In situ simulation of eutrophication and overfishing in a coral reef of Koh Phangan, Thailand

- Effects on algae growth and activity -

Master Thesis

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In situ simulation of eutrophication and overfishing in a coral reef of Koh Phangan, Thailand

- Effects on algae growth and activity -

In cooperation with: Leibniz Center for Tropical Marine Ecology (ZMT), Bremen Center for Oceanic Research and Education (COREsea), Koh Phangan, Thailand





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Declaration of Authorship

I certify that the work presented here is, to the best of my knowledge and belief, original and the result of my own investigations, except as acknowledged, and has not been submitted, either in part or whole, for a degree at this or any other University. Formulations and ideas taken from other sources are cited as such. This work has not been published.

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The important thing is not to stop questioning. Curiosity has it own reason for existing. (Albert Einstein)

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Abstract

Coral reefs worldwide are under pressure of global as well as local threats including overfishing, coastal development, pollution and mass tourism activities. The relative role of factors causing coral reef degradation is still controversially discussed and likely dependent on ecoregions. The Gulf of Thailand is largely under-investigated regarding coral reef responses to anthropogenic stressors although increasingly impacted by pollution and fishing. This study therefore aimed to assess the relative importance of top-down and bottom-up factors on benthic algae growth and activity through in situ simulation of overfishing and eutrophication in a coral reef of Koh Phangan, Thailand. Over a period of 12 weeks, the anthropogenic impacts overfishing and eutrophication were simulated using nets excluding herbivores > 2 cm and/or bags with coated slow-release fertilizer. Cover, dry mass and oxygen fluxes of the algal community growing on terracotta settlement-tiles were investigated every week. Findings revealed that tile cover was dominated by filamentous turf algae in all treatments, with shorter but denser algae on uncaged tiles. Single individuals of macroalgae, most frequently of the genus Padina sp., were identified on caged tiles from week 7, while crustose coralline algae were not observed in any of the treatments. Algae dry mass and cover increased significantly over time in all experimental plots. Effects of nutrients were not visible in any of the monitored parameters. Effects of caging were visible in taller turf and macroalgae on caged tiles, higher respiration on caged tiles compared to uncaged tiles and a significant response in algae dry mass when comparing data of all weeks (twofactorial ANOVA, caging p = 0.045; nutrients p = 0.576; interaction p = 0.607). Therefore, herbivory may be the more important factor in controlling reef algal mass at the study site. However, results of this study did not confirm working hypotheses of largely enhanced algae growth following nutrient enrichment and herbivore exclusion. The reasons for rather minute responses to nutrients and caging were most likely high ambient nutrient concentrations which already satisfied algae growth, and a relatively small herbivorous fish community. High nutrient loads were introduced via a river discharging into the bay, with phosphate concentrations 10 times higher than in the reef. Relatively low C:N ratios and gross primary production also indicated high ambient nutrient concentrations, resulting in dominance of turf algae. Herbivorous fish were obviously able to prevent macroalgal growth on uncaged tiles, but not effective in decreasing total algal mass in a large extent. All results suggest that the investigated reef is already highly impacted by overfishing and sewage pollution. Although macroalgal cover was low and coral cover still relatively high, the obviously intensive human impact on the reef demands better management in order to prevent further reef degradation. Initial management targets should focus on protection of herbivores, as top-down factors were shown to be of higher importance in the studied reef.

1. Introduction

Coral reefs in danger

Coral reefs are among the most diverse and productive aquatic ecosystems on the planet and support the food and livelihood of at least 500 million people worldwide (Wilkinson 2008). Unfortunately, it is now evident that approximately 75 percent of the world's coral reefs are currently threatened by a combination of local and global pressure. This includes climate change, but also regional or local factors like destructive fishing and overfishing, coastal development, pollution and unsustainable tourism activities. These local pressures threaten more than 60 percent of reefs worldwide (Burke et al. 2012). Especially in South East Asia, overfishing has affected the majority of coral reefs, and pollution from land is significant. The future threats from global warming and acidification will exacerbate the problems in this region: by 2030, 99% of reefs are predicted to be threatened with about 80% at high or very high levels (Burke et al. 2011).

Thailand & tourism

The Gulf of Thailand is no exception to this trend. Some of the most important tourist destinations in the Gulf of Thailand are the islands of Koh Samui, Koh Tao and Koh Phangan in the Surat Thani province that attract millions of visitors each year (Vorlaufer, 2005). Tourism can disturb the sensible ecological balance in the benthic reef community by contributing to increased nutrient input and sediment load following urbanization and agricultural development along the coastlines and the construction of new beachfront resorts (Costa et al. 2006; Sandin et al. 2008). As Thailand is usually lacking effective water treatment facilities, hotels often discharge untreated sewage waters directly into rivers or the ocean (Cheevaporn & Menasveta 2003, Paytan et al. 2006).

Anthropogenic eutrophication

Untreated sewage water is a waste product which contains high concentrations of nutrients and, once reaching coastal waters, acts as fertilizer by enhancing primary production (Lapointe 1997; Smith et al. 1981). This may result in blooms of normally nutrient limited phytoplankton and macroalgae. Threshold values of 1 µM nitrate and 0.1 µM phosphate have been proposed by Bell (1992) and Lapointe (1997) to enable dominance of large fleshy algae. However, these values are below the mean nutrient concentrations reported for 1000 coral reefs worldwide (Kleypas et al. 1999) and the concept of nutrient thresholds has been rejected as a cause for shifts to algal dominance by recent studies (Szmant 2002, McClanahan et al. 2004). But fact is, corals typically thrive in oligotrophic waters (Muscatine & Porter 1977), and chronically enhanced levels of nutrients may alter the coral reef community by a combination of direct effects on corals like decreasing calcification rates (Kinsey & Davies 1979; Marubini & Atkinson 1999; Ferrier-Pages et al. 2000) and indirect

through the promotion of algae and bioeroders (studies reviewed by Fabricius 2005). Rather than directly smothering coral colonies, macroalgae typically use the available space after corals were killed by other disturbances. However, once established they prevent coral recovery (Schaffelke et al. 2005) and inhibit coral recruitment by space occupancy, sediment trapping or shading (Sammarco 1980; Connell et al. 1997; Szmant 2002; Schaffelke et al. 2005). Whereas macroalgae are able to outcompete corals and prevent coral settlement (Done 1992, Hughes 1994), crustose coralline algae (CCA) may have the opposite effect. CCA contain chemical cues that facilitate settlement of some coral species (Morse et al. 1996; Heyward & Negri 1999) and thus promote coral recruitment. Littler & Littler (1984) hypothesized CCA to dominate benthic communities under high nutrients and high grazing pressure. Other studies found that CCA are negatively correlated with nutrient availability (Belliveau & Paul 2002). The suppression of CCA is most probably due to the promotion of turf algae through nutrients. Turf algae are small and fast growing species forming a conglomerate of different filamentous green and red algae and cyanobacteria. These algal turfs effectively trap sediment (Purcell 2000) and by this means inhibit survivorship of CCA beneath sediment deposits (Fabricius & De'ath 2001) what, in turn, makes the substratum less suitable for coral recruitment (Birrell et al. 2005).

Overfishing

The seas of South East Asia, especially the Gulf of Thailand, still support a significant proportion of the world's fisheries and constitute the primary source of food protein security and income for most local coastal communities (Moberg & Folke 1999). But fisheries management is inadequate and most target species are considered fully fished or overfished (Myers & Worm 2003, Wilkinson 2008). Especially the excessive removal of large algalgrazing fishes (herbivores), the 'immune system' of a reef, decreases reef resilience that is defined as the ability to absorb recurrent disturbances and rebuild coral dominated systems rather than shifting to algal dominated systems (Nyström et al. 2000; Hughes et al. 2007). Herbivores play a critical role in coral reef resilience by limiting the establishment and growth of algal communities that impede coral recruitment (Green & Bellwood 2009). Especially four herbivorous functional groups of fish play complementary but equally important roles for the resilience of a reef and the recovery of corals after disturbances. These groups, namely bioeroders, scrapers, grazers and browsers, are preventing algae dominance by feeding on turf or macroalgae (Bellwood et al. 2004, Green & Bellwood 2009). The extent to which reefs possess these functional groups is central to their capacity to resist phase shifts (Bellwood et al. 2004). The fact that most grazing thresholds lie near the upper level observed for herbivores in nature suggests that reefs are highly sensitive to herbivore exploitation (Mumby et al. 2007). Other key herbivores in reef ecosystems are sea urchins which play a major

consumer role in Caribbean reefs, but are less important in the Indo-Pacific region due to a greater functional redundancy of herbivores (Roff & Mumby 2012).

The degradation of coral reefs due to eutrophication and overfishing can finally result in alternative stable states where the return to the pristine state is difficult. As nutrients increase and herbivorous fish disappear, coral reef communities change from dominance of nutrient-recycling symbiotic organisms such as corals, to increasing proportions of macroalgae, and further to heterotrophic filter feeders and bioeroders (Birkeland 1987). These changes in coral reef community compositions are known as phase shifts. The typical state after a phase shift in coral reefs is indicated by a high macroalgal cover, reducing the availability of coral settlement space and increasing the frequency and intensity of coral–algal interactions (Done 1992). This leads to reduced coral recruitment and growth of polyps (Mumby et al. 2007) as well as decreased structural complexity in the reef and negatively impacts the functioning of coral reef communities (Wild et al. 2011).

Bottom-up versus top-down factors

Increased nutrient input and overfishing of herbivores are considered to be the main anthropogenic drivers controlling coral reef ecosystem functioning. The relative importance of these bottom-up versus top-down factors is vigorously discussed within the last years of coral reef research and not yet resolved for the Gulf of Thailand. Bell (1992), Lapointe (1997), Littler et al. (2006) and Vermeij et al. (2010) found significant increases in algae biomass after exposure to enhanced nutrient levels only. In contrast, McCook (1999) concluded in his review that nutrient overloads can contribute to reef degradation, but that they are unlikely to lead to phase shifts simply by enhancing algal growth rates and hence allowing overgrowth of corals, unless herbivory is naturally or artificially low. Supportive for this statement is, that numerous studies have shown increases in algal abundance in response to herbivore reduction with no change in nutrient supply, demonstrating that enhanced nutrients are not necessary for phase shifts (Belliveau & Paul 2002; Diaz-Pulido & McCook 2005; Burkepile & Hay 2006; Burkepile & Hay 2009).

A simple model for testing hypotheses concerning the interactive effects of nutrients and herbivory was developed by Littler & Littler (1984). This relative dominance model (RDM) predicts the dominance of turf algae in a system with low grazing pressure and low nutrient concentrations, dominance of CCA under high nutrient concentrations and high grazing pressure and dominance of frondose macroalgae when nutrient concentrations are high and grazing pressure is low. The RDM was opposed in several studies; in fact high nutrients were found to even suppress establishment of large brown algae (McClanahan et al. 2003).

Recent reviews by Burkepile & Hay (2006), Gruner et al. (2008) and Teichberg et al. (2012) concluded that in most cases nutrients and grazers independently affected producer

biomass, but that there may also occur interactive or synergistic effects. The relative role of bottom-up versus top-down controls in structuring benthic reef communities is obviously context-dependent, that means it may differ by type of habitat, by latitude or by functional groups of algae and herbivores. The important role of geographic region in ecosystem responses was emphasized and discussed recently by Roff & Mumby (2012), who suggested Indo-Pacific reefs being more resilient and less likely to undergo phase-shifts due to higher biodiversity and biomass of branching corals and herbivores as well as lower rates of macroalgal growth and recruitment.

Scientific justification and objectives

Anthropogenic as well as natural environmental changes act as stressors on various benthic key groups and impact coral reef resilience. In the last decades, an increasing trend of macroalgal blooms coinciding with decreasing coral cover was observed worldwide (Hughes 1994; McManus & Polsenberg 2004; Nugues & Bak 2008). Algal-dominated reefs usually have lower fish stocks, less tourism appeal and coral biodiversity. And problems for coral reef managers are increasing, as 50% of the world's population will live along coasts by 2015, putting unsustainable pressures on coastal resources (Wilkinson 2008).

To sustain coral reefs worldwide it is inevitable to reduce overfishing and pollution at a local scale. As a scientific basis to implement management strategies for specific regions, detailed knowledge about the respective ecosystems is needed (Hoegh-Guldberg et al. 2007; Daily et al. 2009). Especially the understanding of factors preventing and reversing phase shifts has to be improved (Smith et al. 2010). Small scale experiments have been helpful in determining the mechanisms involved in phase shifts (McManus & Polsenberg 2004) but results are equivocal and differ considerably depending on location and study organisms. Additional factorial experiments focused on the combination of herbivory and nutrient loading would add to the understanding of driving actors (Szmant 2002). Studies on top-down versus bottom-up control of coral reefs have been conducted almost exclusively in the Caribbean, where the ecological system differs considerably from the Indo-Pacific in its stressors and responses (Roff & Mumby 2012). Understanding of resilience in the Indo-Pacific is still in its infancy and studies testing the relative influence of bottom-up and top-down factors on algae or coral growth originated from the relatively healthy Great Barrier Reef only. South East Asian reefs on the other hand have hardly been studied in this regard, although they have been classified as the most threatened reefs in the world with overfishing and pollution as main threats (Wilkinson 2008; Burke et al. 2011).

Therefore, this study aimed to assess linkages between anthropogenic influences and ecosystem functioning in a typical reef of Koh Phangan, Thailand. The Gulf of Thailand is a largely under-investigated area in marine research where *in situ* manipulation experiments focusing on ecologic responses to anthropogenic impacts were conducted for the first time.

The relative importance of bottom-up versus top-down factors and synergistic effects was investigated through *in situ* simulation of eutrophication and overfishing. This allowed the analysis of direct responses in algae community composition, growth and activity to manipulations of bottom-up and top-down controls in a coral reef resembling most South East Asian reefs, impacted by run-off from land, fishing and tourism. Based on similar studies, largely enhanced growth of algae and development of unique algal assemblages in response to caging and nutrients was hypothesized.

The study was closely linked with two other thesis projects using the same experimental setups and looking at responses in different benthic groups. This allowed the comparison of nutrient and caging effects on algae (present study), invertebrate recruitment (Bastian, unpublished data) and surface sediment parameters (Becker, unpublished data). The data obtained were supported by a comprehensive set of background parameters such as benthic composition, herbivorous fish abundance and biomass, nutrient availability, chlorophyll a concentrations, as well as current, light and temperature regime. Abiotic and survey data obtained in 2011 at the same study site were used for chronological comparisons and to develop suggestions for future long-term monitoring programs (Bennecke 2012; Börder 2012; Schwieder 2012; Pusch 2011).

2. Material and Methods

2.1 Study site

This study was conducted from February 7th to May 5th, 2012 at the Center for Oceanic Research and Education (COREsea) on Koh Phangan in the lower Gulf of Thailand.

Koh Phangan, with approximately 168 km² the second largest island of the Samui-Archipelago is less populated than the neighboring islands but also increasingly impacted by tourism (Vorlaufer 2005). The experimental set-up was located in Mae Haad on the northwest coast of Koh Phangan (09⁴7'44.85"N/099⁵ 8'41.51"E). The 0.8 km long bay hosts some bungalow resorts and experiences frequent dive and snorkel tourism (personal observations). Fishing in the area is prohibited, but was observed after sunset by local dive schools once or twice a month. A sandy strip close to the beach merges into a field with coral rubble, small coral colonies and high cover of macroalgae (*Sargassum* sp., *Padina* sp., *Turbinaria* sp.) before sloping down to a patchy fringing reef in 2-5 m depth. Big boulders of the massive coral *Porites* sp. and structurally diverse coral areas alternate with natural sand pools. At about 5 m water depth the reef is slowly replaced by shallow sand flats. Water levels in May were too low to swim over the shallow algae and rubble fringe, but access to the outer reef border was provided through a sandy channel in the center of the bay. Field manipulations were conducted at a water depth of 5 m in sand pools at the outer reef border north of the channel (Fig. 1, Tab. 1).



Fig.1: Koh Phangan and study site Mae Haad with experimental plots 1-4 (adapted from GoogleEarth)

Tab. 1 Location of	f experimental	plots
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Plot	Coordinates
1	09%47'48.24"N / 099%58'41.88"E
2	0947'49.10"N / 09958'42.06"E
3	0947'49.42"N / 09958'42.18"E
4	0947'50.24"N / 09958'42.16"E

2.2 Experimental design

Experiments to test the impact of nutrient supply and herbivore exclusion were run from February 17th to May 13th, 2012 (12 weeks).

The study used 16 cages with two levels of herbivory (caged or uncaged) and two nutrient levels (enriched or unenriched) with a crossed design that allowed testing the interaction between the two factors. Each cage was randomly assigned to one of four treatments: A: control – no manipulation, B: nutrient enrichment, C: herbivore exclusion or D: both nutrient enrichment and herbivore exclusion (Fig. 2). Each of the four treatments (A, B, C, D) had four replications (plot 1, 2, 3, 4). Per plot, the four set-ups were fixed in a sand pool with at least 1 m distance to each other, facing seawards. The distance between the plots was 10-30 m.



Fig. 2: Experimental design showing one of four replicates for each treatment situated at 5 m water depth. A: control – no manipulation, B: nutrient enrichment, C: herbivore exclusion D: both nutrient enrichment and herbivore exclusion.

2.3 Experimental set-up and analyses

The cages (each 50 cm in length, width and height) were constructed using PVC-tubes (\emptyset = 2 cm) and two-way fitting joints (Fig. 3). Two square frames, the lower one filled with concrete, were connected by vertical PVC tubes (\emptyset = 1.5 cm) which protruded downwards by 15 cm to secure the cages in the sand. Plastic nets were fixed between the vertical tubes in an angle of 45°. The angle served to avoid sediment ation on the 15 terracotta settlement tiles fixed to the net with cable ties. Settlement tiles were standardized to 5 x 10 x 0.5 cm from customary floor tiles. The underside, which was used for settlement, was untreated and grooved. Terracotta offers a heterogenous surface enhancing species richness and biomass compared to other artificial substrates (Brock 1979). Tiles were preconditioned in buckets with seawater for 24 h prior to deployment. No metal was used for the construction of cages to exclude the risk of heavy metal poisoning and the precipitation of phosphate with iron ions. Herbivore exclusion was achieved by covering the frames with fishing net of mesh size 2 cm to exclude larger fish and invertebrates. Nets were prepared to form an open cube, put over the cages and fixed to the lower frame with rope, which facilitated weekly exchange and cleaning of nets.

Nutrient enrichment was achieved by displaying bags of polyethylen mesh (40 x 2 cm, mesh size 1 mm) filled with 40 g Osmocote[®] 'controlled release fertilizer' pellets (13% N, 13% P_2O_5 and 13% K_2O). Two bags were fixed to the plastic net in cages assigned to nutrient enrichment, facing land and ocean side to ensure the supply with nutrients independent from current direction. Fertilizer diffusers were exchanged weekly to ensure constant supply with nutrients. The remaining fertilizer was dried and re-weighed to estimate daily nutrient release. This fertilization method was described in a study by Worm et al. (2000) and tested against other methods prior to field work. The preliminary studies revealed that bags with slow-release fertilizer released highest concentrations of nutrients and release was most stable over 7 days compared to other methods (slow-release fertilizer in flower pots (Smith et al. 2001); nutrient enriched agar in perforated tubes (Teichberg et al. 2008)) (Stuhldreier, unpublished data).



Fig. 3: Visualization of cage designs. Left: uncaged, right: caged treatments (figure by Becker, using Maxon Cinema 4D and Adobe Photoshop).

Tiles for analyses were collected randomly from each cage every week, stored in ziplock bags for transportation, rinsed with seawater to remove sediment and mobile invertebrates and analyzed for process- and status-variables in the Center for Oceanic Research and Education, South East Asia (COREsea) on Koh Phangan, Thailand in the following order (1-3). All analyses were carried out on the day of sampling. Afterwards, algae mass was dried and ground to powder for elemental and stable isotope analysis at the Leibniz Center for Tropical Marine Ecology (ZMT) in Bremen, Germany (4).

 The quantitative and qualitative differences in succession and species composition on the tiles were investigated using photo documentation and the software CPCe 4.1 for Windows (Kohler & Gill 2006). All organisms recruited to the tiles were categorized into the following groups: turf algae (filamentous species < 3 cm height and cyanobacteria), macroalgae, crustose coralline algae (CCA) and sessile invertebrates. Cover was observed below 50 random grid points projected on each tile with CPCe 4.1. The number of points was stated to represent the actual cover with high precision assuming around 20 % cover on tiles (Pante & Dustan 2012).

- 2. The differences in respiration and photosynthetic activity of the algal communities on tiles were tested by incubating the tiles in the dark and under light conditions as measured at 5 m water depth (700-1200 LUX) at constant temperature (*in situ* temperature ± 0.2 ℃) in airtight 500 mL Weck[®] jars stored in a 100 L cooling box for 1.5 h. Water for the incubations and the cooling box was collected on the day of sampling from the reef in 5 m water depth with dry-bags. Oxygen concentrations at the start and at the end of incubations were measured with a *HACH multi HQ40d* optode. Jars were not stirred during incubations as the aim was to measure diffusive oxygen flows, but a possible concentration gradient was destroyed prior to measurement by removing the tile from the jar and stirring gently.
- 3. For algae dry mass estimates, tiles were rinsed with fresh water to remove salt, and all algae were scraped from each plate with razor blades. These samples were collected in pre-weighed, pre-combusted tinfoil and dried for 7 days in a solar driven dry oven ensuring 40-48 °C for at least 5 h per day. Dry mass was then determined with a digital precision scale (accuracy 0.001 g).
- 4. Samples were transported to the ZMT and again dried for 24 h at 40 ℃ before ground to powder with mortar and pestle. Elemental analyses for carbon (C), nitrogen (N) and organic carbon content (C_{org}) were performed for weeks 2, 4, 6, 8, 10 and 12 in three replications for each treatment (plots 2, 3, 4) with a CHN elemental analyser (*Eurovector Euro EA 3000*; precision calculated from analyses of standards: ± 0.44 % for C and ± 0.01 % for N (SD)). Analyses were prepared by weighing 1 ± 0.1 mg sample or standard (Apfelblatt) into 10 x 10 mm tin (C/N) and silver cups (C_{org}). For C_{org} analyses, 100 µL 1N HCl was added to remove CaCO₃ and cups were dried for 24 h at 40 ℃.

Stable isotope analyses for quantifying fertilizer uptake ($\delta^{15}N$ signatures) were performed for week 6 in three replications per treatment (plot 2, 3, 4) in a mass spectrometer (*Thermo Finnigan DeltaPlus* with *Flash EA 1112 Series*; precision: ± 0.06 ‰ (SD)). Week 6 was chosen because the number of samples was limited by device utilization and this specific sampling occasion took place before strong winds could possibly reduce nutrient uptake. Results from elemental analyses served as initial value for calculating the algae dry mass weighed into 5 x 12 mm tin cups (target value 40 µg N).

As the study was closely linked with two other projects, the set-up included another set of tiles, fixed to the underside of the net to study the succession of invertebrate recruitment as

part of the Master thesis of Pepe Bastian. Sediment samples from below the cages were analyzed for chlorophyll a concentrations and oxygen production by Soureya Becker for her Master thesis. Sampling took place on two consecutive days each week by scuba diving.

2.4 Environmental parameters

Caging artifacts:

Light availability was monitored using $HOBO^{\textcircled{B}}$ Pendant Temperature/Light Data loggers displayed in all experimental set-ups of plot 2 during the 12 weeks (15 min intervals). Water movement, as a proxy for currents and flow exposure, was measured in open and caged set-ups of different plots as well as on two different occasions using the clod-card technique reviewed by Jokiell & Morrissey (1993). Customary fine plaster from a local construction market was mixed with water, poured in round bottomed egg trays and allowed to harden for 48 h at room temperature. The flat bases were sanded to calibrate all clods to a weight of 20.0 \pm 0.2 g. Then, the clods were glued to terracotta tiles (5 x 10 x 0.5 cm) with *Pattex*[®] epoxy steel filler. Clod cards were weighed after drying prior to deployment in the field for 24 h and after. The calibration was accomplished by simultaneously immersing clod cards in buckets with 20 L seawater for 24 h which allowed the calculation of a dimensionless diffusion-factor (DF = weight loss field/weight loss bucket). This factor was used for comparing water movement at different plots or over time.

Nutrients:

At regular intervals, water samples were taken within the plots to follow the success of nutrient enrichment. Water for nutrient analyses was sampled with 60 mL syringes from around 5 cm above the central tiles. For caged set-ups, syringes were equipped with 30 cm rubber tubes to facilitate sampling from outside the cage without disturbance. Samples were filtered through VWR glass microfibres filters 691 (Ø=47 mm, particle retention 1.6 µm) to remove particles and stored at 4 °C until analysis. The determination of nitrate and phosphate was performed in the COREsea laboratory within 4 h after sampling using spectro-photometric analysis following the standard protocols of Zhang & Fischer (2006): "simplified Resorcinol method for determination of nitrate" for NO_x ($NO_2^- + NO_3^-$) and Murphy & Riley (1962) described in Grasshoff et al. (1999) for PO_4^{3} . The spectrophotometer (Biochrom Libra S12) with a path-length of 5 cm enabled measurement of low nutrient concentrations. The detection limit for phosphate concentrations, determined by calibrations using 7 different standard concentrations, was 0.062 µmol L⁻¹. A two-point-calibration was performed at each day of measurement to ensure constant accuracy of results. The result section of this thesis only includes values for phosphate as determined nitrate concentrations were considered untrustworthy due to 7-point-calibrations with very low precision and highly variable values over time.

Surveys:

Benthic-, fish- and invertebrate-surveys as well as feeding observations on the cages were conducted at the study site to evaluate the environmental state of the ecosystem and to quantify the influence of herbivores.

To evaluate the status and the benthic community composition of the reef in Mae Haad, data were collected using the Line-Point-Intercept (LPI) -Transect method described e.g. in Hill & Wilkinson (2004) and evaluated as most cost effective and accurate method by Nadon & Stirling (2005). Three 50 m transect lines were displayed in a row in 4 m depth and investigated by scuba divers, recording benthic taxa directly below the transect line in 0.5 m steps. The categories classified included hard corals to genus level, macroalgae, turf algae, crustose coralline algae, other invertebrates, rubble and sand.

Fish abundance and biomass was calculated using underwater visual fish census methods described by Green & Bellwood (2009). Three belt transects of 50 m length, 5 m width and around 2.5 m height were observed by scuba divers swimming over the area at a constant swimming speed. Transects were observed in triplicates, the swims were separated by 5 min waiting periods outside the transect area. Key fish species \geq 10 cm were recorded in abundance and assigned to size classes (5 cm intervals). The length was converted into biomass by using abundance, mid value of size class and species specific coefficients available on www.fishbase.org or reviewed in Green & Bellwood (2009).

Sea urchins, sea cumbers and hermit crabs were counted in three 50 x 5 m transects in direct proximity to plots along the reef border during daytime.

Feeding observations on the experimental set-ups were conducted after 14 weeks of exposure, counting fish abundance within the cage at time intervals of 5 min and bites on the tiles for a continuous period of 30 min.

2.5 State of eutrophication

Six stations along a transect running from the experimental plots at the reef border to a river discharging into the bay were analyzed for water and sediment parameters on three occasions in June 2012 (20th, 27th, 30th).

Water parameters investigated included phosphate concentrations, chlorophyll a (Chl a) concentrations, particulate organic carbon (POC), particulate nitrogen (PN) and biological oxygen demand (BOD). Nutrient analyses were carried out as described in 2.2.3. For Chl a analyses, 3 L of seawater or 0.5 L of river-water was filtered on pre-combusted *VWR glass microfibres* filters ($\emptyset = 47$ mm, particle retention 1.6 µm) with an electric vacuum pump (max pressure < 200 mbar) and incubated in 10 mL 90 % Acetone for 24 h at 4 °C before analyzed in a spectrophotometer (*Biochrom Libra S12*) according to *ESS Method 150.1* (Wisconsin State Lab of Hygiene 1991). For POC and PN analyses, 2 L of seawater or 0.5 L of river-water was filtered on pre-weighed, pre-combusted *GF/F Whatman* filters ($\emptyset = 47$ mm, pore

size 0.7 μ m). Filters were dried and stored at 4 % until transported to the ZMT, dried at 40 % for 24 h, weighed again and analyzed in a CHN elemental analyzer (*Eurovector Euro EA 3000*). Water for BOD was incubated in the dark for 24 h and oxygen concentrations were measured as described in 2.2.2.

Sediment parameters monitored included CaCO₃ content, POC, PN, ChI a concentrations and sedimentary oxygen consumption (SOC). For sediment ChI a, 6 g of sediment (wetweight) was incubated in 8 mL 90 % Acetone for 24 h at 4 °C before analyzed in the spectrophotometer according to *ESS Method 150.1*. Volume of filtered water in the given formula was replaced by dry-weight of sediment (~ 4 g). Sediment samples were transported to the ZMT, ground to powder with mortal and pestle and prepared for C/N (15 ± 1 mg in tin cups) and C_{org} analyses (30 ± 1 mg in silver cups + 200 µL 1 N HCl for 3 days). Incubations of 12 g sediment (wet weight) were conducted as described in 2.2.2 for 4 h in the dark to calculate SOC.

Additional transects parallel to the beach and along the outer reef border were sampled to follow the distribution of nutrients within the bay.

2.6 Statistics

Statistical analyses were performed using GraphPad Prism 5.0 and SigmaPlot 12.0 for Windows. Significance level for all tests was set to 0.05. Data were tested for a Gaussian distribution and for homogeneity of variances prior to analyses.

Nutrient enrichment success and caging artifacts were evaluated using one-tailed unpaired ttests. Dry mass, algae cover on tiles, nitrogen content of the algal biomass and oxygen production/consumption in the different treatments were compared for every sampling occasion using non-parametric Kruskal-Wallis-tests with Tukey post hoc tests. For comparison of mean values over time, parametric ANOVA or t-tests were used if requirements of normality and homogeneity of variances were achieved. Otherwise, Kruskal-Wallis- or Mann-Whitney-tests were applied.

The chronological sequence of biomass or cover parameters was depicted graphically to examine different responses to treatments over time by linear regression analyses. Slopes of regression lines were compared using the Kruskal-Wallis-test.

Algae dry mass and total algae cover were tested on responses to treatment and time by using two-way mixed-model ANOVAs in GraphPad Prism 5.0. Caging and fertilization were tested on single and interactive effects on dry-weight and cover with two-factorial ANOVAs in SigmaPlot 12.0.

3. Results

All values given in the text are mean values \pm standard deviations (SD). Values in figures are means \pm SD or means \pm standard error of the mean (SEM) which is stated in the legend of respective figures.

3.1 Environmental parameters & experimental set-up

The bottom water temperature increased from 28 °C in February to 32 °C in April and back to 30 ℃ in June. Water levels during the study period decreased considerably due to seasonal variations in the circulation pattern in the Gulf of Thailand (Buranapratheprat & Bunpapong 1998). Salinity varied between 31.0 and 31.8. Comparison of diffusion factors and personal observations revealed that plot 1 experienced a higher hydrodynamic force than the other plots (Fig. 4), but this difference was not significant. More pronounced differences in hydrodynamics were observed on a temporal scale. When the westerly Pattaya winds started in the end of April, the studied bay was constantly impacted by high surface waves and strong underwater surge. Comparison of diffusion factors from clod cards deployed in March and May revealed a significant difference in conditions (Fig 4; Mann-Whitney-test, p = 0.0286). From week 9 onwards, several cages, especially in plot 1, were lifted, tilted or moved due to high water motion at the reef. When the wind increased in mid May, with constant wind speeds of 15-20 knots, the loosening of the set-ups increased drastically despite the fixation of cages with additional weights. Therefore, the experiment was terminated after the twelfth sampling. Observations on feeding behavior of fish on settlement tiles continued during the following weeks.



Fig. 4: Hydrodynamics [DF] at different plots (1, 3 and 4) in March 2012 (A) and comparison of hydrodynamics [DF] in March vs. May (B) using clod cards made from plaster. DF: dimensionless diffusion factor, calculated from the ratio of weight loss of field tiles versus weight loss of control tiles. Significant differences ($\alpha = 0.05$) between groups are indicated with *. Lines in boxes represent mean values with whiskers indicating min and max values.

Caging artifacts in the present experimental set-up were small. Water movement, measured by the clod card technique, was slightly but not significantly lower in caged plots compared to uncaged plots. The mean total mass lost of fertilizer from the diffusers was not affected by the difference in hydrodynamics and averaged 4.4 ± 1.1 g per diffuser after 7 days of

exposure (Fig. 5). This corresponds to a daily release of around 164 mg N and 164 mg P in enriched plots.

Mean daily light availabilities within experimental plots ranged from 400 to 2200 lux and were on average 23 \pm 9 % lower in caged set-ups (Fig. 6). The difference was significant (Mann-Whitney-test, p \leq 0.0001).

The release of **nutrients** from diffusers in enriched treatments was evidenced by fertilizer weight loss and very high nutrient concentrations in direct proximity of diffusers. But nutrient water concentrations in the center of experimental plots (20 cm distance to diffusers) were not significantly elevated in enriched (0.172 \pm 0.049 µmol PO₄³⁻ L⁻¹) compared to un-enriched treatments (0.157 \pm 0.043 µmol PO₄³⁻ L⁻¹). The analysis of δ^{15} N in algae tissue of the different treatments did not prove uptake of artificially introduced nutrients (lower δ^{15} N-value) by algae in fertilized treatments.



Fig. 5: Hydrodynamics [DF] and loss of fertilizer [g] after 7days exposure in uncaged versus caged treatments. DF: dimensionless diffusion factor, calculated from the ratio of weight loss of field tiles versus weight loss of control tiles. Lines in boxes represent mean values with whiskers indicating min and max values.



date

Fig. 6: Mean daily light availabilites [lux] comparing uncaged (continous line) and caged (broken line) set-ups at 5 m water depth for February and March 2012.

3.2 Effects of treatments on algae growth and diversity

The effects of simulated impacts eutrophication and overfishing on algae dry mass and cover were small. Algae growth did not show consistent responses to treatments on individual sampling occasions. Over time, nutrient enrichment did neither affect algae mass or cover nor macroscopically visible diversity of algal assemblages. Caging on the other hand had a significant effect on algae dry mass (two-factorial ANOVA, caging: p = 0.045) but not on total algae cover and resulted in higher diversity of algal assemblages including different species of fleshy brown macroalgae. An interactive effect was not observed as caging effects dominated responses in combined treatments.

The fouling on tiles was dominated by turf algae, a conglomerate of small filamentous algae and cyanobacteria, in all treatments. **Macroscopic differences** were visible between caged and uncaged tiles. Whereas uncaged tiles experienced short and dense turf with occurrence of small tough red algae but no fleshy brown macroalgae, caged tiles showed less dense fouling by much taller turf algae. The loose aggregates on caged tiles efficiently trapped sediment and were lost more easily during transport and handling than the closely attached fouling on uncaged tiles. From week 7, individuals of fleshy brown macroalgae started to grow on caged tiles. The algae were most frequently of the genus *Padina*, usually represented by small individuals. Other species included small *Sargassum* sp. and one large individual of *Dictyota* sp.. Macroscopic differences in algae diversity or quantity between unenriched and enriched treatments were negligible.

Algae dry mass did not show pronounced differences between treatments for individual sampling occasions (Fig. 7). Algae dry mass on tiles started low with values of 0.1 - 0.2 mg dry mass cm⁻² after week 1. In weeks 2, 3 and 4, tiles in nutrient-enriched treatments showed more fouling than un-enriched treatments, but differences were not significant. From week 4 to week 5, the algae dry mass on caged tiles doubled. Algae growth leveled off during the next two weeks, and the combined treatments showed significant higher values than the controls in week 6 (Kruskal-Wallis-test, p = 0.0321). From week 7, the control treatment showed increased growth. During the next three weeks, treatment C (cage, no nutrients) exhibited highest algae mass due to more frequent occurrence of macroalgae on tiles; the difference to treatment B (nutrients, no cage) was significant in week 10 (Kruskal-Wallis-test, p = 0.0257). After 12 weeks exposure, differences between treatments were still small. From week 1 to week 12, mean algae dry mass in total increased 32 fold in the controls, 64 fold and 30 fold in the eutrophication and overfishing treatments respectively and 75 fold in the combined treatments. These are mean values; replicates of the same treatments showed highly variable results.

The high variability in fouling was most evident by depicting the succession of algae dry mass. Fitted curves over time showed steeper slopes for caged treatments but the standard

error was high and the goodness of fit lower than in uncaged treatments, indicating high fluctuations in herbivore excluding systems (Tab. 2). Especially in plot 1, biomass values varied considerably over time for caged tiles, indicating higher hydrodynamics in the area closest to the channel. Enriched treatments showed slightly smaller slopes than the respective non-enriched treatment. The differences in slopes were not significant.



Fig. 7: Algae dry mass [mg cm⁻²] on settlement tiles over time, comparing different treatments. A: control, B: nutrient enrichment, C: herbivore exclusion, D: nutrient enrichment & herbivore exclusion. Significant differences between treatments ($\alpha = 0.05$) are indicated by *. Columns represent mean values with error bars indicating the SEM.

Tab. 2: Slopes (mean \pm SEM) and r² for linear trend lines approximating the succession of algae dry mass in treatments A-D.

	Α	В	C	D
Slope	0.711 ± 0.087	0.688 ± 0.076	0.946 ± 0.154	0.818 ± 0.134
r²	0.870	0.891	0.790	0.789

There was no consistent trend in responses to treatments based on ANOVAs of individual weeks. The experiment was based on a randomized block design, and tiles from the same cage can be treated as matched set of subjects. A two-way mixed-model ANOVA showed that time had a significant effect on algal growth (p < 0.0001), whereas treatment did not significantly affect results (Tab. 3). The interaction of factors was not significant which showed that treatment did not have an identical influence on algae dry mass at the different time points. The matching of data from the same set-up was considered effective (p = 0.0018); this justifies the use of a matched-data test and suggests that position of cages had a higher influence on algae growth than treatments. Because the matching considered in the test was not based on data from one individual, but from independent tiles within the same cage, there is no problem with assumptions of circularity which usually have to be tested in 'repeated-measures ANOVAs.

When nutrients and caging were tested for independent and synergistic effects on algae dry mass in a two-factorial ANOVA, caging showed a slightly significant effect (p = 0.045), whereas nutrient enrichment and the interaction did not (Tab. 4). The fact that interaction of factors was not significant showed that the effect of different levels of nutrients did not depend on the level of caging.

Tab. 3: Two-way mixed-model ANOVA testing effects of factors treatment and time on algae dry mass. Data were tested for normality with D'Agostino & Pearson-test. Significant p-values are indicated in **bold**.

Source of Variation	DF	MS	F	p-value
Time	11	141.80	21.65	< 0.0001
Treatment	3	27.89	1.51	0.2620
Interaction	33	6.41	0.98	0.5090
Subjects	12	18.47		
(matching)			2.82	0.0018
Residual (Error)	132	6.55		
Total	191			

Tab. 4: Two-factorial ANOVA testing the single and interactive effects of nutrient enrichment and caging on algae dry mass. Data were sqrt-transformed to achieve normal distribution (Shapiro-Wilktest). Significant p-values are indicated in **bold**.

Source of Variation	DF	MS	F	p-value	
Nutrients	1	0,233	0,314	0,576	
Caging	1	3,005	4,062	0,045	
nutrients x caging	1	0,197	0,266	0,607	
Residual	172	0,740			
Total	175	0,747			

Total algal cover on settlement tiles increased significantly over time, but was not affected by treatments when comparing individual weeks with one-way ANOVAs or all data over time (Fig. 8 A, Tab. 5). As matching of subjects was not effective, influences of factors time and treatment were tested by two-factorial ANOVA. A two-factorial ANOVA with factors caging and nutrients did not show significant effects on total algal cover.

Macroalgae on tiles were too rare to be tested statistically as individual algal group. Figure 10 B shows mean tile cover after 12 weeks when macroalgal cover was highest. Macroalgae only occurred in caged treatments.





Tab. 5: Two-factorial ANOVA testing effects of factors treatment and time on total algae cover. Data were tested for normality with Kolmogorov-Smirnoff-test. Significant p-values are indicated in **bold**.

Source of Variation	DF	MS	F	p-value
Time	5	4402	36,80	< 0.0001
Treatment	3	188,3	1,574	0.2032
Interaction	15	195,7	1,636	0.0853
Residual (Error)	72	119,6		
Total	191			

3.3 Effects of treatments on oxygen production and consumption

The effects of simulated impacts eutrophication and overfishing on oxygen fluxes in the algal assemblages were very small and only visible in significantly higher mean respiration of caged compared to uncaged tiles (Mann-Whitney-test, p = 0.002).

The differences in **oxygen production** by algal communities on tiles are shown for week 9 to 12 when light and temperature conditions were most stable (Fig. 9 A). Values from incubations shaded by the walls of the cool box were excluded if they differed considerably from other replicates. Significant differences between treatments were only observed in week 9 (Kruskal-Wallis-test, p = 0.0425). Macroalgae containing tiles produced more oxygen than those with turf cover (e.g. week 11, 3C: large *Dictyota* sp.; week 12, 2D: large *Padina* sp.). Oxygen production was slightly higher for caged treatments and increased over time with increasing algae mass on tiles.

O₂-consumption increased slightly from week 7 to week 12 (Fig. 9 B). Individual weeks did not show considerable distinctions in respiration, but means over the six weeks revealed a significant difference in oxygen consumption on uncaged versus caged tiles (Mann-Whitney-test, p = 0.0002).

The **gross primary production** per area was calculated assuming 12 h light and 12 h darkness in tropic regions. Algae communities on tiles achieved mean gross primary production (GPP) of 0.591 \pm 0.242 g C m⁻² d⁻¹. This is a mean value for weeks 10 to 12, because GPP in week 9 was very small due to low photosynthetic activity caused by lower light levels during incubations. Algal assemblages on caged tiles fixed slightly more carbon than on uncaged tiles. The mean production to respiration ratio was 1.894 \pm 0.378. Values above 1 indicate that produced organic matter in the reef is not efficiently recycled. Results suggest a high proportion of primary producers in the established community (high production : respiration ratio) dominated by turf algae (low GPP).



Fig. 9: Oxygen production (A) and consumption (B) of incubated tiles over time. Treatments A: control, B: nutrient enrichment, C: herbivore exclusion, D: nutrient enrichment & herbivore exclusion. Significant differences between treatments ($\alpha = 0.05$) are indicated by *. Columns represent mean values with error bars indicating the SEM.

3.4 Effects of treatments on %N, C:N, and Ctotal:C

The content of nitrogen and carbon in algal tissue was not affected by simulated impacts eutrophication or overfishing. A more striking difference occurred over time with significantly increasing N-content and slightly decreasing C:N-ratio.

The **percentage of Nitrogen** in algal tissue was very low and ranged from 0.3 to 1.2 % (Fig. 10 A). Whereas an influence of treatments was not visible, the values increased significantly over time (comparison of week 4 and 12 with unpaired t-test, p < 0.0001).

The molar **ratio of organic Carbon to Nitrogen** (C:N) was not influenced by treatments and decreased slightly over time corresponding to the increasing N-content (Fig. 10 B). The mean ratio was 13.786 ± 4.011 , which is almost two times the Redfield-ratio for marine organisms of 6.625. The analysis of a single macroalgae revealed a even higher C:N ratio of 17.725.

The ratio of **total Carbon to organic Carbon** (C_{total} :C) was 2.03 ± 0.67, not influenced by treatment and decreased significantly from week 4 to week 12 (unpaired t-test, p = 0.0011).



Fig. 10: Nitrogen content [%] (A) and molar C:N ratio (B) in algal tissue over time. Columns represent mean values with error bars indicating the SEM.

3.5 State of eutrophication

Six stations on an ocean-land-transect from experimental plots at the reef border into a river discharging into the bay were investigated for water and sediment parameters. The river experienced high loads of anorganic nutrients and organic matter and discharged the polluted water into the bay.

Stations 1-4 were located in the bay, 5 and 6 in the river (Fig. 11 A). The river was not directly connected to open water at this time of the year. Samples for station 4 were taken in shallow water directly where the river water seeps through the sand (Fig. 11 B). Here, the influence of the river is visible in lower salinity and higher phosphate concentrations (Tab.6).



Fig. 11: Location of sampling stations along eutrophication gradient (A) and river discharge at the beach (B) at the study site Mae Haad.

Tab. 6: Abiotic parameters at stations 1-6 (mean \pm SD). Stations 1-4 are located in the bay, 5 & 6 in the river.

station	depth (m)	Temp (℃)	Light (LUX)	salinity	pН	PO ₄ ⁻³⁻ (µmol L ⁻¹)
1	2.4 ± 0.1	30.7 ± 0.5	4819 ± 451	31.3 ± 0.3	7.93 ± 0.04	0.155 ± 0.080
2	0.9 ± 0.2	31.6 ± 1.0	10956 ± 822	31.4 ± 0.3	8.03 ± 0.01	0.097 ± 0.060
3	0.7 ± 0.3	31.8 ± 1.1	10219 ± 3613	31.4 ± 0.3	8.07 ± 0.04	0.111 ± 0.058
4	0.3 ± 0.1	32.8 ± 2.8	12980 ± 5870	30.9 ± 0.6	8.17 ± 0.09	0.179 ± 0.138
5	0.2 ± 0.1	31.6 ± 2.5	13726 ± 2460	0.6 ± 0.2	8.02 ± 0.02	1.873 ± 0.314
6	0.2 ± 0.0	33.4 ± 1.6	14131 ± 2923	0.1 ± 0.0	7.50 ± 0.13	1.521 ± 0.296

The water in the river was highly turbid, the sediment was coarse and of terragenous origin. Phosphate water concentrations in the river were ten times higher than the ones in the reef (t-test, p < 0.001). Parameters indicating high loads of organic matter also had high values in river stations (Fig. 12). POC and PN in the water column were lowest in stations 3 and 4 and increased in both directions. Water Chl a concentrations were significantly higher in river stations (t-test, p = 0.0001). Calcium-carbonate content was high in the reef sediment and low at river stations. POC and PN in sediment samples showed very low values for station 4. Sediment Chl a concentrations were also lowest at station 4; values for station 5 & 6 were significantly higher than stations 1, 2 & 3 (t-test, p = 0.0012).



Fig. 12: Parameters in the water column (left) and the sediment (right) along the sampling gradient. Stations 1-4 were located in the bay, stations 5 and 6 in the river (see Fig. 13 for exact locations). PO_4^{3-} : phosphate; POC: particulate organic carbon; PN: particulate nitrogen, ChI a: chlorophyll a. Columns represent mean values with error bars indicating the SD.



Fig. 13: Biological oxygen demand in water column [μ mol O₂ L⁻¹ d⁻¹] (left) and sedimentary oxygen consumption [μ mol O₂ g⁻¹ d⁻¹] (right) along with regression analyses using particulate organic carbon contents in the water or sediment respectively.

Biological oxygen demand in the water column was highest in samples from station 1 at the reef border, followed by the river stations (Fig. 13). The regression with particulate organic carbon in the water was not significant. Sedimentary oxygen consumption was significantly higher in river stations (t-test, p = 0.0024). Regression analysis with POC in the sediment did not show significant interrelations.

Additional water sampling along a transect parallel to the beach revealed highest phosphate water concentrations where the river discharged into the bay. The river-water seeped through the sand and its influence was detected in the entire southern part of the bay where the river ran parallel to the beach (Fig. 14 A). In the far north of the bay, in a semi-enclosed area east of the island Koh Ma, elevated phosphate concentrations were most probably caused by resorts discharging their wastewater onto the beach as the picture taken at this location suggests (Fig. 14 B).



Fig. 14: Phosphate concentrations $[\mu mol L^{-1}]$ at locations indicated in the map (A). Size of the place marks illustrates the relative amount of phosphate (A). Picture of waste pipes originating from a resort at the location indicated by the arrow (B).

This pattern in phosphate concentrations measured in samples along the beach was slightly reflected in surface water samples from the outer border of the reef (Fig. 15). Stations 5 & 6 with elevated phosphate concentrations were situated at the mouth of the sandy channel where water exchange with the shallower part is relatively high. Stations 9-11 showed elevated phosphate concentrations as well and were located in the northern part of the bay, where water from the semi-enclosed area east of the island Koh Ma entered the bay. All surface water concentrations were overall lower than measured close to the bottom in previous weeks.



Fig 15: Stations of surface water sampling along the outer reef border. The diagram on the right shows respective phosphate concentrations [µmol L¹]. Columns represent values of one sampling occasion.

3.6 Reef status

The reef in Mae Haad is characterized by relatively low coral and high turf algae cover indicating degradation, but macroalgal cover is very low. The importance of invertebrate grazers like sea urchins is negligible but herbivorous fish account for a high share of total fish abundance and biomass (64 %).

Rugosity of the reef surface in the northern part of the bay was 0.649 ± 0.106 , measured as the ratio of the distance covered by a chain which was placed onto the reef structure following caves and crevices versus the chain's actual length. A mean value close to 1 would be measured at a flat surface whereas small values indicated a highly complex structure of the reef surface. The relatively small standard deviation indicated that the reef structure was evenly complex over the measured area.

Live coral cover (LCC) was 20.0 ± 4.4 %, dominated by massive *Porites* colonies (Fig. 5). Hard coral genera not given in the figure as they were not observed in surveys, but frequently during diving included *Platygyra*, *Goniastrea*, *Pavona*, *Montipora*, *Pachyseris*, *Goniopora* and *Alveopora*. CCA and macroalgae were rarely represented with 1.0 ± 1.7 and 4.4 ± 5.9 %, respectively. The most frequent substrate was dead coral rock, often *Acropora* sp., overgrown with filamentous turf algae (41.7 ± 5.9 %). Sand also had a high share in the benthic cover due to natural occurring sand pools within the patchy reef (28.7 ± 9.3 %). Diversity of benthic composition was calculated to be 1.03 ± 0.05 (Shannon-Wiener-Index).



Fig. 5: Benthic composition in Mae Haad given as cover [%]. Values derived from Line-Point-Intercept surveys (3 x 100 points). LCC: live coral cover summing up cover of all scleractinian corals. Columns represent mean values with error bars indicating the SD.



Fig. 6: Abundance of reef fish [individuals m^{-2}] in different size classes (A) and corresponding biomass [g m^{-2}] calculated from abundance, mean of size class and conversion factors (fishbase.com) (B). herb.: herbivorous; carn.: carnivorous. Columns represent mean values with error bars indicating the SD.

Total **fish** abundance was 0.137 ± 0.040 individuals m⁻². The most frequent groups observed were rabbitfish (Siganidae) with 29 %, followed by parrotfish (Scaridae; 28 %), wrasses (Labridae; 16 % carnivorous species, 7 % herbivorous species) and butterflyfish (Chaetodontidae; 20 %) (Fig. 6 A). Herbivorous fish thereby accounted for 64 % of total fish abundance. Fish biomass was dominated by parrotfish (8.5 \pm 5.4 g m⁻²) and carnivorous wrasses due to occurrence of some large individuals \geq 30 cm. Total fish biomass was 21.3 ± 9.2 g m⁻², with herbivorous fish biomass accounting for 13.7 \pm 9.1 g m⁻² (= 64 % of total biomass). Different species of fish were observed to graze on settlement tiles. Main consumers were adult parrotfish (Scaridae), schools of juvenile parrotfish (10-15 cm length), wrasses (Labridae), few damsels (Pomacentridae) and blennys (Blenniidae), which lived in open cage tubes. Also, small and medium sized hermit crabs were observed within set-ups frequently, leaving entire tiles in the open plots cleaned from fouling (survey along reef border: 0.043 ± 0.023 ind. m⁻²). A school of juvenile batfish (*Ephippidae*) was observed resting between cages of plot 2 frequently but did not feed. Rabbitfish (Siganidae) browsed the area, but were not seen to graze on tiles. Small carnivorous damselfish sheltered within or close to cages, but did not feed on algae. Sea urchins Diadema setosum and Echinotrix calamaris were never observed within the plots and hardly seen in the surroundings (survey along reef border: 0.002 ± 0.003 ind. m⁻²). Feeding observations revealed fish abundances of 7-20 individuals in uncaged plots and around 2 individuals, small enough to enter the cages, in caged plots. Tiles experienced 6-12 bites min⁻¹ in uncaged plots, mostly caused by a school of 10 juvenile parrotfish roaming between cages and neighboring coral rock to graze on turf algae. Feeding took place haphazardly on settlement tiles, on the net between tiles and on cage structure. Tiles in closed cages only experienced bite rates of 0-0.5 bites min⁻¹ by small fish able to enter the cages.

4. Discussion

Widespread anthropogenic changes of nutrient cycles and consumer regimes challenge ecologists to predict the response of primary production and ecosystem function to these perturbations (Gruner et al. 2008b). This study aimed to assess the impacts of eutrophication and overfishing through *in situ* simulation in a typical coral reef of South East Asia, impacted by run-off from land, fishing and tourism.

In contrast to most published studies (e.g. reviewed in Burkepile & Hay 2006; Gruner et al. 2008), differences in algae growth and activity in response to nutrient enrichment and/or herbivore exclusion were negligible in this study. Parallel studies on the response of invertebrate recruitment patterns did not show differences between treatments at the present state of data evaluation either (Bastian, unpublished data). Sediment chlorophyll a concentrations were not influenced by nutrient addition, but were significantly higher in caged treatments. If this effect was due to herbivore exclusion or caging artifacts still has to be evaluated (Becker, unpublished data).

4.1 Effects of in situ fertilization

The comparison of algae dry mass, cover and oxygen fluxes in unenriched versus enriched treatments did not show any significant differences. Turf algae were equally abundant on unenriched and enriched tiles. Macroalgae established on caged tiles showed no clear preference to nutrient levels.

Release of nutrients from fertilizer diffusers worked well, nitrate and phosphate concentrations in water samples taken in direct proximity of diffusers were above the upper detection limit of nutrient determination methods. The daily release of nutrients calculated from fertilizer weight loss was higher than in studies by Belliveau & Paul (2002) conducted at 10 m water depth, where a release of 95.2 mg N and P d⁻¹ resulted in significant water nutrient enrichment. However, although *in situ* fertilization in the present study was successful regarding nutrient release from diffusers, water samples from above settlement tiles did not show elevated phosphate water concentrations in enriched compared to unenriched treatments because nutrients in the present study likely diluted quickly due to fast water currents in 5 m water depth. The uptake of artificially introduced nutrients by algae in fertilized treatments could not be evidenced by elevated nitrogen content or a lower δ^{15} N-value in algal tissue. Uptake of nutrients was either not possible because the enrichment of the watercolumn was too low, or background concentrations already satisfied algae growth.

The percentage of nitrogen in algal tissue was increasing over time in all treatments, indicating an increase in available nutrients in the water column. This could be due to seasonal changes in the current-regime (Buranapratheprat & Bunpapong 1998) or resuspension of nutrients from the sediment when the wind increased to 5 beaufort and the waves became stronger during May (www.windguru.cz and personal observations).

Increasing N-contents may have also been caused by increased nitrogen fixation by cyanobacteria which likely occurred in higher abundances when the cover of closely associated turf algae increased.

Contrary to the obtained results, where nutrient enrichment did not lead to changes in algae dry mass or cover, some studies reported significant increases of turf algae (Vermeij et al. 2010) or fleshy algae in response to nutrification (e.g. Lapointe et al. 1997; Smith et al. 2001; Littler et al. 2006). However, in numerous other studies nutrient enrichment did not lead to elevated algal growth (e.g. Thacker et al. 2001; Belliveau & Paul 2002; McClanahan et al. 2003). The contradictory results concerning algal response to nutrification suggest that nutrients may stimulate growth of some macroalgae, while that of others exhibits no change (Thacker et al. 2001) or is even suppressed by high nutrient concentrations (McClanahan et al. 2003a). The suppression of macroalgae may be caused by changing competitive abilities; smaller species with higher surface area to volume ratios, like turf algae, are more likely to benefit from high nutrient conditions (Carpenter 1990), and are thereby able to successfully compete with macroalgae for space. Turf algae can therefore be seen as indicators for high ambient nutrient concentrations.

Elevated nutrient concentrations do usually not only show effects on algae mass, but also on other parameters. The ratio of carbon to nitrogen for instance decreases with increasing ambient dissolved inorganic nitrogen concentrations (Atkinson & Smith 1983). The median C:N ratio for benthic algae is 18.3 (Atkinson & Smith 1983), while the ratios obtained during the present study were approximately 13.8. The relatively low C:N ratio compared to the median value given by Atkinson & Smith (1983) suggests that the algae in Mae Haad thrived in a high inorganic nutrient environment and were not limited by nutrient availability.

Additional nutrient input by *in situ* fertilization could obviously not trigger total algal growth relative to controls as predicted prior to the study. Contradictory to predictions of the relative dominance model (RDM), occurrence of macroalgae or CCA was not supported by nutrient addition. The lack of algal growth response to artificial nutrification, the relatively low C:N ratio and the dominance of turf algae in all treatments suggest that ambient nutrient concentrations were high, supporting the growth of opportunistic algae species.

4.2 Effects of herbivore exclusion

Caging did not have a significant influence on algae dry mass or cover when comparing treatments at individual sampling occasions. A two-factorial ANOVA of all data with factors caging and nutrients showed a significant effect of caging on algae dry mass (p = 0.045), whereas nutrients and the interaction did not. Photosynthetic activity did not differ between treatments, but mean respiration on caged tiles was significantly higher on caged than on uncaged tiles (t-test, p = 0.0002). Besides, macroscopic differences were visible. Whereas

highly grazed turf in open plots remained short, turf on caged tiles was tall and effectively trapped sediment. Macroalgae only grew on caged tiles.

Caging may not have been sufficiently effective in reducing herbivory, because a mesh size of 2 cm does not exclude small fish or micrograzers. However, as the goal was to simulate overfishing of fisheries target species, juveniles and small species as well as invertebrates were tolerated within the caged set-ups. Small fish entering the cages often came for shelter whereas feeding on tiles was rare; open plots experienced much higher feeding rates (feeding observations). Hermit crabs likely accounted for a considerable share in algal reduction on tiles, although often considered carni- or omnivorous (Ramsay et al. 1997). Larger feeding schools of parrot- and rabbitfish, often observed on the reef flat, were rarely seen close to the border of the reef, where cages were located. A reason may be the higher macroalgal cover in shallower parts of the bay. The group of fleshy macroalgae has proven to be particularly attractive to herbivores (Hay 1984, Littler & Littler 1984) and only becomes abundant where grazing pressure is lowered. Macroalgae are therefore considered early warning indicators of reef degradation by overfishing. The fact that in the present study macroalgae only grew on caged tiles indicates that the herbivore community was still able to limit macroalgae biomass in the reef.

The significant effect of caging on algae dry mass confirmed previous experiments that showed that in the presence of significant herbivory, algal biomass was minimized (Hughes 1994; Miller et al. 1999; Smith et al. 2001; Thacker et al. 2001; Belliveau & Paul 2002; Jompa & McCook 2002; Hughes et al. 2007; Burkepile & Hay 2009; Smith et al. 2010). However, algal growth response to caging in the present study was much less pronounced than in other studies where differences in algae mass or cover were highly significant (e.g. reviewed in Gruner et al. (2008)). Recent studies conducted by scientists of the *Coral Reef Ecology* group (ZMT) showed highly significant impacts of herbivore exclusion on algal dry mass or cover in the Caribbean (Rau, unpublished data) and the Red Sea (Jessen & Wild, in review). However, direct comparisons are questionable because ecosystem functions differ considerably between regions. Caribbean systems for instance show a higher rate and extent of macroalgal growth after natural or manipulative herbivore exclusion and blooms of algae appear faster and in a larger extent (Roff & Mumby 2012). This suggests that the algae growth response to caging is comparatively weak in the studied reef because the rate of macroalgal growth is considerably lower than in other study areas.

Another reason for the observed minor differences in algae mass and cover apart from slow algae growth may be low effectiveness of algal removal in uncaged treatments. The most important consumers of macroalgae in coral reefs are herbivorous fish. Roff & Mumby (2012) reviewed fish biomass estimates and stated that Indo-Pacific reefs on average support a herbivorous fish biomass of $29.0 \pm 4.1 \text{ g m}^2$ including parrotfish biomass of $13.1 \pm 2.4 \text{ g m}^2$

(both mean \pm SEM). The herbivorous fish biomass obtained in our study was 53 % and the parrotfish biomass 35 % lower than the mean values given for the Indo-Pacific. Few herbivorous fish implies low grazing pressure what may have enabled high algae cover also in uncaged treatments.

Herbivore exclusion enabled growth of macroalgae in caged treatments, but overall growth response to caging was much less pronounced than assumed. Top-down effects by herbivory in the studied reef were therefore considered effective in removing macroalgae, but insufficient in reducing total algal mass. The only minor differences in algae dry mass and cover comparing caged and uncaged treatments, together with the relatively low herbivorous biomass, suggest that the reef was already impacted by overfishing.

4.3 Interactive or synergistic effects

Synergistic effects of factors nutrients and caging could not been proved statistically, the combined treatments closely resembled caged treatments in all monitored parameters.

Some studies reported highest algae mass and dominance of macroalgae on settlement surfaces exposed to both nutrient enrichment and herbivore exclusion (Burkepile & Hay 2006; Smith et al. 2001; Smith et al. 2010). Several studies reported that nutrients had an effect on algal abundance only when herbivores were excluded (Russ & McCook 1999; Thacker et al. 2001; Jompa & McCook 2002). This suggests that nutrient effects will clearly depend on the intensity of local herbivory. All studies testing factorial combinations of nutrients and herbivory on algae have varied in terms of nutrient effects, but have all emphasized the importance of herbivory (Miller et al. 1999; Smith et al. 2001; Thacker et al. 2002). Likewise, present study's algae assemblages in combined treatments resembled the one in caged, unenriched treatments both optically and in terms of dry mass and cover.

The relative influence of caging was much higher than nutrient addition when combining the two factors. The higher response to caging compared to nutrients implies that top-down controls are more important in the studied reef than bottom-up controls and that synergistic effects are negligible. Dominance of macroalgae in treatments with low herbivory and high nutrients, as predicted in the RDM, was not observed.

4.4 Effects of time

Growth of algae on settlement tiles was not consistent and replicates showed highly variable results. Therefore, the differences between treatments in succession of algae dry mass, evaluated through comparison of trend line slopes, were not significant. Statistical analyses testing the influence of factors time and treatment revealed a highly significant influence of time on algae dry mass (two-way mixed model ANOVA, p < 0.0001), whereas treatment or the interaction did not affect results. The matching of data from identical set-ups was

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considered effective (p = 0.0018), what indicated that the position and/or conditions of individual cages had a high influence on differences in algae dry mass and may be a reason for the high variability between replicates. Small scale changes in reef conditions could thereby override possible effects of simulated eutrophication and overfishing.

The extent and rate of algae growth experienced in this study was less than in similar experiments and the abundance of macroalgae after 12 weeks was low. Most studies manipulating herbivory and nutrient levels have been conducted in the Caribbean where growth rates of macroalgae are much higher than in Indo-Pacific reefs. Roff & Mumby (2012) reviewed that increases in growth after removal of grazing pressure took at least 10 weeks in Indo-Pacific manipulation experiments, with a maximum of 25 % macroalgal cover after 20 weeks compared to increase in growth after 1-4 weeks and 90 % macroalgal cover after 20 weeks in Caribbean systems. The time lag in algal growth response to caging in Indo-Pacific reefs may explain the relatively limited occurrence of macroalgae during this study and suggests that longer experiments would likely result in higher algae mass and higher macroalgal cover.

4.5 State of eutrophication

Water phosphate concentrations measured at the study site seemed high when compared with proposed threshold values for elevated macroalgal growth of 1.0 μ mol NO₃⁻ L⁻¹ and 0.1 μ mol PO₄³⁻ L⁻¹ (Bell 1992, Lapointe 1997). However, mean phosphate concentrations measured in Mae Haad (0.16 ± 0.05 μ mol L⁻¹) were well within the range of mean nutrient concentrations reported for 1000 coral reefs worldwide (0.25 ± 0.28 μ mol NO₃⁻ L⁻¹ and 0.13 ± 0.08 μ mol PO₄³⁻ L⁻¹; Kleypas et al. 1999) and therefore did not indicate a highly eutrophic system. But samples from a river discharging into the bay revealed water phosphate concentrations ten times higher than in the bay and samples along the beach proved the release of nutrients into the reef. The high phosphate concentrations were most likely caused by sewage water from resorts fringing the river, where waste water from showers and laundries was discharged untreated (personal observation of waste pipes). Other parameters indicating high loads of organic material, like POC, PN and chlorophyll a concentrations, were also elevated in river stations.

The calcium-carbonate content in the substrate revealed biogenic origin of reef and terragenous origin of river sediments. Sedimentary oxygen consumption in the river was very high, although the sediment was coarse which is usually correlated with low microbial abundances (e.g. Dale 1974). An explanation could be the presence of diatoms or green algae in the sediment (high chlorophyll a concentrations) or very high loads of organic material due to wastewater input (high POC concentrations). Strikingly, most parameters decreased towards the beach, but increased again at the stations located in greater depth in the reef. This was most likely due to hydrodynamic effects. Whereas the shallow station

close to the beach was highly impacted by wave action preventing the accumulation of organic material on the sediment, relatively sheltered stations in deeper water offer better conditions for microphytobenthos growth or accumulation of organic material from the water column. Parameters in the water column were more even due to mixing of water bodies by tides.

High loads of nutrients and organic material from the river are very likely to influence reef functioning in the bay. High nutrient input may explain the lack of response in algae growth following additional nutrification in enriched treatments because ambient concentrations already satisfied algal growth. Beside the controversially discussed effect of nutrification on algae growth, nutrients are an ecological problem for reefs by reducing calcification rates in corals (Ferrier-Pages et al. 2000, Fabricius et al. 2005) and increasing the abundance of small blue-green algae and other microorganisms eroding the reef substrate (Holmes et al. 2000). The influence of the river discharge may vary depending on water levels, seasonal differences in algal growth or tourist numbers and should be monitored by nutrient analyses to develop management plans for pollution reduction.

4.6 Reef status

The observed coral cover of 20.0 ± 4.4 % was rather minute compared to the majority of reefs in the Indo-Pacific with coral covers of 32.6 ± 18.2 % (Roff & Mumby 2012). Monitoring of the present study's reef in 2011 revealed higher coral cover (30 ± 4 %, Schwieder 2012) and benthic diversity. However, last year's study was conducted in the southern part of the bay where the reef is less patchy with lower sand cover. Surveys in the southern bay during this study were prevented through unexpected changes in weather and visibility conditions. The proportion of dead coral rock overgrown with turf algae was high, indicating a decline in coral cover within the last years, most likely during the last bleaching events in 2003 and 2010. Especially branching species like *Acropora* sp. were severely affected (Yeemin et al. 2006). Frondose macroalgae illustrate the least desirable reef condition reflecting pollution in concert with destructive herbivore fishing practices (Littler & Littler 1984). Macroalgal cover in the studied reef was surprisingly low (4.3 ± 5.9 % instead 9.7 \pm 11.6 % as mean value for the Indo-Pacific and 27.6 \pm 25.1 % for the Caribbean (Roff & Mumby 2012)). This suggests that the reef still has a high resilience to disturbances and is not at risk of a phase shift in a short term, although coral and turf algae cover indicated severe degradation.

Resilience largely depends on the herbivore community in a reef. The abundance of sea urchins monitored in the present study was much lower than obtained during surveys at the study site in 2011 (0.016 sea urchins m⁻²; Pusch 2011) or studies from the northern Gulf of Thailand (e.g. 2.94 \pm 0.24 individuals m⁻²; Buaruang & Yeemin 2006), although filamentous algae covering dead corals provided available food sources. Surveys were conducted during

the day along the seaward border of the reef, close to experimental plots. Much higher densities of sea urchins occurred in the shallow zones of the bay where substrate was dominated by sand and macroalgae, the same was observed during other studies (Ruengsawang & Yeemin 2000). At least along the reef border of the studied bay, sea urchins were quantitatively obviously not key herbivores. The observed fish abundances $(0.137 \pm 0.040 \text{ individuals m}^2)$ were also low compared to data obtained by Pusch in 2011 in the same bay (0.96 individuals m⁻²). The relative abundance of herbivorous fish on the other hand was higher in the present study (64 % compared to only 35 % of total abundances) what could either indicate an increase in herbivore abundance due to increasing availability of food, or the fact that surveys targeted different species. The difference in total fish abundances was most likely caused by the fact that this year's study did not include damselfish in fish counts. Damsels occur in high numbers in the reef, but only account for a low proportion of total biomass due to their small size. They are difficult to identify under water and often carnivorous or with unknown food preference. As we aimed to estimate the influence of herbivorous species important for reef resilience and compare our results with data from other reef check protocols, the quantification of large species like parrot-(Scaridae) and rabbitfish (Siganidae) seemed appropriate. Comparisons of these individual groups is still difficult because the study by Pusch (2011) only gives mean values for all sampling stations from which Mae Haad was considered as one of lowest abundances. However, values for Scaridae (0,20 ind. m⁻²) and Siganidae (0,15 ind. m⁻²) obtained by Pusch are even higher than total fish counts in the present study and therefore likely much higher than parrot-and rabbitfish abundances obtained during this year. Another biasing factor may be the different location of transect deployment; last years surveys have been conducted in the southern part of the bay which is less disturbed by divers and snorkellers. Even including all biasing factors, abundance of fish in the present study seemed relatively low compared to last year. Also, the comparison with mean herbivorous fish biomass in Indo-Pacific reefs (discussed in 4.2) indicated a relatively small herbivorous community with few large individuals, although the relative share of herbivorous fish was quite high.

Low herbivore abundance and biomass compared to last year's and mean Indo-Pacific data indicate that the reef in Mae Haad is impacted by overfishing. The assumption is supported by the fact that local dive schools observed trawlers fishing just off the reef border during nighttime 1-2 times a month although fishing in the area is prohibited.

4.7 Implications and perspectives

Results of this study did not confirm working hypotheses of largely enhanced algae growth following nutrient enrichment and herbivore exclusion. The reasons were most likely high ambient nutrient concentrations caused by sewage water input via a river discharging into the bay and a relatively small herbivorous fish community. Whereas additional nutrient

supply to the experimental plots did not lead to enhanced algal growth, some responses to caging were observed. Herbivory is therefore considered the main environmental driver of benthic community composition in the studied reef. However, responses to exclusion experiments were less pronounced than in comparable studies because fishing had obviously decreased herbivore abundance.

The study revealed that macroalgal cover in the studied reef was very low although the bay was severely impacted by nutrient pollution from land. These results provide evidence opposing the theory that anhropogenic eutrophication alone is a major cause for shifts to macrolgal dominance and stress the importance of a healthy herbivore community in preventing algal dominance. From the obtained results, ubiquitous turf algae are suggested as indicator for high nutrient concentrations. Macroalgae could serve as warning indicator for overfishing as they were obviously rapidly consumed when herbivores were present.

These results for the first time describe responses to *in situ* fertilization and herbivore exclusion in a reef already impacted by pollution from land and fishing. Even relatively small herbivorous communities are obviously able to limit algal abundances despite high ambient nutrient concentration. But although macroalgal cover is still low and coral cover relatively stable, it is questionable how long the suppression of algae by grazing will be effective when anthropogenic impacts overfishing and pollution further decrease top-down and increase bottom-up factors. Therefore, efforts should be made to minimize pollution and the loss of grazers.

Management plans to improve coral reef health should always focus on the key drivers of resilience at a local scale. In the studied system, top-down factors (herbivores) were proven to be of higher importance than bottom-up controls (nutrients). For management plans this would mean to first strengthen the status of the bay as marine protected area and reinforce the existing fishing ban. This secures a healthy herbivore community able to control algae cover in the reef and thereby facilitates coral recruitment. Sewage treatment facilities for resorts should be obligatory, but enforcement by law is unlikely. Responsible tourists could inquire information about sewage and waste disposal at the resorts and thereby urge them to consider eco-friendly tourism. Besides, dive schools and especially snorkel-trip-operators should be educated and encouraged in appropriately cautious handling of the reef ecosystem as snorkellers were frequently observed to rest on coral blocks. Results of so called 'non-regulatory measures' including education and scientific activities have been impressive in terms of behavioral and attitude changes in Thailand in the past (Yeemin et al. 2006). To be successful on a local level, management plans have to link private tourism business and conservation. If education and regulations are able to lower stress on the reefs in Mae Haad and other bays of Koh Phangan, recovery to a state with higher coral and lower algal cover will be much faster and effective. A healthy coral reef will support the extensive

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fishery on Koh Phangan by nursing juvenile target species and the tourism industry by offering a colorful and diverse underwater world. Monitoring of the reef over the next years can help to predict long-term consequences of excessive anthropogenic impacts in this and other reefs and evaluate the success of management plans.

4.8 Improvement suggestions

The experimental set-up was effective in simulating overfishing; additional nutrient uptake in algal tissue could not be evidenced. Caging artifacts were considered to be negligible as cages did not significantly effect water movement or release of fertilizer. Light availability was lower in caged set-ups but as the experimental plots were situated at a shallow depth, light availability is still high and believed to satisfy photosynthesis. Many studies including cage controls concluded that caging did not have significant influences on algae growth (e.g. Russ & McCook 1999 and references therein; Belliveau & Paul 2002). The cage design was stable as long as wave action and under water surge was moderate. Continuous strong westerly winds in May increased hydrodynamics at the reef border drastically and experimental cages were pulled out of the sediment. After the termination of *in-situ* experiments, the fixation method of cages was reconsidered and tested. Large concrete weights secured to lower cage frames were effective in holding the set-ups in place but are considered impracticable due to bad handling and by offering additional hard substrate as well as hiding places for reef organisms. Fastening by means of plastic pegs buried in the sediment and fixed to the cages by rubber bands seem to be an effective method of securing the set-ups without additional weights and despite high water motion for future experiments.

Used settlement tiles were grooved which complicated the removal of algae mass after sampling. Additional it is possible that grazing on tiles was less effective due to the complex structure. Tiles without grooves were not available on Koh Phangan, but the use of flat tiles is highly recommended for future studies, either imported from the mainland or prepared from available tiles with a belt grinder or the like. As the transportation of sampled tiles to the laboratory resulted in loss of algae biomass in some cases, the use of *in situ* photographs is recommended for cover analyses. Due to an incomplete set of *in situ* pictures, photographs from the lab were used for cover analyses in this study.

One goal of the study was to establish a monitoring program for benthic and fish communities at the study site, based on data from 2011 and this study. Unfortunately, results were hardly comparable because last year's monitoring took place in the southern part of the bay Mae Haad, where the reef is less patchy and less disturbed by divers and snorkellers. Furthermore, different survey methods were applied and different species of fish or different organization levels of corals quantified. As guidance for future studies conducted at the study site I suggest following monitoring strategies for better comparability:

- 1. To achieve a better resolution, surveys are conducted in the southern as well as the northern part of the bay.
- 2. Benthic cover is evaluated by divers using the Line-Point-Intercept-method which is fast, cost effective and at least as precise and accurate as comparable methods (Nadon and Stirling 2006). Organization level observed in surveys is the genus level which is sufficiently simple to be applied by less experienced students but allows valuable insight into benthic community composition. An alternative for hardly trained volunteers could be to just evaluate total coral cover compared to sand, rubble, turf algae, macroalgae and CCA. At least three transects of 50 m should be surveyed in 0.5 cm steps over a homogenous area (Nadon and Stirling 2006). Surveying different water depths is not necessary because the entire reef is situated in ≤ 5 m water depth. Care is taken to ensure deployment of transect lines in relatively constant depth, parallel to the shore and close to the substrate. Bias between observers can be minimized by training the identification of corals together and should be evaluated by observing 1-2 identical transects.
- 3. Fish surveys are complex and training takes longer. The easiest and most suitable approach for the evaluation of herbivorous fish communities in a reef is to use the belt-transect-method described in Green & Bellwood (2009). This method was designed to evaluate reef resilience and survey key herbivorous species rather than focusing exclusively on fisheries species. Three 50 m transects are displayed in a row at constant water depth, separated by 5 to 10 m to ensure independence of replicates. The deployment of 3-4 lines of 5 m length perpendicular to each transect helps to estimate belt width during diving. Fish counts over each transect are conducted in triplicates with 5 min waiting period outside the transect area to minimize disturbance. Surveyors should swim with constant speed and note target species slightly in front of his/her own position, taking care not to count the same fish twice. Species observed are key herbivorous species of the families Scaridae, Siganidae, Labridae and Ephippidae. Pomacentridae are often herbivorous but small, numerous and hard to identify and therefore difficult to include in surveys. If surveyors are trained well enough it is desirable to include herbivorous, non-resident damselfish (Centropyge species). Larger carnivorous fish can be included in the survey, e.g. easily identified species of the family Labridae and Chaetodontidae, to evaluate the relative abundance of herbivorous species in the fish community. The estimation of fish sizes requires training, but is highly desirable as herbivorous biomass is a better indicator of coral reef resilience than abundance (Green & Bellwood 2009 and references within). A scale on the underwater writing board helps to estimate sizes during diving. This study used size classes of 5 cm for fish \geq 10 cm. If damsels are

included in the surveys, the addition of a smaller size limit and smaller size classes has to be considered. Herbivorous fish biomass is calculated from abundance, mid value of size class and species-specific coefficients available on www.fishbase.org.

4. Surveys of herbivorous invertebrates like sea urchins are conducted in three 50 x 5 m belt-transects closely observing crevices and cavities in the substratum.

Survey methods can be adapted to serve individual research questions but should at least include parameters as stated above to establish a continuous monitoring program in Mae Haad, Koh Phangan, Thailand and follow chronological changes in benthic composition and herbivorous fish community.

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