Sex and symbionts
New discoveries in local and regional patterns of coral reproduction and ecology

Micaela Hellström
“The sea - once it casts its spell - holds you in its net of wonder forever.”

Jacques Yves Costeau
(1910-1997)
List of papers

The papers in this thesis are referred to in the text by their roman numerals:
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Additional publications by the author of this thesis:


Abstract

Coral reefs belong to the most diverse and the most threatened ecosystems on earth. Anthropogenic stressors and climate change have led to mortalities at levels unprecedented in modern times. The aims of this thesis are to investigate aspects of the corals’ ability to reproduce, disperse, adapt and survive in order to maintain themselves. Papers II-III study reproduction in a common soft coral species, *Sarcophyton elegans*, with previously unknown reproductive modes. Paper IV investigates genetic distribution of coral-symbiont associations in *Galaxea fascicularis* focusing on adaptation to the environment along the coastline of Vietnam.

*S. elegans* is a gonochoric broadcast spawner with a 1:1 sex ratio. Reproduction is strictly size dependent (*Paper II-III, method; Paper I*). Oogenesis takes 19-24 months, with a new cycle commencing every year. Spermatogenesis takes 10-12 months. The majority of gametes were released during the annual austral mass spawning event after full moon in November, but spawning also occur between August and February. The polyps at the outer edge of the colonies released their gametes first, followed by polyps situated closer to the center during subsequent months. Colonies upstream in the prevailing current spawn earlier than those downstream (*Paper II*). The colonies were arranged in clusters of alternating males and females, which spawned simultaneously and were of the same genotype (*Paper III*). Fission and budding is a common mode to expand locally. Additionally, females undergoing fission divided into the most fecund size classes (*Paper III*).

The *G. fascicularis* and their associated symbionts were not genetically coupled to each other but to environmental factors (*Paper IV*). The host displayed an inshore-offshore zonation, with higher diversity offshore. The D1a symbiont exhibited an inshore-offshore zonation. In contrast; the 5 different C symbiont types showed a latitudinal distribution gradient, which shifted in dominance north to south. The study highlights the importance of protecting resilient coral and algal genotypes in stressed areas (*Paper IV*) and the need to understand reproductive modes (*Papers II-III*) for coral conservation.

**Keywords:** Indo-Pacific, *S. elegans*, *G. fascicularis*, *Symbiodinium*, size, reproduction, allozymes, ITS2, mtDNA, geography, environment
Svensk sammanfattning


Korallen G. fascicularis upptar alg symbionter helt oberoende av genetisk uppsättning. Däremot styrs förekomsten av genotyper hos både korallvärd och alg av miljöfaktorer. Reven nära kusten som utsätts för mycket föroreningar, visar genetiska uppsättningar av alg som är stresstålig (typ D) och genotyper av koraller som skiller sig mellan förorenade och renal miljöer. Algtypen C visade sig bestå av många genetiska varianter som visade en ändring av utbredning i nord-sydlig riktning. Mångfalden av alg i G. fascicularis kan bero på att artens larver upptar algerna från vattnet (horisontell upptagning) och inte från föräldralokalier (vertikal upptagning). Detta är en direkt konsekvens av yttre befruktning. Denna studie visar vikten av att förstå korallers reproduktionsstrategier, samt behovet att skydda korallarter i stressade områden eftersom de uppvisar tåliga genotyper hos både alg och korallvärd.
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Introduction

Background
Coral reefs belong to the most diverse and the most threatened (Bellwood et al. 2004) ecosystems on earth, often referred to as rainforests of the sea (Connell 1978), not just because of the estimated 800 to 1000 species of reef building corals themselves (Veron 1993, 1995, 2000, Knowlton 2008) and the thousands of closely related taxa of soft corals (Benayahu and Loya 1977, 1981 Dinesen 1985, Fabricius and Alder slade 2001), but because of the 1-9 million other species living mainly or entirely in relation to them (Readka-Kulda 1997, Knowlton 2008). The reef community includes 32 of the 33 phyla inhabiting marine ecosystems, compared to 17 phyla in total hosted by terrestrial ecosystems (Norris 1993). The coral reefs cover only 0.1-0.5% of the ocean floor, still, an estimated 30% of marine fish live on coral reefs (Moberg and Folke 1999). The reef building corals and a majority of the tropical soft corals exist in a close nutritional partnership with unicellular algae (Protista) of the genus *Symbiodinium* called zooxanthellae. The symbiosis is a key factor behind the high levels of productivity on the reef (Muscatine 1990, Yellowlees et al. 2008). The acquisition strategies of the symbionts are either vertical (transfer to the offspring from the parent colony) or horizontal (acquisition from the surrounding environment) and depend on the reproductive modes of the corals (brooders or spawners) which determine the portfolio of symbiont types within the host (LaJeunesse 2005, Stat et al. 2008, Stat et al. 2011b). Regional differentiation patterns of these taxa may be affected by the type of acquisition strategy (LaJeunesse et al. 2010a).

Coral reefs are of crucial importance for the daily livelihood of the human populations living around them, including some of the poorest populations in the world (Fisher et al. 2011). The reefs provide essential ecological goods such as; food production for both local consumption and export, protection of the shoreline from wave erosion and income from recreational activities (Moberg and Folke 1999, United Nations Environment Programme 2008). Ten per cent of the fish consumed by humans originate from the reefs (Smith 1978). In 2003 the economic net gain from the coral reefs was estimated to exceed USD 30 billion (Cesar et al. 2003). Additionally soft corals on the reefs produce highly complex compounds with medicinal potential, the scope of which is still to be explored (Carte 1996, Sella and Benayahu 2010).
In recent decades the reefs have changed profoundly due to anthropogenic stressors such as; increased sedimentation, nutrient loading, pollution and destructive fishing (dynamite fishing, cyanide fishing and over-fishing) (Hughes et al. 2003, Pandolfi et al. 2003, Nyström et al. 2008, Burke et al. 2011). Since the mid 1970’s and 1980’s climate change and elevated water temperatures have led to local and global coral bleaching followed by mass mortality of reef building corals (Wilkinson 2000, 2004). Negative human impact in combination with increasing and decreasing SSTs (sea surface temperatures) (Hoegh-Guldberg 1999, LaJeunesse 2010a), and increased ocean acidity (Hoegh-Guldberg et al. 2007, Knowlton and Jackson 2008) can make the conditions for the symbionts unsuitable, and they leave their host (mass bleaching; Glynn 1993, Glynn et al. 1996). A consequence of this is high levels of coral mortality with devastating consequences for the organisms living on the reefs (loss of habitats) and for the human populations who depend on them (Moberg and Folke 1999, Nyström et al. 2000). In the late 1990’s and early 2000’s following a period of unusually high SSTs, levels of coral bleaching unprecedented in modern times, caused the demise of 30%-50% of the world’s coral reefs and alterations to coral communities species compositions (Wilkinson 2000, 2004, Hoegh-Guldberg et al. 2007). During the past 30 years a reduction of live coral cover by 50-95% on a global scale has been estimated (Jackson 2008, Jackson 2010, Burke et al. 2011). These events have also locally reduced soft coral cover by 50% (Bastidas et al. 2004) to 95% (Benayahu 2007) compared to the initial area covered before bleaching events.

Increased environmental stressors make the corals more susceptible to pathogens (Palmer et al. 2010, Haapakylä et al. 2011), bleaching, and reduce their reproductive output (Michalek-Wagner 2001, Baird and Marshall 2002, Ward et al. 2002). These events have a long lasting effect on the coral reef ecosystems and raise the questions about ecology, biogeography and evolution of the algal-coral symbiotic partnerships.

If bleaching continues to intensify, entire soft coral species may go extinct before they even are discovered (Benayahu 2007) and their pivotal biological, chemical and physical roles in the ecosystems on the coral reefs may not be fully realized before it is too late. The soft coral taxonomy is even more complex and unknown than that of scleractinian corals, and therefore soft coral research has been overshadowed by scleractinian research. In recent years attempts to farm soft corals in order to extract their secondary compounds for the industry (Sella and Benayahu 2010) have underlined the importance of understanding their life history characteristics.

Surveys on coral reef management and population genetics to date have concentrated on regions in the Caribbean, Hawaii, the Red Sea and the Great Barrier Reef whilst high diversity reefs surrounding poor nations in the cen-
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tral Indo-Pacific and Southeast Asia are heavily underrepresented (reviewed by Fisher et al. 2011). The latter areas are heavily populated and face environmental challenges as rapidly growing human populations (depending on the goods from the reefs) and emerging economies put these ecosystems under pressure. Today more than 60% of the coral reefs worldwide are threatened and 50% of these classified as highly threatened, the corresponding rates in South East Asia are 95% and 50% (Burke et al. 2011).

The close association of corals, bacteria, symbiont algae and fungi together form the ‘holobiont’ (Rohwer et al. 2002, Stat and Gates 2011) which is highly interconnected to processes such as; reproduction of the host (uptake of symbionts determined by brooders or spawners) (LaJeunesse 2005, LaJeunesse et al. 2010b), predation of corallivorous fish (disperse the symbionts through excrements) (Porto et al. 2008) ocean currents for dispersal, and environmental factors such as SST’s and pollution. The importance of understanding the different ecological processes that maintain the coral reef ecosystems is pertinent for efficient conservation efforts of these threatened ecosystems.

Thesis objectives
The objective of this thesis is to gain further insight into two important interlinked aspects of coral reef research crucial for assessing the maintenance and survival of coral populations. The first half of the thesis focuses on sexual and asexual reproduction in a common species of soft coral Sarcophyton elegans on the Great Barrier Reef (Papers II and III) with previously unknown reproductive modes. Reproduction is a key for survival through recombination, ability to disperse and is also reported to determine the uptake of life supporting symbionts. The data collected are based on observations before global coral reef mass mortality events and is therefore a potential baseline study for post bleaching research. The study also suggests a reliable metric of soft corals for repeated measure studies (Paper I). The second part of the thesis focuses on genetic diversity and distribution in a scleractinian coral species, Galaxea fascicularis, and its associated symbionts in relation to environmental factors along a vast latitudinal and environmental range and is the first study of its kind in the South China Sea (Paper IV). The reefs in Vietnam are disintegrating at an alarming rate mainly due to anthropogenic stressors and a rapidly growing population. This thesis highlights the importance of understanding different biological processes such as genetic adaptation to environmental factors and the consequences of modes of sexual and asexual reproduction for dispersal and survival.

Paper I was set out to determine a valid measurement for different growth forms of soft corals, which might be applicable to other erect fleshy soft coral species and other soft bodied invertebrates. Our hypothesis was that the aggregated cemented spicules at the colony base provide more structure to
the colony as the base is closely attached to the substrate and therefore less likely to be affected by water intake and expulsion than other parts of the colonies. The study aimed to find a reliable measure related to biomass and reproductive potential without showing too much temporal fluctuations due to the variations of water intake of the hydroskeleton by:

- Measuring variation of different dimensions in soft corals of contrasting morphology to find a stable measure over 24 hours.
- Establishing the rate of variation/stability in measures used in earlier studies.
- Establishing ecological relevance of measure by correlating the metric to body volume (a proxy for potential fecundity) after water displacement.

The objectives of Papers II-III were to document in detail the sex differentiation, mode and timing of reproduction at different scales in an ecologically important soft coral species *Sarcophyton elegans* by:

- Determining the modes sexual reproduction (Paper II) e.g. broadcast spawning or brooding, and asexual reproduction (Paper III) asexual planulae, fission, buddying, stolon formation and other forms of vegetative growth.
- Estimating the life history characteristics such as sex ratio, gametogenesis (time and mode of oogenesis and spermatogenesis) and size (measured according to Paper I) at first reproduction (Paper II).
- Distinguishing if the prevailing paradigm of one single mass-spawning event characterized the timing of reproduction or not, in monthly surveys over 2.5 years (Paper II).
- Comparing the timing of spawning at different spatial scales, from within a single colony to between colonies and between local sizes and identifying possible correlations (Paper II).
- Establishing possible sex, size and habitat dependent modes of asexual reproduction (Paper III).
- Establishing contribution of asexual and sexual reproduction to one of the study populations by examining genetic differences using allozyme gel electrophoresis (Paper III)

Paper IV tested hypotheses regarding the relationship between several environmental factors and diversity and genetic differentiation among latitudinally separated populations of corals and symbiotic zooxanthellae. The study determined distribution of zooxanthellate ITS2 types within one broadcast spawning coral species, *Galaxea fascicularis* (Harrison 1988) with horizontal symbiont uptake (Baker et al. 2008) in relation to:

- Inshore (anthropogenic stressors) and offshore (less stressed) reef habitats.
- Latitude over a 3200 km range of the coast of Vietnam, covering 11 degrees of latitude.
- Host characteristics (mtDNA genotype).
- Environmental factors (sea surface temperatures and chlorophyll derived from satellite data, depth and visibility).

**Study Species**

*Position in the tree of life: Scleractinia, Alcyonacea and Dinoflagellata*

The common characteristics for corals, jellyfish, sea anemones and hydrozoans belonging to the phylum Cnidaria are radial symmetry, possessing nematocysts which are stinging capsules (cnidae) for capturing prey, true tissue (dipoblastic) and lack of a body cavity. The class Anthozoa includes hard and soft corals, sea pens, sea anemones and zoanthids and possesses tentacles in the adult form. Hard or scleractian corals belong to the subclass hexacorallia (they possess a multiple of 6 tentacles at the oral end of the polyps) and represent approximately 800-1000 species (Veron 2000), and soft corals belong to the subclass octocorallia (they possess 8 tentacles at the oral end of the polyps) with more than 3000 species divided between three main orders; Pennatulacea (sea pens), Helioporacea (blue corals) and Alcyonacea (Daly et al. 2007).

Many of the tropical hard and soft corals live in symbiosis with dinoflagellates (genus *Symbiodinium*) called zooxanthellae. The zooxanthellae types within the gastrodermal cells of the animal host are photosynthetic. The vast majority (approx. 95%) of the assimilated organic carbon (‘photosynthate’) is typically translocated to the coral, contributing substantially to its carbon and energy needs (Trench 1993, Yellowlees et al. 2008). In return the symbionts receive protection from external predators and receive host derived substrates, principally carbon dioxide (CO$_2$(aq)) and ammonium (NH$_4^+$) (Trench 1993, Yellowlees et al. 2008). The organic carbon from the algae enables the host to form large CaCO$_3$ deposits. This relationship is the basis for the coral reefs which are the largest structures on earth constructed by a living organism.

Soft corals do not form large CaCO$_3$ structures like scleractinian corals but form species specific spicules instead. This is due to differences in the three dimensional structure of the CaCO$_3$ molecules in the two subclasses.

Dinoflagellates belong to the Kingdom Protozoa, parvkingdom Alveolata and phylum Dinoflagellata (Cavalier-Smith 1993). About half of all aquatic protists in the phylum Dinoflagellata are photosynthetic, making up the largest group of eukaryotic algae aside from the diatoms (Lin 2011). The species of the *Symbiodinium* genera are both free-living and symbiotic, and cornerstones of the coral-algae-bacteria holobiont complex underlying the possibilities for reef building corals to exist (Patten et al. 2008, Stat and Gates 2011).
**Sarcophyton elegans**
The fleshy soft corals of the genus *Sarcophyton* belong to the order Alcyonacea and are widespread on tropical reefs worldwide. *Sarcophyton elegans* is common in shallow lagoons and reef flats throughout the GBR, often appearing in large monospecific aggregations (Papers I-III). Adults and juveniles of *S. elegans* are relatively easy to distinguish from other *Sarcophyton* species by morphology and by examining the microscopic sclerites, which are species specific (Fabricius and Alderslade 2001). The colonies are mushroom shaped, and the disc-like polyp-bearing region (the polypary) has two different kinds of polyps; large autozooids that bear tentacles and smaller, more numerous siphonozooids lacking obvious tentacles (Fabricius and Alderslade 2001). Prior to this study the reproductive mode of this zooxanthellate soft coral was unknown.

**Galaxea fascicularis**
The colonial scleractinian coral *G. fascicularis* is common on varying reef habitats and shows a wide distribution range. The species does not exist in the Caribbean but has been encountered on reefs everywhere else in the world (Veron 2000). The species has a reproductive mode called pseudogynodioecy: an unusual breeding system where the coral is gonochoric (i.e. separate male and female colonies) and hermaphroditic simultaneously. The female colonies produce viable egg cells in a yearly cycle; however, the males produce normal sperm cells and sterile pseudo-eggs within the same colony (Harrison 1988). The species is a broadcast spawner and during gamete release the pseudo-eggs in the hermaphroditic colonies function as floaters to push the sperm to the surface for fertilization with the viable egg cells from female colonies (Harrison 1988). The species is hermatypic and is reported to be resilient to bleaching (Yamazato 1999, Marshall and Baird, 2000, Huoang et al. 2011) and sedimentation (Philipp and Fabricius 2003). Due to its wide latitudinal distribution and presence in both disturbed and pristine habitats the species is ideal for wider population genetic studies.

**Symbiodinium sp.**
When first discovered in the 1970’s symbiotic zooxanthellae were thought to be only one, pandemic species named *Symbiodinium microadriaticium* (presently classified as A1). Today, because of the advancements in molecular biology, 9 groups (A-I) of *Symbiodinium* have been identified as distinct lineages earlier called clades (based on Rowan and Powers 1991a, 1991b, LaJeunesse et al. 2003, 2004, Sampayo et al. 2007, Stat et al. 2009, Pochon and Gates 2010). Of these; A-D, F and G are known to inhabit corals (Baker 2003). The different endemic *Symbiodinium* lineages can be delineated to types (subclades, genetically designated with ITS1 and ITS2 rDNA, of which close to 100 have been discovered) and are in many cases distinct species; possibly even distinct on a higher taxonomic level (LaJeunesse 2001, 2005, Coffroth and Santos 2005).
It is generally perceived that certain *Symbiodinium* types (based on the ITS2 level) are ‘host generalists’ associating with a wide range of hosts, whereas others are ‘host specialists’ associating with only certain host species or genera (Sampayo et al. 2007, LaJeunesse et al. 2010a, Wicks et al. 2011). Some types are even shown to associate to different genotypes (based on the putative control region) within one host (Bongaerts et al. 2010). The tolerance of corals to environmental stressors are partly attributed to the different symbiont groups with group D being more thermally tolerant than group C (e.g. Rowan and Knowlton 1995, Baker 2001, Berkelmans and van Oppen 2006). However, an increasing number of studies based on ITS2 types suggests varying function of types within the A-F or G group, and provide the possibility to further investigate coral symbiont interactions (i.e. LaJeunesse 2003, Sampayo et al. 2007, 2008, Frade et al. 2008, La Jeunesse et al. 2010b, LaJeunesse 2011).

Given the importance of the algal symbiosis to coral survival the number of symbiont types and the patterns of their occurrence among marine hosts are highly relevant to understanding the ability of the holobiont (coral-host-bacteria-fungi complex) to adapt to environmental change (Buddemeier and Fautin 1993). To date the majority of surveys determining the distribution of *Symbiodinium* in coral hosts have been based on an inclusion of very few colonies per species (1-5) in large multispecies surveys over a variety of geographic scales (e.g. LaJeunesse et al. 2010a, Wicks et al. 2010), or on one species of host within a limited geographic region (Bongaerts et al. 2010, Stat et al. 2011). Only a few studies have looked at within-species diversity of symbionts across broad geographic regions (Howells et al. 2011 (soft coral), Pinzon and LaJeunesse 2011).

**Reproduction in Soft Corals**

*Sexual reproduction:* The mode and timing of reproduction are key life history characteristics influencing the population dynamics, ecology and evolution of organisms (Stearns 1992), but major characteristics of reproduction remain unknown for many marine species. For example, whilst most hard corals are hermaphroditic (male and females in the same colony) (Carlon 1999), it has only been established relatively recently that soft corals are primarily gonochoric (having separate males and females), as reviewed in Benayahu (1997) and in Hwang and Song (2007). Timing of reproduction is also variable among corals, but one general observation from the limited data available is that soft corals in temperate waters tend to show continuous gametogenesis, internal fertilization and brooding of developing embryos (Gohar 1940, Hartnoll 1975, Farrant 1985, Cordes et al. 2001, McFadden et al. 2001), however, recent studies in temperate environments have shown that reproduction in deep sea temperate corals is more complex (Mercier and Hamel 2011). In

Soft corals (Cnidaria: Alcyonacea) have this far shown three different modes of sexual reproduction including internal brooding (Achituv and Benayahu 1990), external surface brooding of planulae larvae (Dinesen 1985, Benayahu et al. 1990) and broadcast spawning of gametes with fertilization taking place in the water column (e.g. Alino and Coll 1989, Babcock 1990, Benayahu 1997). The mode of reproduction and length of breeding season varies both within genus (Hartnoll 1975, Dahan and Benayahu 1997, Hwang and Song 2007) and species (Benayahu and Loya 1986, Schleyer et al. 2004) depending on the geographical location of the corals.

**Asexual reproduction**

Soft corals are reported to possess a large range of mechanisms of clonal propagation such as budding of daughter colonies in *Sarcophyton gemmatum* (Verseveldt and Benayahu 1978); fragmentation in *Junicella fragilis* (Walker and Bull 1983); colony fission in *Capnella gaboenis* (Farrant 1987), *Xenia macrospiculata* (Benayahu and Loya 1985), *Alcyonim digitatum* (McFadden 1986), and *Sinularia flexibilis* (Bastidas et al. 2004); formation of stolons in *Efflatunaria* sp. (Dinesen 1985, Karlson et al. 1996), rapid autotomy of small fragments with root-like processes in *Dendronephthya hemprichi* (Dahan and Benayahu 1997) and production of asexual planulae (Fautin 2002 review). The ecological and biological circumstances in which a particular mode of asexual reproduction is favored are not well understood for any coral.

Asexual propagation is considered to be the most dominant mode of reproduction in soft corals (Verseveldt and Benayahu 1978, Fabricius 1997, Fabricius and Alderslade 2001). However, three recent studies showed that sexual reproduction may play a significant role in the population dynamics in soft coral species including *A. digitatum* (McFadden 1997), *Sinularia flexibilis* (Bastidas et al. 2001) and *Clavularia koellikeri* (Bastidas et al. 2002). Even so, the two latter studies concluded that asexual recruitment played a more important role for the population than sexual recruitment.

The variety of modes of reproduction revealed by a relatively small set of studies, usually limited in spatial and temporal extent, suggests that more detailed research is required to better document the reproductive strategies of soft coral species. However, comprehensive long term datasets on timing and mode of coral reproduction are extremely rare (Bastidas et al. 2002, Fuchs et al. 2006).
Materials and methods

Study Areas

Australia

The studies for this thesis were conducted in different parts of the Central Indo-Pacific. The soft coral studies (papers I-III) took place in the vicinity of Lizard island in the Northern Great Barrier Reef (14°40’S, 145°28’E): Site A was on shallow Loomis Reef at the western entrance of the Lizard Island lagoon. Site B was on a small wave-exposed patch reef between South Island and Palfrey Island of the Lizard Island group (Fig. 1b). The water depth at both sites ranged from 3 m at high tide to total exposure during maximum low tide in the austral spring. All field observations and collections were performed by snorkeling or SCUBA. Additionally for measurement studies in Paper I, specimens of the a zooxanthellate soft coral *Dendronephthya sp.* were collected from the deep wave exposed northern side of the island.

Vietnam

Corals were collected in March 2009, August 2009 and in April 2010 from 11 sites using SCUBA and snorkel from 4 regions (North, North Central, Central and Southern) in Vietnam (Fig. 1a, Table1). Sample locations ranged over several degrees of latitude (09°55N-20°45N) and a maximum distance of 3200 km, and encompassed major gradients in seasonal temperatures and coral species number (Table 1). The distances from the sites to the mainland were recorded (km), and included sites with reduced visibility, lower light levels, and higher sediment load (usually nearer shore) and those with greater visibility, higher light levels and reduced sediment load (usually further offshore). The inshore sites were situated at 50 to 800 meters from the mainland whilst the offshore sites were situated 8-14 km from the shoreline.
Figure 1. Map of study sites in the Indo-Pacific. The sites in (a) Vietnam (inserted enlargement) are denoted 1-11 and described in detail in Table 1. The sites on (b) Lizard Island, Great Barrier Reef, Australia are denoted A (Lagoon site); and B (Exposed site, close to South Island). (Main map: Google Earth™ mapping service, insert of Lizard Island taken by Barry Goldman Australian Museum)

Oceanographic data
Data for monthly Chl a (chlorophyll a, mg m$^{-3}$) (a proxy for turbidity) and SSTs (sea surface temperatures °C) measured between July 2002 and December 2010 were retrieved from the Giovanni online data system, which is maintained by the NASA Goddard Earth Sciences Data and Information Services Center. SeaWiFS, NOAA/AVHRR measurements were averaged over 9 km$^2$ from areas close to the sampling sites (Fig. 2). Local SSTs and additionally water temperatures at 5 and 15 m, and visibility data (kindly provided by local dive operators in Nha Trang, Phu Quoq and Hoi An and by IO and IMER divers on the other sites) were used to confirm the satellite-derived SSTs and Chl a data respectively. This local verification was useful because Chl a satellite data taken from near shore with pixels falling within a land-water interface are more unreliable than data taken offshore (Dien and Hai 2006, Maynard et al. 2008).
Figure 2. Average monthly values (±S.D.) between 2002 and 2010 of SSTs (a) and chlorophyll $a$ concentrations (proxy for turbidity) for the locations based on satellite imaging. Inshore and offshore locations in Cat Ba and Nha Trang showed almost identical SST curves and therefore only one curve each present the SST’s in Cat Ba and Nha Trang. I and O in panel b) denote Inshore and Offshore respectively.
Table 1. Background information on study sites including, region (North-South), number of scleractinian corals per region (Veron et al. 2009), GPS coordinates, distance to mainland (ML) in kilometers (km), inshore-offshore (IS, OS), maximum site depth, average (avg.) sea surface temperature (SST) (average from July 2002 to Dec 2010), average yearly SST range (monthly average SST’s averaged over 2002 – 2010), avg Chlorophyll a (Chl a) (average from July 2002 to Dec 2010), average yearly Chl a range (monthly average Chl a averaged over 2002 – 2010) and avg. visibility range (based on daily data by dive operators averaged over 2005-2009).

<table>
<thead>
<tr>
<th>Region</th>
<th># Coral sp.</th>
<th>Site</th>
<th>Coordinates</th>
<th>ML dist. (km)</th>
<th>Depth max (m)</th>
<th>SST (°C)</th>
<th>SST range (°C)</th>
<th>Chla (mg/m^3)</th>
<th>Chla range (mg/m^3)</th>
<th>Vis range (m)</th>
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<tbody>
<tr>
<td>North</td>
<td>182</td>
<td>1. Cat Ba IS I</td>
<td>20°45'N, 107°04'E</td>
<td>0.8</td>
<td>2.8</td>
<td>25.5</td>
<td>17-32</td>
<td>2.4</td>
<td>0.84-5.94</td>
<td>0.5-4</td>
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<td></td>
<td>2. Cat Ba IS II</td>
<td>20°47'N, 107°06'E</td>
<td>0.1</td>
<td>2.2</td>
<td>25.5</td>
<td>17-32</td>
<td>2.4</td>
<td>0.84-5.94</td>
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<tr>
<td></td>
<td></td>
<td>3. Cat Ba OS I</td>
<td>20°37'N, 107°09'E</td>
<td>14.2</td>
<td>6.7</td>
<td>25.5</td>
<td>17-32</td>
<td>1.6</td>
<td>0.45-3.00</td>
<td>1.5-10</td>
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<td></td>
<td>4. Cat Ba OS II</td>
<td>20°37'N, 107°08'E</td>
<td>13</td>
<td>2.8</td>
<td>25.7</td>
<td>17-32</td>
<td>1.6</td>
<td>0.45-3.00</td>
<td>1.5-10</td>
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<tr>
<td>North</td>
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<td>5. Hue</td>
<td>16°14'N, 108°12'E</td>
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<td>6</td>
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<td></td>
<td>6. Hoi An</td>
<td>15°56'N, 108°29'E</td>
<td>12</td>
<td>12</td>
<td>27.1</td>
<td>20-32</td>
<td>1.2</td>
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<td>1.0-30</td>
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<tr>
<td>Central</td>
<td>397</td>
<td>7. Nha Trang IS I</td>
<td>12°12'N, 109°13'E</td>
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<td>4.2</td>
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<td>24-30</td>
<td>0.5</td>
<td>0.05-1.96</td>
<td>0.5-4</td>
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<td>8. Nha Trang IS II</td>
<td>12°10'N, 109°12'E</td>
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<td>4</td>
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<td>24-30</td>
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<td>0.05-1.96</td>
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<td></td>
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<td>0.16-2.47</td>
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<td>10. Nha Trang OS II</td>
<td>12°04'N, 109°18'E</td>
<td>8.3</td>
<td>10</td>
<td>27.6</td>
<td>24-31</td>
<td>0.5</td>
<td>0.16-2.47</td>
<td>4.0-30</td>
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<td>South</td>
<td>404</td>
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<td>09°55'N, 103°59'E</td>
<td>11</td>
<td>12</td>
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</tbody>
</table>

Methods Paper I: Colony Measurements
To test the hypotheses that the basal circumference is a more precise measure of colony size we used three different morphologies of soft corals represented by; *Sinularia flexibilis* (branching fleshy growth form with a very calcified base), *Sarcophyton elegans* (erect mushroom shaped growth form, with a relatively calcified base) and *Dendronephthya sp.* (erect growth form, with a mainly water filled interior). Ten individual colonies of each species at depths greater than 5 m were measured eight times over 24 hr providing coverage of two tidal cycles, and one diurnal period.

Colony height, oral disc diameter and basal stalk circumference (Fig. 2) were measured to the nearest cm with a measurement tape after the colonies were touched firmly to cause colony contraction (as described in Fabricius 1995).

In December 1993, a second set of observations aimed to determine which field measure of colony size correlated best with colony volume (after water
displacement in formalin). The best predictor of colony volume was examined using linear regression.

![Image](image.png)

Figure 3. Illustration of the measurements of the colony size made for (a) Sarcophyton elegans, (b) Sinularia flexibilis and (c) Dendronephthya sp.

### Methods Paper II: Sexual Reproduction in S. elegans

**Colony sexual identity, sex ratio, and size at onset of sexual reproduction**

At each study site (Fig. 1b), three 1 x 10 m belt-transects were laid down end to end on the reef. In December 1991 a detailed map was drawn and included sizes of all the colonies within the transects (Paper I) to determine colony size distribution.

In October 1991, several weeks before the predicted coral annual mass spawning (e.g., Harrison and Wallace 1990), the sex of the colonies were determined by making a small incision in the polypary which exposed the mesenteries containing the visible gonads (Alino and Coll 1989) The basal circumferences were measured according to Paper I. The collection was biased towards smaller colonies in order to detect the smallest size at maturity. Histological samples in the smaller colonies were processed as described below in order to confirm the absence of gametes in the colonies that appeared to be immature.

**Gametogenic cycle**

To determine the length of the gametogenic cycle, 10 large male colonies and 10 large female colonies (> 20 cm in stalk circumference) outside the transects at sites A and B (Figure 1) were numbered with plastic markers. The marked colonies were sampled approximately monthly between October 1991 and January 1994, by taking a small biopsy as outlined by Benayahu and Loya (1986). All samples were preserved in 10% formalin in seawater (v/v) for three days and then transferred to 70% ethanol for further analysis. The samples were stained with eosin and haematoxylin according to Winsor (1984). Diameters of the oocytes and spermarys were measured with a micrometer under a compound microscope. Fresh samples were examined under a stereomicroscope to establish the color difference between mature
and immature gonads. The gametogenic cycle was determined according to descriptions by Glynn et al. (1991) and Schleyer et al. (2004).

Temporal spawning dynamics
The positions of the colonies along the belt transects were recorded and their reproductive state was noted monthly from October 1991 to January 1994. The colonies were monitored every two weeks for spawning activity after both the full moon and new moon, between August and March 1992 and 1993 and in January 1994 as higher reproductive activity occurred in those months. To obtain information on the spatial distribution of immature, mature and spawned mesenteries in individual polyparies, incisions were made at several points along the radius of the polypary to expose the mesenteries. A colony was recorded to have released its gametes when ripe gametes were recorded as present during a census and absent at the next sampling event. Colonies were observed releasing eggs and sperm on three separate dates, and the detailed times of gamete release within the transects were recorded at site A only, due to logistic reasons.

Methods Paper III: Asexual Reproduction in S. elegans
Modes of asexual reproduction
The study took place at the same sites as Paper II (Fig. 1b). All field observations and collections were performed by snorkeling or SCUBA. Prior to the annual mass spawning event in November 1991, the sex and size of the Sarcophyton elegans colonies were determined as outlined in Papers I and II. Detailed maps of the study sites with the size, sex and position of each colony were drawn for each transect, and the mode of asexual reproduction in each colony was recorded during each observation period. The transects were monitored on a quarterly basis between August 1991 and February 1994.

Genetic analyses
Potential ramets (physically separated colonies of the same genotype) and genets (genetically different colonies) were identified in the field and the spawning groups (Paper II) by using used polymorphic allozyme markers.

The samples from 322 colonies were collected in the field and immediately transferred to liquid nitrogen. The samples were processed according to standard protocols. An initial screening of 32 enzymes using a variety of electrophoretic conditions determined that there were two reliably-scorable polymorphic loci; LGG and GPI.
Methods Paper IV: Genetic distribution of G. fascicularis and its associated symbionts

Sample collection and DNA extractions
Corals were collected in March 2009, August 2009 and in April 2010 from 11 sites using SCUBA and snorkel from 4 regions (North, North Central, Central and Southern) in Vietnam (Fig. 1a, Table1, Fig 2). One individual polyp was collected from each colony and stored in 95% ethanol until further DNA analysis.

DNAs for both coral and plant tissue were extracted simultaneously from the same tissue sample using the DNeasy Blood & Tissue Kit (Qiagen, Santa Clarita, Calif).

Genotyping
The coral host was genotyped using a fragment of a non-coding intergenic region between cyt b and ND2 in the mitochondrial DNA. The Polymerase Chain Reaction (PCR) was conducted using the primers 188-2-F and 188-1-R as outlined in Watanabe et al. (2003) with an annealing temperature of 54°C.

The symbionts were genotyped by amplifying the Symbiodinium ITS2 region using the primers ITSintfor2 and ITS2no-clamp (LaJeunesse & Trench2000). PCR was amplified using a TD (Touch Down) protocol modified after LaJeunesse & Trench (2000) and Porto (2008).

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

All the analyses were performed in R (version 2.10.1, R Foundation for Statistical Computing) if not stated otherwise.

**Paper I:**
Variation of measures over 24 hours were analyzed using the coefficient of variation. Paired student’s t-test was used to compare the CV between the measures. Linear regression analysis was used to analyze correlation between colony volume and the different measures.

**Paper II:**
Sex ratio was tested for any deviation from 1:1 using the Chi-square test.

**Paper III:**
To determine the effects of reef exposure, mode of asexual reproduction, and sex of the colonies on size at time of budding or fission, we used a General Liner Model (GLM) with colony size versus sex (male or female), site (lagoon or exposed), replicate (transect 1, 2 or 3 within each site), signs of colony fission (present or absent) and production of buds (present or absent) as variables.

Allele frequencies and expected genotype frequencies were calculated in BIOSYS1. The probability of getting the observed genotype frequencies under the null assumption of Hardy-Weinberg equilibrium was calculated using a chi-squared statistic (Maddox et al. 1989).

**Paper IV:**
*Genotyping*
The chromatograms were aligned by hand using MEGA5 (Tamura et al 2011) The sequences were identified using BLAST search and aligned to the one existing published reference (AB109376) on GenBank, the other reference genotypes are described in Watanabe et al. (2003). The procedure for the Symbiodinium genotypes was as outlined above for the host; however the alignments were verified by sequences based on LaJeunesse et al. (2003, 2009). The different haplotypes were designated by DAMBE, thereafter for the host; analyses of diversity were performed using Maximum Parsimony (MP) analysis in MEGA5.0 (Tamura et al. 2011) under the delayed transition
Coupling between host and symbiont genotype
Association between the Symbiodinium ITS2 and G. fascicularis mtDNA genotypes was tested by Pearson's Chi-square. Additionally we used SPSS Answer tree's Exhaustive CHAID (CHi-squared Automatic Interaction Detector) and C&RT tree modules to detect potential couplings between Symbiodinium and host genotypes.

Diversity indices
‘True diversity’ (D) denotes the effective number (Jost 2007) of elements which is the effective number of ITS2 sequences. It is calculated by using the Shannon and Weaver diversity index, H’ as follows:
D = exp(H’)
H’ = - Σ i=1^s p_i ln p_i
Where p is either the proportion of ITS2 sequences i out of s sequences in the sample for Symbiodinium, or the proportion of mtDNA sequences i out of s sequences in the sample for the host. Comparisons of D among the 11 different sites were analysed using analysis of variance (ANOVA) with Fisher’s LSD post hoc testing of population pairwise differences.

Environmental parameters
The environmental variables at each site analysed in the study were Chl a (mean, min, max and range), SST (mean, min, max and range), visibility (mean, min, max and range from local dive measurements), site depth (mean, min, max and range), number of coral species in the region (based on Veron et al. 2009) and latitude (Y). The variables were checked for normality by Kolmogorov-Smirnov test, and were log-transformed when needed to obtain a normal distribution. Relationships between the variables were assessed using a principal component analysis (PCA) in R (version 2.10.1, R Foundation for Statistical Computing). PCA results were summarized in a bi-plot containing the distribution of environmental parameters in two-dimensional space, and their correlations with the PCA axes. For each variable the measure with the highest eigenvalue (i.e. Chl a choosing Chl a max etc.) was chosen for further analysis.

The effect of the environmental variables on the presence/absence of different ITS2 types (with n > 30) was also tested separately by using a generalized linear model (GLM). As the ITS2 type data were binary (presence/absence), a binomial regression was applied to the presence/absence data in the construction dataset. All linear and quadratic terms were included as potential predictors in the building of the model. Co-variance between each variable was assessed using pair plots and only variables with co-variance > 0.8 were considered for the GLM. In order to select the model that explained the most variation using the fewest number of variables, a ‘backwards stepwise’ procedure was used (R software version 2.10.1, R Foundation for Statistical Computing). The statistic used to select the final
linear model was the Akaike’s Information Criterion (AIC—Chambers and Hastie, 1997).
Major findings – summary of papers

The most reliable measure of *S. elegans* and the two other soft coral species described in Paper I is basal stalk circumference. The measure correlated strongly with volume after water displacement and additionally showed the smallest variation of change over 24 hours (Figs. 4 and 5).

Figure 4. Histograms showing mean coefficient of variation (CV) and minimum and maximum CV (bars) over 24 hours in *Sarcophyton elegans* (a), *Sinularia flexibilis* (b) and *Dendronephthya sp.* (c). White bars denote basal circumference, dark grey bars colony height and light grey bars oral disc diameter.

Colony size is rather than age (Hughes and Jackson 1985) a key metric in coral life history studies and an important predictor of growth and reproductive potential. The size structure of a population can be used to estimate rates of recruitment and size specific mortality (e.g. Harvell and Grosberg 1988; Babcock 1990; McFadden 1997; Gutierrez-Rodrıguez and Lasker 2004). Previous work has demonstrated that soft coral size estimates vary over a period of days (Cordes et al. 2001) or weeks (Fabricius 1995), whilst Paper I demonstrates that soft coral size estimates vary considerably within a 24 h period. The difficulty in obtaining a reliable measure is acknowledged among soft coral scientist (Fabricius and Alderslade 2001, Cordes et al. 2001). The consequences of an imprecise metric can give inaccuracies in growth, shrinkage and size frequency distribution data. The results from Paper I suggests it may be useful to establish a metric for colony size prior to long term studies on life histories and dynamics in soft corals.
Figure 5. Linear regressions showing the relationship between the linear size measurements (on the x-axis) of basal stalk circumference (filled diamond, solid line), colony height (open diamond, dashed line), and oral disc diameter (open circle, dotted line), with colony volume after water displacement by formalin (y-axis). Regression equations and significance of the relationships are illustrated for (a) *Sarcophyton elegans* ($R^2 = 0.75$, p < 0.0001; $R^2 = 0.74$, p < 0.0001 and $R^2 = 0.48$, p = 0.01) for basal stalk circumference, disc diameter and colony height respectively), (b) *Sinularia flexibilis* ($R^2 = 0.51$, p = 0.01 and $R^2 = 0.42$, p = 0.02 for basal stalk circumference and colony height respectively) and (c) *Dendronephthya* sp. ($R^2 = 0.87$, p=0.0002 and $R^2 = 0.34$, p = 0.66 for basal stalk circumference and colony height).

**Paper II** revealed that *S. elegans* is a typical tropical alcyonarian coral as it is a gonochoric broadcast spawner with a sex ratio of 1:1. Sexual reproduction was strictly controlled by colony size with first reproduction at 130 mm basal stalk circumference for females and 120 mm for males. Oogenesis takes 19-24 months, with a new cycle commencing every year (Figs 6a, 7a). Figure 6a shows the different simultaneous development stages during oogenesis. Spermatogenesis takes 10-12 months (Fig 6b, 7b).

The majority of gametes are released during the annual austral mass spawning event after full moon in November, but gametes are also released each month after the full moon between August and January. This unusual pattern of gamete release was achieved by a novel mechanism of gamete production, reported here for the first time. All autozooid polyps participate in reproduction, but those at the outer edge of the colonies release the gametes first and during subsequent months the polyps situated closer to the center of the colonies release their gametes. This strategy represents a new manner in which to accommodate the demands of feeding and reproduction, in contrast to separation of roles for individual polyps observed for other corals.
Colonies upstream in the prevailing current initiated and ceased spawning, up to one month earlier than those further downstream. \textit{S. elegans} appears to have a strategy that allows for the protection of releasing gametes during mass spawning of a number of species, but to hedge bets by spawning over an extended period to avoid loss of investment in gametes in case of catastrophic events (storms etc.) The results also gave some new insights to the spectacular annual multi species mass spawning event taking place on the Great Barrier Reef in Australia, and indicated that the fecundity concept for future studies needs to be revisited.
During the study we also observed that some scelractinian hard corals of the genus *Acropora* released their gametes from the edge of the polyps during the full moon in the months leading up to the annual mass spawning event. That means that previous calculations of fecundity in corals may be underestimated because most fecundity studies have taken place just before the annual mass spawning event and concluded that some coral species exhibit polyps in sterile zones, while others do not. Our results therefore may indicate that coral species including *Acropora* species may not exhibit a sterile zone at the edge of the colonies (Hall and Hughes 1996).

A more detailed study into the mechanisms underlying ‘split-spawning’ would be of interest as hormonal cues (Atkinson and Atkinson 1992, Slattery 1995, Tarrant 2005) may regulate the timing of spawning and therefore control the repeated spawning patterns. In a larger context it is known that the cues to spawning are regulated by the full moon, water temperature, solar irradiation and recently Levy et al. (2007) found that the expression of the Cry-2 gene is activated by the faint blue light of the full moon. Even this study was conducted close to the main annual mass spawning event, but the gene may as well be activated during other months close to the full moon.

**Paper III** showed that the mode of asexual reproduction in *S. elegans*, were budding and fission (Fig 8). Budding was fairly rare, with only 8% of colo-
nies producing buds over the study period. Fission was commonly used in these populations, with 38% of colonies observed splitting and was strictly size dependent with larger colonies dividing into smaller more fecund size classes. Fission took more than 30 months to complete and the complete process was beyond the time scale of this study, budding took 3-6 months to complete.

**Figure 8. Sarcophyton elegans.** Modes of asexual reproduction observed in colonies. (a) Binary fission starting from the polypary, eventually producing two moderately sized colonies. (b) Binary fission starting from the base of the stalk. (c) Buddying sequence.

The genetic study revealed thirteen (possibly up to 22 if no sex change among colonies is assumed) genotypes in the 30 m² area studied (Fig 10), which complemented the pattern of clonal expansion seen within individual sex-specific spawning groups having genetically identical colonies.

**Papers I-III** are based on data collected prior to the escalating global bleaching events that altered many coral reefs on a global scale (Wilkinson 2000, 2004), and therefore the two papers in this study may function as a reference study for future post-bleaching studies. The discoveries in the thesis showed that soft corals exhibit an even larger spectrum of reproductive traits than described before in the order (Paper II).
Figure 9. Genotypes (a) and sex (b) of single-sex colony-groups of *Sarcophyton elegans* on site A. Each circle corresponds to a spawning group (described in Paper II).

In Paper IV the study sites were visited both in 2009 and 2010 to obtain efficient sample sizes. The differences between sites 1 and 2 in 2009 and
2010 were substantial. Site 1 was subjected to dynamite fishing in 2010 and no live corals remained. In 2009 Site 2 was dominated by *G. fascicularis* (40% of coral cover) and approximately 40 other scleractinian coral species (Ngai et al unpublished data). In 2010 the site was altered to large sand banks covered with plastic baskets used for clam culture, with hardly any corals remaining.

The mtDNA sequences in *G. fascicularis* revealed six new haplotypes which were closely related to those obtained from Japan (Fig 10). The majority of haplotypes represented the S01 type which was even more dominant on in-shore than on offshore reefs (Fig 11). The samples from the inshore reefs from site 1 and 2 fell apart and their skeletons dissolved quickly in alcohol, whereas the samples from other sites remained intact. This may be an effect of heavy pollutants - such as arsenic, dioxins, increasing sediment loads and reportedly untreated acidic waste waters in the nearby Sông Hồng river (Red River) (Duong et al. 2008, 2010, Faxneld 2011, Winkel et al. 2011) - on coral calcification.

![Figure 10. Molecular phylogenetic analysis of host mtDNA haplotypes using the Neighbor-Joining method (MEGA 5.0)](image)

Percentages of replicate trees where associated taxa clustered together in the bootstrap test are shown (500 replicates). Evolutionary distances were computed by Jukes-Cantor method using number of substitutions per site as units. The analysis involved 6 nucleotide sequences from Vietnam (S01-S04, L01 and L02) and 8 from Japan (SA, SB, LA-LE from Watanabe et al. 2005). All ambiguous positions were removed for each sequence pair. There were a total of 696 positions in the final dataset. Evolutionary analyses were conducted in MEGAS5.0. (Tamura et al. 2011).
Figure 11. Observed geographic distribution patterns of *Galaxea fascicularis* mtDNA intergenic genotypes across regions and sites along the coast of Vietnam. Pie charts are scaled by area to match sample numbers (1) Cat Ba Inshore I, Ba Trai Dao (n = 5); (2) Cat Ba Inshore II, Van Boi (n = 12); (3) Cat Ba Offshore I, Vung Cao Bai (n = 16); (4) Cat Ba Offshore II, Vung Tau (n = 12); (5) Hue (n = 24); (6) Hoi An (n = 12); (7) Nha Trang IS I, Nha Trang Port (n = 20); (8) Nha Trang IS II, Diamond Bay, (n = 13); (9) Nha Trang OSI, Mooray-Rainbow (n = 20); (10) Nha Trang OSII, Lan Beach-Mama Hahn (n = 17); (11) Phu Quoc (n = 22).

Rare alleles were restricted to one or two populations, but individual haplotypes did not appear to have any geographical pattern at regional scales (Figure 11) and no significant genetic structure was found on a regional scale ($F_{ST}$ = -0.044, p = 0.91, AMOVA). On the other hand, there was a significant genetic structure differentiating inshore and offshore sites ($F_{ST}$ = 0.093, p = 0.021, AMOVA).

The diversity indices of the hosts showed a strong inshore offshore distribution (Fig 12). The diversity indices of the symbionts showed a strong latitudinal distribution with a tendency of higher diversity further South (Fig 14). There was no coupling between host and symbiont genotypes (Pearson's chi-square test; $\chi^2 = 1.72$, p = 0.19) (No associations detected with Answer tree) and no association between host diversity and latitude (2-way ANOVA; p > 0.40).
Figure 12. Genetic diversity of *G. fascicularis* based on mtDNA (non-coding intergenic region between cyt b and ND2) at inshore and offshore sites along the coastline of Vietnam. Genetic diversity measured by Shannon Weaver’s true diversity index (‘D’) averaged (±S.D.) across sites.

The ITS2 genotypes of the Symbiodinium sequences (Paper IV) belonged to group D (n = 142) and group C (n = 132) which has been reported from China and Spratley Islands (Dong et al. 2009, Huang 2011). The previous studies in China are based on group level only and do not cover the area in Vietnam. There were strong differences in the latitudinal distribution of the *Symbiodinium* ITS2 types (Fig. 13). Group D was present in all regions displaying only one ITS2 type; D1a and was found consistently on inshore sites. Group C was divided into five ITS2 genotypes; C1 (*S. goreaui*), C3, C3u, C21, and C27 (La Jeunesse et al. 2003, 2009). Group C was represented by one ITS2 types in the north region (C1), two types in the North Central regions (type C3u in Hue and type C1 in Hoi An), all six types in the Central Region (Nha Trang) and three types in the South region (types C1, C3, C3u).

The types changed dominance from C1 in the north to C3u in the south. C1 one is reportedly tolerant to temperature fluctuations and regarded as a resilient *Symbiont* type. The C1 presence may be an effect of a more fluctuating temperature regimes and higher turbidity and sedimentation in the north compared to the South Central and South Regions. However the division may also be a result of the different monsoons in northern and southern Vietnam with alternating currents resulting in a cut off at the Hue-Hoi An region, causing a dispersal barrier between symbionts. Further research using microsatellite markers may be needed to evaluate the barrier hypothesis.
Figure 13. Observed geographic distribution patterns of *Symbiodinium* types (ITS-2) across regions and sites along the coast of Vietnam. (details in fig 11 and table 1). Pie charts are scaled by area to match sample numbers (1) n = 23, (2) n = 20, (3) n = 27, (4) n = 21, (5) n = 21, (6) n = 23, (7) n = 27, (8) n = 23, (9) n = 21 (10) n = 13, (11) Phu Quoc n = 21.

Figure 14. Genetic diversity of *Symbiodinium* (ITS-2) types hosted by *G. fascicularis* in the different sites ranging from north to south along the coastline of Vietnam. Genetic diversity measured by Shannon Weaver’s true diversity index (‘D’) across sites in the regions. Site information is provided in full in Table 1.
Reefs closer to the shore are likely to undergo more stress caused by human activities as pollution, eutrophication and siltation as well as natural stress such as freshwater runoff (Latypov 2005). This, in turn, can cause differences in the zooxanthellae composition in the host. The findings in the present study confirm the role of D1a and C1, where D1a took over dominance in extremely stressed environments seemingly unsuitable for other symbionts. Corals with horizontal uptake are thought to be more flexible in their exchange of symbionts than brooding corals with vertical uptake (Baker et al. 2008), therefore horizontal uptake in areas with higher diversity of corals and free living symbionts should result in higher diversity of symbionts within the species. This is provided the conditions are not stressed and of no advantage to the opportunistic D1a type. The results in our study confirm this theory and suggest that dominance of D1a is an indicator of a stressed reef. Interestingly the sampling design in the present study showed that approximately 50% of G. fascicularis colonies in the southern South China Sea harboured clade D due to inshore-offshore patterns possibly created by anthropogenic factors, thus questioning previous conclusions stating that group C is more prevalent in area (Baker 2004, Huang et al 2009, Huang et al. 2011). Barriers, such as strong currents between inshore and offshore areas, different depths and difference in climate between the sites are also plausible to influence the difference in symbiont types (LaJeunesse et al. 2010a). The shifts in dominance of Symbiodinium types over latitudinal and on an inshore offshore gradient is consistent with these general findings, and that some Symbiodinium types appear to be more resilient than others. The different host haplotypes in our study also showed differences in frequency related to anthropogenic stressors. In some instances sampling only a few sites might show an apparent relationship between coral genotype and Symbiodinium type. However, in our study we were able to demonstrate, that while there were regional differences in symbiont type, these were related to environmental differences and not to genetic characteristics in the coral G. fascicularis.
To wrap it up:

In order to understand how reefs adapt and persist or go extinct in the ‘Anthropocene’ (Jackson 2008) it is important to understand the wider aspects of holobiont maintenance and the interactions between reproduction of the host, environment, and presence of symbionts in the water. These interactions need to be determined in detail among both brooders and spawners on large scales of both time and space to enable predictions of the future states of reefs.

Species diversity and abundance are indicators of the present state as well as the future existence of the reef. However, a species may be abundant but exhibit low genetic variation. Low genetic variation is a potential vulnerability to organisms as fewer genotypes have a smaller chance to recombine and adapt to environmental changes, and may be wiped out in the face of environmental change. Preliminary studies elsewhere indicate that the genetic diversity in corals is lower in disturbed areas than in pristine areas (Souter 2007). Therefore, it is relevant to determine genetic variation in soft and reef building corals over larger geographical scales in relation to both geographic distance and varying human disturbance. These types of studies in combination with detailed life history data, and species diversity data, are needed to convey a wider and more accurate picture of the present state of the reefs.

The marine ecosystems are facing rapid changes and enormous threats due to destructive anthropogenic impact. The world population increased from 2 billion people in the 1950’s to 6.8 billion today (Global Footprint Network 2010, Jackson 2010). Renewable resources are already overexploited and a changing climate is a threat for the future of marine ecosystems, of which some have collapsed locally. Toxic pollution, sediment run-off to the oceans, dynamite fishing, cyanide fishing (mainly for export of ornamental fish), overfishing and unsustainable aquaculture take a toll on the reef environment (Worm et al. 2009, Jackson 2010, Burke et al. 2011). These are some factors behind the fast demise of the reefs today, where 60% are threatened globally and 95% in South East Asia (Burke et al. 2011). As discovered at repeated visits among our study sites (Paper IV), human-induced destruction of reef habitats is ongoing in North to South Vietnam. Baseline data before human impact is necessary for defining the more natural states of reefs as well as facilitating detection of ongoing reef destruction. Data collected at the Great Barrier Reef in Australia for Papers I, II and III are also potential baseline studies for future post bleaching research.
Furthermore, this thesis provides a reliable method for estimating central life history traits of soft corals (size in Paper I). Thesis explores fundamental processes (such as reproduction Paper II & III, genetic adaptation and distribution Paper IV) and environmental factors Paper IV (such as potential stressors) that may determine the survival of coral populations. Further knowledge of such fundamentals will be necessary for building effective conservation strategies. However, these efforts are only sufficient if placed in a larger context where this research, politics and economy can connect on local as well as international levels. It is possible to reduce pollution (e.g. eutrophication) in harmony with economic development using modern practices such as waste water treatment, usage of less polluting fish feed in fish farms, shoreline protective zones in farmland and urban areas, and generally less environmentally destructive technologies. While non-sustainable illegal fishing methods with e.g. dynamite and cyanide calls for crucial changes in attitude so that these illegal enterprises are made societally unacceptable. I believe that education and "awareness" campaigns involving local communities, schools, entrepreneurs (local scientists, industry, fishermen, dive operators, and tourism entrepreneurs) may be a first step in the right direction. But can enough joint efforts be mobilized in time to conserve and secure future sustainable use of these reefs?
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You - for reading this. Now you can flick through the rest of this book ➔