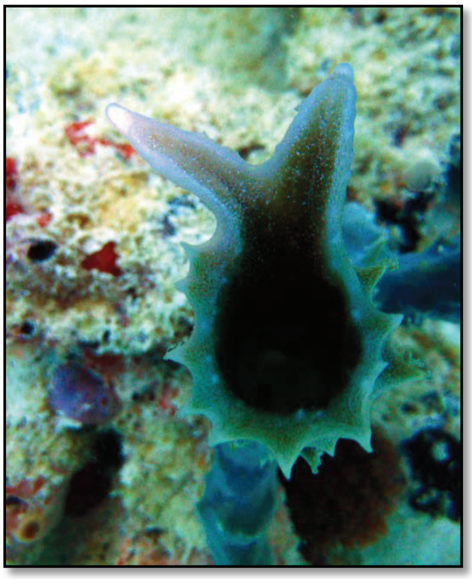
# Chemical ecology of Western Indian Ocean reef sponges



A dissertation by

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*Für meine Mama*

# SUMMARY

Sponges are among the dominant benthic organisms on coral reefs, representing important spatial competitors for reef-building corals. Coral reefs have experienced drastic declines in coral cover and corresponding increases in the abundance of other spatial competitors, such as macroalgae, corallimorpharians and sponges, due to a combination of global and local stressors. The ability of sponges to chemically defend themselves against predators, microbes and other competitors may partially explain their high abundance on reefs worldwide. Nonetheless, studies investigating sponge abundance and chemical ecology are rare, particularly in the Western Indian Ocean, which is considered a hotspot of coral and sponge biodiversity.

Thus, this thesis is the first study that provides insights into the chemical ecology of sponges from the Western Indian Ocean. The thesis consists of a general introduction, three chapters investigating in detail different aspects of chemical defence mechanisms in sponges from Zanzibar and a general discussion.

In **Chapter 1** I assessed the benthic community composition of the reef at Bawe, an island on Zanzibar's West Coast. This reef was dominated by reef-building corals, but sponges also represented a diverse and abundant component of the reef fauna. Moreover, laboratory experiments were conducted to investigate if predation was a key factor in structuring the sponge community. Findings revealed that the reef was dominated by palatable rather than chemically defended sponge species, demonstrating a lack of predatory control on the sponge community likely due to overfishing. In the absence of predation, palatable sponges could potentially undergo uncontrolled growth in the future and thus subject reef-building corals to greater competitive pressure.

**Chapter 2** focused on the antimicrobial and cytotoxic activities of secondary metabolites extracted from the most abundant sponges at the reef around Bawe. The experiments revealed that the most abundant sponges were remarkably well defended against co-occurring marine bacteria and in particular against potential pathogens. Moreover, the majority of the sponge extracts also displayed cytotoxic activities. Metabolites with either antimicrobial or cytotoxic properties can provide sponges with a competitive advantage over corals. Antimicrobial compounds could alter the coral microbiota while cytotoxic compounds are able to impair the cell division of corals. Consequently, the

potential allelopathic properties of sponge crude extracts from the three most abundant and bioactive sponges were further examined in field experiments under natural conditions in **Chapter 3**. These experiments showed that the extracts of all three sponges possessed allelopathic compounds that adversely affected the photosynthetic efficiency of the corals symbiotic zooxanthellae. Despite this, experiments with live sponge fragments were unable to detect significant impairments on the physiology of corals. However, the presence of allelopathic compounds, and their significantly negative effect on coral photo-efficiency, suggests that allelopathy by sponges does play a role in spatial competition with corals. As a result, sponges might be able to exert negative effects on the coral's fecundity, their reproduction or even their associated microbiome making corals more vulnerable towards further natural or anthropogenic disturbances and pathogenesis.

Collectively, the present study demonstrated that sponges on reefs in Zanzibar are serious spatial competitors against reef-building corals. The increased sewage input in combination with other local stressors, such as destructive fishing practices or damage to the reef through tourism activities, will most likely result in more frequently occurring sponge-coral interactions. Thus, management strategies for Zanzibarian reefs should focus on minimizing anthropogenic stressors, like the establishment of a sewage water treatment facility to minimize stress on the benthic community, in order to reduce the risks to coral reef health. Fisheries regulations should also be enforced as an important component of coral reef management plans in order to restore healthy herbivorous as well as spongivorous fish populations on the reefs, which limit the proliferation of competing macroalgae and sponges.

# ZUSAMMENFASSUNG

Schwämme stellen eine der dominierenden Organismengruppe im Ökosystem Korallenriff dar und konkurrieren somit stark um frei verfügbaren Platz mit den riffbildenden Korallen. Aufgrund globaler und lokaler Stressfaktoren ist der Hartkorallenbewuchs in den letzten Jahren global stark zurückgegangen, jedoch ist im Gegenzug die Abundanz von Makroalgen, Scheibenanemonen und Schwämmen, die mit Korallen um frei verfügbaren Lebensraum konkurrieren, drastisch gestiegen. Die Fähigkeit sich chemisch gegen Prädatoren und pathogene Mikroorganismen zu verteidigen, sowie das Potenzial sich gegen andere Konkurrenten einen Platz im Riff zu sichern, erklärt teilweise den großen Erfolg von Schwämmen. Dennoch gibt es sehr wenige Studien, die Abundanzen von Schwämmen im Riff erhoben oder deren chemische Ökologie untersucht haben. Vor allem im westlichen Teil des Indischen Ozeanes, der einen Biodiversitäts-Hotspot von Korallen sowie auch Schwämmen darstellt, wurde bislang wenig an Schwämmen geforscht.

Daher ist diese Dissertation die erste wissenschaftliche Arbeit, die Einblicke in die chemische Ökologie von Schwämmen im westlichen Indischen Ozean liefert. Die vorliegende Dissertation besteht aus einer allgemeinen Einführung in das Thema, drei Kapiteln, in denen die verschiedenen chemischen Verteidigungsmechanismen von Zanzibars häufigsten Schwammarten untersucht wurden, und einer abschließenden Diskussion.

**Kapitel 1** dieser Arbeit untersuchte die Zusammensetzng der benthischen Gemeinschaft von Bawe, einer Insel an Zanzibars Westküste. Obwohl das Riff um Bawe von Hartkorallen dominiert wird, machen Schwämme eine führende und artenreiche Komponente der Rifffauna aus. Darüberhinaus stellte dieses Kapitel fest, dass Prädation als Schlüsselfaktor für die Strukturierung der Schwammgemeinschaft verantwortlich ist. Die Ergebnisse zeigten, dass das Riff von Schwämmen dominiert ist, die für schwammfressende Fische genießbar sind, was eine Folge von Überfischung sein könnte. Genießbare Schwämme könnten zukünftig unkontrolliert wachsen und damit riffbildende Korallen einem höheren Konkurrenzdruck aussetzen.

Der zweite Teil dieser Arbeit analysierte die Extrakte verschiedener Schwämme auf ihre antimikrobiellen sowie cytotoxischen Aktivitäten. **Kapitel 2** demonstrierte, dass die am häufigsten vorkommenden Schwammarten außergewöhnlich gut gegen Bakterien und besonders gegen potentielle Pathogene verteidigt sind. Darüber hinaus besaß die Mehrzahl

der Schwammextrakte auch cytotoxische Aktivitäten. Metabolite mit sowohl antimikrobiellen sowie cytotoxischen Eigenschaften verschaffen Schwämmen einen Konkurrenzvorteil gegenüber Korallen im stetigen Kampf um Lebensraum. Daher wurden die allelopathischen Eigenschaften der Sekundärmetabolite von den drei häufigsten und chemisch am besten verteidigsten Schwämmen in **Kapitel 3** zusätzlich in Feldexperimenten unter natürlichen Bedingungen untersucht. Die Arbeit konnte nachweisen, dass die Extrakte der drei Schwämme allelopathische Verbindungen besitzen, welche die Photosyntheseleistung von Korallen negativ beeinflusste. Obwohl Experimente mit Schwammstücken keinen signifikanten negativen Einfluss auf die Photosyntheseleistung der Korallen gezeigt hatten, schließt das nicht aus, dass Schwämme Allelopathie für den Kampf um Lebensraum nutzen. Daher könnten Schwämme auch negative Auswirkungen auf die Fruchtbarkeit, Fortpflanzung oder auf das Mikrobiom von Korallen ausüben, wodurch Korallen sensitiver auf natürlich oder anthropogene Umweltveränderungen reagieren und anfälliger gegenüber der Entwicklung von Krankheiten sind.

Zusammenfassend zeigte diese Dissertation, dass Schwämme von Zanzibars Korallenriffen ernstzunehmende Konkurrenten für riffbildende Korallen sind. Die erhöhte Abwassereinleitung in Kombination mit anderen lokalen Stressfaktoren, wie etwa destruktive Fischereipraktiken oder Schädigungen des Riffes durch touristische Aktivitäten, werden höchstwahrscheinlich zu einem vermehrten Vorkommen an Interaktionen von Schwämmen und Korallen führen. Daher sollten Managementstrategien für Zanzibars Riffe die Einrichtung einer Abwasseranlage als vorrangiges Ziel haben, damit zusätzliche Risiken für die Gesundheit der Korallen minimiert werden können. Zudem müssen Fischereivorschriften als eine wichtige ergänzende Schutzmaßnahme für die Riffe durchgesetzt werden, damit gesunde herbivore wie auch spongivore Fischbestände wiederhergestellt werden können, die die weitere Ausbreitung von Makroalgen sowie Schwämmen einschränken.

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# ABBREVIATIONS

| | |
|---|---|
| ARP | Ankyrin repeat proteins |
| C | Carbon |
| CCA | Crustose coralline algae |
| Chl *a* | Chlorophyll *a* |
| COTS | Crown-of-thorns starfish |
| DOC | Dissolved organic carbon |
| DOM | Dissolved organic matter |
| ENSO | El Niño Southern Oscillation |
| ENT | Enterococcus |
| GBR | Great Barrier Reef |
| GPP | Gross primary production |
| HMA | High microbial abundance |
| ind. | Individual |
| ITCZ | Inter-tropical convergence zone |
| $LD_{50}$ | Lethal Dose, 50% |
| LMA | Low microbial abundance |
| MPA | Marine protected area |
| NE | Northeast |
| ODT | Optimal defence theory |
| PAM | Pulse amplitude modulated |
| POC | Particulate organic carbon |
| PS II | Photosystem II |
| SE | Southeast |
| TPR | Tetratricopeptide repeat domain- encoding proteins |
| WIO | Western Indian Ocean |
| WW | wet weight |
| YBD | Yellow band disease |

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# CHAPTER 1

# GENERAL INTRODUCTION

## Tropical Coral Reefs - The Rainforests of the Sea

Coral reefs are, together with rainforests, the most productive and biologically diverse ecosystems in the world (Odum and Odum 1955; Connell 1978; Knowlton et al. 2010). While rainforests comprise 2% of the Earth´s surface, coral reefs cover only approximately 0.1% (Reaka-Kudla 1997). Nonetheless, coral reefs harbour approximately one third of all marine species (Reaka-Kudla 1997; Bouchet 2006; Plaisance et al. 2011). It was long assumed that within the Indo-Australian Archipelago, the Coral Triangle constitutes the world's largest marine biodiversity hotspot (Roberts et al. 2002). However, there is increasing evidence of a second high diversity hotspot within the Western Indian Ocean (Obura 2012). Therefore, previous reports on the potential biodiversity of tropical coral reefs are likely underestimated, as many regions such as Eastern Africa remain highly underrepresented in research efforts (Fisher et al. 2011).

Coral reefs are of inestimable value to human societies worldwide due to a range of ecosystem services (Moberg and Folke 1999). Tens of millions of people depend on reef ecosystems for their protein intake (Salvat 1992), as fish caught on coral reefs contribute up to 10% to the worldwide fish consumption (Smith 1978). Other ecosystem services include the provision of nursery areas, tourism, coastal protection, building material and new pharmacological compounds (Done et al. 1996; Peterson and Lubchenco, 1997; Moberg and Folke 1999; Hoegh-Guldberg et al. 2007).

Coral reefs can be found in the upper photic zone in oligotrophic (nutrient-poor) waters along coastline of more than 100 countries (Rahav et al. 1989; Gast et al. 1999; Moberg and Folke 1999). The structural basis of coral reef ecosystems is a complex carbonate framework formed by the scleractinian corals (stony corals). This framework and the corals themselves provide a habitat for a diverse range of other species in the reef community. The majority of scleractinian corals live in symbiosis with photosynthetic dinoflagellate algae of the genus *Symbiodinium*, commonly referred to as zooxanthellae. Symbiont densities within the host tissue range between $0.5* 10^{6}$- $5* 10^{6}$ cells $cm^{-2}$ (Drew 1972; Porter et al. 1984; Hoegh-Guldberg and Smith 1989). The zooxanthellae translocate up to 95% of photosynthetic products (photosynthates), such as sugar and amino acids, to the coral host and receive in return essential inorganic nutrients from the corals' waste metabolism (Muscatine 1990; Trench 1979; Swanson and Hoegh-Guldberg 1998; Furla et al. 2000). This symbiosis is responsible for the high primary productivity as well as for the ecological success of coral reefs (Muscatine and Porter 1977).

Coral reefs are characterized by a remarkably high gross primary production (GPP), ranging from 0.1- 3.0 g C $m^{-2}$ $d^{-1}$, despite them thriving in oligotrophic waters (Crossland et al. 1991). However, as the respiration rates of the reef community are of similar magnitude, there is little net gain within the system (Crossland et al. 1991; Atkinson 2011). Therefore, all the reef derived matter has to be rapidly retained and efficiently recycled by benthic and pelagic reef organisms (Muscatine and Porter 1977, Wild et al. 2004, de Goeij et al. 2013). In this context, especially sponges play a key role in the benthic-pelagic coupling on coral reefs by providing food to various reef organisms through the retention and recycling of reef derived organic matter (Yahel et al. 1998; Richter et al. 2001; de Goeij et al. 2013).

## Reef Degradation and Phase Shifts

Increasing physical stress by a combination of global and local stressors exerted on corals affect their health status and decrease their ability to invest energy in competing with other functional groups (Aronson and Precht 2001; Hoegh-Guldberg et al. 2007). Coral reefs worldwide have already experienced a dramatic decrease in coral cover and species diversity (Hughes et al. 2003; Bellwood et al. 2004; Bruno and Selig 2007; Loh et al. 2015; Suchley et al. 2016; Zaneveld et al. 2016) and are predicted to suffer further losses by a combination of global anthropogenic (effects of global climate change, i.e. warming and ocean acidification) and local anthropogenic (overfishing, sedimentation, eutrophication and habitat destruction) threats. The Global Coral Reef Monitoring Network report from 2008 states that around 19% of coral reefs around the world are past recovery and another 15% are on the verge of being lost (Hughes et al. 2010). It is estimated that by 2030 more than 60% of coral reefs may be lost (Hughes et al. 2003; Wilkinson 2008).

In the Caribbean, coral cover on most reefs was reduced by approximately 80- 90% over the past three decades (Jackson et al. 2014) while the abundances of sponges and macroalgae increased drastically (Loh et al. 2015; Bruno et al. 2009; Hughes et al. 2010). On most Caribbean reefs, macroalgae coverage averages approximately 29%, followed by both sponges and corals with around 16% cover (Loh et al. 2015). The removal of two key functional groups from the reef system, the dominant branching coral *Acropora* and the sea urchin *Diadema antillarum*, was suggested to have led to the drastic loss in coral cover and the high increase in algal abundance (Mumby et al. 2007; Roff and Mumby 2012; Mumby et al. 2013). *Acropora* had the ability to successfully compete with macroalgae for space due to its fast growth rates and *D. antillarum* was able to restrain the proliferation of macroalgae due to its high grazing intensity (Mumby et al. 2013). Functional redundancy on Caribbean reefs was extremely reduced as overfishing had resulted in a low diversity and biomass of grazer species (Hay 1984; Hughes 1994; Graham et al. 2013). After the die-off of *D. antillarum* populations, herbivorous fish biomass was too low and could not exert sufficient grazing pressure leading to the proliferation of macroalgae (Graham et al. 2013). Feedback loops that may further reinforce coral depleted states on reefs in the Caribbean are high nutrient inputs from river discharge and from windblown Saharan dust that promote algae and sponge growth by providing them with an additional food source as well as the interplay/ interaction of sponges and seaweeds (Shinn et al. 2000; Dai et al. 2012; Pawlik et al. 2016). Sponge communities are assumed to have adapted to the enhanced nutrient input and most sponge species are heterotrophic feeders using picoplankton and especially the dissolved organic carbon (DOC) released by seaweeds as food source (de Goeij et al. 2013; Pawlik et al. 2016). Sponges release nutrients gained through their heterotrophic feeding activity from which seaweeds in turn may profit (Southwell et al. 2008; Pawlik et al. 2016).

Similarly, the Great Barrier Reef (GBR) experienced a major loss of around 50% of its initial coral cover (De'ath et al. 2012). The current pace of coral cover decline in the GBR and the Caribbean amount to 1.51 and 1.4 % per year, respectively (Gardner et al. 2003; Côté et al. 2005; De'ath et al. 2012). In contrast to the Caribbean, the main reasons for the decline in coral cover of the GBR were assumed to be tropical cyclones, crown-of-thorns starfish (COTS) outbreaks and coral bleaching (De'ath et al. 2012).

Coral bleaching is a consequence of global warming leading to thermal stress in the coral holobiont. During thermal stress the density of zooxanthellae and/or their photosynthetic pigment concentration are greatly reduced due to the expulsion of zooxanthellae by the coral host as a stress response (Lesser 2006; Baker et al. 2008). Corals that lost approximately 50% or more of their pigmented symbionts become pale or white as their calcareous skeleton becomes visible (Fitt et al. 2000). Zooxanthellae are of great importance for corals since they provide their host with photosynthates and the decline of autotrophic inputs due to reduced densities of zooxanthellae results in high mortality, reduced growth rates, lower fecundity and higher susceptibility to diseases (Baird and Marshall 2002; Douglas 2003; Hughes et al. 2003; Lesser et al. 2007; Baker et al. 2008). The GBR and other reef ecosystems worldwide experienced a reduction of calcification rates by 15-20% since 1990 (Gardner et al 2003; Scheffer et al. 2008). Even coral reefs which are remote from human impacts and well protected are progressively losing their ecological resilience, i.e. their capacity to withstand disturbances (Scheffer et al. 2001; Diaz-Pulido et al. 2009; Hughes et al. 2003).

As a consequence, an increasing number of reefs worldwide have already changed from a coral-dominated state to a state dominated by other organisms, such as macroalgae, corallimorpharians or sponges (Norström et al. 2009). This transition is commonly referred to as phase-shift (Hughes et al. 2010; Norström et al. 2009). On many reefs, especially in the Caribbean, sponges have become the dominant taxa in terms of abundance and biomass (Diaz and Rützler 2001; Pawlik 2011; Bell and Smith 2004). Similarly, reports of shifts from coral- to sponge-dominance have been reported from the Barrier Reef of Madagascar, the Quirimba Archipelago in Mozambique, or the Indonesian Wakatobi region (Barnes 1999; Barnes and Bell 2002; Bell and Smith 2004).

## Sponges

Sponges are among the dominant sessile organisms on many coral reefs (Diaz and Rützler 2001; Barnes and Bell 2002; Bell and Smith 2004; Loh et al. 2015). They are the oldest animal group still extant on the Earth, as the oldest sponge fossils date back around 580 million years ago into the Cambrian period (Brasier et al. 1997; Li et al. 1998; Müller et al. 2004). Sponge populations can be found in marine as well as freshwater habitats in all climate zones worldwide (van Soest et al. 2012). They are divided into four classes: Calcarea (calcareous sponges), Demospongiae (demosponges), Hexactinellida (glass sponges), and Homoscleromorpha (homoscleromorphs) (Hooper and van Soest 2002; Gazave et al. 2011). To date, the global sponge diversity amounts to 8737 valid species with approximately 83% belonging to the class Demospongiae (van Soest et al. 2012; van Soest et al. 2016).

Sponges are among the structurally simplest organized metazoans, but possess a remarkable molecular complexity and are by far not primitive animals (Nichols et al. 2012; Riesgo et al. 2014). Most metazoan genes, such as genes essential for the development of nervous systems, are present in all sponge groups (Gazave et al. 2009; Richards and Degnan 2012; Riesgo et al. 2014). This indicates that genetic complexity is a shared characteristic trait in early metazoans in which sponges may not invest energy as these complex metazoan structures are not necessary for their filter- feeding lifestyle (Riesgo et al. 2014).

Sponges are among the most efficient optimal foragers, as their bodies are highly adapted to their filter-feeding lifestyle (Gili et al. 1998), enabling them to filter vast volumes of water up to 100.000 times their own volume per day (Weisz 2006). Their high feeding efficiency is attributed to three unique physiological and morphological features. First, their pump work is energetically highly efficient, as it accounts for only 0.85% of their respiratory output (Riisgård et al. 1993; Riisgård and Larsen 1995). Second, they possess an extensive network of water canals and chambers lined with flagellated cells, the choanocytes, which create an unidirectional water current to capture food particles and expel waste (Jørgensen 1966; Bergquist 1978; Brusca and Brusca 1990). Third, totipotent cells and dynamic tissue enable sponges to remodel their aquiferous system to optimize the water flow according to changing environmental conditions or after physical damage (Simpson 1984; Ruppert et al., 2003). Moreover, sponges are capable of selectively ingesting food and discriminating between different types of prey (Yahel et al. 2006). This enables them to optimize their diet and thus to maximize their gained energy as well as to avoid harmful food (Yahel et al. 2006).

## Ecological Services of Sponges

Sponges are essential structural components of coral reef ecosystems due to their high abundance in combination with their ability to contribute to bentho-pelagic coupling and act as key filter feeders in many marine and freshwater environments (Pile et al. 1997; Richter et al. 2001; Ribes et al. 2005). Sponges feed on a wide spectrum of prey including dissolved organic matter (DOM), bacteria, as well as pico- and nanoplankton (Reiswig 1971; Yahel et al. 2003; de Goeij et al. 2013). The latter dominates the planktonic biomass (Tremblay et al. 1994) and fixed carbon input through phytoplankton grazing exceeds that of zooplankton predation to a high degree on coral reefs, suggesting that this could be an important allochthonous food source in the otherwise low nutrient environment (Yahel et al. 1998; Richter et al. 2001). Sponges are capable of creating plankton- depleted layers over the benthos of the lake Baikal or coral reefs due to their efficient filtering with high retention efficiencies for phytoplankton ranging between 58- 99% (Pile et al. 1997; Yahel et al. 1998; Richter et al. 2001; Ribes et al. 2005). The estimates of total particulate organic carbon (POC, i.e. living cells and detritus) uptake rates in sponges range from 0.3 – 1.4 µmol C $cm^{-3}$ $h^{-1}$ (Maldonado et al. 2012 and references therein). Sponges are also able to take up DOM which represents the vast majority (>97%) of the total organic carbon pool in the open ocean and in coral reef waters (Benner 2002; Yahel et al. 2003; de Goeij et al. 2008). Sponges transform the captured DOM to particulate detritus, which can be in turn used by other coral reef organisms, via the so-called "sponge loop" (de Goeij et al. 2013). DOM removal rates are twice as high as removal rates for bacterio- and phytoplankton and DOM seems to be therefore a quantitatively more important organic carbon source (de Goeij and van Duyl 2007). Various dominant benthic primary producers such as corals, algae and seagrasses release DOM due to excess photosynthates (Zlotnik and Dubinsky 1989; Haas et al. 2011; Ziegler and Benner 2000; van Duyl et al. 2011). However, only bacteria, bivalves and sponges are able to directly use DOM as a food source, and it is thus easily washed out and the energy lost from the reef system (Azam et al. 1983; Roditi et al. 2000; Carlsson 2002). The assimilated DOM is used by the sponge in the rapid and constant renewal of its filter system by expelling vast amounts of old or damaged filtering cells (choanocytes) as detritus (cell shedding; de Goeij et al. 2013; Rix et al. 2016). Sponges possess the fastest cell cycle of any known animal with cell division rates of only 5- 6 hours, leading to a turnover of up to 35% of their body C per day (de Goeij et al. 2009; de Goeij et al. 2013; Alexander et al. 2014). On Caribbean coral reefs, these turnover rates equal the total GPP rates of the entire reef ecosystem (de Goeij and van Duyl 2007, de Goeij et al. 2013). Recently, the transfer of energy via the sponge loop could be also demonstrated for Red Sea coral reefs as well as North Atlantic cold water reefs, indicating that it is a ubiquitous feature of reef ecosystems worldwide (Rix et al. 2016).

Thus, sponges seem to be an important source of dissolved inorganic compounds for coral reef organisms due to their remineralization of organic matter which takes place in all sponges irrespective of their abundance and composition of their associated microbial populations (Maldonado et al. 2012; Ribes et al. 2012). Additionally, the sponge loop represents a key process in reef biogeochemical cycles, retaining and recycling reef derived matter that would be otherwise lost for the ecosystem (De Goeij et al. 2013). The supply of nutrients derived from the phytoplankton uptake from sponges and more importantly the transfer from energy via the sponge loop likely enables coral reefs to flourish in relatively nutrient-poor waters (Richter et al. 2001; de Goeij et al. 2013; Rix et al. 2016).

## Microbial Symbionts

Sponges not only gain energy from DOM and particle feeding, but also from their symbiotic associations with a diverse range of eukaryotic and prokaryotic organisms, including bacteria, archaea, fungi and unicellular algae (Taylor et al. 2007; Thomas et al. 2010). Sponges are marine holobionts, harbouring a remarkable diversity of inter- and intracellular symbionts of at least 47 bacterial and archaeal phyla, which can comprise up to 35% of their biomass (Hentschel et al. 2006; Taylor et al. 2007; Reveillaud et al. 2014). Sponge symbioses are the oldest known associations between animals and microbes dating back around >635 million years ago by the records of fossil steroid biomarkers (Li et al. 1998; Love et al. 2009). Sponges can be organized based on their microbial abundances into high microbial abundant (HMA) ($10^8$- $10^{10}$ microbes per gram of sponge wet weight) and low microbial abundant (LMA) sponges ($10^5$- $10^6$ microbes per gram of sponge wet weight) (Hentschel et al. 2006; Glöckner et al. 2014). The microbial communities of LMA sponges resemble the ones prevailing in nearby seawater more closely both in concentration (Vacelet and Donadey 1977; Hentschel et al. 2006) and community composition (Schmitt et al. 2007), whereas HMA sponges host microbial communities exceeding the density of microbes in seawater by 2-4 orders of magnitude, and exhibit a much higher diversity on the phylum level (Weisz et al. 2008; Poppell 2013; Bayer et al. 2014). The differences in the microbial communities of LMA and HMA sponges may be attributed to their differences in morphology (Schläppy et al. 2010; Weisz et al. 2008). LMA sponges are characterized by broad aquiferous channels and high pumping rates allowing for a high exchange rate of nutrients and other food sources (Weisz et al. 2008). These stand in marked contrast with the narrower aquiferous canals, denser mesohyl, and considerably lower pumping rates (52- 94%) of HMA sponges which ensure the uptake of nutrients by the microbial communities (Vacelet and Donadey 1977; Siegl et al. 2008; Weisz et al. 2008).

Sponges acquire their microbial symbionts via a combination of horizontal (incorporation from the seawater) and vertical (transfer through reproductive stages) transmission. This complex process was termed 'leaky vertical transmission', since the microbial communities, especially in HMA sponges, are mainly composed of vertically transmitted microbes (Schmitt et al. 2008; Thacker and Freeman 2012; Webster and Taylor 2012; Won et al. 2008). The oocytes, sperm and larvae of HMA sponges contain symbiotic microbes, while LMA sponge larvae are aposymbiotic (Usher et al. 2005; Sharp et al. 2007; Taylor et al. 2007; Vogel 2008; Glöckner 2013). Vertical symbiont transmission explains the low overlap between sponge and seawater microbial communities and why sponges from different regions host the same sponge-specific microbes (Hentschel et al. 2002; Simister et al. 2012a; Taylor et al. 2007; Schmitt et al. 2012). However, even sponges from different orders share a small common microbial core community which they may have taken up from the surrounding seawater via the filter feeding activity or through injuries in the sponge epidermis (Schmitt et al. 2012). Sponges are able to recognize bacterial symbionts and distinguish them from bacteria serving as a food source (Wilkinson et al. 1984; Thomas et al. 2010) as well as to ingest food items selectively after capturing (Yahel et al. 2006). Additionally, sponge-associated microbes have developed mechanisms to avoid phagocytosis, such as thickened cell walls or protective slime capsules (Van Donk et al. 1997; Friedrich et al. 1999; Vogel 2008). Another way to avoid digestion by sponge cells is to interact with surrounding cells and proteins of the sponge host. For this purpose, sponge-associated microbes express high abundances of ARPs (Ankyrin repeat proteins), TPRs (Tetratricopeptide repeat domain- encoding proteins) that are capable of blocking the progression of phagocytosis by communicating with the sponge host cells (Wu et al. 2004; Thomas et al. 2010; Siegl et al. 2011; Webster and Taylor 2012).

The association with microbial symbionts is beneficial for the sponge host due to a multitude of microbial services. For instance, the cyanobacterial photosymbionts provide their host with carbon meeting up to 50% of its energy requirements and 75% of its carbon budget (Wilkinson 1983; Freeman and Thacker 2011). Other benefits include vitamin B12 biosynthesis (Siegl et al. 2011; Thomas et al. 2010), metabolic waste removal (Hallam et al. 2006; Hoffmann et al. 2009), stabilization of the sponge skeleton (Hentschel et al. 2002), and production of secondary metabolites (Piel et al. 2004; Pawlik 2011).

## Secondary Metabolites

The survival of sponges is dependent on the production of chemically active compounds since they are sessile invertebrates lacking any behavioral escape or defence mechanisms (Ianora et al. 2006). Therefore, the production of secondary metabolites by associated microbes is one of the most important benefits for the sponge holobiont and explains partly their success in various marine and freshwater habitats (Hentschel et al. 2012; Piel 2009; Wilson et al. 2014). Secondary metabolites are used by sponges as defence against predators, ultraviolet (UV) radiation, as well as harmful and potentially pathogenic bacteria, and against other benthic space competitors (Pawlik et al. 1995; Kelly et al. 2005; Pawlik et al. 2007).

Sponges produce the greatest diversity of secondary metabolites among marine organisms (Blunt et al. 2009) and until today more than 5300 secondary metabolites are described from sponges (Sinko et al. 2012). The production and storage of secondary metabolites is energetically costly, resulting in a resource trade-off between chemical defences and other physiological processes including recruitment, growth and regeneration (Pawlik et al. 2008; Walters and Pawlik 2005; Leong and Pawlik 2010b). Consequently, the concentration of secondary metabolites in the sponge tissue varies for different sponge species or even within the same sponge species depending on their species-specific microbial communities or recent life history (Rohde et al. 2012, 2015; Simister et al. 2012a; Vicente et al. 2015). Therefore, even sponge conspecifics can differ in their chemical defences. Some secondary metabolites from sponges worldwide serve multiple defensive roles to reduce the high biosynthesis costs (Uriz et al. 1992; Richelle-Maurer et al. 2002; Rohde et al. 2015). For instance, extracts of *Amphimedon compressa*, a Caribbean sponge, inhibits overgrowth by invertebrates, deters predation by a reef fish and exhibits antimicrobial activities (Newbold et al. 1999; Pawlik et al. 1995; Engel and Pawlik 2000). Additionally, triterpene glycosides isolated from the Caribbean sponge *Ectyoplasia ferox* show anti-predator as well as allelopathic activities and prevent the overgrowth by neighbouring organisms (Kubanek et al. 2002). Another mechanism of sponges to increase their efficiency of chemical defences is to concentrate them in specific parts of their body to maximize their fitness in accordance with the optimal defence theory (ODT) (Rhoades 1979). Secondary metabolites may be allocated to apical parts of their body or exhibit higher concentrations in the outer parts of the sponge tissue that are more vulnerable to predation (Becerro et al. 1998; Schupp et al.

1999). These distribution patterns of secondary metabolites in accordance with the ODT are evident in multiple sponge species worldwide (Peters et al. 2009; Freeman and Gleason 2010).

Besides the anti-predatory properties of sponge secondary metabolites, they also serve as a defence against bacteria and viruses (Kelly et al. 2003, 2005; Rohde et al. 2015). This feature is critical, as sponges are constantly exposed to potentially harmful bacteria in the surrounding water column with densities ranging from $6x\,10^4$ $ml^{-1}$ to $2.5x\,10^6$ $ml^{-1}$ in the Indian Ocean (Sorokin et al. 1985; Wiebinga et al. 1997; Goosen et al. 1997). Additionally, sponges are exposed to large quantities of microbes passing through their bodies due to their filter feeding activities (Gili and Coma 1998). Secondary metabolites with antimicrobial properties serve to defend their surface from colonization by fouling organisms as well as by potential pathogens which might initiate diseases (Wahl 1989; Kelly et al. 2003; Littler and Littler 1995).

Sponge secondary metabolites are also used in the competition for space on coral reefs (Porter and Targett 1988; Pawlik et al. 2007; Engel and Pawlik 2000). Space is a highly limited resource for sessile organisms on tropical coral reefs (Dayton 1971; Jackson and Buss 1975). Sponges have the ability to rapidly overgrow and smother other space competitors, but are also able to release chemical compounds that can cause bleaching and tissue necrosis in neighbouring corals, decreasing their chances of survival even without direct physical contact (Porter and Targett 1988; Pawlik et al. 2007). This is attributed to compounds that impair the cell division of competitors through apoptosis, autophagocytosis, and necrosis (Folmer et al. 2009). The ability of those compounds to rapidly kill dividing cells led to an increasing pharmaceutical interest for the use of these compounds in the development of anti- cancer drugs or drugs against diseases, such as Malaria (Singh and Thakur 2016). Thus, the main focus of studies on sponge secondary metabolites initially was their potential pharmacological value. In recent years however a growing number of studies also started to investigate the ecological functions of these compounds.

## Research Gaps

In the Western Indian Ocean (WIO) corals were greatly affected by the 1997/1998 El Ninõ Southern Oscillation (ENSO) resulting in mass coral bleaching and high subsequent mortality (Wilkinson et al. 1999; McClanahan et al. 2007). The subsequent COTS outbreaks during 2002/2003-2006 led to a further reduction in coral cover and the reefs around Zanzibar experienced in consequence of both events a shift in coral communities and decreased species diversity (Muhando and Lanshammar 2008; Muhando 2009; Muthiga et al. 2008). Additionally, reefs on Zanzibar have been affected by destructive fishing practices, overfishing and untreated sewage discharge (Mohammed 2000; Jiddawi and Öhmann 2002; Moynihan et al. 2012). Phase shifts, the role of sponges and their chemical ecology have been widely studied in the Caribbean, Pacific, Mediterranean and Red Sea but not in the WIO region (Pawlik et al. 1995; Burns et al. 2003; Becerro et al. 2003; Rohde et al. 2015). Also sponges in the WIO remain a greatly understudied component of the coral reef ecosystem even though the WIO region was identified as a sponge biodiversity hotspot (Obura et al. 2012). In the face of global change and the increasing frequency of pulse disturbances it is important to investigate the interactions between sponges and corals as sponges are one of the top spatial competitors on coral reefs worldwide.

## AIMS AND SPECIFIC RESEARCH QUESTIONS

This thesis aims to investigate the chemical ecology of sponges on coral reefs off Zanzibar's West Coast. The overall goal is to define the role of sponges and their competitive ability towards corals by examining the chemical defence mechanisms of sponge extracts in the laboratory as well as in the field under natural conditions. The following research questions were addressed to give further insight into the chemical ecology of sponges:

1. How are benthic coral reef communities in Zanzibar structured?
   What are the dominant benthic reef organisms? How abundant and diverse are epi-reefal sponges on Zanzibar's reefs?

2. Is the increasing prevalence of sponges on the reef related to sponge chemical defences against various threats such as predators, microorganisms and competitors?

3. Do sponges contain allelochemicals which negatively affect the health of coral competitors?

4. Is the reef around Bawe resilient enough to withstand further disturbances? Are there indications for possible phase-shifts?

## REEF SITE

The Zanzibar Archipelago, covering approximately 2500 km², belongs to the United Republic of Tanzania and consists of the two main islands Unguja and Pemba as well as some smaller adjacent islands such as Chumbe, Misali, Changuu, Bawe and Mnemba (Johnstone et al. 1998; Gössling 2001; Ngazy et al. 2005). The largest island, Unguja, is located approximately 40 km from the mainland Tanzania between 5°40'E and 6°30'S and is commonly referred to as Zanzibar (Gössling 2001). Zanzibar lies within the inter-tropical convergence zone (ITCZ) which creates large seasonal changes through the southeast (SE; April to October) and northeast (NE; November to March) monsoon winds (McClanahan 1988; Mwaipopo 1988). The SE monsoons are characterized by high wind and wave energy, strong currents, cooler water temperatures around 26°C, reduced marine productivity, higher abundance of benthic algae and enhanced rainfall (McClanahan 1988; Muhando 2002; Muthiga et al. 2008; Muzuka et al. 2010). Nutrient concentrations are also higher during SE monsoons due to water column mixing and influx of terrestrial nutrients after the rainy season (McClanahan 1988). The NE monsoons in contrast are characterized by lighter winds leading to warmer water temperatures around 29-30°C and higher ocean productivity (McClanahan 1988; Muhando 2002; Muthiga et al. 2008). Most coral reefs are situated close to the coast or surround islands and sandbanks due to the narrow continental shelf (Johnstone et al. 1998; Bergman and Öhman 2001). Tides are semidiurnal with mean spring amplitudes of 3- 4 m (Mwaipopo 1988; Bergman and Öhman 2001). Coral reefs on the eastern ocean-facing side are exposed to strong currents and waves. As a result reefs are dominated by massive or encrusting scleractinian as well as soft corals while reefs on the sheltered western coast located in the Zanzibar Channel were dominated by branched corals (Johnstone et al. 1998;).

The reef at Bawe Island, covering 16 km², is currently dominated by large monostands of branching *Porites* as well as massive *Porites* after the El Ninõ in 1997/1998 followed by a COTS outbreak from 2002/2003-2006 (see Fig. 1) (Bergman and Öhman 2001; Muthiga et al. 2008; Muhando and Mohammed 2002; Muhando and Lanshammar 2008; Knudby et al. 2010). However, the reef around Bawe Island still harbours some unique and regionally rare coral taxa (Zvuloni et al. 2010) and a diverse sponge fauna comparable to other reefs around the West Coast of Zanzibar. Zanzibar in general was described as an area of high biodiversity and thus of high conservation priority and the reef at Bawe seems to be a good representative of Zanzibar´s coral reefs (McClanahan et al. 2007; Obura 2012).

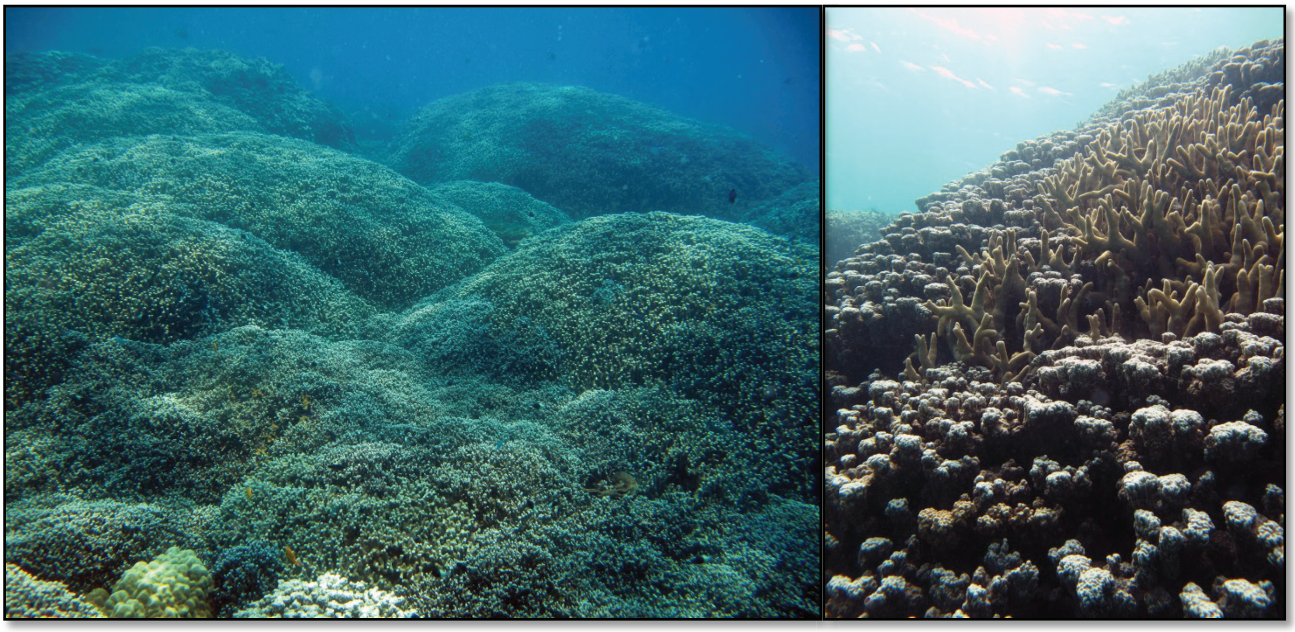

**Fig. 1:** The reef at Bawe Island dominated by monostands of branching Porites .and massive Porites.

The sponges investigated in this thesis were chosen based on their abundance in sponge quadrats. The sponges chosen for the study were also observed to be common members of the reef communities of other island surrounding Zanzibar`s West Coast.

## Introducing my Sponge Species:

### 1) *Haliclona atra* (Pulitzer- Finali, 1993)

This is the most and second most dominant sponge species in terms of coverage and abundance at 5 and 10 m depth, respectively. It was growing in large numbers in between branching corals of the genus *Porites* (Fig. 2).

**Fig. 2:** A stand of *H. atra* growing sprawled over branching *Porites*.

*H. atra* is a green-brown branching sponge. The tissue of this sponge is soft, but dense, and the outer surface is velvety to the touch. Upon physical contact, the sponge exhibits rapid discoloration of the outer surfaces and tissue loss onto the skin or other contact surfaces. Direct physical contact is commonly accompanied by a numbing sensation. Barrow and Cappon (1991) reported for an Australian sponge of the genus *Haliclona* the development of an irritant rash on human skin after a prolonged exposure for 24 hours. Interestingly, this sponge species harbours large amounts of brittle stars that could be commonly observed on the surface of this sponge (Fig. 3).

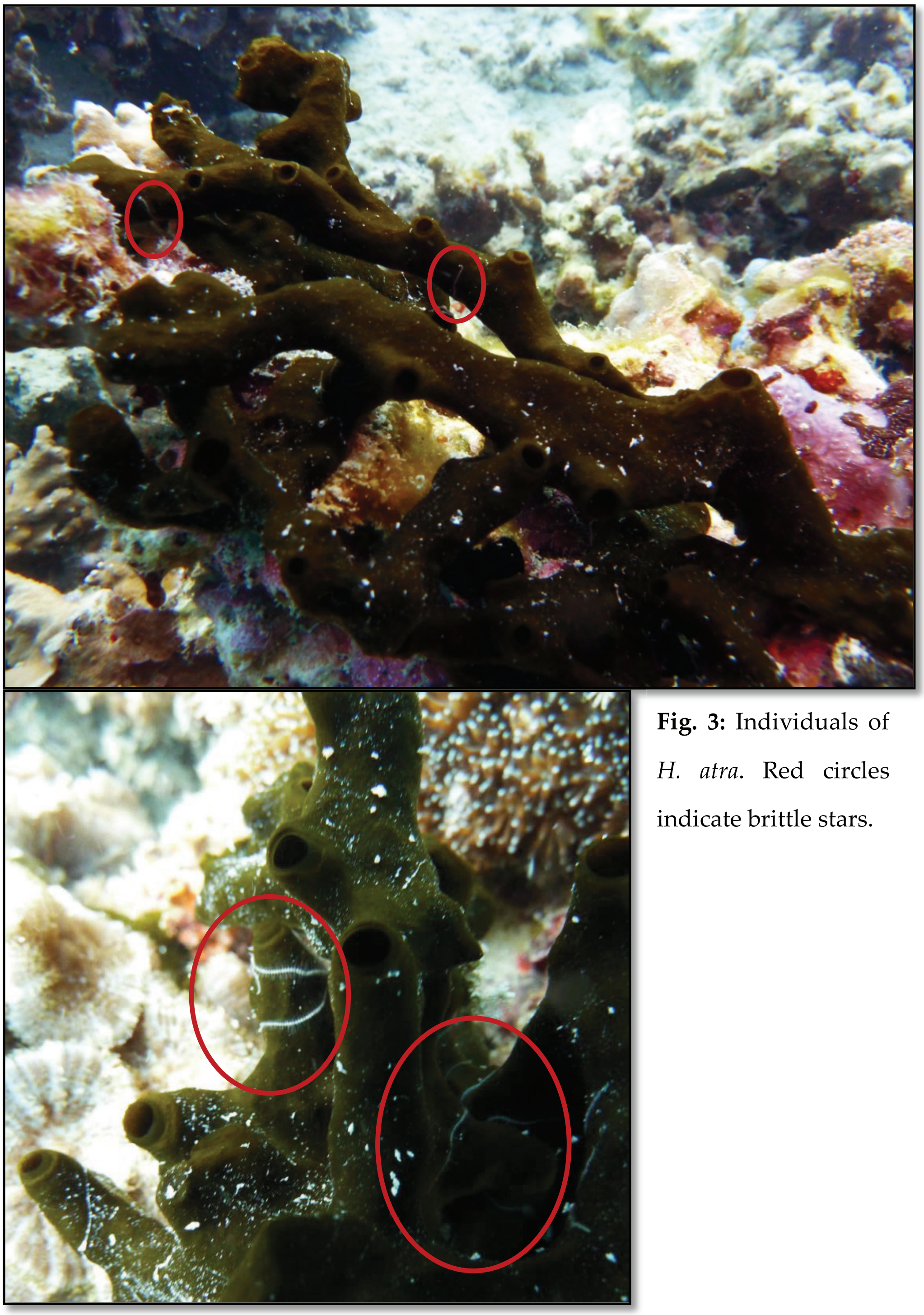

**Fig. 3:** Individuals of *H. atra*. Red circles indicate brittle stars.

### **2) *Haliclona fascigera*** (Hentschel, 1912)

This blue tube sponge has very thin, smooth tissue and grows in single tubes or several tubes clustered together. Its surface is frequently covered in sediment pellets (Fig. 4). *H. fascigera* was observed to grow often on top of coral colonies. This species was absent at 5m depth.

**Fig. 4:** The sponge *H. fascigera* and its surface covered in sediment pellets.

### **3) *Callyspongia* sp.** (Duchassaing and Michelotti 1864)

*Callyspongia* sp. is a branching sponge species which possesses many oscules. It has two colour morphs, one is bright blue and the other is deep purple (Fig. 5). The tissue of *Callyspongia* sp. is firm and hard, but interfused with many water channels on the inside. It grows mostly along the substrate, also often growing over corals, with many attachment points to the substrate. This species was absent at 5m depth.

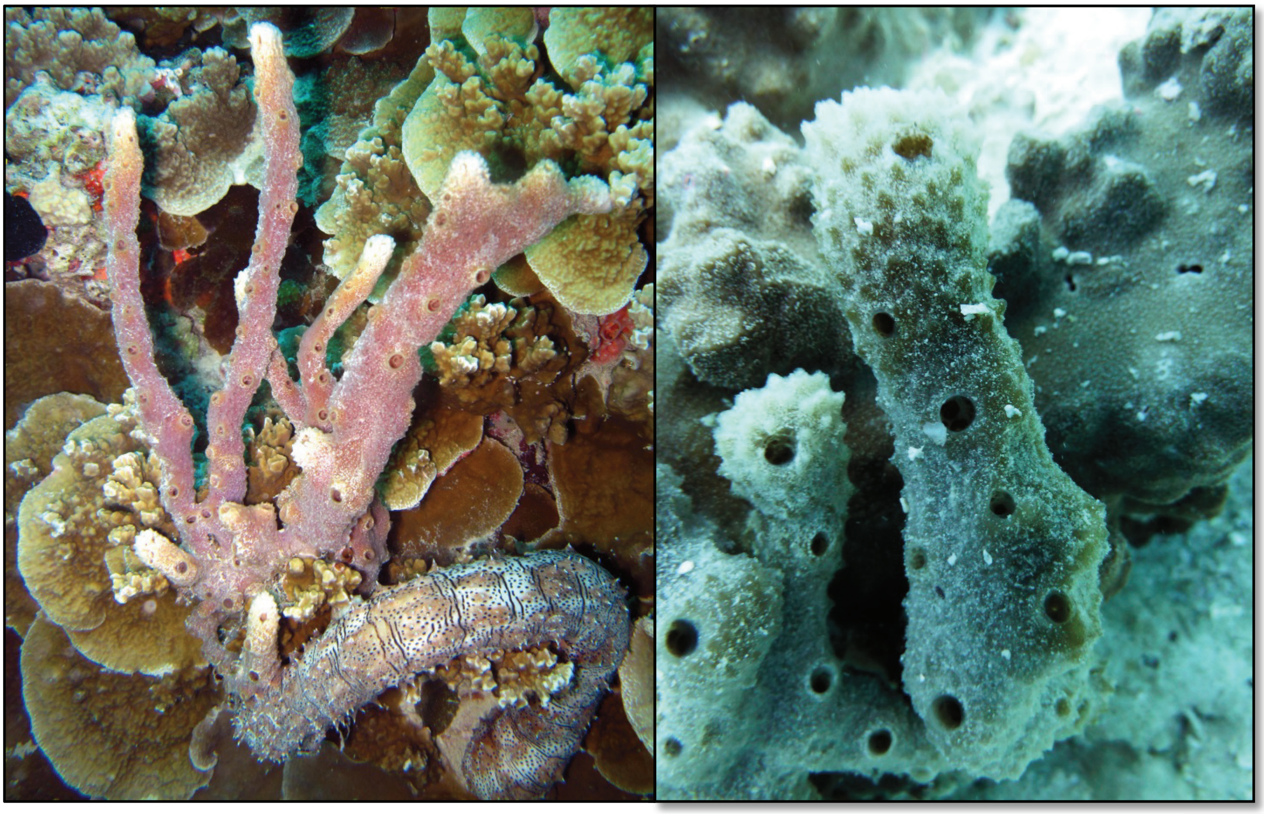

**Fig. 5:** The two colour morphs of *Callyspongia* sp.

## 4) *Callyspongia aerizusa* (Desqueyroux-Faúndez, 1984)

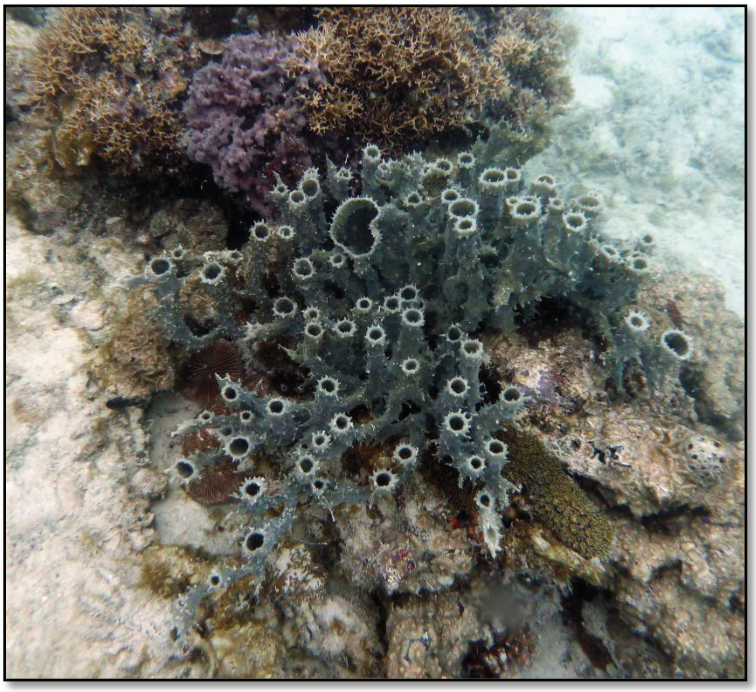

**Fig. 6:** Individual of *C. aerizusa* with multiple spiky tubes.

*C. aerizusa* is a blue tube sponge with many protruding spikes on its surface (Fig. 6). The inner lining of the tube is smooth. The tissue of *C. aerizusa* is very firm and dense. Its surface was covered in sediment pellets in a similar way to *H. fascigera*.

### 5) ***Pseudoceratina* sp.** (Carter 1885)

*Pseudoceratina* sp. is a massive, brightly yellowed coloured sponge species. It´s tissue is very firm and dense and hard to cut. On its surface are numerous irregular villi/projections. This sponge can exhibit locally high abundances whereas it is completely absent from other reef areas (Fig. 7). When exposed to air or injured underwater, the colour of this sponge species turned progressively dark blue within a few minutes (Fig. 8). The reason for this change in colour of their tissue is a result of the production of bromotyrosine derivatives, which can be commonly observed for several other sponges of the order Verongida (Ross et al. 2000; Su et al. 2013). This particular sponge species also releases a blue colored vapour upon injury (Fig. 8) (e.g., when cut during sampling). It grows in between corals and can induce partial coral bleaching upon direct physical contact (Fig. 8).

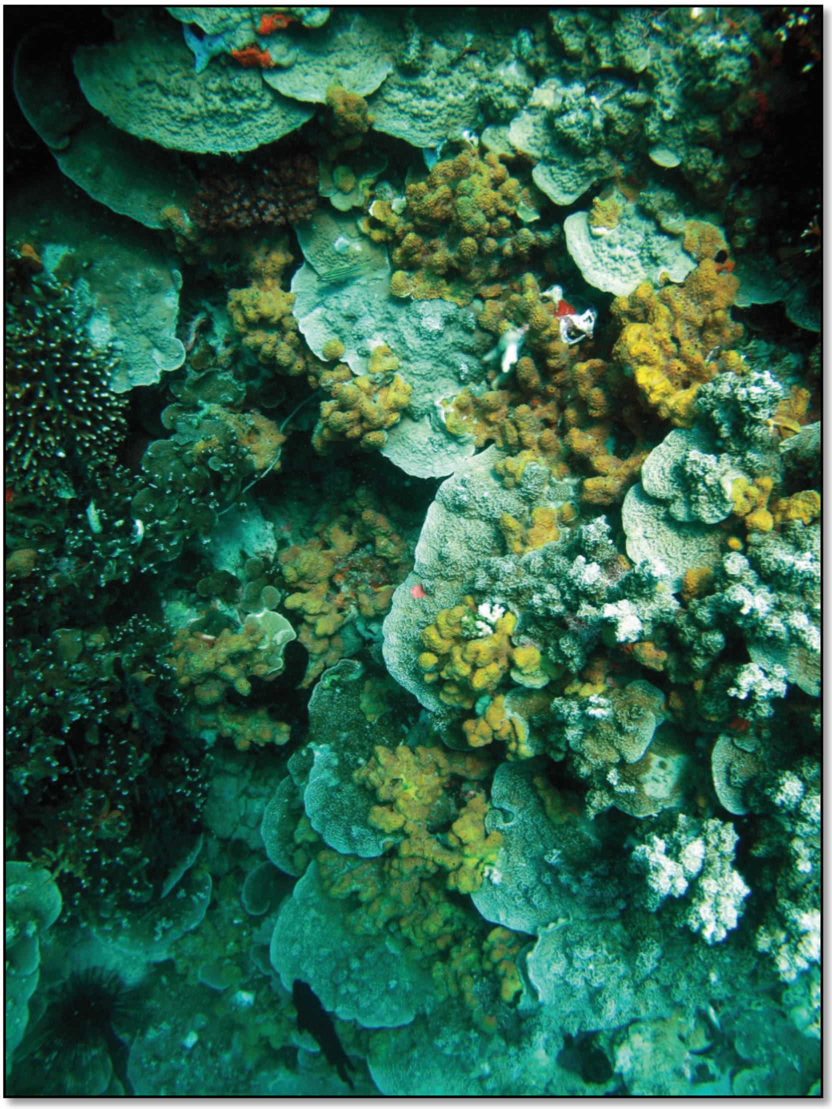

**Fig. 7:** Section of the reef with many *Pseudoceratina* sp. individuals.

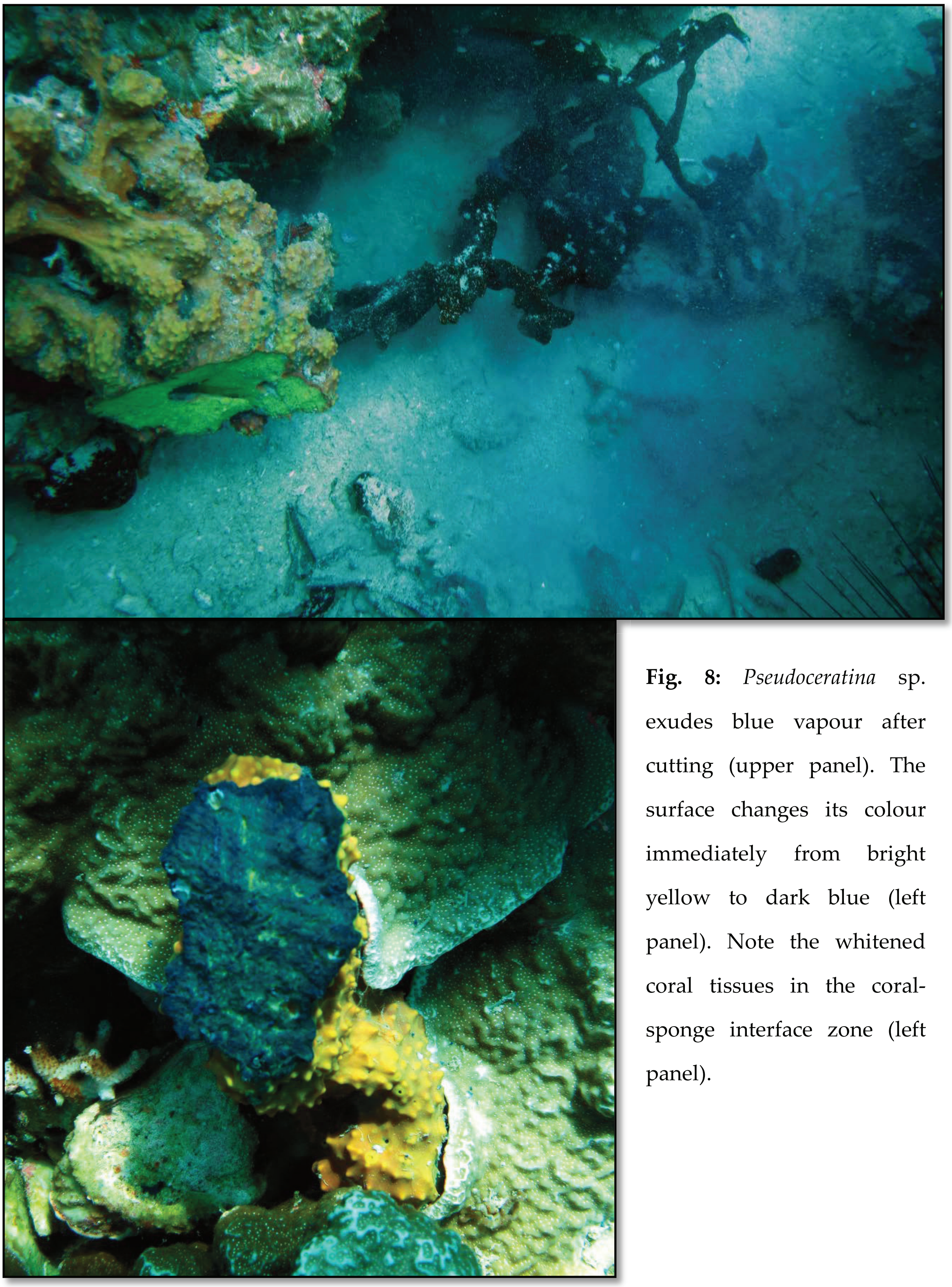

**Fig. 8:** *Pseudoceratina* sp. exudes blue vapour after cutting (upper panel). The surface changes its colour immediately from bright yellow to dark blue (left panel). Note the whitened coral tissues in the coral-sponge interface zone (left panel).

### 6) *Scopalina hapalia* (Hooper et al. 1997)

**Fig. 9:** Individual of *S. hapalia*.

*S. hapalia* is an orange coloured sponge that consists of very soft tissue which moulders easily when cut. It has a transparent skin layer around its oscules. *S. hapalia* can be encrusting, but often forms large cushions (Fig. 9). At Bawe Island it was not commonly observed, but dominant on the impacted site off Changuu Island. This species was absent at 5m depth.

### 7) *Plakortis kenyensis* (Pulitzer- Finali, 1993)

*P. kenyensis* is a khaki coloured massive sponge species. It has a very smooth surface, and is visibly free of epibionts (Fig. 10). Its inner tissue is dense and coloured bright beige. This species was absent at 5m depth.

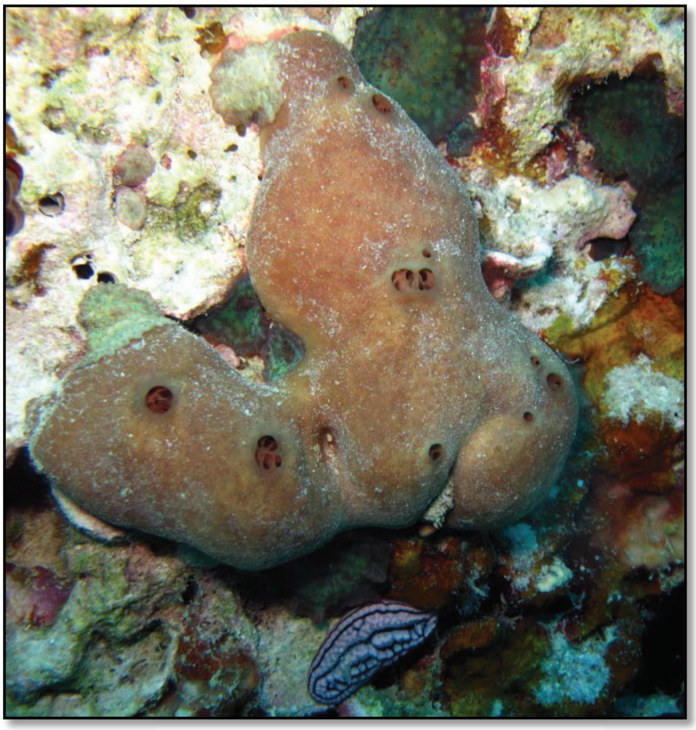

**Fig. 10:** Individual of *P. kenyensis*.

### 8) *Tetrapocillon minor* (Pulitzer- Finali, 1993)

Sponges of the genus *Tetrapocillon* are described as rare and unusual. *T. minor* consists of a very soft, thin and flabby tissue and was characterized by a smooth surface always free of epibionts. It seems to have no skeleton as it immediately collapses when removed out of the water. Individuals of *T. minor* have a blackish colour and a globular morphology. It was observed that this sponge often grew on rubble or lateral of corals. This sponge species senses water movements. If approached not very carefully, *T. minor* starts to rapidly close its big oscules completely.

**Fig. 11**: Individuals of *T.minor* with large oscules growing on dead corals.

### 9) *Biemna* sp.

*Biemna* sp. was the most abundant sponge species on 10m depth and the second most abundant on 5m depth. This species is one of few sponges able to settle on sand which might explain its dominance at 10m depth as there were many sandy patches in the reef (Cerrano et al. 2002). It can have an encrusting or massive, cushion- like, growth form and was observed to induce partial bleaching in corals (Fig. 12). *Biemna* sp. possesses firm and dense tissue which brittles easily when cut. The outer tissue of this sponge is yellow to red or brown, and the inner tissue

**Fig. 12:** *Biemna* sp causing bleaching in a coral.

light beige. This sponge species produces large amounts of thick mucus which can induce dermatitis upon direct physical contact (van Soest et al. 2002).

### 10) *Paratetilla* sp.

*Paratetilla* sp. has a globular morphology, and it is thus also known as the golf-ball sponge (Fig. 13). It has a lot of ostia which are located further inwards and one or two massive oscules. *Paratetilla* sp. has very firm tissue interfused with a lot of large spicules. These large spicules protrude from its surface and may create a "lotus effect" through surface roughness, thereby constituting a self-cleaning mechanism under high sedimentation rates (Schönberg 2015). Sponges of the genus *Paratetilla* are therefore well adapted to perturbed sites with higher sediment loads and common on reefs close to highly populated areas (Mc Donald et al 2002; Fromont et al. 2006). This is consistent with the observation that *Paratetilla* sp. was commonly more observed on the more impacted site at Changuu Island.

**Fig: 13:** Individual of *Paratetilla* sp. displaying its one oscule and several ostia.

## OVERVIEW OF THESIS CHAPTERS

This thesis consists of a general introduction (Chapter 1), three chapters presenting the core research of the present PhD thesis (Chapters 2-4), and a general discussion of the key findings (Chapter 5). Each chapter is intended for publication as an independent research article.

*Chapter 2:*

**Anti-predatory effects of 10 common reef sponges from Zanzibar**

**Helber SB**, de Voogd NJ, Muhando CA, Rohde S and Schupp PJ

This chapter presents a survey of the benthic community composition of the reef at Bawe, an island on Zanzibar´s West Coast, and evaluates the abundance and diversity of the sponge community. Further, this chapter assesses if the ten most abundant sponges on the West Coast of Zanzibar are chemically defended against predators. Laboratory feeding assays with the spongivorous pufferfish *Canthigaster solandri* are performed in order to test the palatability of the sponge extracts. The results of the feeding assays are compared with the sponge benthic cover data to investigate if the observed bioactivity in sponges correlates with their abundance on the reef.

This study was initiated by S.B. Helber, S. Rohde and P.J. Schupp. Transect surveys and sponge sampling were conducted by S.B. Helber with support from C.A. Muhando. N.J. de Voogd identified all sponge species. The experimental work was conducted by S.B. Helber with support of S. Rohde and P.J. Schupp. S.B. Helber analyzed the data and wrote the manuscript with editorial help from all authors. This chapter has been accepted for publication in *Hydrobiologia*.

*Chapter 3:*

# Antimicrobial and cytotoxic activities of reef sponges from Zanzibar

**Helber SB**, Muhando CA, Rohde S and Schupp PJ

Sponges and their associated microbes produce an impressive diversity of secondary metabolites with different biological activities in order to chemically defend themselves against microbial pathogens and invertebrate competitors. Therefore, this chapter investigates if the ten dominant sponge species from coral reefs on Zanzibar's West Coast exhibit antimicrobial defences against 27 environmental and nine pathogenic bacterial strains known to be associated with various coral diseases. The antimicrobial activity of the sponge species are quantified by the disc diffusion assay. Furthermore, the cytotoxic properties of the organic sponge extracts are examined with the brine shrimp mortality assay. The production of these bioactive compounds can give an indication of the competitive abilities of the different sponge species.

This study was initiated by S.B. Helber, S. Rohde and P.J. Schupp. Sponge sampling was conducted by S.B. Helber with support from C.A. Muhando. The experimental work was conducted by S.B. Helber with support of S. Rohde and P.J. Schupp. S.B. Helber analyzed the data and wrote the manuscript with editorial help from all authors. This chapter is in preparation for submission.

*Chapter 4:*

## Allelopathic activities of reef sponges from Zanzibar

**Helber SB**, Hoeijmakers DJJ, Muhando CA, Richter C, Rohde S and Schupp PJ

On many coral reefs worldwide sponge abundances are increasing while coral cover is declining. The competitive abilities of sponges determine if they are able to gain space on the reef. Thus, the aim of this chapter is to investigate the allelopathic activities of three Western Indian Ocean reef sponges *in situ*. Sponge extracts are incorporated into phytagel strips attached directly to corals. The maximum photosynthetic yield of coral branches exposed to phytagel strips treated with sponge extracts and control strips are compared via PAM fluorometry. Further, the effects of biologically active metabolites of live sponge fragments on corals are compared via PAM fluorometry in contact and non-contact situations. The results give insights into the ability of sponges to cause bleaching and tissue necrosis in neighboring corals even without direct contact and generate hypotheses about how sponges decrease the chances of coral survival through impairments of their physiology.

This study was initiated by S.B. Helber, S. Rohde, C. Richter and P.J. Schupp. Field experiments were conducted by S.B. Helber and D.J.J. Hoeijmakers with support from C.A. Muhando. S.B. Helber analyzed the data and wrote the manuscript with editorial help from all authors. This chapter is in preparation for submission.

# CHAPTER 2

# Anti-predatory effects of 10 common reef sponges from Zanzibar

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**Helber S.B.**, de Voogd N.J., Muhando C.A., Rohde S., Schupp P.J. Anti-predatory effects of 10 common reef sponges from Zanzibar. This chapter has been accepted for publication in *Hydrobiologia*.

## ABSTRACT

Predation is a key factor influencing the distribution and community composition of sponges and other benthic organisms. The ability to produce deterrent secondary metabolites may partially explain the high abundances of sponges on coral reefs worldwide. Nonetheless, studies investigating sponge abundances and chemical ecology are rare, particularly in the Western Indian Ocean, which is considered a hotspot of sponge biodiversity. Therefore, this study assessed whether predation is a key factor in structuring the sponge community on reefs in Zanzibar by testing whether chemical defence potential correlates with sponge abundance. Sponge biodiversity and abundances (surface area) were determined at Bawe Island. Sponges were abundant on the reefs at Bawe Island accounting for 4.8 ± 3.8% and 7.5 ± 1.7% of the benthic cover at 5m and at 10m depth, respectively. The palatability of the most abundant sponge species was tested in laboratory feeding assays with the spongivorous pufferfish *Canthigaster solandri*. In the feeding assay 3 sponges (30%) deterred feeding by *C. solandri*. However, the presence of feeding deterrent compounds in sponges did not correlate with their abundance on the reef. This may be due to low predatory fish abundances allowing for the high prevalence of chemically undefended species.

## INTRODUCTION

On many reefs, especially throughout the Caribbean, sponges have become the dominant taxa in terms of abundance and biomass (Bell and Smith 2004; Diaz and Rützler 2001; Pawlik 2011). Sponges can outnumber corals in some locations of the Western Indian Ocean (such as the Barrier Reef of Toliara in Madagascar; Quirimba Archipelago in Mozambique) or sponges can even dominate certain coral reefs, such as in the Wakatobi region in Indonesia in which reefs shifted from coral- to sponge-dominated reefs (Vacelet and Vasseur 1977; Barnes and Bell 2002; Barnes 1999; Bell and Smith 2004). Despite a few studies that quantified the high sponge richness on some locations in the Indian Ocean, there is almost no information on their ecological roles in coral reef ecosystems even though sponges are important spatial competitors for reef building scleractinian corals (Barnes and Bell 2002; Bell and Smith 2004; de Voogd et al. 2004). Sponges, algae and other invertebrates have increased in abundance and biomass at several reefs worldwide correlating with the decline in coral cover caused by overfishing, sedimentation, eutrophication and climate change which has released other benthic organisms from spatial competition with corals (Norström et al. 2009; Bell et al. 2013; Colvard and Edmunds 2011a; Hoegh-Guldberg et al. 2008). Another characteristic factor structuring benthic communities in tropical reef ecosystems is predation pressure (Hay 1991). For sponges, the relative importance of top-down (predation pressure) and bottom-up (availability of resources) processes in shaping sponge communities is contentious (Pawlik et al. 2015a, 2015b; Slattery and Lesser 2015). Pacific oceanic reefs are characterized by low primary productivity in the water column compared to the Caribbean which receives a lot of nutrients from river discharge as well as from windblown Saharan dust (Shinn et al. 2000; Dai et al. 2012; Pawlik et al. 2016). The Caribbean sponge communities have adapted to the enhanced nutrient input and most sponge species are heterotrophic releasing nutrients gained through feeding on picoplankton and dissolved organic carbon (DOC) to the reef community (Southwell et al. 2008; de Goeij et al. 2013; Pawlik et al. 2016). In contrast, the nutrient low environment of the Pacific is dominated by phototrophic sponges that cycle nutrients internally with their with their photosymbionts rather than releasing them to their surroundings (Wilkinson and Cheshire 1990; Freeman and Easson 2016; Pawlik et al. 2016). Therefore bottom-up processes may play a more important role for the Pacific sponge communities, whereas top-down processes such as angelfish and parrotfish predation have a greater influence in shaping sponge communities on Caribbean reefs (Dunlap and Pawlik 1996; Pawlik et al. 2015a). The effects of predation on sponge communities can be complex because spongivores can completely remove certain species from some habitats or restrict them to others (Dunlap and Pawlik 1996; Hill and Hill 2002; Loh and Pawlik 2009; Wulff 2000).

Structural defence mechanisms, such as sponge spicules and sponging filaments, seem to contribute little to the protection against predators (Chanas and Pawlik 1995; Rohde and Schupp 2011). Most generalist predators, such as trunkfishes or parrotfishes (Pawlik 1998; Randall and Hartman 1968), possess strong jaws which are even able to crush coral skeletons (Bellwood and Choat 1990). Others, like angelfishes have relatively long gut retention times allowing them to utilize the proteins in sponging filaments (Chanas and Pawlik 1995; Burns and IIan 2003). Thus, to avoid predation, only the production of chemical compounds seems to offer successful protection (Pawlik et al. 1995).

Sponges yield the greatest diversity of secondary metabolites among invertebrates and currently more than 5300 secondary metabolites have been described from sponges (Sinko et al. 2012). The species composition and chemical ecology of sponges has been widely studied (Becerro et al. 2003; Burns et al. 2003; Pawlik et al. 1995; Rohde et al. 2015), however little is known about sponges of the Western Indian Ocean. The Western Indian Ocean represents a marine biodiversity hotspot (Obura 2012). Along with corals, sponges are also one of the most diverse and abundant organisms of benthic communities in the Western Indian Ocean (Barnes and Bell 2002b; Barnes 1999). Thus, this study examines the organic extracts from the most abundant sponges around the West Coast of Zanzibar for chemical anti-predatory effects. Furthermore, it was evaluated if the prevalence of chemical defences is related to sponge abundance on the reef. Comparisons with fish abundance data can reveal whether chemically defended sponges are more abundant on reefs with high fish abundance while undefended sponge species are more abundant on reefs with high fishing pressure.

## MATERIALS AND METHOD

### Study site

Field surveys were conducted from September to December 2014 at Bawe Island on Zanzibar (Unguja), Tanzania (Fig. 1). Bawe island (06° 09′25.56″ S, 39° 08′0.96″ E) is located on the west side of Zanzibar 7 km away from the capital city Stonetown. Bawe is protected from direct exposure from the Indian Ocean, by being located on the west side of the island in the Zanzibar channel (Bergman and Öhman 2001). The reef at Bawe is heavily influenced by fishing activities and untreated sewage discharge from Stonetown and its harbour (Lokrantz et al. 2010; Moynihan et al. 2012).

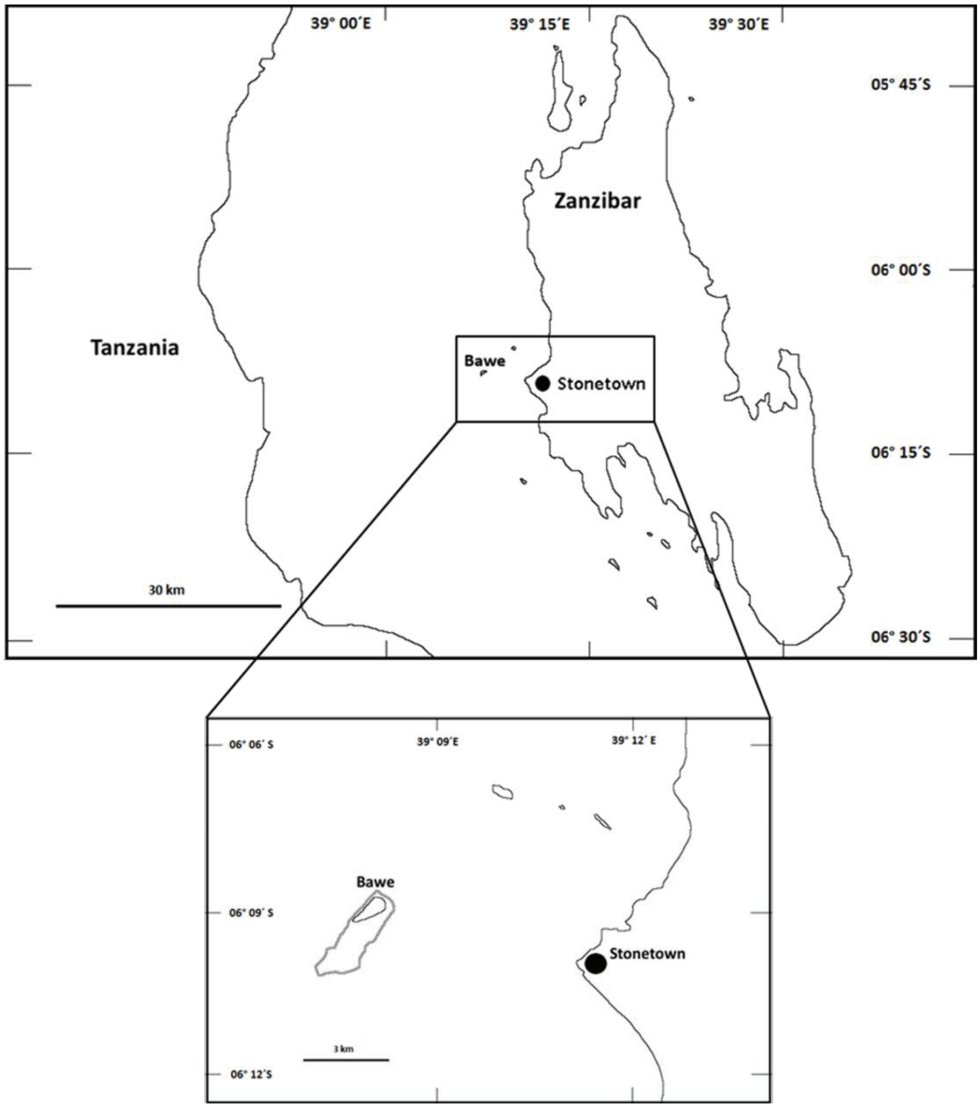

**Fig. 1:** Location of the Study site Bawe Island and its fringing coral reef (06° 09′25.56″ S, 39° 08′0.96″ E) on the West side of Zanzibar Island, Tanzania.

### Benthic reef coverage and sponge cover

The benthic community composition was assessed using Line point intercept transects. The non-cryptic sponge biodiversity, cover and abundance were evaluated in detail using 0.25m² quadrats. Six transects of 50m length were randomly placed by SCUBA divers both at 5 and 10m depth, separated by at least 10m. Benthic substrate categories recorded included: live coral (genera level), corallimorpharians, sponges (species level), soft corals, macroalgae, dead coral, sand, rubble and rock. Starting at 10m, one 0.25m² sponge quadrat was placed every 10m along each transect on alternating sides of the transect line. Within each quadrat the number of non–cryptic sponge species was recorded along with the surface area occupied by each species.

### Sponge collection and Identification

Based on the sponge community survey, ten of the most abundant sponge species were chosen for the investigation of their anti- predatory defences. Specimens of the selected sponge species were sampled randomly by scuba-divers at 10m depth. Replicates (3-5 individuals) of each sponge species were collected at least 20m apart to avoid collection of clones and transferred into zip block bags filled with seawater. At the Institute of Marine Sciences (IMS; Stonetown) a small 5 cm x 3 cm part of the collected sponge individuals was cut with a sterile scalpel blade and transferred into sterile falcon tubes filled with 99.9% Ethanol for species identification. Sponge vouchers used for species identification are stored at the Naturalis Biodiversity Center in Leiden, Netherlands (Table1).

**Table 1:** The most abundant sponge species at Bawe Island, Zanzibar, and their natural tissue extract concentration. Extracts yields in mg extract/g WW are given as the mean of 3- 5 extractions (± STD).

| Order | Family | Species | Number of Replicates | Natural Concentration [mg/g WW] |
|---|---|---|---|---|
| **Haplosclerida** | Chalinidae | *Haliclona fascigera* | 5 | 13.00 (± 6.38) |
| **Haplosclerida** | Chalinidae | *Haliclona atra* | 3 | 26.51 (± 4.53) |
| **Haplosclerida** | Callyspongiidae | *Callyspongia aerizusa* | 5 | 19.35 (± 10.14) |
| **Haplosclerida** | Callyspongiidae | *Callyspongia* sp. | 3 | 22.90 (± 2.18) |
| **Verongida** | Pseudoceratinidae | *Pseudoceratina* sp. | 3 | 31.06 (± 18.63) |
| **Homosclerophorida** | Plakinidae | *Plakortis kenyensis* | 3 | 16.80 (± 13.25) |
| **Scopalinida** | Scopalinidae | *Scopalina hapalia* | 3 | 17.14 (± 5.86) |
| **Poecilosclerida** | Desmacellidae | *Biemna* sp. | 3 | 18.29 (± 2.84) |
| **Tetractinellida** | Tetillidae | *Paratetilla* sp. | 3 | 26.31 (± 3.66) |
| **Poecilosclerida** | Guitarridae | *Tetrapocillon minor* | 4 | 18.39 (± 7.37) |

### Extraction procedure

Samples were immediately transferred to the field IMS laboratory. After gently removing the sponges from the bags, sponge pieces were weighed to the nearest 0.01 g (wet weight) and cut into small pieces. The sampling vials were filled up with 99.9% Ethanol until 1 cm above the sponge pieces. Samples were repeatedly extracted in 99.9% Ethanol over three consecutive days. The extracts were filtered to remove particles every 24 hours and the filtrate was frozen at - 20°C for storage and transfer. Extracts were further processed for chemical analysis at the Institute for Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, Germany. Samples were filtered again and evaporated under reduced pressure using a rotary evaporator (water bath temperature 35°C). Crude extracts were transferred into pre-weighted glass vials and evaporated to complete dryness with a Speed Vac. Natural extract concentrations were calculated as mg extract per g of sponge wet weight (WW) (see Table 1). Pooled extracts of the replicate sponge individuals were used for the experiments. All extracts were stored at -20°C until use.

### Artificial food preparation

Potential feeding deterrence of the ten sponge species was tested by incorporating sponge extracts into an artificial food following the method described in Pawlik et al. (1995). In short, 0.16 g sodium alginate, 0.3 g fish food (Brand) and a pinch of freeze-dried, powdered squid mantle mixed together in distilled water (5 ml). Extracts were dissolved in 100 µl of ethanol and gently stirred into the mixture at natural concentrations (see Table 1). The food mixtures were loaded each in separate 5ml syringes and the contents of the syringes emptied in a 0.25 M calcium chloride solution forming long, spaghetti-like strands. The strands were removed from the calcium chloride solution after a few minutes, rinsed in seawater and cut into 3 to 5 mm long pellets. Control pellets lacking extracts, but also containing 100 µl of Ethanol solvent, were prepared in the same way.

### Fish feeding Assay

Aquarium assays were performed similar to Pawlik et al. (1995). Control and treatment pellets were presented to 19 individuals of the spotted sharpnose pufferfish, *Canthigaster solandri*, (Richardson 1845) held in separate 100l aquaria. This omnivorous pufferfish species is commonly used as a model organism for studies of feeding deterrence in benthic organisms (Pennings et al. 1994; Rohde et al. 2012; Rohde et al. 2015; Rohde and Schupp 2011).The control pellets were first offered to a fish to determine if they were feeding. If it was consumed, a treatment pellet followed. Fish that did not eat the control pellets were not used for the experiment. If the fish ate the treatment pellet, the sample was scored as accepted. A pellet was considered as rejected if one of the following situations arose: (1) The pellet was taken at least three times into the mouth cavity but spat out each time (flushing), (2) it was ignored or (3) if a fish attempted to eat the pellet, but spat it out and subsequently ignored it. After fish showed one of those behaviours a second control pellet was offered to determine if the fish were satiated with food. Pellets were scored as rejected only in assays in which the fish also ate the subsequent control. Fisher's exact test was used in order to evaluate for significant differences in the palatability of treated versus control pellets (Rohde et al. 2012).

## RESULTS

### Sponge abundance and benthic community composition

Hard coral cover at Bawe was high with 77 ± 12.5% coverage at 5m and 44 ± 12.5% coverage at 10m (Fig. 2). At 5m depth, rubble (7%) and sand (5%) were the second most abundant benthic component, while at 10m depth rubble (22%) followed by sponges (15%) and sand (10%) represented the dominant benthic categories.

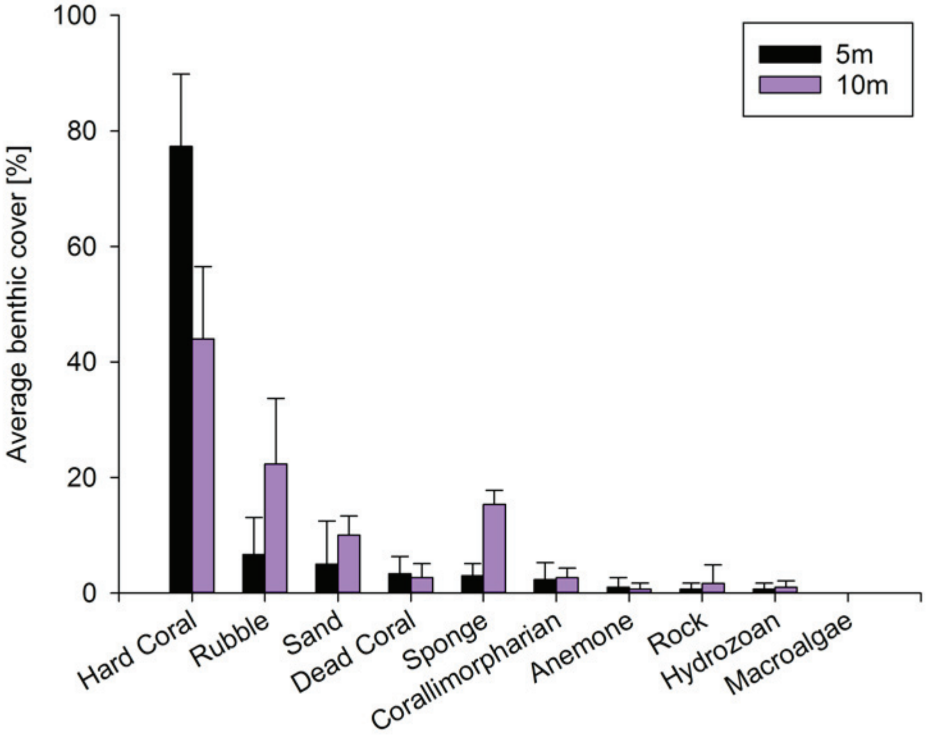

**Fig. 2:** Percentage cover of live hard corals, rubble, sand, dead corals, sponges, corallimorpharians, anemones, rock, hydrozoans and macroalgae along 50 m transects at Bawe Island in 5 m and 10 m depth determined by Line Point Intercept transects (Means + SE, n=6).

A total of 16 and 21 sponge species were recorded at 5m and 10m depth, respectively. Average percent sponge cover at 10m (7.5± 1.7% cover $m^{-2}$) was not significantly higher compared to 5m (4.8± 3.8% cover $m^{-2}$) (t- test, n= 6, df= 10, t= -1,584, p> 0.05). Five sponge species, *Haliclona atra* (Pulitzer-Finali 1993), *Biemna* sp. Gray 1867, *Callyspongia aerizusa* Desqueyroux-Faúndez 1984, *Paratetilla* sp. Dendy 1905 and *Tetrapocillon minor* Pulitzer-Finali 1993 were present at both depths, while *Callyspongia* sp. Duchassaing and Michelotti 1864, *Scopalina hapalia* (Hooper et al. 1997), *Haliclona fascigera* (Hentschel 1912) and *Plakortis kenyensis* Pulitzer-Finali 1993 could only be found at 10m (Fig. 3). *Pseudoceratina* sp. Carter 1885 was not recorded in the sponge quadrats. The average abundance of sponge individuals was also almost twice as high at 10m (13.7± 3.6 ind. $m^{-2}$) compared to 5m (7.6± 3.1 ind. $m^{-2}$; t- test, n= 6, df= 10, t = -3,180, p= 0.01). The ten sponge species used in the feeding experiments accounted for approximately 75% and 81% of total sponge coverage and for 54% and 80% of all sponge species at 5m and 10m depth each.

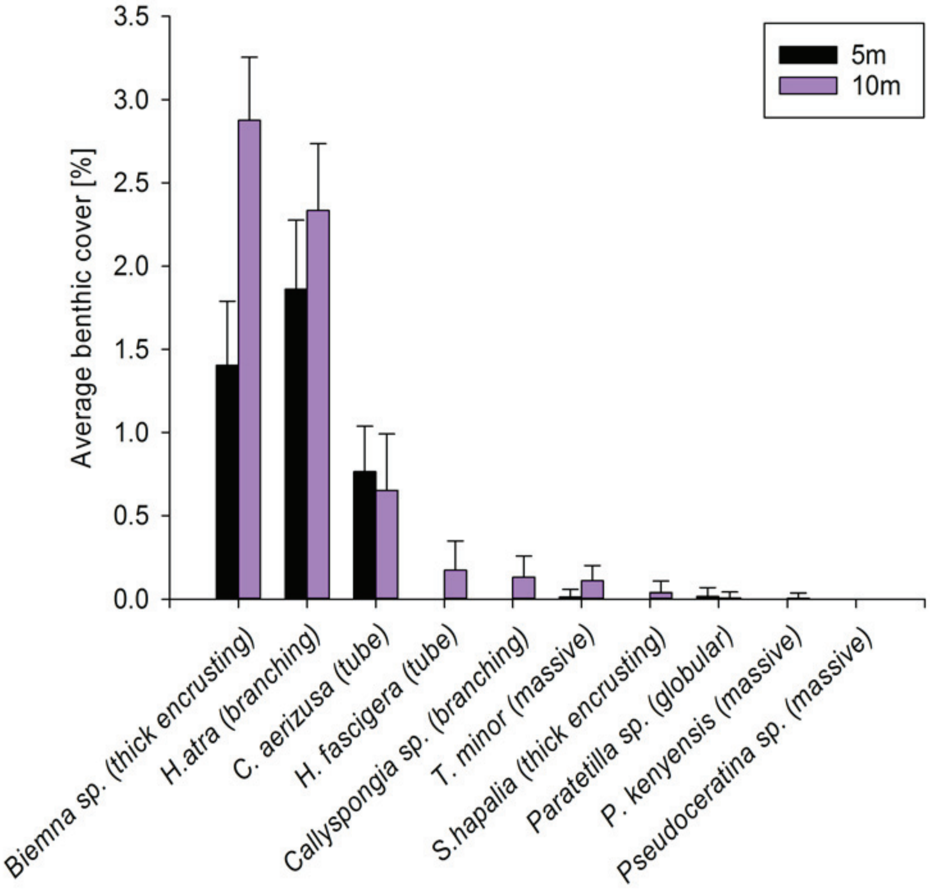

**Fig. 3:** Percent sponge cover at Bawe Island in 5m and 10m depth as determined by sponge quadrats along the line point intercept transects (Means + SE, n= 30 quadrats at 5 m and n= 27 quadrats at 10 m depth).

### Fish Feeding Assay

Extract yields of the tested sponge species are presented in Table 1.

Three of the ten sponge species deterred predation by the sharpnose pufferfish *C. solandri* (Fig. 4). We did not observe a correlation between feeding deterrence and sponge phylogeny. While both *Callyspongia* species did not exhibit any deterrent effects, the two *Haliclona* genera yielded both a highly palatable (no pellets rejected) and also a highly deterrent effect (92% rejected pellets). Deterrence of the sponge extracts was unrelated to their average percent cover (Fig. 3). The slope of the linear regression was not significantly different from 0 (t= 0.5127, slope= 0.0052, p> 0.05). Additionally, the coefficient of correlation, $R^2 = 0.0318$

indicated there was no or only a negligible relationship between anti-predator chemical defences of the sponge species and their abundance on the reef (n=10; p > 0.05).

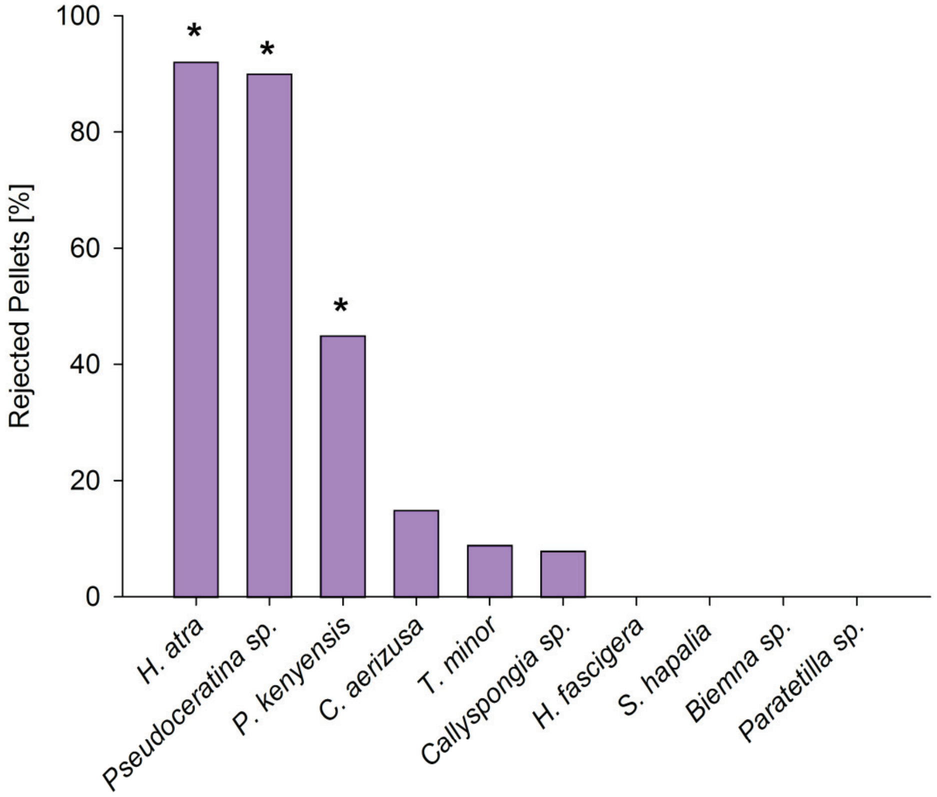

**Fig. 4:** Percent of food pellets rejected by the pufferfish *C. solandri*. Sponge taxa are ordered by rank of deterrence found. Sponge pellets were fed to 11-13 different pufferfish individuals for each sponge species investigated. * indicates significant deterrence (Fisher´s exact test, p< 0.05).

## DISCUSSION

### Sponge abundance and benthic community composition

In the present study, sponge richness was higher at 10m (21 species) than at 5m (16 species) depth. Sponge species richness was similar to other regions in the Western Indian Ocean such as Kenya, Mozambique and Madagascar, where sponge richness has been reported to reach its maximum between 10m and 15m depth (Barnes and Bell 2002; Bell and Smith 2004).

Barnes and Bell (2002b) reported 11- 18 and 15- 25 sponge species at 5m and 10m depth, respectively, for the above mentioned regions. Likewise, sponge surveys in the Wakatobi region, Indonesia, yielded again similar species numbers of 25 and 33 sponge for transects at 10 m and 15m depth (Bell and Smith 2004).

Sponge abundance in this study was with 7.6 ± 3.1 individuals/m² at 5m and 13.7 ± 3.6 individuals/m² at 10m depth similar to sponge density for reefs in the Spermonde Archipelago, Indonesia (1.2-12.7 individuals/m² reported, de Voogd et al. 2006).

Percent sponge cover for reefs at Bawe Island was with 4.8 ± 3.8% cover $m^{-2}$ at 5m and 7.5 ± 1.7% cover/m² at 10m comparable to studies from Indonesia (3-11% sponge cover at 10m depth in the Wakatobi National Park, Powell et al. 2010; 4.4% and 5.4% sponge cover $m^{-2}$ at 5 and 10m depth, respectively, at Derawan Islands, Indonesia, de Voogd et al. 2009).

However, the sponge coverage reported here and in the other studies from the Indo-Pacific (Powell et al. 2010; de Voogd et al. 2009) are much lower compared to Caribbean reefs (15.9% sponge cover; Loh et al. 2015). Reef communities in the Caribbean changed not only its structure in favour of sponge dominance, but also in favour of macroalgae dominance on many reefs (Loh et al. 2015; Mumby 2009). As a consequence Caribbean reefs had lower coral cover than coral reefs in the Western Indian Ocean (Diaz and Rützler 2001; Loh et al. 2015; Lokrantz et al. 2010; McClanahan et al. 2014). In contrast to the Caribbean, macroalgae did not comprise a major benthic category on Zanzibar`s reef (Lokrantz et al. 2010) and sponges were the second most dominant benthic organisms after hard corals at both depths (see Fig. 2).

The absence of *Callyspongia* sp., *S. hapalia*, *H. fascigera* and *P. kenyensis* from the 5m transects show depth related changes in the sponge community. One factor influencing species composition is substrate availability; a particular important factor for *Biemna* sp. This species is one of few sponges able to settle on sand (Cerrano et al. 2002). At 10m depth, several *Biemna* sp. individuals were observed settling on sand which made up 10% of the total benthic categories (Fig. 3). The high abundance of *H. atra* at both depths may be more related to its reproductive strategy and effective predator deterrence. Its branching morphology allows for easy fragmentation, maximizing recruitment in combination with sexual reproduction (Wulff 1984). Furthermore, fragmentation reduces the energy needed for the production of gametes which can be invested in the production of feeding deterrent compounds or in growth, as shown for sponges of the genus *Callyspongia* (de Voogd 2005; Tsurumi and Reiswig 1997; Leong and Pawlik 2010b).

**Palatability**

Only 30% of the sponge species examined deterred feeding by the generalist predator *C. solandri* (Fig. 4). This is much lower than results reported from the West Pacific (87%; Rohde et al. 2015), Caribbean (57.5 %- 69% ; Pawlik et al. 1995; Loh et al. 2015), Eastern Pacific (54%; Padilla et al. 2010) or the Red Sea (41%; Burns et al. 2003). However, the 30% feeding deterrence reported here was higher than the one found in Eastern Mediterranean sponges (20%; Sokolover and Ilan 2007). One explanation for the low percentage of feeding deterrent compounds in sponge extracts could be the method of extraction. Ethanol was used as solvent which would not be sufficient for the extraction of very non-polar secondary metabolites. It was shown for sponges and seaweeds that there were differences in the yield as well as bioactivity of their ethanolic versus their less polar extracts (Amade et al. 1987; Cronin et al. 1995). However this varies from species to species and there are also cases in which the activity of the polar and the semi polar extract did not differ (Safaeian et al. 2009). The majority of our investigated sponge species are in genera previously observed to be palatable, such as *Callyspongia* and *Haliclona* (Pawlik et al. 1995; Burns et al. 2003). This is the result of a resource trade-off in which for instance sponges of the family *Callyspongiidae* invest their energy in longer reproduction times as well as faster growth rates instead of producing feeding deterrent metabolites (Ilan and Loya 1988, 1990; Leong and Pawlik 2010b). However, in the present study, *H. fascigera* produced a palatable extract whereas *H. atra* was highly deterrent. Production of secondary metabolites may vary for different sponge species or even within the same sponge species depending on their species-specific microbial communities or recent life history (Rohde et al. 2012; Rohde et al. 2015; Simister et al. 2012; Vicente et al. 2015). Therefore, even sponge conspecifics can differ in their chemical defences. The other two sponge species, *Pseudoceratina* sp. and *P. kenyensis*, that yielded unpalatable extracts are known for producing secondary metabolites with antifeedant activities, such as brominated tyrosine derivatives (*Pseudoceratina* sp.), crasserides (both) and chondrillin (*P. lita*) (Sakemi et al. 1987; Pennings and Paul 1993; Costantino et al. 1993; Burns et al. 2003;

Pawlik et al. 1995; Walters and Pawlik 2005; Ruzicka and Gleason 2009; Thoms and Schupp 2008; Flórez et al. 2015). The palatable extract of *Paratetilla* sp. could indicate that this species invests its energy in the development of physical rather than chemical defence; i.e. large spicules (commonly 1- 2 mm; Schönberg 2015) as spicules above 250 µm deter predation (Burns and Ilan 2003).

Selecting the appropriate predator for feeding assays is important since potential predators vary in their sensitivity towards deterrent secondary metabolites (Pennings et al. 1994; Burns et al. 2003). The pufferfish *C. solandri* was used in this study because it was shown to be a suitable model organism for feeding preferences of reef fishes in the Indian Ocean (Pennings et al. 1994; Thoms and Schupp 2008; Rohde et al. 2015; Rohde and Schupp 2011). *C. solandri* is very common around Zanzibar and previous studies showed that field feeding assays with reef fish communities and laboratory assays with *C. solandri* provide corresponding results (Gullström et al. 2008; Rohde and Schupp 2011; Tyler et al. 2009). Unfortunately, there is little information on sponge predation from the Indian Ocean. Based on studies from other regions of the world, possible sponge predators other than fish include turtles, starfish, crustaceans and nudibranchs (Powell et al. 2015; von Brandis et al. 2014; Waddell and Pawlik 2000; Wulff 1995). In Zanzibar, starfish were rarely observed on the reef and small crustaceans as well as molluscs are unlikely to affect sponge distribution (Waddell and Pawlik 2000; Gemballa and Schermutzki 2004; Powell et al. 2015), although feeding of the snail *Cypraea tigris* on *Pseudoceratina* sp. was observed during pre-dawn dives (Appendix 1-Fig.1). Sponges represent a major part of the diet of adult hawksbill turtles in the Caribbean as well as the Western Indian Ocean (León and Bjorndal 2002; von Brandis et al. 2014), but this turtle species experienced large population declines (Bourjea et al. 2008; Mortimer and Donnelly 2008). Sea urchin densities on reefs around Zanzibar were high with *Diadema setosum* being the dominant species (Bronstein and Loya 2014). *D. setosum* is a generalist predator that feeds also on sponges, but only 5% of its gut content was composed of non-soluble residues, such as sponge spicules (Meroz and Ilan 1995; Bronstein and Loya 2014).

**No correlation between sponge abundance and feeding deterrence**

The presence of feeding deterrent compounds in sponges did not correlate with their abundance on the reef. The reef around Bawe Island exhibits clear signs of overfishing such as the absence of herbivorous fishes in the largest size class (81- 100% of maximum length calculated for each investigated species) as well as the low abundance (ranging from around 2-4 ind. 250 $m^{-2}$), biomass (ranging from approximately 250- 1100g 250 $m^{-2}$) and diversity of scraper and grazer species (Jennings and Polunin 1995; Lokrantz et al. 2010). Especially fishes belonging to the family of Scaridae, which were reported to be one of the main predators of sponges in the Caribbean (Dunlap and Pawlik 1996; Wulff 1997b, 1997c), were less abundant and had a lower biomass on heavily fished reefs such as Bawe compared to reefs of the ‘no-take’ marine reserve Chumbe Island (de la Torre-Castro et al. 2008; Lokrantz et al. 2010). Chumbe is also located on the west side of Zanzibar, but 13 km away from the capital city Stonetown. Additionally, all reefs on Zanzibar and especially the reefs with high fishing pressure are characterised by an extremely low biomass of parrotfish compared to other areas, such as the Great Barrier Reef or Kenya (McClanahan et al. 1997; Hoey and Bellwood 2008; Lokrantz et al. 2010). The absence of sponge feeding fishes might explain the high prevalence of chemically undefended species in the sponge community on the reefs at Bawe Island. Instead of the production of feeding deterrent secondary metabolites, chemically undefended species are able to invest their energy into reproduction, regeneration and growth which enables them to counterbalance grazing by spongivores (Leong and Pawlik 2010a; Pawlik et al. 2008; Walters and Pawlik 2005). Studies in the Caribbean showed that palatable sponges dominated coral reefs at overfished sites whereas sites with many fish predators were characterized by higher coverage (up to > 90%) of defended sponges (Loh and Pawlik 2014; Loh et al. 2015). The overall benthic sponge cover on overfished reefs in the Caribbean was twice as high, leading to increased sponge-coral interactions resulting often in sponge overgrowth (Loh et al. 2015). Most coral-sponge interactions involved palatable sponges that could undergo uncontrolled growth, likely due to release from predation pressure (Loh and Pawlik 2009, 2014). The lack of sponge predation has implications on the overall health of the coral reef since undefended sponges will recruit more rapidly and grow faster subjecting reef building corals to greater competitive pressure

(Loh et al. 2015).

Both, the removal of spongivorous fish as well as sewage input, could be responsible for an increase in sponge cover as sewage input might lead to an increase in pico- and bacterioplankton abundance which make up a big part of the sponge diet (Rützler 2002; Lesser 2006). In the Caribbean, bioeroding sponges of the genus *Cliona* increased in size and cover on reefs exposed to sewage pollution and it was demonstrated that some sponge species cope better with high sedimentation rates than corals or are even able to metabolise certain contaminants (Perez et al. 2003; Ward-Paige et al. 2005). Thus, even if sewage input might not actively have favoured sponge abundance, high nutrient levels and water turbidity can have caused a decrease in the calcification rates of corals and water turbidity as well as sedimentation could have reduced calcification, photosynthesis and growth (Fabricius 2005).

The reef at Bawe is exposed to untreated sewage discharge from Stonetown which was identified as main initiator leading to a phase- shift from coral to corallimorpharian dominance on the neighbour island Changuu, located 5 km away from Stonetown (Kuguru et al. 2004; Muhando et al. 2002).The reef at Bawe has a lower sewage input than the one at Changuu due to current patterns and its further distance to Stonetown (Kuguru et al. 2004; Muhando and Mohammed 2002). Bawe’s corallimorpharian cover remained unchanged since 1998 ranging from 3- 4% (Kuguru et al. 2004; Muhando et al. 2002), and its sponge cover increased substantially from < 1% up to 7.5%. At the same time, higher sewage exposure at Changuu increased corallimorpharians, but had no effect on sponge cover ranging from 2- 4% (Muhando et al. 2002; Bergman and Öhman 2001; Lokrantz et al. 2010). This indicates that other factors (i.e. predation) must interact with eutrophication effects.

## CONCLUSION

The reefs around Zanzibar have already experienced shifts in coral communities and decreased species diversity following the massive 1997-1998 bleaching event as well as crown-of-thorns-starfish (COTS) outbreaks during 2002/2003- 2006 (Muhando and Lanshammar 2008; Muhando 2009; Muthiga et al. 2008). The reef at Bawe could be vulnerable to a coral-sponge phase shift when faced with another major bleaching event or further removal of spongivorous fishes. The monitoring of sponge-feeding fish abundances and their relationship to sponge and coral communities of Western Indian Ocean reefs offers interesting future research. This is especially true given the paucity of information on spongivory, particularly within the Western Indian Ocean.

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# CHAPTER 3

# Antimicrobial and cytotoxic activities of reef sponges from Zanzibar

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**Helber S.B.**, Muhando C.A., Rohde S., Schupp P.J. Antimicrobial and cytotoxic activities of reef sponges from Zanzibar. This chapter is in preparation for submission.

## ABSTRACT

Sponges are one of the top spatial competitors on coral reefs worldwide as well as one of the most abundant sessile marine invertebrates. The success of sponges is in part dependent on their ability to deter predators, inhibit pathogenic microbes as well as demonstrate competitive dominance towards other sessile benthic organisms. Sponges and their associated microbes yield a wide array of compounds with different biological activities in order to chemically defend themselves. This study assessed if ten of the most dominant sponge species from Zanzibar's coral reefs were chemically defended against a wide array of bacteria. Furthermore, the cytotoxic activities of the organic sponge extracts were investigated. Antimicrobial activity of the sponge species was quantified by disc diffusion assays. Sponge extracts were tested against 36 environmental bacterial strains, 9 of which were known to be associated with various marine diseases. Potential cytotoxicity of sponge extracts was assessed using the brine shrimp lethality assay. We could show that all tested sponge species showed antimicrobial properties with a wide range of activity and that sponges from Zanzibar were better defended against potential pathogens compared to environmental strains. Additionally, five out of ten sponge species demonstrated cytotoxic activities in the brine shrimp assay. The results offer interesting future research in the competition for space between sponges and corals since both antimicrobial as well as cytotoxic properties may provide sponges with a competitive advantage over corals.

## INTRODUCTION

Sponges are one of the top spatial competitors for reef-building scleractinian corals on coral reefs (Barnes and Bell 2002; de Voogd et al. 2004). Their great success in marine environments can be partly explained by their ability to feed on a wide spectrum of prey (Pawlik et al. 2015), the very low energetic costs of their filter-feeding activities (Gili and Coma 1998) and their strong chemical defences (Pawlik 2011). Survival of sponges is often depending on the production of chemically active compounds since they are sessile invertebrates lacking any behavioral escape or defence mechanisms. Sponges produce the greatest diversity of secondary metabolites among benthic marine organisms (Faulkner 1984) and up until now more than 5300 secondary metabolites have been described from sponges (Sinko et al. 2012). The main focus of studies on sponge secondary metabolites has been their potential pharmacological activity, but a growing number of studies also started to investigate the ecological functions of these compounds.

Sponges have the ability to deter predators (Pawlik et al. 1995; Burns et al. 2003; Rohde et al. 2015), inhibit pathogenic microbes (Amade et al. 1987; Kelly et al. 2005, Rohde et al. 2015) as well as to demonstrate competitive dominance towards other sessile benthic organisms (Pawlik et al. 2007; de Voogd et al. 2004; Madduppa et al. 2015). Predation is one of the main factors which structures tropical reef communities (Hay 1991) and it has been well documented that sponges often possess chemical antipredator defences (Pawlik et al. 1995; Rohde et al. 2015). Another factor contributing to their competitiveness is their ability to combat microbial attacks. Sponges defend their surface from colonization by fouling organisms as well as potential pathogenic bacteria (Wahl 1989; Kelly et al. 2003). The growth of detrimental bacteria on their surface or body cavities can have negative consequences such as impairment of their feeding abilities, increased risk of dislodgement and settlement of larger fouling organisms (Wahl 1989; Holmstrom and Kjelleberg 1994). In the case of colonization by potential pathogens, diseases might be initiated leading to the degradation of host tissue or even to death of the host (Littler and Littler 1995). Marine organisms are constantly exposed to potentially harmful bacteria with bacterial abundances in the Indian Ocean ranging from $6x\,10^4\ ml^{-1}$ to $2.5x\,10^6\ ml^{-1}$ in the surrounding seawater (Sorokin et al. 1985; Wiebinga et al. 1997; Goosen et al. 1997). Sponges are additionally exposed to large quantities of microbes passing through their bodies due to their filter feeding activities (Gili and Coma 1998). Given the exposure of sponges to high numbers of bacteria in the marine environment and the low incidence of infection with diseases, chemical defences in sponges are likely playing a crucial role in providing sponges with effective defences against tissue damage by predators or against invasion of pathogenic microbes after wounding (Thoms et al. 2006; Rohde et al. 2015). A third main factor shaping the species composition on coral reefs is the competition for space. Space is a highly limited resource for sessile organisms on coral reefs (Dayton 1971; Jackson and Buss 1975). Sponges are assumed to be competitively superior over other reef organisms not only due to their ability of rapidly overgrowing competitors but also due to the release of chemical compounds (Porter and Targett 1988; Pawlik et al. 2007; Engel and Pawlik 2000). Cytotoxic secondary metabolites produced by sponges may be able to inhibit the growth of other space competing organisms by impairing their cell division and thus providing sponges with an advantage in the competition of space on coral reefs (Aerts 1999; Folmer et al. 2009).

The chemical defences of sponges have been widely studied in the Caribbean, Pacific, Mediterranean and Red Sea (Pawlik et al. 1995; Burns et al. 2003; Becerro et al. 2003; Rohde et al. 2015), however little is known about sponges of the Western Indian Ocean (WIO). Thus, this study is the first to evaluate chemical defences in WIO reef sponges. Organic extracts from ten of the most abundant sponge species on reefs around the West Coast of Zanzibar were examined for ecologically significant antimicrobial and cytotoxic effects of their secondary metabolites.

## MATERIAL AND METHODS

### Study site

The field study was conducted from September to December 2014 at Bawe Island on Zanzibar (Unguja), Tanzania. Bawe (06° 09′25.56″ S, 39° 08′0.96″ E) is located on the west side of the island in the Zanzibar channel. It is sheltered from direct exposure to the Indian Ocean by Zanzibar and situated 7 km away from the capital Stonetown (Bergman and Öhman 2001). The reef at Bawe is heavily influenced by fishing activities and untreated sewage discharge from Stonetown and its harbour (Lokrantz et al. 2010; Moynihan et al. 2012).

### Collection and extraction procedure

Based on a sponge community survey (Helber et al. 2016a), the ten most abundant sponge species were chosen for the investigation of their antimicrobial and cytotoxic properties. This included *Callyspongia aerizusa*, *Callyspongia* sp., *Haliclona atra*, *Haliclona fascigera*, *Biemna* sp., *Paratetilla* sp. *Pseudoceratina* sp. *Scopalina hapalia*, *Plakortis kenyensis* and *Tetrapocillon minor*. Specimens of the selected sponge species were sampled randomly by scuba-divers at 10 m depth. Replicates (3-5 individuals) of each sponge species were collected at least 20 m apart to avoid collection of clones and transferred into zip block bags filled with seawater. Samples were immediately transferred to the laboratory facilities at the Institute of Marine Sciences (IMS, Stonetown). After gently removing water, sponge pieces were weighed to the nearest 0.1 g to determine wet weight (WW), cut into small pieces and extracted three times with 99.9% Ethanol. The extracts were filtered to remove particles and the filtrate was kept at -20°C for storage and transport. Samples were filtered again and evaporated under reduced pressure using a rotary evaporator (38°C) at the laboratories of the ICBM, University of Oldenburg. The crude extracts were transferred into pre-weighted glass vials and evaporated to complete dryness with a Speed Vac. Natural extracts concentration were calculated in mg per g of WW per sponge (see Table 1). Pooled extracts of the replicate sponge individuals were used for the experiments. All extracts were stored at -20°C until use.

**Table 1:** The most abundant sponge species at Bawe Island, Zanzibar, and their natural tissue extract concentration. Extracts yields in mg extract per g WW are given as the mean of 3- 5 extractions (± STD).

| **Order** | **Species** | **Number of Replicates** | **Natural Concentration [mg/g WW]** | **Benthic cover at 10m depth[%]** |
|---|---|---|---|---|
| Haplosclerida | *Haliclona fascigera* | 5 | 13.00 (± 6.38) | 0.17 (±0.90) |
| Haplosclerida | *Haliclona atra* | 3 | 26.51 (± 4.53) | 2.33 (±4.70) |
| Haplosclerida | *Callyspongia aerizusa* | 5 | 19.35 (± 10.14) | 0.37 (±1.32) |
| Haplosclerida | *Callyspongia* sp. | 3 | 22.90 (± 2.18) | 0.13 (±0.48) |
| Verongida | *Pseudoceratina* sp. | 3 | 31.06 (± 18.63) | 0.00 (±0.00) |
| Homosclerophorida | *Plakortis kenyensis* | 3 | 16.80 (± 13.25) | 0.01 (±0.03) |
| Scopalinida | *Scopalina hapalia* | 3 | 17.14 (± 5.86) | 0.04 (±0.14) |
| Poecilosclerida | *Biemna* sp. | 3 | 18.29 (± 2.84) | 2.88 (±4.20) |
| Tetractinellida | *Paratetilla* sp. | 3 | 26.31 (± 3.66) | 0.01 (±0.04) |
| Poecilosclerida | *Tetrapocillon minor* | 4 | 18.39 (± 7.37) | 0.11 (±0.24) |

### Bacterial Panel

The agar disc-diffusion assay was used to test for antibacterial activities of the sponge crude extracts. 36 bacterial strains over a wide phylogenetic range were tested including nine known pathogens for marine diseases (*Aurantimonas coralicida, Acitenobacter baylyi, Vibrio* sp., *Vibrio coralliilyticus, Rhodococcus corynebacterioides, Pantoea eucrina, Ruegeria atlantica, Vibrio shilonii* and one bacteria of the family *Alteromonadaceae*). The bacterial strains were formerly isolated from the crustose coralline alga *Hydrolithon reinboldii* and from two sponges (*Rhabdastrella globostellata* and *Pseudoceratina* sp.) from Guam, except for three marine pathogens (*A. coralicida*, *V. shilonii*, *V. coralliilyticus*) that were ordered from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DMSZ, Braunschweig, Germany.

### Antimicrobial assays

Bacterial strains were grown in liquid marine broth medium for 24 hours at 25°C before each experiment. Crude extracts of the different sponge species were dissolved in aliquots of ethanol at natural concentrations (see Table 1). 15 µl of crude extract were added onto a sterile paper disc (Ø 6 mm, Whatman) and the solvent was allowed to evaporate completely. Control discs were prepared in the same manner with 15 µl of solvent only. Marine broth agar plates were inoculated with 200 µl of liquid culture of the respective test strains and spread evenly to provide an uniform bacteria lawn. Up to seven extract discs and one solvent control disc per plate were placed on the surface of agar plates with the solvent side facing the plate. Three to six replicates of each sponge species were tested, depending on the abundance of the sponges in the field during collection. Following a 24 h and 48 h incubation period, growth inhibition zones were scored as clear halos around the discs. The radius of the inhibition zone (without disc) was measured to the nearest 0.5 mm. Solvent control discs never inhibited bacterial growth. Permutational multivariate analysis of variance (PERMANOVA) was conducted with the Primer software (Version 6.1.13) and the PERMANOVA+ add-on (Version 1.03). The analysis was used to test for significant activities of sponge extracts (Anderson 2001; McArdle and Anderson 2001). Results were categorized as no effect (0), weak inhibition (0–1 mm), moderate inhibition (>1 to 3 mm), strong inhibition (>3 to 7 mm) and very strong inhibition (>7 to 15 mm) after Lippert et al. (2003).

### Brine shrimp lethality assay

Brine shrimp (*Artemia salina*) eggs were placed in a hatching tank containing seawater with strong aeration under continuous light at 24°C for 12 hours. Photophilic nauplii were collected approximately 12 hours after hatching.

A stock solution of 10 mg* $ml^{-1}$ was prepared for each pooled extract of the ten sponge species. From the stock solution 1000, 100 and 10 µl were transferred to different petri dishes and allowed to evaporate. The respective amount of ethanol, served as control. After the solvents had evaporated, 5 ml filtered (0.45 µm) and autoclaved seawater was added to each petri dish. The petri dishes were placed on a shaker for 30 minutes to ensure that the extracts dissolved in the seawater. Subsequently, 10 brine shrimps were added to each petri dish and the total volume got adjusted to 10 ml resulting in a final extract concentration of 1 mg, 0.1 mg and 0.01 mg sponge crude extract per 10 ml seawater. Larvae were not feed during the experiments, as they still feed on their yolk-sac (Pelka et al. 2000) and can survive for up to 48 hours without food (Lewis 1995). Toxicity was determined after 48 hours (instar III/IV stage) of exposure by counting the surviving nauplii. Extracts and controls were prepared in triplicates. Larvae were considered dead if no internal or external movement could be observed. Paired t-test or Mann-Whitney U tests were conducted to test for significant differences in mortality rates of brine shrimp between the individual sponge extracts and the control. A Bonferroni correction was applied in order to deal with multiple testing.

## RESULTS

### Antimicrobial Assay

The antimicrobial activities of the crude extracts of the ten Zanzibarian sponges against 36 bacterial strains are presented in Table 2. All tested sponge extracts showed antimicrobial activity. Antimicrobial effects of the extracts varied widely with respect to the bacterial test strains. The extract of *Pseudoceratina* sp. had the strongest antimicrobial activity in terms of the length of the inhibition zones as well as the number of bacteria inhibited (inhibited all strains). *Callyspongia* sp. and *H. atra* extracts revealed the second and third most antibacterial activity (69% and 47% of all strains inhibited, respectively; Tab. 2). In contrast, *Paratetilla* sp. and *T. minor* were the least chemically defended species inhibiting only 11% of the bacterial strains and also showing the smallest inhibition zones. The other five sponge species displayed all moderate antibacterial activity with 22-33% of the bacterial strains inhibited (Tab. 2). When comparing the activity of the different sponge extracts against environmental versus pathogenic bacterial strains, most sponge species were better defended against potential pathogens (PERMANOVA, $p< 0.05$). All tested sponge species, except *T. minor* and *Pseudoceratina* sp. showed stronger antimicrobial activity against pathogenic bacterial strains with respect to the size of the inhibition zones (Fig. 1) as well as the number of bacterial strains inhibited (Tab. 2).

**Table 2:** Degree of antimicrobial activity by crude extracts of ten sponge species. Radius of inhibition zone: 0 no effect; > 0–1 mm: weak inhibition; >1–3 mm: moderate inhibition; >3–7 mm: strong inhibition; >7–15 mm: very strong inhibition (after Lippert et al. 2003).

| Sponge species | Weak | Moderate | Strong | Very strong | ∑ strains inhibited (% active) |
|---|---|---|---|---|---|
| *T. minor* | 0 | 4 | 0 | 0 | 4 (11%) |
| *Paratetilla* sp. | 0 | 4 | 0 | 0 | 4 (11%) |
| *H. fascigera* | 1 | 7 | 0 | 0 | 8 (22%) |
| *P. kenyensis* | 1 | 8 | 0 | 0 | 9 (25%) |
| *S. hapalia* | 1 | 6 | 2 | 0 | 9 (25%) |
| *C. aerizusa* | 2 | 8 | 1 | 0 | 11 (31%) |
| *Biemna* sp. | 3 | 9 | 0 | 0 | 12 (33%) |
| *H. atra* | 8 | 7 | 2 | 0 | 17 (47%) |
| *Callyspongia* sp. | 2 | 18 | 5 | 0 | 25 (69%) |
| *Pseudoceratina* sp. | 2 | 6 | 19 | 9 | 36 (100%) |

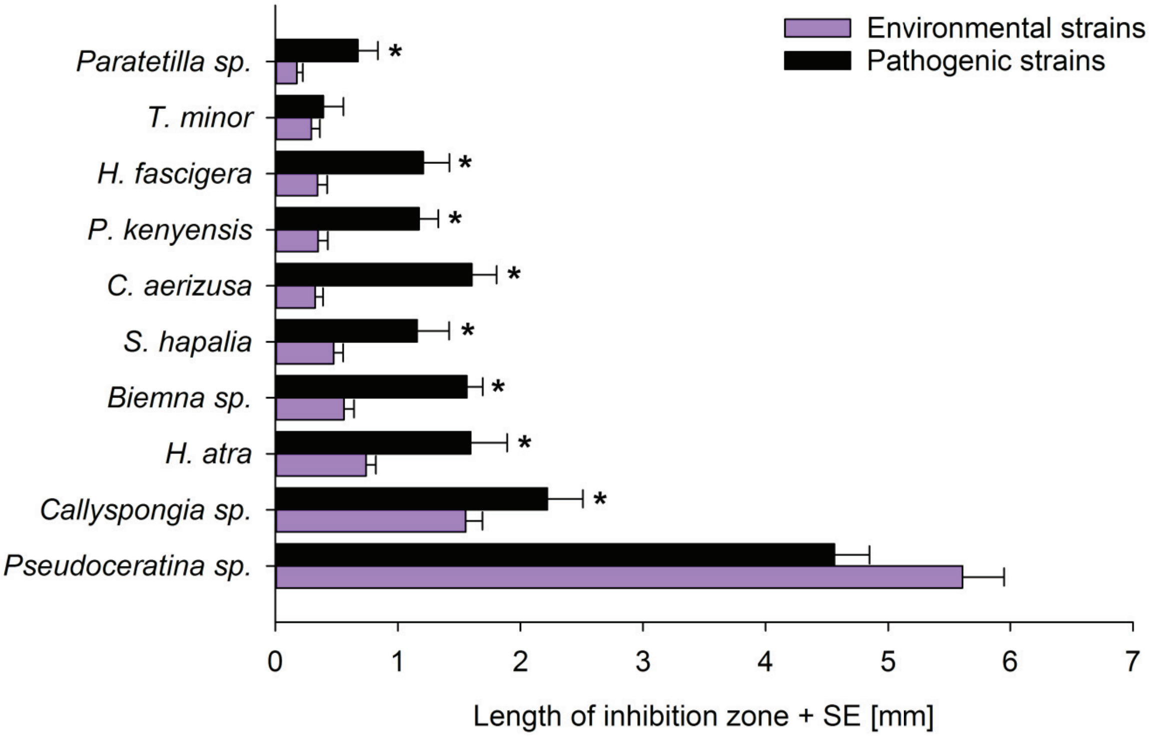

**Figure 2:** Comparison of the length of the inhibition zones (mean radius, mm + SE) for environmental and pathogenic bacterial strains for each sponge crude extract. * indicates a significant difference between the inhibition of environmental vs. pathogenic bacterial strains (PERMANOVA, $p< 0.05$).

## Brine shrimp Assay

The results of the brine shrimp bioassay showed that five out of the ten sponge species were cytotoxic (Fig. 3). Mortality of *Artemia* larvae is shown after a 48 hours exposure to the various sponge extracts. This time was chosen since most extracts displayed an increasing activity up to 48 hours of exposure (Carballo et al. 2002). In addition, *Artemia* nauplii have been shown to exhibit their greatest sensitivity to sponge compounds in the second and third instar larval phase (Sánzchez-Fortún et al. 1996; Lewis 1995).

The highest lethality was found for extracts of *Callyspongia* sp. at both concentrations (Mann-Whitney U test; T=57, n=6, p=0.002), which exhibited *Artemia* mortality rates of 100% after just 12 hours (data not shown). *S. hapalia* revealed the highest mortality with 95% at a concentration of 1000 μg $ml^{-1}$ (Mann-Whitney U test; T=57, n=6, p=0.002), but only a non-significant mortality rate of 5% at a concentration of 100 μg $ml^{-1}$. *H. fascigera* caused moderate mortality rates varying between 40- 45% at both 1000 µg ml$^{-1}$ and 100 µg ml$^{-1}$ of extract concentration (Mann-Whitney U test; T=57, n=6, p=0.002 and paired t-test, t=5.245, df=5, p=0.0017). *Pseudoceratina* sp. showed moderate lethality rates between 24-26% at both concentrations (Mann-Whitney U test; T=57, n=6, p=0.002 and paired t-test, t=4.088, df=5, p=0.0047). *C. aerizusa* exhibited a significant mortality rate of 21% for brine shrimp larvae at a concentration of 1000 µg ml$^{-1}$ (Mann-Whitney U test; T=57, n=6, p=0.002). No significant mortality rates were obtained from extracts of *H. atra*, *Biemna* sp., *Paratetilla* sp., *P. kenyensis*, *T. minor* and for *C. aerizusa* at a concentration of 100 µg ml$^{-1}$. No mortality of *Artemia* larvae could be detected in controls.

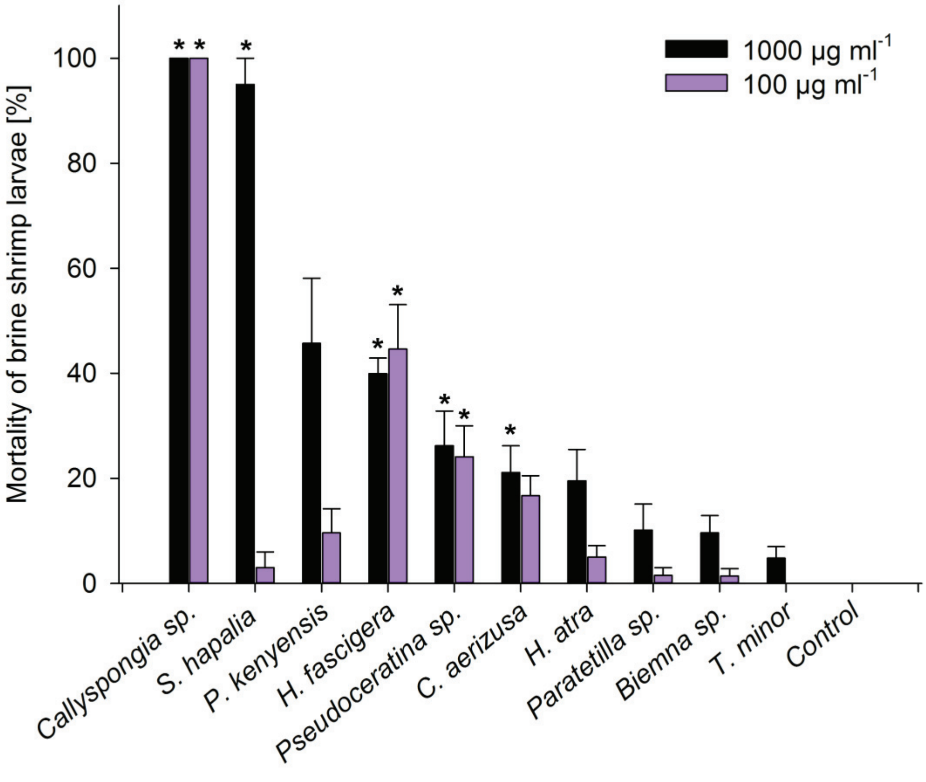

**Figure 3**: Mortality rates (+ SE) of the brine shrimp larvae in response to exposure to the different sponge crude extracts at 1000 µg ml$^{-1}$ and 100 µg*ml$^{-1}$ after 48 h.* indicates a significant mortality rate compared to control (paired t-test or Mann-Whitney U test, $p<0.05$).

## DISCUSSION

The results show that highly potent antimicrobial as well as cytotoxic activities could be detected in extracts of the most abundant sponges of Zanzibar.

### Antimicrobial activity

All investigated sponge species exhibited antimicrobial activity with varying degrees in the number of inhibited test strains (11% up to 100% of strains inhibited). These results are in accordance with other studies examining the antimicrobial activity of sponges in which all sponge species from the Indian Ocean (Ely et al. 2004; Dobretsov et al. 2005) and Antarctica (Lippert et al. 2003) displayed antimicrobial activity. The strong activity against the variety of bacterial strains exhibited by *Pseudoceratina* sp. in this study indicates that this sponge species produces broad-spectrum antimicrobial compounds. Sponges of the genus *Pseudoceratina* have been shown to contain bromotyrosine alkaloids and sterols of the aplystane type which possess cytotoxic (Tsukamoto et al. 1996; Buchanan et al. 2008), antimicrobial (Fusetani 2008; Takada et al. 2001) as well as anti-HIV (Ross et al. 2000) and antimalarial (Lebouvier et al. 2009) activities. Purealin C and its derivatives, which have been isolated from another *Pseudoceratina* species, are known to exhibit broadspectrum antimicrobial activities as well (Salim et al. 2012). The two *Callyspongia* sponges displayed strong to moderate antimicrobial activity. A variety of secondary metabolites with different biological activities have been reported from *Callyspongia* species, with Siphondiol (López et al. 2005), Akaterpin (Fukami et al. 1997) and Utenine (Yamada et al. 1997) displaying antimicrobial properties. The third most active sponge genus was *Haliclona*, with *H. atra* and *H. fascigera* inhibiting 17 (47%) and eight (22%) of the tested bacterial strains, respectively. *Haliclona* spp. produce Manzamine, alkaloids with antitumor, antimicrobial and antimalarial activities (Sakai et al. 1986; Kobayashi et al. 1995), as well as the antifungal and antimicrobial compounds Papuamine, Haliclonadiamide and Halaminoles (Baker et al. 1988; Clark et al. 2001). Additionally, extracts from several Indo-Pacific *Haliclona* species have been shown to inhibit various bacterial strains, emphasizing the overall antimicrobial activity of this genus (Crews and Harrison 2000; Ely et al. 2004; Thompson et al. 1985). However, there are also some *Haliclona* species which possess weak or no antimicrobial activity (Bergquist and

Bedfort 1978; Rifai et al. 2005; Lippert et al. 2003). The variability in sponge secondary metabolites of the same genus or even of the same species is not uncommon as their production is influenced by genetic traits as well as environmental factors and the sponge-associated microbes (Uriz et al. 1995; Edrada et al. 2000; Lippert et al. 2003; Kim et al. 2005; Rohde et al. 2012; Turon et al. 2009). *Biemna* sp. displayed a moderate antimicrobial activity, inhibiting 12 (33%) of the tested bacterial strains. Sponges of the genus *Biemna* have been reported to be a great source for bioactive compounds with antimicrobial as well as cytotoxic activities, which include pyridoacridines (e.g. Labuanine), steroids (e.g. Ehrenasterol and Biemnasterol) as well as polycyclic alkaloids (e.g. Biemnadin, Hydroxyascididemin and Netamines; Govinden-Soulange et al. 2014; Youssef et al. 2015). The sponges *P. kenyensis* and *S. hapalia* were active against nine (25%) of the examined bacterial strains. No reports of secondary metabolites of *S. hapalia* were found in the literature. The most prominent antimicrobial compounds isolated from plakinid sponges are Plakinidones, Plakortide, Manzamenone and Plakortin, which showed activity against several bacteria including *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* (Higgs and Faulkner 1978; Kubota et al. 2013; Kushlan and Faulkner 1991; Mohammed et al. 2010). No bioactive properties have been reported for the sponges *Paratetilla* sp. and *T. minor*.

All sponges, except for *Pseudoceratina* sp. and *T. minor*, were significantly more active against known coral pathogens than against bacteria encountered in their environment. Newbold et al. (1999) reported also weaker inhibition rates for seawater bacteria compared to bacteria inhabiting necrotic sponge tissue or known pathogens. These results suggest that the secondary metabolites in most sponges are highly selective. This selectivity may allow the sponge host to form close associations with beneficial microbes while inhibiting potential pathogens from growing or settling on the surface of the sponge (Kelman et al. 2001; Kelly et al. 2005; Rohde et al. 2012; Rohde et al. 2015). Other than the use of antimicrobial compounds, sponges possess efficient defence mechanisms that recognize pathogens and initiate an immune response. Sponges are known to distinguish between infectious and non-infectious bacteria through molecular responses, receptor molecules and membrane proteins (Perović-Ottstadt et al. 2004; Wiens et al. 2007; Fu et al. 2013). Bacteria associated with sponges are able to produce molecules which act on the sponge cells by inhibiting its immune as well as apoptotic system (Thomas et al. 2010; Siegl et al. 2011; Gardères et al. 2012, 2014).

Growth inhibition, which was tested in the present study, is only one of the three stages in the colonization process by bacteria or potential pathogens (attachment, growth and swarming). Secondary metabolites of sponges might interfere with each of the 3 different colonization stages (Kelly et al. 2003; Kelly et al. 2005). For example, bacteria isolated from sponges showed an enhanced attachment to agar surfaces treated with extracts of their sponge hosts, but were inhibited in their growth, which represents a possible mechanism to control the growth of the microbial population within the sponge host. In Caribbean sponge species, attachment of bacteria onto the sponge surface was the most, and growth the least inhibited characteristic (Kelly et al. 2005). Moreover, there are bacteria, such as *Endozoicomonas* sp., which need host molecules in order to be able to grow (Gardères et al. 2015).

Therefore, it would be interesting to test all colonization stages with the extracts of sponges from Zanzibar since *T. minor* was a sponge that inhibited the growth of only 11% of the tested strains but had always a very clean and smooth surface (personal observation). Several studies have shown that there is often no correlation between the antimicrobial activity of the sponge extract and the epibacterial abundance in sponges or ascidians (Beccero et al. 1994; Wahl et al. 1994). Instead of producing antimicrobial compounds, sponges were found to also attract bacteria to their surfaces that repel other biofilm-forming bacteria and thus maintain a clean and smooth surface (Krug 2006).

*Pseudoceratina* sp. was the only sponge in this survey which showed microbial activities against potential sponge disease-causing bacteria genera, such as *Alphaproteobacteria* (Angermeier et al. 2012; Webster et al. 2002) and *Pseudomonas* (Cervino et al. 2006; Choudhury et al. 2003). There is still a lack of knowledge about the main cause of sponge diseases and the pathogenic bacteria involved (Angermeier et al. 2012; Luter et al. 2010) which is why we used pathogenic bacteria, that are known to cause various diseases in corals, in the present study. However, cyanobacteria, which were associated with coral diseases were as well found to replace the *Synechococcus/ Prochlorococcus* clades in sponges affected by the sponge orange band disease (Angermeier et al. 2011). One bacterium discovered in sponge lesions affected by *Aplysina* black patch syndrome had a 99% similarity to a coral black band disease strain and the same bacterial strains dominated the tissue of diseased sponges and corals (Webster et al. 2008). Previous studies showed that the disruption of the holobiont symbiosis and the subsequent shift in sponge microbiota caused by colonization of opportunistic scavengers or disease causing bacteria is the major cause of sponge diseases (Choudhury et al. 2003; Gao et al. 2015; Olson et al. 2013). Environmental perturbations including urban runoff, nutrient enrichment, anthropogenic pollution and especially increase in temperature are linked to disease outbreaks in marine invertebrates (Lesser et al. 2007; Di Camillo et al. 2012). The brown lesion disease in sponges is most likely caused by terrestrial pathogens used in pest control management of insects and fungi (Cervino et al. 2006). The high prevalence of antimicrobial defences in the investigated sponges suggests that pathogens are a common threat to sponges on Zanzibar's reefs. On Zanzibar, the sponges on the reefs surrounding Stonetown are especially exposed to untreated sewage and runoff from the main city, which could represent a potential source for the introduction of a variety of bacteria and potential pathogens (Moynihan et al. 2012; Limbu and Kyewalyanga et al. 2015). Human fecal bacteria in wastewater are assumed to be the etiological agent of white pox and black band disease in corals (Frias-Lopez et al. 2002, Patterson et al. 2002). Even if wastewater does not harbour etiological agents for diseases of benthic invertebrates, it has been demonstrated that sewage introduces many opportunistic microbial taxa, such as e.g. *Vibrionaceae* and *Rhodobacteraceae*, that can alter the microbial community in coral as well as sponge holobionts resulting in disease onset (Rosenberg and Ben-Haim 2002; Piskorska et al. 2007; Vega Thurber et al. 2009; Stabili et al. 2012; Sweet et al. 2015; Ziegler et al. 2015). Nutrient enrichment, especially increase in nitrogen, has been demonstrated to lead to a faster progression of diseases in corals, since pathogens are normally nitrogen limited (Kim and Harvell 2002; Bruno et al. 2003; Voss and Richardson 2006; Redding et al. 2013). The reef around Bawe Island was reported to have higher phosphate and nitrate concentrations compared to other reefs around Zanzibar, which could help to increase pathogen fitness and virulence (Limbu and Kyewalyanga 2015). Around Bawe only few cases of sponge diseases have been reported, indicating that sponge antimicrobial defences seem to be efficiently in defending sponges from pathogens. The high number of antimicrobial activities in the ethanolic extracts of all investigated sponges could be a response to the high prevalence of potential pathogens.

Even though strong bioactivities could be detected in the ethanolic extracts of the investigated sponge species, it is very likely that those activities might be underestimated since previous studies found bioactivities mostly in lipophilic compounds as these wont dilute easily when exuded into the surrounding seawater (Touati et al. 2007; Sotka et al. 2009;

Peters et al. 2010; Blunt et al. 2014; Angulo-Preckler et al. 2015).

### Cytotoxicity

The brine shrimp lethality assay is considered to be an easy, rapid and inexpensive bioassay to test for general toxicity of organisms. Brine shrimp have been recognized of being the most sensitive organisms in regard to sponge crude extracts (Richelle-Maurer et al. 2002). There is a good correlation of the toxicity in the brine shrimp assay and the potential anti-tumor activities of extracts (Anderson et al. 1991; Carballo et al. 2002; Soapi et al. 2013).

In the present study, five (50%) of the tested sponge species showed cytotoxicity in the brine shrimp assay. Other studies also found that 59-75% of sponges species showed cytotoxic activities in brine shrimp or other cytotoxicity assays (Richelle-Maurer et al. 2002; Uriz et al. 1992; Soapi et al. 2013). The slightly lower activity of sponges in this study could be due to the fact that only ethanolic extracts were investigated which most likely extract the more lipophilic compounds only to a certain degree. Extracts from all four sponges within the order Haplosclerida displayed significant cytotoxic activities in the brine shrimp assay with *Callyspongia sp.* showing the highest cytotoxic activity. The families Callyspongiidae and Chalinidae harbour alkylpiperidine alkaloids, specific chemotaxic markers, which are likely responsible for the observed activities (Fusetani 2008). Cytotoxicity against brine shrimp has also been reported for some *Haliclona* species with $LD_{50}$ values of 65 $\mu g\ ml^{-1}$ (Teruya et al. 2006) and 126 $\mu g\ ml^{-1}$ (Mansoor et al. 2007; Park et al. 2009), which is comparable to the present study. The sponge species with the second highest activity in the brine shrimp assay was *S. hapalia*; however there is no information on secondary metabolites for this species. Sponges of the genus *Plakortis* are known for their cyclic polyketides peroxides which are of great pharmacological interest due to their variety of bioactivities, such as antiparasitic, antineuroinflammatory and cytotoxic activities (Rudi et al. 2003; Kossuga et al. 2008; Feng et al. 2010). However, no significant cytotoxic activities could be found in extracts of *P. kenyensis*. A related species *P. lita* displayed brine shrimp mortality rates of 20-50% at a concentration of 100 $\mu g\ ml^{-1}$ (de Voogd 2005). Other *Plakortis* species displayed $LD_{50}$ values ranging from 15-91.9 $\mu g\ ml^{-1}$ (Sata et al. 2005; Epifanio et al. 2005). The lack of cytotoxic activity in *S. hapalia* extracts at a concentration of 100 $\mu g\ ml^{-1}$ and in *P. kenyensis* at both concentrations was likely due to problems in dissolving these extracts in ethanol. The moderately cytotoxic activity of *Pseudoceratina* sp. was most likely ascribed to bioactive bromotyrosine derivatives in the crude extract (de Voogd 2005; Lira et al. 2011; Su et al. 2013; Shaala et al. 2015). *Biemna* sp. did not demonstrate any bioactivity contrary to previous studies (de Voogd 2005; Sapar et al. 2014). This result is somewhat surprising since the most prominent natural products isolated from sponges of the genus *Biemna* proved to be cytotoxic to human carcinoma cells (Zeng et al. 1993; Sorek et al. 2006; Aoki et al. 2003).

Compounds responsible for sponge toxicity are able to rapidly kill cells of competitors through apoptosis, autophagocytosis and necrosis (Aerts 1999; Folmer et al. 2009; Singh and Thakur 2016), thereby inhibiting the growth of other space competing organisms. It has been demonstrated that allelochemicals with cytotoxic properties have the ability of inhibiting metabolic processes in various coral species as well as killing live coral tissue within a few days (Porter and Targett 1988; Chaves-Fonnegra et al. 2008). Other functions of cytotoxic compounds include the inhibition of photosynthesis in the corals symbiotic zooxanthellae (Pawlik et al. 2007). Furthermore, production of cytotoxic metabolites varies depending on the presence or absence of other competitive organisms (De Caralt et al. 2013). Additionally, compounds that were previously identified in bioassays as having antimicrobial activities, could also possess allelopathic properties, such as Neurymenolide A which plays a role in coral-algae interactions (Andras et al. 2012). The anti-microbial activities of this compound was assumed to alter the microbiome of the coral mucus by inhibiting protective bacteria and promoting the colonization of pathogenic bacteria (Ritchie 2006; Nugues et al. 2004).

The ten sponge species tested in the present study for antimicrobial and cytotoxic activities were also tested for their feeding deterrent properties (Helber et al. 2016a). No relationship could be found between the deterrence of sponge extracts and their antimicrobial activity or toxicity. The same results were reported from sponge species in the Caribbean (Pawlik et al. 1995). However, it has been discussed that secondary metabolites in sponges serve multiple defensive roles since they have high biosynthesis costs (Pawlik et al. 1993). Compounds that serve multiple ecological functions were described from sponges worldwide such as in the Mediterranean, Caribbean, Guam, Indonesia and Oman (Uriz et al. 1992; Kubanek et al. 2002; Richelle-Maurer et al. 2002; Rohde et al. 2015). For instance Halitoxin was found in four sponges of the genus *Haliclona* and was reported to have antimicrobial and cytotoxic properties, to be lethal to mice as well as ichthyotoxic (Schmitz et al. 1978; Kelman et al. 2001). Furthermore, Ceratiphenol A, isolated from the Red Sea sponge *Pseudoceratina arabica*, displayed antimicrobial as well as antifungal and anti-migratory activities against highly metastatic cancer cells (Badr et al. 2008; Shaala et al. 2015). The overall fitness of an organism can be increased by optimizing the efficiency of chemical defences (Kubanek et al. 2002). If one compound has multifunctional roles in the defence against different organisms, then the energetic costs which are saved by utilizing only this compound instead of producing several different ones, can be devoted to other processes, such as reproduction and growth (Bell et al. 2013).

In conclusion we could demonstrate that sponges from Zanzibar possess strong growth inhibitory activities against tropical marine bacteria, in particular against potentially pathogenic bacteria. The remarkable antimicrobial activities could represent an adaptation to the high prevalence of bacteria caused by sewage outflow from Stonetown. Moreover, the medium to strong activities in the brine shrimp mortality assay might indicate that sponges are important space competitors on the reef. From our results, *Pseudoceratina* sp., *Callyspongia* sp., *S. hapalia* and *H. atra* are the species with the strongest biological activities. *H. atra* was the most abundant sponge species while *Pseudoceratina* sp. exhibited patchy but high abundances on the reefs around Bawe Island (Helber et al. 2016a; personal observation). Therefore it would be interesting to investigate if the cytotoxic and antimicrobial properties of these sponges provide them with a competitive advantage over corals and thus explain partly their high abundances on the reef.

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# CHAPTER 4

# Allelopathic activities of sponges from Zanzibar

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**Helber S.B.**, Hoeijmakers D.J.J., Muhando C.A., Richter C., Rohde S.' Schupp P.J. Allelopathic activities of sponges from Zanzibar. This chapter is in preparation for submission.

## ABSTRACT

Reefs worldwide have experienced dramatic losses in coral cover over the last decades along with a replacement of corals with other space-competing organisms. Sponges have become the dominant taxon on many Caribbean reefs and their abundance and biomass is reported to rise also on reefs in other regions. Sponges are assumed to be superior competitors for space due to their use of allelochemicals, but conclusive evidence for this 'chemical warefare' is so far lacking for most regions, including the Indian Ocean. Allelopathic effects between the three most abundant sponge species (*Pseudoceratina* sp., *Callyspongia* sp. and *H. atra*) and the main frame-building hard coral *Porites* sp. were assessed in the field on coral reefs along Zanzibar's West Coast. The photosynthetic performance of the *Porites* sp. was assessed by pulse amplitude modulated (PAM) fluorometry. Therefore tissue extracts of the three sponges were incorporated in phytagel strips touching the coral surface. PAM fluorometry revealed a reduction in the dark-adapted maximum quantum yield of photosystem II (PSII), whereas no significant effects on the photosynthetic performance of the coral could be detected in experiments with live sponge fragments. This is the first report demonstrating sponge allelopathic effects on the photo physiology of coral symbionts, suggesting that allelopathic substances may provide a potential feed-back loop for the encroachment of sponges on hard corals.

## INTRODUCTION

One of the main factors shaping the community composition of sessile, benthic assemblages is the competition for space (Dayton 1971). Especially on coral reefs, free space with adequate irradiance for photosynthesis, exposure to food and currents is one of the most limiting resources for sessile benthic organisms (Jackson and Buss 1975; Birrell et al. 2008). The high biodiversity on coral reefs results in high frequency of competitive interactions between sessile organisms of the same and of different species (Van Veghel et al. 1996; Knowlton and Jackson 2008). Anthropogenic impacts such as global climate change, ocean acidification, eutrophication, sedimentation and overfishing as well as disease outbreaks have led to a decline in reef-building corals worldwide and to an increase in the abundance of other spatial competitors such as macroalgae, corallimorpharians and sponges (Aronson and Precht 2001; Hoegh-Guldberg et al. 2007; Hughes et al. 2007, 2010; Norström et al. 2009). The above stressors have led to severe impairments of the health status of corals, as energy is needed for various maintenance functions (e.g. mucus production for sediment removal) and is therefore not available for growth or production of secondary metabolites to successfully compete for the limited space (Rinkevich and Loya 1985; Foster et al. 2008; Singh and Thakur 2016). Prolonged exposure to the above stressors often results in coral mortality, creating free reef substratum for the colonization by other sessile benthic organisms. Overfishing, and the persistent removal of herbivores and/or spongivores, has released macroalgae and sponges from predation pressure on many reefs. Additionally, eutrophication has resulted in enhanced growth rates of organisms such as algae, corallimorpharians, ascidians and sponges (Chadwick-Furman and Spiegel 2000; Hughes et al. 2007; Shenkar et al. 2008; Chadwick and Morrow 2011). As a result, organisms other than hard corals can undergo uncontrolled growth and out-compete the dominant coral species (Loh and Pawlik 2009; Slattery and Lesser 2014; Hughes et al. 1987). Mechanisms of such interactions and the role of secondary metabolites in spatial competition have been described extensively for coral-coral and coral-macroalgae interactions (Benayahu and Loya 1981; Chornesky 1983; Baird and Hughes 2000; Chadwick and Morrow 2011; Andras et al. 2012; Bonaldo and Hay 2014). However, there is a lack of studies investigating coral-sponge interactions in an ecologically meaningful way, even though sponges replaced corals as the dominant benthic taxon in terms of abundance and biomass on many Caribbean and some East African and Indonesian reefs (Barnes 1999; Diaz and Rützler 2001; Barnes and Bell 2002; Bell and Smith 2004; Loh et al. 2015).

Sponges are assumed to be competitively superior over other reef organisms not only due to their ability of rapidly overgrowing competitors but also because of the release of chemical compounds (Porter and Targett 1988; Pawlik et al. 2007; Engel and Pawlik 2000). Bioactive compounds are released through tissue contact, via sponge mucus or directly into the surrounding water causing bleaching and tissue necrosis in neighbouring corals thereby reducing their chances of survival (Porter and Targett 1988; Pawlik et al. 2007). Allelochemicals that are exuded into the surrounding water are likely to get diluted very fast and will only have an effect at very short distances (Kubanek et al. 2002). In contrast, cytotoxic chemicals that are incorporated in mucus may be able to remain in higher concentrations close to the sponge since they are not easily diluted by water flow (Jackson and Buss 1975; Sullivan et al. 1983). Several sponges are known to use allelopathy in order to gain a competitive advantage over other sessile benthic organisms. For example, extracts of the sponge *Plakortis halichondroides* coated onto cellulose pads and placed in direct contact with the coral *Agaricia lamarcki* bleached coral tissue within 24 hours (Porter and Targett 1988). The sponge *Crambe crambe* from the Mediterranean Sea also employs allelochemicals in order to induce tissue necrosis in neighbouring organisms (Turon et al. 1996). Other studies demonstrated as well that sponge extracts incorporated into gels inhibited the growth of other benthic organisms or were even able to cause bleaching and tissue necrosis in neighbouring corals (Engel and Pawlik 2000; de Voogd et al. 2004; Pawlik et al. 2007). The competitive abilities of sponges may be further enhanced with increasing anthropogenic disturbances that are stressful to corals but tolerable for sponges (Chadwick and Morrow 2011). In the Western Indian Ocean (WIO) corals were greatly affected by the 1997/1998 El Ninõ Southern Oscillation (ENSO) resulting in mass coral bleaching and a decline in coral cover (McClanahan et al. 2007, 2014; Obura 2005). This decline of coral species has led to a shift of the ecological balance in favour of other space-competing functional groups, especially corallimorpharians but also sponges (Norström et al. 2009; Muhando et al. 2002; Kuguru et al. 2004). On the reef around Bawe Island sponge cover increased sevenfold over the last 15 years (Mohammed et al. 1999; Muhando et al. 2002; Muthiga et al. 2008; Lokrantz et al. 2010; Muzucka et al. 2010; Helber et al. 2016a).

The aim of this study was to determine if the three most common sponges observed at Bawe Island, Zanzibar had allelopathic effects on co-occurring corals. Pulse amplitude modulated (PAM) fluorometry was used to detect possible effects of sponges on the photosynthetic efficiency of the coral symbionts (zooxanthellae). This non-invasive *in situ* technique is seen as a useful proxy for intra- and interspecific comparisons of coral health, especially if coral tissues from the same coral individuals are investigated (Beer et al. 1998; Ralph et al. 2002). From our results in previous studies (Helber et al. 2016a, 2016b) the sponges *Pseudoceratina* sp., *Callyspongia* sp. and *Haliclona atra* were chosen for the field experiments on the basis of their known bioactivity and abundance. The bioactivity, especially the cytotoxicity, of sponge extracts seems to be a good proxy for their ability to overgrow corals in the field (Aerts 1999). The four most bioactive sponges in the Spermonde Archipelago were also reported to cause necrosis of corals in more than 85% of interactions observed *in situ* (de Voogd et al. 2004). Therefore, field experiments are necessary to determine the ecological functions of the compounds under natural conditions. Thus, we incorporated sponge extracts into phytagel at natural concentrations and placed them around branches of the coral *Porites*. In addition, live sponge fragments were placed in contact and in non-contact situations (approximately 1 cm away) with massive corals of the genus *Porites*. *Porites* was chosen for the field experiments since the reefs at Bawe Island are dominated by large monostands of branching *Porites* as well as massive *Porites* after the El Ninõ in 1997/ 1998 followed by a Crown-of-Thorn Starfish (COTS) outbreak from 2002/2003-2006 (Bergman and Öhman 2001; Muthiga et al. 2008; Muhando and Mohammed 2002; Muhando and Lanshammar 2008). Furthermore, all three sponge species were observed to grow adjacent or even in between branching *Porites* corals (Appendix 3- Fig.1), making the study of interactions among these two organisms ecologically relevant.

## MATERIAL AND METHODS

### Study site

The field study was conducted in November 2015 at Bawe Island on Zanzibar (Unguja), Tanzania. Bawe (06° 09´25.56″ S, 39° 08´0.96″ E) is located on the Western side of the island in the Zanzibar channel sheltered from direct exposure to the Indian Ocean (Bergman and Öhman 2001). The island is situated 7km away from the capital Stonetown. The reef at Bawe is heavily influenced by fishing activities and untreated sewage discharge from Stonetown and its harbour (Lokrantz et al. 2010; Moynihan et al. 2012).

### Preparation of crude organic extracts for the phytagel experiments

The sponges *Pseudoceratina* sp., *Callyspongia* sp. and *H. atra* were chosen for the field experiments on the basis of their known bioactivity and abundance (Helber et al. 2016a; 2016b). Specimens of the selected sponges were sampled randomly by scuba-divers at 10 m depth in November 2015. Three replicates of each sponge species were collected at least 20 m apart to avoid collection of clones. Samples were immediately transferred to the field laboratory (Institute of Marine Sciences, Stonetown). Sponges were drip dried, weighed, cut into small pieces and repeatedly extracted in 99.9% Ethanol over three consecutive days. The extracts were filtered through coffee filters every 24 hours to remove particles and the filtrate was frozen at -20°C. At the laboratory facilities of the Institute for Chemistry and Biology of the Marine Environment (ICBM) in Wilhelmshaven, the samples were filtered again and evaporated under reduced pressure using a rotary evaporator (35°C). The crude extracts were transferred into pre-weighted glass vials and evaporated to complete dryness with a Speed Vac. The extract yield in mg per g of wet weight (WW) per sponge is referred to as the natural concentration. Pooled samples of the replicate sponge individuals were used for the experiments. All extracts were stored at -20°C until use.

**Preparation and experimental set up of Phytagel strips**

Assays with sponge extracts incorporated into phytagel were adapted from past studies of sponge overgrowth experiments and sponge-coral interactions (Pawlik et al. 2007; Engel and Pawlik 2000). Treatment phytagel strips with natural gravimetric concentrations of sponge extracts and control phytagel strips with only solvent were prepared as described in Engel and Pawlik (2000). Ethanol was used as solvent. The phytagel was poured into a rectangular mold backed with a gaze. After cooling and hardening the phytagel covered gaze was cut into strips with a small square patch of phytagel in the center of each strip (2 cm x 2 cm). Strips were transported in zip block bags on the same day to the reef at Bawe. One control (solvent only) and one treatment strip (with sponge crude extract) were fixed with rubber bands around adjacent coral branches of the same *Porites* colony at depths of 4-6 m (Fig. 1). Eight to 12 colonies that displayed no signs of bleaching were chosen for the experiment and marked with numbered tags. For extracts of *H. atra* and *Pseudoceratina* sp. six treatment and control strips, and for the extract of *Callyspongia* sp. four treatment and control strips were fixed around six or respectively four different coral colonies on the same fringing reef between 13:00 and 15:00 h. Measurements of the photosynthetic efficiency of the zooxanthellae were taken with a diving PAM fluorometer (Walz, Germany) after 16-18 hours, before sunrise of the next day, in order to get the maximum quantum yield of the dark-adapted coral tissue. Under each phytagel patch, 6-8 measurements were taken as well as on a coral branch that had neither treatment nor control phytagel strips attached. PAM readings under each sponge extract and control gel were taken from two adjacent coral branches (Fig. 1). Measurements were also recorded from coral tissue of the same colony that were neither exposed to sponge extract gels nor to control gels, to account for effects of shading, abrasion and the physical presence of the gel. For all readings with the PAM fluorometer, a clip was used that kept the probe at a distance of 1.0 cm from the coral surface. Permutational multivariate analysis of variance (PERMANOVA) was conducted with the Primer software (Version 6.1.13) and the PERMANOVA+ add-on (Version 1.03). The analysis tested for significant differences in maximum quantum yield of coral symbionts exposed to phytagel sponge extracts, phytagel solvent controls and no treatment (Anderson 2001; McArdle and Anderson 2001).

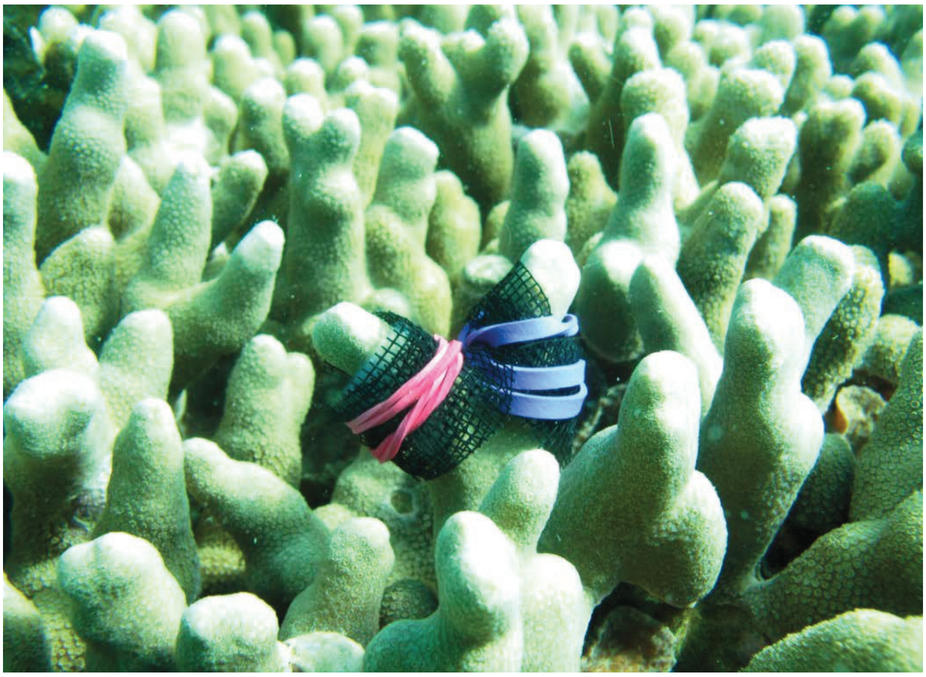

**Figure 1:** Set-up of the phytagel experiments. Sponge extract and control gel were attached to two adjacent coral branches.

### Preparation and experimental set- up of live sponge fragments

Two sponge pieces from ten different individuals of *Callyspongia* sp. and *H. atra* were cut in the reef and attached with cable ties to numbered tiles. Tiles with the sponge fragments on top were fixed on crates mounted in the reef. Sponge fragments were allowed to recover for 7-9 days on the crate before experiments were started. Only sponges that showed no sign of necrosis were used for the experiments. Tiles with two sponge pieces from the same sponge individual on top were placed with the aid of rubber bands at the base of a massive *Porites* colony. One sponge fragment was attached directly in contact with the coral whereas the other fragment was positioned approximately 1 cm next to the base of the coral (Fig. 2). Eight to ten healthy-looking *Porites* colonies that displayed no signs of bleaching were chosen for the experiment. Polyester kitchen sponges (sponge mimics) were attached to tiles and placed also in direct contact or in approximately 1 cm distance to the base of the coral to control for shading and abrasion. Kitchen sponges as well as the tiles were soaked in seawater for three days prior to the experiments to avoid the leakage of any potential harmful substances. Possible effects of the live sponge fragments or the kitchen sponge controls on the *Porites* colonies were assessed after 18-20 hours using PAM fluorometry. Measurements were taken before sunrise of the next day as described above for the phytagel experiments.

**Figure 2:** Set-up of the live sponge fragments. One sponge fragment as well as a sponge mimic was attached directly in contact with the *Porites* coral whereas the other fragment as well as sponge mimic was positioned approximately 1 cm next to the base of the coral.

## RESULTS

### Allelopathic activities of sponge extracts

Extracts of all three investigated sponges, *H. atra*, *Callyspongia* sp. and *Pseudoceratina* sp., showed significant effects on the photosynthetic yield of branching *Porites* corals (Fig. 3; PERMANOVA, p= 0.0001, df= 2). Metabolites produced by *Callyspongia* sp. had the strongest effect on the photosynthetic yield, decreasing the maximum quantum yield by 41% (PERMANOVA, p=0.0039, t=5.6076, df=3). Extracts of *H. atra* and *Pseudoceratina* sp. had comparable negative effects on the effective quantum yield, reducing it by 22% and 19% each (PERMANOVA, p=0.0014, t=5.1455, df=5 for *H. atra* and p=0.0018, t=6.1752, df=5 for *Pseudoceratina* sp.). Sponge metabolites in the gel did not only affect the maximum photosynthetic yield of the corals but caused also bleaching of the underlying coral tissue. Coral tissue exposed to metabolites of *Callyspongia* sp. and *H. atra* extracts showed the most visibly bleaching.

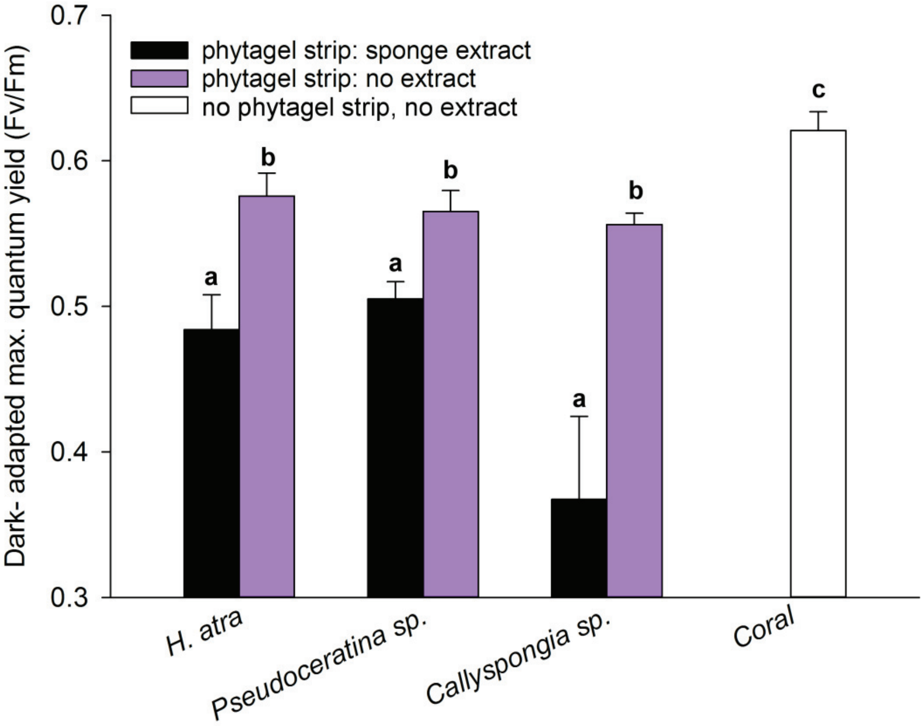

**Fig. 3:** Maximum photosynthetic quantum yield (bars) of symbiotic algae (zooxanthellae) in the coral *Porites* sp. after 16-18-h exposure to phytagel strips containing natural concentrations of secondary metabolites of sponges (mean + SE, n= 6, except *Callyspongia* sp., n= 4). Letters indicate significant differences between treatment, control strips and unexposed coral tissue (Coral).

Control phytagel strips had also significant effects on the maximum photosynthetic yield of corals. Nonetheless, the extracts of the sponges exhibited much stronger impairments on the corals photosynthetic performance and were also significantly different from the control strips.

### Allelopathic activities of sponge fragments

Sponge fragments of *H. atra* and *Callyspongia* sp. had no significant effect on the maximum photosynthetic yield of massive *Porites* corals, in either contact or non-contact situations (Fig. 4). Even though it seems like fragments of *H. atra* exerted a negative impact on the photosynthetic yield, the values were not significantly different from the sponge mimic or the coral control, most likely due to the low number of replicates (n=3), as the PAM fluorometer broke down after initial measurements, limiting the replicates to three instead of the installed ten.

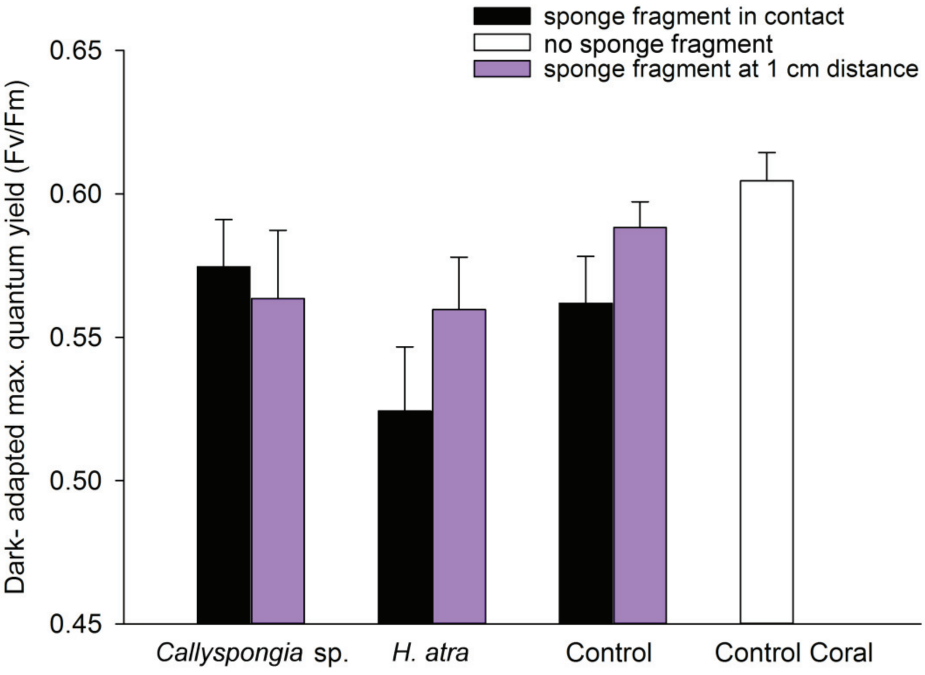

**Fig. 4:** *In situ* effects of sponge fragments and sponge mimics (Control) on the maximal photosynthetic quantum yield (bars) of symbiotic algae (zooxanthellae) in a massive reef coral of the genus *Porites* after 18-20h exposure (mean + SE, n= 7, except *H. atra*, n= 3).

## DISCUSSION

### Allelopathic activities of sponge extracts

Compounds in the extracts of *Pseudoceratina* sp., *Callyspongia* sp. and *H. atra* incorporated into phytagel had negative effects on the maximum photosynthetic quantum yield of the zooxanthella that live within the coral tissue. All three sponge species produced secondary metabolites that caused a decrease of the photosynthetic efficiency by 19-41%. Additionally, *H. atra* as well as *Callyspongia* sp. also caused bleaching in branching *Porites* corals. The observed effects may be considered conservative since the bioactive compounds in our experiment were distributed evenly in the gel in contrast to the situation in natural sponges, where the allelopathic agents had higher concentrations near the surface (Kubanek et al. 2002; Lane et al. 2009; Rasher et al. 2011; Andras et al. 2012). This is in accordance with the optimal defence theory (ODT) in which organisms concentrate defensive compounds in parts that are especially exposed to predation and/or parts that are important for the fitness of an organism, e.g reproductive organs (Rhoades 1979; Becerro et al. 1998; Schupp et al. 1999; Peters et al. 2009; Freeman and Gleason 2010; Rhode and Schupp 2011). Moreover, allelopatic effects of the sponges could be also underestimated since only Ethanol was used for the extraction of secondary metabolites due to constraints in available chemicals in the field. Thus, only polar compounds were extracted while non-polar compounds could not be incorporated into the phytagel strips. There are reports in which sponges or seaweeds exhibited a big difference in their bioactivity of their ethanolic versus their less polar extract (Amade et al. 1987; Cronin et al. 1995) However this varies from species to species and there are also cases in which the activity of the polar and the semi-polar extract did not differ (Safaeian et al. 2009).

### Allelopathic activities of sponge fragments

Live sponge fragments of *Callyspongia* sp. and *H. atra* did not significantly reduce the photosynthetic efficiency of massive *Porites* corals. The inert sponge mimics produced no detectable effects on the photosynthetic performance either. *H. atra* seemed to have more negative effects in contact situations compared to *Callyspongia* sp., even though the differences were not significant. A possible reason could be that *H. atra* secretes mucus. The incorporation of allelochemicals in mucus is one way to avoid their dilution by water currents (Jackson and Buss 1975). The burrowing sponge *Siphonodictyon* sp. secretes mucus down its oscular chimney carrying siphonodictidine, which spreads around its base and inhibits coral growth in a zone of approximately 1-2 cm (Sullivan et al. 1983). Another reason may be associated with the observation of tissue abrasion (green-brown coloured spots on the coral surface after removal of the sponge fragment) which could have caused a stronger leak of allelopathic substances to the coral. Besides, sponges of the genus *Haliclona* are mainly reported to produce cytotoxic secondary metabolites including allelochemicals that are lipophilic and deployed on its surface which would affect corals more during contact interactions (Sakai et al. 1986; Green et al. 2002; Kubanek et al. 2002; Lane et al. 2009; Rasher et al. 2011; Andras et al. 2012; Yamazaki et al. 2013).

The lack of significant results was most likely caused by the lack of proper replication due to the breakdown of the PAM fluorometer.Another reason may be also the insufficient exposure. Long term field experiments with live sponge fragments are needed in order to draw conclusions as whether to coral-sponge interactions are static and sponges use their allelochemicals only to defend their substrate space or if they actively harm corals in order to gain new space. On reefs in Colombia it was observed that sponges only overgrow corals in 2.5% of contact situations and all other interactions were described as standoff interactions (Aerts and van Soest 1997). Furthermore, corals possess also defensive strategies and some competitive interactions between sponges and corals may reverse after longer periods of time, depending on the present external influences (Lang and Chornesky 1990, Chadwick-Furman and Rinkevich 1994, Langmead and Chadwick-Furman 1999b). Corals started to form “domes” as a response to sponge contact in order to avoid further contact but also to try and overtop sponges (López-Victoria et al. 2006; Hoeksema et al. 2014; García-Hernández et al. 2016). Corals also need several weeks to develop defensive organs such as sweeper tentacles and mesenteric filaments (Dai 1990; Lang and Chornesky 1990; Chadwick and Morrow 2011; Cruz et al. 2016). The latter are digestive structures that are discharged to attack neighbouring organisms (Lang and Chornesky 1990; Chadwick and Morrow 2011). Sweeper tentacles are elongated tentacles with a bigger nematocysts battery compared to those of ordinary tentacles (Peach and Hoegh-Guldberg 1999; Chadwick and Morrow 2011;

Cruz et al. 2016). Sweeper tentacles are used as a preventive mechanism to identify and harm encroaching opponents that grow or settle within their reach and one of the few defensive mechanisms of corals that are able to damage corallimorpharians (Langmead and Chadwick-Furman 1999b; Lapid et al. 2004; Lapid and Chadwick 2006). Other than defensive organs corals are also known to deploy allelochemicals in space competition some of which were previously identified as used by sponges as well (Gunthorpe and Cameron 1990a; Rinckevich et al. 1992; Koh and Sweatman 2000).

There is only one long-term study from Colombia (López-Victoria et al. 2006) that observed coral-sponge interactions over 13 months and demonstrated that sponges can be superior over corals dependent on the angle of contact between the competitors and dependent on the coral species encountered. Moreover, fragments of the bioeroding sponge *Cliona orientalis* needed 2-3 months until they invaded the tissue of corals on the Great Barrier Reef (Schönberg and Wilkinson 2001).

### Effects of sponge allelochemicals

Sponge extracts incorporated into phytagel had negative effects on the photosynthetic performance of corals while the negative effects on the corals photosynthetic performance by sponge fragments in contact or non-contact situations were not significant. The results in the present study are similar to closed aquaria experiments in which damaged sponges were used to imitate release of allelopathic compounds in the field. Even though the release of allelopathic compounds was tenfold or hundredfold increased in injured sponges and those exudates were highly concentrated in the closed seawater systems, corals were able to recover after a short time (Aerts 1999). This can be explained by differences in the diffusion rates of allelopathic compounds as neither the duration nor the frequency of allelopathic compound release by sponges is known (Aerts 1999; Pawlik et al. 2007). Other possible reasons could be that allelochemicals are unstable and may lose their activities after certain periods of time, that sponges did not release allelochemicals at all during the time of the experiment or strong water currents diluted exuded metabolites (Aerts 1999; Smith et al. 2006; Pawlik et al. 2007; Chaves-Fonnegra et al. 2008). This could indicate that the major parts of allelopathic agents are lipophilic or at least partly lipophilic and deployed at the surface, such as triterpene glycosides, thus impacting corals only in contact situations (Kubanek et al. 2002; Lane et al. 2009; Rasher et al. 2011; Andras et al. 2012). This makes the defence more efficient as it reduces the loss of compounds to seawater since exuded, hydrophilic compounds would be rapidly diluted whereas lipophilic compounds are retained at sponge-coral contact zones (Kubanek et al. 2002). Furthermore, creation of unfavourable conditions at the sponge surface will also ensure that settling fouling organisms get deterred (Wahl 1989). Additionally, surface deployment of compounds could serve as an anti-predator defence as well (Lindquist and Hay 1995). *Callyspongia* sp. had the strongest, negative effect on the photosynthesis of the coral *Porites*. This sponge species has been demonstrated to lack chemical defences against predation, but possesses strong antimicrobial and in particular cytotoxic activities (Helber et al. 2016a, 2016b). The extracts of *H. atra* and *Pseudoceratina* sp. impaired the photosynthetic performance of *Porites* significantly and both were defended against predators as well as bacteria and showed weak to moderate activities in the brine shrimp lethality assay (Helber et al. 2016a, 2016b). Sponges that are chemically defended against multiple threats (e.g. predators and bacteria) seem to be more likely to have detrimental effects on competitors because produced secondary metabolites serve often multiple ecological roles (Pawlik et al. 2007; Kubanek et al. 2002; Engel and Pawlik 2000). In contrast, sponges lacking chemical defences were shown to have less pronounced effects on the photosynthetic efficiency of corals (Pawlik et al. 1995, 2007; Engel and Pawlik 2000). Assuming biosynthesis of such secondary metabolites is costly (Pawlik 1993), sponges could increase overall fitness by producing metabolites with multiple defensive roles, devoting saved energy to other processes, such as reproduction and growth (Pawlik, 2011; Bell et al. 2013). For example, compounds that have antimicrobial activities may also function as allelopathic agents by altering the coral microbiota, inhibiting the growth of protective bacteria and promoting the colonization by pathogenic bacteria (Nugues et al. 2004; Ritchie 2006; Andras et al. 2012). Furthermore, the growth form of sponges can also have an influence on their toxicity. Massive and encrusting sponges could be more aggressive in spatial competition than branching sponges since the latter are able to avoid competition for new substrate by vertical growth (Aerts 2000; de Voogd et al. 2004). *Pseudoceratina* sp. is a sponge with a thick encrusting growth form while the other two species are branching sponges. However, *Callyspongia* sp. was growing mainly close to the substrate with many attachment points (S. Helber, personal observation). *H. atra* was the most abundant sponge species on the reef at 5m and the second most abundant species at 10m depth (Helber et al. 2016a). This sponge species was often observed to grow between branches of *Porites* corals, thus being in constant contact with the coral. Allelopathic compounds of sponges showed great variability in their toxicity to neighbouring organisms. Sponge individuals of one species were able to cause necrosis to one invertebrate space competitor while not damaging another type of neighbour (de Voogd et al. 2004). Furthermore, some coral species seem to be more susceptible to allelopathic damage. Experiments with extracts and live fragments of several algae species demonstrated that corals of the genus *Acropora* and *Pocillopora* are especially sensitive to algal allelopathic agents while corals of the genus *Porites* were not as much affected (Carpenter et al. 2008; Rasher et al. 2011). Some coral species differed also in their recovery potential. Corals of the genus *Porites* recovered quickly while corals of the genus *Pocillopora* showed no signs of recovery after the removal of aggressive alga individuals *in situ* (Bonaldo and Hay 2014). These branching corals species are also more sensitive to natural and anthropogenic disturbances and experience high mortality rates on reefs worldwide, which could further increase through the expansion of aggressive spatial competitors (Muko et al. 2001; Carpenter et al. 2008).

The maximum photosynthetic quantum yield is a proxy for the health of the zooxanthellae which provide corals with energy for growth and reef formation (Muscatine et al. 1981; Davies 1984). Thus, impairments in the health of these symbiotic algae can lead to reduced growth and less available energy that corals could invest otherwise in recruitment or spatial competition. It has already been demonstrated that corals experience a reduction in their growth, fecundity, egg size, recruits and survival during competition with algae or other sessile benthic organisms (Romano 1990; Tanner 1995; Tanner 1997; Idjadi and Karlson 2007; Foster et al. 2008; Chadwick and Morrow 2011). As reefs are becoming increasingly overfished the abundance of spongivores that control sponge growth decreases, which results in additional increases of sponge-coral interactions (Loh and Pawlik 2009; Loh et al. 2015). The frequency of sponge-coral interactions will be further enhanced by the continuing nutrient input, in particular on Zanzibar’s reef through the untreated sewage discharge (Moynihan et al. 2012; Limbu and Kyewalyanga 2015). Additional nutrient input creates favourable conditions for filter-feeders such as sponges by promoting the production of bacterioplankton, which make up a large part of the sponge diet (Rogers 1990; Aerts and van Soest 1997; Holmes 2000; Rützler 2002; Fabricius 2005; Chadwick and Morrow 2011). Sponge cover on the reef at Bawe Island already increased substantially from < 1% up to 7.5% leading to more sponge-coral interactions over the last 15 years (Mohammed et al. 1999; Muhando et al. 2002; Muthiga et al. 2008; Lokrantz et al. 2010; Muzucka et al. 2010; Helber et al. 2016a). The present study could demonstrate that sponges on reefs in Zanzibar are potentially strong spatial competitors for reef-building corals. We could show that sponge extracts of the three most abundant sponge species have a negative impact on coral physiology. As a result, sponges may be able to exert negative effects on the fecundity, reproduction or the coral microbiome making corals more vulnerable towards further natural or anthropogenic disturbances and pathogenesis. Therefore, reef management around Zanzibar should focus on mitigating anthropogenic disturbances which enhance sponge populations, e.g. with the establishment of a wastewater treatment facility.

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# CHAPTER 5

# GENERAL DISCUSSION

# KEY FINDINGS

The aim of this thesis was to investigate the chemical ecology of sponges in the WIO, especially in regard to their chemical defences and competitive abilities towards reef-building corals. The key findings of a series of field and laboratory experiments investigating the chemical ecology of WIO sponges are summarized as answers to the four questions formulated in the general introduction (Chapter 1).

## 1) How are benthic coral reef communities in Zanzibar structured?

### What are the dominant benthic reef organisms?

The benthic community on the reef around Bawe is still dominated by hard corals both at 5m and 10m water depth. Coral rubble was the second most abundant benthic category due to destructive fishing activities and anchor damage by tourist boats (Muzuka et al. 2010; Chapter 2). Soft corals as well as macroalgae were virtually absent from the reef around Bawe Island and reported coverage of these two benthic organisms in the last 15 years never exceeded 5% (Mohammed et al. 1999; Mbije et al. 2002; Lokrantz et al. 2010; Bronstein and Loya 2014; Chapter 2). Corallimorpharians, which are one of the dominant benthic organisms (14% coverage) at reefs of the neighbouring island Changuu, represented a minor component of the benthos at Bawe with coverage remaining unchanged between 2-4% since 1998 (Muhando et al. 2002; Kuguru et al. 2004; Chapter 2).

### How abundant and diverse are epi-reefal sponges on Zanzibar's reefs?

Sponges represented one of the most abundant benthic categories besides corals. Sponge cover increased sharply over the last 15 years from <1% up to 7.5% cover (Mohammed et al. 1999; Muhando et al. 2002; Muthiga et al. 2008; Lokrantz et al. 2010; Muzucka et al. 2010; Chapter 2). Sponge richness varied with depth between 16-21 species around Bawe Island and was comparable to other WIO regions as well as Indonesia (Barnes and Bell 2002b; Bell and Smith 2004; Powell et al. 2014). Sponge richness as well as sponge cover was lower on reefs in the Red Sea or the Pacific (Adjeroud 1997; Desmet 2009; Hall et al. 2013; Rix 2015; Freeman and Easson 2016). Sponge densities as well as sponge cover in this study were comparable to values reported from different areas in Indonesia (de Voogd et al. 2006, 2009; Powell et al. 2010). However, coral reefs in the Caribbean yielded the highest sponge cover averaging 15.9% for coral reefs in twelve countries (Loh et al. 2015). Reef community structure in the Caribbean has changed not only in favour of sponge dominance, but also in favour of macroalgae dominance on many reefs (Loh et al. 2015; Mumby 2009). As a consequence coral cover on Caribbean reefs is around 16%, considerable lower than coral cover in the Western Indian Ocean, which ranges from 18% to 77% (Kenya: 18-40% and Zanzibar: 44-77%) (Diaz and Rützler 2001; Loh et al. 2015; Lokrantz et al. 2010; McClanahan et al. 2014; Chapter 2).

In conclusion, we could demonstrate that the reef around Bawe Island is still dominated by reef-building corals. Moreover, the study shows that sponges are an important and diverse component of the benthic community. Sponge cover might continue to increase if trends from the last years continue. Thus sponges may likely further gain in importance for the benthic community, as anthropogenic pressures, such as overfishing and nutrient pollution, and global climate change will promote increases in sponge and decreases in coral cover.

## 2) Is the increasing prevalence of sponges on the reef related to sponge chemical defences against various threats such as predators, microorganisms and competitors?

Ten of the most abundant sponges on the reef around Bawe Island were investigated for their chemical defence mechanisms (anti-predatory and antimicrobial) as well as their cytotoxic activities.

### Anti-predatory defences

The present study showed that only 30% of the sponge species examined deterred feeding by the pufferfish *C. solandri*. Thus, Zanzibar´s reefs are dominated by palatable sponge species unlike most reefs, which are dominated by chemically defended species such as reefs from the West Pacific (87%; Rohde et al. 2015), Caribbean (57.5%-69% ; Pawlik et al. 1995; Loh et al. 2015), Eastern Pacific (54%; Padilla et al. 2010) or the Red Sea (41%; Burns et al. 2003). Higher proportions of palatable sponges are most likely the result of predator removal on reefs. Since the production of feeding deterrent secondary metabolites is energetically costly (Pawlik 1993), palatable species invest their energy into reproduction, regeneration and growth (Leong and Pawlik 2010a; Pawlik et al. 2008; Walters and Pawlik 2005). The dominance of palatable sponge species on coral reefs has implications for overall reef health since they are able to grow uncontrolled on overfished reefs and might start to overgrow reef-building corals, as already observed on reefs in the Caribbean (Loh and Pawlik 2009, 2015). However, there is no information about the importance of sponge predation in the Western Indian Ocean and further research is needed in order to understand the role of spongivory in regulating the distribution and community composition of sponges.

### Antimicrobial defences

Antimicrobial activity of the sponge species was quantified by the disc diffusion assay. Sponge extracts were tested against 27 environmental and 9 pathogenic bacterial strains known to be associated with various coral diseases. All investigated sponge species exhibited antimicrobial activities with varying degrees in the number of inhibited test strains

(11% up to 100% of strains inhibited) as well as in the strength of the bacterial inhibition. These results were in accordance with other studies examining the antimicrobial activity of sponges from the Indian Ocean (Ely et al. 2004; Dobretsov et al. 2005) and Antarctica (Lippert et al. 2003). When comparing the activity of the different sponge extracts by environmental versus pathogenic bacterial strains, most sponge species were better defended against potential pathogens. These results suggest that the secondary metabolites in most sponges are highly selective. This selectivity may allow the sponge host to form close associations with microbes while inhibiting potential pathogens from growing or settling on the surface of the sponge (Kelman et al. 2001; Kelly et al. 2005; Rohde et al. 2012; Rohde et al. 2015). Environmental perturbations including urban runoff, nutrient enrichment, anthropogenic pollution and increased temperatures are linked to disease outbreaks in marine invertebrates (Lesser et al. 2007; Di Camillo et al. 2012). On Zanzibar, the sponges on the reefs surrounding Stonetown are especially exposed to untreated sewage and runoff from the main city, representing a potential source for the introduction of a variety of bacteria and potential pathogens (Moynihan et al. 2012). Nonetheless, this study could demonstrate that sponges around Bawe Island seemed to be remarkably well-defended against co-occurring marine bacteria.

### Cytotoxic activities

The cytotoxic activities of the ten most abundant sponges were assessed with the brine shrimp mortality assay as it is considered to be a very effective test to detect bioactive compounds in sponges (Richelle-Maurer et al. 2002; de Voogd 2005). In the present study, five (50%) of the tested sponge species showed bioactivity in the brine shrimp assay. This result is in accordance with other studies in which 59-75% of sponges showed cytotoxic activities in the brine shrimp or other cytotoxicity assays (Richelle-Maurer et al. 2002; Uriz et al. 1992; Soapi et al. 2013).

However, no pattern was found between sponge abundance on the reef and the degree of predatory deterrence, antimicrobial and cytotoxic activity. *Pseudoceratina* sp., *Callyspongia* sp., *H. atra* and *S. hapalia* demonstrated the strongest bioactivities, however only *H. atra* was also the most abundant sponge at 5m and the second most abundant at 10m water depth. *Pseudoceratina* sp. exhibited locally high abundances on reef sections around

Bawe Island, but overall percent benthic cover was low (Chapter 2; personal observation).

## 3) Do sponges contain allelochemicals which negatively affect the health of coral competitors?

Three of the most bioactive and abundant sponges were chosen for examining the presence of allelochemicals via PAM fluorometry to assess if possible allelopathic compounds have negative effects on the photosynthetic efficiency of the coral symbionts (zooxanthellae). Results from the field experiments revealed that sponge extracts of the most abundant sponge species had a negative impact on coral physiology. Extracts of *Pseudoceratina* sp., *Callyspongia* sp. and *Haliclona atra* incorporated into phytagel reduced the photosynthetic efficiency of branching *Porites* corals by 19-41% (Chapter 4). Additionally, the extracts of *H. atra* as well as *Callyspongia* sp. also caused bleaching in *Porites*. Other studies have also demonstrated that sponge extracts incorporated into gels inhibited the growth of other benthic organisms or were even able to cause bleaching and tissue necrosis in neighbouring corals (Engel and Pawlik 2000; Pawlik et al. 2007).

Short term experiments using live sponges for sponge-coral allelopathic interactions did not show significant results, similar to previous reports in the literature (Aerts 1999; Chaves-Fonnegra et al. 2008; Chapter 4). However, the results do not rule out that sponges use allelopathy in space competition. Reasons for the insignificant results could be that 1) exuded allelochemicals were unstable and lost their activities, 2) sponges might not have released allelochemicals during the experiment or 3) strong water currents diluted exuded metabolites (Aerts 1999; Smith et al. 2006; Pawlik et al. 2007; Chaves-Fonnegra et al. 2008). Coral species also exhibited different susceptibilities and recovery potentials to allelopathic damage with corals of the genus *Porites* recovering quickly, while corals of the genus *Pocillopora* or *Acropora* were especially sensitive (Aerts 1999; Carpenter et al. 2008; Rasher et al. 2011; Bonaldo and Hay 2014). These branching coral species are known to be more sensitive to natural and anthropogenic disturbances and in the past experienced high mortality rates on reefs worldwide, which could further increase through the expansion of aggressive spatial competitors such as the investigated sponges (Muko et al. 2001; Carpenter et al. 2008).

Thus, long term field experiments with live sponge fragments are needed in order to draw conclusions as to whether coral-sponge interactions are static, and sponges use their allelochemicals only to defend their substrate space, or if they actively harm corals in order to gain new space. Nonetheless, the present study demonstrated that the three sponge species have the potential to impair the health of coral symbionts (zooxanthellae). Sponge cover on the reef at Bawe Island already increased substantially from < 1% up to 7.5% and will likely lead to more sponge-coral interactions if this trend continues in the future (Mohammed et al. 1999; Muhando et al. 2002; Muthiga et al. 2008; Lokrantz et al. 2010; Muzucka et al. 2010; Chapter 2). The frequency of sponge-coral interactions on Zanzibar's reef will also likely be further enhanced by the continuing increased nutrient input, due to the undischarged sewage output (Moynihan et al. 2012; Limbu and Kyewalyanga 2015). Extensive nutrient input creates favourable conditions for filter-feeders such as sponges by promoting the production of bacterioplankton, which make up a large part of the sponge diet (Rogers 1990; Wilkinson and Cheshire 1990; Aerts and van Soest 1997; Rützler 2002; Chadwick and Morrow 2011). The increasing frequency of sponge-coral interactions could enhance the vulnerability of corals towards future natural or anthropogenic disturbances and pathogenesis.

## 4) Is the reef around Bawe resilient enough to withstand further disturbances? Are there indications for possible phase-shifts?

Increasingly more reefs are experiencing phase-shifts from coral dominance to states dominated by macroalgae, soft corals, corallimorpharians or sponges. Is the reef around Bawe resilient enough to withstand further disturbances? What are the main factors on Zanzibar that may lead to a lower resilience towards phase-shifts?

## Top down factors (fish and sea urchin grazing)

### Role of sea urchins on coral reefs

Even though the abundance of herbivorous fishes is low, sea urchin densities are very high on the reef at Bawe (Lokrantz et al. 2010; Bronstein and Loya 2014). Densities of the sea urchin *Diadema setosum* doubled over the last 10 years from approximately 2.6 ind. per $m^2$ to 6 ind. per $m^2$ (Mohammed et al. 1999; Bronstein and Loya 2014). Contrary to the Caribbean where densities of >16 *D. antillarum* per $m^2$ caused higher coral recruit mortalities (Sammarco 1980, 1982; Edmunds and Carpenter 2001, Carpenter and Edmunds 2006), it is assumed that sea urchin densities of around 5 *Diadema savignyi* per $m^2$ are sufficient to exert negative effects on post-settlement mortality of coral larvae on Kenyan coral reefs (O'Leary et al. 2013).

Even though grazers, including herbivorous fishes and sea urchins, promote the abundance of CCA on coral reefs as they remove competitively dominant fleshy algae, they can also reduce the cover of CCA when they exceed certain threshold abundances (Steneck 1986; Estes et al. 1998). Overfishing in East Africa has led to the removal of sea urchin predators and thus to an increase in sea urchin populations which erode the reef structure, reduce CCA cover by at least four fold as well as remove coral recruits (McClanahan 2000; Carreiro-Silva and McClanahan 2001; O'Leary and McClanahan 2010, O'Leary et al. 2012; O'Leary et al. 2013). This could also be a future threat for the reef at Bawe Island and other reefs around Zanzibar as they are heavily overfished and sea urchin densities of Bawe already exceed densities of 5 ind. per $m^2$, which have been reported to negatively impact coral recruitment at Kenyan reefs (Lokrantz et al. 2010; O'Leary et al. 2013; Bronstein and Loya 2014). Therefore it would be important to restore fish communities, including sea urchin predators and herbivorous fishes, since higher densities of herbivorous fishes seem to be more efficient in removing algae as well as more beneficial for coral recruitment (Jessen and Wild 2013; O'Leary et al. 2013).

**Importance of parrotfish as herbivores and spongivores**

On some reef systems in the Pacific and in cryptic habitats smaller herbivorous fishes may be sufficient to clear spaces of algae and thus enable the settlement of corals as well as preventing the overgrowth of newly settled recruits (O'Leary et al. 2013; Mumby et al. 2015). Nonetheless, the most abundant and influential of all roving herbivorous fishes on tropical coral reefs are large individuals of parrotfish as well as surgeonfish (Lewis and Wainwright 1985; Hoey and Bellwood 2008; Lokrantz et al. 2008; Aferworki et al. 2013; Johansson et al. 2013). Parrotfish are considered as a key functional group due to their intense grazing activity and their unique feeding mode (Bellwood and Choat 1990; Feitosa and Feirrera 2008; Bonaldo et al. 2014). They possess one of the highest feeding rates of herbivores taking at least double or three times as many bites per minute as rabbitfishes or surgeonfishes (Bellwood and Choat 1990; Goatley and Bellwood 2010; Fox and Bellwood 2013). Additionally, the beak-like jaws of parrotfishes enable them to completely remove algae even with parts of its attached substratum, ensuring that the cleared space will stay free for over 8 days, which gives sessile organisms such as corals the possibility for settlement (Bellwood and Choat 1990; Hoey and Bellwood 2008; Bonaldo and Bellwood 2009). All reefs on Zanzibar, and especially the reefs with high fishing pressure (e.g. Bawe and Changuu), are characterised by an extremely low biomass of parrotfish compared to other areas, such as Kenya or the Great Barrier Reef (McClanahan 1997; Hoey and Bellwood 2008; Lokrantz et al. 2010). Biomass of herbivorous fish, mainly parrotfish, on the heavily fished reef of Bawe amounted to 10-44 kg per ha while reefs of the nearby 'no-take' marine reserve at Chumbe Island harboured herbivore biomasses of 68-140 kg per ha (Lokrantz et al. 2010). Biomass of herbivores is more comparable to the low values in the Caribbean of approximately 20 kg per ha in Jamaica and up to 170 kg per ha in Barbados than the high estimates of Kenyan reefs ranging from 500- 700 kg per ha (McClanahan 1997; Williams and Polunin 2001; O'Leary and

McClanahan 2010).

Parrotfish do not only prefer algae or corals as their main food sources, but are also together with angelfishes the major sponge predators (Chanas and Pawlik 1995; Dunlap and Pawlik 1996, 1998; Wulff 1997c). Parrotfish are able to control the distribution and abundance of sponges and can completely remove certain sponge species from some habitats (Dunlap and Pawlik 1996; Hill and Hill 2002; Loh and Pawlik 2009; Wulff 2000). The low abundance and biomass of parrotfish, as well as the dominance of parrotfish in the smallest size classes, make it unlikely that they will have a profound impact on the present sponge community (Lokrantz et al. 2008, 2010). The low abundance of spongivores and high sea urchin densities seem to inhibit the proliferation of macroalgae and promote sponge growth. Even though *D. setosum* is known for feeding on sponges as well, it was revealed that only 5% of its gut content was made up of non-soluble residues, such as sponge spicules (Bronstein and Loya 2014). A similar pattern could be observed on a coral reef in Belize, which changed its community structure from coral to sponge dominance after high coral mortality caused by a bleaching event and the subsequent inhibition of macroalgal growth by sea urchin grazing (Aronson et al. 2002; Gonzalez-Rivero et al. 2011).

Even though herbivory as well as spongivory may be one of the main drivers of phase-shifts, it is suggested that water quality might be an equally or even more important driver (Halpern et al. 2008; Fabricius 2005; Suchley et al. 2016; Zaneveld et al. 2016).

**Potential Crown-of-thorns starfish (COTS) outbreaks**

COTS are the main coral predator in the Indo-Pacific region and are estimated to be responsible for 42% of coral cover loss in the GBR and one of the main factors further contributing to coral cover decline (De´ath et al. 2012). Coral reefs around Tanzania and Zanzibar experienced a major COTS outbreak starting in 2002, during which densities of COTS increased by a hundred-fold from 0.01 COTS per m² to 1 COTS per m² within almost 1.5 years (Obura 2005). For comparison, COTS densities of 0.005- 0.01 per m² were able to reduce coral cover by 46% in 2.5 years at the GBR (Pratchett et al. 2010). The reef around Bawe experienced a dramatic reduction in live *Acropora* cover after repeated COTS outbreaks from 2002/2003-2006 (Muhando and Lanshammar 2008; Muthiga et al. 2008). *Acropora* populations only recovered on the reef around Chumbe Island since park managers of the marine protected area (MPA) immediately initiated a COTS removal program (Muhando and Lanshammar 2008). Large populations of COTS could be observed on the reefs around the Zanzibar Archipelago up until 2009 and have been linked to global warming, overfishing and eutrophication due to sewage discharge (Muhando and Lanshammar 2008; Muthiga et al. 2008; Grimsditch et al. 2009).

## Bottom-up factors

### Sewage outflow

The water quality of the reefs on the West Coast of Zanzibar close to the main city of Stonetown is heavily impacted by untreated sewage discharge (Moynihan et al. 2012; Limbu and Kyewalyanga 2015). Multiple pipes coming from currently 2289 septic tanks, which extend 55m from the coast along the sea bottom, discharge daily $2.2 \times 10^6$ l of wastewater (Moynihan et al. 2012). The water quality on Zanzibar deteriorated sharply over a 12 year period as indicated by a proportionally higher amount of $^{15}N$ in the ratio of $^{15}N$:$^{14}N$ of common benthic organisms and an increased amount of fecal indicator bacteria, such as Enterococcus (ENT) (Heikoop et al. 2000; Noble et al. 2003; Risk 2009; Baker et al. 2010; Moynihan et al. 2012). In 2000, hard corals from Changuu and Chumbe (MPA) both had an isotopic signature $\delta^{15}N$ of 4.2‰, and 12 years later the value for Chumbe stayed in a similar range ($\delta^{15}N$= 3.5‰) whereas the one for Changuu experienced a nearly three-fold increase up to 12.2‰ (Heikoop et al. 2000; Moynihan et al. 2012). The reef around Bawe also exhibited higher nitrate and phosphate concentrations caused by the inflow of untreated sewage from Stonetown (Limbu and Kyewalyanga 2015). Sewage input and the resulting increase in nutrients has strong impacts on the potential resilience of coral reefs. It has already been demonstrated that a rise in nutrient concentrations can lower bleaching thresholds for corals by 2°C (Wooldridge and Done 2009). High nutrient levels and water turbidity were reported to cause a decrease in the calcification rates of corals by over 50% and water turbidity as well as sedimentation resulted in reduced calcification, photosynthesis and growth (Anthony and Hoegh-Guldberg 2003; Fabricius 2005).

### Influence on coral recruitment

Enhanced turbidity has been associated with lower coral recruitment rates as sewage discharge disrupts the chemo-sensory cues in corals vital for reproductive synchrony, fertilization success as well as larval settlement and metarmophosis (Maragos et al. 1985; Tomascik and Sander 1985; Richmond 1993; Nzali et al. 1998; Fabricius 2005). Unfortunately there is a paucity of information about coral recruitment on Zanzibar. Rostad (2006) reported that there are only differences in recruitment during the South East Monsoon season between Bawe and Chumbe (MPA) with Bawe having a lower recruit density. Otherwise, recruitment densities of 5-6 recruits per m² (Bawe) and 7-8 recruits per m² (Chumbe) did not display any differences nor did the survival rates of the recruits (Rostad 2006). However, there is no current information about recruitment densities and success rates on reefs around Zanzibar.

### Initiation of diseases through disruption of the host-symbiont relationship

Furthermore, the exposure to sewage discharge and increased nutrients was shown to be responsible for the development and progression of coral diseases (Patterson et al. 2002; Bruno et al. 2003; Voss and Richardson 2006; Vega Thurber et al. 2009, 2014; Sutherland et al. 2010, 2011). Even if wastewater does not harbour etiological agents for diseases in benthic invertebrates it could be demonstrated that sewage introduces many opportunistic microbial taxa, such as e.g. *Vibrionaceae* and *Rhodobacteraceae*, that can alter the microbial community in coral as well as sponge holobionts resulting in the onset of diseases (Rosenberg and Ben-Haim 2002; Vega Thurber et al. 2009; Ziegler et al. 2016).

Sponges are able to cope better with increased nutrient input or sewage discharge since a large part of their diet consists of bacterioplankton, the growth of which is promoted by organic pollution, whereas corals are adapted to low-nutrient environments (Rützler 2002). However, sponges can also only flourish and tolerate high nutrient concentrations to a certain extent (Rützler 2002; Gochfeld et al. 2007, 2012; Fiore et al. 2010; Easson et al. 2014). Sponge community composition is assumed to shift under nutrient stress with a reduction in sponge diversity (Gochfeld et al. 2007; Easson et al. 2015). HMA sponges, harbouring more photosymbionts compared to LMA sponges, are assumed to dominate sponge communities in high nutrient environments whereas oligotrophic environments were comprised of a mix of HMA and LMA sponges as well as of sponges containing different amounts of photosymbionts (Easson et al. 2014, 2015; Zhang et al. 2015). Excessive nutrients benefit the sponge symbionts while repressing sponge holobiont health (Easson et al. 2014). Similar patterns have been observed in coral symbiosis, where coral metabolism is disrupted under elevated nutrient concentrations when zooxanthella grow uncontrollably and respire the photosynthetically gained carbon themselves instead of translocating it to their animal host (Muscatine et al. 1989; Dubinsky et al. 1990; Falkowski et al. 1993).

### Combination and importance of overfishing and sewage discharge

Both overfishing and sewage discharge are expected to decrease the resilience of reefs around Zanzibar's West Coast by reducing the competitive abilities of corals against other space competing benthic organisms as well as by decreasing coral recruitment, growth and survival (Hughes et al. 2007; Birrell et al. 2008; Zaneveld et al. 2016). Further, sewage input has the ability to disrupt the coral microbiome through enhanced concentrations of potential pathogens, increasing the prevalence of coral diseases (Burkepile and Hay 2006; Smith et al. 2006, 2010; Zaneveld et al. 2016). Additionally, elevated seawater temperatures as a consequence of global climate change may exacerbate the effects of overfishing and nutrient input by disrupting the coral microbiome as well as enhancing pathogen fitness, thereby amplifying physiological stress responses in the coral (Thurber et al. 2009; Zaneveld et al. 2016). Thus, it is not only vital to restore and manage healthy herbivore populations on reefs, but at the same time to improve the water quality to avoid negative impacts on coral physiology as well as prevent the initiation of coral diseases (Suchley et al. 2016; Zaneveld et al. 2016).

## Possible Phase-Shift Scenarios on the reef at Bawe

Global reductions in coral cover were often associated with changes in the benthic reef community structure and the emergence of a new dominant benthic organism. The change in community structure to an alternate state can be reinforced by feedback loops (e.g. inhibition of coral recruitment or settlement) and result in an alternate stable state (phase-shift) which is difficult to reverse (Suding et al. 2004; Mumby and Steneck 2008; Norström et al. 2009; Mumby et al. 2009; Nyström et al. 2012). Most phase-shifts from coral to macroalgal dominance are reported from the Caribbean, while the stability of alternate states in the Indo-Pacific is contentious due to the lower concentrations of nutrients and lower fishing pressure (Bellwood et al. 2004; Hughes et al. 2010; Graham et al. 2013). One key factor leading to phase-shifts in the Caribbean was the loss of functional redundancy (Hughes et al. 2010; Graham et al. 2013). Overfishing led to a drastic decline in reef fish populations and only one sea urchin species, *Diadema antillarum*, replaced herbivorous fish as the main grazer (Hay 1984; Hughes 1994). After disease-induced mass mortalities of this sea urchin followed by its slow recovery and a subsequent coral bleaching event, macroalgae have dominated many reefs in the Caribbean until today (Hughes et al. 2010; Graham et al. 2013; Loh et al. 2015). Similarly, the reefs around Bawe Island may be vulnerable to a phase-shift if the high fishing pressure as well as high sewage input continues, since the functional redundancy of the grazing community on its reef is greatly reduced. There are several different possibilities of an alternate state or even phase-shift, which could result in the reef possibly being dominated by algae, corallimorpharians or sponges.

### Algae

It is unlikely that the reef around Bawe will shift to algal dominance, since algae cover remained constant around 1-2% over the last 15 years (Mohammed et al. 1999; Mbije et al. 2002; Lokrantz et al. 2010; Bronstein and Loya 2014; Chapter 2). Moreover the high densities of sea urchins seem to be sufficient to inhibit macroalgal proliferation (Aronson et al. 2002; Bronstein and Loya 2014). However, the intense grazing of sea urchins could impede future coral recruits as well as result in slow bioerosion of the reef structure.

**Corallimorpharians**

It is also not very likely that corallimorpharians will take over the reef at Bawe since their cover has remained stable since 1998, ranging from only 3-4%, which is comparable to values for the Chumbe MPA (Kuguru et al. 2004; Muhando et al. 2002; Rostad 2006; Chapter 2). However, the reef on the neighboring Island Changuu experienced a phase-shift from coral to corallimorpharian dominance within 2-3 years after the 1997/1998 El Nino event (Kuguru 2002; Muhando et al. 2002; Kuguru et al. 2004), as corallimorpharians were able to out-compete the reef-building corals. Corallimorpharians are superior competitors to corals because corallimorpharians are capable of actively damaging space competitors via tentacles containing nematocysts and smothering by mucus enabling them to overgrow scleractinian corals (Chadwick 1987, 1991; Chadwick and Adams 1991; Miles 1991; Chadwick-Furman and Spiegel 2000; Kuguru 2002), corallimorpharians can tolerate high temperatures, turbid waters, desiccation and high UV radiation (Chadwick-Furman and Spiegel 2000; Muhando et al. 2002; Kuguru et al. 2007, 2010), as well as higher concentrations of nutrients, especially phosphate and nitrate, which result in enhanced asexual reproduction of corallimorpharians (Chadwick 1987, 1991; Chadwick and Adams 1991; Spiegel 1998; Rushingisha 2012; Chadwick-Furman and Spiegel 2000). Increased input of sewage was identified as the main initiator leading to the phase-shift of corallimorpharian dominance on the reef of Changuu by causing high water turbidity due to suspended solids as well as high concentrations of nutrients (Muhando et al. 2002; Kuguru et al. 2004). New findings demonstrate that corals are able to coexist with corallimorpharians in oligotrophic waters, but are out-competed when nitrate and phosphate values are elevated (Rushingisha 2012).

The reef at Changuu has a higher sewage input than the one at Bawe due to the prevailing water current patterns and its closer proximity to Stonetown (Mohammed et al. 1993; Kuguru et al. 2004; Muhando and Mohammed 2002). Additionally, the reef around Bawe is still characterized by clear waters with visibilities between 10-11m and low turbidity (Muhando et al. 2002; personal observation). Thus, it seems unlikely that corallimorpharian dominance poses an imminent threat to the reef around Bawe.

### Sponges

On reefs around Bawe sponge cover increased sharply from < 1% to 7.5%, contrary to the stable and low macroalgal as well as corallimorpharian cover (Mohammed et al. 1999; Muhando et al. 2002; Muthiga et al. 2008; Lokrantz et al. 2010; Muzucka et al. 2010; Chapter 2). The high increase in sponge cover but not in macroalgae or corallimorpharians over the last 15 years might be the result of overfishing, high sea urchin abundance and declining water quality.

Studies showed that macroalgae are able to out-compete sponges under low grazing pressure, since sponges might represent an alternative food source for herbivores thereby further lowering algal grazing pressure (Gonzalez-Rivero et al. 2011, 2015). Additionally, it was demonstrated that algae responded positively to sponge contact due to the transfer of nitrogen from the sponge to the algae (Maldonado et al. 2012; Davy et al. 2002; Pile et al. 2003; Easson et al. 2014; Pawlik et al. 2016). Nonetheless, high grazing pressure limits the growth of algae, thus facilitating the proliferation of sponges, as observed on reefs in the Mediterranean, Caribbean and GBR (Sammarco et al. 1987; Aronson et al. 2002; Cebrian and Uriz 2006; Cebrian 2010). In Belize sponges were able to take over the reef after the 1997/1998 El Niño event since the high coral mortality resulted in depressed coral recruitment and macroalgae were kept at low densities through sea urchin grazing (Aronson et al. 2002). Furthermore, sponges were able to out-compete algae even on overfished reefs with low numbers of herbivores, when sponges were released from predation pressure (Loh et al. 2015). The possibilty that sponges have been released from predation pressure on the reef at Bawe Island is supported by fact that the majority (70%) of the sponge community consists of species that are palatable to fish (Chapter 2). In contrast, coral reefs in the Caribbean with abundant predatory fishes are characterized by a lower coverage (< 10%) of palatable sponges (Loh and Pawlik 2014; Loh et al. 2015). Palatable sponges will recruit more rapidly and grow faster subjecting reef building corals to greater competitive pressure since they do not have to invest energy in the production and storage of complex feeding-deterrent secondary metabolites (Pawlik 1993; Loh and Pawlik 2009, 2014; Loh et al. 2015).

Moreover, there are several reports from reefs in the Caribbean and one from the GBR which shifted from coral to sponge dominance due to increased nutrient input (Schönberg

2000; Aronson et al. 2002; Rützler 2002; Ward-Paige et al. 2005). In one case the shift occurred even though high abundances of spongivorous fish were present (Rützler et al. 2000; Wulff 2000, Aronson et al. 2002). Additionally, a bacterial species characterized as a fecal bioindicator is assumed to have killed corals in Florida Keys, whereas sponges were not affected and able to rapidly increase in cover (Ward-Paiges et al. 2005). Moreover, sponge cover was even positively correlated with the abundance of fecal bacteria and nutrients in the water column (Rose and Risk 1985; Sammarco 1996; Holmes et al. 2000; Diaz 2005). Additionally, the occurrence of overgrowth by the sponge *Terpios hoshinota* was observed more frequently in pollution-stressed zones (Rützler and Muzik 1993). The reefs on Zanzibar´s West Coast are heavily exposed to untreated sewage and runoff from the main city, which could represent a potential source for the introduction of a variety of bacteria and potential pathogens (Moynihan et al. 2012). However, this study demonstrated that the sponge community of the reefs around Bawe are exceptionally well-defended against bacteria and especially against potential pathogens (Chapter 3). The present study could further show that extracts of three of the ten most abundant sponges were able to produce allelochemicals which caused bleaching and tissue necrosis in neighbouring corals (Chapter 4). Thus, the sponges were able to impair the photosynthetic efficiency of corals thereby decreasing the coral health (Porter and Targett 1988; Pawlik et al. 2007; Chapter 4).

In conclusion, this study could show that sponges are well-defended against bacteria and potential pathogens and are probably more resistant than corals to the current sewage input to the reef at Bawe (Chapter 3). Additionally, the investigated sponges were demonstrated to be highly competitive compared to the dominant coral species and both of these factors may have been reasons for their increasing abundance on the reef in recent years (Chapter 2 and Chapter 4).

# CONCLUSION

The reef around Bawe Island still maintains a very high hard coral coverage and supports a locally high diversity of coral species with some unique taxa present when compared to other corals reefs around Zanzibar, as shown by the present and previous studies (Mbije et al. 2002; Muthiga et al. 2008; Muhando 2009; Zvuloni et al. 2010; Chapter 2). Moreover, the reef showed a remarkable potential for recovery following the massive 1997-1998 bleaching event as well as the crown-of-thorns-starfish (COTS) outbreaks during 2002/2003- 2006, even though its species diversity remains reduced (Mbije et al. 2002; Muhando and Lanshammar 2008; Muthiga et al. 2008; Muhando 2009; Zvuloni et al. 2010). The increased sewage input in combination with other local stressors, such as destructive fishing practices or damage to the reef through tourism activities, as well as global stressors, might make the reef around Bawe Island vulnerable to a phase-shift. As shown in earlier studies and in this thesis, sponges display strong resistance to environmental stress and they are often competitively superior compared to corals (Carballo 2006; Schönberg 2006; Pawlik et al. 2007; Chapter 3; Chapter 4).

Several studies (Grigg 1995; Anthony et al. 1997; Edinger et al. 1998; Aronson et al. 2002; Rützler 2002; Ward-Paiges et al. 2005) showed that sewage discharge resulted in coral mortality or in a reduction of species diversity, but most reefs were able to recover after the sewage input was stopped. Thus, management strategies for Zanzibar should focus on the establishment of a proper sewage water treatment facility as the top priority to minimize risks for coral reef health. Regulations for fisheries also have to be enforced as an important complementary reef protection measure to restore healthy herbivorous as well as spongivorous fish populations on the reefs.

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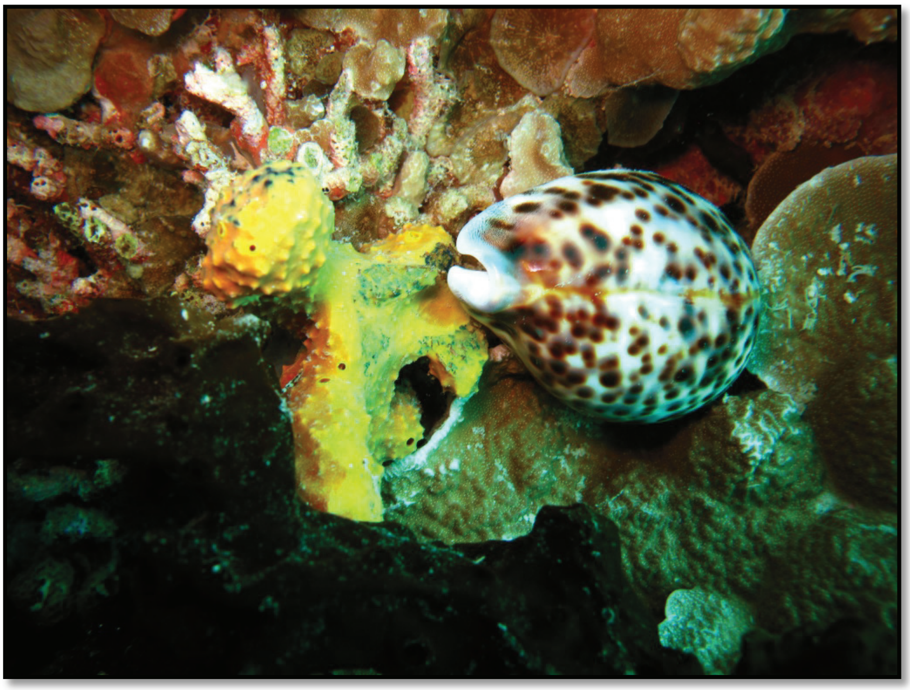
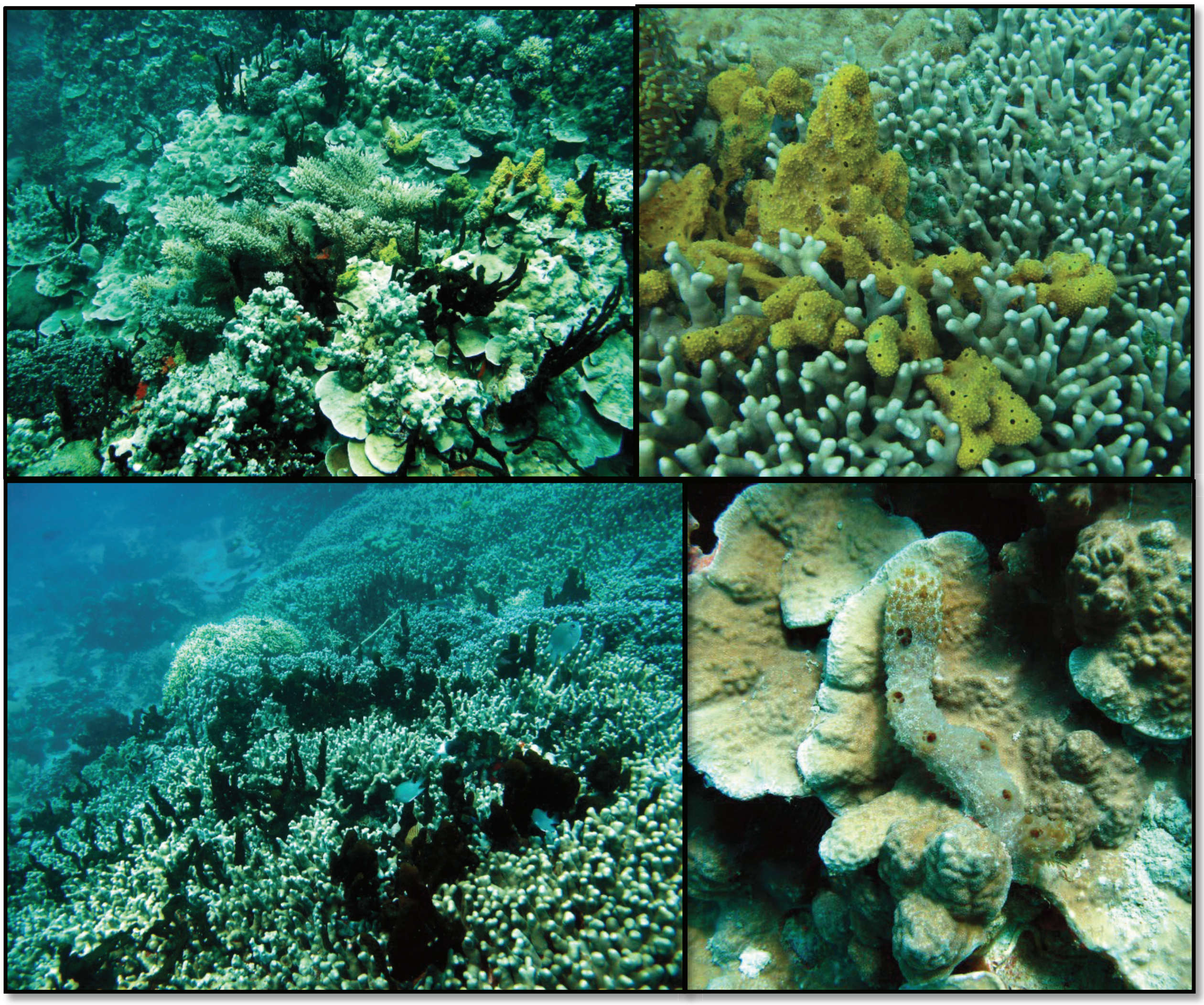
# APPENDICES

## APPENDIX 1



**Appendix 1-Figure 1:** Feeding of the snail *Cypraea tigris* on *Pseudoceratina* sp. during pre-dawn dives.

# APPENDIX 3



**Appendix 3-Figure 1:** The three sponge species *Pseudoceratina* sp., *Callyspongia* sp. and *H. atra* on the reef at Bawe Island. All sponges were observed to grow adjacent or even in between branching and massive *Porites* corals.

Name: ________________________________ Ort, Datum: ________________________

Anschrift: ______________________________________________________________

# **ERKLÄRUNG**

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

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selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

__________________________

(Unterschrift)