Understanding coral reefs in an impacted world

Physiological responses of coral reef organisms to coastal pollution and global warming

A dissertation by

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Summary

In the past coral reefs have been able to adapt to changing environments, present reefs however are facing a multitude of anthropogenic disturbances at much higher frequencies and magnitudes that might hamper further adaptations. As a consequence, these ecologically and economically important ecosystems are degrading worldwide at an unprecedented pace. Especially in tropical countries within the Coral Triangle region, such as Indonesia, growing populations and coastal development raise the need of effective management plans to protect the sensitive coral reef ecosystems. Reef managers until now were restricted by limited knowledge on the effects of certain local stressors, such as common chemical pollutants, on reef organisms and how these local stressors interact with global ones. Furthermore, there are large uncertainties on the effects of environmental disturbances on natural reef recovery by larvae, although the evaluation of larval settlement should play an essential role in reef management.

This thesis investigated isolated and combined effects of selected local stressors and global warming. In initial experiments, the influence of habitat loss on the structure dependent reef fish *Amphiprion ocellaris* was determined and automated intermittent-flow respirometry for the determination of metabolic condition evaluated as a standard method. Using this respirometry set-up, the isolated and combined effects effect of two common pollutants, diesel fuel and a surfactant (linear alkylbenzene sulfonate, LAS), on the metabolic condition of two important reef organisms, the rabbit fish *Siganus guttatus* and the scleractinian coral *Pocillopora verrucosa*, were analyzed. In addition, this thesis determined potential in-situ effects of anthropogenic disturbances on the recruitment of coral larvae and the composition of bacterial biofilm communities that play a major role in this recruitment process.

While loss of shelter had no effect on fish metabolism, chemical pollution caused significant negative effects on metabolic condition of both fish and corals. In isolation the surfactant increased metabolic rates in *S. guttatus* while diesel fuel resulted in a metabolic depression. *P. verrucosa* responded to the surfactant with a decrease in photosynthesis and a severe tissue loss. Diesel alone had no effects on the coral. An interactive effect between diesel fuel and the surfactant was found for *S. guttatus*. Combined pressure of either pollutant in combination with high temperature resulted in mostly additive effects for both species. While organisms are able to tolerate stress to a certain limit, the exposure to combined stressors

poses severe additional threats to their metabolic condition. Since metabolic impairment can affect growth and reproduction, it subsequently affects entire populations and the coral reef ecosystem. These results indicate the necessity to reduce local stressors such as pollution in order to increase the resilience of reefs to global stressors such as a increased sea surface temperature.

In the second part of this thesis in-situ studies on coral recruitment were performed in the Spermonde Archipelago, Indonesia. The area is influenced by different magnitudes of local stressors, such as nutrient and pollutant inputs, due to the close proximity to the large urban area of Makassar. Water quality decreased from offshore towards Makassar at a regional scale, as well as closer to individual islands at a smaller spatial scale. These changes in water quality resulted in shifts of bacterial community compositions on potential settlement substrates for coral larvae. Settlement of larvae, as determined by numbers of recruits, was more influenced by microhabitats of the settlement substrates than by declining water quality. Nevertheless, at the site closest to Makassar, which is heavily influenced by local stressors, no coral recruitment could be recorded. These findings indicate the severe future coral reefs close to large urban areas are facing unless management actions are taken.

This thesis reveals the significance of pollution from highly localized sources, which gain in importance due to their widespread and daily utilization. Coral reef management needs to address this issue more specifically in future and reduce the discharge of pollutants immediately, to give coral reefs the chance for adaptation to global stressors. Reducing local stressors will further benefit the recovery of coral reefs via recruitment through coral larvae. This work has shown that environmental conditions can alter the recruitment, indicating the need for effective implementation of monitoring strategies for local stressors.

Zusammenfassung

In der Vergangenheit waren Korallenriffe in der Lage sich veränderten Umweltbedingungen anzupassen. Gegenwärtig sind Riffe jedoch menschlichen Störungen in größerem Ausmaß und in immer kürzeren Abständen ausgesetzt, was eine Anpassung erschwert. Dies hat zur Konsequenz, dass diese ökonomisch und ökologisch wichtigen Ökosysteme weltweit mit beispielloser Geschwindigkeit zerstört werden. Besonders in tropischen Ländern innerhalb des Korallendreiecks, wie zum Beispiel Indonesien, erhöhen eine stetig wachsende Bevölkerung und fortschreitende Küstenentwicklung den Bedarf an effektiven Managementstrategien zum Schutz der empfindlichen Korallenriffe. Bisher sind Schutzbemühungen oft durch unzureichenden Wissenstand über die Auswirkungen von lokalen Stressoren, wie zum Beispiel die Verschmutzung mit chemischen Schadstoffen, auf Rifforganismen limitiert. Hinzu kommt, dass in vielen Fällen unklar ist, wie sich die lokalen und globalen Stressoren gegenseitig beeinflussen. Des Weiteren gibt es noch große Wissenslücken welche Effekte veränderte Umweltbedingungen auf die natürliche Wiederherstellung von Riffen durch Ansiedlung von Korallenlarven haben. Diese Ansiedlung sollte einen essentiellen Bestandteil von zukünftigen Managementvorhaben in Korallenriffen ausmachen.

In dieser Dissertation werden ausgewählte einzelne, sowie miteinander kombinierte lokale Stressoren bei gleichzeitigen, simulierten Erwärmungsszenarien untersucht. In ersten Experimenten wurde der Einfluss von Lebensraumzerstörung auf den struktur-abhängigen Rifffisch *Amphiprion ocellaris* untersucht. Die Verwendung von automatisierter Sauerstoffverbrauchsmessung wurde als Methode zur Bestimmung der Kondition des Stoffwechsels getestet. Diese Sauerstoffmessungen wurden verwendet um den Einfluss von zwei einzelnen, sowie miteinander kombinierten, häufigen Schadstoffen zu untersuchen: Dieseltreibstoff und ein Tensid (linear alkyliertes Benzolsulfonat, LAS). Untersucht wurde der Einfluss auf den Metabolismus von zwei wichtigen Korallenrifforganismen, dem Kaninchenfisch *Siganus guttatus* sowie der Steinkoralle *Pocillopora verrucosa*. Zusätzlich wurden im Zuge dieser Dissertation Feldstudien durchgeführt, die tatsächlich vorkommende anthropogene Störungen auf die Ansiedlung von Korallenlarven und die Zusammensetzung von bakteriellen Gemeinschaften, welche eine wichtige Rolle bei der Larvenansiedlung spielen, bestimmt haben.

Während der Verlust von Versteckmöglichkeiten keinen Einfluss auf den Metabolismus von *A. ocellaris* zeigte, verursachten die chemischen Schadstoffe deutliche negative Auswirkungen auf beide untersuchten Organismen. In Isolation erhöhte das Tensid die Stoffwechselraten von *S. guttatus* während Diesel zu einer Unterdrückung führte. *P. verrucosa* reagierte auf LAS mit einer Verminderung der Photosyntheserate sowie einem massiven Gewebeverlust. Im Gegensatz dazu hatte Diesel allein keinen Einfluss auf die Koralle. Im Falle von S. guttatus konnte eine Interaktion beider Schadstoffe festgestellt werden, wenn der Fisch beiden Schadstoffen gleichzeitig ausgesetzt wurde. Gemeinsam mit erhöhter Temperatur wurden für die meisten Kombinationen der beiden Schadstoffe additive Effekte auf die beiden Versuchsorganismen herausgefunden. Obwohl die Tiere in der Lage sind Stress bis zu einem bestimmten Limit zu ertragen, so bedeutet das Auftreten von mehreren Störungen gleichzeitig eine Bedrohung für den Gesamtzustand der Individuen. Verschlechterungen des Gesamtzustandes führen zu verringertem Wachstum und verminderter Reproduktion. Dies wiederum hat Auswirkungen auf die gesamte Population sowie das Ökosystem Korallenriff. Die Ergebnisse dieser Arbeit verdeutlichen die Notwendigkeit, dass lokale Störungen der Umwelt, wie z.B. durch Chemikalieneinfluss, verringert werden müssen, um die Widerstandskraft von Korallenriffen gegenüber globalen Effekten des Klimawandels zu erhalten.

Im zweiten Teil dieser Dissertation wurden Feldstudien zur Ansiedlung von Korallenlarven im Spermonde Archipel in Indonesien durchgeführt. Diese Gegend wird unterschiedlich stark durch den lokalen Einfluss der Großstadt Makassar beeinflusst, besonders durch erhöhte Nährstoff- und Schadstoffeinträge. Die Wasserqualität verschlechtert sich in der Nähe zu Makassar, ebenso wie auf einem kleineren Maßstab in der direkten Umgebung von einzelnen, bewohntenInseln. Diese Änderungen der Wasserqualität bewirken Veränderungen der Zusammensetzung von Bakterien-gemeinschaften auf potenziellen Siedlungsflächen für Korallenlarven. Die Ansiedlung von Korallenlarven selbst wird dabei eher durch Mikrohabitate als durch die Wasserqualität beeinflusst. Jedoch konnte am Standort mit der geringsten Entfernung zu Makassar durch die starken Einflüsse der Stadt keinerlei Ansiedlung beobachtet werden. Dies verdeutlicht wie unsicher die Zukunft von Korallenriffen in der Nähe von großen Städten ist, sollten keine geeigneten Schutzmaßnahmen getroffen werden.

Diese Dissertation deckt die Bedeutung von besonders lokalen Schadstoffen auf, die durch ihre weitverbreitete Nutzung oft einen starken, negativen Einfluss auf Korallenriffe haben. Das Management von Korallenriffen muss diesen Punkt in Zukunft stärker beachten und

den Eintrag dieser Schadstoffe in Küstensysteme umgehend vermindern, um den Riffen die Chance zu geben, sich den globalen Umweltveränderungen anzupassen. Die Verminderung von lokalen Störungen wird außerdem die Chance auf Wiederherstellung von Korallenriffen durch Larven von Steinkorallen erhöhen. Diese Dissertation zeigt, dass veränderte Umweltbedingungen die Ansiedlung von Larven beeinflussen, und wie wichtig Überwachung von Schadstoffeinträgen für die Zukunft von Korallenriffen ist.

Acknowledgements

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General Introduction

Global and local stressors on coral reefs

Coral reefs are economically and ecologically important ecosystems (Moberg & Folke 1999) that face a multitude of stressors (Hoegh-Guldberg et al. 2007, Burke et al. 2012). These stressors can be divided into global as well as local ones, depending on their origin and spatial extent.

Global stressors are climate change related alterations, such as ocean acidification and global warming, resulting from greenhouse gas emissions by industries and anthropogenic settlements (Pandolfi et al. 2011). Ocean acidification is caused by higher atmospheric CO_2 concentrations resulting in an increase of carbonic acid in the sea, which leads to a lowering of pH (Kleypas et al. 1999, Bindoff et al. 2007, Doney et al. 2009). Such changes in ocean chemistry can weaken the calcium carbonate skeletons in calcifying organisms like scleractinian corals (Kleypas et al. 1999, Hoegh-Guldberg et al. 2007) and alter juvenile recruitment (Munday et al. 2008). Another result of CO_2 emissions is global warming as the atmosphere is heating up due to an accumulation of greenhouse gases (Lashof & Ahuja 1990). 80% of this heat has been absorbed by the oceans over the past years, increasing water temperatures as far down as 3000 m (IPCC 2007). Several emission scenarios were developed by the International Panel on Climate Change (IPCC) to predict world climate in the future. Within these future emission scenarios, sea surface temperatures are expected to increase between 0.3 - 4.8 °C until the end of this century, while pH in the oceans will decrease in a range of 0.06-0.32 (IPCC 2014). Although natural daily or seasonal fluctuations in temperature and pH can occur (Lough 1997, Pelejero et al. 2005), both acidification as well as ocean warming are increasing due to human influence since the beginning of the industrial revolution (IPCC 2013). Not only these changes themselves, but also the rate at which they are occurring is critical (Hoegh-Guldberg et al. 2007). Coral reefs in the tropics usually face very little variation in temperature compared to those in other parts of the world. Therefore even small increases in water temperature can lead to a high stress in reef organisms living there (Maina et al. 2011, Morgan 2011, Lesser 2013). A widely recognized phenomenon, in most cases connected to rising temperatures or increased solar radiation is coral bleaching. During high stress the symbiotic relationship between coral host and zooxanthellae is disrupted, the algae are degenerated or expelled and the loss of the algae

pigments makes the coral appear white (Brown 1997). If the stress is only of short duration, the corals may recover. Otherwise high mortalities have been observed (Wilson et al. 2010).

Besides those global stressors, there are multiple stressors that are spatially restricted and originate from local sources, mostly related to urbanization and agricultural development. One major issue is increased nutrient input to coral reef systems (Fabricius 2005, Cooper et al. 2007). These are mainly phosphorus and nitrogen inputs from fertilizers and sewage runoff, which commonly lead to enhanced algal growth, referred to as eutrophication (Smith et al. 1999, Smith & Schindler 2009). In the generally oligotrophic conditions of coral reefs (Schlager 1981, Nelson et al. 2011), these added nutrients can lead to severe changes in water quality (Smith et al. 1999, Kroon et al. 2011), increase coral diseases and bleaching (Vega Thurber et al. 2014) and in turn alter community compositions (Haas et al. 2009). Terrestrial runoff further increases sedimentation on coral reefs, altering light availability (Weber et al. 2006) as well as transporting various chemical pollutants to the sea (Kroon et al. 2011). These chemical pollutants originate from a wide array of anthropogenic activities such as fertilizers (Burke et al. 2012), motor fuels (Ocean Studies Board and Marine Board 2003, Haapkylä et al. 2007) or detergents (Chupa et al. 2007, Ivancović & Hrenović 2010). Fishing activities on different scales can lead to overfishing (Jackson et al. 2001), depriving coral reefs of essential community members (Cinner & McClanahan 2006). In many cases these are herbivores, which are key players in shaping the benthic community composition of the reefs by grazing on turf and macroalgae (Hughes et al. 2007). Where fish resources have already become scarce, destructive fishing methods such as bottom trawling or dynamite fishing are applied to get fish in the already fish deprived habitats (Edinger et al. 1998, Pet-Soede & Erdmann 1998, Jackson et al. 2001). Further habitat destruction takes place when coral reef materials are used as building materials for the local communities.

Usually multiple disturbances act simultaneously on a reef ecosystem (see Fig. 0.1). When multiple stressors occur at the same time, their effects can either simply be added or there can be interactive effects (Crain et al. 2008). They can either enhance each other's responses (= synergistic effects) or weaken them (= antagonistic effects) (Dunne 2010). Interactions can occur when stressors act directly with another, or when organisms' responses to one factor are altered by the occurrence of another (Crain et al. 2008). Synergistic effects are most likely to occur when stressors act through alternative and dependent pathways (Crain et al. 2008). As organisms are able to tolerate stress up to a certain extent, this exposure to multiple stressors poses a severe threat (Wilson et al. 2006), potentially causing a

higher sensitivity to further stress (Beyer et al. 2014). Thermal stress for example is known for its potential to influence the sensitivity of organisms to other stressors (Hoar et al. 1988) such as light and sedimentation stress (Anthony & Connelly 2007), hypoxia (Ekau et al. 2010) and sensitivity to pollution (Beyer et al. 2014). Poor water quality and high nutrient inputs enhance the bleaching susceptibility of corals (Wooldridge 2009). While reef ecosystems in the past have been able to adapt to changing conditions, present reefs are heavily influenced by human impacts, reducing their resilience and leaving them more susceptible to ever faster occurring climate change (Wilson et al. 2010).
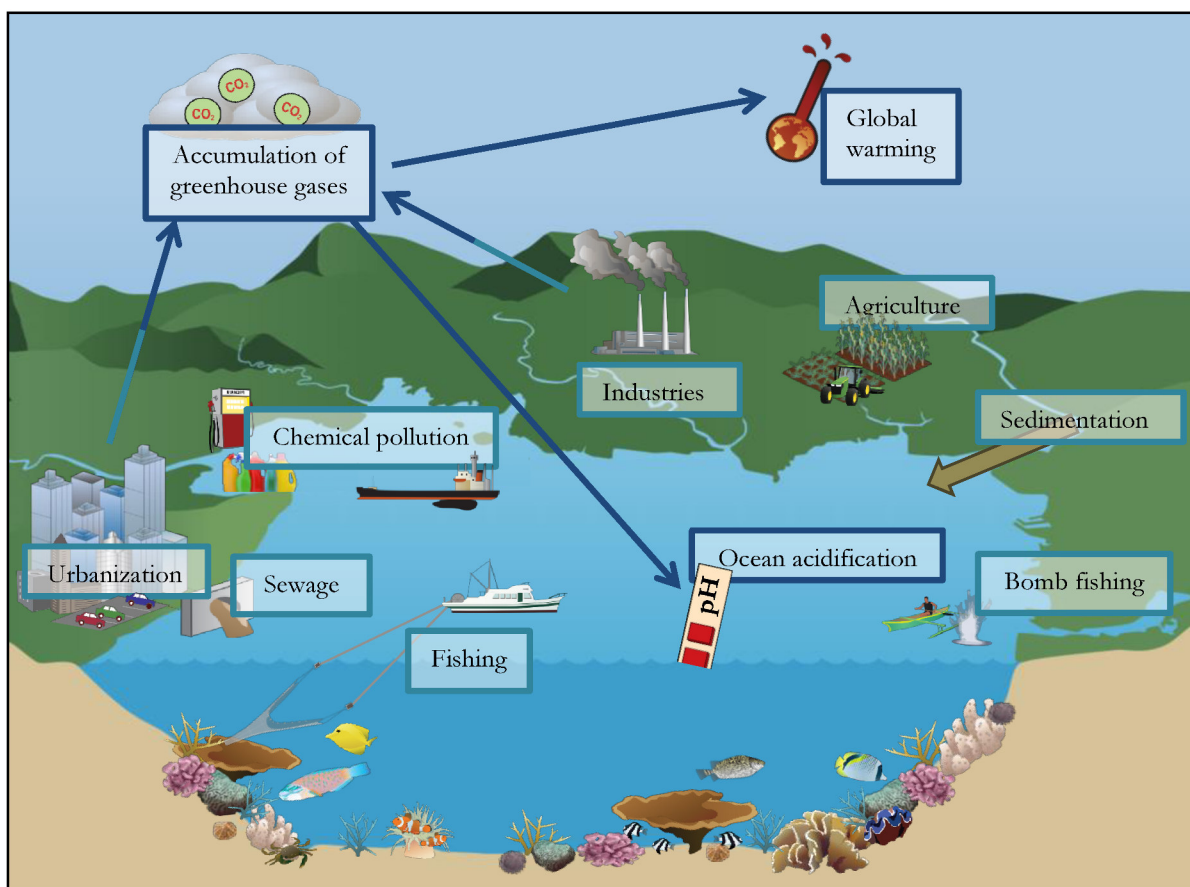
Fig. 0.1: Overview of global (dark blue) and local (light blue) stressors affecting coral reefs. Clockwise starting left: Urbanization of coastal areas, industries and agriculture cause stress mainly via associated sewage and chemical pollutant inputs (as well as nutrients and suspended particles). Human settlements and industries are further leading to production of greenhouse gases that accumulate in the atmosphere and cause ocean acidification and global warming, lead to increased sea surface temperatures. Fishing activities and bomb fishing directly affect members of the coral reef ecosystem.

29 % of all coral reefs worldwide are situated within the Coral Triangle, a region in the Indonesian/Philippines Archipelago where the center of coral reef biodiversity is situated, with 76% of all coral reef species occurring there (Veron et al. 2009, Burke et al. 2012). Reefs

in this area were largely unprotected for a long time, while human populations were growing and exposing them to the entire array of anthropogenic stressors (Veron et al. 2011). Several studies predict this area as one of the most sensitive to further stressors (Teneva et al. 2012), making it a focal area for coral reef research and protection (Bruno & Selig 2007).

Measuring stress - physiological responses of reef organisms

Effects of stressors on ecosystems or populations can be predicted from the knowledge of how those stressors affect individuals on the organism level, making them important indicators to measure environmental stress (Maltby 1999). Organisms can react to stressors in different ways and scientists have used a wide array of response parameters when investigating stress (Moberg 2000). These parameters depend on the organism under study and include a range of biochemical, physiological and morphological responses (Beitinger & McCauley 1990, Depledge & Fossi 1994, McPherson et al. 2010, Van Dam et al. 2011). With emerging new technologies the use of genomic methods has increased over the past decades. More common stress indicators are RNA:DNA ratios, enzyme activities, reproduction success, growth and metabolism (Niimi 1990, Barton & Iwama 1991, McPherson et al. 2010). Metabolism combines all processes controlling the performance of organisms in terms of behavior, survival, growth and reproduction, therefore changes in physiology are appropriate indicators to measuring effects of stressors (Maltby 1999, Kingsolver & Huey 2003, Biro & Stamps 2010). Decreased metabolic rates over a longer time period can lead to growth inhibition, decreased reproduction success and lower offspring fitness (Burt et al. 2011).

Fish and reef-building corals are among the most studied groups within coral reef organisms. As they are key players in the ecosystem this is highly justified.

Fish constitute a large portion of the economic value in coral reefs and fulfill several ecological key functions (Hixon 2011). Most important among these are certainly control of the food web and regulation of algae growth by herbivorous species (Holmlund & Hammer 1999, Hixon 2011). Measuring physiology in fish is a common tool to increase the ecological understanding of changing environments (Wilson et al. 2010). The overall physiological status or health of an individual is often referred to as condition or fitness (McPherson et al. 2010). Fish condition is affecting predation risk as well as competition for resources, such as food and shelter that would be beneficial to the condition

(Booth & Beretta 2004). Fish metabolism is usually determined in terms of oxygen consumption per biomass as a measure for metabolic rate (Schmidt-Nielsen 1997). Standard metabolic rates in animals at rest provide insights on the minimal metabolic energy required to sustain life and can be used to compare metabolism between species or between different environmental conditions (McNab 1997, Zimmermann & Kunzmann 2001, Careau et al. 2008).

Reef-building corals of the order of scleractinia provide the foundation and three dimensional structures of coral reefs (Munday 2004). Next to a large array of microorganisms (Rohwer et al. 2002, Bourne et al. 2009), reef building corals are associated with zooxanthellae (unicellular symbiotic dinoflagellates) that provide them with energy fixed during photosynthesis. This symbiont photosynthesis together with the host respiration forms a close carbon cycling in the holobiont (Al-Horani et al. 2003). Under optimal light conditions up to 95% of all carbon fixed by zooxanthellae may be transferred to the coral host, mainly in forms of glycerol, glucose, amino acids and lipids (Stambler 2011). Photosynthesis can be measured as the maximum quantum yield (F_v/F_m) and is a common measure to determine stress in corals (Stambler 2011). Together coral respiration and photosynthesis are measures of the holobionts' basal metabolic functions and can be used to determine non-lethal stress effects on corals (Porter et al. 1999, Osinga et al. 2012).

Reef degradation and potential recovery

Due to the multitude of stressors coral reef areas worldwide are declining. Already now there are no pristine reefs left and many are substantially threatened by the mostly anthropogenically induced stressors (Carpenter et al. 2008, Halpern et al. 2008). Coral cover is reduced, fundamentally altering the composition of the entire ecosystem (Wilson et al. 2010). Large areas of coral reefs are entirely lost while in other cases substantial changes in reef structure occur, that affect all reef associated organisms (Munday 2004). Especially fish communities are depending on the three dimensional structure provided by scleractinian corals and are altered in times of coral decline (Jones et al. 2004, Munday 2004, Jones 2013).

Even if coral reef ecosystems are not entirely lost, communities might shift towards species that are more tolerant to the new conditions (Hughes et al. 2003), leading to changes on global and local scales (Wilson et al. 2010). These changes need to be understood in order to

determine appropriate management strategies (Graham et al. 2014). Resilience of coral reefs is a measure of the ability to recover to a particular state after disturbance events (Hoegh-Guldberg et al. 2007). If certain tipping points are reached the ecosystem changes into an alternative state. In many cases phase shifts towards algae dominated reefs have been documented, that have lower ecological and economic value for the surrounding communities (Hoegh-Guldberg et al. 2007).

Once reef areas are degraded by stress events they can recover if appropriate recovery times and favorable conditions are given. A key process in natural recovery of coral reefs is recruitment with coral larvae (Sawall et al 2013). For most scleractinian species the sexual reproduction via planktonic larvae is the primary means of recolonization and the recombination of genotypes may further enhance species survival and the overall tolerance of the population to stressors (Ritson-Williams et al. 2009, Harrison 2011). Especially after one-time destructive events such as bomb fishing or severe storms the dispersal of coral larvae from adjacent reefs can help the recovery. As the density of larvae arriving at reefs determines the recruitment success (Ritson-Williams et al. 2009), reefs in very isolated locations have lower chances for recovery after stress events (Wilson et al. 2010). The transition of free swimming planula larvae to sessile coral recruits includes the settlement and metamorphosis of the larvae (Ritson-Williams et al. 2009, Tebben et al. 2015), which highly depend on environmental stimuli, most importantly bacterial communities on reef surfaces (Hadfield 2011, Tran & Hadfield 2011). As coral reef ecosystems face diverse environmental changes, these can interfere with the settlement of coral larvae, a process of vital importance for sustaining coral reef ecosystems and their resilience (Ritson-Williams et al. 2009). It may also change the bacterial communities on reef surfaces (Lau et al. 2005, Salta et al. 2013), which potentially could have further indirect alterations on coral larvae settlement (Sawall et al. 2012).

Gaps of knowledge

In order to provide sustainable management plans for coral reefs, the effects of different environmental disturbances and their potential interactions on reef organisms need to be understood (Ban et al. 2014). Especially in the tropics, where growing populations consequently lead to increasing stress on reefs, efficient management plans are vital for the ecosystem, as well as for the local people relying on reefs for their livelihoods. Although

extensive research has been carried out on coral reefs, due to the multitude of organisms and stressors, there are still many questions unanswered at different organization levels.

One of the most important gaps is the lack of knowledge on how certain organisms react to certain stressors. Due to highly species- and stressor-specific responses, this presents a large challenge that can only be answered one part at a time. Chemical pollutants form a relatively new threat to coastal ecosystems and the effects of many pollutants and their potential interactions with climate change have not been determined (Ban et al. 2014). Metabolism as the basis of life plays an important role on the organism level, but information on the effect of multiple stressors such as habitat loss, chemical pollutants and global warming on metabolism of common key players in reefs are still largely missing.

Another important gap on how global and local stressors affect the coral reef ecosystem is their effects on recruitment with coral larvae. Especially on already degraded reefs, the replenishment of the benthic community with coral larvae is of vital importance. Nevertheless the key process, how larvae choose places to settle, is still heavily discussed and many questions still remain regarding how settlement is affected by changing environments. Especially how bacterial communities are changing due to environmental stressors needs to be evaluated in order to determine the potential effects on larval recruitment.

Objectives and research questions

The aim of this thesis was to determine how two important coral reef organisms deal with combinations of global and local stressors. The focus was on the two key players fish and scleractinian corals due to their importance in shaping the ecosystem. The thesis had three main objectives. First the metabolic responses to different disturbances on the organism level were to be determined, analyzing a combination of global and local stressors. Then the effect of multiple disturbances on the population level was investigated by analyzing the ability for natural reef recovery via settlement of coral larvae. Assuming that the composition of bacterial communities is vital for coral larvae settlement, the effect of environmental differences on these was analyzed as well to determine indirect influences on the larval settlement. The following research questions were addressed:

1. What effects on fish and coral metabolism are caused by coastal pollution and habitat loss?

2. How do key coral reef organisms react to local changes in combination with global warming?

3. Are there direct or indirect impacts of local stressors and global warming on coral reef recovery via coral larvae?

Approach

This thesis was conducted at the Leibniz Center for Tropical Marine Ecology in Bremen. Fieldwork was carried out in Indonesia, the country with the largest coastline of all countries within the coral triangle, with growing populations that heavily rely on coral reef products and services. The thesis was conducted in collaboration with three local partners; the Indonesian Institute of Sciences (LIPI), the Ministry of Marine Affairs and Fisheries (KKP) and the Universitas Hasanuddin, Makassar (UNHAS). The first experimental study at the ZMT laboratory aimed to adjust the method for respiration measurements to the requirements of the thesis. An automated intermittent flow system was implemented to determine metabolism in terms of oxygen consumption in individual non-stressed fish. The effect of shelter loss, as occurring by habitat degradation, on metabolism of the false clown anemonefish *Amphiprion ocellaris* was determined. The automated intermittent flow respirometry method was then applied for the following two experiments carried out in Indonesia. Local stress by chemical pollution with motor fuels and surfactants was combined with high temperatures to investigate the effects on metabolism of fish and scleractinian corals. For the second study *Siganus guttatus* was chosen as model fish species over *A. ocellaris,* due to its occurrence and economic importance in the study area. *Pocillopora verrucosa* was analyzed as a member of the scleractinia in the third chapter. While these first chapters focused on selected stressors and their effect on metabolism of individual organisms in controlled laboratory experiments, the last two chapters focused on an in-situ approach. Field surveys and experiments were carried out in the Spermonde Archipelago in south Sulawesi, Indonesia, an island chain with varying anthropogenic influence stretching out from the highly populated city of Makassar. In this setting with an authentic combination of naturally occurring stressors, water quality, bacterial communities in different reef compartments and the aggregate formations in the water column were determined at different spatial scales in chapter four. These results were the basis for chapter 5, where the

settlement of coral larvae and bacterial community compositions were determined to investigate the potential for coral reef recovery after disturbances.

Chapter and Publication outline

This thesis includes this general introduction, followed by five chapters, based on the scientific publications listed below, and concludes with a general discussion. The **first chapter** introduces the experimental method and determines the effect of shelter on the metabolism of structure dependent reef fish. The **second chapter** contains the experiments on reef fish exposed to chemical pollutants, either in isolation or in combination with high temperature, while the **third chapter** includes similar experiments with a scleractinian coral. The **fourth chapter** is setting the stage for the in-situ studies, determining bacterial community compositions in water and sediments along the two different spatial scales of disturbance. **Chapter five** builds on these differences along the island chain and investigates bacterial community compositions on reef surfaces and coral larvae settlement.

The individual publications contained in this thesis are listed below, with authors contribution indicated.

Publication 1:

Kegler P, Kunzmann A, Bröhl S, Herbert NA. (2013) No evidence of shelter providing a metabolic advantage to the false clownfish *Amphiprion ocellaris*. J Fish Biol 82(2): 708-713.

The concept for this study was developed by P. Kegler, A. Kunzmann and N. Herbert. The experiments were carried out by P. Kegler, S. Bröhl and N. Herbert. Analysis and interpretation of data was carried out by P. Kegler, N. Herbert and A. Kunzmann. The manuscript was written by P. Kegler with critical revision from all authors.

Publication 2:

Baum G, **Kegler P**, Scholz-Böttcher BM, Alfiansah YR, Abrar M, Kunzmann A. Metabolic performance of the coral reef fish *Siganus guttatus* exposed to combinations of water borne diesel, an anionic surfactant and high temperature. (In preparation for Marine Pollution Bulletin)

The concept for this study was developed by G. Baum, P. Kegler and A. Kunzmann. The experiments were carried out by G. Baum and P. Kegler with help of Y. Alfiansah and M. Abrar. Analysis of PAH samples was aided by B. Scholz-Böttcher. The manuscript was written by G. Baum with critical revision from all authors.

Publication 3:

Kegler P, Baum G, Indriana LF, Wild C, Kunzmann A. Physiological response of the hard coral *Pocillopora verrucosa* from Lombok, Indonesia, to two common pollutants in combination with high temperature (in review after revisions PLoS ONE, 2015)

The concept for this study was developed by P. Kegler, G. Baum and A. Kunzmann. The experiments were carried out by P. Kegler and G. Baum, aided by L. Indriana. Data analysis and interpretation was carried out by P. Kegler, G. Baum, C. Wild and A. Kunzmann. The manuscript was written by P. Kegler with critical revision from all authors.

Publication 4:

Schwieder HF, **Kegler P**, Jennerjahn TC, Jompa J, Hassenrück C, Gärdes A. Shifts of bacterial community composition in the water column, on aggregates and within the sediments along water quality gradients: the effect of different spatial scales. (In preparation for Frontiers in Microbiology)

The concept for this study was developed by H. Schwieder, T. Jennerjahn and A. Gärdes. Field work was carried out by H. Schwieder, P. Kegler, A. Gärdes. Data analysis and interpretation was carried out by H. Schwieder with support by C. Hassenrück and A. Gärdes. The manuscript was written by H. Schwieder with revision from all authors.

Publication 5:

Kegler P, Schwieder H, Gärdes A, Ferse S, Alfiansah YR, Lukman M, Hassenrück C, Kunzmann A. Coral larvae settlement and of bacterial biofilm communities in changing environmental conditions of the Spermonde Archipelago, Indonesia. (In preparation for Marine Ecology Progress Series)

The concept for this study was developed by P. Kegler, H. Schwieder, A. Gärdes and A. Kunzmann. The experiments were carried out by P. Kegler and H. Schwieder, aided by M. Lukman and Y. Alfiansah. Analysis and interpretation of results was performed by P. Kegler together with C. Hassenrück, H. Schwieder, A. Gärdes and S. Ferse. The manuscript was written by P. Kegler with critical revision from all authors.

Further publications not included in this thesis:

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Kroll S, Soltmann C, Koch D, **Kegler P**, Kunzmann A, Rezwan K (2014) Colored ceramic foams with tailored pore size and surface functionalization used as spawning plates for fish breeding. Ceram Int 40: 15763-15773.

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Chapter 1: Shelter and Metabolism of *A. ocellaris*
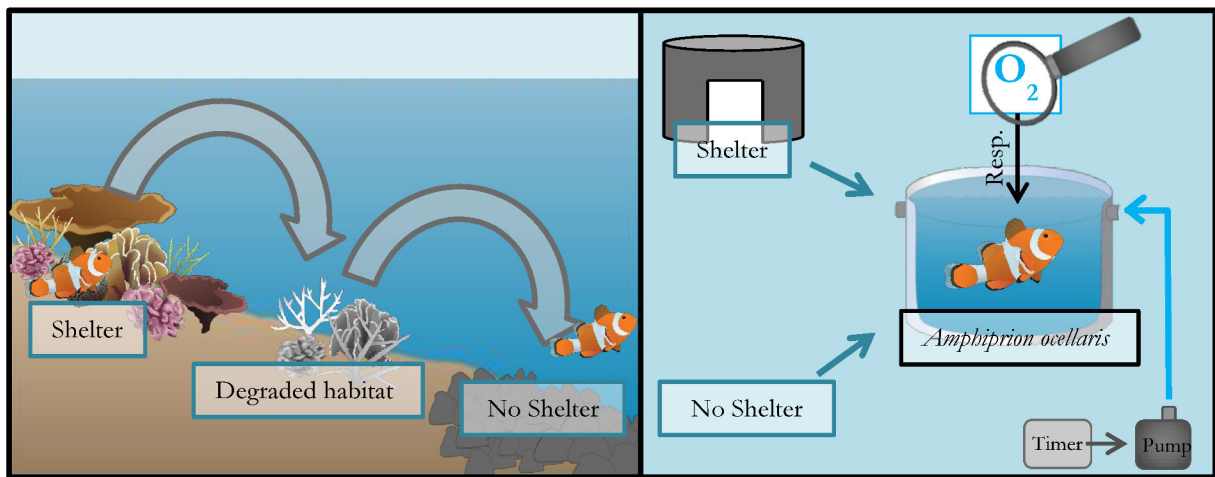
Chapter 1: Habitat degradation due to decreasing live coral cover leads to a loss of shelter for reef fish. Laboratory experiments were performed to investigate the effect of habitat loss on *Amphiprion ocellaris*. Further the initial experiments were used to determine the use of automated intermittent-flow respirometry for long-term metabolism measurements.

Kegler P, Kunzmann A, Bröhl S, Herbert NA. (2013) No evidence of shelter providing a metabolic advantage to the false clownfish *Amphiprion ocellaris*. J Fish Biol 82(2): 708-713.

No evidence of shelter providing a metabolic advantage to the false clown anemonefish, *Amphiprion ocellaris*

Kegler P, Kunzmann A, Bröhl S, Herbert NA

Abstract

Shelter reduces the standard metabolic rate (maintenance cost) of salmonids, suggesting that the energetic benefit of lowered metabolism could extend to other shelter-dependent species. There was no evidence, however, that shelter conveyed a metabolic advantage to the false clown anemone fish, *Amphiprion ocellaris*, in terms of standard and routine rates of oxygen uptake. The metabolic and fitness benefit of shelter might not, therefore, be widespread among all fish species.

Introduction

Energy is the fundamental currency of life and animals have evolved intricate ATP production pathways for the purposes of maintenance, biosynthesis (growth) and preforming external work (Perrin & Sibly 1993). Although not a direct measure of energy metabolism, the rate of oxygen consumption (MO_2) provides a practical estimate of energy expenditure and metabolic rate partitioning in aquatic organisms (Steffensen 1989). For example, the standard metabolic rate (SMR) realistically estimates the basal costs (maintenance) of resting post-absorptive fish (Cook et al. 2011) and routine metabolic rate (RMR) estimates the costs of a spontaneously active fish (Herbert et al. 2001).

Through evolution, organisms are expected to allocate energy in an optimal pattern, but this appears to depend heavily on environmental forces that influence the way in which fitness costs and benefits are imposed (Perrin & Sibly 1993). For example, variability in SMR is apparently linked with personality and may influence fitness through effects on dominance and risk taking (Biro & Stamps 2010). The SMR of juvenile salmonids also shows considerable intraspecific variation which appears to favor individuals with high or low maintenance costs on a context-dependent basis involving, for example, variations in food, water flow and conspecific density (Armstrong et al. 2011; Burton et al. 2011). Interestingly,

the provision of shelter appears to lead to differences in patterns of energy allocation, with evidence of salmon showing a 30% lower SMR, presumably due to lowered costs of vigilance (Millidine et al. 2006). This reduction in SMR is ecologically important because it could expand aerobic metabolic scope, through a greater difference in SMR and maximal metabolic rate. So it is theoretically possible that salmon and other shelter-dependent fish would benefit from greater growth potential (Dupont-Prinet et al. 2010), and improved hypoxia tolerance (Cook et al. 2011). Unfortunately it is not yet known whether other shelter-dependent fish exhibit metabolic changes as a function of shelter. Since many tropical fishes are already believed to be hypoxia-tolerant (Nilsson & Östlund-Nilsson 2004) and the proposed metabolic effect of shelter by Millidine et al. (2006) could theoretically improve this further, this study set out to resolve whether shelter has any metabolic benefit for the false clown anemonefish, *Amphiprion ocellaris* (Cuvier 1830). Being a common tropical reef fish that naturally forms strong associations with shelter (Allen 1975, Fautin 1991), *A. ocellaris* is a good model species. Intermittent flow respirometry was employed, with simultaneous records of activity, to quantify the effect of shelter on *A. ocellaris* SMR and RMR.

Material and Methods

Thirteen wild caught, adult clownfish (*Amphiprion ocellaris*) (mean ± SD; mass = 6.7 ± 2.0 g; mean length = 6.4 ± 0.7 cm) were obtained from a commercial supplier and kept in the recirculation system at the MAREE (MARine Experimental Ecology) unit at the Leibniz-Center for Tropical Marine Ecology (ZMT, Bremen). Fish were held for at least one year prior to experimentation, with easy access to flowerpots as artificial shelter. They were fed a mixed diet of Mysis and Artemia each day, but were starved for at least 24 h prior to respirometry to exclude the effects of specific dynamic action (SDA) on SMR (Jordan & Steffensen 2005). Only females were used in experiments to exclude any sex effect.

The mass-specific rate of O_2 consumption (MO_2, in mg O_2 h^{-1} g^{-1}) of 6 fish with shelter and 7 fish without shelter was compared (n = 13 fish in total), with respirometry starting every day in the early afternoon and running for about 11 h. The MO_2 of individual fish was measured in a temperature controlled room at 24 °C using an automated intermittent flow respirometer according to the general protocol of Cook et al. (2011) with specific modifications to accommodate fish of different size and a shelter (Fig. 1.1). A circular Perspex respirometer with a flat base (18.9 cm Ø and 9.2 cm high internal) was housed in a 40 L reservoir tub filled with aerated, filtered (0.8/0.2 μm, PALL Corporation; www.pall.com) UV sterilized seawater.

A small pump (MiMouse from Sicce; www.sicce.com) was used to flush aerated water from the reservoir through the chamber and was connected to a custom built data acquisition system (DAQ) and a computer for control over the 25 min respirometry cycle that consisted of a phase of flush (5 min), wait (1 min) and measure (19 min). The decline in O_2 saturation within the sealed measuring state was recorded using a fibre-optic O_2 needle-type microsensor connected to a Microx TX3 meter (Presens; www.presens.de). Water was continuously recirculated from the respirometer through an external loop of TygonTM tubing and the O_2 microsensor was fitted into this line via a cuvette. MO_2 was calculated according to the methodology described by Cook et al. (2011). The respirometer was designed to house one of two different inserts that either did or did not provide shelter to fish (Fig. 1.1). The shape of the two inserts was different but the volume was almost identical and therefore provided a comparable volume of water within the respirometer and associated tubing (1.94 L and 1.97 L with and without shelter respectively). The shelter insert provided a solid darkened roof to the central region of the respirometer under which fish could hide. The non-shelter insert had no such roof.
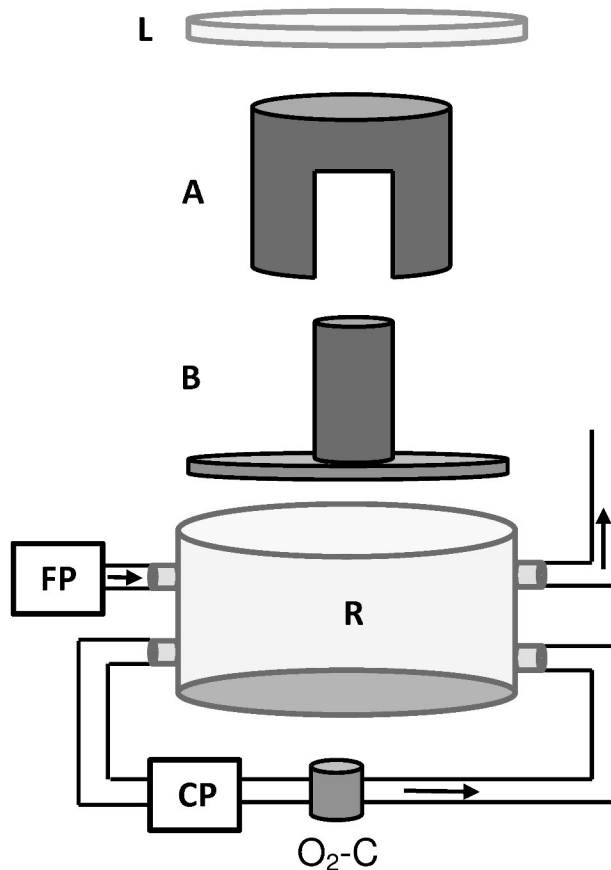
Figure 1.1. Schematic overview of the static respirometer. A circular respirometer chamber (R) was equipped with one of two inserts that either provided shelter (A) or did not provide shelter (B). The respirometer and insert (A or B) was sealed with a lid (L). A flush pump (FP) was under the control of respirometry software and flushed the respirometer at defined intervals. A circulation pump (CP) passed water continuously over the O_2 sensor housed in the O_2 cuvette (O_2-C) within a closed loop. Not to scale.

Fish were transferred in a net to the respirometer (equipped with either the shelter or non-shelter insert) and MO_2 was repeatedly measured by applying 25 flush/wait/measure cycles to each fish which took about 11 h to complete. The area was screened off with black plastic to prevent external disturbance but a miniature CCD camera did record the behavior of the fish remotely. SMR was resolved by plotting the frequency distribution of MO_2 data and calculating the 15th percentile according to the methodology of Chabot and Claireaux (2008), Dupont-Prinet et al. (2010), Cook et al. (2011) and Nelson and Chabot (2011). The number of cycles taken for fish to reach SMR was also calculated and used as an estimate of recovery by fish with and without shelter. RMR was taken as the average of all MO_2 values after the first 4 h of respirometry. The first 4 h were omitted because fish had not fully settled and acclimated to the chamber within this timeframe (see below and Fig. 1.2). Ethovision XT Tracking software (v. 8.0 from Noldus Information Technologies; www.noldus.com) was used to provide basic information on fish swimming speed and the amount of time fish used the shelter. The whole respirometry system was detached after each experiment and scrubbed with ethanol to preclude bacterial respiration in subsequent runs. Metabolic differences between fish with and without shelter (n = 6 and 7 respectively) were examined with t-tests in SigmaPlot version 11, with significance accepted at P < 0.05.
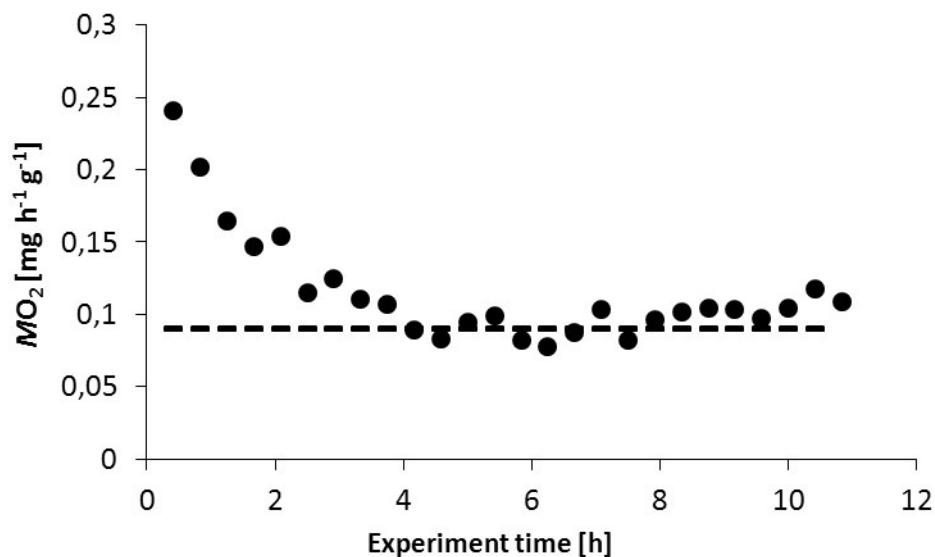
Figure 1.2. Exemplary overview of MO_2 (closed circles) and SMR measures for a single female *A. ocellaris* across 11 h (i.e. 25 cycles). The dashed line indicates SMR, estimated using the 15th percentile method of Chabot and Claireaux (2008).

Results and Discussion

A. ocellaris always showed their highest metabolic rates immediately after being introduced to the respirometer, although they recovered and rapidly reached SMR within ~ 10 cycles (~4 h) when left undisturbed (see example in Fig. 1.2). Preliminary experiments with the same species showed that SMR was indeed reached within this period because lower MO_2 was never recorded with experiments up to and exceeding 24 hours in duration. The rate of recovery from handling *by A. ocellaris* is therefore impressive but more rapid rates have been seen in sedentary sub-tropical species using a similar respirometry setup (Khan & Herbert 2012). Automated intermittent flow respirometry provides several other advantages and ultimately ensured that our metabolic measures were accurate and free of experimental artefacts. The collection of many MO_2 measures contribute to robust measures of SMR and the automated intermittent cycling respirometry pattern reduces experimentor interference, thus providing fish the opportunity to recover rapidly under quiet predictable conditions. The mean swimming velocity of all clownfish was very slow (0.47 ± 0.2 cm s-1, corresponding to 0.04 ± 0.02 cm BL s-1, with BL= body length) and provides evidence that fish settled well within the confines of the respirometer. The settled nature of *A. ocellaris* is further supported by the measured RMR (0.139-0.155 mg h-1 g-1. Table 1.1) being slightly less than the routine MO_2 of 14 Pomacentrid species (0.16 – 0.56 mg h-1 g-1) in the study of Nilsson and Östlund-Nilsson (2004). To ensure that the strength of our MO_2 signal was also not affected by the loss of shelter, we deliberately changed the shape versus volume of the chamber inserts. In- and outflow tubing was also positioned to ensure a good flow of water throughout the chambers.

Table 1.1. The metabolism of female *A. ocellaris* with and without shelter. All values are means with 95 % Cl in parentheses. NS= not significant

	Shelter	no Shelter	df	t	*P*-value
SMR [mg O_2 g^{-1} h^{-1}]	0.142(0.025)	0.120 (0.015)	11	1.51	0.16[NS]
RMR [mg O_2 g^{-1} h^{-1}]	0.155 (0.038)	0.139 (0.021)	11	0.99	0.35[NS]

After taking particular care to obtain robust metabolic measures, no significant difference was observed between fish with and without shelter in terms of SMR or RMR (Table 1.1). Millidine et al. (2006) observed a 30 % reduction in the SMR of Atlantic salmon parr with shelter but, given the results of the current study, this clearly is not a universal response for all shelter-dependent fishes. It is interesting to note that salmon parr often opted to reside next to, or at least within tactile range of the perceived safety structure and so did not always use available shelter directly (Millidine et al. 2006). Such behavior is consistent with our observations of individual *A. ocellaris* that used shelter for variable amounts of time (mean ± SD; 34 ± 21 % of time). This pattern of behavior is also typical of *A. ocellaris* in the wild on reefs where they commonly move in and out of shelter at regular intervals, with plausible differences between individuals (pers obs). However, despite the number of movements in and out of shelter, fish did spend a meaningful amount of time hiding under the roof of the sheltered insert where the level of security was clearly greater than the control context. The plastic inserts used do not provide an accurate representation of natural shelter for *A. ocellaris*, but the individuals interacted with the plastic shelter in the same way they would with an anemone, i.e. constantly moving in and out of the shelter. The plastic inserts were chosen as a simple, surrogate form of shelter because it was not possible to separate the metabolic rate of *A. ocellaris* from living anemone, nor was it possible to control the build-up of bacterial respiration from complex non-living structures across lengthy trials. Future studies should therefore attempt to solve these issues and aim to use more biologically relevant forms of shelter. Because of a limited supply of suitably sized females and maintenance of the wild caught fish in captivity for a year, the relatively low sample size and the possible domesticated nature of the fish might have influenced the lack of metabolic difference to shelter. It is therefore also acknowledged that a greater sample size would have strengthened our conclusions and fish receiving minimal periods of laboratory acclimation may possibly show a different metabolic response to shelter as a result of retained anti-predator reactions.

Conclusion

To conclude, the routine use of shelter by *A. ocellaris* does not appear to adjust standard or routine metabolism and is thus unlikely to confer any fitness benefit in terms of improved growth and improved low O_2 tolerance. More research should ascertain whether shelter

provides a metabolic benefit to other shelter-dependent species and, if so, resolve what the ecological ramifications might be.

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Chapter 2: Fish Response to Pollutants and Temperature
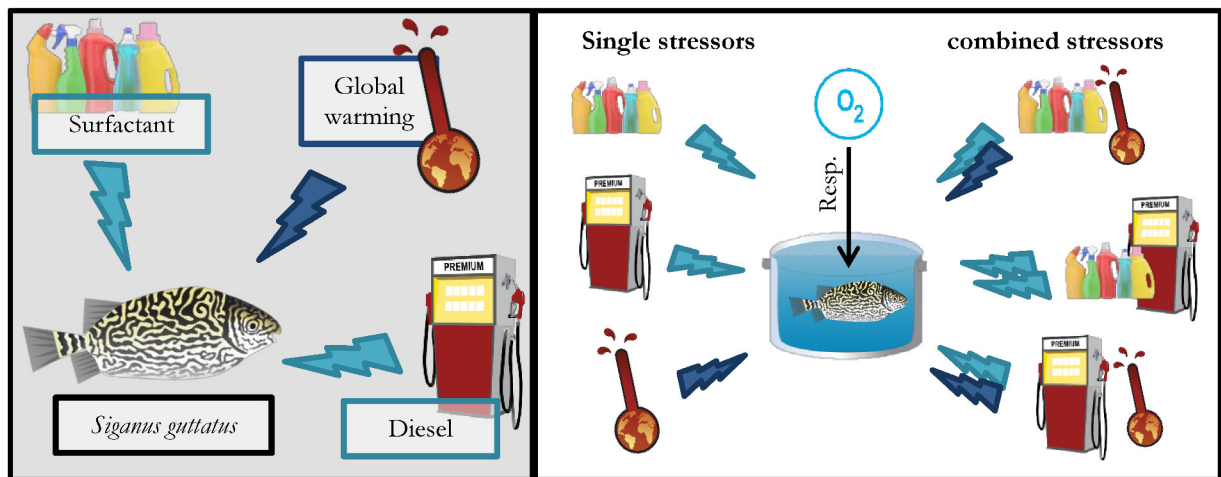
Chapter 2: Coral reef organisms frequently face local and global stressors. Laboratory experiments were performed to investigate the effect of local stressors (surfactant and diesel pollution) and global warming on *Siganus guttatus* metabolism. Standard metabolic rates were measured in individual fish subjected to either single or combined stressors.

Baum G, **Kegler P**, Scholz-Böttcher BM, Alfiansah YR, Abrar M, Kunzmann A. Metabolic performance of the coral reef fish *Siganus guttatus* exposed to combinations of water borne diesel, an anionic surfactant and high temperature. (In preparation for Marine Pollution Bulletin)

Metabolic performance of the coral reef fish *Siganus guttatus* exposed to combinations of water borne diesel, an anionic surfactant and high temperature

Baum G, Kegler P, Scholz-Böttcher BM, Alfiansah YR, Abrar M, Kunzmann A

Abstract

Jakarta Bay in Indonesia and its offshore island chain, the Thousand Islands, are facing extreme pollution. Surfactants and diesel-borne compounds from sewage and bilge water discharges are common pollutants. However, knowledge of their effects on reef fish physiology is scarce. This study investigated combined and single effects of a) the water accommodated fraction of diesel (WAF-D, determined by Σ EPA polycyclic aromatic hydrocarbons (PAH)) and b) the surfactant linear alkylbenzene sulfonate (LAS) on metabolic performance of the coral reef fish *Siganus guttatus*. Responses to combinations of each pollutant with high temperature (+3 °C) were determined. Short-term exposure to WAF-D led to a significant decrease in standard metabolic rates, while LAS led to an increase. During combined exposure, metabolic depression was observed. Effects of pollutants were not amplified by higher temperature. This study highlights the need to reduce import of these pollutants and to avoid negative long-term effects on fish health.

Introduction

Coral reefs are increasingly under pressure due to the simultaneous impact of multiple environmental stressors. As a result of growing urbanization and industrialization in coastal areas, especially in many developing countries, coral reefs are degrading at an enormous speed. At least 19 % of reefs worldwide have been permanently lost (Wilkinson 2008) and over 60 % are considered at immediate risk from direct human activities (Burke et al. 2012). Coral reefs are of huge economic and environmental importance in many developing countries, supporting fisheries and tourist sectors and providing many different habitats with high productivity and diversity. About one third of all fish species worldwide occur in coral reefs and many pelagic fish of high fishing value need coral reefs as breeding grounds

(Crabbe 2009). Some of the most pressing stressors on coral reefs are local stressors such as eutrophication due to intense sewage and terrestrial run-off, increased sedimentation, pollution with toxic chemicals and overfishing as well as global stressors such as ocean warming. These stressors influence overall species abundances, as well as composition and diversity of coral reef communities.

Research into cumulative and interactive impacts of multiple stressors is still not very frequent (Crain et al. 2008) and even less so on coral reef fish. The intensity and diversity of anthropogenic stressors has however increased rapidly over the last decades, especially in the field of chemical stressors such as organic pollutants. Effects of multiple stressors have mostly been assumed to be additive (Halpern et al. 2007). However, current literature indicates that multiple stressors interact with each other and tend to have synergistic effects on communities meaning that the combined effect of multiple stressors is often worse than expected (Crain et al. 2008). This interaction can be a synergism (i.e. amplification) or an antagonism (i.e. reduction) (Dunne 2010).

In Jakarta, a megacity with around 25 Million inhabitants in the Greater Jakarta Metropolitan Area, multiple stressors have caused severe degradation of coral reef ecosystems within the bay (< 5 % cover) (Cleary et al. 2014, Baum et al. 2015). Jakarta Bay faces extreme eutrophication coupled with intense sedimentation (Baum et al. 2015). Several rivers with a combined catchment area of 2000 km2 discharge directly into the bay and transport large amounts of sewage and industrial effluents with high pollutant levels (Rees et al. 1999). Along the offshore island chain Pulau Seribu ("Thousand Islands"), a spatial patchwork of differentially degraded reefs is present along the islands as a result of localized anthropogenic effect, especially factors related to eutrophication (Rachello-Dolmen & Cleary 2007, Baum et al. 2015). With a total population of around 22,700 people, the island chain is densely populated (BPS 2012).

Along the islands and in Jakarta Bay (JB), numerous stakeholders are presently involved in fishing (around 40,000 fishermen, BPS 2012), sand mining, tourism and aquaculture and transport (Tanker). This has caused intensive boat traffic, both from smaller boats such as fishermen boats and from larger vessels and tankers. The major port Tanjung Priok in JB has now become one of the leading harbors in South East Asia and tanker routes go directly through the island chain (Bengen et al. 2006). Through the release of bilge and ballast water from boats, both from large tankers and small fishing boats, organic contaminants such as

polycyclic aromatic hydrocarbons (PAHs) can enter marine waters as part of the water accommodated fraction (WAF) of fossil fuels such as for example diesel used by boats.

Another ubiquitous pollutant class are surfactants. In untreated effluents, certain classes of surfactants can occur in concentrations that are toxic to aquatic organisms (Ankley & Burkhard 1992), ranging between 0.4 and 40 mg/L (Abel 1974). Anionic surfactants such as linear alkyl benzene sulfonates (LAS) are widely used as domestic detergents. LAS are quickly degraded in water and often found below detection limits, however high amounts of linear alkylbenzenes can be found after short-term exposure (washing of boats in reef areas) and in areas with extremely high population densities and lack of efficient sewage treatments. In JB high amounts of linear alkylbenzenes were detected, indicating that the bay receives very poorly treated sewage (Rinawati et al. 2012).

Local anthropogenic stressors such as the above mentioned organic chemicals are often accompanied with global stressors due to climate change which in combination result in enhanced vulnerability of the ecosystem (Risk et al. 2001, Knowlton & Jackson 2008, Pörtner et al. 2014). A global rise in sea surface temperature of up to 4.8 °C within this century has been predicted (IPCC 2013). Higher water temperature can enhance reaction rates and in turn increase the sensitivity of organisms to contaminants (Falahudin et al. 2012, Beyer et al. 2014).

Because of the key position of fish in many marine food webs and their economic importance, fish are suitable indicator species. Fish can be exposed to diesel-borne compounds and LAS in the water column (Logan 2007). Numerous studies have addressed biochemical responses of fish to hydrocarbons, either as WAF of fossil fuels (e.g. Agamy 2012, 2013) or as single PAHs (Baussant et al. 2001, Dos Santos et al. 2006) as well as to LAS (Zaccone et al. 1985, Lewis 1991) at the cellular level, however very few have looked at whole-body responses (e.g. Maki 1979, Christiansen et al. 2010, Davoodi & Claireaux 2007). Metabolic rates reflect the overall energetic requirements of an individual fish and thus detect overall stress levels, even when organisms are exposed to sublethal concentrations of contaminants. An increase in respiration can indicate acute stress and higher oxygen demand, while a decrease can either occur due to acclimation or depression due to the toxic effects of a stressor (Guppy et al. 1999).

Respirometry is a well-established and acknowledged method to estimate the metabolic rate and identify stress levels caused by pollutants and temperature stress in fishes (Schreck 1990). The standard metabolic rate (SMR) refers to respiration rates for basal physiological

processes in resting and unfed fish, while the routine metabolic rate (RMR) reflects respiration that includes energy for locomotion, digestion etc. (Sokolova et al. 2012). By measuring the maximal metabolic rate (MMR) under high stress, the aerobic metabolic scope (AMS), i.e. the energy that is available for fitness-related functions (Fry 1971), can be estimated as the difference to the SMR.

Organic pollutants are of growing concern to marine ecosystems due to their increasing presence close to large urban areas, their high number of different individual compounds and the high likelihood of interactive effects. Hence, future research should focus more on detecting interactive effects in order to predict changes in ecosystems such as coral reefs more accurately. Considering that of Indonesia´s 252 million inhabitants around 95 % live at the coast (Martinez et al. 2007), frequent use of organic pollutants all over coastal areas represents a significant pollution problem on a regional scale.

To our knowledge, there are no publications describing effects of diesel-borne compounds and LAS in combination with increased water temperature on fish metabolism. This study investigates in acute exposure experiments the potentially interactive effects of the two stressors WAF-D (water accommodated fraction of diesel) and LAS combined with increased temperature, on whole animal oxygen consumption rates in juvenile *Siganus guttatus* (Siganidae, Rabbitfishes), a common food fish in Indo-Pacific regions (Lam 1974). With regard to the short-term exposure of diesel-borne compounds and surfactants close to coral reefs with high boat traffic (bilge water discharge) and sewage run-off, this study aimed to determine how respiration rate of *S. guttatus* are affected by WAF-D and LAS a) in isolation and b) in combination as well as c) under increased temperature reflecting a global warming scenario.

Material and Methods

Experimental fish

Specimens of *S. guttatus*, collected along the Seribu Island chain situated north of Jakarta, were bought from the ornamental fish trader PT Dinar in Jakarta (http://dinardarumlestari.blogspot.de; 04.03.2015). All fish were juveniles with an average wet weight of 23.4 g ± 4.5. Two large semi-flow through cylindrical tanks (500 L) were used to acclimatize the fish for 14 days prior to experiments. 50 % of the water in the tank was changed daily with filtered sea water (0.2 µm). Water circulation within the keeping tank was

created by using two aquaria pumps (Hydor korallia, www.hydor.com). The water used for treatment tanks and for the experiments was obtained directly from a nearby reef and UV-sterilized, as well as treated with calcium hypochlorite solution before storage. The water parameters salinity, temperature, pH and dissolved oxygen (DO) were monitored daily in the morning using a WTW 340i Multiparameter system. Additionally, temperature data loggers (HOBO Pendants from www.onsetcomp.com) were deployed in all tanks to detect any daily fluctuations in temperature. All specimens were exposed to a constant 12 h light: 12 h dark cycle and were fed daily.

Experimental protocol

Fish were first exposed to either of three different treatments at 28 °C to resemble temperature conditions found in the reef: "control" or exposure to one of the two different pollutants linear alkylbenzene sulfonate "LAS" or the water accommodated fraction of diesel "WAF-D". These treatments were then repeated under a "global warming scenario" with three degrees above control temperature (31 °C): increased water temperature "temp" (31 °C) and a combination of either LAS or WAF-D with increased temperature; "LAS + temp" and "WAF-D + temp".

Salinity, pH and dissolved oxygen were measured at the start and end of each experiment using a WTW 340i Multiparameter system. Additionally a temperature data logger (HOBO Pendant from www.onsetcomp.com) was deployed in the glass aquarium. Temperature in treatments with higher temperature was maintained using Eheim Jäger 150W aquarium heaters (www.eheim.com). A 12 h light: 12 h dark cycle was adjusted to simulate the natural conditions. Each incubation chamber was shielded at the sides with a black plastic bag to prevent visual contact between fish. After each experiment, the entire experimental set-up was cleaned thoroughly with a mild hypochlorite solution followed by rinsing with fresh- and distilled water. All specimens were starved for 24 h prior to respirometry to remove any confounding effects of feeding on metabolic rate (Ross et al. 1992, Jordan & Steffensen 2007).

Respirometry

Automated intermittent-flow-through respiration runs (Fig. 2.1) were conducted with always three fish running in parallel replicates for each treatment. Experiments started at around

6:30 pm and ended at around 4:30 pm the following day (total duration: 16.7 h ± 0.5). Experiments were conducted at the Pulau Pari Research Station (5°51.756´S, 106°36.716`O) in a 100 L aerated glass aquarium containing four circular acrylic glass incubation chambers (total system volume per chamber: 885 mL) for parallel measurements of three individual fish and one blank measurement, respectively. The glass aquarium served both as a reservoir used for flushing the incubation chambers with oxygenated water and to equalize the temperature between the replicates.

Oxygen concentration within each incubation chamber was recorded continuously every 20 s using optical oxygen sensor spots and a 4-channel Firesting oxygen meter (www.pyro-science.com) calibrated prior to each experiment. A small pump (Eheim compact 300, www.eheim.com) ensured water flow within the chamber for the 10 min measurement phase, while a second pump (aquabee UP 300, www.aquabee-aquarientechnik.de) was set by a timer to flush the chambers after each measurement phase (10 min) with oxygenated water from the surrounding tank for 3 min (flush phase) (see Fig. 2.1). The experimental set-up was placed in a separate room to minimize human disturbances. The reservoir was aerated rigorously using several air stones to ensure high levels of dissolved oxygen. During respiration measurements, oxygen saturation was maintained above 85 % and water was re-oxygenated to ~95 % during the flush phase.
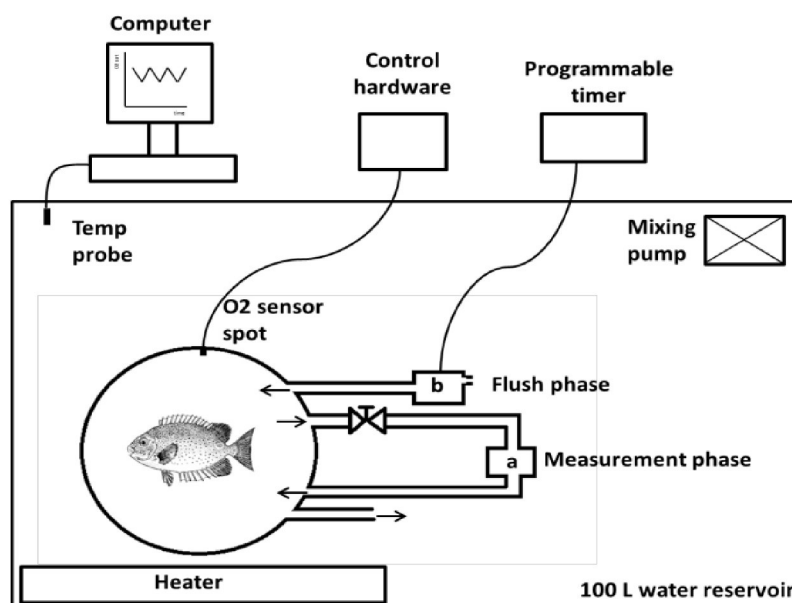
Fig. 2.1 Schematic illustration of the experimental set-up to determine whole-body oxygen consumption rates of *Siganus guttatus*. Oxygen concentration within the chamber was recorded continuously using a oxygen sensor spot. A small pump (a) ensured water flow within the chamber

for the 10 min measurement phase, while a second pump (b) controlled by a timer was set to flush the chamber with surrounding oxygenated water every 10 min for 3 min (flush phase).

Mass-specific whole body oxygen consumption rates by single fish (MO_2, mg O_2 g^{-1} h^{-1}) were calculated from the temporal decline in oxygen concentration (i.e. depletion rate = slope) for each single measurement phase. The first four hours were used for the fish to acclimatize to the new surroundings before MO_2 values were used for calculations of metabolic rates. In order to test for differences between day and night measurements, calculations were performed separately for each 4 hours during the night (00:00-04:00) and during the day (09:00– 12:00). Routine metabolic rate (RMR) was determined by averaging MO_2 values of overall measurement phases for day or night time measurement phases. Similar, the 15 % quantile method was used to estimate standard metabolic rate (SMR) (Chabot & Claireaux 2008, Franklin et al. 2013).

At the end of the experiment fish were removed from the chambers and subjected to a chase protocol (after Roche et al. 2003) where they were chased for 3 min with a net in a 100 L tank containing the same water conditions of the respective treatments followed by a 1 min air exposure. Fish were then immediately transferred back to the incubation chambers where MO_2 determination was ensured within 30 s after the end of air exposure. MO_2 was determined for another 8.8 +/-1.4 cycles until MO_2 decreased towards normal levels again. From these MO_2 values, maximum metabolic rate (MMR) was calculated by using the 85 % quantile method. AMS was then calculated as the difference between SMR during day and MMR for each specimen. A postblank (3-4 cycles) was run at the end of each experiment for each fish and bacterial respiration accounted for.

<u>Analysis of pollutants</u>

LAS:

Linear alkylbenzene sulfonate (LAS) was purchased from a local company in Jakarta (PT. Findeco Jaya, www.findeco.com) and stored at 4 °C until further usage. LAS-stock solutions (2 mg/L) were prepared daily and administered directly into the water of the experimental aquarium of each experiment respectively. 50 mL water samples for LAS analysis were taken at the start and end of each experiment (n = 3 for each). Samples were stored at 4 °C until further analysis. LAS was subsequently analyzed the same day spectrophotometrically (SQ 300 Merck Millipore Filterphotometer) using the methylene blue active substances

(MBAS) method modified after George & White (1999). For the assay, 0.2 mL methylene blue - solution (preparation of stock solution: 0.062 g boric acid + 37.5 mg methylene blue + 10 mL chloroform filled to 100 mL with saltwater (35 PSU)) were added to a 1 mL sample in a glass tube and vortex mixed 5 times for about 3 s. Then 6 mL chloroform were added, the solution vortex mixed for another 2 min and immediately afterwards the optical density of the chloroform layer measured at 665 nm wavelength (n = 4 absorbance measurements per sample). Calibration curves (8-point calibration with a concentration range between 0 – 4 mg/L) using LAS as standard and seawater from each experimental tank (i.e. to ensure that exactly the same salinity is used) were constructed for each treatment. All calibration curves followed linear functions with $r^2 \geq 0.97$.

To determine LAS contamination during short-term exposure, water from 1 m depth and in 1 m distance to a small boat was sampled directly after a fisherman cleaned his boat with soap. These tasks are common practice by local fishermen while they are anchoring at a reef (pers. observation). In addition, to reflect natural exposure conditions (sewage run-off), LAS surface water samples were taken in 1, 10, 50 and 150 m distance to the harbor at Pari Island. All samples were analyzed for LAS the same day.

WAF-D:

To reflect the local exposure with diesel-borne compounds, the water accommodated fraction of diesel (WAF-D) was prepared. The diesel used (Indonesian: "solar") was bought at a local gas station (state-owned Indonesian oil and natural gas corporation PT. Pertamina) and stored in a brown glass bottle. WAF-D- stock solutions were prepared for each experiment separately by weighing 5 g diesel on a precision scale (Sartorius ME 235 S) and adding it to 1 L of filtered sea water in a 1 L volumetric flask. The solution was capped, placed in the dark and mixed for 24 h on a magnetic stirrer, and allowed to settle for 5 min (as recommended by Singer at al. 2000). The lower phase (i.e. the water accommodated phase containing the water soluble PAHs) was then used as a pollutant for the experiments and the concentration of the EPA (US Environmental Protection Agency) PAHs determined to reflect WAF-concentrations (as superscription) (Netherlands National Water Board 2008, Christiansen et al. 2010). For each experiment 2 L WAF-D- stock solution were added to the experimental tank containing 100 L sea water. EPA PAH-concentration was determined in the diesel, in WAF-D - stock solution and in experiments (one sample from the start and end of each experiment, respectively).

For each sample 1 L were filtered (0.7 μm filter, VWR) and poisoned with 50 mL 2-propanol until further analysis. Samples were then pre-concentrated by solid phase extraction (SPE) by passing it through a preconditioned CHROMABOND® C18 PAH cartridge (6 mL, 2000 mg) by gravitation, following the elution of PAH´s from the cartridge using 5 mL dichloromethane. The dichloromethane was phase reduced to ˜1mL and 250 μl of dimethylformamid added as a keeper. As a procedure blank, bi-destillated water samples were prepared using the same procedure. The measured Σ EPA PAH-concentration in those blanks (15.3 μg/L) reflect the local background and were subtracted from the samples analyzed. Furthermore, as a method validation procedure, the standard addition method was applied by adding 100 μl of an EPA PAH standard with known concentration (Σ EPA PAH: 1ng/L) twice to selected samples (linear functions yielded an $r^2 \geq 0.99$). (see Table S2.1 for sample list). Additional reference surface water samples for PAH-determination were taken at sites within JB to reflect natural exposure conditions (see Fig. 2.2 for study area).
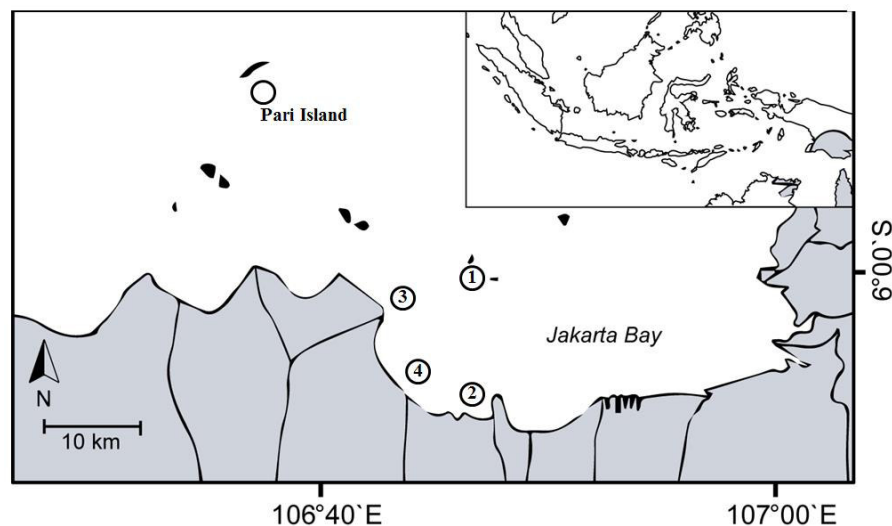
Fig. 2.2 Study area. Map includes sampling stations (1-4 and Pari Island) for PAH water samples reflecting natural exposure conditions in Jakarta Bay.

Furthermore, to determine PAH-contamination during short-term exposure (bilge water discharge), water from 1 m depth and in 1 m distance to a small fisherman boat (diesel driven) was sampled directly after bilge water that had accumulated at the bottom of the boat was dumped to the surrounding water. This is common practice by local fishermen while they are anchoring at a reef (pers. observation). EPA PAH-concentration was analyzed directly in the bilge water as well.

Table 2.1: Time program for excitation and emission wavelength of the fluorescence detector (FLD) for each detected compound: 15 different polycyclic aromatic hydrocarbons (PAH). The solubility in water (mg/L) for each PAH is given (ATSDR 2005).

Time [min]	Excitation wavelength [nm]	Emission wavelength [nm]	Detected Compound	Solubility [mg/L]
0	275	350	Naphthalene	31
			Acenaphthene	3.8
			Fluorene	1.9
			Phenanthrene	1.1
3.8	260	420	Anthracene	0.045
4.15	270	440	Fluoranthene	0.26
			Pyrene	0.132
4.7	260	420	Benzo(a)anthracene	0.011
			Chrysene	0.0015
5.2	290	430	Benzo(b)fluoranthene	0.0015
			Benzo(k)fluoranthene	0.0008
			Benzo(a)pyrene	0.0038
			Dibenzo(ah)anthracene	0.0005
			Benzo(ghi)perylene	0.00026
6.62	300	500	Indeno(1,2,3cd)pyrene	0.062

All samples (see Table S2.1 for full sample list) were prepared for PAH-determination the same day. Samples were transferred to Germany for further analysis at the Institute for Chemistry and Biology of the marine environment (ICBM) in Oldenburg, Germany. The analysis of 15 EPA-PAHs (acenaphthylene was not measured) was performed on a Waters ACQUITY UPLC (ultrahigh performance chromatography) system coupled to a FLD (fluorescence detector). A list of measured PAHs as well as their solubility in water (mg/L) (ATSDR 2005) can be found in table 1. For fluorescence detection, excitation and emission wavelengths were time programmed to receive the maximum sensitivity for each compound (c.f. Table 2.1). The PAHs were separated on a RP18 analytical UPLC column (ZORBAX Eclipse PAH Rapid Resolution HD 2.1 mm (I.D.) x 100 mm; 1.8 μm, Agilent) equipped with a C18 guard column (Vanguard 2.1 mm (I.D.) x 5 mm, Waters) at 35°C. The mobile phase

consisted of water (A), and acetonitrile/water (v/v, 9/1) (B) and was run at a flow rate of 0.35 mL/min with the following gradient elution program: starting with 50 % of (A) and 50 % of (B), the mixture had 35 % of (A) and 65 % of (B) after 2.9 min and 1 % of (A) and 99 % of (B) after 4.2 min. This final proportion was held for 2.9 minutes. Subsequently it was changed to 50 % (A) and 50 % (B) reached after 1.9 min (9 min runtime) and held for 1 minute. All single steps were performed with a linear mixing gradient. Column pressure was approx. 500 bar. The injection volume was 2 μl in all cases. Samples were analyzed directly or after dilution with acetonitrile (1+1 or 1+9, respectively).

The standard solutions for the 10 point calibration contained three different concentrations of distinct PAH groups each regarding different detection sensitivities, e.g. 100 ng/mL for naphthalene and acenaphthene, 20 ng/mL for fluorene, phenanthren, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, dibenzo(ah)anthracene and indeno(1,2,3cd)pyrene, and 10 ng/mL for benzo(k)fluoranthene, benzo(a)pyrene and benzo(ghi)perylene. The standard solutions used contained 100/20/10, 80/16/8, 60/12/6, 40/8/4, 20/4/2, 10/2/1, 8/1.6/0.8, 6/1.2/0.6, 4/0.8/0.4 and 2/0.4/0.2 ng/mL. All calibration curves followed linear functions with $r^2 \geq 0.98$.

Data treatment

Univariate statistics were performed with SigmaPlot 12.5. Differences between treatments and between day- and night- measurements for SMR, MMR and AMS values, respectively, were analyzed using one-way ANOVA. To test for any single or combined effects of the stressors LAS, WAF-D and temperature, two-way ANOVAs for SMR, MMR and AMS values, respectively, were performed. Data were checked for normality and homogeneity of variances in all cases. Significant differences were then compared pairwise with the post-hoc Fisher LSD test (in case of one way ANOVAs) and Tukey test (in case of two-way ANOVAs).

Results

Experimental water conditions

Water conditions (Table 2.2) of the keeping system and during experiments were similar to ambient reef conditions with 95-100 % oxygen saturation and a salinity of 33 PSU ± 0.3. The

water temperature in the keeping system (28.2 ± 0.5 °C) was similar to the control temperature treatments (27.4 ± 0.4 °C). High temperature treatments (31.2 ± 0.2 °C) were conducted 3.8 °C above control temperature treatments. No significant differences were detected for either DO, salinity or temperature for experiments within control and high temperature treatments, respectively.

Table 2.2: Physical water parameters in the fish keeping system and during the control and high temperature experiments, respectively. Dissolved oxygen (DO), salinity, pH and temperature were measured at the start and end of each experiment and once daily in the keeping system. Data are means ± SD.

Physical water parameters	Keeping	Experiments	
		Control temp	High temp
DO [% sat]	98.5 ± 2.7	102 ± 2.2	99 ± 1.7
Salinity [PSU]	32.9 ± 0.4	33.1 ± 0.7	33.6 ± 0.5
pH	7.9 ± 0.4	n.a.	n.a.
Temperature [°C]	28.2 ± 0.5	27.4 ± 0.4	31.2 ± 0.2

Concentrations of pollutants

Natural exposure conditions:

Measurements within the harbor at Pari island revealed a LAS concentration between 0.6-0.8 mg/L within the first 50 m, while directly at the reef (150 m distance to the harbor of Pari island) values were below the detection limit and thus considered to be zero (Table 2.3). Σ EPA PAHs concentrations varied within JB and at Pari island. While they revealed 70 - 385 µg/L within the bay, the water samples at Pari island contained 10.2 µg/L. All samples were on average mainly comprised of the PAHs phenanthrene (61.7 %), naphthalene (15.2 %) and fluorene (9.2 %), comprising together > 85 % of the 15 measured EPA PAHs (Table 2.3, Fig. 2.3).

Short-term exposure conditions:

Measurements taken directly after the washing of a boat revealed LAS concentrations of up to 5.7 ± 0.4 mg/L. Within 10 min however, values were diluted to 0.5 ± 0.3 mg/L. This

shows that enhanced LAS levels occur temporary (Table 2.3). Samples taken directly after bilge water discharge, revealed a Σ EPA PAHs concentration of up to 13374 μg/L. However, within 10 min, values fell to 294 μg/L due to dilution processes. In analogy to the LAS determinations locally elevated PAH-levels have to be anticipated temporary. The bilge water mainly consisted of the three PAHs naphthalene (31.7 %), acenaphtene (4.8 %) and fluorene (31.2%) and phenanthrene (32.3 %). The PAH composition between water samples taken 5 min (sample 1) as well as 10 min (sample 2) after bilge water was dumped into the water and the bilge water did not differ significantly, however sample 1 contained small amounts of pyrene, benzo(a)anthracene and chrysene even though these were not detected in the bilge water (Table 2.3, Fig. 2.3).

Table 2.3: PAH [Σ EPA, μg/L; n = 1 or 2] and LAS [mg/L; n = 3] concentrations during the experiments as well as under natural and short-term exposure conditions. Start and end measurements are given separately for each experimental treatment as well as for control and high temperature experiments. PAH concentrations reflecting natural exposure conditions were measured at different sites in Jakarta Bay (JB) and LAS concentrations in the harbor area at Pari Island. PAH concentrations reflecting short-term exposure conditions were measured after bilge water was dumped into the water and for LAS after the washing of a fisher boat. Data are means ± SD in case of n > 2.

Group	PAH [Σ EPA, μg/L]			LAS [mg/L]		
Experiments	Control temp	start	490 ± 96	Control temp	start	2.0 ± 0.1
		end	434 ± 176		end	1.6 ± 0.1
	High temp	start	1077.2	High temp	start	2.1 ± 0.1
		end	603.1		end	1.6 ± 0.1
	WAF-D	start	394	LAS	start	2.1 ± 0.1
		end	57.7		end	1.7 ± 0.1
	WAF-D + LAS	start	585.7	WAF-D + LAS	start	2.0 ± 0.1
		end	810.2		end	1.5 ± 0.1
	WAF-D + temp	start	1077.2	WAF-D + temp	start	2.1 ± 0.1
		end	603.1		end	1.6 ± 0.1
Natural exposure conditions	Jakarta Bay (JB) sites	Pari Island	10.2	Pari Island: Distance to harbor	1m	0.8 ± 0.1
		1	99.2		10m	0.8 ± 0.1
		2	384.6		50m	0.6 ± 0.1
		3	226.9	150 m (reef area)		0
		4	69.7			
Short-term exposure conditions	Time after bilge water discharge	30 s	13374.7	Time after washing of boat	30 s	5.7 ± 0.4
		5 min	733.3		5 min	1.3 ± 0.1
		10 min	294.3		10 min	0.5 ± 0.3
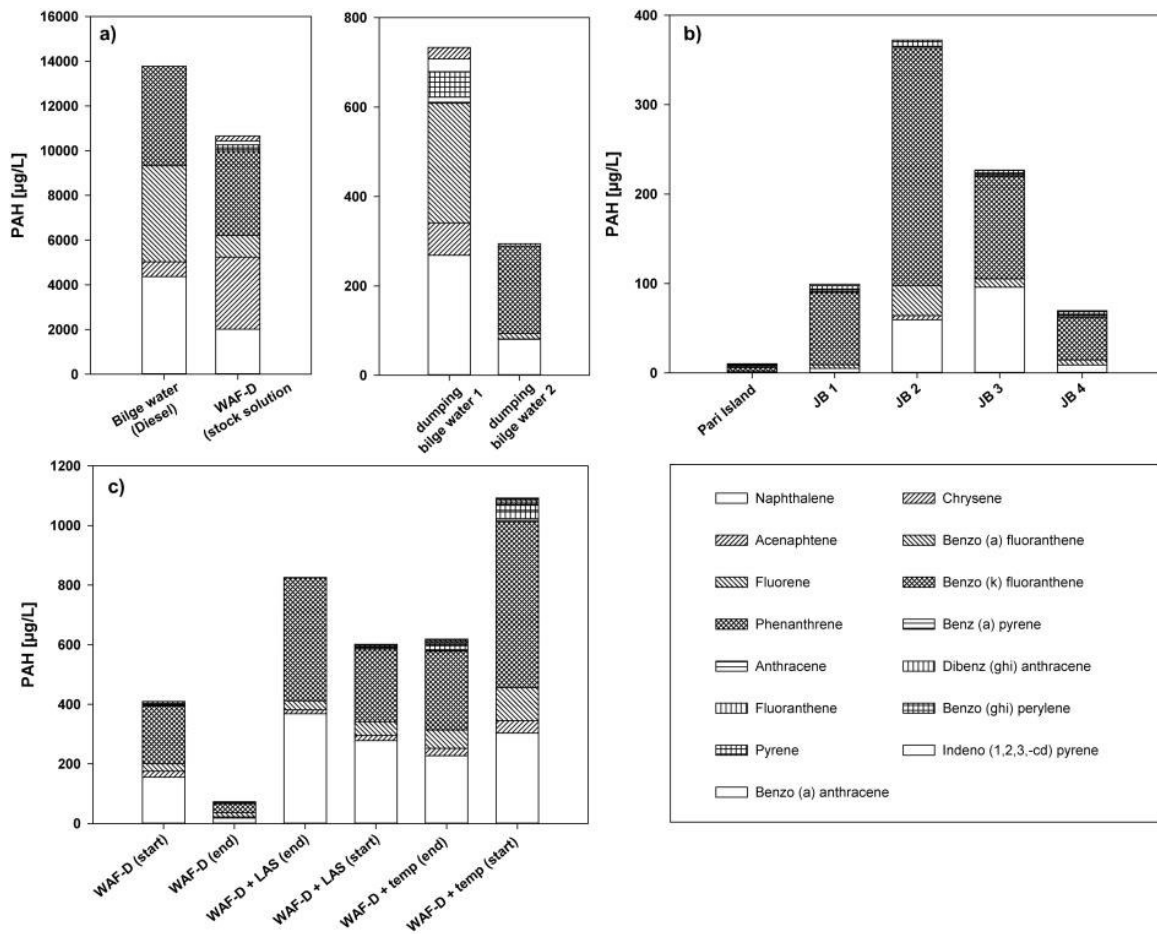
Fig. 2.3: Composition of polycyclic aromatic hydrocarbons (PAH) [µg/L] under a) short-term and b) natural exposure conditions as well as during c) the experiments. 15 EPA (US Environmental Protection Agency) PAHs were measured. PAH concentrations reflecting short-term exposure conditions were measured 5 min (dumping 1) and 10 min (dumping 2) after bilge water was discharged into the water. PAH concentrations reflecting natural exposure conditions were measured at different sites in Jakarta Bay (JB) (see Fig. 2.2). Start and end measurements are given separately for each experimental treatment. The PAH composition of the WAF-D stock solution used in the experiments is shown in a) as well.

Experiments:

LAS concentrations at the start of both the control and high temperature treatments were around 2 mg/L $\pm$ 0.05 mg/L and decreased by 20 % to 1.6 $\pm$ 0 mg/L at the end of the experiment. No differences were detected between high and control temperature treatments. Neither were any significant differences detected between the start and end concentrations among the treatments (Table 2.3). The Σ PAH concentrations in the experiments (mean: 588 µg/L) was above the total concentrations found in JB (natural exposure; mean =

195 µg/L) but falls within the range of levels found during the bilge water discharge (294-733 µg/L). The WAF-D stock solution contained 10653.41 µg/L Σ PAH and was mainly composed of the PAHs phenanthrene (35.2 %), acenaphtene (30.3 %), naphthalene (18.9 %) and fluorene (9.1 %), together accounting for > 90 %. This PAH composition did not differ significantly from the composition in the bilge water (p = 0.214). The four main PAHs found in the WAF-D stock solution together comprised 100 % of the total composition in the bilge water: phenanthrene (32.3 %), naphthalene (31.7 %), fluorene (31.2 %) and acenaphtene (4.8 %). The PAH composition between the WAF-D stock solution and any of the treatment samples was not significantly different. Mean concentrations of the fours PAHs in the experiments were: phenanthrene (45.5 %), naphthalene (36.3 %), fluorene (9.8 %) and acenaphtene (3.8 %) (Table 2.3, Fig. 2.3). Σ PAH concentrations were higher in WAF-D + temp and WAF-D + LAS treatments compared to the control. In the WAF-D treatment Σ PAH concentrations decreased by 85 % to the end of the experiment, in the WAF-D + temp treatment by 44 % and in the WAF-D + LAS Σ PAH increased by 38.5 %. The overall PAH composition in samples from the different treatments (both start and end samples) differed significantly (p = 0.028), however the post hoc Student-Newman Keuls test revealed only a significant difference between the treatments WAF-D + Temp (start) and WAF-D + LAS (end) as well as between WAF-D + Temp (start) and WAF-D (end). In addition, the PAH composition of the four main PAHs was not significantly different between start and end samples of the treatments, however in end samples, most low-molecular PAHs of the 15 EPA-PAHs were not present any longer (Table 2.3, Fig. 2.3).

Effect of WAF-D and LAS in isolation

Mean SMR values for the control were 163.5 ± 5.45 mg O_2 kg^{-1} fish h^{-1}. There were no significant differences in metabolic rates detected between day and night measurements, in any of the treatments (p > 0.05) for SMR and RMR rates (see Fig. 2.4). Therefore only total (day + night) values are discussed in the following (see Table S3). Mean SMR values differed significantly between treatments (p < 0.001). SMR decreased significantly during the WAF-D treatment to 132 ± 5.3 mg O_2 kg^{-1} fish h^{-1} (p 0 0.001) and increased significantly during LAS treatment to 208.6 ± 6.8 mg O_2 kg^{-1} fish h^{-1} compared to the control (p < 0.001). RMR values were in all cases slightly above SMR values and followed the same significant pattern (p = 0.006) as for SMR values. Mean RMR values for the control were

192.3 ± 14 mg O_2 kg^{-1} fish h^{-1}. Mean MMR and AMS values differed as well significantly between treatments ($p < 0.001$ and $p = 0.015$, respectively). However, neither AMS nor MMR of the WAF-D treatment were significantly different to the control and under LAS exposure a significant increase was only found in MMR rates. Mean MMR values for the control were 334.3 ± 117.5 mg O_2 kg^{-1} fish h^{-1} and for AMS values 171.8 ± 113.6 mg O_2 kg^{-1} fish h^{-1}, respectively (Fig. 2.4 for post hoc analysis, Table S3).
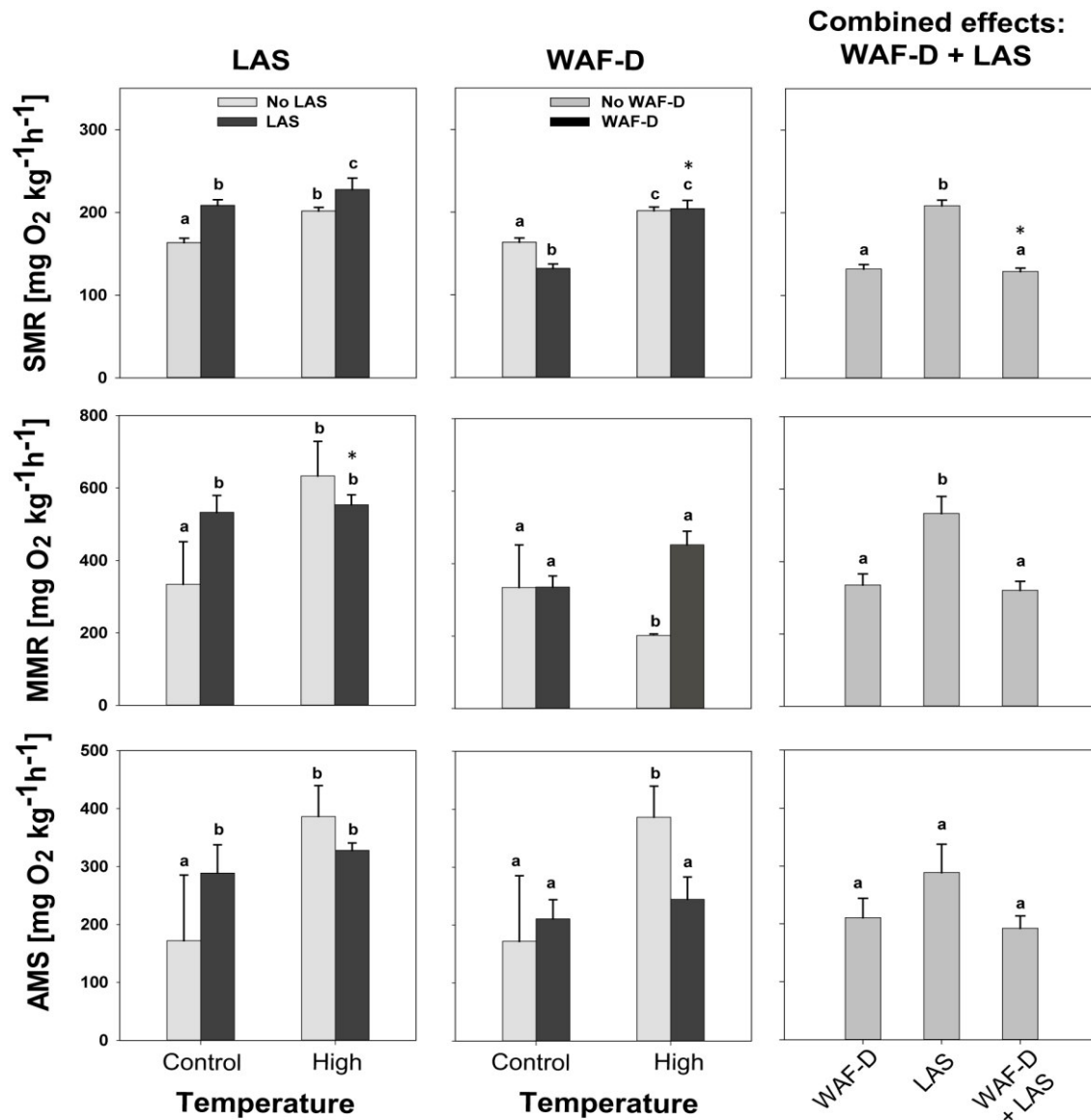
Fig. 2.4: Metabolic stress responses of *Siganus guttatus* to short-term exposure of a) LAS and b) water accommodated fraction of diesel (WAF-D) in isolation and in combination with high temperature respectively, as well as to c) LAS and WAF-D in combination. Metabolic rates are given as means ± SD in mg O_2 kg^{-1} fish h^{-1} (n = 3) at control and high temperature experiments: standard metabolic rates (SMR), maximum metabolic rates (MMR) and aerobic metabolic scope rates (AMS). Dissimilar letters in each of the plots represent a significant difference between treatments ($p < 0.05$, one-way ANOVA). Asterisks indicate significant interactions ($p < 0.05$; two-way ANOVA).

Effect of WAF-D and LAS in combination

SMR and RMR values under combined exposure of LAS and WAF-D were significantly reduced compared to the control ($p < 0.001$ and $p = 0.001$, respectively) but not significantly different to the WAF-D treatment ($p = 0.716$ and $p = 0.532$, respectively). A significant interaction for SMR ($p = 0.001$) and RMR ($p = 0.02$) was found for the LAS + WAF-D. This interaction was not significant for MMR and AMS values (Fig. 2.4, Table S2 and S3).

Effect of WAF-D and LAS in combination with temperature

Mean SMR values of 201.8 ± 4.4 mg O_2 kg^{-1} fish h^{-1} under the temperature treatment were significantly higher compared to the control ($p < 0.001$). The combination of WAF-D with high temperature revealed a significant interaction ($p = 0.007$). The combination of LAS with high temperature however revealed no significant interaction ($p = 0.146$). However, SMR values of the temp + LAS treatment (227.8 ± 13.7 mg O_2 kg^{-1} fish h^{-1}) were significantly higher compared to all other treatments ($p < 0.05$).

A similar trend was observed for RMR, MMR and AMS values with a significant increase during the temperature treatment compared to the control ($p > 0.05$). Significant interactions were found for the combination of temp and LAS ($p = 0.026$) and for temp and WAF-D ($p = 0.009$) for RMR values. There was a significant interaction detected for MMR values concerning the combined Temp + LAS treatment ($p = 0.04$), however not for the temp and WAF-D treatment ($p = 0.096$). A significant interaction for AMS values between either WAF-D or LAS with high temperature were not observed.

Mean MMR values of the treatments for temp (633.5 ± 4.4 mg O_2 kg^{-1} fish h^{-1}) and temp + LAS (554 ± 13.7 mg O_2 kg^{-1} fish h^{-1}) and LAS (533.2 ± 6.8 mg O_2 kg^{-1} fish h^{-1}) were significantly increased by 28 % compared to the control (mean: 334.3 ± 117.5 mg O_2 kg^{-1} fish h^{-1}) (Fig. 2.4, Table S2 and S3).

Discussion

Results from this study show that both LAS concentrations and diesel borne compounds such as PAHs, especially under short-term exposure conditions (i.e. bilge water discharge and washing of boats above reef areas) can show significantly increased levels in the JB area and

the Thousand Islands. Short-term exposure (16 h) to WAF-D and to sublethal concentrations of LAS significantly affected the metabolic condition of *S. guttatus*, with a decrease in SMR for WAF-D and an increase for LAS, respectively. Significant impairment in SMR can lead to trade-offs towards lower growth rates and reproduction efficiency (Calow 1991, Calow & Forbes 1998, Van Straalen & Hoffmann 2000, Logan 2007). Under combined exposure to both stressors, metabolic depression was observed as well. LAS led to a significantly higher PAH concentration in the water therefore suggesting that the effect of WAF-D (decrease in respiration) may have counteracted and neutralized the effect of LAS (increase in respiration). Results further show that a 3.8 °C increase in temperature (reflecting predicted global warming effects) may not necessarily cause further metabolic stress with regard to LAS and WAF-D toxicity. A synergistic, i.e. amplified reduced (WAF-D) or increased (LAS) change in metabolic rates was not observed. This study nevertheless highlights the need to reduce the import of these pollutants in coastal areas if long-term effects on fish health are to be avoided.

Pollutant concentrations

Results from this study show that both LAS concentrations and PAH concentrations (Σ EPA PAH) under both natural exposure conditions in JB and the harbor area at Pari Island as well as under short-term exposure conditions (i.e. bilge water discharge and washing of boats above reef areas) can show significantly increased levels.

Within the harbor area at Pari Island, LAS concentrations between 0.6 - 0.8 mg/L were found and after washing of boats up to 5.7 mg/L for short time periods. These values are far above the predicted no effect concentration of 0.12 mg/L for LAS in marine fish (Tattersfield et al. 1996, van de Plassche et al. 1999). Surfactants are ubiquitous, but often regarded harmless due to their high biodegradability and speculated ontrol concentrations in the environment (Rebello et al. 2014). However, many studies have reported highly increased LAS levels in marine environments, e.g. LAS was found in untreated sludge at high concentrations of up to 30,200 mg/kg dry weight (Berna et al. 1989), in surface waters at concentrations of up to 0.416 mg/L (Fox et al. 2000) and a few hours after massive discharges in the Gulf of Eilat of up to 5 mg/L (Shafir et al. 2014). Ivancovic & Hrenovic (2010) stated that LAS concentrations in untreated waste water close to reefs can reach 1.1 mg/L. Even though LAS is degraded relatively fast in seawater (half-life time (T50) for

LAS in seawater is 6 days (Shafir et al. 2014)), pollution with LAS should not be underestimated.

LAS concentrations at the start of both the control and high temperature treatments were around 2 mg/L $\pm$ 0.05 mg/L, thus within the range of observed LAS concentrations in JB and at Pari Island. Similar to natural degradation of LAS in seawater over time by bacterial communities (see review Rebello et al. 2014), LAS concentrations decreased on average by 21.9 % during the course of each experiment. During the high temperature treatments no significant increase in LAS degradation could be found, possibly since the 3.8 °C increase in temperatures were not enough.

The Σ PAH concentrations found in surface waters in JB (70-385 µg/L) and 10 min after bilge water discharge (294 µg/L) suggest that PAH contamination is significant in the area and could pose significant stress for fish (Logan 2007). Similar levels of the same 15 EPA PAHs were found in other parts of Indonesia in surface waters as well, e.g. Timor Sea: 54-213 µg/L (Falahudin et al. 2012) and Lampung Bay: 50-411 µg/L (Munawir 2007). PAH concentrations of up to 68.8 µg g^{-1} dry weight (PAH 15 EPA) (Falahudin et al. 2013) and 1252 ng g^{-1} dry weight (PAH 14 EPA) (Rinawati et al. 2012) have been reported for sediments in Jakarta Bay.

Bilge water discharge in the JB/Thousand Islands area should also not be underestimated, especially considering the extremely high population density and boat traffic in the area. In the Netherlands, a bilge water discharge of 22,889 m^3 had been estimated for 2006 (Netherlands National Water Board 2008). A similar high discharge could be assumed for the JB/Thousand Islands area.

Overall, the four PAHs phenanthrene, acenaphtene, naphthalene and fluorene dominated (> 80 %) the PAH composition in WAF-D and in the experiments as well as from samples collected in JB and after the bilge water discharge. In addition, the Σ EPA PAH concentrations in the experiments (mean: 588 µg/L) fell within the range of levels found during the bilge water discharge. Thus it can be concluded that WAF-D is suitable to reflect the local exposure with diesel-borne compounds. However, it should be noted that WAF-D contains a number of other water soluble substances which may have affected metabolic conditions of *S. guttatus* as well, i.e. diesel oil is commonly mixed with lubricating oil (grease), both contain sulphur (< 1.5 %) and mineral oil saturated hydrocarbons (MOSH) (Netherlands National Water Board 2008, EFSA 2012). Further experimental studies are

necessary to deduce individual effects of these other substances on metabolic performance of *S. guttatus*.

Similar to natural degradation of PAH in seawater over time, Σ EPA PAH concentrations decreased on average by 19.7 % during the course of the experiment. On the one hand, PAHs are naturally degraded by bacteria (see review Rebello et al. 2014), on the other hand temperature effects, especially during the high temperature treatments and surface effects may have occurred. The solubility of PAHs in water is enhanced three- to four-fold by a rise in temperature from 5 to 30 °C (Neff 1979). PAHs are non-polar, hydrophobic compounds, which do not ionize. As a result, they are only slightly soluble in water (ATSDR 2005). Especially larger PAHs disappear faster since PAH solubility in water decreases as the molecular weight increases. In addition, PAHs with a higher molecular weight have a stronger surface tension and stick to surfaces more easily. Therefore, during end samples of the experiments hardly any of the larger PAHs were found. Especially, obtained concentrations in samples for naphthalene have to be considered with caution due to its extremely low solubility in water and high evaporation at high temperatures.

Effects of WAF-D

Siganus sp. are herbivorous, diurnal fish (Lam 1974) and often found schooling in coastal sandy or muddy areas. Mean SMR values of 163.5 ± 5.45 mg O_2 kg^{-1} fish h^{-1} for the control fall within the range of other reported values for tropical coral reef fish, i.e. between 100 and 800 mg O_2 kg^{-1} fish h^{-1} (Nilsson & Östlund-Nilsson 2004, Nilsson et al. 2009).

The present study found a 19 % decrease in SMR in fish exposed to WAF-D compared to the control. Fish are able to take up the four main PAHs found in the WAF-D either through the gills during respiration (Baussant et al. 2001), the gut via ingestion of food, sediment and detritus or directly through absorption via the skin (Logan 2007). Once taken up, these PAHs are distributed in the body via the bloodstream (Logan 2007) and accumulate in fatty tissues such as liver and bile, where they are further oxidized, and which increases their water solubility and reactivity with enzymes (Pampanin & Sydnes 2013). Generally, PAHs are rapidly eliminated in fish, either through diffusion across the gills and skins or actively through biotransformation in the liver with an elimination rate of 2 to 33 days, depending on their alkylation degree (Jonsson et al. 2004).

The observed decrease in SMR during WAF-D exposure may indicate that oxygen limitation occurs for the fish (Calow 1991). Especially under long-term exposure, this could lead to mortality and reduced digestion, as well as reduced growth rates (Christiansen & George 1995, Barron et al. 2004). Similar narcotic effects have been reported by other studies. For instance, Christiansen et al. (2010) exposed polar cod to WAF of petroleum with PAH concentrations between 0.1 and 40 μg/L (Σ 16 EPA PAHs and priority PAHs naphthalenes, phenanthrenes and dibenzothiophenes) and observed reduced RMR rates after chronic (4 weeks) and acute (60 min).

The four main PAHs found in the WAF-D are known to be toxic, especially for phenanthrene and naphthalene a wide range of studies exists on physiological effects on fish (e.g. Jee et al. 2004, Sun et al. 2006, Oliviera et al. 2008). For instance, Dos Santos et al. (2006) found a narcotic effect of the PAH naphthalene on the oxygen consumption of the fish Trachinotus carolinus (Florida pompano) after chronic exposure (12 days) and high concentrations (300 μg/L). However they also found that the response was time and dose dependent, since oxygen consumption increased after short-term exposures (50 min) and lower concentrations (150 μg/L).

In this study, neither AMS nor MMR were significantly reduced under WAF-D treatment compared to the control. All of the fish regained relatively normal SMR values after the chase protocol within 1 to 2 hours, suggesting that WAF-D may not cause a severe long-term dysfunction of metabolism. Other studies have though reported effects on AMS by WAF of fossil fuels or oil, e.g. in the common sole, SMR was unaffected after exposure to WAF of petroleum but MMR was significantly depressed resulting in a reduced aerobic scope (Davoodi & Claireaux 2007).

Nevertheless, the underlying physicochemical processes responsible for the reduced SMR rates in *S. guttatus* observed in this study are far from clear and require further investigation. The wide range or reported metabolic responses in fish, shows that mechanisms of the toxicity on fish by oil related components are still poorly understood. For instance, many studies have reported tendencies to increasing oxygen consumption rates in fish after exposure to hydrocarbons, either as WAF (Correa & Garcia 1990, Davison et al. 1992) or as single PAHs (Brocksen & Bayley 1973, Anderson et al. 1974, Vargas et al. 1991) and attributed this to higher energy costs due to an overall increase in stress level and detoxification processes. Fish are able to metabolize and eliminate PAHs using various enzymes, however these processes differ for each individual PAH (Hahn et al. 1994). In

addition, metabolism often varies greatly between fish species, which may lead to species dependent PAH accumulation rates (Baussant et al. 2001, Jonsson et al. 2004).

Effects of LAS

Findings revealed that metabolic rates significantly increased by 28 % during short-term exposure to LAS (1.6-2 mg/L) compared to the control. Since surfactants reduce the water surface tension, they can penetrate mitochondrial membranes causing structural damage in the branchial respiratory epithelium (Engelhardt et al. 1981, Rosety-Rodriguez et al. 2002) which limits oxygen transfer (Agamy 2013). Aerobic processes are diminished and anaerobic oxidation takes place (Brage & Varesche 2014). Other studies have reported effects of LAS on respiration and metabolism e.g. increases in respiratory rate of bluegills fish (0.39 to 2.2 mg/L; Maki 1979).

Overall, LAS causes lowered functional capacity of various organs, such as gills and skin (Abel 1974), oxidative stress and mucus layer damage of fishes (Susmi et al. 2010) as well as disruption of chemoreceptors (Bardach et al. 1965). Zaccone et al. (1985) reported enhanced mucus production in epidermal cells and a decrease in activity of respiratory enzymes after exposure to sublethal concentrations of the anionic surfactant sodium alkylbenezensulphonate in catfish.

The observed increase in SMR rates during exposure with LAS confirms that even environmentally realistic concentrations may pose metabolic stress in *S. guttatus*. However short-term exposure with LAS did not cause a significant decrease in AMS in this study, suggesting that, similar to WAF-D, LAS may not cause a severe long-term dysfunction of metabolism. Nevertheless, under long-term exposure the increase in SMR may lead to significant impairment and trade-offs towards lower growth rates and reproduction efficiency (Calow 1991, Calow & Forbes 1998).

Combined effects of WAF-D and LAS

Under combined exposure of both LAS and WAF-D, SMR rates were significantly reduced compared to control. In general the solubility of PAHs is enhanced with increasing amount of dissolved and colloidal organic fractions such as LAS by incorporating the PAHs into micelles (Neff 1979). This means that in the presence of LAS, the concentration of

hydrocarbons increases temporarily in the water phase and as result their bioavailability (Middaugh & Whiting 1995). For instance Bajpai & Tyagi (2007) found that a laundry detergent concentration of 2 mg/L in the water can cause fish to absorb double the amount of chemicals than normally. Therefore adding for example dispersants to oil may create a more toxic solution than the non-dispersed oil (Papathanassiou et al. 1994, Ramachandran et al. 2004).

In this study, *S. guttatus* were exposed to a higher Σ PAH concentration in the WAF-D + LAS treatment compared to the WAF-D treatment. Therefore it could be postulated that the metabolic depression observed during the WAF-D treatment may have been even more severe during the WAF-D + temp treatment and neutralized/counteracted against the effect of LAS (increase in respiration). Agamy 2013 found that gills of juvenile *Siganus canaliculatus* showed the strongest histopathological response when exposed to dispersed WAF of light Arabian crude oil. Responses to only WAF or dispersant were less severe. The overall reduction in SMR during the WAF-D + LAS treatment was not stronger than during the WAF-D treatment, however a metabolic depression nevertheless occurred. In addition, all of the fish regained relatively normal SMR values after the chase protocol within 1 to 2 hours, suggesting that the contamination of WAF-D and LAS may not cause a severe long-term dysfunction of metabolism.

In the literature a vast range of reported effects of fish to WAF of fossil fuels/PAHs and LAS has been reported and understanding cumulative effects of both stressors is very difficult. Fish are a highly diverse group with various different life stages and behavioral patterns (i.e. different gill structures and respiration rates) which affect physiological responses (Neff 2002, Logan 2007). In addition, individual fitness of each fish influences responses. Furthermore, WAF-D is composed of many different water soluble substances that may affect *S. guttatus*. This may explain the observed often highly species-specific responses of fish to WAF of fossil fuels/PAHs and LAS.

Effects of WAF-D and LAS at high temperature

Under elevated temperature, the oxygen demand of fish increases and as result their standard and routine metabolic rate as well (Pörtner & Knust 2007). This was also observed in this study for *S. guttatus*. A reduction in aerobic scope (AMS) for *S. guttatus* under increased temperature however could not be observed as has been reported for other coral reef fish at

elevated water temperatures (31- 33 °C) compared to controls (29 °C) (Nilsson et al. 2009). At higher temperatures, the circulatory and ventilatory systems cannot keep up with the increased oxygen demand which then leads to the reduction in AMS (Fry 1971, Pörtner & Knust 2007). *S. guttatus* may have a higher tolerance towards increased temperatures. Nilsson et al. (2009) varying thermal tolerance levels found for different reef fish species.

Higher temperature has shown to increase the uptake or detoxification rate of contaminants (Beyer et al. 2014). For instance, an elevated toxicity of metals at higher temperatures has been reported and metal uptake and accumulation increases with increasing temperature (Cairns et al. 1975, McLusky et al. 1986). Under the combined exposure of high temperature and LAS, SMR rates were higher than during either high temperature or LAS exposure alone, suggesting an increased uptake rate. However no significant interactive effect (no synergism) could be found, i.e. higher temperature did not increase the negative effect of LAS and cause even more metabolic stress.

The combined exposure of both high temperature and WAF-D however had a significant interaction effect on SMR. WAF-D did not cause a decrease in SMR rates under high temperature. This may suggest possibly increased detoxification rates of the components of WAF-D under higher temperature. Thus in both cases, LAS and WAF-D exposure, an increase in water temperature as expected from global warming, may not necessarily cause further metabolic stress with regard to LAS and WAF-D toxicity.

Conclusion

In this study, short-term exposure of sublethal concentrations of both WAF-D and LAS significantly caused metabolic stress in *S. guttatus*. Even though metabolic rate analysis does not give exact answers to the physiological mechanisms disrupted by the pollutants, it gives answers to the severity, since it is an indicator at the level of whole organisms with implications for populations and communities (Johns & Miller 1982). Further studies looking at trade-offs of energy allocated to detoxification processes, as well as at the underlying detoxification mechanisms using molecular indicators represent complex topics for future research (Logan 2007). In addition, further experiments are needed to determine lethal threshold values of the pollutants in *S. guttatus*, and by varying exposure times and increasing measurement intervals, additional information on the risks of low concentrations during long-term vs. high concentrations during short-term exposure can be gained. For instance,

specific experiments could be designed to simulate bilge water discharge and washing of boats by e.g. exposing fish once an hour for a few minutes.

Even though both LAS and diesel-borne pollutants such as PAH are degraded naturally in marine waters in relatively short time periods (Ivancović & Hrenović 2010, Pampanin & Sydnes 2013), large-scale sewage run-off from densely populated islands and high boat traffic in the JB/Thousand Islands reef complex, suggest that these two pollutants pose a significant threat to reef organisms. For instance, assuming 10.000 boats of varying size in the area which at least once a day dump their bilge water into the water, the likelihood that fish are exposed to diesel-borne pollutants possibly even several times a day, is very high and may therefore constitute a regional problem rather than a local problem. The long-term effects on fish metabolism have to be studied. Considering that short-term exposure of WAF-D and LAS can cause significant changes to standard metabolic rates in fish, trade-offs towards lower growth rates and reproduction efficiency are highly likely, which eventually will lead to reduced fish catches. Ultimately a negative feedback to human livelihoods and food security may be the consequence, not only in local areas but also regional areas considering the frequency and area exposed to these pollutants.

Indonesia´s continuing growth in population, especially along the coast, poses severe problems for ecosystems. The installation of effective sewage treatment plants, use of green surfactants (new class of biodegradable and biocompatible products; Patel et al. 1999, Rebello et al. 2014) and stricter guidelines for industrial waste could significantly reduce pollutant levels in the water (Clara et al. 2007). Similar, large tanker routes should not pass by close to islands with coral reefs, as is presently the case in the Thousand Islands. Parallel, marine awareness and local education campaigns could help to change the washing habits of local fishermen and reduce WAF-D and LAS pollution in the region. Without a better understanding of impacts of combined stressors on marine organisms and underlying mechanisms of WAF-D/PAH and LAS toxicity, these mitigation efforts and management strategies such as marine planning and conservation are however void.

Acknowledgements

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Chapter 3: Coral Response to Pollutants and Temperature
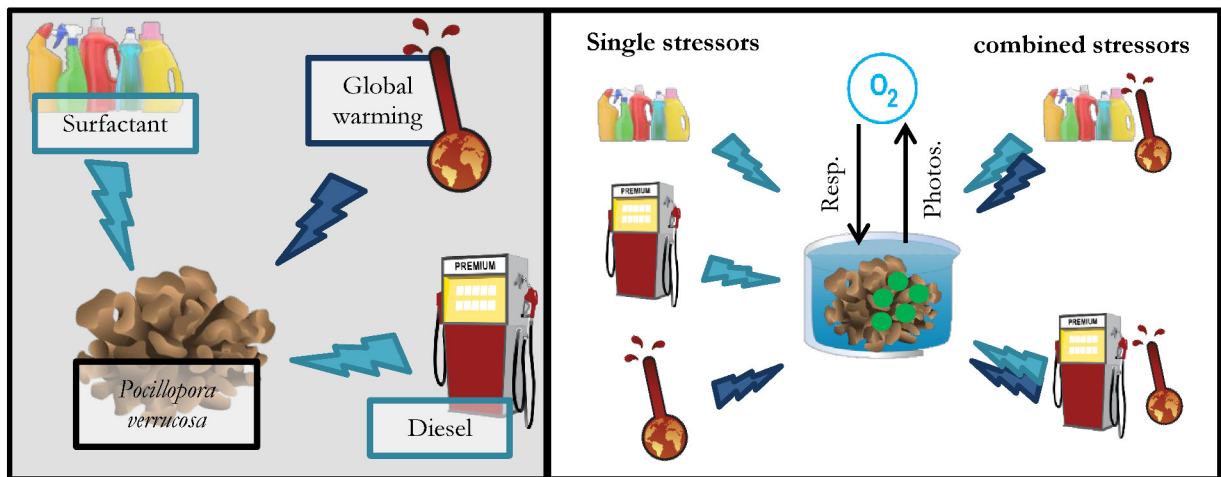
Chapter 3: Coral reef organisms frequently face local and global stressors. Laboratoy experiments were performed to investigate the effect of local stressors (surfactant and diesel fuel pollution) and global warming on *Pocillopora verrucosa* metabolism. Dark respiration and maximum quantum yield of photosynthesis were measured in corals subjected to either single or combined stressors.

Physiological response of the hard coral *Pocillopora verrucosa* from Lombok, Indonesia, to two common pollutants in combination with high temperature

Kegler P, Baum G, Indriana LF, Wild C, Kunzmann A

Abstract

Knowledge on interactive effects of global (e.g. ocean warming) and local stressors (e.g. pollution) is needed to develop appropriate management strategies for coral reefs. Surfactants and diesel are common coastal pollutants, but knowledge of their effects on hard corals as key reef ecosystem engineers is scarce. This study thus investigated the physiological reaction of *Pocillopora verrucosa* from Lombok, Indonesia, to exposure with a) the water-soluble fraction of diesel (determined by total polycyclic aromatic hydrocarbons (PAH); 0.69 ± 0.14 mg L^{-1}), b) the surfactant linear alkylbenzene sulfonate (LAS; 0.95 ± 0.02 mg L^{-1}) and c) combinations of each pollutant with high temperature (+3 °C). To determine effects on metabolism, respiration, photosynthetic efficiency and coral tissue health were measured. Findings revealed no significant effects of diesel, while LAS resulted in severe coral tissue losses (16-95 % after 84 h). High temperature led to an increase in photosynthetic yield of corals after 48 h compared to the control treatment, but no difference was detected thereafter. In combination, diesel and high temperature significantly increased coral dark respiration, whereas LAS and high temperature caused higher tissue losses (81-100 % after 84 h) and indicated a severe decline in maximum quantum yield. These results confirm the hypothesized combined effects of both diesel and LAS with high temperatures. Our study demonstrates the importance of reducing import of these pollutants in coastal areas in future adaptive reef management, particularly in the context of ocean warming.

Introduction

With growing human populations, the anthropogenic influence on coastal ecosystems is increasing. Halpern et al. (2008) found that no marine areas are unaffected by anthropogenic influences and 41 % are even strongly affected. About 275 million people live in close vicinity to coral reefs and most of them depend on their ecosystem services for their

livelihoods (Burke et al. 2012). One third of the world's coral reefs are located in the Coral Triangle in the Indonesian/Philippines Archipelago (Burke et al. 2012), where hard coral cover declined significantly within the past decades due to a multitude of global and local stressors (i.e. factors that are diverging from the natural conditions) (Bruno & Selig 2007). About 85 % of all reefs within the coral Triangle are threatened by local stressors, up to 90 % in combination with global stressors (Burke et al. 2012). Global stress is generated by climate change, which is usually accompanied by local anthropogenic drivers, such as overfishing, pollution, sedimentation and eutrophication, which in combination result in enhanced vulnerability of the ecosystem (Knowlton & Jackson 2008, Pörtner et al. 2014, Risk et al. 2001). Global sea surface temperatures are estimated to increase up to 4.8 °C within this century (IPCC 2013). Low variances in surface temperature in tropical regions such as Southeast Asia, where organisms live already close to their upper thermal limits, leave organisms there more susceptible to climate change (Maina et al. 2011, Lesser 2013). Bleaching of corals due to a breakdown of the symbiosis between corals and their symbiotic algae is strongly associated with high sea surface temperatures, as shown in field and laboratory studies (Fournie et al. 2012, Hoegh-Guldberg et al. 1999, Wild et al. 2011). The majority of studies concerning stressors on coral reefs have focused on ocean acidification and global warming associated coral bleaching. Several studies have investigated combined effects where stressors can have additive, synergistic or antagonistic effects, and it is important that we understand these in order to develop appropriate management strategies for coral reefs (Beyer et al. 2014, Wilson et al. 2006). Synergistic effects have been found for example between temperature and light stress on photosynthesis in corals (Bhagooli & Hidaka 2004), while increased CO_2 levels and temperature had antagonistic effects (Reynaud et al. 2003).

One important pollutant in the ocean is diesel, used to fuel machines and ships all over the world. Although diesel is not the only source of oil pollution (there are many others, both from anthropogenic, as well as natural sources, see Ocean Studies Board and Marine Board 2003), it is a very important one. In 2012 each day over 3.5 billion liters of motor fuels were consumed all over the world and while an effort is made to reduce this number, in growing countries like in the coral triangle region, there was a steady increase in diesel consumption over the past decade (e.g. in Indonesia from 40 million L d^{-1} to 84 million L d^{-1}) (US Energy Information Administration). Diesel is introduced to the environment via oil spills from ships and harbors, from discharge of routine tanker operations and from municipal and urban runoff (Isobe et al. 2007, Santos et al. 2010). Among the water soluble constituents in

diesel, polycyclic aromatic hydrocarbons (PAHs) pose the highest threat to the environment (Haapkylä et al. 2007, Santos et al. 2013). Several studies have discovered toxic effects of PAH on aquatic organisms, mainly fish (Logan 2007, Simonato et al. 2007, Vanzella et al. 2007, Santos et al. 2010). A range of physiological responses to oil pollution by corals, depending highly on the type of oil used, were found in previous studies, including growth impairments, mucus production and decreased reproduction (for an overview see Haapkylä et al. 2007). But most of these studies were performed before 1990 investigating effects of large oil spills and due to the many different types of oils, concentrations and durations it is hard to compare the results.

Another group of frequently used chemicals that regularly end up in the ocean are surfactants which are applied by households and industry in large quantities in detergents and soaps. In 2003 18.2 billion kg of surfactants were used all over the world (Chupa et al. 2007). Linear alkylbenzene sulfonate (LAS) is one of the most common surfactants in use (Ivanović & Hrenović 2010), the consumption of LAS alone in 2003 was 2.9 billion kg (Chupa et al. 2007). Although to some extent surfactants are eliminated from water by biodegradation within a few hours up to several days, significant proportions of surfactants attach to suspended solids and remain in the environment (Lara-Martín et al. 2010). This sorption of surfactants onto suspended solids depends on environmental factors, such as temperature, salinity or pH (Lara-Martín et al. 2010). The important role of water temperature in combination with pollution is mainly due to enhanced reaction rates at higher temperatures, which leave organisms more sensitive to chemicals (Falahudin et al. 2012, Beyer et al. 2014).

Indonesia, the country with the largest area of coral reefs within the coral triangle, has a large and growing number of human settlements clustered along the entire coastline in close vicinity to coral reefs, and in most cases no waste water treatment is occurring (Isobe et al. 2007, Burke et al. 2012). In areas without sufficient sewage- and waste water treatments, concentrations reaching 1.1 mg L^{-1} of LAS and 0.2 mg L^{-1} of PAH from diesel can be entering the reefs (Ivanović & Hrenović 2010, Falahudin et al. 2012). Thus, there is a continuous contamination of coastal waters with these two very commonly used pollutants, turning the local stressor into a regional threat.

While several studies have investigated responses to contaminants on the cellular level, it is important to understand the effects of stressors on physiological performance on the whole-organism level (Maltby 1999). Metabolic rates are indicators of the overall energy budget of

organisms and can indicate non-lethal stress responses (Porter et al. 1999). Metabolism of the coral holobiont (Rohwer et al. 2002, Bourne et al. 2009) includes host and symbiont respiration, as well as symbiont photosynthesis and metabolic energy is needed among other processes to transport calcium to the host skeleton (Beer et al. 1998, Al-Horani et al. 2003). Kaniewska et al. (2012) showed that effects in coral physiology become apparent before any changes in calcification processes can be detected. Thus, measurements of respiration in combination with photosynthesis are a common method in coral physiological research (Lesser 2013). An increase in respiration can indicate acute stress, while a decrease can indicate either an acclimation or depression due to a stressor (Guppy & Withers 1999). To investigate photosynthetic capacity, the quantum yield of linear electron transport is a useful tool to determine coral health and serves as a diagnostic tool for the analysis of pollutants (Jones et al. 1999, Lesser 2013). Further, the ratio of photosynthesis to respiration (P:R) provides an estimate, whether a coral can live on the energy obtained from its zooxanthellae (Coles & Jokiel 1977).

To our knowledge, there are no publications describing effects of the pollutants diesel and LAS combined with high temperature on coral metabolism. This study investigates this potentially interactive effect on the physiology of a tropical reef coral *Pocillopora verrucosa* in acute exposure experiments. *P. verrucosa* is common in the study area and therefore a good representative of the scleractinian corals that are of vital importance for coral reefs. The main objective was to determine if and how the coral is affected by pollutants in isolation and combination with increased temperature. Special focus was put on whether there are combined effects between the pollutants and high temperature, because their simultaneous occurrence in the reef is likely. Oxygen consumption and photosynthetic activity were chosen as response parameters, to determine the response of the coral holobiont metabolism. The hypothesis is that the metabolism of *P. verrucosa* will be negatively affected by both pollutants and that there will be combined effects with temperature.

Material and Methods

Coral sampling and rearing

Pocillopora verrucosa fragments of approx. 5 cm height (average surface area ± SD: 112 cm^2 ± 29 cm^2) were sampled using Scuba diving at two sites (S 08°20.259', E 116°02.260' and S 08°21.768', E 116°01.897') during 4 days in July 2013 on Gili

Trawangan north of Lombok, Indonesia. Both sites were similar in reef habitat, environmental conditions and measured physical water parameters. The research permit for the study area was approved by the Indonesian ministry for research and technology (RISTEK, permit no. 176/SIP/FRP/SM/V/2013). Two fragments each from a total of 60 colonies were sampled from the two sites combined. All fragments were glued onto 5x5 cm ceramic tiles directly after sampling and brought to a rearing station located in the reef in front of the sampling island (S 08°20.750', E 116°02.608'). They were left in the reef to recover from sampling for two weeks. Water parameters (salinity, temperature, pH and dissolved oxygen) at all sites were measured before and after each dive using a Eureka Manta 2 multiprobe (Eureka Water probes, Austin, USA) and water samples were taken for environmental LAS and PAH determination. After two weeks all healthy coral fragments (two fragments each from 42 colonies) were taken to a laboratory of the Indonesian Institute for Science (LIPI) at Pemenang, Lombok. Fragments were placed in a 600 L semi-flow-through outside tank, located in a larger pool to buffer temperature fluctuations during the day. Water flow through the tank was adjusted that the entire water volume was exchanged once a day with fresh water from the reef, supplied by a pump ca. 200 m away from the shore. Water circulation within the rearing tank was created by using two circulation pumps (Hydor korallia, Hydor Ind., Sacramento, USA). Light conditions at the sampling sites were measured using a LI-1400 data logger with a Li-192 underwater quantum light sensor (LI-COR Biosciences, Lincoln, USA). They ranged from 90 to 500 μmol quanta m^{-2} s^{-1} and averaged around 200 μmol quanta m^{-2} s^{-1} during midday, therefore were adjusted to these intensities in the rearing system by shading the tank from direct sunlight. Corals received additional heterotrophic feeding with designated coral food (Coral V Power by Preis Aquaristik, Bayerfeld, Germany) once a day. Temperature, salinity, pH and dissolved oxygen were monitored daily, always at the same time, using a WTW 340i Multiparameter system (WTW GmbH, Weilheim, Germany).

Experimental protocol

Four main experiments were performed with four replicates for each treatment (see Table 3.1). Corals from the two sampling sites were randomly chosen for the different experiments. Treatments were control, increased water temperature, diesel and combination of diesel with increased temperature. For the control treatment the same protocol as for the other treatments was applied, but neither pollutant nor high temperature were applied. Two

additional experiments were performed with the surfactant LAS (linear alkylbenzene sulfonate) alone and in combination with increased temperature. A reduced experimental protocol was used for these two treatments, where no respiration, but only photochemical yield was measured. Temperature was increased using Eheim Jäger 150W aquarium heaters (Eheim GmbH & Co. KG, Deizisau, Germany). The control temperature was adjusted to 28 °C, to resemble the temperature measured in the reef. Increased temperature was 31 °C, three degrees above the control temperature. Diesel was bought at a local gas station and a water accumulated fraction (WAF) (Singer et al. 2000, Simonato et al. 2008) was produced from 5 g diesel in 1 L of filtered seawater. This solution was stirred for 24 h, then left to settle for 20 min before the lower phase with the waterborne diesel constituents was retrieved. 490 mL of this 0.5 % WAF were immediately administered to each tank at the start of the experiments. LAS was purchased from a local supplier in Indonesia (PT. Findeco Jaya, www.findeco.com) and stored at 4 °C. For each experiment 190 µL LAS were administered to each tank, resulting in a final concentration of 0.00019 %.

Table 3.1: List of all treatments administered to *Pocillopora verrucosa* during the study.

Treatment	Description
Control	control reef temperature ~28 °C, without pollutant addition
High Temperature	+3 °C, without pollutant addition
Diesel	490 mL of 0.5 % water accumulated fraction (WAF) of diesel
LAS	190 µL Linear alkylbenzene sulfonate
Diesel and Temperature	490 mL of 0.5 % WAF, +3 °C
LAS and Temperature	190 µL, +3 °C

All treatments were applied for a total of 84 h; first corals were subjected to 48 h of pre-treatment without measurements to increase the exposure time of the corals to the stressors. This was followed by 36 h of continuous oxygen measurement, during which also analysis of photosynthetic yield took place. During the pre-treatments the tanks were subjected to natural daylight adjusted to the same intensities as in the rearing tank, while the measurement

tanks were artificially illuminated from 7:00 to 19:00, using a 2x20 W aquarium light (MW1-Y20X2 from Guangdong Zhenhua Electric Appliance Co. Ltd, Zhonhshan, China). The light intensities were 60 µmol quanta m^{-2} s^{-1} and a 12 h light: 12 h dark cycle was adjusted to simulate natural conditions. This difference in light intensity compared to the rearing and pre-treatment period occurred due to practical reasons of the laboratory set up, but were the same for all experiments. Pre-treatments always started in the evening and lasted for 48 h during which no heterotrophic feeding of the corals took place. Prior to the measurement phase all epiphytes and debris were removed from the coral fragments and the tiles. Then the fragments were moved to the respiration set-up (see Fig. 3.1), which was prior to the start of measurements filled with filtered seawater and the same stressors as in the pre-treatment tanks.
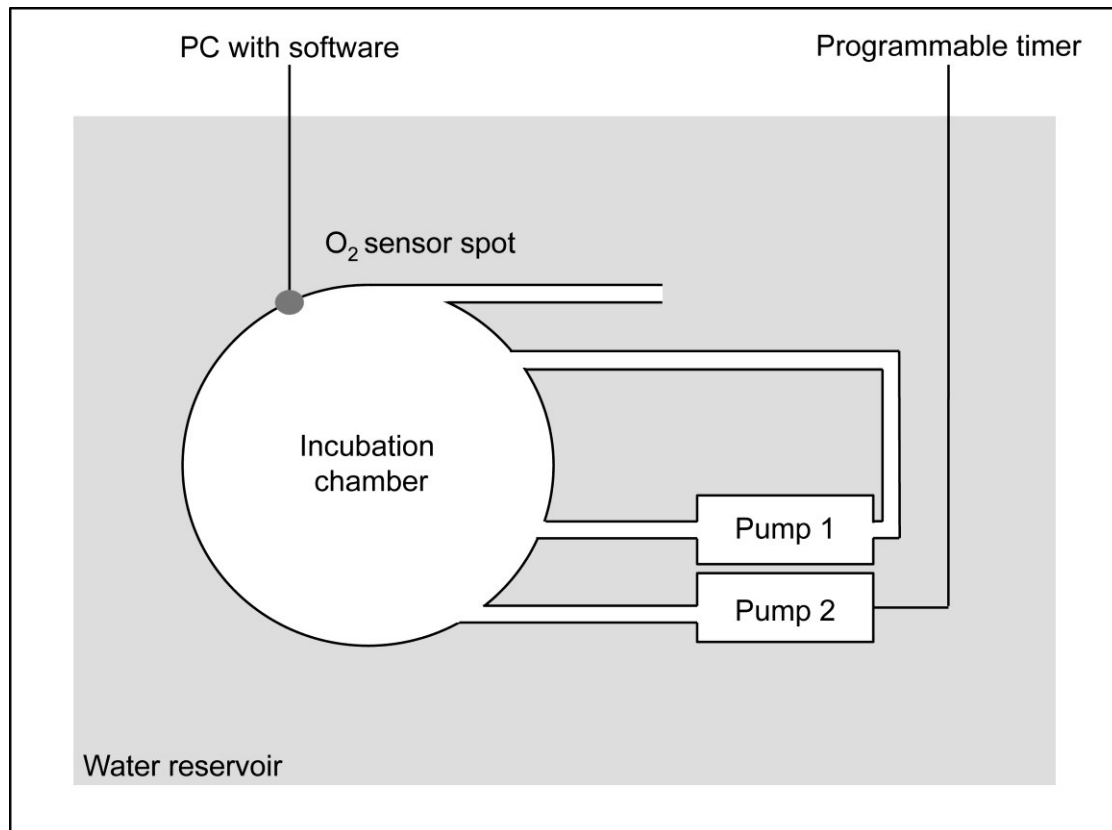
Fig. 3.1: Schematic drawing of the experimental setting. Oxygen concentrations within the incubation chambers were continuously monitored using a sensor spot and optic fiber cable. A recirculation pump (pump 1) was used to ensure continuous water flow within the chamber, while Pump 2 was turned on at programmed intervals to flush the chamber with oxygenated water from the surrounding water reservoir. Four incubation chambers were placed in the reservoir simultaneously, which served also as a temperature control.

Physical water parameters were measured every day for each pre-treatment and measurement phase, using a WTW 340i Multiparameter system (WTW GmbH, Weilheim, Germany). Water samples from pre-treatment and measurement tanks were analyzed for pollutants as described below. After each experiment the entire experimental set-up was cleaned with 70 % Ethanol and rinsed with distilled water to remove bacterial contamination. Surface area of the coral fragments was determined using the aluminum foil method described by Marsh (1970). In this method pieces of aluminum foil are fitted closely to the coral skeleton and the weight of these pieces is compared to a calibration curve with aluminum foil pieces of known size. After each experiment all fragments were photographed from two sides and the pictures analyzed using Image J software (v1.47, National Institutes of Health, Bethesda, USA) to determine the amount of tissue loss during experiments.

Respirometry and PAM fluorometry

Measurements of oxygen fluxes took place in acrylic incubation chambers. To provide stronger oxygen fluxes and reduce the error due to individual variance, two fragments from one colony were measured in the same incubation chamber. Four of these chambers were situated within a 100 L tank, which served both as a reservoir used for flushing the incubation chambers with oxygenated water and to equalize the temperature between the replicates. All chambers were connected to a pump ensuring water circulation within the chamber and to a flush-pump, supplying oxygenated water from the surrounding water bath at programmed time intervals using a custom build timer (see Fig. 3.1 for details). 30 min measurement periods were followed by 3 min flush periods to allow for continuously high oxygen levels (>90 % saturation) within the chambers. During the entire measurement phase oxygen content within the incubation chambers was recorded using optical oxygen sensor spots and a 4-channel Firesting oxygen meter with the associated software (Oxygen Logger v.3.12.4, Pyro Science GmbH, Aachen, Germany). The system was calibrated prior to each experiment. After the coral fragments were removed from the incubation chambers blank respiration in the system was measured for another 1.5 h to determine bacterial respiration at the end of the experiment. Oxygen fluxes from respiration and photosynthesis were calculated as described below. Photosynthetic capacity was determined by measuring the chlorophyll fluorescence of photosystem II (PS II), using a pulse-amplitude modulated fluorometer (DIVING-PAM, Heinz Walz GmbH, Effeltrich, Germany). Maximum quantum yield (F_v/F_m) (Walz 1998) was measured in the beginning and end of the measurement phase

in corals that were dark-adapted (1 h at the beginning of the experiment, approx. 10 h at the end of the experiment as it took place following the dark measurements). All fragments were measured three times with approx. 5 min between each measurement.

LAS and PAH determination

Water samples from each experiment were taken at the beginning and end of each pre-treatment and measurement phase. To quantify the diesel WAF within the samples total polycyclic aromatic hydrocarbons (PAHs) were determined, which are among the major soluble toxic constituents in diesel and a practical measure for the toxicity of a PAH mixture (Logan 2008). Samples for PAH determination were filtered (0.7 μm filters, VWR International, Radnor, USA) and stored with 2-propanol (50 mL) before pre-concentrating them using solid-phase extraction (SPE) by passing it through a CHROMABOND © C18 PAH cartridge (6 ml, 2000 ng; Macherey-Nagel GmbH & Co. KG, Düren, Germany) and elution of the PAHs with 5 mL dichlormethane from the cartridge. The dichloromethane was evaporated to 1 ml and 250 μl of dimethylformamid was added as keeper. For analysis of total EPA-PAH concentrations Ultra performance liquid chromatography (UPLC) was performed at the Institute for Chemistry and Biology of the Marine Environment (ICBM) in Oldenburg, Germany. A methodological quality control was performed, using a deuterated internal standard and the standard addition method. For LAS analysis triplicate samples of 50 mL were taken and stored at 4 °C until further analysis. All LAS determination took place <24 h after sampling. Spectrophotometric analysis (SQ300 from Merck Millipore, Billerica, USA) was performed applying a modified version of the methylene blue assay for anionic surfactants (MBAS) standard method (George & White 1999). Prior to each LAS determination, a calibration curve was obtained using sodium-dodecyl sulfonate as a standard and filtered seawater from each experimental tank to ensure the same salinity.

Data analysis

Oxygen fluxes were calculated using Microsoft Excel 2010. For each 30 min measurement period the decline in oxygen concentration within the incubation chamber was calculated and standardized to the consumption in 1 h. Fluxes were determined for each 12 h dark and light period (day and night) separately, taking averages from 18-25 measurement periods. All values were further standardized to surface area of the coral and values for bacterial

respiration were accounted for by including the oxygen consumption values from the blanks, measured after the experiments. Gross photosynthetic rate was estimated as the difference between dark and light respiration, assuming the same respiration rates during light and darkness (see discussion). To avoid any confounding effects due to handling stress after the pre-treatment, the first 12 h in each experiment were excluded from the analysis. Statistical analysis was performed in R (R v.3.0.2 using R Studio v.0.98.1056). All data were checked for normal distribution using the Shapiro Wilk test and for heterogeneity of variance with Levene's test. Two-way ANOVA was carried out with diesel and temperature as fixed factors for dark and light periods separately to determine significant effects of the stressors and their interaction (see Table 4 for the results). To determine differences between the individual treatments, a post-hoc Tukey HSD test was applied (see S2.1 table in the supporting information). In case of the photosynthetic yield in the LAS treatments, data were not normal distributed and multiple Wilcox rank sum tests were applied to detect differences between treatments.

Results

Water parameters

Physical water parameters measured within the rearing tank and during the experiments resembled those determined at the sampling sites (Table 3.2). LAS and PAH concentrations at the sampling sites were below detection limit, thus considered to reach zero. During the experiments there was no significant difference in the LAS or PAH concentrations between pre-treatment and measurement phase or between control and high temperature ($p > 0.05$). The pollutant concentrations for both diesel (as measured by total PAH analysis) and LAS decreased significantly from the beginning to the end of each pre-treatment and measurement period. The values decreased from 0.69 ± 0.14 mg L^{-1} to 0.25 ± 0.05 mg L^{-1} and from 0.95 ± 0.02 mg L^{-1} to 0.87 ± 0.05 mg L^{-1} for PAH and LAS, respectively.

Control

As a control corals were subjected to the same experimental protocol (including pre-treatment and measurement period) like the other treatments. Without any stressor present dark respiration rates were 0.019 ± 0.005 mgO$_2$ h^{-1} cm^{-2} and light respiration rates

0.082 ± 0.003 mgO$_2$ h^{-1} cm^{-2}. The photosynthesis rate calculated from the difference between dark and light respiration was 0.011 ± 0.003 mgO$_2$ h^{-1} cm^{-2}, concluding in a P:R ratio of 0.58 ± 0.12. Maximum quantum yield did not differ between the start and end of the experiment, in both cases being 0.71 ± 0.02.

Table 3.2: Physical water parameters. Salinity, dissolved oxygen (DO), pH and temperature at the sampling stations (averages of both stations are shown), the rearing system and the experiments (average of the daily measured parameters at midday). Temperature for the experiments is given for the control and high treatments separately.

	Sampling stations	Coral rearing	Pre-treatments	Measurement phase
Salinity [PSU]	34.0 ± 0.2	33.7 ± 0.1	34.0 ± 0.2	34.1 ± 0.2
DO [% sat]	102.2 ± 3.0	102.8 ± 5.0	102.0 ± 1.1	96.3 ± 2.8
pH	8.1 ± 0.0	8.3 ± 0.0	8.3 ± 0.0	8.1 ± 0.1
Temp [°C]	28.3 ± 0.2	28.3 ± 0.5	28.9 ± 0.3	27.9 ± 0.1
			31.1 ± 0.2	30.9 ± 0.1

Table 3.3: Summary of *Pocillopora verrucosa* responses. Physiological responses of the coral holobiont for all treatments. Given are holobiont respiration measured during dark and light periods, photosynthesis rate estimated from the difference between the respiration values and maximum quantum yield measured after 48 h and 84 h. The 84 h results for treatments containing LAS are given in brackets as they are not reliable due to high tissue loss (see LAS results and discussion section). Tissue loss as seen in treatments containing LAS is given as determined at the end of the experiment.

	Dark Respiration [mgO$_2$ h^{-1} cm^{-2}]	Light respiration [mgO$_2$ h^{-1} cm^{-2}]	Photosynthesis [mgO$_2$ h^{-1} cm^{-2}]	Maximum quantum yield 48 h [F$_v$/F$_m$]	Maximum quantum yield 84 h [F$_v$/F$_m$]	Tissue loss after 84 h [% loss]
Control	0.019 ± 0.005	0.082 ± 0.003	0.011 ± 0.003	0.71 ± 0.02	0.71 ± 0.02	-
High temperature	0.012 ± 0.003	0.003 ± 0.001	0.009 ± 0.003	0.74 ± 0.01	0.72 ± 0.01	-
Diesel	0.015 ± 0.001	0.006 ± 0.003	0.010 ± 0.003	0.71 ± 0.02	0.71 ± 0.02	-
LAS	-	-	-	0.73 ± 0.01	(0.58 ± 0.22)	52.5 ± 30.15
Diesel + high temperature	0.023 ± 0.003	0.008 ± 0.002	0.014 ± 0.005	0.74 ± 0.01	0.71 ± 0.01	-
LAS + high temperature	-	-	-	0.63 ± 0.13	(0.14 ± 0.08)	92.25 ± 7.26

Diesel

When corals were exposed to the water accumulated fraction of diesel, the dark and light respiration rates were 0.015 ± 0.001 mgO$_2$ h^{-1} cm^{-2} and 0.006 ± 0.003 mgO$_2$ h^{-1} cm^{-2}, respectively (Fig. 3.2). Gross photosynthesis was 0.010 ± 0.003 mgO$_2$ h^{-1} cm^{-2} with a P:R ratio of 0.61 ± 0.17. The maximum quantum yield gave exactly the same results as in the control treatment with 0.71 ± 0.02 (Fig. 3.3). No significant effects of diesel exposure were detected on either of these parameters (see Table 3.3 for ANOVA results).

Table 3.4: ANOVA results for diesel and temperature experiments. Two way analysis of variance (ANOVA) for maximum quantum yield (F_v/F_m) and respiration values. Effects of temperature (control or high) and pollutant (no pollutant or with diesel) were analyzed in isolation and in combination with each other. Significant values ($p<0.05$) are indicated by an asterisk.

	Df	(SS)	(MS)	F value	Pr (>F)
F_v/F_m (Beginning of measurement period)					
Temperature	1	3.03E-03	3.03E-03	8.963	0.011 *
Pollutant	1	0.00E+00	0.00E+00	0.000	1.000
Temperature:Pollutant	1	2.50E-05	2.50E-05	0.074	0.790
F_v/F_m (End of measurement period)					
Temperature	1	2.50E-05	2.50E-05	0.090	0.770
Pollutant	1	1.00E-04	1.00E-04	0.358	0.561
Temperature:Pollutant	1	1.00E-04	1.00E-04	0.358	0.561
Light respiration					
Temperature	1	8.93E-06	8.93E-06	1.100	0.315
Pollutant	1	1.02E-05	1.02E-05	1.252	0.285
Temperature:Pollutant	1	6.17E-05	6.17E-05	7.601	0.017 *
Dark respiration					
Temperature	1	1.00E-08	1.00E-08	0.001	0.982
Pollutant	1	4.46E-05	4.46E-05	2.947	0.112
Temperature:Pollutant	1	2.11E-04	2.11E-04	13.939	0.003 *

Temperature

Similar to diesel, temperature on its own had no effect on the oxygen fluxes or P:R ratio of the coral. Dark respiration was 0.0121 ± 0.0034 mgO$_2$ h^{-1} cm^{-2}, light respiration 0.003 ± 0.001 mgO$_2$ h^{-1} cm^{-2} (Fig. 3.2), concluding in a gross photosynthesis of 0.009 ± 0.003 mgO$_2$ h^{-1} cm^{-2} and a P:R ratio of 0.76 ± 0.11. At the start of the measurement

phase, after corals were exposed to high temperature for 48 h, maximum quantum yield was significantly increased compared to all other treatments (p=0.0112, confirmed by Tukey HSD, supplementary information table S3.1). At this time the yield was 0.74 ± 0.01, but subsequently decreased to control levels of 0.72 ± 0.01 within 24 h until the end of the experiment (Fig. 3.3).
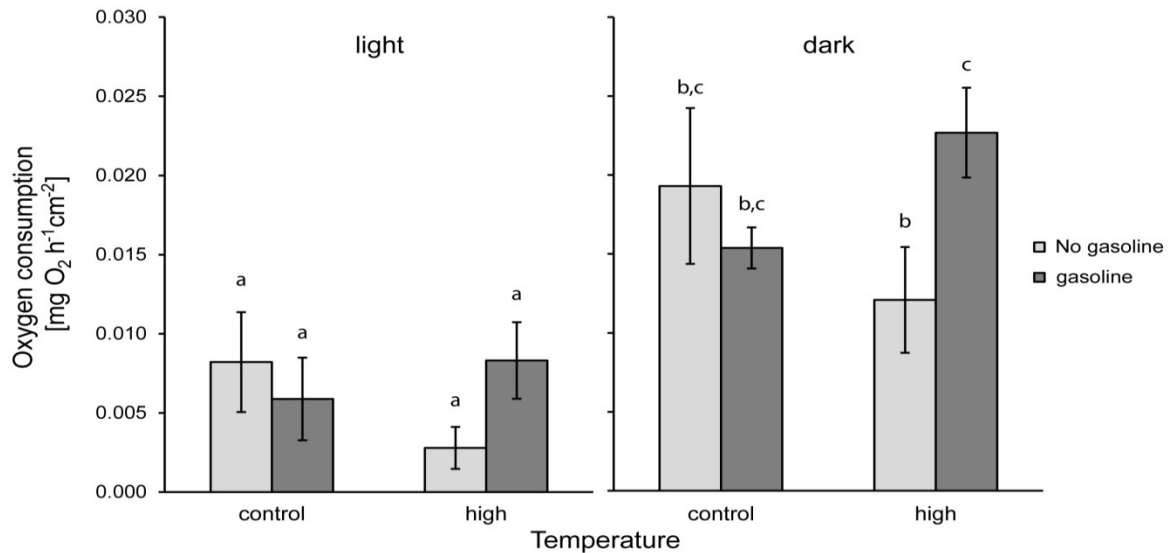
Fig. 3.2: Oxygen consumption. Consumption of oxygen by *Pocillopora verrucosa* in diesel and high temperature treatments for the light and dark period. Differences between the two periods (60-80 % lower oxygen consumption during the light period) are due to the occurring photosynthesis in light. Given are averages for each treatment (n=4) with standard deviations. Different letters indicate significant difference as found in Tukey HSD post hoc test (Supplementary information table S3.1).

LAS

Exposure (>24 h) to LAS caused tissue ablations in the coral fragments (see Fig. 3.4). This ranged from 16 % tissue loss in some fragments up to 95 % in others at the end of the experiment (84 h). On average the tissue loss in the isolated LAS treatment was 53 ± 30 % (n=8). The amount of tissue ablation was always similar in both fragments originating from the same coral colony. The tissue loss hampered the measurements of photosynthetic activity. During the first measurements all coral tissues were still intact, while values from the end of the experiment can only serve as a rough prediction of actual values. Although the PAM fiber optic was always placed at positions where tissue was still visibly unimpaired, the results obtained are not reliable due to the high tissue loss. Maximum quantum yield in the

treatments with LAS was 0.73 ± 0.01 at the beginning of the measurement period and 0.58 ± 0.22 at the end of the experiment (Fig. 3.3).
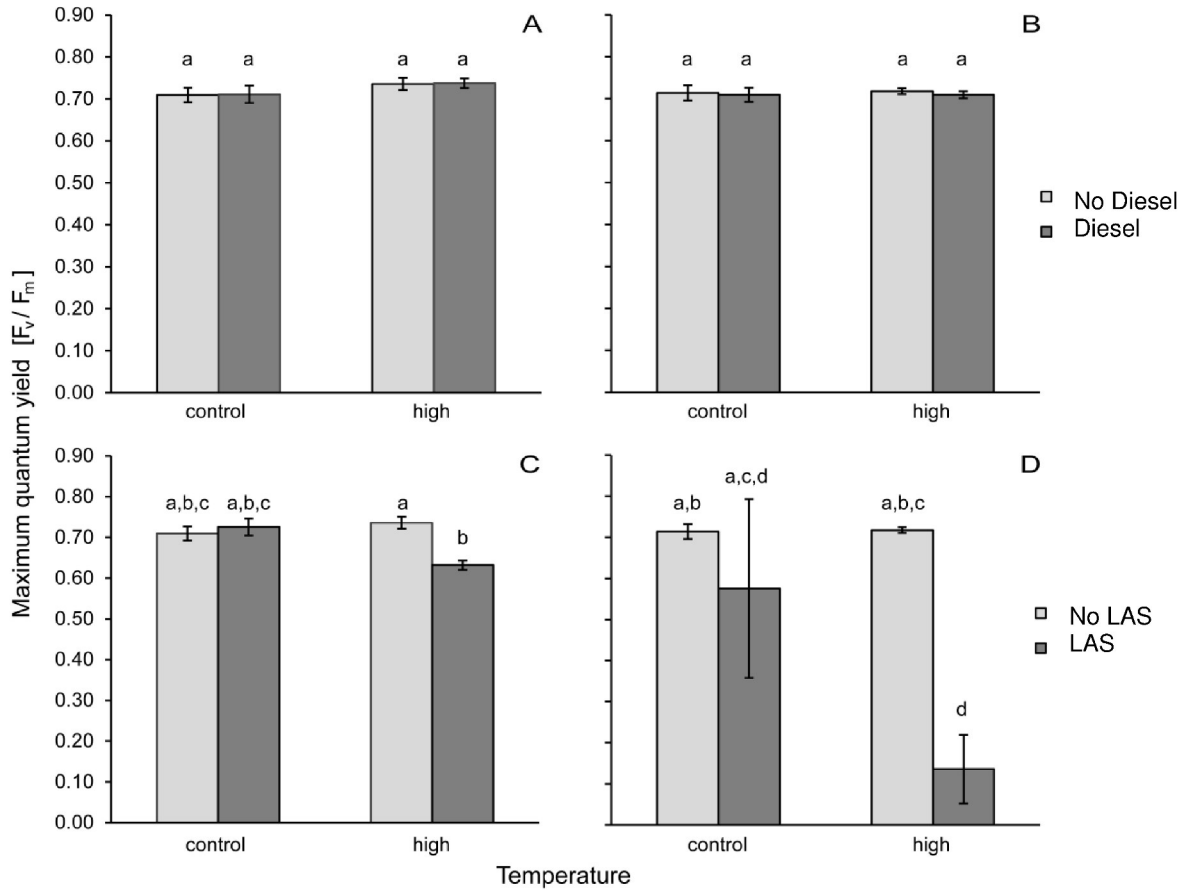
Fig. 3.3: Maximum quantum yield (F_v/F_m) of *Pocillopora verrucosa* subjected to pollutants and high temperature. WAF of diesel (A+B) and linear alkylbenzene sulfonate, LAS (C+D) were administered either individually or in combination with high temperature. Measurements took place after 48 h and 84 h (A+C and B+D, respectively). Given are averages for each treatment (n=4) with standard deviation. Different letters indicate significant differences as determined by Wilcox rank sum tests.

Combined effects

Even though neither diesel nor temperature alone had a significant effect on respiration, significant effects were found in the combined treatment. A significant interaction between diesel and temperature was detected (p=0.0174 in light and p=0.0029 in darkness, see Table 4). The Tukey test validated this effect only for the dark period (dark period p=0.0107, light p>0.07, see supplementary information table S3.1). The light respiration rate was

0.008 ± 0.002 mgO$_2$ h^{-1} cm^{-2}, dark respiration was 0.023 ± 0.003 mgO$_2$ h^{-1} cm^{-2}and thus higher than in the treatment with increased temperature alone (Fig. 3.2). But there was no significant difference between the combined and the control treatment. Despite the difference in oxygen consumption values, there was no difference in gross photosynthesis (0.014 ± 0.005 mgO$_2$ h^{-1} cm^{-2}), P:R ratio (0.61 ± 0.15) or maximum quantum yield (0.72 ± 0.02).The combination of high temperature and LAS led to even higher tissue ablations than LAS in isolation (on average 92 ± 7 %, with n=8, in a range from 81 to 100 %), thus results of maximum quantum yield are highly unreliable and are merely given to show the decreasing trend in the values. At the start of the measurement phase, when no ablations of coral tissue were yet visible, maximum quantum yield in the combined LAS and high temperature treatment was 0.63 ± 0.13 and thus significantly lower than the other treatments (p=0.0265, Wilcox rank sum test). At the end of the experiment the values measured for maximum quantum yield decreased to 0.14 ± 0.08 (p=0.0294, Wilcox rank sum test), but at this point tissue loss was already at its maximum.

Fig. 3.4: Documentation of tissue loss due to LAS. *Pocillopora verrucosa*, subjected to LAS treatment, showing severe tissue loss after 84 h exposure.
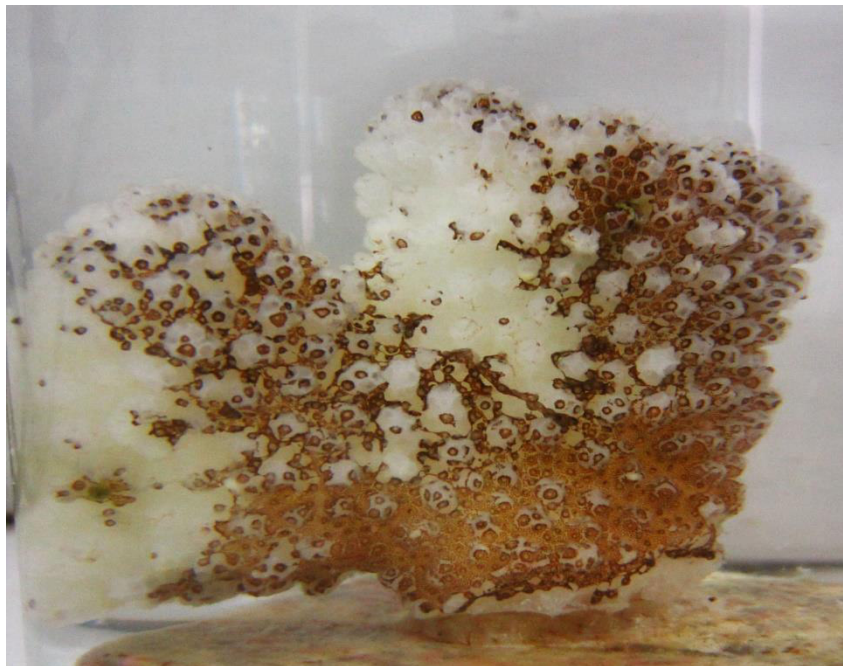
Discussion

This study showed that the physiology of *Pocillopora verrucosa* was influenced by the three stressors temperature, diesel and LAS, but resulted in different responses. While diesel and temperature had a significant interactive effect on dark respiration, LAS on its own and in combination with temperature resulted in a severe tissue loss of coral fragments. Temperature on its own led to a significant decrease in maximum quantum yield only in the beginning of the measurement period, while diesel had no significant effect at all. Thus our working hypothesis is only partly supported by the findings, but proved right in the context of combined effects with high temperature. This confirms other studies, which have predicted a negative effect of global warming on organisms' sensitivity to chemical stressors (Beyer et al. 2014). In addition, our study highlights the importance of measuring several response parameters, as different stressors can result in diverse responses.

The analysis of ambient LAS and diesel concentrations around Gili Trawangan showed no measureable amounts of these pollutants in the water. This can be explained by the fact that the island as a tourism hotspot is kept clean and strong currents would quickly dilute any pollutants entering the water. A contrasting situation occurs in more densely populated areas, such as the Thousand Island chain off the Indonesian capital Jakarta, where also the boat traffic is much higher, including large commercial vessels passing through the island chain. At the Thousand Islands, PAH values up to 0.23 mg L^{-1} and LAS of up to 0.9 mg L^{-1} were detected (Baum et al. in prep.). Other studies in the Indo-Pacific measured total PAH concentrations between 0.05 to 0.21 mg L^{-1} (Falahudin et al. 2012), and LAS concentrations in the Red Sea ranging from 0.001 to 0.03 mg L^{-1} (Shafir et al. 2014). During short periods, the concentrations can reach higher values close to pollutant sources (Braga & Varesche 2014, Shafir et al. 2014). One source of diesel and PAH is the regular evacuation of water from the bilge in ships. Total PAH concentrations next to a boat after discharging bilge water were still higher than the PAH concentrations in our experiments (0.9 mg L^{-1} 10 min after discharging), but would further dissolve after a longer time period. Similarly, LAS concentrations next to a boat after cleaning were still 1.3 mg L^{-1} (G. Baum et al., in prep). This shows that the pollutant concentrations used in this study are relevant in the environmental context. The decreasing PAH and LAS concentrations during the course of our experiments resemble the natural exposure conditions of corals in the reef, with initially higher concentrations that are decreasing over time. Fast degradation has been described before for LAS (Ivancović & Hrenović 2010) and to some extent also for diesel

(Pampanin & Sydnes 2013). Half-life time of LAS in seawater is ca. 6 days due to biodegradation and adsorption to suspended particles (Kemp et al. 2011).

The respiration and photosynthetic yield values measured in the experiment were comparable to those measured by other authors for different coral species (Muscatine et al. 1984, Porter et al. 1999, Kemp et al. 2011, Ulstrup et al. 2011). The values given in our paper for photosynthesis are estimations for the actual rates of oxygen produced, calculated from the difference between dark and light oxygen consumption. This assumes that holobiont respiration during dark and light are the same, which is not always the case as mentioned by Lesser (2013). Therefore gross photosynthesis might be slightly underestimated by this method. Compared to other studies, the calculated P:R ratio of 0.63 was very low. Generally, a P:R ratio below 1.0 indicates lack of photosynthetically fixed carbon (Davies 1984). In our study, this is due to the low light intensities from the artificial aquarium light. The light intensities were only one third of light intensities measured in the reef, explaining why photosynthesis rates during the experiments were quite low. Still 60 µmol quanta m^{-2} s^{-1} were enough to result in photosynthesis in *P. verrucosa*.

Diesel exposure had no effect on the coral physiology. Other authors have reported negative effects of different sources of oil on corals, but the literature on the effects of diesel, other oil sources and PAH on corals is contradictory. While several studies report decreases in maximum quantum yield (Mercurio et al. 2004), tissue alterations (Harrison et al. 1990) and damages to reproductive systems (Rinkevich & Loya 1979, Negri & Heyward 2000), there are also other studies where no effect on corals was found (Braga & Varesche 2014). In a recent study on cold water corals, DeLeo et al. (2015) also could not detect effects of crude oil treatments on coral health and proposed that initial negative effects could be mitigated, when the coral holobiont uses the hydrocarbon components as a nutrition source, as indicated before by Al-Dahash and Mahmoud (2013). In coral tissues, total PAH concentrations of 0.004 - 0.1 µg g^{-1} dry mass were determined, higher than in the surrounding sediments, indicating a bioaccumulation (Ko et al. 2013, Whitall et al. 2014). Most studies on threshold values of PAH were performed on fish and crustaceans. In general LC50 concentrations for various PAH types and exposure times range from 0.0005 to 32.5 mg L^{-1} (Ministry of environment BC). In sole morphological and physiological effects occurred at PAH concentrations in sediments ranging from 0.054 to 4 µg g^{-1} dry mass (Johnson et al 2002). No threshold values for corals are described in the literature. PAH metabolic products can bind to the organisms DNA and cause severe carcinogenic damage, as well as lead to alterations of

the immune system (Logan 2007), change blood composition and tissue (Simonato et al. 2008).

The significantly higher photosynthetic yield due to increased temperature alone was only detected in the beginning of the experiment, indicating a high stress during the first hours that the coral could mitigate over time. Exposure time has a strong influence on the effect of temperature. While longer exposure reduces the respiration rates in corals, short term exposures can increase both respiration and photosynthetic rates (Coles & Jokiel 1977). In *Montastrea annularis* elevated temperature reduced both photosynthesis and respiration after 6 h of exposure (Porter et al. 1999). Caribbean corals showed minor decreasing effects on photochemical efficiency due to higher temperature in experiments lasting for 10 days (Fournie et al. 2012). Other studies detected negative effects on photosynthesis and respiration due to temperature stress as well (Kemp et al. 2011), although this could not statistically be replicated in the current study, where only negative trends of high temperature were measured.

Even though no significant differences to either diesel or temperature alone could be detected, when diesel was combined with temperature, dark respiration significantly increased compared to the temperature as a single stressor and was similar to the control. This combined effect can be explained by altered membrane properties due to the higher temperature, which affect fluidity and diffusion rates and thus chemical toxicity (van Dam et al. 2011), In hard corals from the Florida Keys, Porter et al. (1999) found that increases in both temperature and salinity reduced respiration rates and if administered at the same time, the effect was mitigated during the first 36 h, but still led to death of all corals after longer exposure. Synergistic effects on maximum quantum yield were also demonstrated before between temperature and light intensities in Japanese corals, where yield decreased even stronger at higher temperatures (Bhagooli & Hidaka 2004). Reynaud et al. (2003) found an antagonistic effect of temperature in combination with increased pCO2 on calcification and photosynthesis of *Stylophora pistillata*, but not on respiration (Reynaud et al. 2003).

LAS resulted in a significant reduction of the photosynthetic yield. Even though maximum quantum yield values obtained at the end of the experiment were not reliable, in combination with high temperature decreases in yield were already seen in corals without tissue loss, showing the importance of yield measurements as early warning indicator. Declines of photosynthetic yield in corals were also measured after cyanide exposure and sedimentation,

where yield values down to 0.1 during stress were recorded (Jones et al. 1999, Philipp & Fabricius 2003). The effect LAS had on the coral tissue was surprisingly severe. First tissue ablations were already visible after only 24 h exposure to 0.9 mg L^{-1} and after 84 h large parts of the coral tissue were detached from the skeleton. Trials with higher concentrations of LAS (results not shown) even lead to tissue losses up to 100 % within the first 24 h. These tissue ablations pose a severe threat to coral health, as the regeneration of tissue needs a lot of energy and time. Generally, marine species tend to be more sensitive to LAS than freshwater species. Surfactants reduce the water surface tension that aquatic organisms depend on (Braga & Varesche 2014), explaining the severe effect on coral tissue as the membrane properties are disrupted (Abel 1974, DeLeo et al. 2015). Anionic surfactants such as LAS can bind to proteins and peptides, resulting in an alteration of their structure and function (Braga & Varesche 2014). Thereby, these pollutants can alter enzymatic activities within the metabolic pathways and affect the DNA (Cserháti et al. 2002, Ivancović & Hrenović 2010). Temara et al. (2001) determined average LC50 values for marine species to be 4.3 mg L^{-1} with no observed effect concentrations at 0.3 mg L^{-1}, which is 2 mg L^{-1} lower than for freshwater species. There are only very few studies on the effects of LAS on corals, most experimental and monitoring work focused rather on fish and other invertebrates than corals. Shafir et al. (2014) performed the first study on the toxicology of detergents on hard corals and found them to be much more sensitive than many other marine organisms. LC50 for *Pocillopora damicornis* was determined to be 2.2 mg L^{-1}, for *Stylophora pistillata* even 1.0 mg L^{-1} in 24 h exposures. In their experiments genotype-specific mortality and adaptation in *P. damicornis* after several exposures was observed. Such a genotype-specific response could explain the finding that in our experiments always both fragments from the same coral colony showed similar ablation rates.

Further experiments with different concentrations of pollutants, especially of LAS, would increase our knowledge on their effects on coral physiology and the impacts on coral reefs. It will be essential to determine threshold values to those common pollutants also for corals. Experiments with multiple pollutants with and without the increase of temperature could reveal more on interactions between them. Varying exposure times and increasing measurement intervals in the experiments would give additional information about the risks of low concentrations during long term vs. high concentrations during very short term exposure. In future research with multiple response parameters should regularly be considered, because while diesel had an effect on respiration and no effect on photosynthetic yield, significant effects on yield were detected during LAS and temperature exposure.

Different stressors can evidently modify the coral physiology in several ways, and some parameters such as respiration may react faster and stronger to pollutants than others. The physiological parameters proved to react fast to environmental changes, which is supported by Kaniewska et al. (2012), who also reported faster effects of physiological responses compared to biomineralisation processes. In order to find appropriate parameters, it is further necessary to understand the underlying mechanisms of PAH and LAS toxicity in corals. Due to high variations in all response parameters, not all trends that were visible could be validated with statistical significance. Kaniewska et al. (2012) also had n=4, but proposed higher replicate numbers in order to get more robust results. Future experiments with higher replication could strengthen our findings.

This study gives further confirmation about the need for better local management in the face of global warming. Even if CO_2 emissions will be reduced, global warming will continue in future (IPCC 2013). For coral reef organisms in tropical areas, a seemingly minor increase of a few degrees can result in severe stress, particularly if other local stressors are present as shown in this study. While removing thermal stress is not achievable in the near future, coral reefs are able to recover when other stressors are removed, thus it will be critical to support resilience of reefs by changing human destructive activities (Hughes et al. 2002). Indonesia's population is strongly reef-associated, and most of the country's coastline is populated without effective sewage treatments in place (Burke et al. 2012). However, every family in each of the villages along the coast is using diesel and soap. Although at the moment no obvious effects of diesel or surfactants are visible on coral reefs, this could potentially become a problem in near future, taking growing populations and climate change into account, and expressing the need for effective local management (Baskett et al. 2009, Burke et al. 2012). Understanding the effect of different stressors and their combinations on key organisms can strengthen the decision support needed for coral reef management (Maina et al. 2011). One way to reduce the outflow of pollutants from human settlements is the implementation of sewage treatments. Experiments on the effectiveness of sewage treatments have shown that from influent waters with LAS concentrations up to 6.7 mg L^{-1}, only max. 0.005 mg L^{-1} remained in the effluent water (Clara et al. 2007). In areas like the Netherlands, where 83 % of all waste water is treated, LAS concentrations of only 1-9 μg L^{-1} were measured in nearshore estuaries (Temara et al. 2001). Further controls on the direct discharge of diesel at least for commercial ships are necessary. At the same time, effective reef management can be achieved by communication of findings, education, training and

outreach of populations living close to reefs to make them aware of the risks and the positive outcome when protecting the reefs (Burke et al. 2012).

Acknowledgements

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Chapter 4: Local Effects on Bacterial Communities
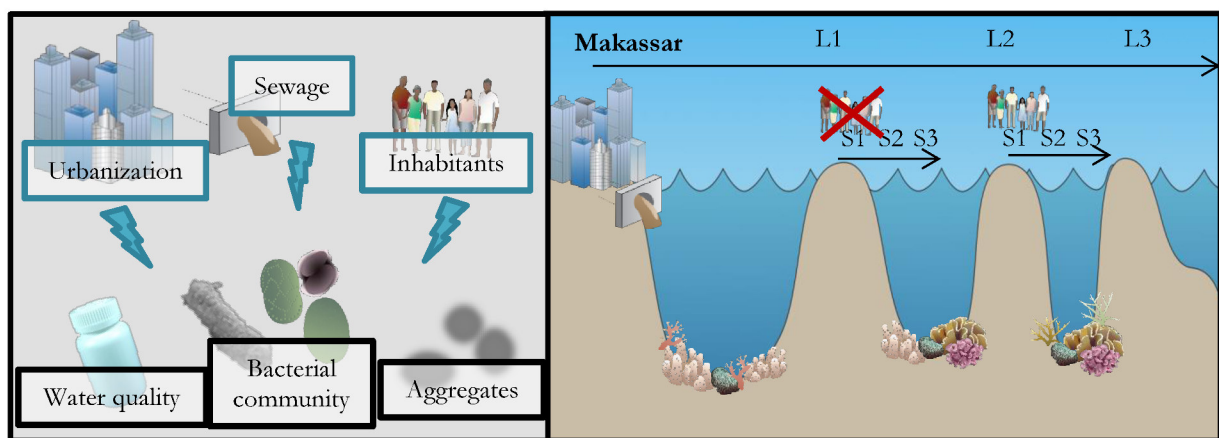
Chapter 4: In-situ effects of a large urban area and connected sewage inputs on water quality, bacterial community compositions and aggregate formations. Effects were determined on two different spatial scales. A large scale (L) across several islands in the Spermonde Archipelago with increasing distance from Makassar. A smaller scale (S) with increasing distance from an inhabited or an uninhabited island.

Schwieder HF, **Kegler P**, Jennerjahn TC, Jompa J, Hassenrück C, Gärdes A. Shifts of bacterial community composition in the water column, on aggregates and within the sediments along water quality gradients: the effect of different spatial scales. (In preparation for Frontiers in Microbiology)

Shifts of bacterial community composition in the water column, on aggregates and within the sediments along water quality gradients: the effect of different spatial scales

Schwieder HF, Kegler P, Jennerjahn TC, Jompa J, Hassenrück C, Gärdes A

Abstract

The Spermonde Archipelago, Sulawesi, Indonesia is heavily influenced by local impacts such as sewage discharge from the metropolitan area of Makassar, and riverine effluent inputs. These lead to strongly elevated phytoplankton biomass in inshore areas of the island chain. This can cause strong shifts in the bacterial community, increase the formation and settling of organic matter and subsequently disturb the benthic ecosystems, which can suffer from excessive sedimentation. Additionally the islands of the archipelago are densely populated and have the potential to add more pressure to the fringing reefs.

Samples for determination of water quality and bacterial community composition in the water column and on sandy sediments were taken along five stations, 1 to 19 km away from Makassar. To additionally elucidate how increased primary productivity influences bacterial activity and community composition in the water column, we incubated water from those stations in rolling tanks and observed changes in biological oxygen demand, as a measure of bacterial activity, aggregate formation rates and differences in the bacterial community composition on the sinking aggregates. On a smaller scale the effect of human populations inhabiting the islands on water quality parameters and bacterial community composition from the shore (25 m) to the reef crest were determined on a pair of islands at a similar distance from Makassar, one inhabited, one uninhabited.

Along the cross-shelf gradient, the inshore station closest to Makassar exhibited the highest concentrations of chlorophyll a and transparent exopolymer particles (TEP). There were also pronounced shifts in the bacterial community composition, especially in the reef sediments and on the sinking particles collected from aggregation experiments. On aggregates collected from waters from the inshore site, *Gammaproteobacteria*, a large group of heterotrophic bacteria known to include relevant pathogens, were almost three times more abundant compared to the sites furthest away from Makassar, with a relative abundance of more than 60 %.

Contrastingly, *Alphaproteobacteria* almost doubled in abundance from ~15 % inshore to around 30 % at the site 19 km away. On the smaller scale there were significant differences between the uninhabited and inhabited islands in terms of water quality. On both islands we also observed distinct differences between the sampled bacterial habitats (free-living and particle attached in the water column, as well as sediments) but neither between inhabited and uninhabited islands, nor along the gradient to the reef crest.

With the current dataset we can show distinct changes along both sampled spatial scales. Interestingly these were not reflected in shifts of the bacterial community composition, where we rather encountered differences between the sampled habitats. The only exception were the sites closest to Makassar, where the relative abundance of potential pathogens in the sediments and on aggregates was significantly higher compared to sites further offshore. In a coastal ecosystem where the human population largely relies on fishing for their daily diet and income, disturbing the coral reefs with local stressors such as intensive sedimentation and nutrient inputs can jeopardize the livelihood of millions of people.

Chapter 5: Coral Larvae and Bacterial Communities
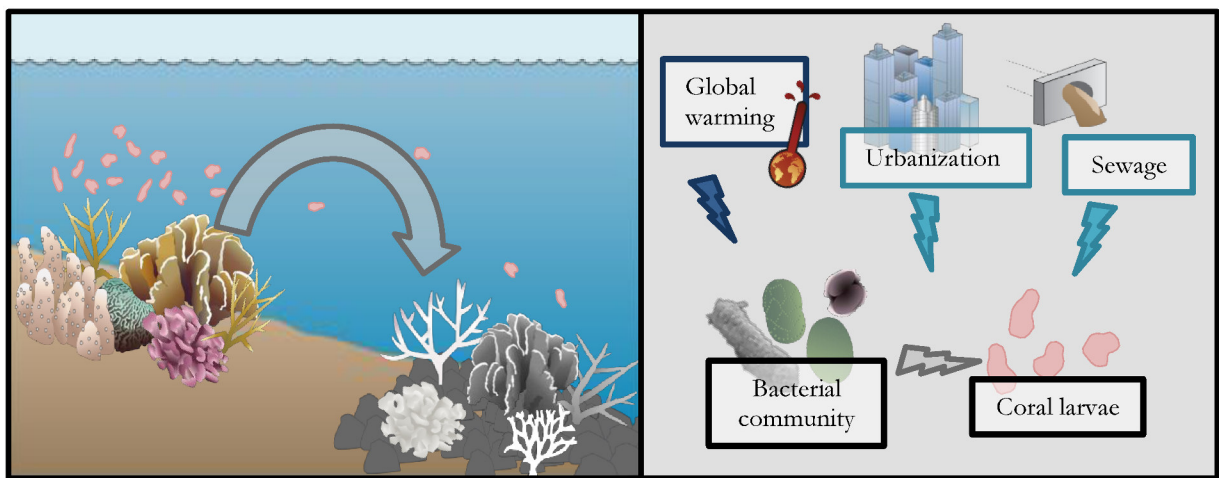
Chapter 5: Natural recovery of degraded reef areas by coral larvae from adjacent communities is vital for coral reefs. Effects of occurring local stressors were determined on bacterial community and coral larvae settlement in-situ. The proposed influence of bacterial communities on coral larvae and the effect of global warming on bacterial communities was investigated.

Kegler P, Schwieder H, Gärdes A, Ferse S, Alfiansah YR, Lukman M, Hassenrück C, Kunzmann A. Coral larvae settlement and of bacterial biofilm communities in changing environmental conditions of the Spermonde Archipelago, Indonesia. (In preparation for Marine Ecology Progress Series)

Coral larvae settlement and bacterial biofilm communities in changing environmental conditions of the Spermonde Archipelago, Indonesia

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

Abstract

Recruitment of coral larvae is one of the key factors for coral reef recovery. Biological settlement cues emitted from bacterial biofilms play a vital part in larval settlement and metamorphosis. They depend largely on the composition of biofilms, which are thought to change drastically with changing environmental conditions and may alter larval settlement behavior in turn. This study investigated bacterial community composition and coral larval settlement at three sites with differing magnitudes of human influences, in the Spermonde Archipelago, Indonesia. Both parameters were determined on natural reef substrate and on artificial ceramic tiles. Results show that the three sites differed in water quality parameters as well as benthic community composition. No coral recruits were found at the most anthropogenically influenced inshore site. Coral recruitment on the other sites was significantly different between sites, but similar in comparison of natural reef substrate and artificial tiles, with differences on a temporal scale. The spatial settlement pattern at both sites was the same with highest numbers of coral spat on the shaded, lower side of the tile. Bacterial communities on artificial tiles were similar to those on natural substrate and were comprised of *Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria* and *Flavobacteria* as the most dominant classes. The bacterial communities were strongly correlated with the sites as determined by water quality and benthic community composition and shifted during incubations at increased temperature. Higher numbers of settlement inducing bacteria of the genus Pseudoalteromonas were found on the site furthest from shore. This study shows that both coral larvae recruitment and bacterial communities are altered by environmental conditions and need to be taken into account in evaluations of the recovery potential of coral reefs.

Introduction

Reefs worldwide are declining due to the multitude of anthropogenic stressors that frequently act in combination. The Spermonde Archipelago in south Sulawesi, Indonesia, was selected as a case study to research bacterial community changes and coral recruitment, as it is characterized by various environmental and anthropogenic influences, mainly related to the city of Makassar with 1.5 million inhabitants (Sawall et al. 2012, 2013, Polónia et al. 2015). More than 100 small, mostly inhabited islands fringed by coral reefs provide the unique possibility to study reef bacterial communities and larval settlement subjected to various environmental conditions and an eutrophication gradient with increasing distance from the city (Cleary et al. 2005, Polónia et al. 2015). Water quality and benthic community compositions as foundations for the overall reef status are recorded to differ markedly between different islands within the Archipelago (for an overview see Polónia et al. 2015). Previous studies in Spermonde have shown declining coral cover, habitat degradation and reefs impacted by blast fishing activities (Edinger et al. 1998, Pet-Soede & Erdmann 1998).

Scleractinian corals play an essential role in coral reef ecosystems as they provide the foundation and three-dimensional structure of the reef (Veron 2000). The loss of this structure reduces habitat for reef associated species resulting in a loss of diversity and functionality of the ecosystem (Stanley 2003, Munday 2004). Coral reefs around the world are experiencing this loss due to a multitude of mostly anthropogenic disturbances (Carpenter et al. 2008, Halpern et al. 2008). One of the key factors in the recovery of coral reefs is the sexual recruitment via coral larvae (Harrison 2011, Sawall et al. 2013). This sexual reproduction via coral larvae can lead to an enhanced fitness of reef communities through adaptation of coral genotypes (Harrison 2011). Knowledge on coral reproduction and recruitment is critical in understanding how different stressors may affect reef populations and can be used for effective coral reef management (Richmond & Wolanski 2011).

Settlement of coral larvae depends on specific and very complex environmental stimuli that relay information about the respective habitat. Settlement and metamorphosis are not necessarily linked to one another. In laboratory experiments certain isolated stimuli were able to induce metamorphosis, but no settlement of larvae (Negri et al. 2001, Tebben et al. 2011). Next to physical cues such as light and depth (Price 2010), chemical cues emanating from biological sources appear to be the most relevant. Among these are conspecific cues from individuals of the same species (Harrison 2011), as well as heterospecific cues from predators

or algae (Price 2010, Dixson et al. 2014). These can either be inhibiting or inducing settlement. In the context of biological cues bacteria, especially those in biofilms, play an important role (Hadfield 2011).

Bacteria and other microorganisms form biofilms, covering most surfaces in the sea (Qian et al. 2003). The role of bacteria, especially those in biofilms, in the settlement process of invertebrate larvae received initial attention as early as the 1950's (Wilson 1955). The hypothesis that settlement was influenced by bacteria was supported by findings that settlement rates were significantly reduced after autoclaving surfaces or the use of antibiotics (Huggett et al. 2006, Sneed et al. 2014). Reports of interactions between larvae and bacteria exist for many marine invertebrates such as sponges, echinoderms, bryozoans, ascidians, crustaceans and corals (Hadfield 2011). Although the majority of studies on settlement of invertebrates have investigated biofouling species like barnacles and polychaetes, the number of studies focusing on coral larvae is increasing. For a large range of coral species crustose coralline algae (CCA) and their associated bacteria have been recorded to induce both settlement and metamorphosis of larvae (Heyward & Negri 1999, Price 2010, Webster et al. 2011). Each CCA species hosts a very unique bacterial community on its surface (Sneed et al. 2015). In many coral species there is high specificity of these cues. In a study with *Pocillopora damicornis*, 3 out of 52 bacterial strains isolated from reef surfaces were able to induce settlement and metamorphosis of larvae (Tran & Hadfield 2011). One genus of bacteria mentioned frequently in the context of inducing larval settlement and metamorphosis is *Pseudoalteromonas* (Negri et al. 2001, Hadfield 2011, Tran & Hadfield 2011). Tetrabromopyrrole (TBP), a metabolite produced by this genus, was identified as an inducer of metamorphosis, although in many cases no settlement occurred (Tebben et al. 2011, Sneed et al. 2014). While Tebben et al. (2015) conclude from their findings, that not bacteria, but live CCA produce the important settlement cues, larvae also settle on surfaces not covered by CCA (pers. observation) and there are reports that no single species, but rather the community structure of bacteria in biofilms is of high importance for larval settlement (Qian et al. 2003, Chung et al. 2010).

Due to their short generation times, bacterial community compositions in biofilms can shift rapidly with environmental conditions (Bourne & Webster 2013). Significant differences in bacterial community structure were observed when biofilms were grown in different environmental conditions such as temperature and salinity (Lau et al. 2005) or eutrophication conditions (Meyer-Reil & Köster 2000). Lau et al. (2005) recorded a significant response of barnacle larvae to biofilms altered by high temperature, while settlement of polychaete larvae

was more affected by changed salinities. The microbial community associated with a tropical CCA species shifted significantly when subjected to high temperature, with an increase in Bacteroidetes and reduction of Alphaproteobacteria, which in turn led to a reduction of the ability to induce metamorphosis (Webster et al. 2011). Similar effects of bacterial community changes due to high temperatures were found on the settlement of barnacle larvae. Sawall et al. (2012) reported bacterial communities on artificial surfaces to be most affected by microhabitat, season and anthropogenic changes in nutrients, where higher nutrients lead to a shift from autotrophic to heterotrophic and sulfur-reducing bacteria. They found that the community depended significantly on the orientation and exposure of ceramic tiles and recorded higher operational taxonomic units (OTU) numbers on tiles located in eutrophied near-shore reefs.

The use of new sequencing techniques is rapidly enhancing the knowledge on bacterial community compositions and provides opportunities to study them in changing environments. A major obstacle in gaining knowledge on coral larvae and bacteria interactions is due to limitations imposed by the logistical restrictions of field research. One way to tackle this is by drawing on laboratory studies, using isolated and cultivated bacteria, which provides the advantage of being able to control experimental conditions but has the major drawback that only a fraction of all bacteria occurring in nature can be cultivated (Zarraonaindia et al. 2013). The other approach are field observations and experiments in combination with recent sequencing techniques, which has the advantage of detecting all bacterial groups involved, but with less control over experimental conditions.

Settlement of coral larvae is one of the essential mechanisms for reef health and recovery but also one of the most difficult to study, therefore many open questions remain. Processes that influence larvae settlement need to be further investigated in terms of effects of water quality, habitat composition and settlement cues. Although the important role of bacteria in the settlement process of coral larvae is widely accepted, the underlying mechanisms and key players still remain unclear. It is still not clear which bacteria are affecting settlement of coral larvae and what community compositions favor or alter the settlement behavior. Further very little information exists on the influence of environmental conditions on bacterial biofilm communities (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) without being able to actually identify the groups of bacteria within their samples. However to determine the

bacteria most affected by environmental changes would be important in order to understand what implications community changes will have for larvae settlement.

The current study investigated settlement of coral larvae and bacterial biofilm communities in the Spermonde Archipelago. The aim was to determine coral recruitment at three different sites and analyze which factors influence scleractinian larvae settlement. Factors of which a potential influence was expected were distance from shore as a proxy for anthropogenic impacts, benthic community, nutrient levels, physical water parameters and especially the bacterial biofilm composition. Temporal and spatial settlement patterns on artificial settlement substrates were to be analyzed. To determine the influence of bacterial communities, this study further investigated how bacterial biofilm communities differ with increasing distance from the shore and which environmental factors influence the community composition. This study is among the first to determine settlement of coral larvae under various environmental influences in the Spermonde Archipelago and to simultaneously investigate bacterial communities under the same conditions, using molecular sequencing methods.

Material and Methods

<u>Study area</u>

The study was conducted between April and June 2014 in the Spermonde Archipelago in southern Sulawesi, Indonesia. This time falls right in between the wet NW monsoon (Dec-Feb) and the dry SE monsoon (June-Sept) in this area (Sawall et al. 2013). Three islands with varying distance from the city of Makassar were chosen for comparisons. These islands were Lae-Lae ("inshore", LL, approx. 1 km from Makassar, S 05° 07', E 119° 20'), Barrang Lompo ("near-shore", BL, 11 km from Makassar, S 05° 02.53', E 119° 19.41') and Badi ("mid-shelf", BD, 19 km from Makassar, S 04° 58.23', E 119° 16.95'). All islands are located on the continental shelf with reefs at different depths surrounding them. All islands are inhabited, with the lowest population density on the mid-shelf island (BD) with ~19000 people per km^2 (total of 1680, BPS Kota Makassar 2010), intermediate on the near-shore island (BL) with ~20000 people per km^2 (4200 in total, BPS Kota Makassar 2010) and highest on the inshore island (LL) with ~22000 people per km^2 (total of 1600, BPS Kota Makassar 2010).

<u>Environmental parameters</u>

Physio-chemical water parameters (salinity, temperature, pH, chlorophyll a concentration, dissolved oxygen concentration and turbidity) were collected once per minute using an Eureka 2 Manta multiprobe (Eureka Environmental Engineering, Texas, USA) for 20-30 minutes during each sampling and during the transect work (a total of 5 times at each site during the 2 month sampling period). Water samples for chemical parameters were collected in 6 replicates each at a depth of 5m, which was about 1 m above the reef, using a 5 L Niskin bottle (HydroBios, Kiel, Germany). Samples were stored in the dark and transported to the laboratory at Barrang Lompo for immediate analysis.

For inorganic nutrient analysis (combined nitrate and nitrite NO_x, phosphate PO_4 and silicate Si) 50 mL were filtered directly on the boat through a 0.7 μm syringe filter and poisoned with 200 μl of a 3.5 g/100mL $HgCl_2$ solution. The samples were stored at -20 °C and transported back to the ZMT, Germany for further analysis using a continuous flow analyzer (Flowsys by Unity Scientific, Brookfield, USA).

For measurements of dissolved organic carbon (DOC), 30 mL samples were filtered through 0.45 μm pore GF/F filters (Whatman GF/F, GE Healthcare, Pittsburgh, USA) and acidified with concentrated HCl (ph below 2). Analysis took place at the ZMT, Germany with high-temperature oxic combustion (HTOC) method using a TOC-VCPH TOC analyzer (Shimadzu, Mandel, Canada). For calibration and quality control artificial seawater standards (Hansell laboratory, RSMAS University Miami, USA) and ultrapure water blanks were used.

Suspended particulate matter (SPM) was measured as dry mass on pre-combusted GF/F filters before and after filtration of a known volume of water sample (2-3 L). Weight of the filters was determined using a precision balance (ME 36S, Satorius, Göttingen, Germany) after drying the filters for 24 h drying at 40 °C.

<u>*In - situ* surveys and benthic transects</u>

For in-situ determinations of coral recruitment and bacterial communities on natural reef substrate three 50 m transects were surveyed at each site. The transects were installed parallel to the shore between 3.5 and 5.5 m water depth, which was chosen due to the high number of hard corals in this region of the reef.

To determine the benthic community composition, 50 x 50 cm quadrats were photographed every 2 m of the transects, alternating to the left and right (n=25 for each transect). Analysis of these pictures was performed with Coral Point Count with Excel extensions (CPCe, version 4.1, Kohler & Gill 2006) with 50 random points per picture. 11 major categories were differentiated based on English et al. (1997). Main live categories included non-Acroporid corals, Acroporid corals, soft corals, coralline algae, macroalgae, turf, others and unknown live, while non-living categories were substrate, dead coral and equipment (frame of the quadrat or shadow). All hard corals were further subcategorized depending on morphology type.

Coral recruitment on natural reef substrate along all transects was determined during night dives (starting at 18:00), using fluorescence census techniques (Baird et al. 2006, Schmidt-Roach et al. 2008). By exciting host and symbiont pigments with blue or ultraviolet light they fluoresce and are thus easier to detect at smaller sizes than by the naked eye (Piniak et al. 2005). A 20 x 20 cm quadrat was placed within a 2 m belt from the transect, wherever the substrate was suitable for settlement (i.e. not on live corals and sandpatches, n=10 for each transect) and checked with a fluorescence dive light (Bluestar and GoBe, Nightsea, Bedford, USA) and a yellow filter in front of the mask. For this study all young corals below a size of 3 cm were counted as recruits (generally recruits were detectable starting from approx. 0.3 cm). All number of coral recruits within the quadrats was recorded.

From the middle of each transect samples to assess the bacterial community composition on natural reef substrate were taken. Small rocks of approx. the same size, covered with crustose coralline algae, were retrieved from the reef. The surface of each rock was scraped immediately after the respective dive with a scalpel and the material stored in 2 mL Eppendorf tubes and preserved with ~1.5 mL of "RNA later" (following Ambion, Texas, USA). Samples were stored in the dark and transported back to the laboratory where they were frozen at -20 °C until further analysis.

Settlement tiles

At each station three metal frames containing ceramic settlement tiles were positioned for the subsequent analysis of coral larvae settlement and bacterial biofilm composition and their temporal development. The frames were placed on sand patches separated by approx. 5 m from each other and always within close vicinity to live corals. Tiles were placed at an angle of ~ 30° to reduce covering by sediments (English et al. 1997). Ceramic settlement tiles

glazed on one side and bare on the other were mounted in pairs on the frames, with the glazed sides facing each other, leaving a small gap of 0.5 cm (Maida et al. 1994). Each frame could hold 16 tile pairs. Four pairs from each frame were sampled every two weeks during the two months sampling campaign. During the first two samplings the sampled tiles were replaced with new ones, which were then taken out during the third sampling. Due to practical reasons the samplings at the near-shore (BL) and mid-shelf (BD) took place on the same day, followed by sampling inshore (LL) the following day.

After each sampling the tiles were transported to the laboratory at Barrang Lompo in clean individual zip-lock bags. At the laboratory all tiles were checked with fluorescent light (GoBe Nightsea) and the number of coral recruits on both sides of each tile noted. After determination of the recruit numbers, two of the four tile pairs from each frame were chosen to be sampled for bacterial community composition. All organic material from a 1 cm wide patch on the side of each of those tiles (containing no coral recruits) was scraped off with a scalpel. The obtained organic material was rinsed into Ependorf cups using ~1.5 mL of "RNA later" (following Ambion, Texas, USA). The samples were frozen at -20 °C until further analysis (described below).

After sampling all tiles were bleached in a 5 % sodium hypochloride solution for 12-24 h and subsequently dried in the sun. Subsequently all coral skeletons on the tiles were then marked and numbered before photographs of all tiles and individual skeletons were taken for later analysis. All tiles were cross-checked with the fluorescence photographs and locations where coral recruits were noted before, but no skeleton was found, were marked as well. Identification to the family level of all recruits was done using Babcock et al. (2003).

Temperature tile experiment

To determine the development of bacterial communities under a global warming scenario an extra frame with settlement tiles was positioned at the near-shore island (BL). After 25 days in the reef eight tile pairs were transported to the laboratory, where samples for bacterial community analysis were taken (see above for details) and stored at -20 °C. The tiles were then placed in one of two clean 10 L plastic tanks containing unaltered water from BL. Temperature in the tanks was controlled by Eheim Jäger aquarium heaters (150 W, Eheim GmbH, Weilheim, Germany) and continuously logged using a Pendant HoBo temperature logger (Onset USA). Two temperature treatments were implemented; a minor temperature increase of +1 °C and a major increase of +3 °C. The tiles were positioned in the same

orientation as before with a 0.5 cm gap between each pair. A small pump (Eheim compact 300, Eheim GmbH, Weilheim, Germany) was installed to provide water movement within the tanks. The experiment started in the afternoon after the sampling and was ended 70 h later. Samples for bacterial community analysis were taken again, making sure not to sample the same patch on the tile as before the experiment.

Bacterial community analysis

All frozen samples for bacterial analysis were transported to the ZMT in Germany. DNA was extracted for 82 samples using the PowerSoil® isolation Kit from MoBio (www.mobio.com) following their extraction protocol. DNA concentration was measured and checked for purity before extracts were sent to LGC Genomics (Berlin, Germany) for PCR and Illumina-sequencing.

DNA 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) with the primer set S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Klindworth et al. 2013). Following the removal of the primers by LGC Genomics the sequences were processed in multiple steps. In the first step all sequences were quality trimmed with a sliding window of 4bp and an average quality of at least 12, using the program Trimmomatic (v.0.33, Bolger et al. 2014). Forward and reverse reads for each sample were merged using PEAR (v.0.96, Zhang et al. 2014). The overlap was set to 10bp with a minimum and maximum length of the merged reads of 350 and 500bp, respectively. The quality of the merged reads was checked using the application FastQC (v.0.11.3, Andrews 2011) before the DNA sequence information was extracted for further processing using BBMap (v.35.43, Bushnell; sourceforge.net/projects/bbmap/). The sequences were dereplicated and clustered into OTUs using the fastidious algorithm of swarm (v.2.1.2, Mahe et al. 2014) with a cutoff between heavy and light amplicons of 3. A representative sequence of each OTU was used for the taxonomic classification using SINA (v.1.2.11, Pruesse et al. 2012) based on the Silva 119 database (Quast et al. 2013). The data were further curated in R with custom functions written for this purpose (MPI Bremen, http://www.mpi-bremen.de/Page8678.html#Section28356). Unwanted lineages (such as Archaea, chloroplasts and mitochondria) were removed from the dataset. In a final step all singleton OTUs were removed, reducing the number of OTUs by 94 % while retaining more than 70 % of sequences per sample. Alpha- and betadiversity of the microbial communities was assessed

using the R package vegan. Samples with fewer than 500 sequences were removed from the dataset before alpha diversity was calculated.

Data analysis

Statistical analysis of all data was performed in R (R v.3.0.2 using R Studio v.0.98.1056). Water parameters were analyzed using Kruskal-Wallis test with a post-hoc multiple pairwise comparison (Siegel & Castellan 1988). Water parameters were Wisconsin double standardized for principle component analysis (PCA). Benthic communities were assed using PCA the same way and the Shannon-Weaver and Simpson diversity indices were calculated. Coral recruitment between near-shore and mid-shelf islands were compared using the Wilcoxon rank sum test. Graphical analysis was performed using R and the functions provided by Calypso (v.3.4, http://bioinfo.qimr.edu.au/calypso/faces/multivariat.jsp) to prepare plots for correlation heatmaps and principle component analysis.

Results

Water parameters

The environmental parameters at the three islands are shown in Tab. 5.1. Chl. a, suspended particulate matter (SPM) and the measured nutrient parameters (NO_x, PO_4 and Si) differed significantly between the three sites (Kruskal Wallis Test $p < 0.05$, see Tab. 5.1). Post-hoc multiple pairwise comparisons showed that NO_x differed only between mid-shelf (BD) and inshore (LL), SPM differed only between near-shore (BL) and inshore (LL), while significant differences in PO_4, Si and Chl. a concentration were observed between inshore (LL) and both of the other islands (Supplementary table S5.1). No changes in water parameters were found between near-shore (BL) and mid-shelf (BD). Principle component analysis of the water parameters was used to visualize this separation of the sites by water parameters (Supplementary Fig. S5.1). The first principle component (PC1) explained 37.6 % of the variance in the results and mainly showed the separation between inshore (LL) and the other two islands.

Tab 5.1: Water parameters measured at the three sites. Given are averages (n=5) with standard deviation for all sampling days for temperature, pH, salinity, HDO and Chl. a, and averages with standard deviation from one sampling day (n=5) for DOC, NO$_x$, PO$_4$ and Si. SPM was determined at each site with n=6. Kruskal- Wallis test results were used to show differences between the islands, test results are presented in the last three columns with asterisks marking significant differences. Different upper-case letters indicate significant differences from post-hoc multiple pairwise comparisons.

	Inshore (LL)	Near-shore (BL)	Mid-shelf (BD)	chi-squared	df	p-value
Temperature [°C]	29.64 ± 0.50	29.73 ± 0.57	29.60 ± 0.52	0.5298	2	0.7673
pH	8.06 ± 0.03	8.09 ± 0.05	8.06 ± 0.04	0.8538	2	0.6525
Salinity	33.36 ± 0.64	33.33 ± 0.75	33.32 ± 0.98	0.0468	2	0.9769
HDO [mg/l]	5.65 ± 0.33	6.35 ± 0.53	5.73 ± 0.63	5.0994	2	0.0781
DOC [μM]	84.97 ± 10.82	69.32 ± 7.70	86.41 ± 18.63	4.4327	2	0.1090
NOx [μM]	0.21 ± 0.02 ^{A}	0.48 ± 0.06 ^{AB}	0.71 ± 0.09 ^{B}	15.1579	2	0.0005 *
PO4 [μM]	0.18 ± 0.01 ^{A}	0.11 ± 0.01 ^{B}	0.12 ± 0.01 ^{B}	11.8383	2	0.0027 *
Si [μM]	2.76 ± 0.27 ^{A}	4.51 ± 0.48 ^{B}	4.44 ± 0.58 ^{B}	11.8017	2	0.0027 *
Chl. A [μg/l]	0.73 ± 0.77 ^{A}	0.04 ± 0.03 ^{B}	0.02 ± 0.01 ^{B}	11.6608	2	0.0029 *
SPM [mg/L]	7.76 ± 0.79 ^{A}	2.85 ± 1.12 ^{B}	5.18 ± 1.12 ^{AB}	14.0000	2	0.0009 *

<u>Benthic transects</u>

Benthic communities differed between the three islands (see Supplementary Fig. S5.2). The most dominant group inshore (LL) was turf algae (44.0 %), with some macroalgae (9.7 %) and soft corals (5.6 %) and only few live corals (6.3 % non-Acropora + 0.1 % Acropora). With further distance from the shore, live coral cover increased, while turf decreased. Near-shore (BL) live coral cover was 27.4 % (non-Acropora, + 0.1 % Acropora) and only 3.7 % were covered by turf algae. 57.1 % of the reef was bare substrate. The most dominant groups at the mid-shelf (BD) were live corals (38.4 %) including a large number of Acroporids (20.0 %). Only 35.9 % was composed of bare substrate. This separation of the islands by benthic community composition can also be seen in the principle component analysis (Supplementary Fig. S5.3). PC1 explained 62.7 % of the variance, mostly between inshore (LL) and the other two islands, while PC2, explained 24.8 % of the variance, mostly between near-shore (BL) and mid-shelf (BD). Permutational multivariate analysis of variance (PerMANOVA, adonis in R) showed a significant difference between the sites (p=0.009, see Tab. 5.2).

Tab 5.2: PerMANOVA for benthic community differences between sites.

PERMANOVA	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
site	2	0.76233	0.38117	24.604	0.89132	0.009 **
Residuals	6	0.09295	0.01549		0.10868	
Total	8	0.85529		1		

Tab 5.3: Benthic community composition determined from photoquadrats using CPCe (n=25 for 3 transects at each site). Average Simpson diversity and percentages of cover for each major category are presented. C.nA = non-Acropora corals, C.A= Acropora corals, DC= dead corals, SC= soft corals, CCA= Crustose coralline algae, MA= macroalgae, T= Turf, O= other live organisms, S=substrate.

	Simpson diversity	C.nA	C.A	DC	SC	CCA	MA	T	O	S
LL	0.66	6.26	0.09	0.06	5.61	0.00	9.70	44.00	1.48	32.78
BL	0.59	27.41	0.06	4.98	0.09	1.36	0.46	3.70	4.85	57.06
BD	0.67	38.41	19.95	2.89	0.00	1.65	0.03	0.53	0.61	35.93

Diversity of the benthic communities was determined by Simpson diversity index (Morris et al. 2014) (see Tab. 5.3). Simpson diversity was lowest at the near-shore site (BL), meaning that this was the island with the highest diversity. There were significant differences to both other sites (see Tab. 5.4). No significant differences in diversity of the benthic community were found between mid-shelf (BD) and inshore (LL).

Tab. 5.4: ANOVA and post-hoc Tukey HSD results for Simpson diversity of benthic communities. Asterisks mark significant results.

ANOVA					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
site	2	0.0125	0.0062	9.8720	0.0127 *
Residuals	6	0.0038	0.0006		
Post hoc Tukey HSD					
	diff	lwr	upr	p adj	
LL-BL	0.0693	0.0064	0.1323	0.0343 *	
BD-BL	0.0860	0.0230	0.1490	0.0136 *	
LL-BD	0.0166	-0.0463	0.0796	0.7105	

There was a significant difference also in distribution of coral morphologies at the three sites (Tab 5.5). Distributions (see Supplementary Fig. S5.4) were similar between the inshore (LL) and near-shore (BL) sites with mostly massive corals (>50 % of live coral) and only few other morphologies (encrusting and tabular inshore (LL) and branching and submassive near-shore (BL)). The diversity of coral morphologies at the mid-shelf (BD) was much higher, with branching Acropora as the most abundant morphology (28.4 %), followed by other branching corals (17.8 %), but many other morphologies also present (encrusting, foliose, massive, submassive and tabular).

Tab 5.5. PERMANOVA for coral morphologies at the three sites.

PERMANOVA	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
site	2	1.29676	0.64838	11.183	0.78847	0.001 *
Residuals	6	0.34788	0.05798		0.21153	
Total	8	1.64464			1	

Coral recruitment

During the 2 month sampling period no hard coral recruitment was recorded at the inshore site (LL). At this site no young recruits (<3 cm) were recorded on natural reef substrates and no coral settlement occurred on settlement tiles. Recruitment of coral larvae was however recorded for both of the other sites. On natural reef substrate at the near-shore reef (BL) 2.9 ± 1.7 hard coral recruits (<3 cm) were counted per 20 x 20 cm quadrat $(0.73 \pm 0.44$ recruits per 100 cm^2), at the mid-shelf (BD) numbers were slightly higher with 3.6 ± 2.0 per quadrat $(0.90 \pm 0.49$ recruits per 100 cm^2) were found. There were no significant differences between coral recruits on natural substrate between the two sites (p=0.18).

The numbers of coral spat on artificial tiles compared to natural reef surfaces was significantly different at both sites (Wilcoxon rank sum test p<0.001). This was even more pronounced at the mid-shelf site (BD), where coral recruitment on the reef was much higher than on the tiles, while near-shore (BL) settlement was more similar on both surfaces. Settlement on artificial tiles at the near-shore island (BL) was significantly higher than at the mid-shelf (BD). A total of 667 spat were recorded near-shore (BL), while 199 were recorded at the mid-shelf (BD). On average this were 0.80 ± 0.12 spat per 100 cm^2 for the near-

shore (BL) and 0.24 ± 0.02 spat per 100 cm^2 for the mid-shelf (BD). Analysis of the temporal settlement pattern showed that at the mid-shelf (BD) the number of settled coral spat on tiles increased with increasing exposure time at all frames (Fig. 5.1). The tiles placed in the reef at later time points differed not markedly from the others. A different picture for the temporal settlement pattern was presented near-shore (BL) (Fig. 5.2), where in addition a difference between the three frames was seen. Almost no larvae were found on tiles placed in the reef during May 2014, but only on those tiles placed in the reef in the end of April. At two frames at the near-shore site (BL) the highest number of coral spat settled during the first two weeks in the beginning of May (on the tiles sampled after 19 days) and decreased during the following samplings. The numbers at the third frame were stable during the first 6 weeks and then increased again during the 8th week. 19 of the total 20 Acropora larvae identified on all tiles had settled at the near-shore island (BL).

A total of 781 coral recruits were counted on the tiles using the fluorescence method, while 824 skeletons were found after drying the tiles. But the differences between the two methods were not significant. This difference includes on one hand spat that could not be detected using fluorescence (either due to missing fluorescent pigments or due to coverage by other macrofouling organisms) and thus were only counted after drying or a number of spat that were lost during the handling procedure of the tiles (bleaching and drying) and thus were only counted by fluorescence. On tiles from the mid-shelf (BD) the number of skeletons found after bleaching was generally lower than the number of spat counted using fluorescence. On tiles from the near-shore (BL) the opposite trend was observed, with always higher numbers of skeletons recorded than live spat counted using fluorescence.

The majority of the skeletons found (669 of the recruits) were identified as Pocilloporidae (81.2 %), with only 20 Acroporidae (2.4 %) and 6 recruits from other families (0.7 %). 15.9 % of the skeletons could not be identified due to adhering materials, broken skeletons or very early stages that could not be identified with certainty. The spatial settlement pattern on the artificial tiles was the same at both sites with coral recruitment (Fig. 5.5 + 5.6); most coral spat settled on the lower side of the lower tile (65.0 % of all spat), with the second-most preferred place being the lower side of the upper tile (30.6 %). Only 0.6 % of larvae settled on the exposed side of the upper tile and only few more (3.8 % of all larvae) on the upper side of the lower tile.
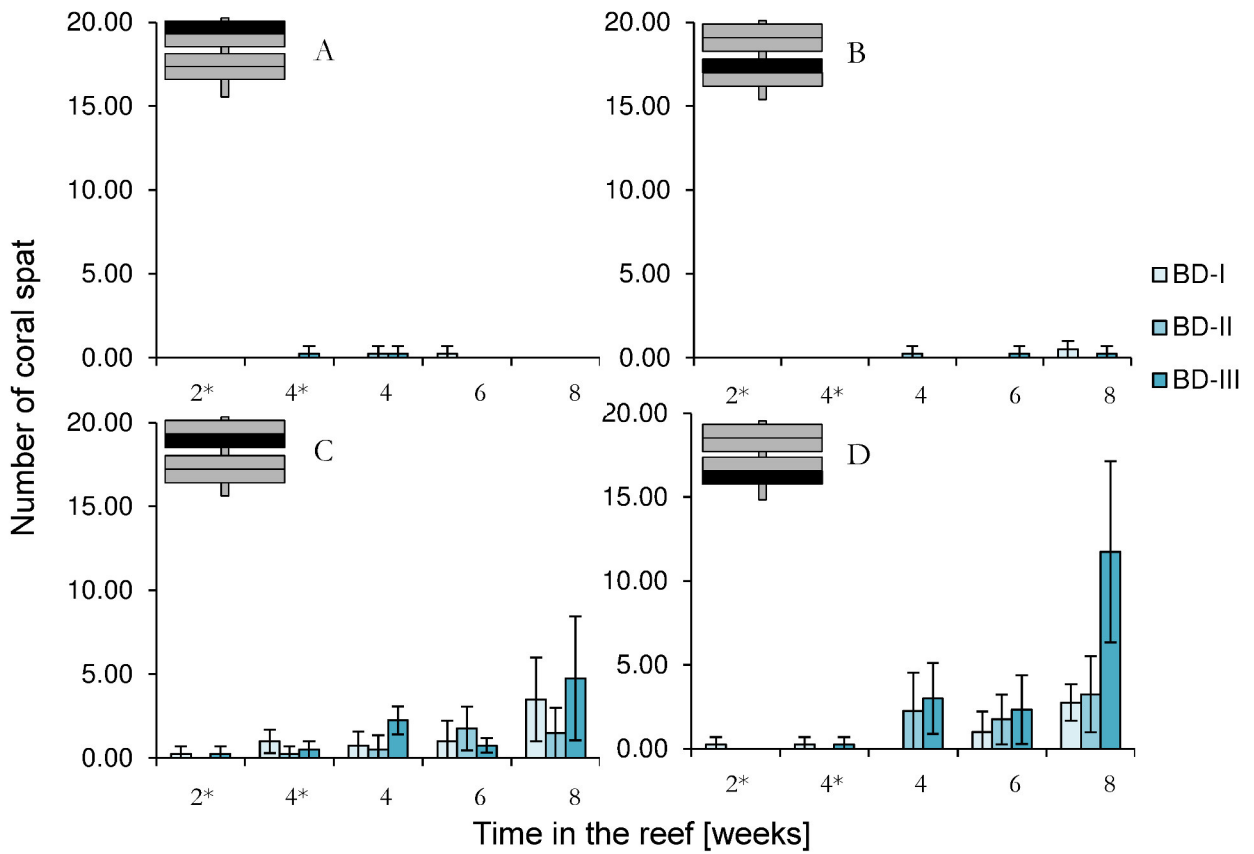
Fig. 5.1 Coral skeleton counts on artificial settlement tiles at the mid-shelf station (BD). Settlement on tile pairs with upper and lower sides of lower and upper tile was recorded. The graphs A-D represent counts on the settlement sides indicated by the schematic of the tile pair in each left corner. Roman numbers indicate the three different frames. Averages for each frame (n=4) are given with standard deviation, sorted by time spent in the reef. Tiles were sampled every 2 weeks and replaced with new tiles during the first two samplings. Week numbers marked with an asterisk indicate replaced tiles, deployed in May 2014, while those without asterisk are the tiles places in the reef End of April 2014.
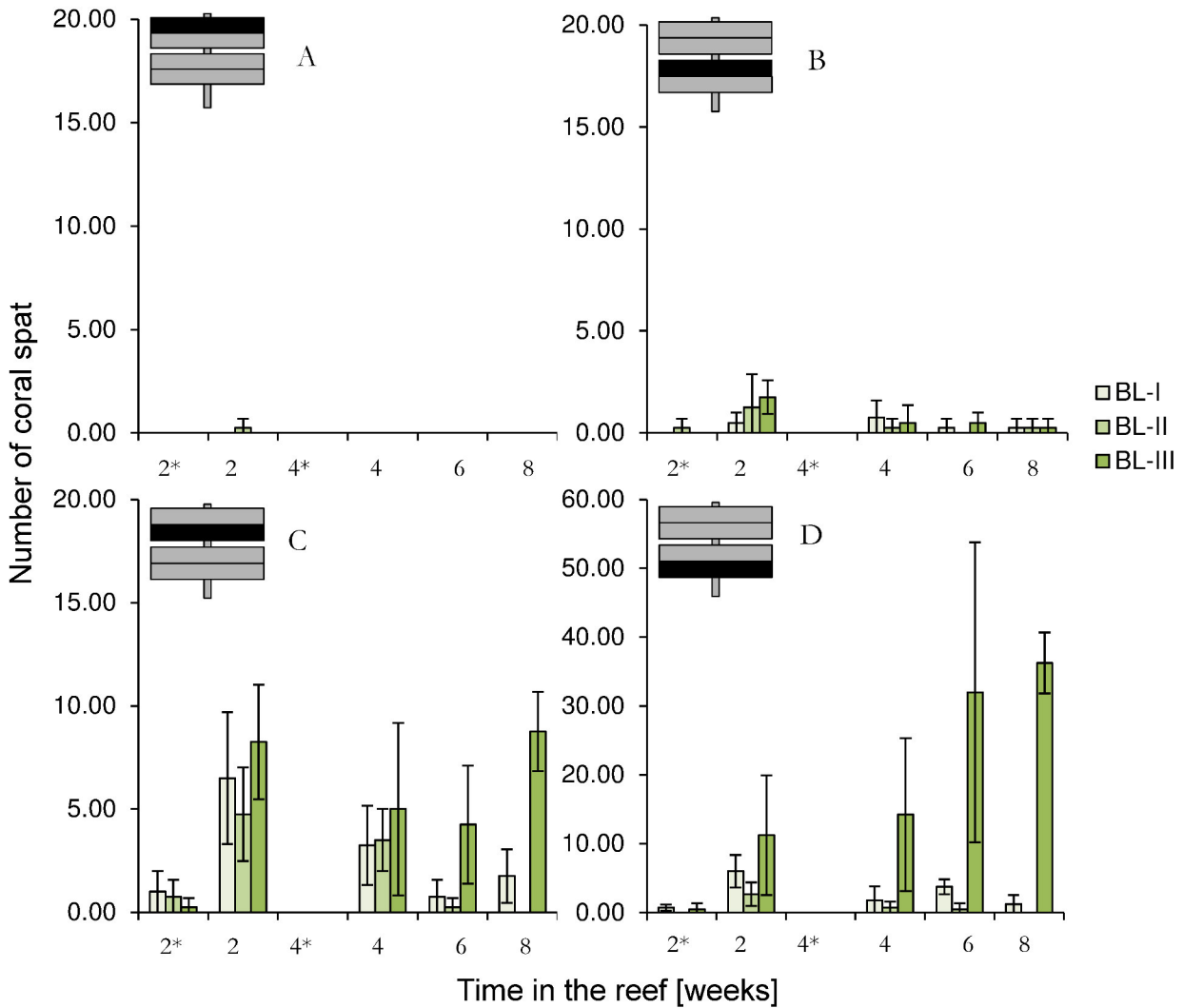
Fig. 5.2 Coral skeleton counts on artificial settlement tiles at the close near-shore site (BL). Settlement on tile pairs with upper and lower sides of lower and upper tile was recorded. The graphs A-D represent counts on the settlement sides indicated by the schematic of the tile pair in each left corner. Roman numbers indicate the three different frames. Averages for each frame (n=4) are given with standard deviation, sorted by time spent in the reef. Tiles were sampled every 2 weeks and replaced with new tiles during the first two samplings. Week numbers marked with an asterisk indicate replaced tiles, deployed in May 2014, while those without asterisk are the tiles places in the reef End of April 2014.

Bacterial communities

For bacterial communities on natural reef substrate diversity (inverse Simpson) was lowest inshore (LL), but no significant differences were found between the other islands. The most abundant classes on natural reef substrates were *Alphaproteobacteria, Cyanobacteria* and *Gammaproteobacteria* at all three sites (see Fig. 5.3). The mid-shelf (BD) community was characterized by higher numbers of *Alphaproteobacteria* than the other islands, but lower *Gammaproteobacteria*, while the inshore island (LL) had the highest abundance of Cyanobacteria and near-shore (BL) the highest number of *Gammaproteobacteria*. While the bacterial communities on reef substrate from inshore (LL) and mid-shelf (BD) were quite similar to another, there was a high variability in the samples from near-shore (BL) (see Fig. 5.4). PERMANOVA showed a significant difference of bacterial communities on natural substrate between the three sites (p=0.002, see Tab 5.6).
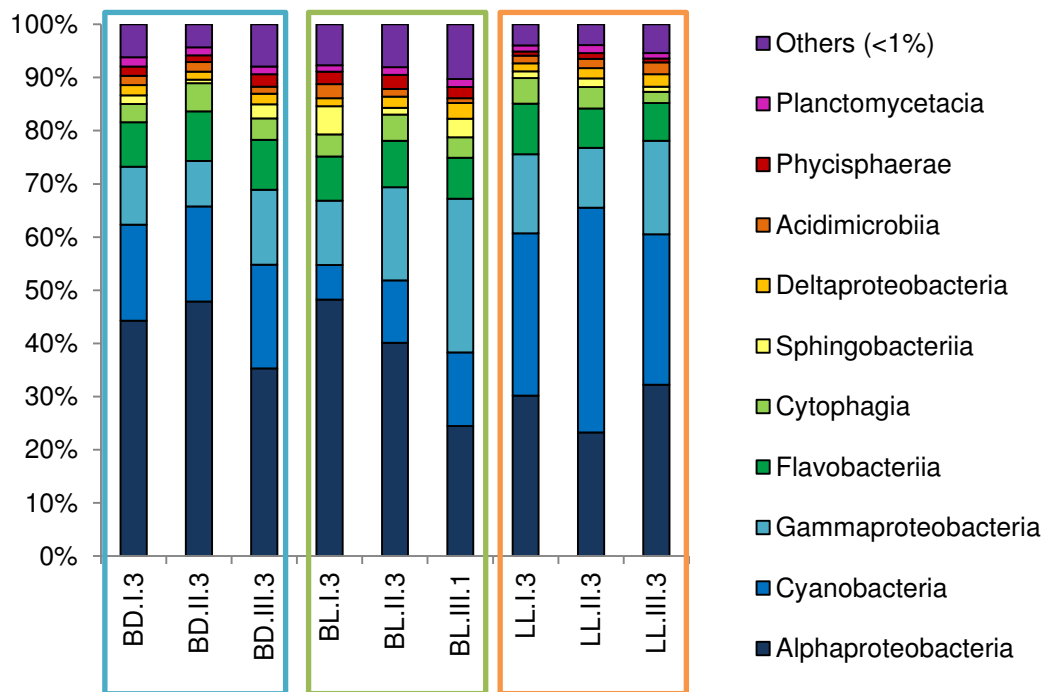
Fig. 5.3 Relative abundance of bacteria classes on natural substrate. Shown are bacterial communities from inshore (LL), near-shore (BL) and mid-shelf (BD) reefs. The most abundant classes are presented with all classes comprising of less than 0.7 % in total were summarized as "others".

Tab. 5.6: PERMANOVA results for bacteria on natural substrate. Bacterial communities were tested on OTU level to test for any effect of site.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
site	2	0.93755	0.46878	1.5156	0.3356	0.002 *
Residuals	6	1.85577	0.3093	0.66436		
Total	8	2.79332	1			
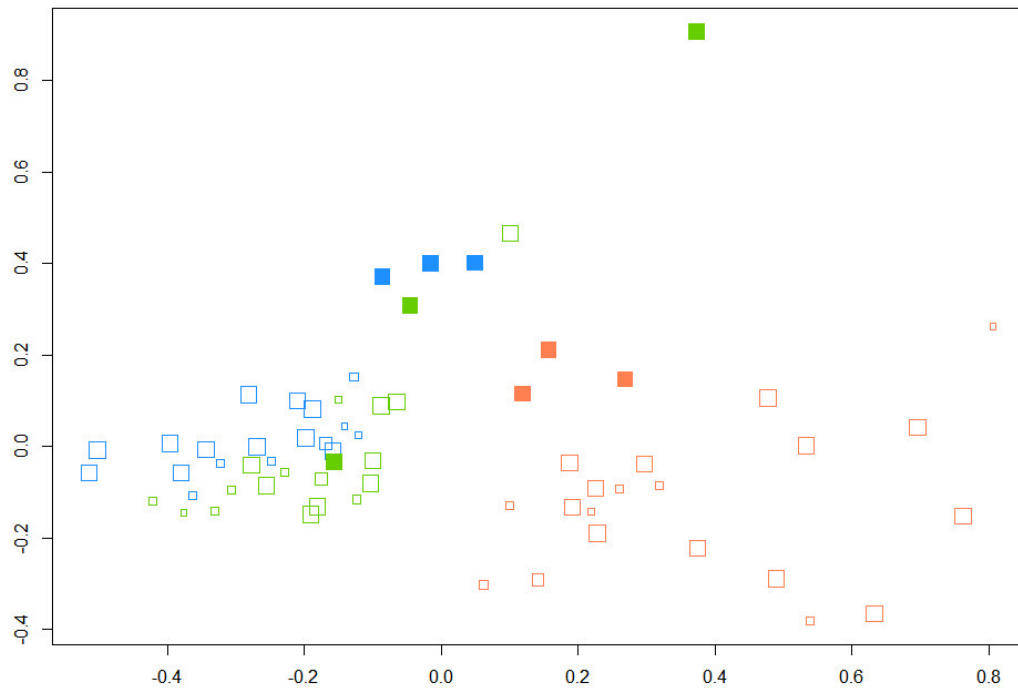
Fig. 5.4: NMDS plot for bacterial communities on artifiical ceramic tiles (open symbols) and natural reef substrates (filled symbols). The different sites are represented by different colors; inshore (LL)= orange, near-shore (BL)= green and mid-shelf (BD)= blue. Artificial substrates were sampled after varying exposure time in the reef, represented by different symbol sizes; smaller symbols represent samples after 2 and 4 week exposure, larger symbols samples after 6 and 8 weeks.

Bacterial communities on natural substrate were correlated with site specific characteristics of water quality (see Fig. 5.5 + 5.6) and benthic community composition (Supplementary Fig. S5.5). Among the most abundant bacteria, *Cyanobacteria* were highly correlated with PO_4, Chl. a and DOC, while *Alphaproteobacteria* with all but DOC, and *Gammaproteobacteria* were only weakly correlated to the water quality in terms of NO_x, SPM and DOC.

On the artificial substrates similar bacterial communities to those on natural reef substrate were found. The most abundant groups were the same as on natural substrate (*Gammaproteobacteria, Alphaproteobacteria* and *Cyanobacteria*). Differences in community composition were only seen between the inshore site (LL) and the other islands (BL+BD) (Fig. 5.4). No difference of either tile orientation or surface on bacterial community composition could be detected.
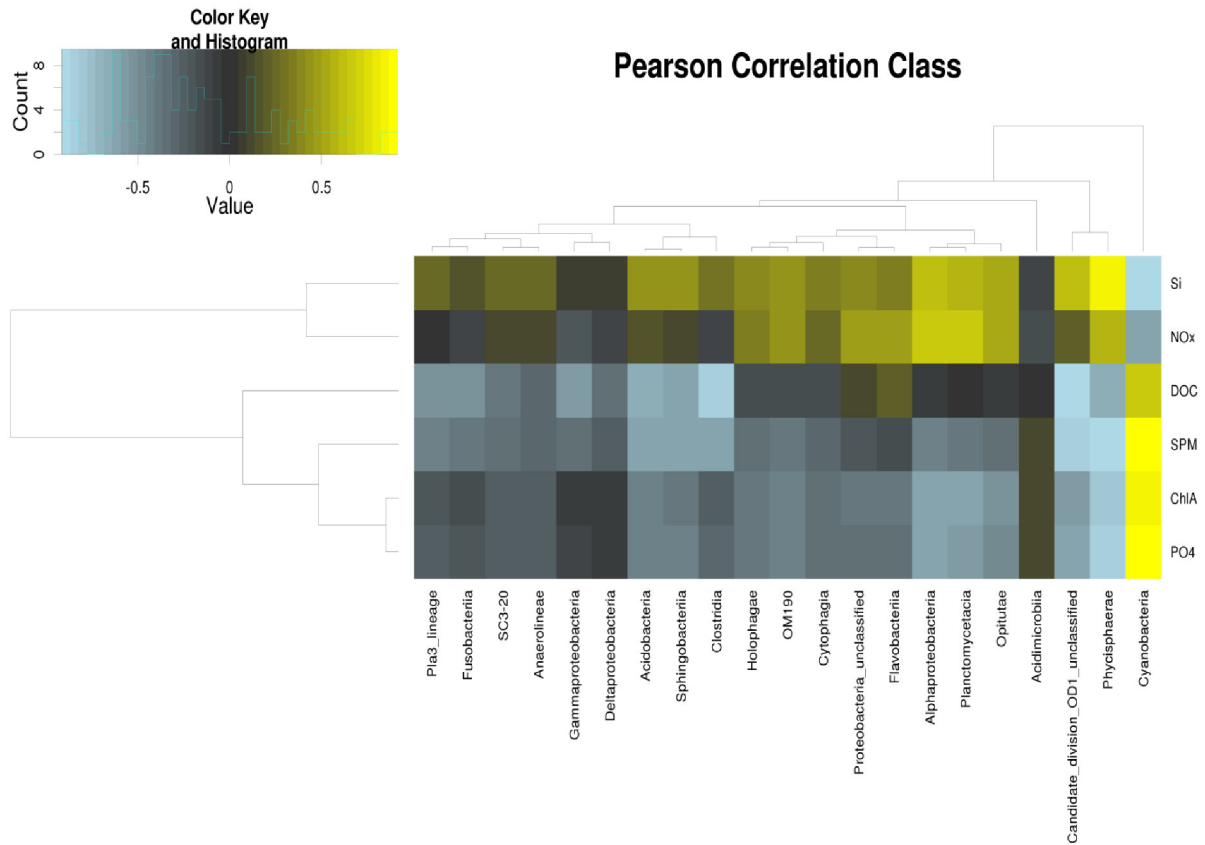
Fig. 5.5: Correlation between water quality parameters and bacterial communities on natural substrate.

Fig. 5.6: Principle component analysis of the bacterial community on natural substrates with influences by A) water quality and B) benthic groups indicated.

Highest numbers of coral settlement inducing *Pseudoalteromonas* were found on tiles at the mid-shelf island (BD) while lowest abundances were found at the near-shore island (BL). *Roseobacter*, a genus recorded to have inhibitory properties towards known coral pathogens (Nissimov et al. 2009) were found as well near-shore (BL) and mid-shelf (BD), but only in very low numbers at the inshore site (LL). Bacteria of the genus Vibrio also had the highest abundance at artificial tiles at the mid-shelf site (BD).

Bacterial communities on artificial settlement tiles were correlated with water quality and benthic community composition similar to those on natural substrates (Fig. 5.7 + 5.8 + Supplementary Fig S5.6). There were clear groups of bacteria correlating with Chl. a and PO_4 as well as with macroalgae, soft corals and turf algae, all specific to the inshore site (LL). The very abundant *Alphaproteobacteria* were among them. *Cyanobacteria* on tiles were highly correlated with all water parameters, while the occurrence of *Gammaproteobacteria* could, although only to a small part, be explained by SPM, DOC and NO_x.
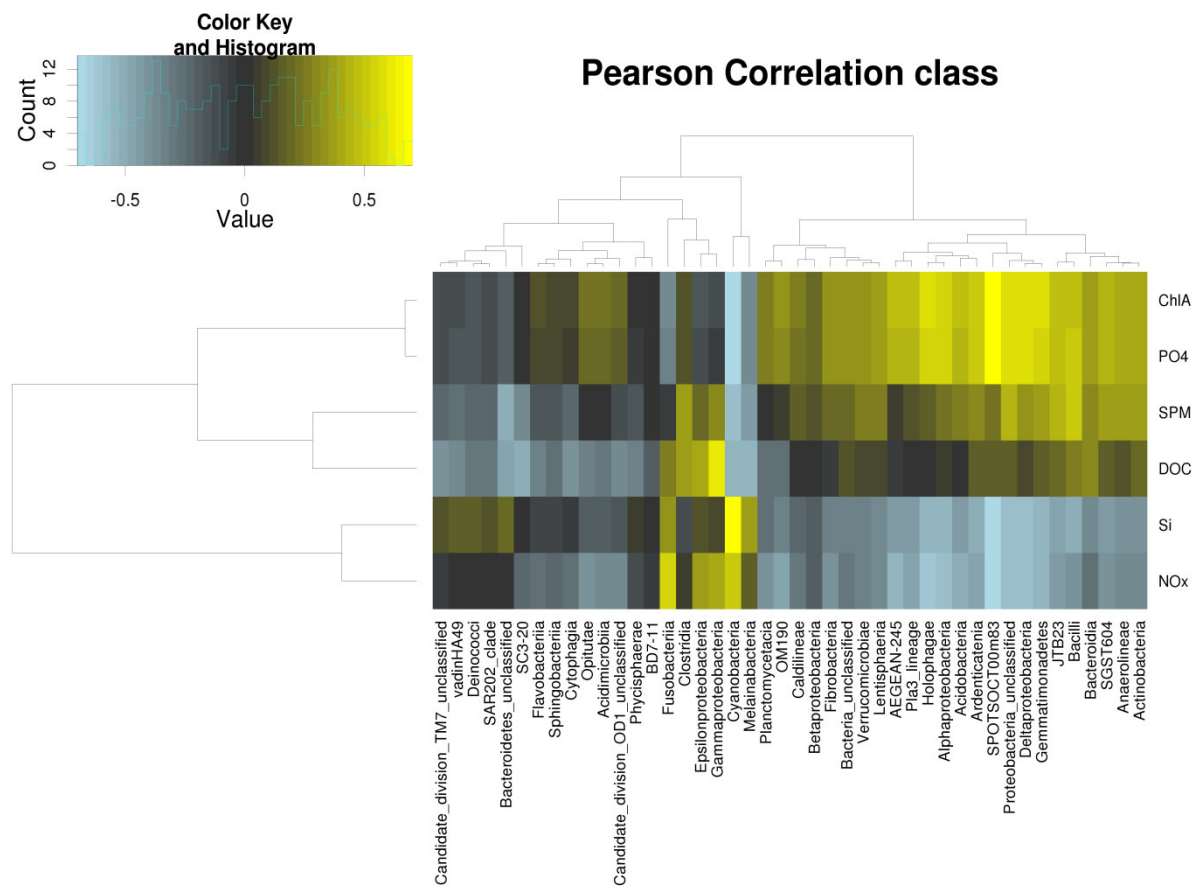
Fig. 5.7 Correlation between water quality parameters and bacterial communities on artificial settlement tiles exposed for 8 weeks in the reef.
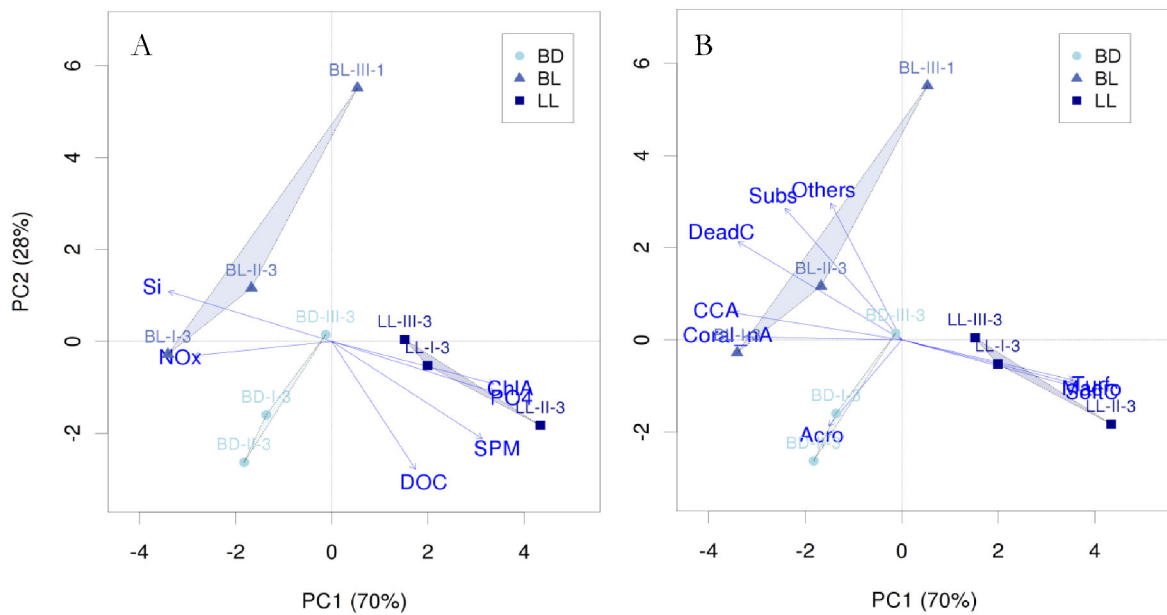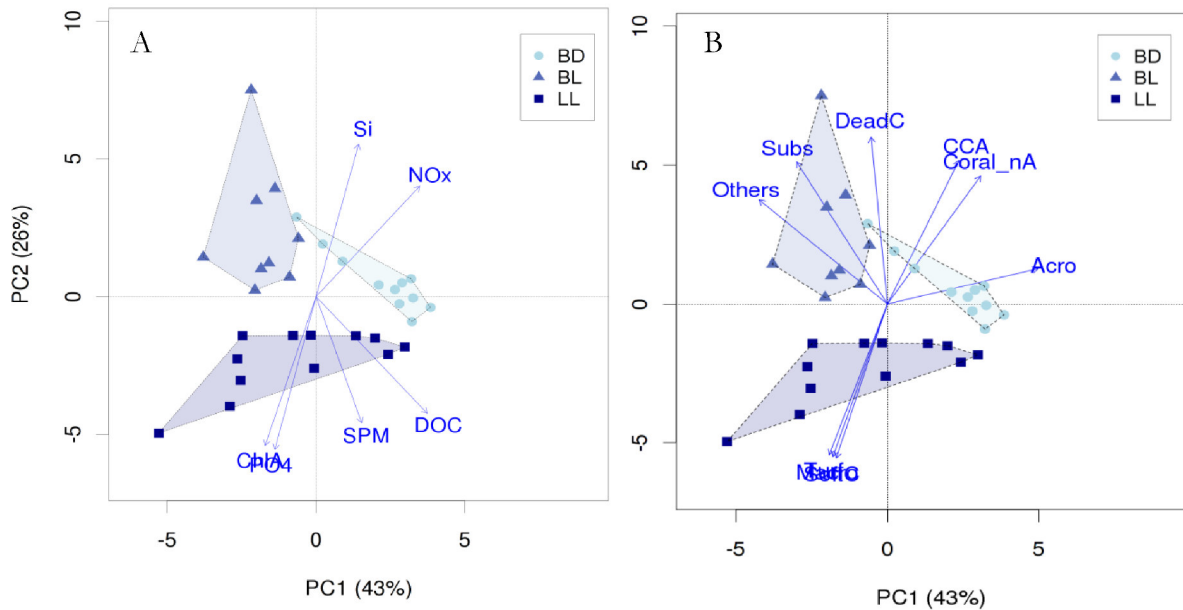
Fig. 5.8 Principle component analysis of the bacterial community on natural substrates with influences by A) water quality and B) benthic groups indicated.

Temperature- Tile Experiment

Due to high ambient temperatures the control temperature treatment also resulted in a temperature increase and was ~1 °C warmer compared to the normal reef temperature (31.1 ± 0.6 °C). Therefore it was considered as a minor temperature increase. It was still significantly lower than the high temperature treatment which had a + 3 °C and was at 32.9 ± 0.9 °C.

At the end of the experiment (i.e. after 70 h at increased temperature), alpha diversity (inverse Simpson) increased slightly in both treatments, but was highest in the +1 °C increase treatment. Relative abundances of bacteria classes were altered by the temperature treatments (Fig 5.9). In both temperature treatments the number of *Gammaproteobacteria* and *Cyanobacteria* was decreasing while the number of *Alphaproteobacteria* and *Flavobacteria* was increasing. Minor changes were also seen in the increase in *Sphingobacteria* and decrease in *Fusobacteria*. There seemed to be no difference between the effect of +1 °C and +3 °C temperature increases on bacterial community.
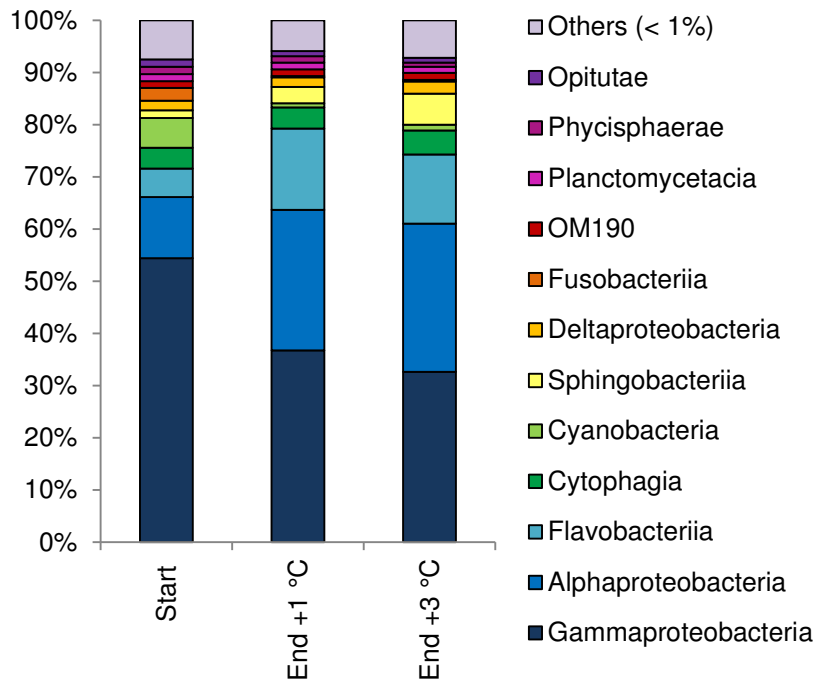
Fig. 5.9 Relative abundances of bacteria classes in the experiment. Tiles were exposed to either a minor or a major temperature increase (31.1 ± 0.6 °C or 32.9 ± 0.9°C respectively) for 70 h. All bacteria classes occuring less than 1 % within the samples are summarized as "others".

Discussion

Differences between sites / Benthic transects

Water quality clearly separated the inshore island (LL) and the two others (BL + BD). Inorganic nutrients (NO_x, PO_4 and Si), Chlorophyll a and suspended particulate matter all showed significant differences, with lower water quality (higher PO_4, Chl. a and SPM) inshore. This is in conformation with previous findings in the area (Sawall et al. 2013, Polónia et al. 2015). No changes between the near-shore (BL) and mid-shelf (BD) islands were found in terms of water quality. Other studies determined that impacts from the coast reached much further during wet season (Dec-May) (Polónia et al. 2015), which explains that during our sampling in the dry season the anthropogenic impacts were restricted to inshore site (LL). In our results we saw no difference in water parameters between near-shore (BL) and mid-shelf (BD) that could be explained by the higher population density on the near-shore island. This implies that the influence from the coast and Makassar with the Jene

Berang river close by have a higher impact on the water quality surrounding the islands, than the communities living on the islands themselves.

The results from the analysis of the benthic community compositions were as expected. The inshore island (LL) had the lowest hard coral cover while a large part of the site was covered by turf algae and the highest number of macroalgae found at all sites. These were not seen at the near-shore island (BL), but there large bare substrate and rubble fields indicated strong destructive impacts in the past. At the offshore island (BD) the live coral cover was the highest, with a large proportion of Acropora corals that were not seen at the other sites.

Similar benthic community compositions were found in other studies. Sawall et al. (2013) found an increase from in live coral cover from 10 % to 18 % between 2007 and 2009 at the inshore site (LL). Unfortunately our study could not confirm this increase 5 years later, but shows a threefold decrease again compared to previous coral covers. Sawall et al. (2013) also found high cover of coral rubble (38-56 %) at blast fishing impacted near-shore islands, coinciding with the large amount of substrate and rubble found at our near-shore island (BL) as well. These findings show that also in the Spermonde Archipelago reefs are subjected to severe stresses leading to degradation of the ecosystem.

Larval recruitment:

The numbers of recruits (young corals < 3 cm) found on the natural reef substrate at the outer shelf island (BD) were slightly higher, but similar to those near-shore (BL), while no recruits were detected along transects at the inshore island (LL) at all. This was the case as well for coral recruitment on artificial settlement tiles. Although recruitment of larvae was recorded five years previously (Sawall et al. 2013) and live coral colonies were found inshore (LL), these seem to be very restricted in their reproduction, indicating a severe threat for these reefs in the future. Recruitment in the reef at the other two islands was in the range of recruitment recorded previously in the area with 1.46 $\pm$ 0.50 spat per 100 cm^2 over a 3 month period (Sawall et al. 2013) and also similar to other regions (Salinas-de-León et al. 2011 and Glassom et al. 2004 for an overview)

The recruitment of corals onto artificial settlement tiles was significantly different from that at on natural reef substrate. At the near-shore reef (BL) higher numbers of coral spat were recorded on artificial tiles than on natural substrate, while at the mid-shelf reef (BD) lower numbers were found on artificial tiles. When assuming that there is no effect of the substrate

itself as presented in other studies (Burt et al. 2009, Salinas-de-León et al. 2011), the difference at the near-shore site (BL) can be explained by post-settlement mortality. While the recruits on tiles could be detected at a very early stage, recruits in the reef were already > 3 mm in diameter. Following settlement, recruits face high pressure due to grazers, overgrowth by algae and sedimentation (Price 2010). Thus coral recruits at the near-shore island (BL) might face higher post-settlement mortalities than those at the outer-shelf island (BD). Further higher numbers on settlement tiles could be due to "trapping effects" from altered hydrodynamic flows due to the raised frame structure compared to natural reef surfaces (Mundy 2000). While at the near-shore reef (BL) the higher numbers of recruits recorded on the tiles compared to the reef can be explained with post-settlement mortalities, the much higher numbers of recruits on reef surfaces at the outer-shelf island have another reason. Although there are no clear reports on times for coral spawning in Spermonde (Sawall et al. 2013), the evidence is large that it occurs between February and April (Salinas-de-León et al. 2013, Yusuf et al. 2013), so slightly before our sampling began. Thus especially mass-spawning species would have already settled and there was lower larvae supply in the waters.

The numbers of coral recruitment on artificial settlement tiles is comparable to those in other studies (Ferse et al. 2013, Salinas-de-León et al. 2013, Sawall et al. 2013). The temporal settlement pattern on the tiles was different at the two sites where recruitment was recorded. At the mid-shelf reef (BD) number of recruits on tiles increased with increasing exposure time in the reef, indicating a constant supply of larvae as by brooding corals. At the near-shore island (BL) most larvae had settled during the first two weeks of tile deployment, which was around the full moon period. The number of recruits on the tiles reduced after this period, further reinforcing the theory on higher post-settlement mortality at this site mentioned above. On one of the frames at the near-shore site (BL) another increase in number of coral spat on tiles was found during the last sampling, which again was right after the full moon.

Most of the spat found on the tiles were Pocilloporidae with only few exceptions. These high abundance of Pocilloporidae is even higher than in other studies (Maida et al. 1994, Sawall et al. 2013), but it most likely the cause of the sampling period being slightly in between the spawning periods. A clear spatial settlement pattern on the settlement tiles was observed at both sites where one third of all recruits settled on the lower side of the lower tile. 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.

Bacterial communities

The lowest diversity in bacterial communities was found inshore (LL), where the major groups (*Gamma-* and *Alphaproteobacteria* and *Cyanobacteria*) made up around 80 % of the entire community. There were significant differences between the bacterial communities 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, Cytophaga-Flavobacterium-Bacteroides, Planctomycetacaeae, Verrucomicrobia* and *Acidobacteriaceae* (Uthicke & McGuire 2007). *Alpha-, Gammaproteobacteria* and *Actinomycetes* were further the most abundant bacteria on crustose coralline algae surfaces (Sneed et al. 2015).

While the bacterial communities at the near-shore (BL) and mid-shelf (BD) islands were very similar, the community at the inshore site (LL) differed from them. This is similar to the water quality parameters, which were also mainly different at the inshore site (LL) and not different between the others. The bacterial communities were correlated with the site specific characteristics of water quality and benthic community composition. Another recent study in the Spermonde area discovered that bacterial communities in sediments are determined by water quality, while benthic communities are more influenced by habitat and community composition (Polónia et al. 2015). As discussed above, the effect of water quality in the Spermonde Archipelago is intensified during the wet season, thus stronger effects of water quality on bacterial community compositions would be expected than during our sampling in the intermediate season. This was found to be true for total OTU numbers, where most pronounced differences between sites were found during the wet season (Sawall et al. 2012). On artificial tiles, the same most abundant groups were found as on natural reef substrate.

Corals are associated with a range of beneficial as well as harmful bacteria (Krediet et al. 2013). The abundances of these were determined on the settlement tiles. The bacterial genus most often recorded to influence settlement of coral larvae, *Pseudoalteromonas* (Negri et al. 2001, Hadfield 2011, Tran & Hadfield 2011), was detected in highest numbers on settlement tiles at the mid-shelf island (BD). Also *Roseobacter*, that have inhibitory properties against several coral pathogens (Nissimov et al. 2009) were found at the mid-shelf reef (BD). Sneed et al. (2015) documented that CCA species that facilitated settlement of coral larvae had high abundances of bacteria inhibiting coral pathogens (Sneed et al. 2015). While the occurrence of these bacteria is beneficial for coral larvae, the genus Vibrio contains many known coral pathogens (Ben-Haim 2003, Sussmann et al. 2008), but also includes some species with larvae settlement inducing properties (Huggett et al. 2006, Tran & Hadfield 2011) and thus

the high abundance of Vibrio at the mid-shelf site (BD) and lowest abundance inshore (LL) can be either positive or negative and cannot be generalized.

While the bacterial community on natural substrates was highly correlated to the water quality and benthic community characteristics separating the sampling sites, the bacteria on the tiles were correlated to different parameters. This indicates a fast colonization of new substrates in the reef by opportunistic bacteria, while the establishment of stable communities, adapted to site-specific conditions, requires longer time periods than observed during this study.

Bacterial communities can change rapidly under varying conditions to adapt to new environments. This was also shown in our temperature experiment, where both temperature increases led to a shift in the community composition within 70 h. In experiments with bacterial communities on CCAs, high temperatures also resulted in a change in community, although there a reduction of Alphaproteobaceria was observed (Webster et al. 2011) contrary to the increase in the present study. Changes in bacterial community structure are expected to alter settlement of coral larvae. The reduction of Alphaproteobacteria on CCA surfaces in the study by Webster et al. (2011) reduced the settlement of coral larvae. Sawall et al. (2013) suggested a preference of coral larvae towards bacterial biofilms with lower bacterial diversity as occurring during the dry season in Spermonde.

In the context of expected changes in coral reef ecosystems the findings of this study determine several factors that need to be considered in evaluations of future reef management strategies. Benthic communities reflect water quality conditions, which are also influencing bacterial communities. Coral larvae recruitment, a necessary requirement for stabilizing coral communities depends strongly on the microhabitat and post-settlement mortalities. In the Spermonde Archipelago the mid-shelf (BD) was recorded to have high coral covers and good water quality, less influenced by anthropogenic impacts. While the benthic community composition at the near-shore island (BL) indicated stronger anthropogenic influences, settlement of coral larvae was plentiful, although post-settlement mortalities seem to be higher than at the mid-shelf site (BD). The inshore island (LL) on the other hand was severely impacted by multiple disturbances. Water quality was very low which was reflected in the benthic community and the complete absence of coral recruitment detected over the sampling period between wet and dry season in this study. If the conditions at this reef are not improved, it will lose its ability to cope with future changes with severe consequences for the ecosystem (Bellwood et al. 2004). Management plans are required in

order to avert further negative impacts on the other islands to prevent ecosystem changes as inshore to be repeated.

Conclusions

This study on the complex interactions of multiple stressors on settlement of coral larvae and bacterial communities has shown that, although no clear correlation between larvae and bacteria could be determined, both are altered by habitat and environmental conditions. Bacterial communities reacted to increased temperatures with fast shifts in relative species abundances. Although different bacterial communities on the settlement tiles were found at near-shore (BL) and mid-shelf (BD) reefs, there were enough positive settlement cues for larvae present. While bacterial communities were highly correlated with the water quality, which also determined the benthic community composition, settlement of coral larvae depended on the microhabitat. Exposure to environmental conditions such as light and sedimentation had a higher impact on larval settlement on artificial tiles, while recruitment in the field was further determined by post-settlement mortalities, varying due to different environmental stressors between the two sites. The results show that coral larvae settlement and bacterial community composition are factors that hold great potential in investigating anthropogenic stress on coral reefs and opportunities to approach coral reef management.

Acknowledgements

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General Conclusions and Outlook

Key findings and significance

This thesis was divided into two parts. The first part investigated the effects of selected local and global stressors and determined their combined influence on fish and scleractinian corals as key players in coral reef ecosystems. The second part investigated the effect of local anthropogenic influences on coral larvae settlement and bacterial communities in-situ in the Spermonde Archipelago, Indonesia, and evaluated their susceptibility to change in response to environmental stress. Chemical pollution and global warming resulted mainly in negative effects on metabolism of the studied species. Combined effects were found, altering the effects of single stressors on coral reef organisms. Gradients in naturally occurring combinations of local stressors in the Spermonde Archipelago caused shifts in the bacterial community compositons in the water column, in carbonate sand sediments and on solid reef surfaces. Bacterial communities on reef surfaces were highly correlated with water quality as well as benthic community structures and showed the ability for rapid shifts in their composition. Coral recruitment was severly hampered by the conditions at the most heavily influenced site and, while settlement at other sites was influenced by microhabitats on settlement surfaces, their post-settlement survival depended more on environmental conditions.

The key results of this thesis and their significance are summarized in the following chapter, referring back to the original research questions from the general introduction.

1. *What effect on fish and coral metabolism are caused by coastal pollution and habitat loss?*

Local stress on coral reefs is caused by a multitude of sources. Stressors such as pollution, can have direct effects on organisms, like fish, as well as indirectly for instance through coral cover decline as a result from the pollution (Hughes et al. 2003). Both types can be detected

at the level of individual metabolism. This thesis investigated the loss of shelter as an indirect stressor due to the degradation of the three dimensional reef structures, as well as the direct effect of coastal pollution on metabolism of three model species.

While biochemical stress responses and cellular bioindicators can yield important information on the underlying mechanisms of stress responses, it is important to consider the whole body response as an overall stress indicator in focus (Beitinger & McCauley 1990, Maltby 1999). A decreased metabolic condition of an individual organism due to stress can lead to lower reproductive output, altered behavior and ultimately mortality, thereby affecting the entire population (Maltby 1999).

In **chapter 1** the effect of shelter loss on the metabolism of a reef-dependent fish was determined. This study was also used to establish a method for the determination of oxygen metabolism. Automated intermittent flow respirometry proved accurate in measuring the metabolism in tropical reef fish. Results showed a fast recovery to standard metabolic rates (SMR) within the first hours of the experiment. The set up was suitable for long term measurements without any methodological and handling interference. Such long term measurements are necessary to determine the effect of stressors on organisms while maintaining high oxygen contents within incubation systems. Thus this intermittent-flow set-up was deemed appropriate for the use in the following chapters as well.

Furthermore, the absence of shelter had no negative effect on the metabolism of *Amphiprion ocellaris*. Depressed metabolic rates would have suggested a conservation of energy for the fish (Caulton 1977), but at least for *A. ocellaris* no apparent fitness advantage by shelter was found. On the other hand there appears to be no disadvantage in terms of stress response by the loss of shelter. Although in similar experiments a metabolic advantage was found for salmonids (Millidine et al. 2006), it seems that loss of shelter does not necessarily lead to a decrease in fitness in all fish species. Shelter in the coral reef habitat is provided by the three-dimensional structure of the reef by living scleractinian corals and can have strong impacts on fish communities (Beukers & Jones 1997, Graham et al. 2006). Although live coral cover is not the only factor providing structure in coral reefs, it plays a relevant part. Direct correlations were found between coral cover and fish biodiversity (Syms & Jones 2000, Jones et al. 2004), indicating that even though no fitness benefits were found due to shelter in our study organism, fish communities still heavily depend on intact reef structures.

While at least some reef fish may tolerate the loss of habitat structure, other local stressors could have more significant effects on fish metabolism. One major problem associated with urbanization, industry, shipping and agricultural development around the world, is that chemical pollution is becoming one of the major threats to marine organisms (Van Dam et al. 2011). Commonly used pollutants by inhabitants of coastal areas are surfactants and motor fuels. Due to the immense utilization of surfactants and motor oils and the large number of small local sources of these pollutants, they can affect reefs at larger regional scales. The experiments presented in **chapters 2 and 3** were aimed to address the effects of chemical pollution and increased temperature on the fish *Siganus guttatus* and the hard coral *Pocillopora verrucosa*. While the metabolism of the fish was depressed by gasoline, it had no visible effect on hard coral metabolism after 84 h of exposure. For fish, negative organism responses due to oil derivatives have been reported (Johnson et al. 2002, Logan 2007, Simonato et al. 2008). The literature on the effects of diesel products on corals is ambiguous. In most studies effects of diesel and other oil sources were found (Rinkevich & Loya 1979, Harrison et al. 1990, Negri & Heyward 2000, Mercurio et al. 2004), while there are also some studies where, similar to this study no effects were found (Braga & Varesche 2014, DeLeo et al. 2015).

The surfactant LAS had a severe negative effect on the metabolic condition of both organisms during the course of these experiments. SMR in *Siganus* was significantly increased, a clear indicator of increased energy demand as a result of high stress (Caulton 1977, Sloman et al. 2000). In corals, this stress became clearly visible by tissue loss in the coral fragments and a decrease in maximum quantum yield. Decreasing photosynthetic efficiency as measured by maximum quantum yield is a common response of sclereactinian corals under stress and was also found in response to high sedimentation rates and cyanide exposure (Jones et al. 1999, Philipp & Fabricius 2003). The findings of this thesis are in accordance with those from the only other study reporting effects of detergents on hard corals (Shafir et al. 2014).

The results from the **first three chapters** show that while no effects of habitat loss were found in a structure dependent reef fish, different effects of the isolated common local stressors chemical pollutants as well as increased temperature on metabolic conditions of two key players in coral reefs, fish and corals, were detected. While the organisms may be able to mitigate some of the effects by single stressors, the exposure to multiple stressors is expected to have even more severe consequences. Chemical pollutants can influence each others toxicity and combined exposures become more complex when taking non-chemical stressors

into account (Beyer et al. 2014, Ban et al. 2015). Such combined effects were investigated in the second research objective in this thesis.

2. How do key coral reef organisms react to local changes in combination with global warming?

The responses to the isolated stressor treatments in **chapters 2 and 3** already indicated their negative influence on *S. guttatus* as well as *P. verrucosa*. One of the major threats to coral reefs is the amount of multiple stressors affecting these ecosystems simultaneously, and their potential interaction with each other (Dunne 2010, Van Dam et al. 2012, Ban et al. 2014). The local stressors investigated in this thesis are very likely to occur in combination with global warming. Thus the responses of fish and scleractinian corals to these combinations were determined in **chapters 2 and 3**. In combination with higher temperatures, in a range expected within future global warming scenarios, the responses of both fish and scleractinian corals became more severe. In the experiments presented in this thesis higher temperature alone caused an increase in SMR in *S. guttatus*. Thus the energy required by the fish to cope with this temperature change had increased, a good indicator for stress. In corals exposed to high temperature dark respiration was also increased at first, but the effect was abated towards the end of the experiments similar to findings by (Coles & Jokiel 1977). While in our experiments no effect of temperature on maximum quantum yield was observed, several other studies have found a decrease in response to higher temperatures, often accompanied by coral bleaching in both field and laboratory observations (Jones et al. 2000, Fitt et al. 2009). This indicates the severe effect global warming on its own will have on corals and ultimately on reef communities.

Although no interaction between ocean warming as a global stressor and the chemical pollutants LAS and diesel could be determined, there were additive effects due to high temperature, further decreasing the metabolic condition of the organisms. In *S. guttatus*, LAS in combination with high temperature caused a stronger increase in SMR compared to the surfactant in isolation. Similar, the combination of gasoline and temperature caused a rise in SMR in the fish. In the coral *P. verrucosa* the combination of gasoline and high temperature also led to an increase in dark respiration. While temperature alone resembled a relatively acute stress, this combined stress was constant and detectable also after 84 h. The combination of the surfactant in combination with high temperature led to the most alarming effects on the coral, causing a severe tissue loss and resulting high mortality at

concentrations that were shown to occur close to surfactant sources in the reef (Baum et al. in prep.).

Coral reef organisms are expected to face an increasing number of combined stressors in the future and interaction effects are likely to occur (Ban et al. 2014). Synergistic effects have been shown for temperature with light intensity or pCO$_2$ (Reynaud et al. 2003, Bhagooli & Hidaka 2004). In fish interactive effects of metals and high temperature have been observed (Cairns et al. 1975). However knowledge on cumulative effects in marine and especially coral reef organisms is still scarce (Crain et al. 2008) and in the past the terms of synergism and antagonism have been used with different studies on combined effects (Dunne 2010). Investigations of combined effects of stressors that are likely to occur simultaneously in the reef are important to determine the actual threat inflicted on coral reef organisms.

The experiments in **chapters 2 and 3** showed that for the studied species, belonging to the groups of the most important key players in coral reefs, the response to the measured chemical pollutants was influenced by simultaneous temperature stress. Responses to stressors such as chemicals are highly species specific, thus these findings cannot automatically be generalized to all species, but it can still be assumed that other scleractinian corals and ectotherm fish with similar lifestyles would react alike.

Thus the major threat to coral reefs that causes the observed degradation worldwide at the present rate is a result of the combination of multiple stressors acting simultaneously (Edinger et al. 1998, Sale 2008). Since many members of coral reef communities depend on living corals, the necessity to reduce pollution on reefs to "buy time" for reefs to adapt to inevitable global warming (Knowlton & Jackson 2008, IPCC 2013) becomes evident. While studies in the past focused primarily on several other pollutants, such as heavy metals (Howard & Brown 1984, Guzmán & Jiménez 1992) or pesticides (Richmond 1993), and their effect on reef organisms, the focus in the future should also be on less studied pollutants, such as surfactants and gasoline, which gain importance due to the extent of their usage. Cumulative effects of multiple stressors are still barely understood (Ban et al. 2014) and studies such as this thesis are needed to determine responses of organisms and ecosystems to these stressors.

3. *Are there direct or indirect impacts of local stressors and global warming on coral reef recovery via coral larvae?*

As coral reefs face an increasing number of stressors simultaneously, smaller acute events, such as blast fishing or storm events, can lead to degradation of entire reef areas. If the conditions are still favorable for coral growth, such areas could recover through the recruitment of coral larvae. Coral larvae settlement strongly depends on the bacterial communities living on the surfaces of settlement substrates. These bacterial communities may however change in response to local and global stressors (Bourne & Webster 2004). **Chapter 4** showed that local anthropogenic influences can affect water quality at different spatial scales (10^1-10^2 km as well as 10^{-1}-10^0 km) in the Spermonde Archipelago. Bacterial communities in the water column and in carbonate sand sediments were only altered in very close proximity to the large urban area of Makassar. Similar results were found in **chapter 5** for bacterial communities on hard reef surfaces, which were mostly different between the examined inshore island, close to Makassar, and islands further away. In short term temperature experiments bacterial communities exposed to thermal stress showed their potential for rapid shifts in community compositions, indicating the effect of future warming scenarios. While bacterial communities were highly influenced by water quality and environmental changes, coral recruitment was rather influenced by microhabitats; at both investigated sites in the Spermonde Archipelago coral larvae displayed distinct preferences to settle on lower, less exposed sides of artificial spawning tiles. In addition there were differences in the effects of environmental parameters on coral recruitment before and after settlement. While the number of newly settled larvae on the artificial tiles at a near-shore island was much higher than at the other, the number of older coral recruits and general coral cover was lower, indicating higher post-settlement mortalities.

The recovery of reefs via coral larvae has been shown to enhance the fitness and resilience of reef communities (Harrison 2011) and has therefore been subject to several previous studies. Most studies on coral larvae and settlement have been performed in Australia at the Great Barrier Reef due to the well documented spawning times for the area. Studies such as the present one that determine settlement of larvae in not so well documented areas are necessary to determine the potential recovery of reef systems. Factors that determine settlement and post-settlement survival of larvae need to be understood to decide appropriate management strategies.

In future not only the number of stressors on coral reefs will increase, also temperature anomalies and storm events are expected to occur more frequently (Hoegh-Guldberg 1999, Timmermann et al. 1999). The potential of coral reefs to recover after such events, given sufficient time for recovery and favorable conditions, will be essential for the ecosystem to cope with the more frequent and stronger environmental changes. Nevertheless, if local stressors become too severe, as seen at the inshore island of Lae Lae in the Spermonde Archipelago, this chance for recovery is lost. Water quality in terms of organic and inorganic nutrients affects coral recruitment in terms of post-settlement survial of larvae as well as acting on the reproduction of adult colonies. As seen in **chapter 3**, chemical pollution and global warming can lead to decreased metabolic conditions of sclereactinian corals which in turn may lead to a reduced sexual reproduction, indicating the different levels how anthropogenic global and local stressors are affecting the reef status as well as its recovery.

Conclusions and future perspectives

Coral reefs are under severe threat due to the combination of global and local stressors (Hoegh-Guldberg et al. 2007, Burke et al. 2012). Human influence is affecting coral reef ecosystems in a multitude of ways. This thesis shows that both fish and corals can tolerate stressors to a certain extent, but succumb when too many stressors occur simultaneously. It also demonstrates that the chance for recovery of the reefs is reduced not only due to the magnitude of single stressors, but also the numbers of environmental stressors. If the reef ecosystem is altered too much, the chances for self-rehabilitation via coral larvae are diminished.

Although coral reef degradation and climate change are global problems, local coral reef management can protect reefs by maintaining their resilience and enhancing resistance to global stressors (Wooldridge 2009, Wilson et al. 2010, Burke et al. 2012). The ultimate goal in coral reef management should be the reduction of threats posed by global stressors, but this will need more time and global action. In the meantime, immediate action concerning local stressors is needed. Reducing local stressors such as coastal pollution and overexploitation of fish resources could assist coral reefs to face global changes (Hoegh-Guldberg et al. 2007). This means, for example, a reduction of pollutants and nutrients entering the reefs by implementation of effective sewage treatments. Marine spatial planning as an alternative to current management strategies could help to relieve some stressors due to over-exploitation on coral reefs (Douvere 2008).

Coral reefs in developing countries in South-East Asia are under constant anthropogenic pressure (Edinger et al. 1998). Growing human populations and an immense number of livelyhoods depending on coral reef resources pose severe threats to reef oranisms. In Indonesia, as in most adjacent countries in South-East Asia, treatment of sewage is still largely missing (Burke et al. 2012). Increasing stress on the organism level is shifting the responses to populations and ecosystem levels. Reduced survival, fitness and growth of individuals transfer responses to the entire population. Lower reproductive output is one of the severe consequences of chronic stress (Knowlton 2001, Baird & Marshall 2002) and reduces reef resilience and the potential for recovery. Changing environmental conditions due to global and local stressors are altering the reproduction success of individuals and populations and interfere with recruitment success of larvae in different taxa (Dixson et al. 2014).

While networks of marine protected- and no-take areas (MPA's and NTA's) are often deemed to be the best option in managing coral reef ecosystems (Hoegh-Guldberg et al. 2007, Wooldridge 2009, Wilson et al. 2010), there are several necessary additions. Especially as most of the described local stressors are land-based, all coastal ecosystems need to be manged in an integrative approach. Marine protection areas can be very helpful tools, but their implementation needs to be effective to avoid "paper parks", which are existing without any change in human behavior due to a lack in enforcements (Sale 2008). Further the connectivity between such protected areas needs to be considered in management strategies, taking spillover of juveniles and adults as well as distribution of larval organisms into account to replenish adjacent areas (Symy & Jones 2000, Cowen & Sponaugle 2009, Christie et al. 2010). Continuous monitoring of protected areas to determine their effect on the ecosystem is necessary to ensure their efficiency. Modelling approaches can and should be used to determine how management of coral reefs can affect the ecosystem, testing alternative management strategies (Gurney et al. 2013). In order for such modelling approaches to be successful, information needs to be gathered on different stressor responses, as it happened in this study. Further research is needed on which stressors are affecting key organism in which ways and how they interact with each other (Knowlton & Jackson 2008). Management strategies need to focus not only on how reefs should be protected, but also determine which areas need protection and how large they need to be in order to have a beneficial effects the area.

In addition to scientific studies and effective reef management strategies, the involvement of local communities into reef protection is needed. Only if the effects and reasons behind coral

reef declines are transported to a broader public, human populations may change their behavior (Inglehart 1995). Education and social events to raise awareness on why and how coral reefs can be protected will aid the enforcement of protection areas and thus should be included in all management plans.

Further research should also focus on how organisms will be able to adapt to changes. All measurements on stress are hampered by the fact that most studies cannot investigate potential adaptations organisms might experience with time, especially if this adaptation covers multiple generations. Reef fish were found to be very sensitive to temperature increases, but these effects vanished over several generations (Donelson & Munday 2011). But studies on multiple generations are still the exception. Nevertheless, environmental changes are occurring at speeds much greater than ever before in the Earth's past (Hoegh-Guldberg et al. 2007, IPCC 2013), making adaptation for organisms harder. Therefore any relief from stressors would help the ecosystem and give it the chance to recover and provide livelihoods also for future generations.

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Colored ceramic foams with tailored pore size and surface functionalization used as spawning plates for fish breeding

Kroll S, Soltmann C, Koch D, Kegler P, Kunzmann A, Rezwan K

Abstract:

The growing demand for reliable porous substrate materials for aquaculture of marine ornamental fishes (i.e. substrate spawners) requires a controllable processing in combination with a straightforward functionalization to adjust both, the material properties and the surface physico-chemical characteristics. In this study, highly porous ceramic foams made of alumina obtained by direct foaming are presented, which are conditioned for controlled aeration of egg clutches laid by clownfishes serving as model organisms for marine substrate spawners. Increasing stirring velocities during preparation of high alkane phase emulsified suspensions (HAPES) lead to decreased μm-scaled foam pore sizes, while similar high open porosities of around 80% are achieved. Wet-chemical silanization using hexadecyltrimethoxysilane (HDTMS) followed by subsequent oxygen plasma treatment of the foam surface is applied to generate bifunctionalized foams with hydrophobic matrix and hydrophilic surface properties. Under marine aquarium conditions, external aeration through the hydrophobic matrix of the foam is applied marginally below bubble point pressures resulting in a significant increase of the dissolved oxygen concentration of the surrounding water close to the foam surface without formation of undesired air bubbles. In accordance with increased foam pore sizes oxygen saturation rates of 0.26, 0.49 and 0.85%/min are obtained ensuring high oxygen concentrations at the spawning plate within a few hours. Assemblies of five bifunctionalized spawning plates to a ceramic housing are accepted by clownfish pairs, laying their eggs directly on the aerated ceramic substrate. The presented spawning plates are highly promising for sustainable aquaculture of marine ornamental fishes aiming to improve the survival rate of early life stages.

Functionalised ceramic spawning tiles with probiotic *Pseudoalteromonas* biofilms designed for clownfish aquaculture

Weseling W, Wittka S, Kroll S, Soltmann C, Kegler P, Kunzmann A, Riss HW, Lohmeyer M

Abstract:

To prevent marine fish egg clutches of substrate spawners from bacterial and fungal infestation, functionalised ceramic spawning tiles with probiotic bacterial biofilms were designed for clownfish aquaculture in this study. Therefore, mechanically stable ceramic spawning tiles made of alumina were fabricated with convenient substrate properties for *Pseudoalteromonas* immobilisation, i.e., hydrophobic matrix and hydrophilic surface properties. An effective biofilm formation was achieved by equilibration of these ceramic tiles in marine liquid medium containing 1 wt % starch and subsequent inoculation with two different *Pseudoalteromonas* strains. Biofilm formation was confirmed qualitatively by scanning electron microscopy (SEM) and quantitatively by photometrical measurements according to Safranin O staining. Furthermore, antagonistic effects originating from *Pseudoalteromonas* biofilms against *Paecilomyces lilacinus* were detected by SEM, which are possibly due to bioactive molecules. Consequently, these innovative microbiologically conditioned ceramic spawning tiles are promising candidates to prevent fish egg clutches from pathogenic infestation, which leads to an improved aquaculture of substrate spawners such as clownfishes.

Supplementary information

For chapter 2:

Table S2.1: List of samples taken for analysis of EPA PAH (polycyclic aromatic hydrocarbons) concentration under natural exposure conditions in Jakarta Bay (JB) and under short-term exposure conditions (bilge water discharge) and during the experiments (start and end samples). Concentrations for total EPA PAH and for each of separate PAH are given in µg/L. The letters A) and B) refer to samples taken twice. As a procedure calibration, the standard addition method was used by adding an EPA PAH standard with known concentration twice to selected samples. These samples are labeled "1x" and 2x".

Group	Sample	Total PAH [µg/L]	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Benzo(a)pyrene	Dibenzo(ah)anthracene	Benzo(ghi)perylene	Indeno(1,2,3cd)pyrene
Natural conditions	Pari South	10.3	0	0	2.1	3.8	0.3	1.5	1.1	0.5	0	0.2	0	0.3	0.2	0.3	0
	JB 1	99.2	5.1	0	3.9	81	0.9	1.8	4.7	1.8	0	0	0	0	0	0	0
	JB 2 (B)	359.8	59.4	5.6	35.6	250.2	2.3	0	6.7	0	0	0	0	0	0	0	0
	JB 2 (A)	384.5	59.3	4.1	31.8	280	2.2	0	7.1	0	0	0	0	0	0	0	0
	JB 3	227	95.9	0	9.4	114.5	1.1	1.6	4	0	0	0	0	0.2	0	0.3	0
	JB 4	69.7	8.9	0	5.6	47.8	0.6	1.6	4	1.2	0	0	0	0	0	0	0
Short-term exposure	Bilge water	13774.7	4364.35	665.2	4297.2	4447.95	0	0	0	0	0	0	0	0	0	0	0
	Time after bilge water discharge: 10 min	733.255	268.28	72.78	267.4	0	13.855	0	56.765	28.73	25.445	0	0	0	0	0	0
	Time after bilge water discharge: 5 min	294.325	80.395	0.955	12.505	195.44	0	0	5.03	0	0	0	0	0	0	0	0
	Blank 1 (JB)	112.4	84.7	0	4.2	21.3	0.3	0.7	1.2	0	0	0	0	0	0	0	0
	Blank 2 (JB)	89.3	77.3	0	3.1	8.9	0	0	0	0	0	0	0	0	0	0	0
Experiments	Treatment: WAF-D (start, A)	440	160.2	21.3	50.8	190	1.1	0.9	1.5	4.9	8.1	0.1	0	0.4	0.5	0	0.2
	Treatment: WAF-D (start, B)	378.4	150.3	18.5	0	197	1.1	0	5.3	2.5	3.7	0	0	0	0	0	0
	Treatment: WAF-D (end, A)	84.2	18.8	5	16.7	37	0.6	0.3	0.8	1.8	3.2	0	0	0	0	0	0
	Treatment: WAF-D (end, B)	61.5	17.1	3.6	10.8	23.5	0.4	0.4	0.5	1.7	3.5	0	0	0	0	0	0

	Treatment: WAF-D (start, 1x)	1770.5	445	314.1	109.9	283.8	57.3	82.7	86	61.9	62.8	60.4	30.1	25.7	60.9	29.7	60.2
	Treatment: WAF-D (start, 2x)	2900.3	668	573.2	163.4	339.4	116.9	134.6	141.6	118.9	121.2	119.9	58.7	49.2	119.9	58.3	117.1
	Treatment: WAF-D + LAS (end)	825.5	368.1	12.9	30.6	412.1	0	0	1.8	0	0	0	0	0	0	0	0
	Treatment: WAF-D + LAS (start)	601.1	278.4	17.1	46.1	243.9	0.7	4	0.9	2.8	4.2	2.5	0.5	0	0	0	0
	Treatment: WAF-D + temp (end)	618.4	227	24.9	61.7	264.6	4.7	13.8	8.4	1.3	4.7	5.7	1.2	0.1	0	0.3	0
	Treatment: WAF-D + temp (start)	1092.5	303.5	40.7	112.1	556.7	10	23.2	22.7	2.9	6.8	8	1.8	1.1	1.1	0.4	1.5
	Treatment: WAF-D + temp (start, 1x)	1818.7	596.5	303.3	111.9	231	55.8	58.9	58.1	61.9	63	64.9	30.8	26.3	63.2	30.7	62.4
	Treatment: WAF-D + temp (start, 2x)	3050.6	827.9	580.7	176.8	292.6	120.1	134.7	145.8	118.3	121.1	121.5	59.7	50.6	122.8	59.7	118.3
	Blank 3	17	1	0.9	5.3	7.6	0.3	0.1	0.1	0.5	1.1	0	0	0.1	0	0	0
	Blank 1	13.1	5.3	0.5	3.3	3.4	0	0	0	0.2	0.4	0	0	0	0	0	0
	Blank 2	15.6	6.6	0.7	4.3	3.2	0	0	0	0.3	0.5	0	0	0	0	0	0
	WAF-D (stock solution)	10653.3	2014.3	3226	973.4	3753.8	0	92.1	201.3	171.4	221	0	0	0	0	0	0
	Diesel	149785.6	47081.3	269.2	25802.2	76165.9	0	0	0	467	0	0	0	0	0	0	0

Table S2.2: Two-Way Analysis of variance for standard metabolic rates (SMR), routine metabolic rates (RMR), maximum metabolic rates (MMR) and aerobic metabolic scope (AMS) to test for significant effects of the stressors LAS, WAF-D and temperature as well as for interactions between the stressors. Post-hoc test was done with Tukey (95 % confidence interval).

Factor	Source of Variation	DF	SS	MS	F	P
	LAS	1	1334.6	1334.6	29.9	**<0.001**
	WAF-D	1	9236.2	9236.2	207.0	**<0.001**
	LAS x WAF-D	1	1727.1	1727.1	38.7	**<0.001**
	Temp	1	2478.4	2478.4	23.6	**0.001**
SMR	LAS	1	3796.7	3796.7	36.2	**<0.001**
	Temp x LAS	1	271.4	271.4	2.6	0.146
	Temp	1	9139.9	9139.9	137.0	**<0.001**
	WAF-D	1	635.1	635.1	9.5	**0.015**
	Temp x WAF-D	1	861.2	861.2	12.9	**0.007**
	LAS	1	25584.6	25584.6	10.8	**0.011**
	WAF-D	1	101120.4	101120.4	42.7	**<0.001**
	LAS x WAF-D	1	19664.5	19664.5	8.3	**0.02**
	Temp	1	8727.4	8727.4	3.1	0.119
RMR	LAS	1	23767.5	23767.5	8.3	**0.02**
	Temp x LAS	1	21320.3	21320.3	7.5	**0.026**
	Temp	1	2921.0	2921.0	1.0	0.344
	WAF-D	1	74438.9	74438.9	25.7	**<0.001**
	Temp x PAH	1	34369.3	34369.3	11.9	**0.009**
	LAS	1	25549.6	25549.6	3.9	0.084
	PAH	1	33412.0	33412.0	5.1	0.054
	LAS x WAF-D	1	34085.0	34085.0	5.2	0.052
	Temp	1	76868.3	76868.3	7.9	**0.023**
MMR	LAS	1	10699.6	10699.6	1.1	0.324
	Temp x LAS	1	58093.0	58093.0	6.0	**0.04**
	Temp	1	129933.3	129933.3	13.7	**0.006**
	WAF-D	1	24330.3	24330.3	2.6	0.147
	Temp x WAF-D	1	24905.2	24905.2	2.6	0.143
	LAS	1	7293.5	7293.5	1.2	0.314
	WAF-D	1	2509.8	2509.8	0.4	0.546
	LAS x WAF-D	1	13762.8	13762.8	2.2	0.178
	Temp	1	48424.7	48424.7	7.1	**0.029**
AMS	LAS	1	2568.3	2568.3	0.4	0.558
	Temp x LAS	1	23115.5	23115.5	3.4	0.104
	Temp	1	46383.9	46383.9	6.7	**0.032**
	WAF-D	1	8011.7	8011.7	1.2	0.312
	Temp x WAF-D	1	24562.7	24562.7	3.6	0.096

Table S2.3: Metabolic stress responses of *Siganus guttatus* during short-term (16 h) exposure to the treatments control, WAF-D (water accommodated fraction of diesel), LAS (linear alkyl benzene sulfonate), temperature, temp +WAF-D, temp + LAS. Metabolic rates are given as means ± SD in mg O2 kg-1 h-1 (n = 3): standard metabolic rates (SMR), routine metabolic rates (RMR), maximum metabolic rates (MMR) and aerobic metabolic scope rates (AMS). p-values are given for differences between day and night measurements of SMR and RMR (one-way ANOVA).

	Metabolic rate	Control	WAF-D	LAS	WAF-D + LAS	Temp	Temp + WAF-D	Temp + LAS
SMR [mg/h/g]	Total	163.5 ± 5.3	132.0 ± 5.3	208.6 ± 6.8	129.1 ± 4.0	201.8 ± 4.4	204.2 ± 10.1	227.8 ± 13.7
	Day	162.5 ± 3.8	124.7 ± 2.7	244.3 ± 14.2	128.9 ± 3.2	246.9 ± 46.8	207.9 ± 5.3	226.0 ± 17.9
	Night	181.6 ± 31.8	143.4 ± 1.5	218.6 ± 13.8	138.9 ± 20.4	229.4 ± 36.5	217.5 ± 21.1	246.6 ± 14.8
	Day/Night comparison (p-value)	0.651	0.163	0.477	0.384	0.057	0.544	0.1910
RMR [mg/h/g]	Total	192.3 ± 14.0	155.7 ± 10.4	246.9 ± 8.5	144.3 ± 7.7	282.0 ± 38.0	231.5 ± 9.1	277.3 ± 5.6
	Day	181.4 ± 10.2	147.7 ± 113.5	274.6 ± 13.6	138.9 ± 4.9	300.0 ± 47.6	231.6 ± 4.8	259.2 ± 7.8
	Night	190.0 ± 27.6	160.5 ± 7.6	246.0 ± 17.3	152.6 ± 19.7	285.0 ± 75.2	235.4 ± 24.3	266.4 ± 20.0
	Day/Night comparison (p-value)	0.258	0.089	0.164	0.4	0.069	0.497	0.257
	AMS [mg/h/g]	171.8 ± 113.6	210.6 ± 33.3	288.8 ± 48.8	192.2 ± 21.6	386.6 ± 53.4	244.5 ± 38.4	328.1 ± 12.7
	MMR [mg/h/g]	334.3 ± 117.5	335.4 ± 30.7	533.2 ± 46.7	321.0 ± 24.4	633.5 ± 95.3	452.4 ± 37.2	554.1 ± 27.6

For chapter 3:

Table S3.1: Results from post-hoc tests for diesel and temperature treatments. Tukey HSD tests performed for the control, high temperature and diesel treatments. Temperature was either "norm" (28 °C) or "high" (31 °C) and pollutant was either "none" or "diesel" (490 mL of 0.5 % WAF). Asterisks indicate significant effects ($p < 0.05$).

		diff	lwr	upr	p adj
PAM Beginning of measurement period					
$Temperature	high-norm	0.0275	7.49E-03	0.047514	0.0112 *
$Pollutant	none-diesel	0.0000	-2.00E-02	0.020014	1.0000
$`Temperature:Pollutant`	norm:diesel-norm:none	0.0025	-3.61E-02	0.041067	0.9973
	high:diesel-norm:none	0.0275	-1.11E-02	0.066067	0.2026
	high:none-norm:none	0.0300	-8.57E-03	0.068567	0.1503
	high:diesel-norm:diesel	0.0250	-1.36E-02	0.063567	0.2689
	high:none-norm:diesel	0.0275	-1.11E-02	0.066067	0.2026
	high:none-high:diesel	0.0025	-3.61E-02	0.041067	0.9973
PAM End of measurement period					
$Temperature	high-norm	0.0025	-0.015702	0.020702	0.7699
$Pollutant	none-diesel	0.0050	-1.32E-02	0.023202	0.5606
$`Temperature:Pollutant`	norm:none-high:diesel	0.0025	-3.26E-02	0.037576	0.9965
	norm:diesel-high:diesel	0.0025	-3.26E-02	0.037576	0.9965
	high:none-high:diesel	0.0100	-2.51E-02	0.045076	0.8315
	norm:diesel-norm:none	0.0000	-3.51E-02	0.035076	1.0000
	high:none-norm:none	0.0075	-2.76E-02	0.042576	0.9188
	high:none-norm:diesel	0.0075	-2.76E-02	0.042576	0.9188
Light respiration					
$Temperature	norm-high	0.0015	-1.61E-03	0.004597	0.3149
$Pollutant	diesel-none	0.0016	-1.51E-03	0.004697	0.2850
$`Temperature:Pollutant`	norm:diesel-high:none	0.0031	-2.89E-03	0.009067	0.4494
	norm:none-high:none	0.0054	-5.59E-04	0.011399	0.0803
	high:diesel-high:none	0.0055	-4.59E-04	0.011499	0.0739
	norm:none-norm:diesel	0.0023	-3.65E-03	0.008312	0.6627
	high:diesel-norm:diesel	0.0024	-3.55E-03	0.008412	0.6338
	high:diesel-norm:none	0.0001	-5.88E-03	0.006079	1.0000

Dark respiration

|					
$Temperature	high-norm	0.0000	-4.19E-03	0.004281	0.9824
$Pollutant	diesel-none	0.0033	-8.99E-04	0.007576	0.1117
$`Temperature:Pollutant`	norm:diesel-high:none	0.0033	-4.87E-03	0.011461	0.6395
	norm:none-high:none	0.0072	-9.49E-04	0.015384	0.0899
	high:diesel-high:none	0.0106	2.43E-03	0.018766	0.0107 *
	norm:none-norm:diesel	0.0039	-4.24E-03	0.012089	0.5079
	high:diesel-norm:diesel	0.0073	-8.61E-04	0.015471	0.0853
	high:diesel-norm:none	0.0034	-4.78E-03	0.011549	0.6210

For chapter 5:
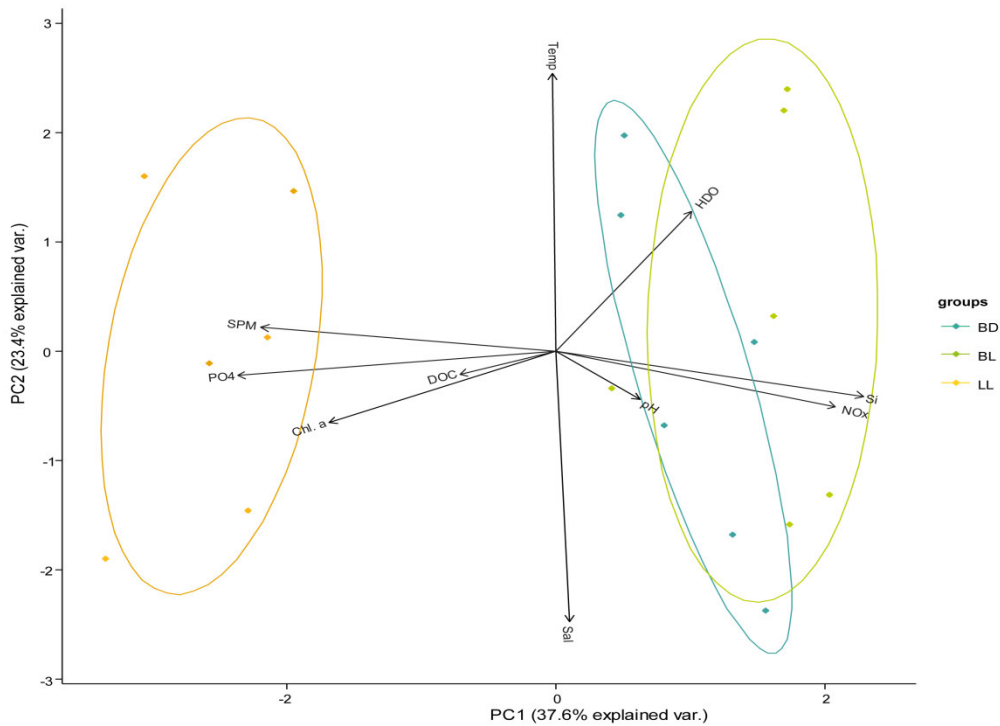
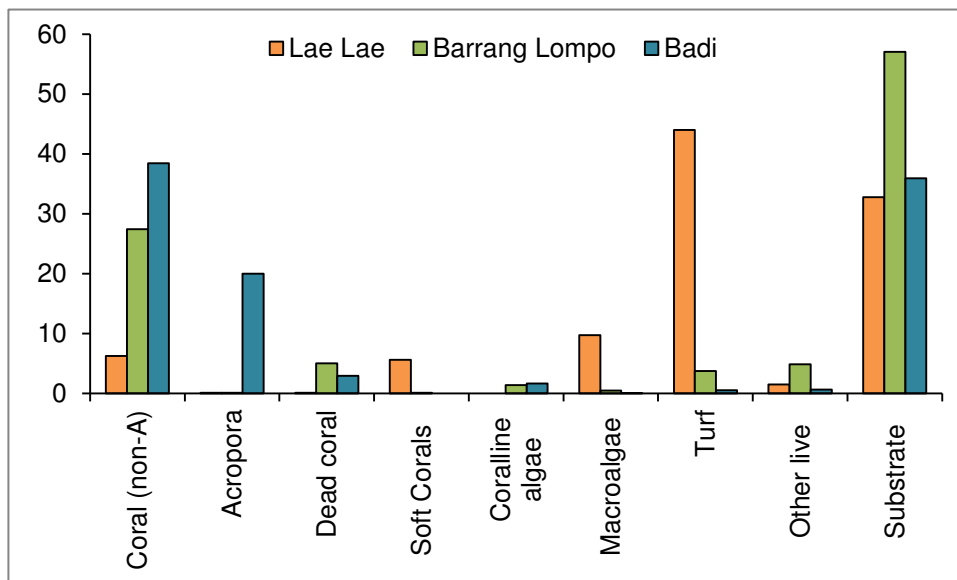Fig. S5.1 Principle component analysis of the water parameters

Fig. S5.2 Benthic cover by taxonomic groups at the reefs inshore (=Lae Lae), near-shore (Barrang Lompo) and mid-shelf (Badi).

S5.1: Post hoc multiple pairwise comparisons for the Kruskal Wallis test on water parameters.

|		obs. dif	critical dif	difference
	BD-BL	2.166667	7.378741	FALSE
Temp	BD-LL	0.583333	7.378741	FALSE
	BL-LL	1.583333	7.378741	FALSE
	BD-BL	2.833333	7.378741	FALSE
pH	BD-LL	1.166667	7.378741	FALSE
	BL-LL	1.666667	7.378741	FALSE
	BD-BL	0.333333	7.378741	FALSE
Sal	BD-LL	0.666667	7.378741	FALSE
	BL-LL	0.333333	7.378741	FALSE
	BD-BL	5.666667	7.378741	FALSE
HDO	BD-LL	0.666667	7.378741	FALSE
	BL-LL	6.333333	7.378741	FALSE
	BD-BL	4.833333	7.378741	FALSE
DOC	BD-LL	1.333333	7.378741	FALSE
	BL-LL	6.166667	7.378741	FALSE
	BD-BL	6	7.378741	FALSE
NOx	BD-LL *	12	7.378741	TRUE
	BL-LL	6	7.378741	FALSE
	BD-BL	2	7.378741	FALSE
PO4	BD-LL *	8	7.378741	TRUE
	BL-LL	10	7.378741	TRUE
	BD-BL	2	7.378741	FALSE
Si	BD-LL *	8	7.378741	TRUE
	BL-LL *	10	7.378741	TRUE
	BD-BL	1.666667	7.378741	FALSE
Chl. A	BD-LL *	9.833333	7.378741	TRUE
	BL-LL *	8.166667	7.378741	TRUE
	BD-BL	5	7.378741	FALSE
Susp. part.	BD-LL	6.5	7.378741	FALSE
	BL-LL *	11.5	7.378741	TRUE
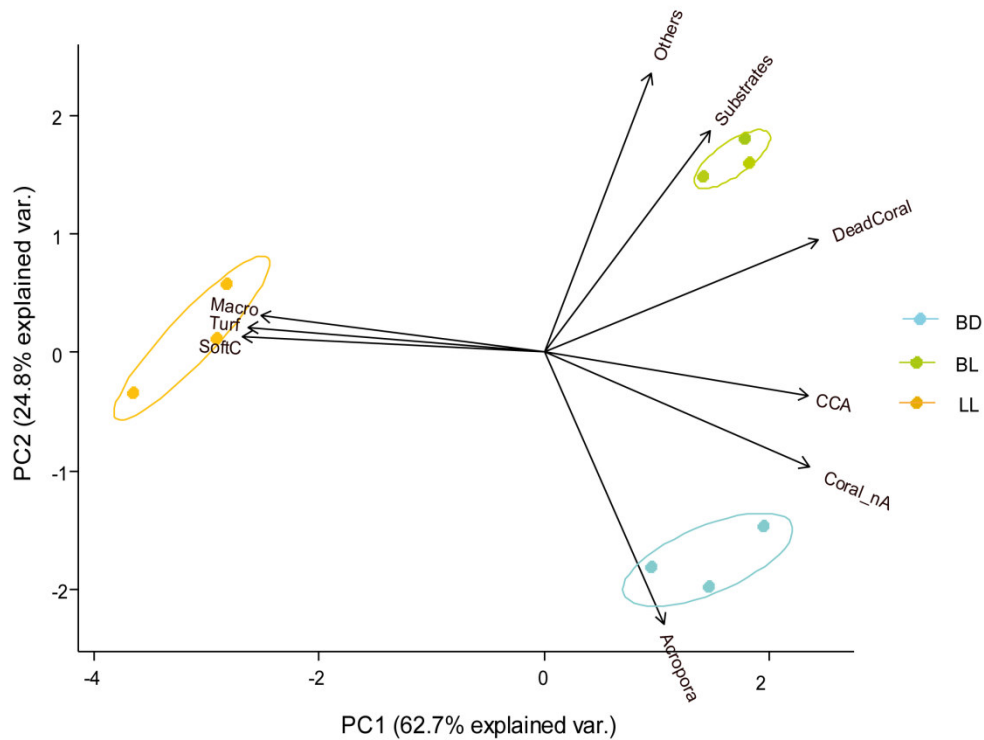
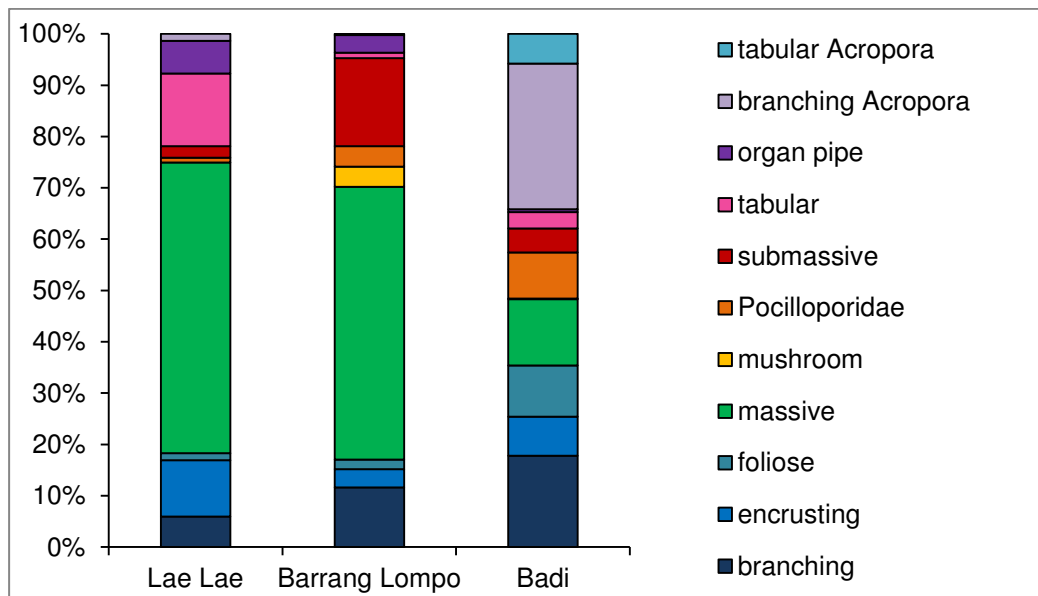Fig. S5.3 Principle component analysis for the benthic groups

Fig. S5.4 Coral morphologies by site. Percentages of coral morphologies at the reefs inshore (=Lae Lae), near-shore (Barrang Lompo) and mid-shelf (Badi).
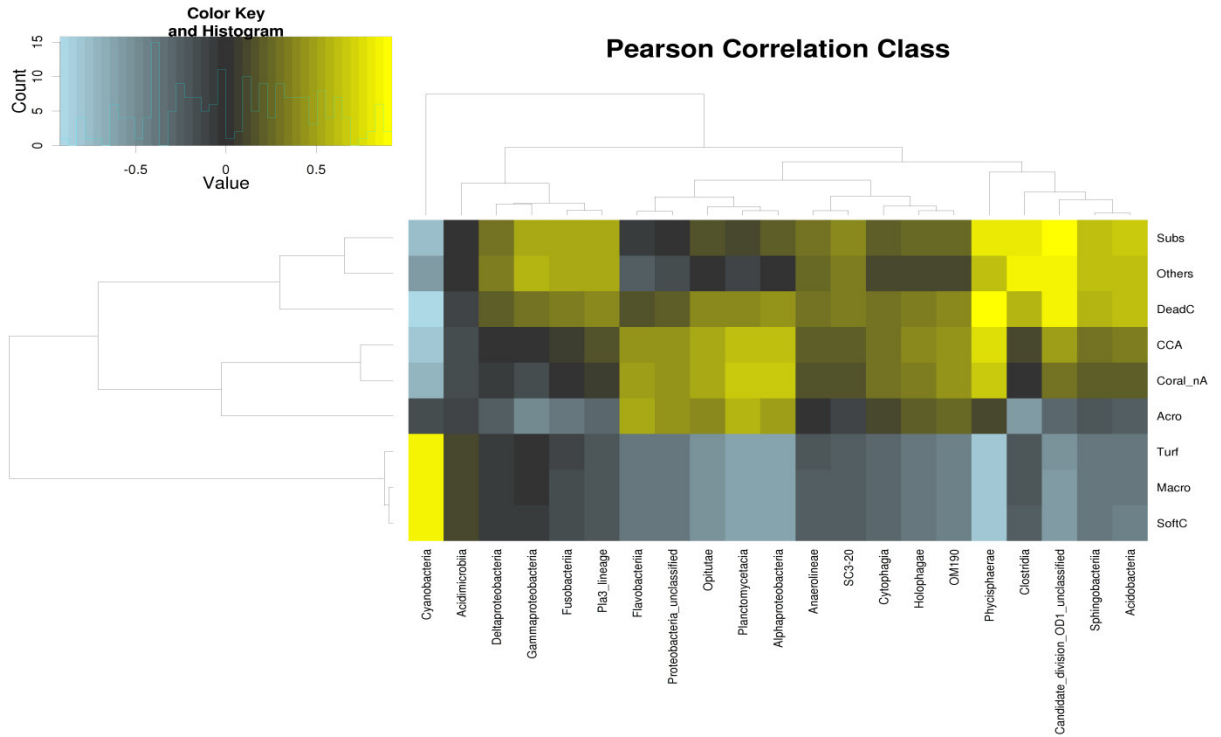
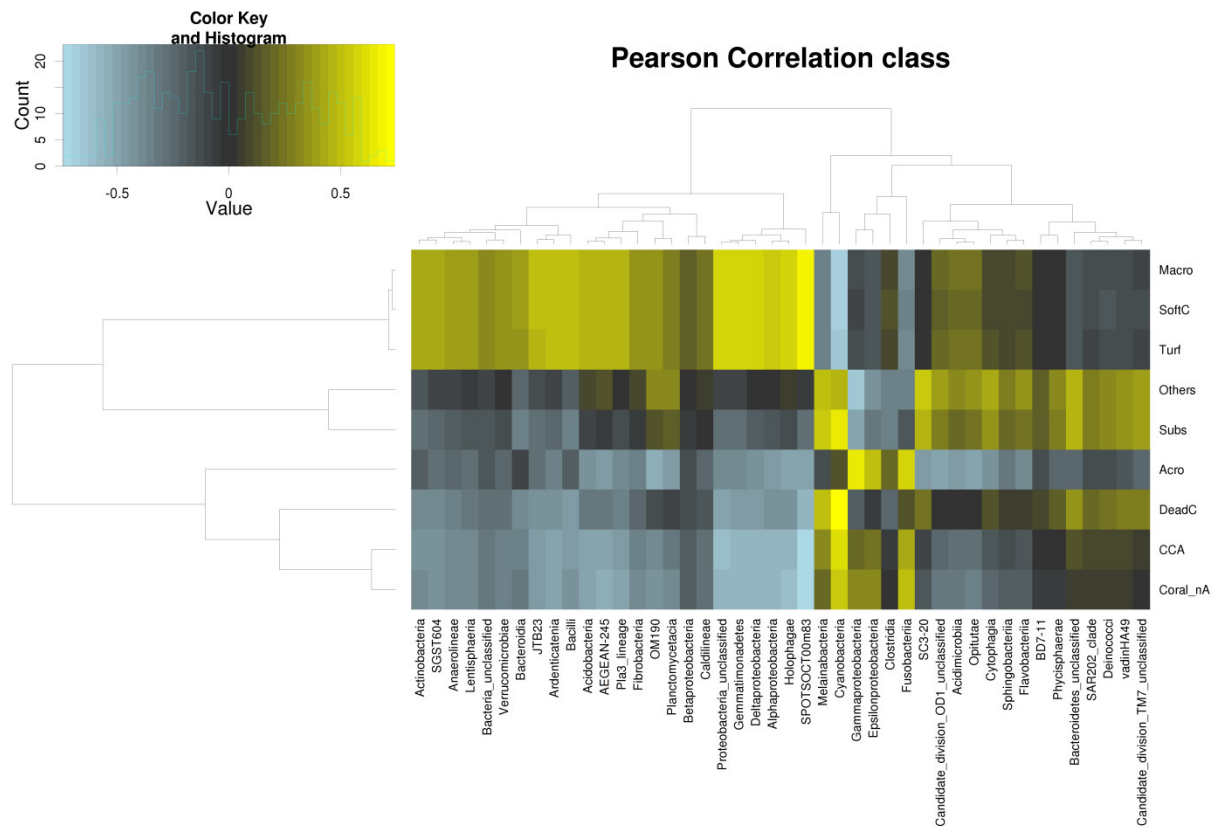Fig. S5.5 Correlation between benthic community and bacterial communities on natural substrate.

Fig. S5.6 Correlation between benthic community and bacterial communities on artificial settlement tiles.

Erklärung

Gemäß § 6 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14.3.2007 versichere ich, dass die vorliegende Arbeit mit dem Titel

"Understanding coral reefs in an impacted world - Physiological responses of coral reef organisms to coastal pollution and global warming"

1. ohne unerlaubte fremde Hilfe selbstständig verfasst und geschrieben wurde.
2. keine anderen als die angegebenen Quellen und Hilfsmittel genutzt wurden.
3. die den benutzen Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden.
4. es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Bremen, 10. September 2015

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Pia Kegler