Consequently it was hypothesised that at inshore reefs, light reduction, through sediment input from runoff, has a stronger influence on triggering bacterial community shifts with a concomitant reduction in $O_2$ fluxes than increased nutrient input (Witt*, et al.*, 2012).

Despite the importance of bacterial communities, the knowledge of abiotic factors controlling the growth of specific functional groups within coral reef-associated biofilms and sponges and whether microbial community shifts consequently affect biofilm and sponge functioning (sponge larval settlement, biogeochemical processes, sponge diseases) is presently very limited, but of great concern from a management aspect. Hence, it is important to investigate the diversity and function of bacteria in order to investigate their potential as bioindicators and furthermore to understand the resilience of coral reef ecosystems.

Marine pollution is a global concern and most developing nations continue to pollute the oceans at increasing rates. Coral reefs are threatenend by human impacts such as coastal development and land-based pollution, in parallel with coral bleaching and crown-of-thorns outbreaks. Coral reefs in Australia, Hawaii and French Polynesia are

the best monitored reefs: management and conservation strategies include long-term monitoring, creating local regulations and zoning, and addressing land-based threats. Although much financial and research effort is invested, for example, in monitoring and conserving the GBR in Australia, the reef degrades at rapid rates and there is still a need for bioindicator research and coastal management improvement (De'ath*, et al.*, 2012). For this reason, knowledge from previous own work from the GBR will be extended to the French Polynesian region (Moorea). Understanding qualitative and metabolic responses of biofilms to terrestrial runoff will contribute importantly to understanding coral reef ecosystems and further, may contribute to future coastal management which may be readily adaptable to other tropical regions.

**Project description**

The project therefore aims to investigate responses of biofilms and sponges along a water quality gradient with respect to bacterial composition, diversity and function (metabolic and gene expression) on a spatial scale (changes in nutrient- and light availability and salinity caused by terrestrial runoff from flooding events). For this purpose, *in situ* transplant experiments will be performed on biofilms established on artificial substrates in aquaria and sponges collected from the field. Both specimens will be deployed along a water quality gradient comprising of sites in the bay highly exposed to terrestrial runoff (low light, high nutrient availability) and more pristine sites (high light, low nutrient availability). These will then be transplanted from impacted to pristine locations and vice versa. Sampled biofilms will subsequently be analysed for bacterial diversity, to identify indicator species (rare or abundant species) using molecular tools (DGGE, 454-pyrosequencing). Diversity data will be combined with measurements of $O_2$ fluxes to determine whether community diversity shifts alter metabolic functioning in response to environmental stressors (e.g., terrestrial runoff). Additionally, a metagenomic approach may further enable evaluating biofilm and sponge function. This study aims to reveal resilience of bacteria, meaning to what extent biofilm communities retain and/or adapt their composition, diversity and/ or function from their original environment to the new environment. Further, it is investigated whether community shifts also alter biofilm functioning and finally to identify key bacterial species used as bioindicators of coral reef health. Detecting changes in biodiversity and understanding consequent

changes in metabolic activity of bacterial biofilms and sponges affected by terrestrial runoff may contribute importantly to future coastal management. Biofilms may serve as bioindicators for water quality on coral reefs and on a greater ecological scale, bacterial biofilms affect invertebrate larval settlement, and may ultimately also alter coral reef recruitment and resilience. Knowledge on bacterial biofilms may therefore add to the development of monitoring impacts and conservation planning.

*Methdology*

Replicate glass microscope substrates (Witt*, et al.*, 2011) fixed into custom-made holders will be incubated in aquaria for 4 weeks prior to arrival (substrates and holders will be sent to research station for pre-incubation prior to arrival, will occupy 2 aquaria) to enable biofilm formation. Biofilms will be deployed along a water quality gradient at the north end of Moorea comprising of 2 sites near the bay head exposed to terrestrial runoff (e.g., Cook's (Paopao) Bay) and 2 pristine sites (sites away from the bay entrance to the ocean end). Star pickets wrapped in plastic foil (to minimize metal contamination) will be deployed into the reef sediment at 3 – 5 m depth and substrate holders will be fastened to the pickets with cable ties. One or more of the following sponges will be collected (to be determined after on-site inspection) (Class Demospongiae): *Neopetrosa sp.* 'blue' is an abundant species and has proven transplantation success (Schiefenhövel & Kunzmann, 2012); Golf-ball sponges: *Cinachyrella sp.* and *Paratetilla sp.* found at shallow depths and turbid waters (Putchakarn, 2007)). All chosen sponges are common so that gained results can also be used in future comparative studies with the same genera from other locations from the Indo-Pacific-Region. All sites will be equipped with light loggers, temperature and salinity data will be provided by LTER and water samples from each site will be taken for nutrient analysis to provide water quality background data (chlorophyll, DOC, DIN etc.). The fieldwork is preferably carried out shortly after the wet season when runoff more intense.

Biofilm control triplicates will be sampled at T=0 before being exposed to the field. The remaining replicates (9) will be deployed at selected field sites. After 3 d (T=1) control replicate biofilms (3) will be sampled, 3 replicates will be transplanted from each site (from impacted to pristine sites and vice versa), 3 control replicates will

remain for final sampling event. After 7 d of exposure to the new site, final sampling will occur at 10 d (T=2). One half of the samples will be sampled for further molecular analyses as described in (Witt*, et al.*, 2011) to be carried out at the LMU Faculty of Geosciences in Germany. DNA extraction will be carried out in the laboratory of the CRIOBE research station. The other half of the replicate samples from each sampling point will be determined for $O_2$ fluxes at the CRIOBE research station.

| Location | Sampling points | Analysis | Inshore | Offshore |
|---|---|---|---|---|
| Replicate sites | | | 2 | 2 |
| Biofilm and sponge | control before | Molecular | 3 | 3 |
| sample replicates | transplant (T=0) | $O_2$ | 3 | 3 |
| | transplanted (T=1) | Molecular | 3 | 3 |
| | | $O_2$ | 3 | 3 |
| | control at site of | Molecular | 3 | 3 |
| | origin (T=2) | $O_2$ | 3 | 3 |
| Total 72 | | | 36 | 36 |

*$O_2$ analyses*

Net photosynthesis ($P_{net}$) and dark respiration (R) measurements will be carried out in sealed glass chambers filled with seawater. Each organism (n = 3) will be incubated in a separate glass container with seawater. After transferring the organisms into the chambers, the initial dissolved oxygen concentrations ($t_0$) will be determined the glasses will be placed in flow-through aquaria to ensure constant ambient temperature conditions during the incubation procedure incubated for 24 h in dark- and light conditions. At the end of the incubation ($t_1$), the oxygen concentration in the glasses will be measured. According to $P_{net} = O_2 * (t_1) – O_2 * (t_0)$, the net photosynthesis will be determined from the oxygen concentrations at the beginning and end of each incubation. Immediately following the light incubation, the same fragment will be incubated again in darkened, in PVC-foil wrapped, glass chambers to measure the dark respiration. The oxygen consumption will be determined according to the formula $R = O_2 * (t_1) – O_2 * (t_0)$. With the data from net photosynthesis and dark respiration, the gross photosynthesis ($P_{gross}$) rates can be determined, according to $P_{gross} = P_{net} + R$. In addition to both incubations, seawater-

control chambers (without organisms) will be incubated in the light and in the dark to measure the planktonic metabolism in the water. The seawater-control values (without organisms) will then be subtracted from the $P_{net}$ and R values (with organisms) to receive oxygen production or consumption from the incubated organism only. The dissolved oxygen concentrations will be determined using a Luminescent Dissolved Oxygen Sensor (HAC HQ10) and normalized to the chamber volume. These $O_2$-fluxes will be related to the surface area and biomass of the organisms. Biofilms and sponges will be sampled on GF/F filters for chlorophyll *a* and carbon and nitrogen elemental analyses according to protocols specified in Witt et al. 2011b and 2012a (measurements carried out in the lab at the LMU Faculty of Geosciences in Germany).

*Molecular analyses*

Biofilms and sponges sampled for molecular analyses will be determined for bacterial community composition and diversity from the transplanted sites and sites of origin. DNA will be extracted and purified using MoBio Biofilm DNA extraction and purification kits. The samples will then be screened using the fingerprinting method of denaturing gradient gel electrophoresis (DGGE). Differences in community composition will be a selection criterium for which samples (with replicates) will further be analysed for diversity determined using 454-Tag pyrosequencing (Illumina at the LMU Faculty of Biology Martinsried, Munich, Germany). Depending on whether changes in bacterial community composition, diversity and $O_2$ production are significantly different will determine which samples will further be analysed using a metagenomic approach to provide insight into the structure and function of whole microbial communities.

Retrieved sequences will be trimmed and aligned using the CLC Genomics and Geneious (Kearse*, et al.*, 2012) platforms available in Munich. Diversity analyses including rarefaction curves, calculating diversity and eveness indices, LIBshuff statistical analysis etc. will be generated using MOTHUR (Schloss & Handelsman, 2008) and multivariate statistics (Analysis of Similarity, Permutational Analysis of Variance, Principal Component Analysis, distance-based Redundancy Analysis) will be performed using PAST. Metagenome sequence data will be processed using two

fully automated open source systems: (1) the MG-RAST v3.0 pipeline (http://metagenomics.anl.gov) (Meyer*, et al.*, 2008) and (2) the Rapid Analysis of Multiple Metagenomes with a Clustering and Annotation Pipeline (RAMMCAP) (Li, 2009), available from the Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA, http://camera.calit2.net). Taxonomic relationships between metagenomes will be analyzed by two complementary analyses using the MG-RAST pipeline.

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## Cost plan

| Item | Details | Amount (€) |
|---|---|---|
| 1. Travel | | |
| International | Return flight Munich – Tahiti | 1900 |
| Domestic | Faa'a<br>Return ferry transit Tahiti – Moorea | 50 |
| 2. Bench Fees | Boat hire, dive gear rent (4 trips)<br>Accommodation (16 days à € 40) | 640 |
| 3. Research consumables | DNA extraction and purification kits | 250 |
| | Chemicals for preservation, ethanol, gloves, pipette tips, cryovial tubes, razor blades, safety disposal containers, microfuge tubes, cable ties, zip-lock bags, star pickets, duck-tape | 200<br>130 |
| | Custom-made holders | 100 |

| | |
|---|---|
| | 1200 |
| Incubation chambers/vials | |
| Sequencing costs | |
| Total | **4470** |

254
255
256

# Curriculum Vitae

**Dr. rer. nat. Verena Witt**
Ludwig-Maximilians University
Department of Earth and Environmental Sciences, Palaeontology and Geobiology
Richard-Wagner-Str. 10 80333 Munich, Germany
Tel.: +49 (0) 89 2180 6615
Fax: +49 (0) 89 2180 6601
Email: v.witt@lrz.uni-muenchen.de

## Scientific Interests

- General Marine Sciences, Biology and Microbiology
- Microbial Ecology and Biogeochemistry
- Genomics
- Effects of climate change and pollution on microbial biofilms and sponges

## Education and Research Activities

08/2008 – 05/2012 **Doctor of Natural Sciences (Dr. rer. nat.)**
magna cum laude
Specialization: Marine Microbiology, Ecology, Molecular Genetics
University of Bremen, Bremen, Germany
Faculty of Biology/Chemistry

03/2011 – 05/2012 based at the Leibniz Center for Tropical Marine Ecology (ZMT)
Coral Reef Ecology (CORE)Prof. Dr. Christian Wild

07/2008 - 03/2011 based at Australian Institute of Marine Science, Townsville, Australia, Water Quality and Ecosystem Health Team
Dr. Sven Uthicke

10/ 2007- 02/2008 **Research Assistant**
Max-Planck-Institute for Marine Microbiology, Bremen, Germany
Microbial Habitat Group, Prof. Dr. Antje Boetius

07-09/ 2007 **Research Assistant**
GeoBio-Center of the Ludwig-Maximilians University (LMU), Munich, Germany, Coral Reef Ecology (CORE)
Prof. Dr. Christian Wild

07/ 2006- 04/2007 **Diploma Degree in Biology**
Ludwig-Maximilians University (LMU), Munich, Germany
Department of Biology II
Specialization: Human Genetics, Cell Biology and Biochemistry
Prof. Dr. med. Thomas Cremer; PD Dr. Stefan Müller

2001 - 2006 **Biology studies**
Ludwig-Maximilian University (LMU), Munich, Germany

## Publications

**Witt V**, Wild C and Uthicke S (2012)
*Terrestrial runoff controls bacterial community composition of biofilms along a water quality gradient in the Great Barrier Reef*
*Applied and Environmental Microbiology* **78**:7786

**Witt V**, Wild C and Uthicke S (2012)
*Interactive climate change and runoff effects alter $O_2$ fluxes and bacterial community composition of coastal biofilms from the Great Barrier Reef*
*Aquatic Microbial Ecology* **66**:117-131

**Witt V**, Wild C, Anthony KRN, Diaz-Pulido G and Uthicke S (2011)
*Effects of ocean acidification on oxygen fluxes through, and microbial community composition of biofilms from the Great Barrier Reef*.
Environmental Microbiology **13**:2976-89

**Witt V**, Wild C and Uthicke S (2011)
*The effect of substrate type on bacterial community composition in biofilms from the Great Barrier Reef*.
*FEMS* Microbiology Letters **323**:188–195

**Witt V,** (2012)
*Effects of disturbances on microbial community composition and activity in biofilms from the Great Barrier Reef*
PhD Thesis Department of Biology, Universität Bremen

**Witt V,** (2007)
*Nuclear Topology of the Bcl11a locus and flanking ultra-conserved sequences in vertebrate development*
Diploma thesis Department of Biology II LMU

## Presentations

*Oral presentations*

**Witt V**, Wild C, Anthony KRN, Diaz-Pulido G, Uthicke S (2011)
*The effects of ocean acidification on microbial diversity and activity in biofilms from the Great Barrier Reef.*
13 th International Symposium on Microbial Ecology (ISME), 22 - 27 August 2010, Seattle, WA, USA. Abstract book: CT10.008

**Witt V**, Wild C, Uthicke S (2010)
*Biofilms as indicators of water quality: changes in microbial diversity and activity in coastal biofilms along a water quality gradient in the Great Barrier Reef.*
Annual Conference of the Australian Government's Marine and Tropical Sciences Research Facility (MTSRF), 18 - 20 May 2010, Cairns, Australia

**Witt V** (AIMS/CORE)
*Diversity and function of biofilm bacteria as indicators for water quality in the Australian Great Barrier Reef*

2nd CORE Mini-Symposium on Biogeochemical Coral Reef Research 2nd May 2008 Munich, Germany

**Witt V** (AIMS/CORE)
*Diversity and activity of microbial biofilm communities in the Great Barrier Reef*
BWG Workshop on Coral reefs and Climate Change 18-30 May 2009 Heron Island, Australia

*Poster presentations*

**Witt V,** Wild C and Uthicke S (2012)
*Interactive climate change and runoff consequences alter $O_2$ fluxes and bacterial community composition of coastal biofilms from the Great Barrier Reef*
14th International Symposium on Microbial Ecology (ISME) 19 - 24 August 2012 Copenhagen, Denmark. Abstract book: PS03 347A P.28

**Witt V**, Wild C, Anthony KRN, Diaz-Pulido G, Uthicke S (2011)
*Effects of ocean acidification on microbial community composition and $O_2$ fluxes in biofilms from the Great Barrier Reef.* 4th Congress of European Microbiologists FEMS, 26 - 30 June 2011, Geneva, Switzerland.

Grasser F, **Witt V**, Lanctôt C, Thormeyer T, Cremer T, Müller S (2007)
Nuclear topology of trans-dev genes and flanking ultra-conserved non-coding sequence clusters in mouse and chicken development. 16th ICC- International Chromosome Conference, 25- 29 August 2007 Amsterdam, the Netherlands
Abstract book: Chromosome Research Vol.15 Suppl. 2, P. 240)

## Field experience

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|---|---|
| 2008 - 2010 | **RV Cape Ferguson**<br>Research vessel of the Australian Institute of Marine Science<br>8 x 2 week field trips to the Whitsundays Islands, Central Great Barrier Reef, Australia |
| 2009 - 2010 | **RV Apollo and RV Aquila**<br>Research vessels of the Australian Institute of Marine Science<br>10 day trips to Magnetic Island |
| 05/2009 | Fieldwork at the Heron Island Research Station (HIRS), Australia |

## Additional Qualifications

- Editorial Services for: Applied and Environmental Microbiology and FEMS Microbiology Letters
- PADI Rescue Diver (220 logged dives, 100 logged scientific dives)
- Australian boating and radio license
- Certifications in Patent- und trademark protection law for natural sciences
- European and Queensland driver's licences