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Flexibility of the coral-algal symbiosis in the face of climate change

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Chapter 1

General introduction

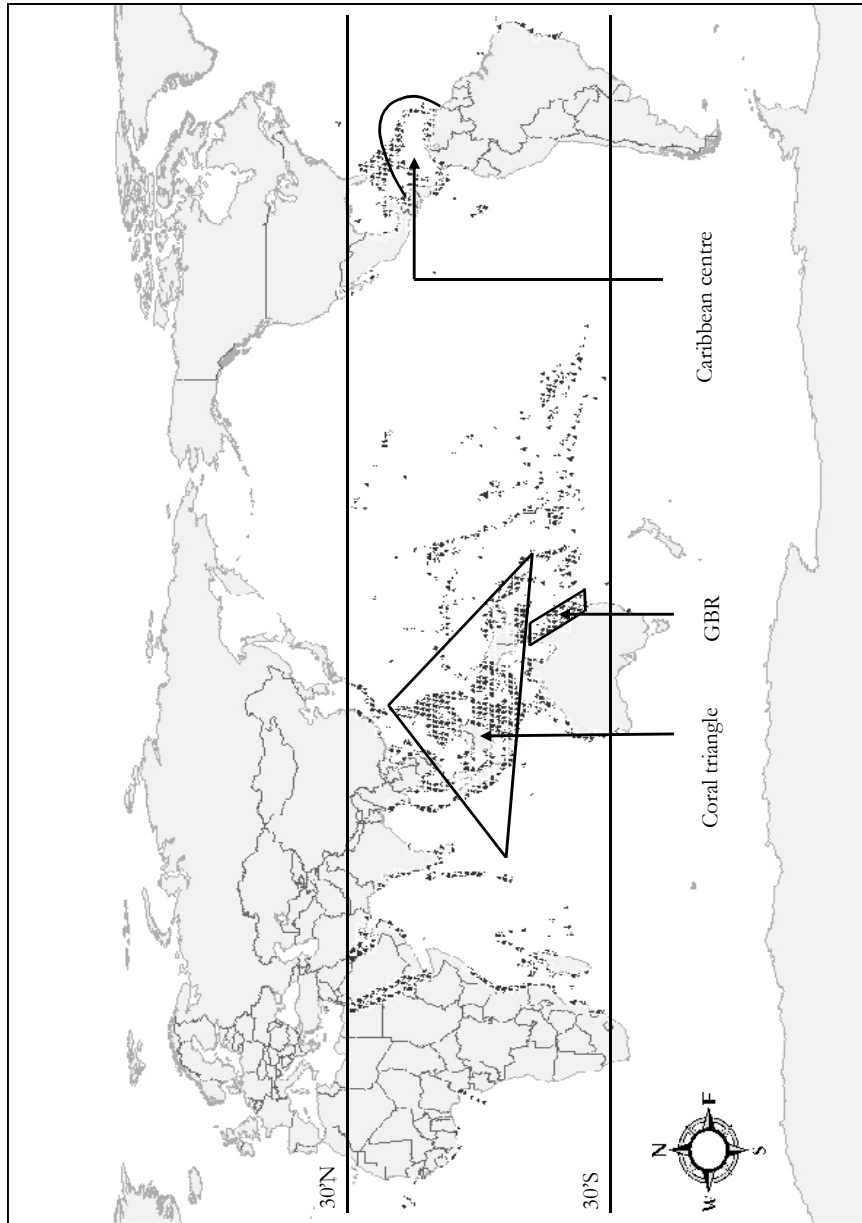


Fig. 1. World map showing the distribution of tropical coral reefs, the locations of the two main centers of reef biodiversity (coral triangle and Caribbean center) and the Great Barrier Reef (Map from ReefBase, <http://reefbase.org>).

Tropical coral reefs are among the richest ecosystems on Earth, whether measured by biodiversity, productivity, biomass or structural complexity. Apart from their ecological value they also provide a vast number of goods and services to people, including food, tourism, coastal protection, aesthetic and cultural significance (Moberg & Folke 1999; Hoegh-Guldberg 2004; Wilkinson 2004). The vast majority of the coral reef systems are situated in the warm tropical seas between 30°N and 30°S (Fig. 1), where they fringe shorelines, form offshore barriers and atolls (Veron & Stafford-Smith 2000). Covering only about 0.1 % of the Earth's surface (Smith 1978; Spalding & Grenfell 1997), they accommodate almost 30% of the world's marine fish species and supply about 10% of the fish consumed by humans (Smith 1978). Annual revenues from tourism are estimated at US\$105 billion for the Caribbean (Burke & Maidens 2004) and AU\$7 billion for the Great Barrier Reef (Access Economics 2007).

Coral reefs have been in an unprecedented decline over the last century, as indicated by significant reductions in species abundances and degradation of reef communities (Hughes *et al.* 2003; Pandolfi *et al.* 2003). Reef losses are currently estimated at 20%; 24% of coral reefs are under imminent risk of collapse and a further 26% are threatened on the longer term (Wilkinson 2004). Numerous human-induced disturbances have been identified as causes of this decline, some of which started to affect reefs as early as a millennium ago (Jackson 2001; Pandolfi *et al.* 2003). The main threats to reef biodiversity include: (1) fishing and harvesting, (2) habitat destruction and fragmentation, (3) pollution, (4) disease, and (5) climate change. Fishing practices have long been unsustainable (Jackson 2001) and have resulted in the loss of key reef organisms such as herbivores that normally reduce the competition between corals and macroalgae (Hughes *et al.* 2007). Destructive practices such as dynamite fishing, coastal development and coral harvesting have directly impacted coral reef integrity. Pollution-based threats include sedimentation (which smothers coral and makes substrata unsuitable for juvenile settlement), eutrophication and herbicides/pesticides (Wilkinson 1999; Wilkinson 2004). The sources of pollution are often land-based (e.g. agriculture) and reach the reef through run-off as a result of poor land-management. Last but not least, climate change has been linked to a recent increase in coral diseases (Harvell *et al.* 2004), as well as to reduced coral calcification in conjunction with ocean acidification (Hoegh-Guldberg *et al.* 2007; Cooper *et al.* 2008). However, the main climate-induced threat to coral

reefs today is the heat-induced disruption of the delicate symbiotic relationship between corals and their algal endosymbionts (Coles & Brown 2003).

Stony corals (Order: Scleractinia) are the ecosystem engineers (Jones *et al.* 1994; Coleman & Williams 2002) of coral reefs. They produce hard calcium carbonate exoskeletons, which give rise to the characteristic structure of coral reefs. Stony corals form an obligate symbiosis with unicellular algae of the genus *Symbiodinium* (Division: Dinophyceae). The algal endosymbionts are photosynthetically active and transfer up to 95% of their photosynthate to the coral host (Muscatine *et al.* 1984); they also stimulate rates of calcification (Pearse & Muscatine 1971; Gattuso *et al.* 1999). In return, the coral host provides protection from predators and an environment optimal for photosynthesis, including the provision of several essential nutrients (Pearse & Muscatine 1971; Muscatine & Porter 1977). The evolution of tropical coral reefs in oligotrophic waters is heavily dependent on this successful partnership and anything that disrupts the partnership will, therefore, cause serious harm. Abnormally high seawater temperatures have been identified as the main cause for the periodic loss of the dark-pigmented algae from the host tissues (Glynn 1993; Berkelmans 2002) as witnessed by an increased frequency and severity of this phenomenon in the last 20 years. As the coral host tissue is mostly transparent, the calcium carbonate skeleton becomes visible making the coral appear white; hence, this response is called “coral bleaching”. Bleached corals may recover after weeks or even months but their growth and reproductive output will be significantly reduced (Szmant & Gassman 1990; Baird & Marshall 2002). If bleaching is too severe and/or prolonged, the corals will die.

In 1998, thermal bleaching of an unprecedented magnitude affected both the Pacific and the Caribbean, resulting in an estimated 16% loss of the world’s coral reefs (Wilkinson 2000). Since then, mass bleaching events have occurred (on a smaller scale) in 2002 (Indo-Pacific), 2005 (Caribbean) and 2006 (Indo-Pacific). Temperatures of as little as 1°C above the average summer maximum are already sufficient to cause bleaching (Glynn 1993; Berkelmans 2002). With expected increases of 1.5-4°C in the tropics by the end of this century (IPCC 2007; Fig. 2), coral reefs are projected to undergo major changes (Hughes *et al.* 2003)—to the extent that only remnant populations of corals may be left by the year 2050 (Hoegh-Guldberg 1999). However, a largely unknown variable in these “doomsday” predictions is the capacity of the coral holobiont (the coral plus the algal symbiont)

to increase its thermo-tolerance, either by adaptation or acclimatization (Coles & Brown 2003; Hoegh-Guldberg 2004; Box 1). Although very little is known about the capacity for thermal adaptation of the present-day coral-algal associations, their evolutionary response in a time frame of several decades is considered to be limited (Hoegh-Guldberg *et al.* 2007). Instead, it has been proposed that the flexibility of the coral-algal association provides a window of response to temperature increases that may allow it to cope with the at least some of the stress.

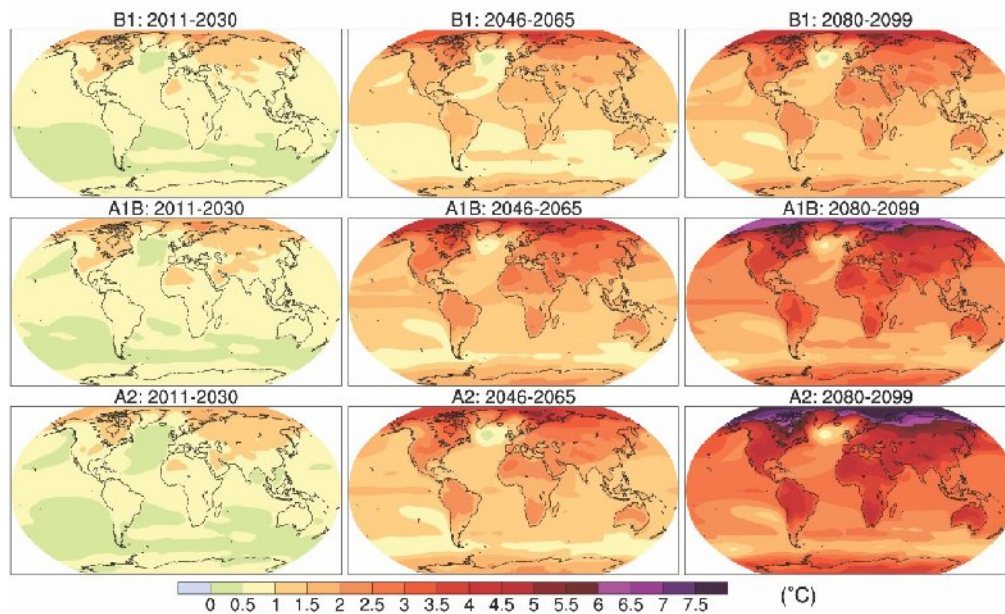


Fig. 2. Predictions of annual mean surface warming (°C) for three scenarios: low CO₂ emission (scenario B1 – top row), medium CO₂ emission (scenario A1B – middle row) and high CO₂ emission (scenario A2 – bottom row) and three time periods: 2011 to 2030 (left column), 2046 to 2065 (middle column) and 2080 to 2099 (right column). Anomalies are relative to the average of the period 1980 to 1999. Warming rates are fastest at the north pole but the tropical regions are expected to face temperature increases of 1.5°C (low CO₂ emissions scenario) to 4°C (high CO₂ emissions scenario) by the end of this century (Source: IPCC Fourth Assessment Report 2007).

The fact that many different *Symbiodinium* types form symbiotic relationships with corals has only been established in the last two decades (Blank & Trench 1985; Rowan & Powers 1992; Rowan 1998; Baker 2003) and it is this observation that has led to the formulation of the “adaptive bleaching hypothesis” (ABH) (Buddemeier &

Fautin 1993; Buddemeier *et al.* 2004; Fautin & Buddemeier 2004). The ABH hypothesizes that bleaching provides an opportunity for corals to re-establish a symbiosis with a different, more stress-tolerant (e.g. heat-tolerant) *Symbiodinium* type, resulting in a coral holobiont better suited to the altered environmental circumstances. Initially, it was thought that uptake of new symbiont types could only take place from the environment and that this adaptive response involved a genetic change in the coral holobiont (Buddemeier & Fautin 1993). Later, it was recognized that change could also take place by an increase in the low abundance, pre-existing *in hospite* populations of symbionts (Fautin & Buddemeier 2004). In order to capture both modes of symbiont change in the ABH, the term “adaptation” was reverted to its meaning in common usage (Box 1) causing considerable confusion in the literature (Hoegh-Guldberg *et al.* 2002; Hughes *et al.* 2003). In this thesis, any changes predicted by the ABH are considered “acclimatizations” (or acclimations when in an experimental setting), as they happen within the life-time of a coral colony and no new genes need to evolve.

Box 1: Definitions of adaptation/acclimatization

The adaptive bleaching hypothesis (ABH) employs the common usage definition of the term “adaptation” : modification of an organism or its part in a way that makes it more fit for existence under the conditions of its environment..... (Fautin & Buddemeier 2004).

This definition is more relaxed than those applied in evolutionary biology:

Adaptation: physiological, biochemical, or anatomical modifications within a species, directed by selection, that happen over generations. Adaptations involve a genetic change and are heritable.

Acclimatization: physiological, biochemical, or anatomical modifications in an individual’s lifetime due to phenotypic plasticity. Acclimatizations do not involve a genetic change and are not heritable. For example, changing to a more thermo-tolerant symbiont type provides a wider envelop of plasticity to increased temperature for the coral holobiont.

Acclimation: similar to acclimatization, but in an experimental, human-manipulated setting.

The central aim of this thesis was to assess the potential of the mechanisms described by the ABH to: (1) induce changes in the symbiotic communities of coral, and (2) mitigate the effects of global warming on coral reefs.

General questions included:

1. Where do heat-tolerant *Symbiodinium* types come from — from already present, low-abundance-algal cells in the coral host before bleaching, or taken-up anew from the environment — and how can we detect and quantify them?
2. To what extent does the symbiont type shape coral fitness — in terms of heat-tolerance, growth and mortality — and what are the relative contributions of host-factors and the local environmental conditions?
3. What are the *Symbiodinium*-related trade-offs between thermo-tolerance and growth/survival, and how are they shaped by coral host and environmental factors?
4. What is the field evidence for the ABH?

To address these questions, a combination of lab and field experiments were designed to increase the sensitivity of *Symbiodinium* detection and ease of quantification, and to experimentally partition and quantify the coral-algal and holobiont-environmental interactions.

BACKGROUND

Scleractinian corals

The fossil record shows that scleractinian corals have been around since the early Triassic, about 237 my BP (Stanley Jr & Fautin 2001). These early corals did not build reefs and the formation of reefs in the late-Triassic is thought to have coincided with the evolution of the coral-dinoflagellate symbiosis (Stanley 2006).

Scleractinian corals have survived massive climatic perturbations, including the mass-extinction events at the end of the Triassic (200 my BP) and Cretaceous (145 my BP).

Corals belong to the metazoan phylum Cnidaria, Class Anthozoa to which hydroids, jelly fish and sea anemones also belong. Their body-plan is simple: radially symmetrical polyps, containing a sac-like body cavity (the stomach) with one opening that serves as both mouth and anus (Fig. 3A). The tentacles surrounding the opening contain stinging cells (nematocysts), which they use to filter particulate matter or small organisms (immobilized by the nematocyst stings) from the water column. The “zooxanthellae” (the symbiotic algal cells) live in specialized vacuoles inside the host’s endodermis cells (Fitt & Trench 1983). Most corals form modular colonies consisting of thousands to even millions of polyps (Veron & Stafford-Smith 2000).

Reef-building corals are highly diverse: 25 living families are currently recognized, containing 246 genera and ~1000 species (Veron 2000). The genus *Acropora* is the most species-rich, containing over a hundred species with a large variety of growth forms (Fig. 3B). Branching forms are the most common in the genus *Acropora* and these dominate Indo-Pacific reefs. Their relatively fast growth rate allows them to outcompete other corals, especially in clear waters. However, the branching habit of many *Acropora* species makes them particularly sensitive to environmental disturbances and breakage. They are among the first taxa to exhibit a bleaching response (McClanahan *et al.* 2004; Carpenter *et al.* 2008). For these reasons, *Acropora* species are often used as model organisms in physiological and ecological studies of reef coral responses.

New coral colonies can be formed asexually through simple mitosis and eventual colony fragmentation or by one of two modes of sexual reproduction. Broadcast spawning corals (~75% of the reef-building corals including all *Acropora* spp.) release massive numbers of gametes (eggs plus sperm) into the water column in a process called mass spawning. Many species can spawn synchronously within a period of several days, once a year (Harrison *et al.* 1984; Babcock *et al.* 1986; Guest *et al.* 2005), although this multi-species reproductive synchrony is not a characteristic of all coral communities (Richmond & Hunter 1990). To accomplish synchronized spawning, broadcasters rely on seasonality in light (solar radiance,

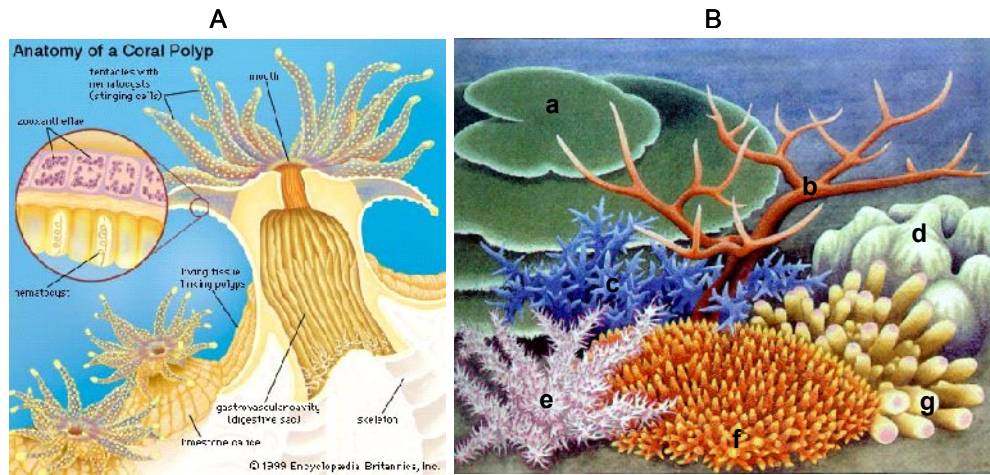


Fig. 3. A: schematic representation of a coral polyp (Courtesy of Encyclopædia Britannica, Inc., copyright 1999, used with permission), B: examples of *Acropora* growth forms: a) tables and plates, b) staghorn, c) bushy, d) massive, e) bottlebrush, f) corymbose, g) digitate (Figure from Geoff Kelley).

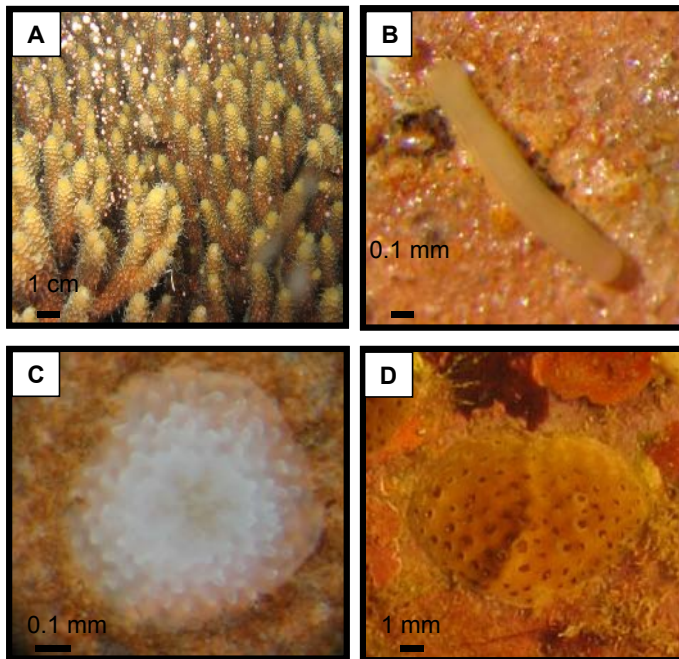


Fig. 4. Sexual reproduction in *Acropora millepora*. A: adult colony releasing egg-sperm bundles, B: planula larva searching for a suitable settlement site, C: newly metamorphosed coral polyp, D: juvenile coral colony ~6 months old (Photos by Jos Mieog).

night/day cycles and lunar radiance) and temperature (Fadlallah 1983; Babcock *et al.* 1986; van Woesik *et al.* 2006). Eggs are fertilized at the water surface where they develop into planula larvae over the course of a few days. Once at the planula stage, the larvae become negatively buoyant, sink and attach themselves via chemical cues (Heyward & Negri 1999) to the substratum. There, they undergo metamorphosis into the recognizable coral polyp. Subsequently, the coral grows into a colony by asexual budding and clonal growth (Fig. 4).

Brooding corals fertilize their eggs internally. Sperm is received from neighboring colonies and fertilization occurs inside the coral polyps. Mature planulae are released, which are mostly capable of immediate settlement. Brooding corals generally produce much smaller numbers of eggs at any one time, but the planula larvae are larger and have higher survival rates. Brooding corals tend to release larvae over longer period (several months) (Fadlallah 1983).

The genus *Symbiodinium*

The unicellular, symbiotic algae of corals belong to the Dinophyceae — an ancient class of protist algae—and are commonly referred to as zooxanthellae. The algal life history involves a vegetative and a motile phase (Schoenberg & Trench 1980; Fig. 5). The vegetative phase ranges in size from 5-15 μm and is the dominant form when in symbiosis with corals. The flagellate stage (zoospore) is known from cultures and is particularly active under illumination. Multiplication of *Symbiodinium*—*in vitro* and *in hospite*) occurs mainly through mitotic divisions. Sexual reproduction has, so far, not been observed in *Symbiodinium*, although it has been inferred from molecular data (Stat *et al.* 2006). Until recently, only a few studies attempted to address the free-living phase of *Symbiodinium* (Carlos *et al.* 1999; LaJeunesse 2001; LaJeunesse 2002), because data collection was hampered by technical difficulties associated with detection in the natural environment.

Encouragingly, significant progress has recently been made by using azooxanthellate polyps as “environmental samplers” (Coffroth *et al.* 2006), sensitive automated particle counters (Littman *et al.* 2008) and better water filtering techniques (Manning & Gates 2008), as well as culturing *Symbiodinium* from water samples taken from macroalgal beds and fish feces (Porto *et al.* 2008). These studies show that the free-living forms are genetically diverse, mainly reside in the benthos

and that corallivorous fish may aid the dispersal through their feces. Future studies are urgently needed to increase our limited understanding of the distribution and dispersal of free-living *Symbiodinium* spp.

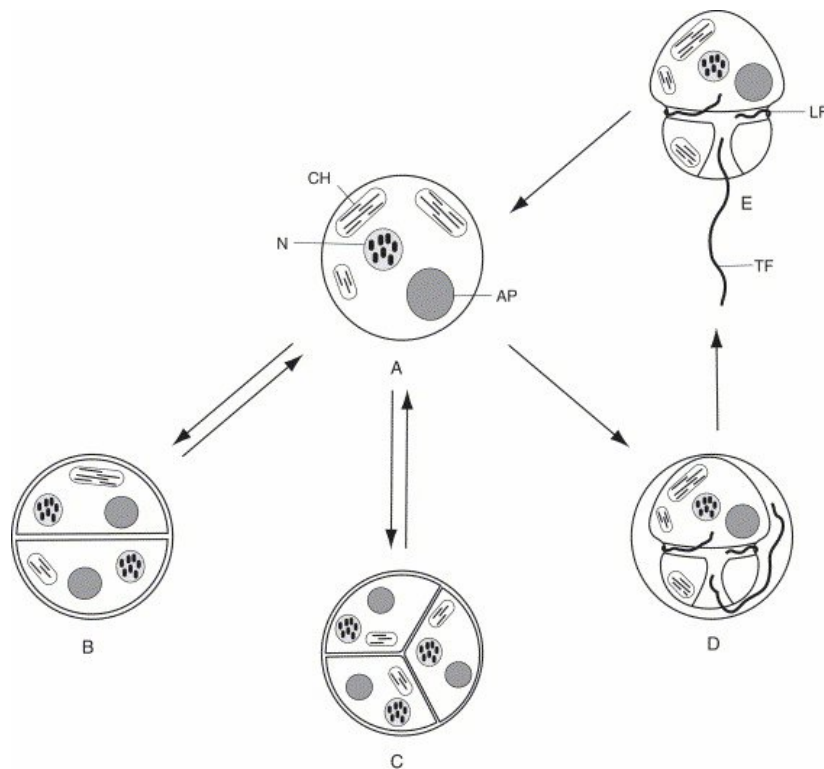


Fig. 5. *Symbiodinium* life cycle. A: vegetative cell, B: dividing vegetative cell producing two daughter cells, C: dividing vegetative cell producing three daughter cells, D: developing zoospore and E: zoospore. CH: chloroplast; N: nucleus; AP: accumulation product; LF: longitudinal flagella; TF: transverse flagella (Reprinted from Stat *et al.* 2006, with permission from Elsevier).

CORAL BLEACHING

The whitening of corals by the loss of zooxanthellae was first described in detail by Yonge and Nicholls (1931). Bleaching is considered a general stress response, as it can be induced by a variety of stimuli including abnormal temperatures, salinities, irradiance levels and various diseases (reviewed by Douglas

2003). Causes for bleaching are likely to be complex and vary per stressor. In the case of thermal bleaching, it is known to be associated with a disruption in the photomachinery that operates in the zooxanthellae (Jones 1998; Smith *et al.* 2005). The typical rise in the excitation pressure over photosystem II is thought to be caused by increased damage to the D1 protein of the photosystem II reaction centers (Warner *et al.* 1999) and/or disruption of the Calvin cycle (Jones *et al.* 1998). Large quantities of reactive oxygen species (ROS) are produced (Smith *et al.* 2005) that swamp the protective mechanisms available and induce, through poorly understood interactions between the coral host and the symbiont, the loss of up to >90% of the symbiotic cells from the coral host tissues (Fitt *et al.* 2000; Weis 2008).

Corals are extremely sensitive to increases in temperature because their upper temperature tolerances are locally adapted to only 1-3°C above the long-term average summer seawater temperature (Hoegh-Guldberg 1999; Berkelmans 2002). Worldwide, temperatures which induce coral bleaching range over 8°C, from 27°C at cool, high latitudes, to 35-36°C in the very warm Arabian gulf (Coles & Brown 2003). Living close to the temperature threshold probably conveys advantages in the absence of large temperature fluctuations—as seen in the last 8,000 years—but is detrimental in an era of rapid warming.

The variability in bleaching thresholds suggests a capacity for reef corals to adapt to higher temperatures, although the time period needed for this adaptation is unknown (Coles & Brown 2003). It is feared that the increases in global temperature are happening so rapidly that there will be insufficient time available for thermal adaptation (Hoegh-Guldberg *et al.* 2007). Corals are believed to evolve slowly, as they have long life spans, overlapping generations and high incidences of asexual reproduction (Hughes *et al.* 2003). In contrast, *Symbiodinium* may be able to provide the needed adaptive potential as a suite of thermo-tolerant *Symbiodinium* genotypes already exist which corals may be able to take advantage of.

THE ADAPTIVE BLEACHING HYPOTHESIS

The Adaptive Bleaching Hypothesis (ABH) poses that the loss of photosymbionts allows some coral species to re-establish a symbiosis with a different dominant *Symbiodinium* type, resulting in a new coral holobiont that is better suited to the altered environmental circumstances (Buddemeier & Fautin

1993; Ware *et al.* 1996; Buddemeier *et al.* 2004; Fautin & Buddemeier 2004). Such a change has the potential to enhance long-term survival of the hosts and may explain how coral reefs have survived repeated climatic fluctuations over geological time (Buddemeier & Smith 1999). There are five fundamental assumptions that underpin the ABH: (1) multiple types of both coral host and *Symbiodinium* species commonly co-exist, (2) both symbiont and coral host have a degree of flexibility in their associations, (3) symbiont types are physiologically different and influence important aspects of the coral's physiology (especially stress-responses), (4) coral bleaching provides an opportunity for repopulation of a host with a different dominant algal symbiont, and (5) stress-sensitive holobionts have competitive advantages in the absence of stress, which implies a reversion to stress-prone combinations under non-stressful conditions. In the next paragraphs these assumptions are discussed in detail.

(1) Diversity and co-existence

There are two areas of exceptional coral reef biodiversity in the world. The primary center, containing the highest coral diversity in the world, lies within the “coral triangle” in the Indo-Pacific (Fig. 1) with an outward attenuation in all directions. A secondary center lies in the southern Caribbean (Fig. 1). Only a few genera are shared between the two areas illustrating the extent of the geographic and temporal isolation between the two oceans (Briggs 2005). Such deep differences were not appreciated until fairly recently because of the notoriously difficult taxonomic identification of many coral species and lack of molecular-based phylogenies, especially those which exhibit high levels of morphological plasticity. The complexities and uncertainties in identifying the coral hosts has also hampered the correct identification of symbiotic combinations (e.g. Santos *et al.* 2001).

All symbiotic dinoflagellates that form symbiotic relationships with corals were once considered to be a single cosmopolitan species, *Symbiodinium microadriaticum* (Freudenthal 1962). This misconception was mainly due to the lack of distinguishing morphological features of both the motile and vegetative stages of *Symbiodinium*. However, subsequent morphological, biochemical, physiological and karyotypic (e.g. Schoenberg & Trench 1980; Blank & Trench 1985), and especially comparative DNA sequencing (e.g. Rowan & Powers 1991a,b) have since shown

that the genus *Symbiodinium* is extraordinarily diverse, exhibiting much higher sequence diversity than observed between genera of non-symbiotic dinoflagellates.

Studies of *Symbiodinium* diversity have mainly utilized the nuclear ribosomal DNA (nrDNA) cistron and associated spacers which provide several regions with different evolutionary rates (Fig. 6). Based on analyses of the relatively slowly evolving 18S, 5.8S and 28S units, *Symbiodinium* has been divided into eight (A-H) phylogenetic clades (Fig. 7) (Rowan & Powers 1991a; LaJeunesse 2001; Pochon *et al.* 2006). The evolutionary position of each clade has received further support from analyses based on mitochondrial (Takabayashi *et al.* 2004) and chloroplast DNA (Santos *et al.* 2002; Pochon *et al.* 2006).

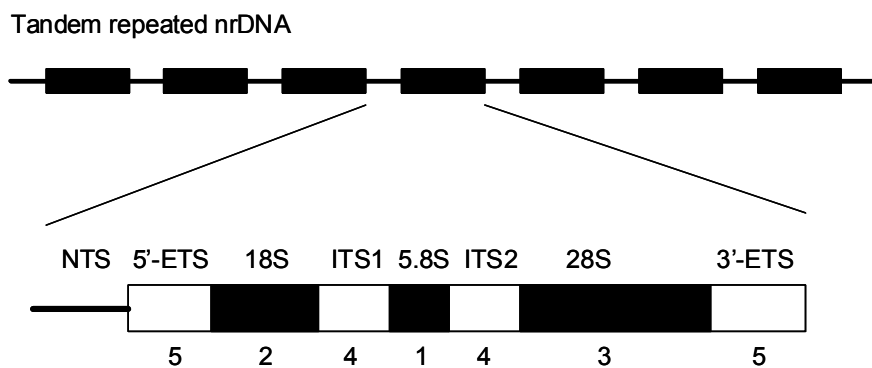


Fig. 6. Organization of the nuclear ribosomal genes. Each consists of three coding units (18S, 5.8S and 28S), which are separated by the internal transcribed spacers (ITS) 1 and 2 and the external transcribed spacers (ETS) on both ends. The repeats are separated from one another by the non-transcribed spacer (NTS). The regions differ in their evolutionary rates, as indicated below each region, with 1 representing the slowest and 5 the fastest rate. The number of repeats per cell is variable both within and between taxa, ranging from 1 to several thousands (Long & Dawid 1980; Rogers & Bendich 1987; Loram *et al.* 2007).

Scleractinian corals are mainly associated with *Symbiodinium* from the clades A, B, C, D (Baker 2003); associations with clades F and G are rare (LaJeunesse 2001; van Oppen *et al.* 2005b; Pochon *et al.* 2006; Goulet 2007). Clade E may represent a free-living subgenus of *Symbiodinium* and H is a lineage specific to the Foraminifera (Pochon *et al.* 2006). Biogeographic and habitat surveys of *Symbiodinium* in scleractinian corals have established two main patterns. First,

clades A, B and F are more common at higher latitudes and clade C is more abundant at tropical latitudes. Second, clades A and B are much more common in the Caribbean than in the tropical Indo-Pacific (LaJeunesse *et al.* 2003; LaJeunesse 2005). The first pattern is thought to be the result of adaptations to the environment (Savage *et al.* 2002; Baker 2003; LaJeunesse *et al.* 2003), whereas the second may be the result of different vicariant histories following the closure of the Central American Isthmus 3.1-3.5 million years ago (LaJeunesse *et al.* 2003; Pochon *et al.* 2004; LaJeunesse 2005). In the Caribbean, an intercladal habitat pattern is present in relation to depth and, thus, irradiance. In general, clades A and B have been found in shallower waters (0-3 m) than clade C (3-14 m) (Rowan & Knowlton 1995; Rowan *et al.* 1997; Toller 2001; LaJeunesse 2002). However, these patterns are dependent on the species and the location. For instance, Diekmann *et al.* (2002) surveyed the symbiont community of five morphospecies of *Madracis* at Curaçao over a depth gradient from 2 to 45 meter and found no differences correlated to habitat.

Clade D has been given special attention, as it is mostly found in marginal habitats where other clades struggle, indicating an opportunistic character and a high stress-tolerance (Baker 2003). Importantly, clade D has increased in abundance in scleractinian corals after bleaching events (Glynn *et al.* 2001; Toller *et al.* 2001; Baker *et al.* 2004; van Oppen *et al.* 2005a) and is favored at reefs exposed to unusually high temperature regimes (Fabricius *et al.* 2004), indicating that symbionts from this clade may play an important role on future reefs.

Below the cladal level, subclades, strains and types have been distinguished. Molecular markers that have been used to resolve within-clade diversity include microsatellites and flanking regions (Santos *et al.* 2004), DNA fingerprinting (Goulet & Coffroth 2003) and, especially, the nrDNA ITS regions (LaJeunesse 2001; van Oppen *et al.* 2001b). In this thesis, I will refer to “types” as the taxonomic unit below the cladal level. Which taxonomic rank (clade, subclade, strain, type) should be considered “species” is open to discussion. Importantly, the many types turn out to have the most ecological relevance and these have not always been identified in ecological studies. For example, in the Indo-Pacific, different types within clade C show zonation with depth/light (van Oppen *et al.* 2001b; LaJeunesse *et al.* 2003).

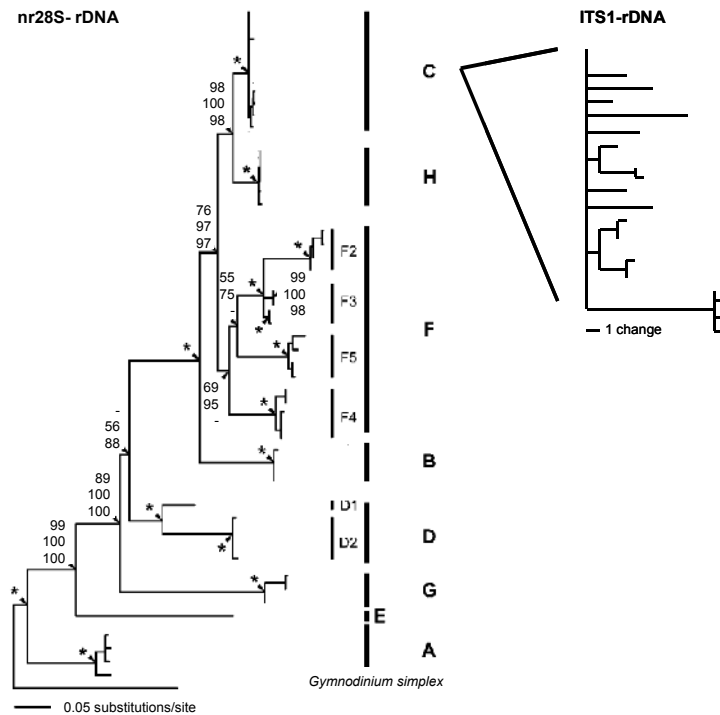


Fig. 7. Maximum likelihood (ML) phylogram of *Symbiodinium*, based on 28S nrDNA. The principle eight clades are A-H. Numbers at nodes are, from top, the bootstrap values obtained with ML, Bayesian posterior probabilities (in percentage) and the bootstrap values obtained with maximum parsimony; * = 100% for all three. Clade C (right) remains unresolved even with ITS1, one of the most variable markers currently available (adapted from Pochon *et al.* 2006).

The use of several different molecular markers at the within-clade level has resulted in considerable confusion about type designations (C1, C2, C2*, C2a, etc.). Lajeunesse (2001) identifies each unique, dominant ITS2 sequence as a type with >100 ITS2 types having been distinguished within clade C alone (Fig. 7). These types do not always correspond to the ITS1 designations used by van Oppen *et al.* (2001b). In this thesis I will use the types *sensu* van Oppen and link these to the types *sensu* Lajeunesse where relevant.

(2) Specificity and flexibility

The specificity of the coral-*Symbiodinium* interactions varies considerably (reviewed in Baker 2003) (Fig. 8). Some symbiont types are widely distributed and found associated with many hosts (called generalists), whereas others are only found in one or a few hosts and/or locations (called specialists). Similarly, some coral hosts can associate with many different symbiont types, whereas others are limited to only one or a few. Hence, a symbiosis can be both flexible (defined here as the ability to associate with multiple partners) and specific, depending on the partner. The mode of symbiont transmission from one generation to the next also plays an important role in shaping specificity/flexibility, as described below.

In horizontal transmission (Richmond 1997) the eggs do not contain algal symbionts and each generation must acquire their own symbionts anew from the environment. This mode of transmission is generally found in broadcasting corals and, hence, is the main mode of transmission in scleractinian corals. Horizontal transmission is likely to involve a certain degree of flexibility of the coral host, as symbiont uptake is dependent on what is available in the environment. Indeed, several studies have described the initial uptake of symbionts by azooxanthellate coral juveniles as relatively non-specific (Coffroth *et al.* 2001; Goulet and Coffroth 2003; Little *et al.* 2004; Gomez-Cabrera *et al.* 2008).

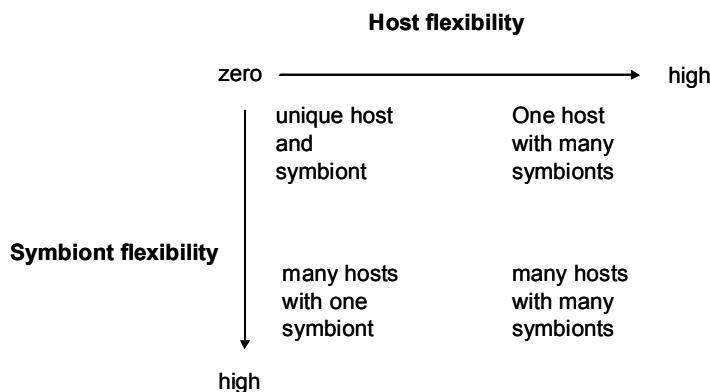


Fig. 8. Conceptual framework for symbiosis specificity (adapted from Baker 2003).

In vertical transmission, the eggs already contain symbiont cells from the parent colony and this mode of transmission is generally found in brooding corals (Fadlallah 1983). Vertical transmission reduces the risk of not finding a suitable symbiotic partner and favors the evolution of specialist symbiont lines (Douglas 1998). Specialist symbiont types are often found associated with vertical transmitters (LaJeunesse *et al.* 2004).

In general, one or a few generalist types and many specialist types of *Symbiodinium* dominate the population at a given site (LaJeunesse 2001, 2002, 2005; Diekmann *et al.* 2002). Both specialists and generalists are spread over the different clades. For clade C, two main generalists have been identified, designated C1 and C2 (C1 and C3, respectively, based on ITS2 *sensu* LaJeunesse 2001). C1 and C2 are common to both the Indo-Pacific and the Caribbean, but other than these two, each ocean has a unique assemblage of locally evolved specialist types. It has been proposed that this pattern may be the result of a worldwide selective sweep of C1 and C2, or their progenitor, before the closure of the Central American Isthmus (3.1-3.5 million years ago), followed by bursts of diversification (LaJeunesse 2005).

Coral species may host different symbionts depending on the depth (e.g. Rowan & Knowlton 1995), geographic location (e.g. van Oppen *et al.* 2005a) and sampling time (e.g. Thornhill *et al.* 2006). Variation within a single colony is also possible (e.g. Ulstrup & van Oppen 2003). Importantly, several studies have documented bleaching-related changes in the symbiont community composition of reef-building corals (Baker 2001; Toller *et al.* 2001; Berkelmans & van Oppen 2006; Thornhill *et al.* 2006). A recent meta-analysis (Goulet 2006) suggested that 29% of the scleractinian coral species associate with more than one symbiont type. This notion was challenged by Baker and Romanski (2007), who re-analyzed Goulet's (2006) data from a different perspective. They concluded that the majority (>>50%) of scleractinian coral species, including virtually all of the dominant reef-building taxa, may be able to host multiple symbiont types — even at the clade level — pointing to the fact that: (1) almost two-thirds of the coral species investigated were represented by a very low number of samples ($N \leq 5$), (2) within-clade flexibility was not taken into account, and (3) the standard molecular typing techniques used were only able to detect the dominant symbiont type within a coral colony, with types with abundances below 5-10% being overlooked (Diekmann *et al.* 2002; LaJeunesse 2002; Goulet & Coffroth 2003; Fabricius *et al.* 2004).

In this thesis I elaborate on the above discussion, particularly in relation to the last point. I hypothesize that many multi-clade associations have been overlooked because of the insufficient sensitivity of the molecular typing techniques used. To test this hypothesis, I develop a new, highly sensitive real-time PCR assay to re-screen samples already typed by commonly used molecular methods (Chapter 2).

(3) Symbiont type affects coral performance

Patterns in the distribution of *Symbiodinium* types — across and especially within coral species — are indicators of holobiont acclimatization. For example, zonation with depth indicates that corals can acclimatize to irradiance levels by associating with certain *Symbiodinium* types (Iglesias-Prieto *et al.* 2004), which can be classified as “high-light” and “low light” types (Baker 2003). Geographic zonation (Rodríguez-Lanetty *et al.* 2001; Fabricius *et al.* 2004; van Oppen *et al.* 2005a; Ulstrup *et al.* 2006) may reflect acclimatization to local environmental conditions such as water quality and temperature regime. Importantly, patterns in the natural bleaching response are strongly correlated with symbiont type (Rowan *et al.* 1997; Rowan 2004; Berkelmans & van Oppen 2006; Sampayo *et al.* 2008). The notion that holobiont physiology is affected by the symbiont identity is supported by differences in the physiological performance of symbiont types. Differences in photo-acclimatory responses to light (Iglesias-Prieto & Trench 1997), growth (Kinzie *et al.* 2001; Robinson & Warner 2006) and heat-tolerance (Bhagooli & Hidaka 2003) have been found between types isolated from different coral hosts. However, extrapolating these findings to the physiology of the holobiont is questionable (Bhagooli & Hidaka 2003), as the symbiont culture conditions are very different from the *in hospite* situation and the holobiont physiology is shaped by the coral host as well. Recently, however, the genetic identity of the symbionts *in hospite* has been unambiguously linked to a 2-3 fold difference in growth rates (Little *et al.* 2004) and in a 1-1.5°C difference in heat-tolerance (Rowan 2004; Berkelmans & van Oppen 2006) in a few coral species. This highlights the influence of the symbiont on important proxies of fitness.

The significance of the symbiont type for holobiont fitness is further examined in this thesis. I hypothesize that within a coral species, harboring different symbiont types leads to stronger differences in coral fitness than can be induced by differences

between coral host populations or their local environmental conditions during development. To test this hypothesis, I construct custom holobionts to partition symbiont type, host population and environmental effects (Chapter 5).

As a spin-off of Chapter 5, the connection between symbiont type and coral growth is further examined in a collaborative study with fellow PhD student Neal Cantin (Chapter 7). By using two custom coral groups and several different techniques (PAM fluorometry, radio-labelling, DCMU exposure, HPLC analyses), we compare the photosynthetic efficiency of the algal symbiont to the photosynthate incorporation by the coral host.

(4 & 5) Bleaching as a trigger for changes and post-bleaching reversal

Annual fluctuations in algal densities, correlated with seasonal changes in light and temperature, appear to be a common feature of scleractinian corals (Fitt *et al.* 2000). This seasonal fluctuation can be coupled with a shuffling of different *Symbiodinium* types (Chen *et al.* 2005). Bleaching may, therefore, not always be required for symbiont changes. With increasing temperature, flexibility of this degree can result in a smooth change towards more heat-tolerant symbionts with little to no coral mortality. However, most scleractinian corals (including those which are known to be flexible) are dominated by a single *Symbiodinium* type (Diekmann *et al.* 2002; Baker 2003; Goulet 2006). Symbiont types that are heat-tolerant (e.g. clade D symbionts) may be weak competitors (e.g. low growth rates) compared to heat-sensitive types in the absence of heat-stress (Little *et al.* 2004), explaining why they generally do not dominate the coral hosts. Bleaching, therefore, provides an opportunity for a symbiont change, as heat-tolerant types can take advantage of the space made available by the loss of the dominant symbiont (Baker 2001; Fautin & Buddemeier 2004). However, when conditions return to normal for prolonged periods of time, the heat-susceptible type may return to dominance as it is competitively superior under those conditions (Thornhill *et al.* 2006). Such a post-bleaching reversal, predicted by the ABH, would leave the corals susceptible to the next bleaching event.

In a second collaborative study with fellow PhD student Alison Jones, we follow the *Symbiodinium* community of an *Acropora millepora* population through a natural mass bleaching event (Chapter 6). We investigate whether bleaching can

induce a temporal change in coral-algal associations resulting in a higher coral thermo-tolerance on the scale of a reef and, if a change occurs, whether post-bleaching reversal is evident within six months after the bleaching event.

IMPLEMENTING CHANGE: SWITCH OR SHUFFLE?

There are two proposed ways by which changes in the symbiont population can occur (Baker 2003):

(1) Symbiont switching involves the uptake of a new algal type from the environment, which then becomes the dominant symbiont. It allows the coral to sample the present pool of *Symbiodinium* that may contain different symbiont types compared to what the coral initially established in symbiosis. Uptake of exogenous zooxanthellae by adult individuals has been shown experimentally in anemones (Kinzie *et al.* 2001), in a soft coral (Lewis & Coffroth 2004) and in the scleractinian coral *Porites divaricata* (Coffroth *et al.* 2008). Coffroth *et al.* (2008) exposed bleached *P. divaricata* from the Caribbean to four atypical types and recovered one atypical type after several weeks, as well as the original type. They suggested that secondary infections are restricted by the limited flexibility of the symbiosis. There is also evidence to suggest that the temporal window for uptake of zooxanthellae in *Acropora* spp. is narrow and restricted to the juvenile stage (Little *et al.* 2004).

In this thesis, I investigate whether adult, experimentally bleached *Acropora millepora* were able to take up atypical symbionts, that were provided in large dosage in their environment, to establish a new symbiosis (Chapter 4).

(2) Symbiont shuffling requires the new dominant symbiont to already be present in low abundance in the coral tissues prior to the bleaching event. During bleaching, the dominant symbiont is expelled, allowing the more stress-tolerant, background symbionts to proliferate during recovery. Symbiont shuffling may play an important role in scleractinian corals, but may be limited in its potential, as it requires a background presence of stress-tolerant symbiont types. As most corals have been found to harbor only one *Symbiodinium* type based on standard assays (Diekmann *et al.* 2002; Goulet 2006), the potential for shuffling has been thought to be small.

I hypothesize here that this notion is incorrect because the standard techniques used in symbiont genotyping have a very low sensitivity for background symbiont

populations and the majority of symbiont backgrounds may have eluded detection. I develop a new real-time PCR assay to investigate how prevalent background clades are in four common scleractinian corals on the Great Barrier Reef (Chapter 2).

EXPERIMENTAL TECHNIQUES & ASSAYS

Raising of specific coral-zooxanthella associations (custom holobionts)

Broadcast spawning of scleractinian corals (such as *Acropora millepora*) allows the collection of huge numbers of (azooxanthellate) eggs and sperm with little environmental impact. These in turn can be used to raise large numbers of specific coral-algal associations, which in this thesis will be referred to as custom corals or custom holobionts. Custom holobionts can be used to partition the influence of the host and symbiont on the coral colony's physiological characteristics. Host (population) effects can be investigated by raising custom holobionts from different coral species (or populations of the same species) with identical symbiont types. Similarly, symbiont effects can be investigated by raising offspring from the same parent colonies, but associated with different symbiont types. Lastly, identical custom holobionts can be outplanted at different locations to establish the effects of the local environmental conditions. A reciprocal design including multiple hosts, multiple symbiont types and multiple field locations allows optimal statistical analyses of all three effects in a factorial ANOVA framework.

The first step in the raising of custom holobionts is the acquisition of coral spawn. As the timing of broadcast spawning is known for many species on the GBR (Babcock *et al.* 1985), fecund colonies of the species of choice can be collected a few days before spawning and kept in tanks. Once spawning has occurred, the eggs are fertilized in the water column within the first four hours (at least two colonies need to spawn synchronously, as self-fertilization generally does not occur). The embryos are then gently washed and transferred to large rearing tanks containing filtered seawater (1 μ M pore) and allowed to develop into planula larvae. After four days, meta-morphosis of the larvae into sessile coral polyps is induced by adding adequate substratum, e.g. autoclaved terra-cotta tiles that have been preconditioned in the field. The coral juveniles are able to establish symbioses with zooxanthellae from the water column, and specific types of *Symbiodinium* (either from culture or

freshly isolated from a host) are added to produce the desired custom holobionts. Once the symbiosis is established (ca. three weeks after the symbiont introduction), the custom holobionts can be outplanted to the field location. The terracotta tiles, to which the custom holobionts are attached, are mounted on a steel rod via a hole in the middle of the tile, and the steel rod is placed in the field suspended between two metal pickets in an area where *Acropora* spp. are common (Fig. 9).



Fig. 9. Terracotta tiles with custom holobionts placed at a field location where *Acropora* spp. are common.

Identifying closely related *Symbiodinium* types

Single Strand Conformation Polymorphism (SSCP) analyses (Fig. 10) of the ITS1, in combination with sequencing, has been a standard approach to identify *Symbiodinium* types in coral samples (van Oppen *et al.* 2001b). Small fragments of coral, containing zooxanthellae, are collected and the total DNA is extracted. Using *Symbiodinium*-specific, fluorescent-labeled primers, the *Symbiodinium* ITS1 region can be amplified by Polymerase Chain Reaction (PCR). After amplification, the PCR product is heated to dissociate the complementary strands, after which the sample is snap-cooled to cause the DNA strands to fold back on themselves. The acrylamide-based gel used for SSCP separates the molecules based on the 3-D structure of the folded, single DNA strands, which depends on their sequence — not their length.

By comparing the obtained bands with previously sequenced reference bands, the *Symbiodinium* type can be resolved. Advantages of this approach include the screening of many individuals in a cost-effective manner prior to sequencing

(although recent advances in sequencing technology have significantly reduced this advantage) and the ability to distinguish multiple symbiont types within one sample.

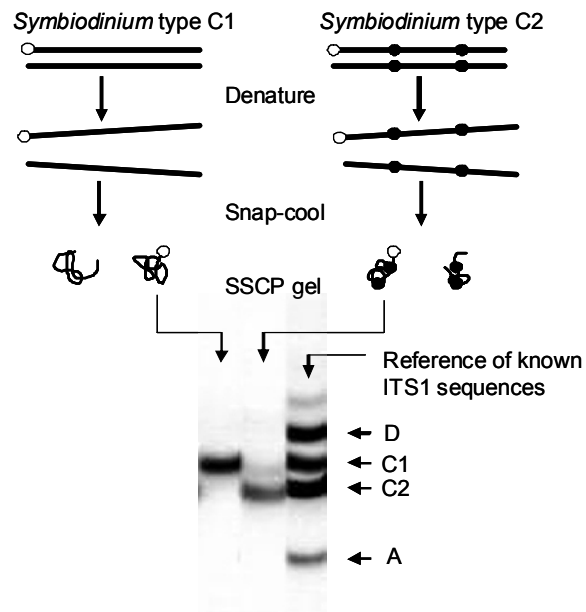


Fig. 10. Schematic representation of an SSCP analysis. ITS1 sequences of types C1 and C2 differ from each other by two base pairs (~ 350 bp long fragment) and produce different bands on the SSCP gel. • = mutation, o = fluorescent label (only on forward primer).

Detection and quantification of *Symbiodinium* background clades

Real-time PCR is a very sensitive assay for detection and quantification of low-abundance background clades in multi-clade associations. Each clade has its own specific primer pair, and a separate PCR reaction is set-up for each primer pair per sample. The reaction mixture includes a fluorescent reporter that only gives off a signal when interacting with double-stranded DNA. During the PCR reaction, the amount of DNA is measured through the fluorescence after each cycle (Fig. 11). In the initial cycles, the fluorescent signal is too low to be detected. After a number of cycles, an exponential increase in the fluorescent signal is measured as the target DNA area doubles every cycle until the PCR reagents run out and a plateau is reached. The cycle in which the sample fluorescence crosses a fluorescent threshold

(set somewhere in the exponential phase) is called the threshold cycle (C_T) and this value is dependent on the amount of target DNA initially present in the sample. The relative abundance of different clades can be calculated by the difference in C_T of the different clade-specific reactions.

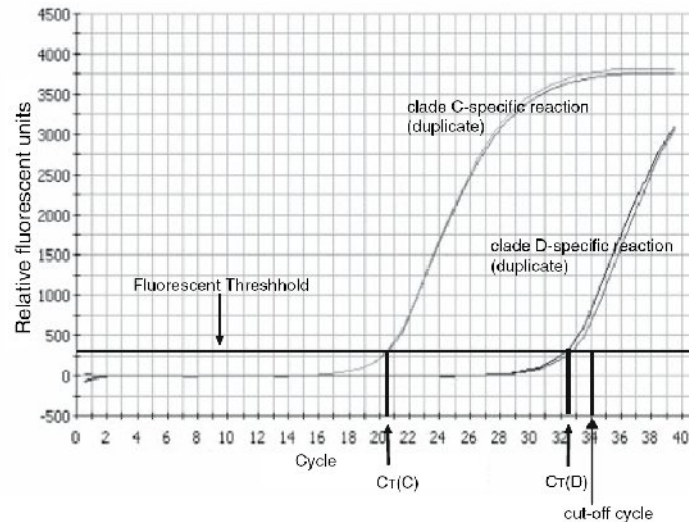


Fig. 11. Real-time PCR profile for the detection of *Symbiodinium* clades C and D in a coral sample. In this example, the average difference in C_T between the clade C and D-specific reactions (run in duplicate) is 12, indicating that there is $2^{12} = 4096$ times more clade C DNA than clade D DNA in the coral sample. The coral under investigation is, therefore, dominated by clade C with a very low (background) presence of clade D.

Measuring photosynthetic performance as a proxy for bleaching

Pulse Amplitude Modulated (PAM) fluorometry is a popular, non-invasive technique for measuring the “health” of *Symbiodinium* cells while in symbiosis with corals. The most commonly measured parameters are the maximum quantum yield (F_v/F_m) and the effective quantum yield (F/F_m'), which give indications of the efficiency of the photosynthetic machinery of the algal symbiont (Ralph *et al.* 2005). The technique involves the measurement of the fluorescence of chlorophyll a under different irradiance conditions (Fig. 12). F_v/F_m is measured after a period of darkness and with the photomachinery at rest. First, F_o is measured by exposing the

coral surface to a pulsating red light at a very low intensity (the measuring light), capable of inducing fluorescence but not inducing photosynthesis. Next, the coral is exposed to an ~1 sec burst of very high, white light, called a saturating pulse, and the maximum fluorescence (F_m) is measured. From these two measurements, the maximum quantum yield (F_v/F_m) can be calculated through the formula: $F_v/F_m = (F_m - F_o)/F_m$. F_v/F_m gives an indication of the maximum number of reaction centers in photosystem II (PSII) that are “open” (ready for light capture for photosynthesis) and heat-stress will lead to a reduction in this parameter.

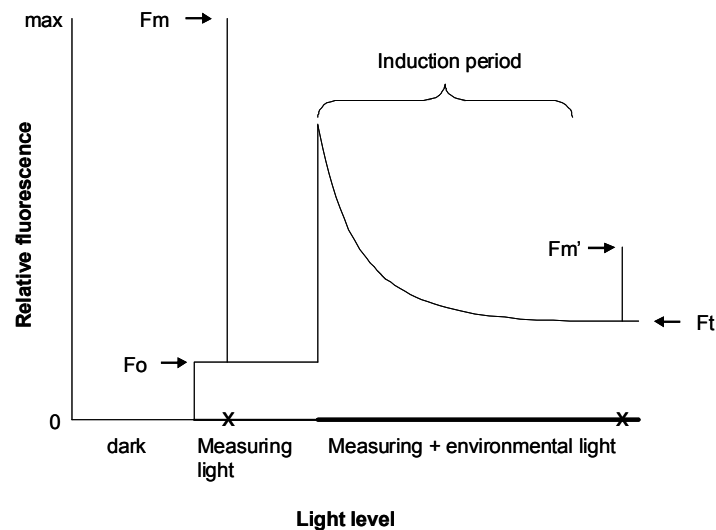


Fig. 12. Measuring maximum and effective quantum yields. X indicates the saturating pulse. F_o and F_m are measured in dark-adapted corals, F_t and F_m' are measured in light-adapted corals after the induction phase has passed.

Effective quantum yield (F/F_m') is measured similarly, but these measurements take place when the corals are under ambient environmental light conditions (either experimental or natural) and the photomachinery is active. Instead of F_o , F_t is measured, which is the fluorescence under the environmental light conditions (+ measuring light). Similarly, using the saturating pulse, F_m' is measured instead of F_m . Care must be taken to wait until the induction period has passed, during which the photomachinery has started up. The effective quantum yield is calculated according to the formula: $F/F_m' = (F_m' - F_t)/F_m'$ and is typically

lower than the maximum quantum yield as a portion of the PSII reaction centers remain closed because of ongoing light capture. Again, heat-stress will cause a reduction in F/F_m' .

When combining F_v/F_m and F/F_m' , the excitation pressure over photosystem II (Q) can be calculated using the formula: $Q = 1 - ([F/F_m']/[F_v/F_m])$, which gives an indication of the percentage of reaction centers that are closed under the environmental light conditions during the effective quantum yield measurements (Iglesias-Prieto *et al.* 2004). Q is considered a very important measure of heat-stress as the accumulation of excitation pressure over photosystem II is inherently associated with coral bleaching (Abrego *et al.* 2008).

OUTLINE OF THE THESIS

The popular view that most scleractinian corals harbor only one clade of *Symbiodinium* per colony is evaluated in **Chapter 2**. To do this required the development of a novel real-time PCR assay which boosted the detection sensitivity for multi-clade symbioses >100 fold. Application of this assay on a sample collection of four common scleractinian corals collected across the Great Barrier Reef revealed that previous studies have missed many multi-clade symbioses and indicated that the potential for symbiont shuffling is large.

Further technical refinements of the real-time PCR assay are described in **Chapters 3 and 4**, aimed to: (1) overcome the limitations associated with variability in ITS1 copy number, (2) include a measure of symbiont cell density per coral unit, and (3) increase the resolution of the assay to the type level.

Adult *Acropora millepora* colonies were experimentally bleached using a herbicide and exposed to high levels of an atypical *Symbiodinium* type over a 6-week recovery period (**Chapter 4**). Screening of post-exposure DNA samples with the optimized real-time PCR assay revealed that no new symbioses had been established. However, results were inconclusive due to the presence of the “atypical” symbiont type at low levels at the start of the experiments in 30% of the colonies and the use of a low-infectious symbiont type (both issues were not known at the start of the experiment). Improvements to the experimental design are discussed.

In order to test specific host-symbiont responses (**Chapter 5**), custom holobionts were produced in the laboratory using two *A. millepora* populations and

six *Symbiodinium* types. The custom holobionts were reciprocally outplanted to two field sites differing in ambient temperature. It was established that the fitness (using growth, survival and heat-tolerance as proxies) of *A. millepora* on the GBR was primarily affected by the symbiont types present and secondarily by environmental factors working on growth and survival. In contrast, host population origin (and hence possible host genetic differences) had little to no effect and no environmental effect was found on the thermo-tolerance. As a result, trade-offs between thermo-tolerance and growth/survival related to *Symbiodinium* type were found to be dependent on the local environmental conditions.

We investigated the effect of a natural bleaching event on the symbiont community within an *A. millepora* population in **Chapter 6**. Before bleaching, 93.5% of the coral colonies harbored the thermo-sensitive *Symbiodinium* type C2, whereas up to six months after bleaching, 71% of the same coral colonies were dominated by the thermo-tolerant *Symbiodinium* type D. The high prevalence of background clades indicated that this change was due to symbiont shuffling. Indications for a drift back to the heat-sensitive C2 (post-bleaching reversal) were found after six months.

In **Chapter 7** the effects of *Symbiodinium* types C1 and D on growth of *A. millepora* is further investigated using custom holobionts outplanted at Magnetic Island. PAM fluorometry (rapid light curves) revealed that that C1 symbionts had a 87 % higher photosystem II (PSII) capacity than D symbionts *in hospite*. Incorporation of photosynthate by the host (as measured by ¹⁴C-labelling) was 121 % higher for C1 holobionts than for D holobionts under identical environmental conditions. The advantage for C1 holobionts was lost in the presence of the herbicide diuron (DCMU), which blocked the electron transport and caused damage to PSII. Therefore, a strong link was suggested between photosynthetic capacity and nutritional benefit to the coral host, which explained why C1 holobionts grew twice as fast as D holobionts at the outplant location (as found in Chapter 5).

In **Chapter 8** the overall results, conclusions and prospects are summarized. The Dutch translation of this chapter is given in **Chapter 9**.

REFERENCES

- Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proc Royal Soc London, B* **275**:2273-2282
- Access Economics (2007) Measuring the economic and financial value of the Great Barrier Reef Marine Park 2005/2006. Report by Access Economics Pty Limited for the Great Barrier Reef Marine Park Authority
- Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous Spawnings of 105 Scleractinian Coral Species on the Great-Barrier-Reef. *Mar Biol* **90**:379-394
- Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. *MEPS* **237**:133-141
- Baker AC (2001) Ecosystems: Reef corals bleach to survive change. *Nature* **411**:765-766
- Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Ann Rev Ecol Evol Syst* **34**:661-689
- Baker AC, Romanski AM (2007) Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: Comment on Goulet (2006). *MEPS* **335**:237-242
- Baker AC, Starger CJ, McClanahan T, Glynn PW (2004) Corals' adaptive response to climate change. *Nature* **430**:741
- Berkelmans R (2002) Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. *MEPS* **229**:73-82
- Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc Royal Soc London, B* **273**:2305-2312
- Bhagooli R, Hidaka M (2003) Comparison of stress susceptibility of in hospite and isolated zooxanthellae among five coral species. *J Exp Mar Biol Ecol* **291**:181-197
- Blank RJ, Trench RK (1985) Speciation and Symbiotic Dinoflagellates. *Science* **229**:656-658
- Briggs JC (2005) Coral reefs: Conserving the evolutionary sources. *Biol Conserv* **126**:297-305
- Buddemeier RW, Baker AC, Fautin DG, Jacobs JR (2004) The adaptive hypothesis of bleaching. In: Rosenberg E, Loya Y (eds) Coral Health and Disease. Springer-Verlag, Berlin, pp 427-444
- Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism. *Bioscience* **43**:320-326
- Buddemeier RW, Smith SV (1999) Coral adaptation and acclimatization: A most ingenious paradox. *Am Zool* **39**:1-9
- Burke L, Maidens J (2004) Reefs at risk. World Resources Institute. http://www.wri.org/biodiv/pubs_description.cfm?pid=3944, Washington D.C. (USA)
- Carlos AA, Baillie BK, Kawachi M, Maruyama T (1999) Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (*Bivalvia*), cardiids (*Bivalvia*), a sponge (*Porifera*), a soft coral (*Anthozoa*), and a free-living strain. *J Phycol* **35**:1054-1062
- Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortes J, Delbeek JC, DeVantier L, Edgar GJ, Edwards AJ, Fenner D, Guzman HM, Hoeksema BW, Hodgson G, Johan O, Licuanan WY, Livingstone SR, Lovell ER, Moore JA, Obura DO, Ochavillo D, Polidoro BA, Precht WF, Quibilan MC, Reboton C, Richards ZT, Rogers AD, Sanciangco J, Sheppard A, Sheppard C, Smith J, Stuart S, Turak E, Veron JEN, Wallace C, Weil E, Wood E (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **321**:560-563
- Chen CA, Wang AT, Fang LS, Yang YW (2005) Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. *MEPS* **295**:113-121

- Coffroth MA, Lewis CF, Santos SR, Weaver JL (2006) Environmental populations of symbiotic dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians. *Curr Biol* **16**:R985-R987
- Coffroth MA, Petrou E, Poland D, Holland L (2008) Can Scleractinians take up new symbionts after a bleaching event? *11th Int Coral Reef Symp Abstract* **25**:25
- Coleman FC, Williams SL (2002) Overexploiting marine ecosystem engineers: potential consequences for biodiversity. *Trends Ecol Evol* **17**:40-44
- Coles SL, Brown BE (2003) Coral bleaching - Capacity for acclimatization and adaptation. *Adv Mar Biol* **46**:183-223
- Cooper TF, De 'Ath G, Fabricius KE, Lough JM (2008) Declining coral calcification in massive Porites in two nearshore regions of the northern Great Barrier Reef. *Global Change Biol* **14**:529-538
- CRC (Reef Research Center) <http://www.reef.crc.org.au/discover/coralreefs/coralgbr.html>
- Diekmann OE, Bak RPM, Stam WT, Olsen JL (2001) Molecular genetic evidence for probable reticulate speciation in the coral genus *Madracis* from a Caribbean fringing reef slope. *Mar Biol* **139**:221-233
- Diekmann OE, Bak RPM, Tonk L, Stam WT, Olsen JL (2002) No habitat correlation of zooxanthellae in the coral genus *Madracis* on a Curacao reef. *MEPS* **227**:221-232
- Douglas AE (2003) Coral bleaching - how and why? *Mar Poll Bull* **46**:385-392
- Fabricius KE, Mieog JC, Colin PL, Idip D, van Oppen MJH (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol Ecol* **13**:2445-2458
- Fadlallah Y (1983) Sexual reproduction, development and larval biology in scleractinian corals. *Coral Reefs* **2**:129-150
- Fautin DG, Buddemeier RW (2004) Adaptive bleaching: a general phenomenon. *Hydrobiol* **530-31**:459-467
- Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol Oceanogr* **45**:677-685
- Fitt WK, Trench RK (1983) Endocytosis of the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal by endodermal cells of the scyphistomae of *Cassiopeia xamachana* and resistance of the algae to host digestion. *J Cell Sci* **64**:195-212
- Freudenthal HD (1962) *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle, and morphology. *J Protozool* **9**:45-52
- Gattuso JP, Allemand D, Frankignoulle M (1999) Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: A review on interactions and control by carbonate chemistry. *Am Zool* **39**:160-183
- Glynn PW (1993) Coral-Reef Bleaching - Ecological Perspectives. *Coral Reefs* **12**:1-17
- Glynn PW, Mate JL, Baker AC, Calderon MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997-1998 El Niño-Southern oscillation event: Spatial/temporal patterns and comparisons with the 1982-1983 event. *Bull Mar Sci* **69**:79-109
- Goulet TL (2006) Most corals may not change their symbionts. *MEPS* **321**:1-7
- Goulet TL (2007) Most scleractinian corals and octocorals host a single symbiotic zooxanthella clade. *MEPS* **335**:243-248
- Goulet TL, Coffroth MA (2003) Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. *Mar Biol* **142**:233-239
- Guest JR, Baird AH, Goh BPL, Chou LM (2005) Seasonal reproduction in equatorial reef corals. *Invertebrate Reprod Developm* **48**:207-218
- Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass Spawning in Tropical Reef Corals. *Science* **223**:1186-1189

- Harvell D, Aronson R, Baron N, Connell J, Dobson A, Ellner S, Gerber L, Kim K, Kuris A, McCallum H, Lafferty K, McKay B, Porter J, Pascual M, Smith G, Sutherland K, Ward J (2004) The rising tide of ocean diseases: unsolved problems and research priorities. *Frontiers Ecol Environm* **2**:375-382
- Heyward AJ, Negri AP (1999) Natural inducers for coral larval metamorphosis. *Coral Reefs* **18**:273-279
- Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshwat Res* **50**:839-866
- Hoegh-Guldberg O (2004) Coral reefs in a century of rapid environmental change. *Symbiosis* **37**:1-31
- Hoegh-Guldberg O, Jones RJ, Ward S, Loh WK (2002) Is coral bleaching really adaptive? *Nature* **415**:601-602
- Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatzioalos ME (2007) Coral reefs under rapid climate change and ocean acidification. *Science* **318**:1737-1742
- Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. *Science* **301**:929-933
- Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskij N, Pratchett MS, Steneck RS, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr Biol* **17**:360-365
- Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proc Royal Soc London, B* **271**:1757-1763
- Iglesias-Prieto R, Trench RK (1997) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. *Mar Biol* **130**:23-33
- IPCC (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment
- Jackson JBC (2001) What was natural in the coastal oceans? *PNAS* **98**:5411-5418
- Jones CG, Lawton JH, Shachak M (1994) Organisms as Ecosystem Engineers. *Oikos* **69**:373-386
- Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO₂ fixation mechanism in zooxanthellae. *Plant Cell Environm* **21**:1219-1230
- Kinzie RA, Takayama M, Santos SR, Coffroth MA (2001) The adaptive bleaching hypothesis: experimental tests of critical assumptions. *Biol Bull* **200**:51-58
- LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: In search of a "species" level marker. *J Phycol* **37**:866-880
- LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar Biol* **141**:387-400
- LaJeunesse TC (2005) "Species" radiations of symbiotic Dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Mol Biol Evol* **22**:1158-1158
- LaJeunesse TC, Loh WKW, van Woesik R, Hoegh-Guldberg O, Schmidt GW, Fitt WK (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnol Oceanogr* **48**:2046-2054
- LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG, Fitt WK, Schmidt GW (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral Reefs* **23**:596-603

- Lewis CL, Coffroth MA (2004) The acquisition of exogenous algal symbionts by an octocoral after bleaching. *Science* **304**:1490-1492
- Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science* **304**:1492-1494
- Littman RA, van Oppen MJH, Willis B (2008) Methods for sampling free-living *Symbiodinium* (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef) *J Exp Mar Biol Ecol* **364**:48-53
- Long EO, Dawid IB (1980) Repeated Genes in Eukaryotes. *Ann Rev Biochem* **49**:727-764
- Loram JE, Boonham N, O'Toole P, Trapido-Rosenthal HG, Douglas AE (2007) Molecular quantification of symbiotic dinoflagellate algae of the genus *Symbiodinium*. *Biol Bull* **212**:259-268
- Manning MM, Gates RD (2008) Diversity in populations of free-living *Symbiodinium* from a Caribbean and Pacific reef. *Limnol Oceanogr* **53**:1853-1861
- McClanahan TR, Baird AH, Marshall PA, Toscano MA (2004) Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia **48**:327-335
- Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. *Ecol Economics* **29**:215-233
- Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proc Royal Soc London, B* **222**:181-202
- Muscatine L, Porter JW (1977) Reef Corals - Mutualistic Symbioses Adapted to Nutrient-Poor Environments. *Bioscience* **27**:454-460
- Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClanahan L, Newman MJH, Paredes G, Warner RR, Jackson JBC (2003) Global trajectories of the long-term decline of coral reef ecosystems. *Science* **301**:955-958
- Pearse VB, Muscatine L (1971) Role of symbiotic algae (zooxanthellae) in coral calcification. *Biol Bull* **141**:350-363
- Pochon X, LaJeunesse TC, Pawlowski J (2004) Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; *Dinophyta*). *Mar Biol* **146**:17-27
- Pochon X, Montoya-Burgos JI, Stadelmann B, Pawlowski J (2006) Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. *Mol Phyl Evol* **38**:20-30
- Porto I, Granador C, Retrepo JC, Sanchez JA (2008) Macroalgal-Associated Dinoflagellates Belonging to the Genus *Symbiodinium* in Caribbean Reefs. *PLOS One* **3**:1-5
- Ralph PJ, Schreiber U, Gademann R, Kuhl M, Larkum AWD (2005) Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. *J Phycol* **41**:335-342
- Richmond R (1997) Reproduction and recruitment in corals: critical links in the persistence of reefs. In: Birkeland C (ed) *Life and death of coral reefs*. Chapman Hall, New York, pp 175-197
- Richmond RH, Hunter CL (1990) Reproduction and Recruitment of Corals - Comparisons among the Caribbean, the Tropical Pacific, and the Red-Sea. *MEPS* **60**:185-203
- Robinson JD, Warner ME (2006) Differential impacts of photoacclimation and thermal stress on the photobiology of four different phlotypes of *Symbiodinium* (Pyrrhophyta). *J Phycol* **42**:568-579
- Rodriguez-Lanetty M, Loh W, Carter D, Hoegh-Guldberg O (2001) Latitudinal variability in symbiont specificity within the widespread scleractinian coral *Plesiastrea versipora*. *Mar Biol* **138**:1175-1181
- Rogers SO, Bendich AJ (1987) Ribosomal-RNA genes in plants - variability in copy number and in the intergenic spacer. *Plant Mol Biol* **9**:509-520

- Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. *J Phycol* **34**:407-417
- Rowan R (2004) Thermal adaptation in reef coral symbionts. *Nature* **430**:742
- Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *PNAS* **92**:2850-2853
- Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* **388**:265-269
- Rowan R, Powers DA (1991a) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. *Science* **251**:1348-1351
- Rowan R, Powers DA (1991b) Molecular Genetic Identification of Symbiotic Dinoflagellates (Zooxanthellae). *MEPS* **71**:65-73
- Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). *PNAS* **89**:3639-3643
- Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *PNAS* **105**:10444-10449
- Santos S, Shearer TL, Hannes AR, Coffroth MA (2004) Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, *Dinophyceae*) of the Caribbean. *Mol. Ecol.* **13**:459-469
- Santos SR, Taylor DJ, Coffroth MA (2001) Genetic comparisons of freshly isolated versus cultured symbiotic dinoflagellates: implications for extrapolating to the intact symbiosis. *J Phycol* **37**:900-912
- Santos SR, Taylor DJ, Kinzie RA, Hidaka M, Sakai K, Coffroth MA (2002) Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. *Mol Phylogeny Evol* **23**:97-111
- Savage AM, Trapido-Rosenthal H, Douglas AE (2002) On the functional significance of molecular variation in *Symbiodinium*, the symbiotic algae of Cnidaria: photosynthetic response to irradiance **244**:27-37
- Schoenberg DA, Trench RK (1980) Genetic variation in *Symbiodinium* (= *Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates .2. morphological variation in *Symbiodinium microadriaticum*. *Proc Royal Soc London, B* **207**:429-&
- Smith DJ, Suggett DJ, Baker NR (2005) Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? *Global Change Biol* **11**:1-11
- Smith SV (1978) Coral reef area and contributions of reefs to processes and resources of worlds oceans. *Nature* **273**:225-226
- Spalding MD, Grenfell AM (1997) New estimates of global and regional coral reef areas. *Coral Reefs* **16**:225-230
- Stanley GD (2006) Ecology - Photosymbiosis and the evolution of modern coral reefs. *Science* **312**:857-858
- Stanley Jr GD, Fautin DG (2001) Paleontology and evolution: the origins of modern corals. *Science* **291**:1913-1914
- Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of *Symbiodinium* and scleractinian hosts - Symbiosis, diversity, and the effect of climate change. *Persp Plant Ecol Evol Syst* **8**:23-43
- Szmant AM, Gassman NJ (1990) The effects of prolonged bleaching on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. *Coral Reefs* **8**:217-224
- Takabayashi M, Santos SR, Cook CB (2004) Mitochondrial DNA phylogeny of the symbiotic dinoflagellates (*Symbiodinium*, Dinophyta). *J Phycol* **40**:160-164

- Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar Biol* **148**:711-722
- Toller W (2001) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biol Bull* **201**:348-359
- Toller WW, Rowan R, Knowlton N (2001) Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *Biol Bull* **201**:360-373
- Ulstrup KE, Berkelmans R, Ralph PJ, van Oppen MJH (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. *MEPS* **314**:135-148
- Ulstrup KE, van Oppen MJ (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Mol Ecol* **12**:3477-3484
- van Oppen MJH, Mahiny AJ, Done TJ (2005a) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs* **24**:482-487
- van Oppen MJH, McDonald BJ, Willis B, Miller DJ (2001a) The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? *Mol Ecol Evol* **18**:1315-1329
- van Oppen MJH, Mieog JC, Sanchez CA, Fabricius KE (2005b) Diversity of algal endosymbionts (zooxanthellae) in octocorals: the roles of geography and host relationships. *Mol Ecol* **14**:2403-2417
- van Oppen MJH, Palstra FP, Piquet AT, Miller DJ (2001b) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proc Royal Soc London, B* **268**:1759-1767
- van Woesik R, Lacharaise F, Koksals S (2006) Annual cycles of solar insolation predict spawning times of Caribbean corals. *Ecol Letters* **9**:390-398
- Veron JEN (1995) Corals in space and time. The biography and evolution of the Scleractinia. Australian Institute of Marine Science, Townsville, Australia
- Veron JEN, Stafford-Smith M (2000) Corals of the world. Australian Institute of Marine Science, Townsville, Australia
- Ware JR, Fautin DG, Buddemeier RW (1996) Patterns of coral bleaching: modeling the adaptive bleaching hypothesis. *Ecol Model* **84**:199-214
- Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *PNAS* **96**:8007-8012
- Weis MV (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *J Exp Biol* **211**:3059-3066
- Wilkinson C (2000) Status of coral reefs of the world: 2000. Australian Institute of Marine Science, Townsville, Australia
- Wilkinson C (2004) Status of coral reefs of the world. Australian Institute of Marine Science, Townsville, Australia
- Wilkinson CR (1999) Global and local threats to coral reef functioning and existence: review and predictions. *Mar Freshwater Res* **50**:867-878
- Willis BL, van Oppen MJH, Miller DJ, Vollmer SV, Ayre DJ (2006) The Role of Hybridization in the Evolution of Reef Corals. *Annu Rev Ecol Evol Syst* **37**:489-517
- Yonge CM, Nicholls AG (1931) Studies on the physiology of corals. IV. The structure, distribution and physiology of the zooxanthellae. *Scientific reports of the Great Barrier Reef Expedition* **1**:178-210