In situ coral nurseries in the Gulf of Thailand: Growth rates in different nutrient conditions and a comparison of attachment methods.

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Abstract

Coral reef health is declining worldwide and 'coral gardening', using nurseries, is being employed to increase the success of restoration projects. Here, different conditions and techniques for nurseries are described and coral growth over five weeks was measured between April and June 2013. Two mid-water floating nurseries in different bays, Mae Haad and Ao Thong Lang, in the Gulf of Thailand were stacked with coral fragments. Mae Haad is the site of intermediate tourism and eutrophic river inflow, while Ao Thong Lang is more remote and has no river inflow. Three hypotheses were tested: (1) Mae Haad was expected to show higher nutrient- and algae concentrations and lower salinity; (2) the bay with lower nutrient and algae concentrations should show higher coral growth rates and (3) Ao Thong Lang was expected to have higher Live Coral Cover and coral biodiversity. (1) Small differences in nutrient- and algae concentrations and salinity were found between the bays, inconsistent with the hypothesis; (2) growth during several weeks was higher at the site with lower nutrient and algae concentrations and (3) Ao Thong Lang shows higher Live Coral Cover and biodiversity. Furthermore, three different attachment techniques were tested: (a) garden hose tubes, (b) PVC tubes and (c) plugs made of plaster and coral rubble. No significant differences were found between detachment rates for these techniques, but suggestions for improvements are made.

Keywords: coral nurseries, techniques, growth rates, nutrients.

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1. Introduction

Coral reefs are very complex and diverse ecosystems (Jaap, 2000; Rinkevich, 2008; Yeemin et al., 2006). They provide habitats for nearly one third of the marine fish species worldwide and harbour 10% of the amount of fish consumed by humans (Rinkevich, 2008). These valuable resources have been subject to increasing pressure due to human population growth (Yeemin, Sutthacheep & Pettongma, 2006), causing threats like overfishing, climate change, disease and pollution (Bellwood et al., 2004; Epstein, Bak & Rinkevich, 2003; Hoegh-Guldberg et al., 2007). This has resulted in unprecedented and alarming rates of reef degradation (Yeemin et al., 2006; Rinkevich, 2005). Moreover, it has been suggested that reef degradation may cause coral reefs to undergo phase-shifts to different states and to become barren (Bellwood et al., 2004; Epstein et al., 2003; Rinkevich, 2008). According to Jaap (2000), barren areas have very low rates of stony coral recruitment because coral recruitment is mostly from local populations. Consequently, these reefs may not be able to restore naturally. This has led authors to argue that measures should be taken to prevent the demise of coral reefs worldwide.

According to Jaap (2000) and Rinkevich (2000, 2005 & 2008), coral reef restoration is an essential tool for protecting and managing these ecosystems. Restoration of natural environments is a concept originally developed for terrestrial ecosystems and has only recently been proposed for marine ecosystems (Epstein et al., 2003). Coral reef restoration consists of transplanting healthy coral colonies to a denuded reef to benefit recruitment, accelerate recovery and improve aesthetics. The goal is to replace dead corals with new ones, speeding up natural recovery and facilitating re-colonization (Jaap, 2000).

An improvement to this strategy is the coral gardening concept, where isolated fragments are cultured *in situ* in shallow underwater nurseries, allowing them to grow before being transplanted into a degraded reef (Epstein et al., 2003). The transplantation of these fragments into their original reef counteracts local genet and species extinction, causing a local rescue effect. Furthermore it preserves some of the genetic variation within the area (Rinkevich, 2005).

Nurseries allow small coral fragments to grow in near ideal conditions: With enough sunlight and sufficient water flow. If the nursery is regularly maintained, predators and macro algae are prevented from disturbing coral growth. These factors increase survival and growth rates before placing the fragments in denuded reefs (Rinkevich, 2005). Several studies have shown that large coral colonies can be grown within three years from small fragments (Clark and Edwards, 1995; Bowden-Kerby 1997; cited in Rinkevich, 2000).
Recently, advances have been made using mid-water floating coral nurseries (Levy et al., 2010; Rinkevich, 2005; Shaish, 2008), most notably in the Red Sea by the National institute of Oceanography in Israel. These designs showed to greatly increase coral growth by providing excellent conditions: Enhanced water flow, decreased sedimentation, optimal light conditions and the elimination of corallivorous organisms.

1.1 Reef restoration in Thailand

Thailand's coral reefs are overall in fairly good condition, however, 6.2% of reefs have been rated as 'poor' (Chou, 1998). One reef with the same classification is Mae Haad on Koh Phangan (Figure 1) (Vetter, 2011), an island in the western Gulf of Thailand. As discussed by Schwieder (2012), Stuhldreier (2012) and Vetter (2011, all unpublished), this fringing reef has low coral cover, high algae densities and low fish densities, possibly due to a high rate of disturbances caused by tourism. Furthermore, a eutrophic river flows into this bay, presumably increasing local nutrient concentrations (Stuhr, 2013, work in progress). Consequently, a local marine conservation centre, COREsea, has started a restoration project utilizing mid-water floating coral nurseries between four and six meters depth, in order to aid local reef restoration.
In the past, several reef restoration projects have been carried out in Thailand. An example of a successful project is the experiment carried out by Sirirattanachai (1994, cited in Yeemin et al., 2006) with *Porites lutea, Acropora sp.* and *Pocillopora damicornis.* The project involved transplanting fragments of these corals to barren areas. The growth rate of *Acropora sp.* was particularly high; up to 6-10 cm per year with an average of 6.5 cm/yr after one year with a survival rate of 88% after two months.
However, except several transplantation projects, the only described coral nursery project within the Gulf of Thailand is written by Saengpaiboon (2003, cited in Yeemin et al., 2006). In this project from 1995, students transferred 58 live coral fragments (*Acropora sp.*) to nurseries. The survival rates of the transplanted fragments were 90-95% after six years, which is higher than the survival rates found after one year by Sirirattanachai (1994, cited in Yeemin et al., 2006), indicating the increased survivability of coral fragments in nurseries.

1.2 Research questions and hypotheses

The present research focuses on finding the best attachment method for fragments in nurseries and investigating conditions for coral growth. The questions to be answered: is there a measurable influence of nutrient and algae concentrations on short-term coral growth rates and which attachment methods lead to low detachment rates?
Three attachment methods were tested to find the method with the lowest detachment rate: PVC tubes, garden hose tubes and coral plugs. It was expected that garden hose would show the lowest detachment rate, due to findings by Shaish et al. (2008) who used plastic tubes and superglue for attachment experiments. They found that only 0.6% *Acropora formosa* fragments detached after one year. In the current research, glue wasn't used for garden hose in order to make replantation after the nursery period easier.
Conditions for coral growth were investigated by comparing coral growth in, nurseries, between areas with different nutrient concentrations. Two bays were selected: one with a lot of tourism as well as eutrophic river water inflow and one with neither river inflow nor tourism. Respective salinity and phosphate- (PO_4^{3-}), nitrate/nitrite- (NOx: NO_3^- & NO_2^-), ammonium- (NH_4^+) and chlorophyll a (Chl a) concentrations as well as coral growth were determined. It was hypothesized that the nursery site at the bay with eutrophic water inflow would show higher

nitrate/nitrite-, phosphate-, ammonium- and Chl a concentrations while having a lower salinity due to the river influx. Coral fragments were expected to show lower growth rates at the site with higher nutrient- and Chl a levels because Chl a concentrations serve as a proxy for primary production and are correlated to higher turbidity, creating light restriction for coral growth (Nybakken & Bertness, 2004).
Additionally, ecosystem health was compared between the two bays to give more insight into local conditions. This was done by determining *Live Coral Cover* and biodiversity at both bays. The bay with increased tourism and river water inflow was expected to have lower *Live Coral Cover* (LCC) and biodiversity, mostly because of damage from divers and snorkelers, but also due to presumed higher eutrophication.

2. Materials and Methods

2.1 Study sites
The two bays selected for the research are Mae Haad (MH) (9°48'06"N, 99°59'22"E) and Ao Thong Lang (ATL) (9°47'50"N, 99°58'42''E), situated on the northern coast of Koh Phangan, a small island in the Gulf of Thailand (Figure 1). Mae Haad, the southern bay, is the site of touristic development with many amateur snorkelers and SCUBA divers, as well as input from a eutrophic river. On the other hand, Ao Thong Lang, the northern bay, is not accessible by land, has no touristic development and lacks river influx. Mae Haad is a bay approximately 1km wide and has a fringing reef along the entire beach. The bay gently slopes down to 12 meters (Vetter, 2011). Ao Thong Lang is about 500 m wide and slopes down to 12 meters within the first 100 m.
All measurements and analyses were carried out within a 6-week timeframe between May 6th and June 14th, 2013.

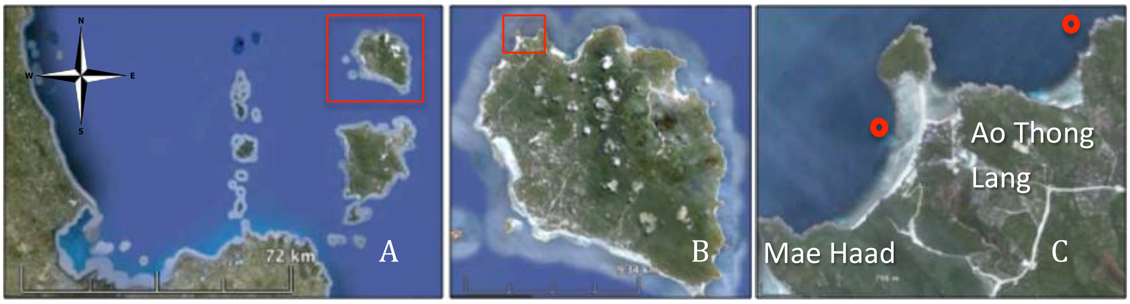

Fig. 1. (A) Western Gulf of Thailand, including the Samui Archipelago[1]. (B) Koh Phangan[2]. (C) Close-ups of the two bays[3], nursery placement indicated by red dots.

2.2 Nursery construction & coral material
The nurseries were made and placed in February 2013 (Figure 2). They were made by connecting PVC tubes to form 1 m^2 squares and a fine (ø 0.5 cm), plastic, flexible mesh was spanned over the square and connected using cable-ties. Four of these squares were joined using cable-ties to form a frame. These were subsequently secured to the bottom using ropes and PVC tubes which served as anchors. Air- filled bottles were attached at several places to lift the construction off the substrate.
Both nurseries were placed on a horizontal surface at a depth between four and six meters, depending on the tides. The nursery at Mae Haad was placed right behind the fringing reef, while the nursery at Ao thong Lang was placed within the reef.
Acropora sp. was chosen to be studied for several reasons. Firstly*, Acropora sp.* cover in Thailand has severely declined after the 1998 bleaching event and specifically in the Samui archipelago after the 2010 bleaching event (Yeemin et al., 2004 & 2009; Hoeksema et al., 2012), increasing the need for replantation; it is fairly resistant to mechanical stress as it may use breaking as a way for colonization (Lirman, 2000) and it's easily harvestable due to its branching growth form.

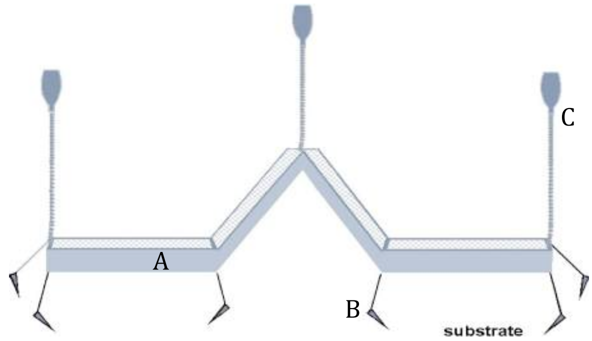

Fig. 2. Nursery design. (A) PVC tubes form squares. (B) Ropes connect to PVC 'anchors'. (C) The framework is suspended mid-water with air-filled plastic bottles.

2.3 Water quality

For the Water Quality experiment, water samples were taken weekly at both nurseries during five weeks between May 13th and June 11th 2013. Seven replicates were taken every week on two consecutive days, depending on weather conditions but always at 4-6 meters depth.

Immediately after transport, salinity and pH were measured using a Lutron WA-2017SD. For chlorophyll a analysis, 3 L (or until filter saturation had been reached) of each replicate was filtrated using pre-combusted VWR glass microfiber filters 691 (ø=47 mm, particle retention 1.6 µm) and an electric vacuum pump (max. p < 200 mbar). The filters were incubated with 10 ml of 90% acetone for 24 hours (4°C) and analyzed according to ESS method 150.1: Chlorophyll – Spectrophotometric (Wisconsin State Lab of Hygiene, 1991).

Filtrated water samples were analysed for nutrient concentrations following different methods: Nitrate/nitrite using a simplified resorcinol procedure (Zhang & Fischer, 2006); phosphate following an adjusted version of the method proposed by Murphey and Riley (1986); ammonium using a method based on Grasshoff et al. (1999). A Biochrom Libra S12 photometer (with 5 cm path length) was used for all measurements.

Salinity and chlorophyll-, ammonium- and nutrient concentrations were analysed with SPSS 21 for Mac, using Mann-Whitney tests after Kolmogorov-Smirnov and Shapiro-Wilk showed data were not normally distributed.

2.4 Ecosystem health

Seven 50 m *point intercept transect surveys* were carried out in Ao Thong Lang bay at random locations within the reef. One person laid out the transect while the other navigated, following a certain compass heading (both in SCUBA gear). After the transect was laid out, pictures were taken at every 50 cm mark. Substrate composition and coral family composition were found by determining the substrate on every picture and calculating the ratios. The data were compared with a similar study by Vetter (2011) in Mae Haad. The Shannon Wiener index and health categorization according to Chou (1994) were calculated for both locations.

2.5 Fragment growth rates

33 And 49 coral fragments were installed in garden hose tubes on the nurseries at Ao Thong Lang and Mae Haad respectively. To determine growth, fragments were measured weekly for five (Ao Thong Lang) or six (Mae Haad) weeks using rulers. Fragments attached to plugs were excluded from the experiment because measuring proved to be much more difficult. Measurements were carried out underwater while SCUBA diving. To determine the average cumulative growth, values from week one were subtracted from consecutive values. Results from both locations were compared with SPSS 21 for Mac using Mann-Whitney non-parametric tests, as Kolmogorov-Smirnov and Shapiro-Wilk tests showed significant deviations from a normal distribution.

At the end of the measuring period, fragments were rated for the amount of bleaching, turf algae cover and blue discoloration. Fragments that were bleached or covered with algae were rated according to a scale – from slightly to completely: slightly meaning small spots under 1 cm^2; significantly meaning spots larger than 1 cm^2; and completely meaning the entire fragment was bleached or covered with algae.

2.6 Nursery attachment methods

10 cm long flexibel garden hose tubes (ø 2 cm) were attached to the nursery mesh using cable-ties (Figure 3D). 33 and 49 fragments, for Ao Thong Lang and Mae Haad respectively, were inserted by gently pressing the fragments into the tubes until they seemed secure. 46 Coral plugs were made by mixing two-thirds coral sand (made by grinding rubble found along the beach) with one-third plaster-powder and mixing it with water to get a homogenous paste. This substance was poured into a sand mould, made by pressing a cup (ø 5 cm) 3 cm deep into a sand-filled container followed by piercing the resulting hole with a pencil (to get an enlarged golf tee-like shape, see Figure 3A). The plugs were cured for 72 hours and coral fragments were attached to the surface using superglue. The plugs – with coral fragments attached – were connected to the nursery by pressing the tip through the mesh (Figure 3B-C).

PVC tubes were only inserted during the first pilot study when it was found that they didn't work well enough and were excluded from the experiment. The detachment rates of fragments in garden hose tubes and of plugs were determined weekly before measuring commenced.

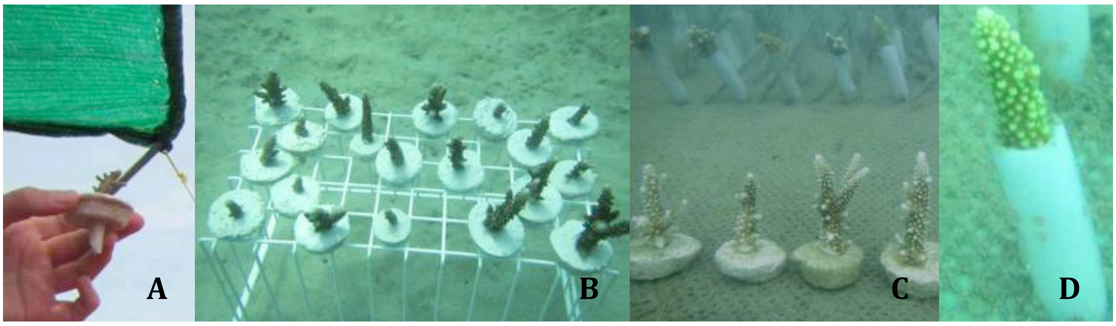

Fig. 3. (A) Fragments were glued to the plugs on the boat, (B) transported using a freezer rack and (C) inserted into the mesh. (D) Close-up of fragment in garden hose tube.

3. Results

3.1 Water quality

The concentrations and salinity values are shown in figure 4. Fluctuations of the parameters over time are shown in the graphs on the left while averages over time are shown on the right side. One of the replicates for Ao Thong Lang week two was unusable due to leakage of the container.

For chlorophyll three data points were excluded: two failed and one had an extreme negative value ($Z \geq 3.29$).

Comparing average concentrations, significant differences between the two locations were found for NOx concentration (Figure 5F-G) ($N = 69$, Mann-Whitney test $p < 0.001$) and salinity (Figure 5A-B) ($N = 69$, Mann-Whitney test $p = 0.031$). In contrast to the hypothesis, NOx values are significantly higher at Ao Thong Lang and salinity is significantly lower.

No significant differences were found between ammonium-, phosphate-, and chlorophyll concentrations of the two locations. However, average nutrient concentrations are higher at Ao Thong Lang. Values for chlorophyll concentrations show a similar pattern: higher values at Ao Thong Lang, although not significant.

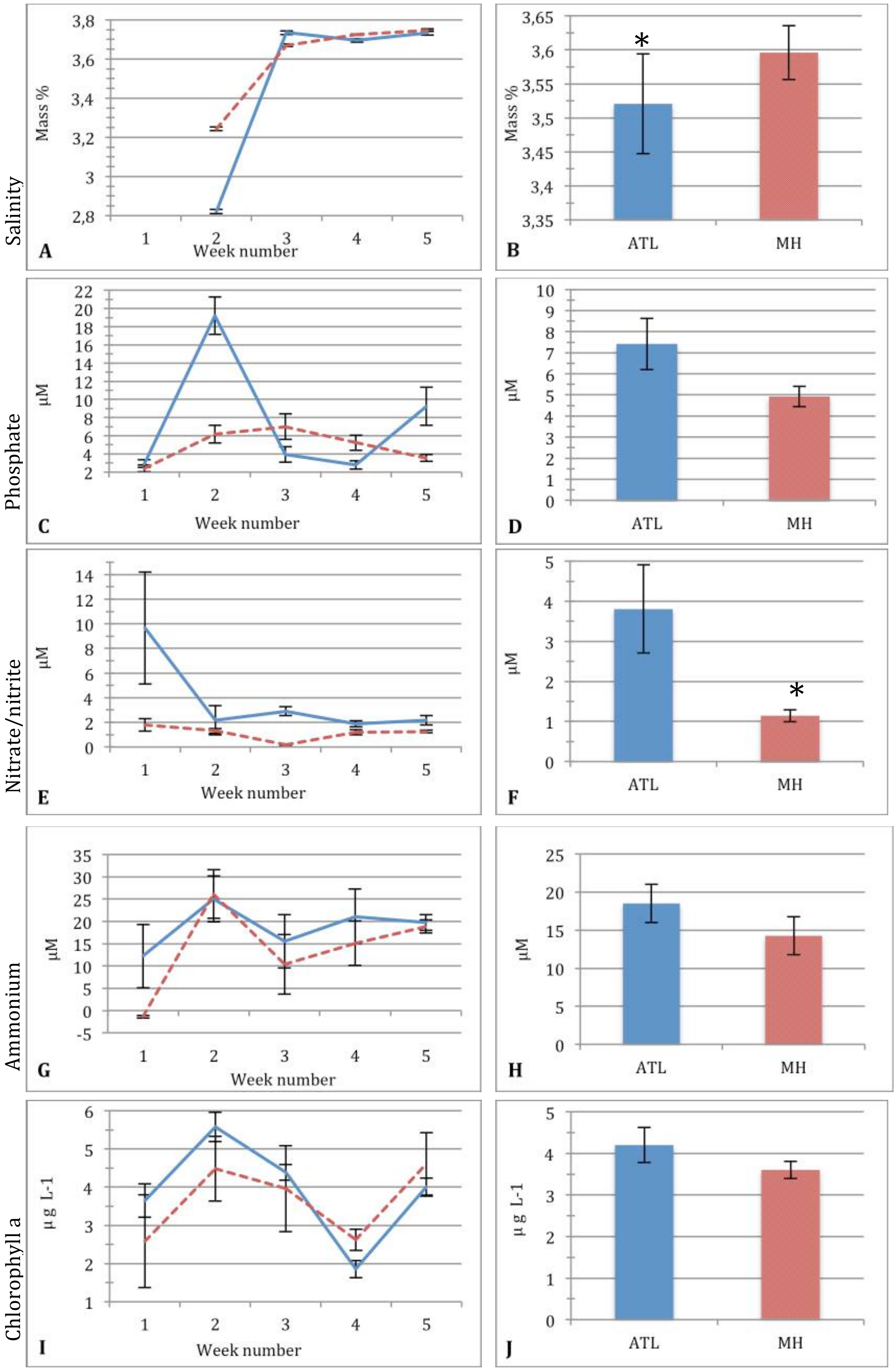

Fig. 4. Chlorophyll a- and nutrient concentrations (conc.) and salinity. ATL is indicated in blue, MH in red (dashed). N = 69 for NOx, PO_4^{3-} & salinity; N = 67 for NH_4^+; N = 66 for Chl a. (A) Weekly salinity in mass percentage ± SE. (B) Average salinity in mass percentage ± SE. (C) Weekly PO_4^{3-} conc. in µM ± SE. (D) Average PO_4^{3-} conc. in µM ± SE. (E) Weekly NOx conc. in µM ± SE. (F) Average NOx conc. in µM ± SE. (G) Weekly NH_4^+ conc. in µM ± SE. (H) Average NH_4^+ conc. in µM ± SE. (I) Weekly chl a conc. in µg L^{-1} ± SE. (J) Average Chl a conc. in µg per litre ± SE.

3.2 Ecosystem health

The substrate composition from this study (Ao Thong Lang) and the study by Vetter (2011) (Mae Haad) are shown in figure 5, and the coral genera composition in figure 6. No data could be statistically tested as the original data from Mae Haad was not available. The most interesting results show that Live Coral Cover at Ao Thong Lang is triple the value of Live Coral Cover at Mae Haad (ATL: 32.2% ± 4.74. MH: 9.5%) while Mae Haad has three times as much sand for substrate (ATL: 6.4% ± 1.2. MH: 32%). Furthermore, Ao Thong Lang showed ten coral families while only six coral families were found at Mae Haad.

The Shannon Wiener index was calculated (Table 1) and the classification according to the system proposed by Chou at al (Table 2) determined. Respective Shannon Wiener index values of 1.61 and 1.05 were found for Ao Thong Lang and Mae Haad, and classifications according to the system by Chou et al. (1994) of 'fair' and 'poor' resp.

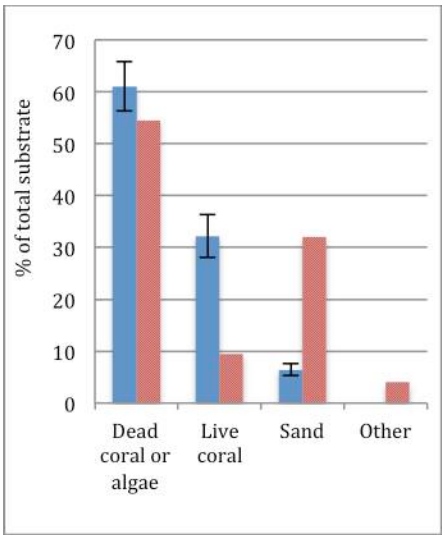

Fig. 5. Substrate composition in % (±SE for ATL).

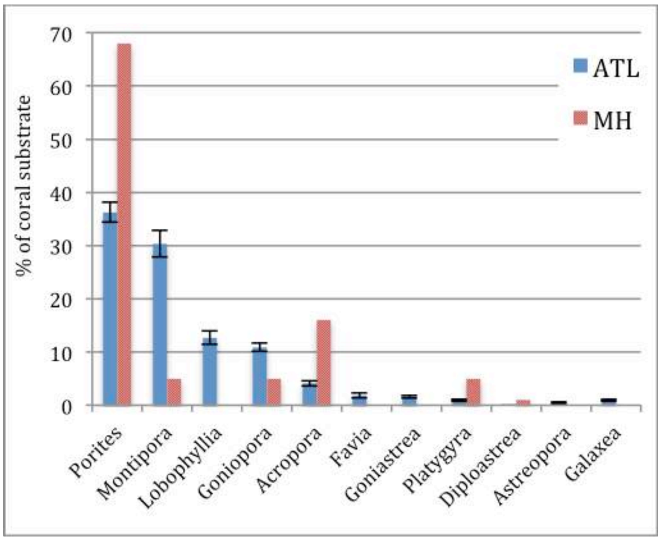

Fig. 6. Coral family composition in % (±SE for ATL).

Location	*Index value*
ATL	1,61
MH	1,05

Table 1. Shannon Wiener index.

Location	*Live Coral Cover*	*Reef rating*
ATL	32,2% ± 4,74 (SE)	Fair (26-50%)
MH	9,5%	Poor (0-25%)

Table 2. Reef classification according to Chou et al. (1994).

3.3 Fragment growth rates

Nine fragments at Ao Thong Lang were excluded entirely from the analysis due to detachment. Two fragments were lost after week 2, so no data could be added during the last week for this fragment. Furthermore, the data of 14 fragments in week 1 had to be excluded due to measurement error. Additionally, measurements couldn't start until one week after measurements started at Mae Haad because fragment harvesting took much longer due to bad visibility and lower *Acropora sp*. densities. At Mae Haad, seven fragments were excluded entirely from the analysis due to detachment, two data points were excluded in week 1 due to measuring error, eleven fragments were excluded after week 2, ten fragments after week three and four fragments after week four, all because of detachment. Furthermore, baseline measurements of 13 fragments had to be redone one week later, so no data is available for week five for these fragments.

Because the data is spread over different timeframes, it was decided to categorize the data into week numbers after baseline measurements (where baseline measurement = week 0). The resulting data for weekly cumulative growth is shown in figure 7.

The graph suggests slow growth at Ao Thong Lang during the first two weeks followed by one week with an increased growth rate, while for Mae Haad, quick growth during the first week is followed by two weeks of slow growth and subsequent faster growth for another two weeks.
Because of this growth pattern, week 1 and week 2 at Mae Haad differ significantly from week 1 and 2 at Ao Thong Lang (week 1: N=50; Mann Whitney test $p < 0.05$ & week 2: N=66, Mann Whitney test $p < 0.05$) as can bee seen in table 3.
Furthermore, at Mae Haad, cumulative growth at week five differs significantly from week one, two and three (Kruskal-Wallis test, N=44, 46 & 35; $p < 0.05$) and, at Ao Thong Lang, week three differs significantly from week one (Kruskal-Wallis test, N=32 $p<0,05$).

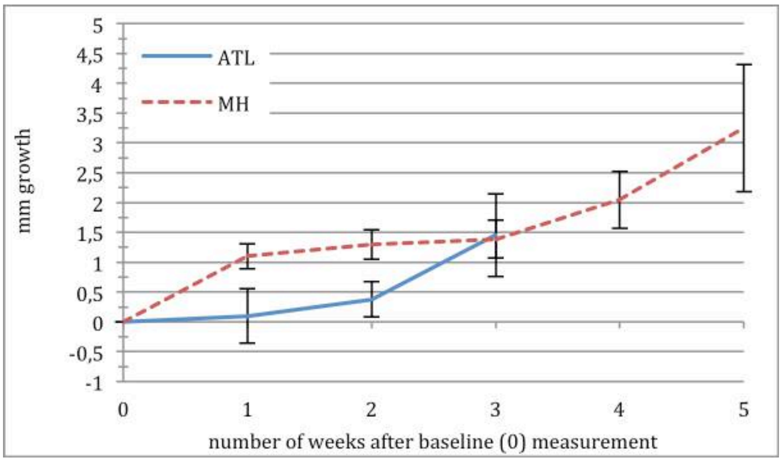

Fig. 7. Average cumulative growth in mm ±SE.

	Week 0	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*
ATL ± SE	-	0,1 ± 0,46	0,38 ± 0,30	1,46 ± 0,70	-	-
N (ATL)	24	10	24	22	-	-
MH ± SE	-	1,1 ± 0,21	1,3 ± 0,25	1,39 ± 0,32	2,05 ± 0,48	3,25 ± 1,07
N (MH)	42	40	42	31	21	4
Significance (P)	**-**	**0,001**	**0,029**	0,689	-	-

Table 3. Average cumulative growth of ATL and MH and significance of differences.

The ratings for bleaching, turf algae cover and blue discoloration of the fragments are shown in figure 8. It must be taken in mind that the fragments at Ao Thong Lang were inserted one week later than the fragments at Mae Haad, so their time spent in the nursery was shorter and they may have been subjected to different weather conditions. Fragments with a classification other than 'completely bleached or covered with algae' are considered to be alive. This leads to 74% and 51% healthy coral fragments; and survival rates of 97% and 95% for Ao Thong Lang and Mae Haad respectively.

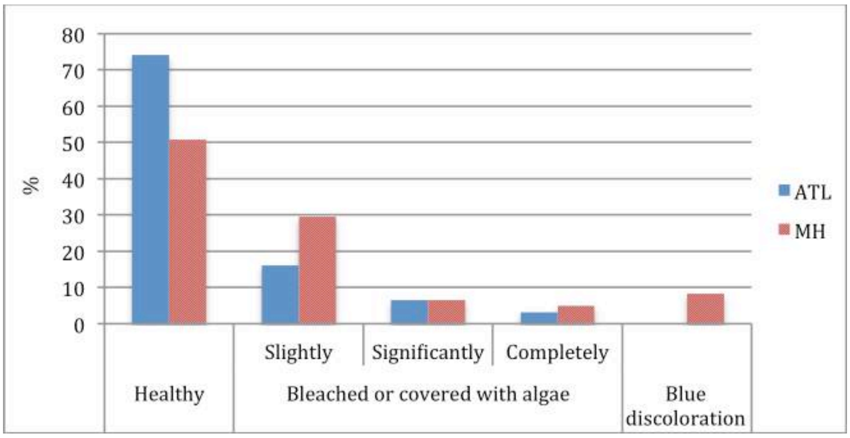

Fig. 8. Health of coral fragments after the measuring period.

3.4 Nursery attachment methods

Coral plugs were only used during the last two weeks of measurements, so detachment rates were calculated over these two weeks (Figure 9). Average daily detachment rates of 2.7% ± 1.0 (SE) and 1.5% ± 0.7 (SE) were found for garden hose and coral plugs respectively. There is no significant difference between the two results (N = 15, Mann-Whitney test $p > 0.05$). The difference is, however, that in the case of garden hose tubes, only the fragment detached from the tube; while in the case of coral plugs, the entire plug disconnected including the fragment: There was only one case where a fragment detached from a coral plug.

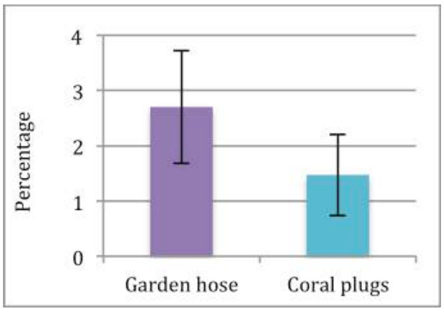

Fig. 9. Daily detachment rate: Percentage of fragments lost per day.

4. Conclusions & Discussion

4.1 Water quality

Cheevaporn and Menasveta (2003) set up water analysis stations at river mouths in the northern Gulf of Thailand. Nitrate concentrations between 1.29 and 10.3 μM and phosphate concentrations between 0.948 and 3.79 μM were found. The nitrate/nitrite concentrations (Figure 4B) found within the present study fall within the lower spectrum of their findings, suggesting slight eutrophication in both Ao Thong Lang and Mae Haad. Phosphate levels found especially at Ao Thong Lang in week two, and to a lesser degree week 5, greatly exceed the values found by Cheevaporn and Menasveta. Compared to previous findings within Mae Haad and Ao Thong Lang (Stuhr, work in progress), these findings seem unrealistic and may be caused by contamination of a reagent. Levels of ammonium found, on average 18.5 and 14.3 μM, or 0.315 and 0.244 mg L^{-1}, are well over the concentration of 0.100 mg L^{-1} established as highly toxic by the FAO (2013), and beyond the concentrations found in previous research in the same locations (Stuhr, 2013), again leading to the conclusion that this may be caused by contamination.

Although phosphate and ammonium concentrations seem unrealistic, it is still possible to compare the values found, as analyses were done on the same day using one source of reagent. Significantly higher nitrate/nitrite concentrations combined with insignificantly higher phosphate, ammonium and chlorophyll concentrations may indicate increased nutrient availability at Ao Thong Lang. However, not enough data was collected during this research to draw this conclusion and, looking at figure 4C and E, significant differences in phosphate and nitrate concentrations between the two locations can be explained by large deviations at Ao Thong Lang, possibly indicating more fluctuating nutrient concentrations. Furthermore, there is no clear cause for increased nutrient concentrations at Ao Thong Lang. More research should be done to find if these findings are correct and if so, what causes higher or equal nutrient and algae load at Ao Thong Lang, contrary to the hypothesis.

A possible cause for the lack of differences in nutrient- and chlorophyll a concentrations between the bays could be that the eutrophic river water doesn't mix in Mae Haad due to lower salinity or higher temperature of the river water. Alternatively, the river water does mix in the bay, but the effects are not measurable at the nursery, possibly because the distance from the river is too great. Furthermore, weather is a really important parameter that has not been taken into account. The effects of rainfall, winds and currents may cause large fluctuations in nutrient distributions. The effect of these parameters in this area should be investigated further.

The higher salinity at Mae Haad (4A & B) can be explained by the differences in geography between both bays: Mae Haad is a wide-stretched bay that gently slopes of to a maximum of 12 (Vetter, 2012, unpublished) meters. In contrast, Ao Thong Lang slopes off to 12 meters depth within the first 50 meters. Because Mae Haad is shallower and larger, it's possible that the bay is subjected to increased warming, promoting evaporation and causing higher salinity.

4.2 Ecosystem health

Although no statistical tests could be performed on the data, it seems highly likely that Ao Thong Lang is in a better state, with higher Live Coral Cover. Ao Thong Lang also represents more genera and the fraction covered per family seems to be more equally distributed, resulting in a 60% higher Shannon Wiener index (Table 3). Additionally, research has shown that coral genera have different calcification sensitivities to thermal stress, leading to shifting coral community composition based on temperature and other environmental factors (Carricart-Ganivet et al., 2012). This could be an explanation for the high high *Porites sp.* cover at Mae Haad as it has been shown to tolerate increased thermal stress due to thicker outer tissue (Fitt et al., 2009; Hoegh-Guldberg et al., 1999). This supports the idea that Mae Haad may be subjected to increased warming because of its geography.

The low coral cover at Mae Haad is most likely the effect of increased diving and snorkelling tourism, causing frequent mechanical stress, as nutrient and chlorophyll a concentrations were not higher during the period of the study. Coupled with increased coral bleaching, this could explain the patterns found: Much lower coral cover and relatively high presence of thermal stress resistant species.

However, much more research should be carried out to draw this conclusion. Parameters that should be investigated include sedimentation rates from the river in Mae Haad and suspended matter in both bays. Corals may be smothered by sediment carried in by rivers and high levels of suspended matter decrease light availability (Rogers, 1990). From personal observation can be said, however, that turbidity at Ao Thong Lang seemed to be consistently much higher than at Mae Haad.

4.3 Fragment growth rates

It is clear that significant coral fragment growth has occurred at both locations. A significant difference in average growth between the locations was even found over the first two weeks of measurements, with higher growth at the site with significantly lower average nitrate/nitrite concentrations and seemingly lower phosphate, ammonium and chlorophyll a concentrations, as hypothesized. However, after three weeks, the average growth was similar in both bays (MH: 1.46 ± 0.7. ATL: 1.39 ± 0.32), so a significant difference between growth rates cannot be concluded. A longer timeframe of measurements may give conclusive results.

The average fragment growth found at Mae Haad was 3.25 ± 1.07 (SE) mm after five weeks. This can be compared to results from Sirirattanachai et al. (1994, cited in Yeemin et al., 2006). The authors placed cement blocks with small fragments of *Acropora sp.* in a denuded reef, west of Koh Krok, in the North Eastern Gulf of Thailand. They found 6-10 cm growth within the first year. This suggests 5.8 – 9.6 mm growth per five weeks, leading to the calculation that growth rates at Mae Haad are equal to 34 – 56% per cent of their growth rates. This is disappointingly low, but growth rates may increase after the timeframe of the study. It must be taken into account that measuring fragments under water with a 1 mm accuracy can be quite difficult and may have resulted into a lot of noise in the data. Measuring fragments less frequently and over longer periods of time may result in higher growth rates.

A lower percentage of healthy fragments were found in Mae Haad, although this could not be statistically tested. Also, coral fragments with blue discoloration on the fracture surface were found only at Mae Haad. These discolorations have been suggested to indicate increased concentrations of green fluorescent protein homologs. It is an immune system response to wounding, disease and infestation with parasites and is associated to higher growth rates and cell proliferation (D'Angelo et al., 2012). Combined with the fact that there are a lower percentage of healthy fragments in Mae Haad, this could indicate that *Acropora sp.* at Mae Haad are in poor condition or under increased stress.

Survival rates of 97% and 95% were found for Ao Thong Lang and Mae Haad respectively. Sirirattanachai et al. (1994, cited in Yeemin et al., 2006) found survival rates of only 88% after two months in a reef restoration experiment and Saengpaiboon (2003, cited in Yeemin et al., 2006) found survival rates between 90 and 95% survival after six years in a nursery. Although it is impossible to compare the results directly, as different time-lengths were used, it seems survival rates found within the present study are at least as good as found by Sirirattanachai.

4.4 Nursery attachment methods

Shaish et al. (2008) found an average detachment rate of 0.6% for *Acropora formosa* after one year using plastic tubes and super glue. The present study differs in that no superglue was used to secure fragments and the resulting daily detachment rate, 2.7% ± 1.0 (SE), is higher than the yearly detachment rate for the method by Shaish et al. (2008). Conclusively, using garden hose tubes without glue is not advisable. However, from personal observation it can be said that long, thin (length > 60 mm, diameter < 20 mm), unbranched, predominantly straight fragments work best for this method because these can be inserted deeper into the tubes, securing them more.

Coral plugs seem to show a lower detachment rate than garden hose tubes, although no significant results were found. However, an essential difference between the two techniques is how detachment takes place. In the case of garden hose tubes, the fragment is detached from the tube, while in the case of coral plugs, the whole plug disconnects from the nursery mesh. This indicates that super glue works well for attaching the fragments to the plugs; however, improvements should be made to the way plugs are attached to the nursery mesh.

A problem that was encountered with the use of glue while installing coral plugs is that fragments have to be taken out of the water, dried with a cloth and then glued to a plug. Because coral fragments can survive only shortly out of the water, there was limited time for gluing and drying. Consequently, the amount of fragments that could be glued to plugs in one session was very limited. During the second session of installing coral plugs, coral fragments were taken out of the water for approximately 20 minutes, this proved to be too long, however, as all fragments had died the successive week. Precautions should be taken to prevent this in future studies.

5. Recommendations

Near the end of the measuring period, both nurseries started to show signs of wear. The mesh disconnected from the PVC framework at several locations and anchors occasionally released from the substrate. Some weeks the detachment rates would be unusually high, leading to the assumption that they were sometimes shoved, possibly by boat anchors, SCUBA-divers or snorkelers. It would be advisable to use cement anchors like Shaish et al (2008) and to try using an alternative for cable-ties to attach the mesh to the frame. Additionally, to avoid wasting time searching in bad visibility and to avoid people accidentally touching them, the nurseries should be marked with floating buoys.

Both nurseries were placed directly behind or within the reef. This is a clear deviation from practices by Levy et al. (2010), Rinkevich (2005), Shafir et al. (2006) and Shaish et al. (2008), who explicitly recommend placing nurseries away from the reef to prevent anthropogenic and marine life disturbances, for example by corallivorous fish. Placing the nursery elsewhere may also prevent contamination with diseases from nearby reefs and reduce sedimentation and turbidity. It would be interesting to see if higher growth rates will be found if different locations are used.

Another recommendations that can be made is using a set team for fragment measurements, as training volunteers requires a lot of time and a lot of mistakes are made in the beginning, causing noise in the data.

For attachment methods, using coral plugs seems to be the best option, as they are easy to be transplanted to the reef once the nursery period is over and detachment rates seem lower, especially when a way is found to attach them to the nursery more securely. However, when gluing the fragments to plugs, precautions should be taken to make sure fragments aren't out of the water for too long, for example by collecting the fragments, then keeping them in water-filled boxes and only taking them out shortly for gluing.

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7. References

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