Coral reefs in the Anthropocene: The effects of stress on coral metabolism and symbiont composition

Suzanne Faxneld
Coral reefs in the Anthropocene

The effects of stress on coral metabolism and symbiont composition

Suzanne Faxneld
To my daughter Cornelia,
who always makes me smile
List of papers

This thesis is based on the following four papers, which are referred to in the text by their roman numerals:

**Paper I**

**Paper II**

**Paper III**
Faxneld S., Hellström M., Tedengren M. Symbiodinium spp. composition in nearshore and offshore Porites lutea and Galaxea fascicularis in northern Vietnam. (Manuscript)

**Paper IV**
Hellström M., Gross S., Faxneld S., Hedberg N., The CC., Nguyen DN., Lan HL., Grahn M., Benzie JAH., Tedengren M. Symbiodinium spp. diversity in a single host species, Galaxea fascicularis, Vietnam; impact of environmental factors, host traits and diversity hot spots. (Manuscript)

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My contributions to the papers:

(I) – Experimental design, laboratory work, statistical analysis, main writer of the paper. 
(II) – Participated in collection of organisms, experimental design, laboratory work, statistical analysis, main writer of the paper. 
(III) – Collection of organisms, field design, lab work, data preparation, main writer of the paper. 
(IV) – Participated in collection of organisms, lab work, data preparation, and writing.
Abstract

Coral reefs constitute some of the most prolific and diverse ecosystems on our planet, but also among the most threatened.

This thesis investigates the effects of environmental stressors on corals’ metabolism and symbiont diversity. Paper I shows that the coral *Turbinaria mesenterina* withstood a single stressor while a combination of two stressors (decreased salinity and increased seawater temperature) lead to decreased metabolism. Increased seawater temperature in combination with two stressors (enhanced nutrients and decreased salinity) lead to rapid mortality of all specimens. Paper II shows that chronic stress in combination with increased seawater temperature affects coral species differently. *Porites lutea* did not show any difference in stress response to temperature increase, regardless of environmental disturbance history, while *Galaxea fascicularis*’ metabolism was negatively affected in chronically disturbed corals but not in corals from less disturbed areas. The main explanation for the difference in response between the two species is different compositions of endosymbionts as found in paper III. *P. lutea* only harboured the symbiont C15, regardless of environment, whilst D1a dominated the nearshore *G. fascicularis* and C1 dominated offshore corals. In paper IV there was a clear inshore-offshore pattern of D1a along the whole coast of Vietnam, where D1a dominated inshore. In contrast, the five symbionts belonging to group C displayed a strong latitudinal gradient, with diversity increasing from north to south. The coral host showed higher diversity offshore than inshore.

The thesis emphasizes the importance of improving water quality (paper I and II) and protecting marginal areas since tolerant coral hosts and symbionts can be found there (paper III and IV), as well as safeguarding areas with high symbiont diversity (paper IV) to increase the ability of corals to withstand future environmental changes.

Keywords: Corals, Disturbance, *Galaxea fascicularis*, ITS2, Metabolism, mtDNA, Pollution, *Porites lutea*, *Symbiodinium*, Temperature, *Turbinaria mesenterina*, Vietnam
Sammanfattning

Korallreven är bland de mest artrika ekosystemen på jorden. De är mycket viktiga, då de bland annat fungerar som habitat för många betydande matfiskar, fungerar som vågskydd mot kusten samt bidrar till inkomster för turistnäringen. Dessvärre håller korallreven på att försvinna världen över. Olika mänskliga aktiviteter ligger till grund för att korallreven har förstörts och inte kunnat återhämta sig. Dessa är bland annat överfiske, utsläpp från till exempel fabriker och jordbruksbutik, hotel som inte renar sitt avloppsvatten, sedimentation som beror på exploateringar på land samt klimatförändringar. Tidigare kunde ofta korallerna återhämta sig efter att ha utsatts för en typ av störning, men i dag kommer inte störningarna ensam, utan flera olika typer kommer samtidigt, vilket resulterar i att korallernas återhämtningsförmåga minskas och till slut kan de inte klara av mer störningar utan dör. I denna avhandling har jag undersökt hur koraller påverkas fysiologiskt och hur symbionterna (zooxantheller som koralerna lever i symbios med) påverkas av kroniska störningar som sker längs med Vietnams kustremsa.

I papper I har jag undersökt hur korallers fysiologi påverkas av kombinationen av flera störningar. Resultaten visar att dålig vattenkvalitet påverkar korallerna negativt när de samtidigt utsätts för ökad temperatur. I papper II har jag undersökt hur olika korallarter som är utsatta för kronisk stress jämfört med icke stressade påverkas av ökad temperatur. Resulten visar att det inte är någon skillnad mellan stressade och icke stressade Porites lutea medan Galaxea fascicularis påverkas negativt av kombinationen av de två störningarna. Förklaringarna till skillnaden i resulten ses i papper III, där korallernas symbionter har undersömts och resultaten visar att P. lutea enbart har samma symbionter avsett om de kommer från kroniskt störda eller mer rena områden, och denna symbiont gör Porites väldigt tålslig, medan G. fascicularis från de kroniskt störda reven domineras av en symbiont tillhörande klad D medan de från det rena revet framför allt domineras av en klad tillhörande C-gruppen. Vidare visar papper IV att symbionterna hos G. fascicularis framför allt har D-gruppen på de mer störda område-na längs med hela Vietnams kustremsa, medan de rena reven har olika typer av C-grupper, där de i norr har en lägre diversitet av C än de i söder.

Denna avhandling visar således att det är oerhört viktigt att förbättra vattenkvaliteten eftersom den bidrar till hur väl korallerna kan klara klimatförändringarna, samt att det är viktigt att bevara även störda rev eftersom tolerant symbionter och korallarter kan finnas där.
Tóm tắt

Rạn san hô là một trong những hệ sinh thái có tính đa dạng sinh học cao, nhưng cũng là hệ sinh thái bị đe dọa nhiều nhất trên trái đất. Các hoạt động danh nhân và biến đổi khí hậu là những yếu tố chủ yếu tân phát các rạn san hô.

Luận án này trình bày về những biến động môi trường do con người và tự nhiên đáng tạc động như thế nào đến quá trình đổi chất và tính đa dạng sinh học của san hô trong san hô. Chương thứ I cho rằng loại san hô Turbinaria mesenterina có thể chống chịu đốn tát tại đối với một tác nhân lớn, tuy nhiên khi có hai yếu tố tác động cùng lúc làm giảm quá trình đổi chất diễn ra trong san hô. Hơn nữa, nhiệt độ nước biển tăng lên kết hợp với hai tác động khác nhau (gồm các chất dinh dưỡng tăng và độ mủi giảm trong nước biển) làm san hô chết nhanh hơn. Chương thứ II đề cập đến các phản ứng khác nhau của từng loại san hô khi bị tác động bởi các yếu tố bất lợi của môi trường kéo dài kết hợp với nhiệt độ nước biển tăng lên. Loại san hô Porites lutea, phản ứng rộng ở các vùng có chất lượng môi trường khác nhau, khi nhiệt độ nước biển tăng lên, chúng đều không biểu hiện bất cứ phản ứng sinh hóa nào. Trong khi đó, với cùng tác động nhiệt độ, loại san hô Galaxea fascicularis cho thấy sự suy giảm của quá trình tạo đổi chất, tuy nhiên sự suy giảm này xuất hiện rõ nét hơn trên những san hô phân bố ở vùng có chất lượng môi trường tốt. Những phản ứng khác nhau của hai loại san hô đối với những tác động bên ngoài được lý giải chủ yếu là do sự khác biệt giữa các loại tạo san sinh zooxanthellae sống trong môi của san hô như được trình bày trong chương III. Chương này chỉ ra rằng tạo san sinh C15 tồn tại trong mô của loại san hô P. lutea phân bố ở các điều kiện chất lượng môi trường sống khác nhau, trong khi đó loại tạo san sinh D1a chiém tự thể trong mô của loài G. fascicularis sống ở các đảo gần đất liền, C1 lại chiếm tự thể trong mô của cùng loại san hô do những phân bố ở vùng biển xa đất liền. Trong chương IV phân hiện rằng không có sự kết hợp nào giữa loại san hô G. fascicularis và các loại tạo san sinh của chúng, do đó sự khác biệt được thấy ở trong các nhóm tạo san sinh là do các yếu tố môi trường gây ra. Một kiểu phân bố gần bờ xã bờ nhiệt độ của nhóm tạo san sinh D1a được thấy ở các ven biển miền Nam, D1a chiếm tự thể ở vùng biển gần đất liền. Trải lại 5 loại tạo san sinh thuộc nhóm C phù thuộc vào phân vùng địa lý, với tính đa dạng thấp ở vùng biển phía Bắc và tăng dần về phía Nam Việt Nam. Thêm vào đó, tính đa dạng sinh học của san hô ở vùng biển cao hơn khi so với san hô vùng gần bờ.
Nhưng nghiên cứu trong luận án này cho rằng chất lượng nước suy thoái hiện nay đang ảnh hưởng đến khả năng chịu đói và yếu tố bất lợi về môi trường như quá trình tăng lên của nhiệt độ nước biển; và tính đa dạng sinh học của san hô và tạo công sinh đang suy giảm cùng với suy giảm về chất lượng nước. Bởi vậy, luận văn này nhằm mảnh tầm quan trọng của chất lượng nước (Chương I và II) và việc cần thiết bảo vệ các loại san hô mang tạo công sinh có khả năng chịu đói đối với tác động từ bên ngoài (Chương III và IV) nhằm làm tăng khả năng sinh sao phục hồi đói đối với những rạn san hô.

Từ khóa: Việt Nam, san hô, Turbinaria mesenterina, Porites lutea, Galaxea fascicularis, tạo công sinh, các tác động từ hoạt động nhân sinh, quá trình trao đổi chất, ITS2, mtDNA.
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Photo 1. A typical coral reef in southern Vietnam. Photo: S. Gross
INTRODUCTION

On our planet, coral reefs constitute some of the most prolific and diverse ecosystems (Connell 1978). Despite that the area covered by coral reefs only represents 0.09% of the world’s oceans, almost one quarter of all marine fish species use it as a habitat and around 800 species of hermatypic (i.e. reef-building) corals have been described (Spalding et al. 2001). Moreover, another 9 million species live on or close to the reefs (Knowlton 2008).

Coral reefs provide a vast number of ecosystem goods and services (Moberg and Folke 1999), which are essential to coastal human societies in tropical regions, e.g. food production for consumption and export, shoreline protection, income from tourism, etc. Many people in developing countries are fully dependent on the coastal resources for their daily livelihood (MEA 2005) and in some countries the only animal protein source available to the local communities comes from small-scale artisanal fisheries (Russ 1991; Lundin and Lindén 1993).

However, coral reefs are being degraded at an alarming rate due to human activities. Globally 50-95% of the reefs have been lost over the last 30 years (Jackson 2008; 2010). Anthropogenic disturbances, such as overfishing (Jackson et al. 2001; Jackson 2008; 2010), land-based pollution and sediment releases (Wilkinson 2000; Jackson 2008; 2010) and climate change (increased seawater temperature and increased CO₂) (Hoegh-Guldberg 1999; IPCC 2007) are considered to be the main drivers behind the decrease in coral cover. Some of the evident changes on coral reefs include framework destruction, loss of biodiversity and invasion of fleshy algae. Many of these disturbances act in tandem (Hughes et al. 2003; Pandolfi et al. 2005). Loss of resilience (e.g. loss of different functional groups or different functions) is affecting the capacity of coral reefs to recover after disturbances (Nyström et al. 2000). Subsequently, ecosystem services are lost, which will impact on societal and economic development in the tropical coastal zone.
Photo 2. Disturbances on coral reefs. Clockwise from top left: a “healthy” coral reef, crown of thorn starfish, land-use changes, coral bleaching, a coral reef smothered by sediment, a coral reef patch dominated by macroalgae, shrimp farms and fishing boats. Photo: S. Faxneld, N. Olsson, M. Hellström.
FRAMEWORK OF THE THESIS

Stress and disturbances on coral reefs
A stress or a disturbance is an event that leads to a disruption of the structure or function of ecosystems, communities, populations or organisms, and furthermore changes resources, substrate availability, or the physical environment (White and Pickett 1985; McManus et al. 2000). On the organism level, stress is often considered as a sublethal effect acting on the physiological performance, leading to less favourable energetic status and possibly to e.g. a decline in growth and reproduction, thereby reducing the fitness of an organism (Hughes and Connell 1999; Gardeström 2008) (Fig. 1).

Disturbances can be both natural and anthropogenic. Natural disturbances can be physical (e.g. storms, hurricanes) or biological (e.g. disease and predator outbreaks) and may operate across a range of different spatial and temporal scales and differ in both frequency and magnitude (Paine et al. 1998). Natural disturbances are usually acute, which means that they are short-term, whereas many anthropogenic disturbances are more chronic, such as pollution, sedimentation etc. (Connell et al. 1997). A chronic disturbance is often more harmful, compared to acute disturbances intervened by periods of recovery (Grigg and Dollar 1990; Brown 1997b). Coral reef recovery is likely to be faster or more complete after an acute disturbance event compared to when being exposed to more prolonged ones (Connell 1997).
Reduced water quality

Nearly 40% of the world’s human population lives within 100 km of the coast (Burke et al. 2011), which leads to an increase in land-run off and deterioration of the water quality (McClanahan et al. 2002; McCulloch et al. 2003; Devlin and Brodie 2005). The increased levels of nutrients and sedimentation caused by coastal development and land-use changes have resulted in chronic exposure (e.g. Szmant 2002; McCulloch et al. 2003). Chronic stress (e.g. high levels of nutrients, pollution and sedimentation) has a negative impact on corals’ fitness in terms of reduced growth rates (Tomascik and Sanders 1985), inhibition of recruitment (Tomascik and Sanders 1987; Hunte and Wittenberg 1992) and mortality (Fabricius 2005). This in turn may lead to reduction in coral richness (Fabricius et al. 2005) and, due to competition with algae, eventually an increase in macroalgal cover and richness can occur (van Woesik et al. 1999; Fabricius and De’ath 2004).

Elevated seawater temperature

Many tropical corals live close to their upper thermal limits and small increases in temperature may be detrimental (Hoegh-Guldberg 1999). During unusually
warm conditions corals will become pale and loose colour through the loss of their symbiotic zooxanthellae and/or degradation of algal and host specific pigments, a stress response referred to as bleaching (Hoegh-Guldberg and Smith 1989; Brown 1997a; Douglas 2003). Bleaching occurs when the temperature increases to higher temperatures than normal for the season rather than when a fixed temperature is reached. Small temperature increases (0.5-1.5°C) over several weeks or large increases (3-4°C) over a few days will bleach corals (Glynn and D’Croz 1990). Bleached corals also lose nitrogen, proteins, lipids, carbohydrates, and weight (Porter et al. 1989). This means loss of energy and thereby a reduction in growth and reproduction for bleached corals (Porter et al. 1989). Depending on the duration and level of temperature stress, corals can either survive or die. The effect of climate change (e.g. increased seawater temperature) is predicted to increase in the future (Donner et al. 2005; Hoegh-Guldberg et al. 2007; IPCC 2007).

Bleaching susceptibility in corals also differs between latitude, depth, morphology, taxa, and zooxanthellae composition (Spencer et al. 2000; Baker et al. 2004; McClanahan et al. 2004; Rowan 2004; Kemp et al. 2006; Ulstrup et al. 2006). Some coral species are more tolerant than others (McClanahan 2004; McClanahan et al. 2004; Ulstrup et al. 2006) and massive corals seem to be more tolerant than other morphological types (McClanahan 2000; Spencer et al. 2000; Edwards et al. 2001). Corals living in more shallow waters seem to be less affected by bleaching (Brown 1997b; Warner et al. 1999; Spencer et al. 2000; Craig et al. 2001) which might be due to that they are more acclimatized to large temperature fluctuations (Cook et al. 1990; McClanahan and Maina 2003). Also corals from high latitude reefs seem to be more tolerant to bleaching compared to equatorial corals, which also could be due to the larger temperature fluctuations at higher latitudes (Kemp et al. 2006). Differences in bleaching susceptibility in corals might also be explained by varying temperature sensitivity of different kinds of zooxanthellae (Glynn et al. 2001; Baker et al. 2004; Rowan 2004). Thus, this difference in bleaching susceptibility can lead to a shift towards more temperature tolerant species (i.e. ‘winners and losers’; Loya et al. 2001).

**Multiple stressors**

Since anthropogenic disturbances have increased, multiple stressors are likely to be the norm within the coming decades (Brown 1997b). These stressors act in tandem and operate on different spatial and temporal scales and may potentially generate synergistic effects (Jackson et al. 2001; Pandolfi et al. 2005), which in turn can make an ecosystem more vulnerable to changes that previously could be absorbed. If an ecosystem is healthy and seldom subjected to any disturbances, the system will subsequently recover after a single disturbance and return to a ‘baseline’ condition. However, there are few, if any, coral reefs in the world that are found in ‘pristine’ conditions (Jackson et al. 2001; Pandolfi et al. 2003;
Knowlton and Jackson 2008). This means that if an ecosystem is already altered by e.g. overfishing or chronic pollution, a major disturbance will be superimposed on the already existing stress (Paine et al. 1998) and as a consequence an ecosystem can shift from a desired to a less desired state (Folke et al. 2004).

Photo 3. Macroalgae-dominated reef, Vietnam. Photo: M. Hellström
Aims and Scope of the Thesis

In order to be able to predict how environmental stress may have an impact on coral communities, it is necessary to study coral physiology. Although disturbances seldom come alone (Brown 1997b), most lab experiments on sublethal effects on corals focus on a single stressor (Coles and Jokiel 1977; Moberg et al. 1997; Råberg et al. 2003). In only a few studies two stressors have been combined (Coles and Jokiel 1978; Alutoin et al. 2001; Nyström et al. 2001; Norde-mar et al. 2003; Anthony et al. 2007). However, it is pertinent to explore how corals respond to compounded stressors since that will be the norm during the coming decades. Moreover, it is important to investigate the genetic diversity (i.e. local adaptation) of the coral host and its associated symbionts since it may explain differences in physiological responses.

The objective of this thesis is to investigate how different stressors affect the coral and associated symbionts, since this information will be of importance to the management of coral reefs in the future.

In paper I, I worked with acute stressors that may occur on coral reefs during e.g. monsoon periods, such as freshwater inflow (i.e. decreased salinity), eutrophication (enhanced nutrients) and increased seawater temperature, and how this affects the physiological performance in Turbinaria mesenterina.

In paper II, I worked with chronic stress, represented by turbidity, together with increased seawater temperature, and how the physiological performance in Porites lutea and Galaxea fascicularis are affected.

In paper III, I investigated if the Symbiodinium diversity in Porites lutea and Galaxea fascicularis could explain the results in paper II.

In paper IV, I investigated how chronic stress and latitude are affecting the Symbiodinium and host diversity in Galaxea fascicularis.
The aim of this thesis is to investigate:

- How single and multiple acute stressors affects the physiological performance in corals (paper I)
- How chronic stress in combination with increased seawater temperature affects the physiological performance in corals (paper II)
- If the Symbiodinium diversity in corals is affected by chronic stress (papers III, IV) and differences in latitude (paper IV)
STUDY ORGANISMS

The zooxanthellae symbiont *Symbiodinium* spp.

**The coral-zooxanthellae symbiosis**

The evolutionary success of reef building corals lies in the tight symbiosis between the coral host and unicellular algae, zooxanthellae. Zooxanthellae are Dinoflagellates (*Dinophyceae*) of the genus *Symbiodinium*, and they consist of a coccoid cell (Taylor 1987). The zooxanthellae are located in the endodermal cells in the gastrovascular cavity of the coral (Muscatine and Porter 1977). The host receives photosynthetic products such as sugar, amino acids, peptides and complex carbohydrates (Trench 1979; Sutton and Hoegh-Guldberg 1990; Swanson and Hoegh-Guldberg 1998) and the algae in turn get important nutrients (ammonia and phosphate) from the host’s waste metabolisms (Trench 1979). The coral also provides the algae with shelter. The energy from the photosynthetically fixed carbon from the algae also assists in the calcification and formation of the coral reef framework (Falkowski et al. 1984). Therefore reef-building corals are almost never found in deeper water where the light intensity is low. Corals behave as both primary producers, i.e. the zooxanthellae carry out photosynthesis and release excess carbon, and as primary consumers, since they utilize the photosynthetic products translocated from the algae (reviewed in Muscatine and Porter 1977; Goreau et al. 1979). Reef-building corals rely on the photosynthetic products of zooxanthellae for the major part of their energy requirement. However, they may also be considered detritus feeders and carnivores (Goreau et al. 1979).

**Symbiont types**

Until the 1970s, it was believed that the zooxanthellae consisted of only one single species, *Symbiodinium microadriaticum* (Taylor 1974 c.f. Coffroth and Santos 2005). However, modern molecular techniques have shown that *Symbiodinium* is a diverse group consisting of 9 different lineages (A-I) (on the level of order and family), which were earlier referred to as 'clades' (based on Rowan and Powers 1991a, 1991b; LaJeunesse et al. 2003, 2004a; Sampayo et al. 2007; Stat et al. 2009; Pochon and Gates 2010). Of these 9 lineages 6 have been found in corals: A-D, F and G (Baker 2003). Additional genetic diversity has been found within the different lineages, which gives a resolution down to species level (sometimes referred to as 'types') (LaJeunesse 2001, 2005; Coffroth and Santos 2005) and several hundreds of *Symbiodinium* types have been found in the
world’s oceans (Coffroth and Santos 2005). The C lineage has been found to be the most genetically diverse (Correa and Baker 2009).

Coral host species

*Turbinaria mesenterina*

*T. mesenterina* is a common scleractinian coral with colonies composed of unifacial laminae. The growth forms differ depending on the habitat; in subtidal habitats the laminae are highly contorted and fused, on upper reef slopes the laminae are upright or tiered and in deeper waters they are horizontal (Veron 2000). This species is tolerant to elevated water temperatures (Marshall and Baird 2000; Floros et al. 2004; McClanahan et al. 2005), and can dominate in shallow turbid environments (Veron 2000). The reproductive mode of the coral is gonochoric broadcast spawning (Baird et al. 2011).

Photo 4. *Turbinaria mesenterina* Photo: www.freeimages.co.uk
Porites lutea

*P. lutea* is a massive scleractinian coral species, which is common worldwide (Veron 2000). The colonies can be as large as 4 m in diameter and it is an important reef-builder. The coral is found on back-reef margins, lagoons and fringing reefs (Veron 2000). *P. lutea* is considered to be tolerant to bleaching (Riegl 1999; Loya et al. 2001; Obura 2001) and sedimentation (Rogers 1990). The coral is a gonochoric broadcast spawner (Fadlallah 1983; Shlesinger et al. 1998; Kerr et al. 2011), and the symbionts are transmitted vertically, i.e. they are transferred from the mother (LaJeunesse et al. 2003).

Galaxea fascicularis

*G. fascicularis* is a massive scleractinian coral species and it is found worldwide, except in the Caribbean (Veron 2000). They are regarded as tolerant to e.g. increased water temperature (Marshall and Baird 2000; McClanahan et al. 2004; Huang et al. 2011) and sedimentation (Philipp and Fabricius 2003). The reproductive mode of the species is called pseudogynodioecy, which is an unusual breeding system where the coral is gonochoric (i.e. separate male and female colonies) and hermaphroditic concurrently (Harrison 1988). The coral has horizontal uptake of symbionts (i.e. acquisition from the surrounding environment) (LaJeunesse et al. 2003).
STUDY AREA

All studies were conducted in Vietnam (Fig 2), where data for paper 1-3 were collected in Halong Bay, northern Vietnam, while in paper 4 samples were taken along the whole coast of Vietnam, from north to south.

Fig 2. Map of Vietnam showing areas for coral collection. Roman letters (I-IV) refer to collection sites with regards to the different papers.
Vietnam’s coastline is over 3200 km long, and stretches from the tropical south (09°55N) to the sub-tropical north (20°45N). This causes a gradient in seasonal temperature and also coral species diversity.

Vietnam is highly dependent on its coast: 80% of the population, or approximately 70 million people, are living within the coastal zone (FAO 2011). The coastline of Vietnam is the most densely populated area in Southeast Asia (Sekhar 2005). Millions of people are both directly (e.g. food) and indirectly (e.g. fishery) dependent on coastal resources for their livelihood (Burke et al. 2002), and many of these people are very poor. The coastal waters are important for fisheries since they are important breeding and spawning grounds, which are essential to sustain a natural fish population. However, the fish stocks at these important fishing grounds have decreased by up to 25-30% and in some places even 50% (Thanh et al. 2004).

Along the coast there are many big cities as well as industrialized zones. Mining areas and aquaculture activities are also located in the coastal areas. These are the main anthropogenic sources causing pressure on the marine environment (Country report on land-based pollution 2004).

Northern Vietnam is regarded as a sub-tropical area and the temperature over the year fluctuates dramatically (winter 16°C and summer 30-31°C), thus affecting the species composition: only the most tolerant organisms can survive (Nguyen 2004; Latypov 2005). Halong Bay in the north of Vietnam (Fig. 2) is a heavily polluted area, which receives land-run offs from the second largest river system in Vietnam, the Red River. The Red River Delta is a densely populated area where 17 million people live on only 17*10³ km². Furthermore, 20% of Vietnam’s rice production takes place there (Thanh et al. 2004), affecting the water quality.

Due to high human pressure most coastal marine areas are heavily disturbed throughout Vietnam.
METHODS

Paper I and II
Corals were collected in the field, using SCUBA and directly transported to an outdoor laboratory where they were allowed to acclimate in seawater tanks for 48 hours before being exposed to different stressors.

Paper I: *Turbinaria mesenterina* were exposed to increased nitrogen, reduced salinity and increased seawater temperature for 24 h in a factorial design with 1, 2 or 3 factors.

Paper II: *Porites lutea* and *Galaxea fascicularis* collected at chronically (nearshore) disturbed reefs were exposed to gradually increased seawater temperatures for 96 h, and compared with the responses of corals collected at less disturbed reef sites (offshore).

The corals’ response to the different stressors was investigated as changes in metabolism. The metabolism was studied by placing the corals in transparent plastic jars with tight-fitting lids without extra supplies of oxygen, thereafter changes in dissolved oxygen concentration in light (net primary production) and darkness (respiration) were measured using an oximeter. By using this approach it is possible to detect stress at an early phase (i.e. sublethal) despite a short exposure period. Net production- and respiration rates were expressed per hour and coral surface area. Respiration rate was added to the net production in order to achieve gross primary production rate. Gross primary production to respiration ratio (GP/R ratio) over 1 h and 24 h was also calculated. This ratio is a dimensionless estimate of the physiological status of the corals. A ratio equal to or above one over 24 h indicates that an organism is self-supporting while a value below one indicates that the organism needs to import energy to fulfil its energy demands (Hopkinson and Smith 2005).

Paper III and IV
Coral pieces were collected using SCUBA. One individual polyp of *Galaxea fascicularis* and small pieces of *Porites lutea* were sampled and stored in 95% ethanol until further DNA analysis.

2-4mm² of coral tissue was grounded in an eppendorf tube in order to break the symbionts’ cell walls and then DNAs were extracted using DNeasy Blood & Tissue Kit (Qiagen, Santa Clarita, Calif.).
The *G. fascicularis* host was genotyped using a fragment of non-coding intergenic region between Cyt b and ND2 in the mitochondrial DNA. The polymerase Chain Reaction (PCR) was performed using the primers 188-2-F and 188-1-R as outlined in Watanabe et al. (2005).

The symbionts of both *G. fascicularis* and *P. lutea* were genotyped by amplifying the *Symbiodinium* ITS2 region using the primers ITSintfor2 and ITS2noclamp (LaJeunesse and Trench 2000). PCR was amplified using a Touch Town (TD) protocol modified after LaJeunesse and Trench (2000) and Porto et al. (2008).

The PCR products were sequenced by direct sequencing in both directions using the reverse sequence as reference for *G. fascicularis* and the forward sequence for the *Symbiodinium* to avoid unambiguities.

All chromatograms were aligned by hand using MEGA5.0 (Tamura et al. 2011). The sequences were identified using BLAST search on NCBI. For the symbionts (paper III and IV) the alignments were verified by sequences based on LaJeunesse et al. (2003) and for the host (paper IV) the alignments were verified by the only existing published reference (AB109376) on GenBank.
Paper I: Physiological effects on corals after exposure to single and multiple acute stressors

The aim of this study was to investigate how the physiology of one coral species was affected by acute stressors that may occur on coral reefs and if this response was more pronounced if the corals were also exposed to increased water temperature.

In this study the coral Turbinaria mesenterina was exposed to increased nitrogen (+5 μM NO₃⁻) and/or reduced salinity (20 psu), in combination with increased water temperature (31°C) to sub-bleaching levels and compared to ambient water (25°C, 30 psu, 0.3μM NO₃⁻). The exposure was 24 h and thereafter the net production and respiration was measured with an oxymeter. Gross primary production rate and GP/R ratios over both 1 h and 24 h were then calculated.

The results showed that all corals exposed to increased temperature in combination with reduced salinity and increased nitrogen died after the exposure, whereas those that had been exposed to reduced salinity and increased nitrogen in ambient temperature did not show any stress response in gross primary production rates, respiration rates or GP/R ratio. Furthermore, corals exposed to increased temperature in combination with reduced salinity showed a decrease in GP/R ratio (Fig. 3a). The mortality rate was 50% compared to reduced salinity at ambient temperature where no mortality occurred. In addition, all corals exposed to increased temperature alone or in combination with another stressor displayed a GP/R²₄h ratio below 1.0 (Fig. 3b), suggesting that they depend on heterotrophic feeding or stored energy to cover their metabolic requirements. Single stressor treatments (i.e. increased temperature or reduced salinity or increased nitrogen) had no effect. The study shows that corals may be able to withstand short periods of exposure to increased nitrogen and/or reduced salinity, but when also faced with increased temperature their stress response increased dramatically. This highlights the importance of eliminating factors that can aggravate the effects of climate change, i.e. improving water quality.
Fig 3. Turbinaria mesenterina (mean values ± SE) a) GP/R\textsuperscript{1h} ratios b) GP/R\textsuperscript{24h} ratios. The dashed line indicates GP/R=1. A=ambient, HT=high temperature, HN=high nitrate, LS=low salinity. *p<0.05, †=all specimens in this treatment died.
Paper II: Physiological responses of corals exposed to increased temperature when chronically stressed

The aim of this study was to investigate how two coral species (Porites lutea and Galaxea fascicularis), exposed to different water quality (i.e. nearshore vs. offshore corals), were affected by increased seawater temperature. Corals were collected from two areas characterized by differences in water quality: nearshore (i.e. exposed to chronic stressors such as high sediment load and increased chlorophyll \( \alpha \) concentrations, and turbidity) and offshore (i.e. less disturbed) reefs. The corals were exposed in the lab to gradually increased water temperatures (25.5-33.5°C) for 72 h and thereafter the temperature was set back to the control temperature (25.5°C) for 24 h. Net production and respiration was measured with an oxymeter. Measurements were conducted after 24 h, 48 h, 72 h and 96 h. Gross primary production rate and GP/R\(^{1h}\) ratio were then calculated.

The results showed that \( P. \) lutea in nearshore reefs responded by an initial decrease in GP/R ratio after 24 h, after a moderate temperature increase (+2°C, from 25.5 to 27.5°C), but there were no differences in GP/R ratio for \( P. \) lutea between heat-exposed and control corals the other days. In comparison, nearshore \( G. \) fascicularis showed a decrease in GP/R ratio after 48 h, 72 h and 96 h of exposure (Fig. 4a). Also, after 72 h these corals had withdrawn their polyps (Fig. 4b), which is a common stress response in corals. There were no differences between the heat-treated and control offshore \( G. \) fascicularis. In conclusion, this study shows that chronic stress had no effect on \( P. \) lutea’s thermotolerance while the chronically stressed \( G. \) fascicularis had a lower thermotolerance when exposed to increased water temperature. Hence, the corals’ response to increased temperature varies both between different species and environmental background disturbance history.
Fig 4. *Galaxea fascicularis*. a) GP/R ratio (mean ± SE) nearshore corals. The dark bars show the controls (25.5°C) and the light bars are the heat-exposed corals (heat-exposure: day 1=27.5°C, day 2=30.5°C, day 3=33.5°C, day 4=25.5°C) *p<0.05. b) Polyp extension in nearshore (upper picture) and offshore (lower picture) corals when exposed to 33.5°C.
Paper III: Difference in *Symbiodinium* composition in two coral species from nearshore and offshore reefs

The aim of this study was to investigate potential differences in *Symbiodinium* diversity in two coral species, *Porites lutea* and *Galaxea fascicularis*, in areas with different environmental background, and how this might explain the results obtained in paper II.

Small pieces of *P. lutea* and one polyp of *G. fascicularis* were collected from nearshore (i.e. exposed to chronic stress such as high sediment load and increased chlorophyll α concentrations, and turbidity) and offshore (i.e. less disturbed) reefs, the same locations as used in paper II. The distribution of zooxanthellae ITS2 types was determined in the corals.

The results showed that all *P. lutea* was associated with the symbiont C15 (Fig. 5a), regardless of environmental disturbance history, while *G. fascicularis* was harbouring the symbionts C1 and D1a, and showed a strong nearshore-offshore pattern, with D1a dominating nearshore and C1 offshore (Fig. 5b).

*P. lutea* has normally been found to associate with C15, so this study further supports these findings. In addition, the study also confirms that the host specific association with C15 in *Porites* gives the coral tolerance to disturbances, since also the nearshore corals were populated by that symbiont. *G. fascicularis*’ association with D1a in the nearshore reefs is not surprising since D1a is normally found in extremely disturbed and marginal reefs, unsuitable for other symbionts. C1 has also been suggested to be a tolerant symbiont and that might explain why it was found in the offshore, not disturbed but still stressful, environment (affected by the large temperature fluctuations over the year). The results from this study indicate that nearshore more stressed *P. lutea* harbouring the host specific C15, is more tolerant to increased water temperatures, compared to *G. fascicularis* harbouring D1a, which normally is considered to be a temperature tolerant symbiont. Hence, nearshore *P. lutea* will be a ‘winner’ during times of environmental changes.
Fig. 5. The distribution of ITS2 types at the four different reefs in a) *Porites lutea* and b) *Galaxea fascicularis*. The different colours represent different ITS2 types; dark grey: C15, light grey: D1a, black: C1.
Paper IV: Difference in *Symbiodinium* diversity with regards to environmental gradients and latitude

In this study the coral *Galaxea fascicularis* was collected in four different regions along the coast of Vietnam (north, north-central, central and south), comprising different seasonal gradients, environmental gradients and coral species diversity. The distribution of zooxanthellae ITS2 types was determined in the coral. Host traits (mtDNA) and environmental factors (visibility, Chlorophyll a, sea surface temperature, distance from land (inshore/offshore) and coral species diversity) were measured.

The host showed an inshore/offshore zonation (Fig. 6a), where the higher diversity was found offshore. Six different ITS2 types of *Symbiodinium* were discovered in total (Fig. 6b). There was no genetic coupling between the host and the symbionts; hence, the differences in symbiont distribution were due to environmental factors. In all regions group D was found, consisting only of D1a, and it was consistently found inshore. Five ITS2 types belonging to group C (C1, C3, C3u, C21 and C27) were found, and they exhibited a strong latitudinal pattern. Group C was represented by one ITS2 type in the northern region (C1), two types in the north-central (C3u in Hue and C1 in Hoi An), all six types in the central region and three types in the southern region (C1, C3, C3u).

There was a change in dominance of ITS2 types, from C1 in the north towards C3u in the south. C1 has been suggested to be a temperature tolerant and resilient zooxanthellae type. The dominance of C1 in the north might be due to the higher temperature fluctuations and higher turbidity and sedimentation in northern Vietnam compared to the other regions. D1a showed a strong environmental zonation, and took over the dominance in the extremely stressed inshore reefs probably due to reduced competition from other symbiont types. The higher diversity of symbionts in central Vietnam might correlate with higher coral species diversity but it might also be caused by the temperature being more suitable for corals. A further explanation for the shift in symbiont diversity from north and north-central to central and south could be the strong currents that act as dispersal barriers.
Fig. 6 Genetic diversity of a) *Galaxea fascicularis* (based on mtDNA) and b) *Symbiodinium* (ITS2) types hosted by *G. fascicularis*, at inshore and offshore sites along the coastline of Vietnam.
DISCUSSION

*Turbinaria mesenterina* was not physiologically affected by a single acute stress, such as enhanced levels of nutrients, reduced salinity or increased water temperature (paper I). In contrast to this paper, other studies have recorded decreased GP/R ratios and/or production rates in *Pocillopora damicornis* and *Porites lutea* which were exposed to the same amplitude of decrease in salinity as in this paper (Moberg et al. 1997; Alutoin et al. 2001). On the other hand, Muthiga and Szmant (1987) did not find any effects on either gross production or respiration rates of *Siderastrea siderea* exposed to the same salinity. Hence, this shows that the effects of a salinity decrease may vary between coral species and/or geographic areas (Moberg et al. 1997). Paper I also showed that when combining two stressors (i.e. decreased salinity and increased temperature), the GP/R ratio decreased, demonstrating that two stressors are more harmful than one. This result is supported by the study by Coles and Jokiel (1978), which recorded a higher mortality rate in corals exposed to the combination of decreased salinity and increased water temperature. However, the most striking result in paper I was that a combination of all three stressors (i.e. enhanced nutrients, decreased salinity and increased temperature) resulted in 100% mortality. This indicates that increased water temperature might have a synergistic effect with the other two stressors. To my knowledge no other experimental studies have combined two stressors plus increased seawater temperature. Thus, paper I shows that ecological surprises can occur when an organism is exposed to multiple stress. In the real world, disturbances seldom come alone and therefore results like these are important for making predictions of how corals that are already exposed to several stressors (e.g. nearshore reefs) might respond to an increase in seawater temperature. These results will then be of importance for decisions regarding management of coral reefs. Moreover, this thesis shows that chronic stress in combination with increased water temperature affected corals differently (paper II). The thermotolerance in *Porites lutea* was not affected by chronic stress, while the chronically stressed *Galaxea fascicularis* on the other hand were more sensitive to a temperature increase. This shows that corals’ response to increased water temperature differs both with regards to species and their environmental history. The most likely explanation for the difference in response between these two species is due to the *Symbiodinium* diversity within the corals (paper III). *P. lutea* was found to only harbour ITS2 type C15, regardless of environmental disturbance history, while *G. fascicularis* was dominated by
ITS2 type D1a in the nearshore reefs and C1 in the offshore. Group D is normally regarded as being tolerant to temperature (e.g. Baker 2003; Rowan 2004; Fabricius et al. 2004; Mieog et al. 2009; LaJeunesse et al. 2010a; 2010b; Stat and Gates 2011) and other environmental disturbances (Toller et al. 2001; LaJeunesse et al. 2010). This could explain why the nearshore G. fascicularis mostly were associated with D1a, since this extremely stressed environment was unsuitable for other symbionts. However, the association with group D is a trade off between tolerance and growth (Little et al. 2004; Jones and Berkelmans 2010) and therefore corals harbouring this group might already live on the margin of what they can handle. Thus, this may be the explanation for G. fascicularis’ negative response to the temperature increase in paper II. Nearshore P. lutea only harboured C15, which indicates that this symbiont was also able to survive in this stressful environment. G. fascicularis reproduce by horizontal uptake, which means that they take up new symbionts from the water column, while P. lutea reproduce by vertical uptake (i.e. the symbionts are transferred from the mother) (LaJeunesse et al. 2003), and thus natural selection has favoured the evolution of symbiont specialization (Douglas 1998). This can be the reason why the two species had different types of symbionts. Porites spp. are mostly harboured by C symbionts (LaJeunesse et al. 2004a; Forsman et al. 2009) and are normally associated with C15 (LaJeunesse et al. 2003; Fabricius 2004; LaJeunesse et al. 2004b; Stat et al. 2009) hence, this association is very host-specific and the holobiont (host-symbiont-bacteria-virus-fungi; Correa and Baker 2011) is tolerant to several types of disturbances (LaJeunesse et al. 2003; Fabricius et al. 2004). Also in paper IV, there was a strong inshore-offshore pattern for group D in G. fascicularis along the whole coast of Vietnam, where D1a dominated on all inshore reefs, confirming other studies regarding group D’s tolerance to disturbances (Baker 2004; Rowan 2004; Fabricius et al. 2004; Mieog et al. 2009; LaJeunesse et al. 2010a; 2010b; Stat and Gates 2011). It was interesting that only one C type (C1) was found in G. fascicularis from northern Vietnam and that the diversity increased with latitude. C1 has also been categorized as a tolerant symbiont (Abrego et al. 2008; Jones et al. 2008), which might explain why this was the only C-symbiont found there. The highest diversity of symbionts was found in Nha Trang, central Vietnam, which also has the highest diversity of coral species and the most suitable water temperatures. So, it does not seem surprising that the highest diversity of symbionts were also located there. No other studies have investigated symbiont diversity on ITS2 level in the South China Sea. Thus, this study contributes to the knowledge of how different ITS2 types are distributed and may be important from a management perspective. Furthermore, paper IV shows the importance of investigating the symbiont diversity within one coral species on a large spatial scale. To date, only a few studies have been carried out on a large scale (Howells et al. 2009; LaJeunesse et al. 2010a; Pinzon and LaJeunesse 2011).
Symbionts and response diversity

This thesis shows that environmental stress has aggravated corals’ physiological response to other stressors (i.e. increased seawater temperature) (paper I and II) and also reduced the symbiont diversity (paper III and IV). The physiological responses (especially in paper II) are probably due to the corals’ association with different symbionts. This I base on the results from paper III and IV.

Studies have shown that different ITS2 types display variegated environmental responses, where some are more tolerant to e.g. increased temperature or sedimentation (Rowan and Knowlton 1995; Baker 2001; Rowan 2004; Garren et al. 2006; Abrego et al. 2008; Jones et al. 2008). Many of the symbiont types have the same ecological and physiological functions and can therefore be clustered together (Correa and Baker 2009). If a coral species is associated with several different symbionts that show varying sensitivity to different types of disturbances, there will be a larger chance that this coral will be able to withstand or survive several types of disturbances, i.e. a high response diversity is possible (Nyström 2006). On the other hand, Porites spp., which is almost always harbouring only the C15 type, is very tolerant to several disturbances.

The corals in central Vietnam, Nha Trang, showed high symbiont diversity, especially in the offshore reefs. Thus, these corals will probably have a higher chance to survive several kinds of disturbances, since the types of symbionts possibly perform differently depending on what disturbance they are exposed to. These reefs whose corals might have high response diversity are thus important to preserve. The reefs can additionally work as an important source population for other reefs in the surrounding area, thereby increasing the ecosystem resilience.

The diversity of symbionts in northern Vietnam was low yet, the most tolerant symbiont types were found there. However, with an improved water quality, perhaps other symbionts might be able to populate the area and then increase both the diversity of symbionts and eventually the ecosystem resilience. On the other hand, the lower symbiont and host diversity in the inshore compared to offshore reefs in northern Vietnam, dominated by very tolerant types, might also indicate that these corals have adapted to a harsher environment. Therefore, these reefs might be very important in the future since they can source an important gene pool. P. luttea has been shown to be the ‘winner’ during times of environmental changes (Loya et al. 2001). This species is very tolerant to several types of disturbances, both chronic stress and increased water temperatures (Philipp and Fabricius 2003; Marshall and Baird 2000; McClanahan et al. 2004; Huang et al. 2011), which might imply that this is one of the species that will be found on nearshore disturbed reefs yet in the future.

To increase the resilience of coral reefs it would be important to preserve offshore reefs with a higher diversity of species and symbionts, since they can act as a source population for other areas. However, at the same time it is important not to forget the nearshore, disturbed reefs, which normally have a
lower species and (especially in the north) symbiont diversity, since the most tolerant species will be found there.

CONCLUSION
In this thesis I have shown that water quality is important for corals’ tolerance to further stressors, and that degraded water quality might act synergistically with increased water temperature causing mortality of corals. In addition, this thesis also demonstrates that the physiological responses may differ depending on coral species and also what symbionts they harbour.

To increase the capacity of a coral reef to recover after disturbances, it is not only important to try to preserve different reef areas, both in order to increase the diversity of coral symbionts and to conserve tolerant symbionts, but it is also very important to improve the water quality, since that is a major problem for coral reefs and further increased sea surface temperatures might cause synergistic effects with already existing disturbances.


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