University of Miami
Scholarly Repository

Open Access Theses | Electronic Theses and Dissertations

2011-05-11

Latitudinal Patterns in the Distribution of Algal Symbionts (Symbiodinium spp.) in Reef Corals of Madagascar, and their Response to Thermal Disturbance

Roxane K. Boonstra
University of Miami, rboonstra@rsmas.miami.edu

Follow this and additional works at: https://scholarlyrepository.miami.edu/oa_theses

Recommended Citation
Boonstra, Roxane K., "Latitudinal Patterns in the Distribution of Algal Symbionts (Symbiodinium spp.) in Reef Corals of Madagascar, and their Response to Thermal Disturbance" (2011). *Open Access Theses*. 269.
https://scholarlyrepository.miami.edu/oa_theses/269

This Open access is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarly Repository. It has been accepted for inclusion in Open Access Theses by an authorized administrator of Scholarly Repository. For more information, please contact repository.library@miami.edu.

UNIVERSITY OF MIAMI

LATITUDINAL PATTERNS IN THE DISTRIBUTION OF ALGAL SYMBIONTS (*SYMBIODINIUM* SPP.) IN REEF CORALS OF MADAGASCAR, AND THEIR RESPONSE TO THERMAL DISTURBANCE

By

Roxane K. Boonstra

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

May 2011

©2011
Roxane K. Boonstra
All Rights Reserved

UNIVERSITY OF MIAMI

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science

LATITUDINAL PATTERNS IN THE DISTRIBUTION OF ALGAL SYMBIONTS (*SYMBIODINIUM* SPP.) IN REEF CORALS OF MADAGASCAR, AND THEIR RESPONSE TO THERMAL DISTURBANCE

Roxane K. Boonstra

Approved:

_____
Andrew C. Baker, Ph.D.
Associate Professor of
Marine Biology and Fisheries

_____
Terri A. Scandura, Ph.D.
Dean of the Graduate School

_____
Diego Lirman, Ph.D.
Assistant Professor of
Marine Biology and Fisheries

_____
Timothy R. McClanahan, Ph.D.
Senior Conservation Zoologist
Wildlife Conservation Society
Mombasa, Kenya

BOONSTRA, ROXANE K. (M.S., Marine Biology and Fisheries)
(May 2011)

Latitudinal Patterns in the Distribution of Algal Symbionts (*Symbiodinium* spp.) in Reef Corals of Madagascar, and their Response to Thermal Disturbance

Abstract of a thesis at the University of Miami.

Thesis supervised by Associate Professor Andrew Baker.
No. of pages in text. (76)

The island continent of Madagascar spans nearly 13.5^{o} of latitude in the SW Indian Ocean. Its coastline includes a number of well developed coral reefs, ranging from tropical Nosy Bé (NW Madagascar, 12^{o}S) and Vohemar (Volhmarina, NE Madagascar, 13^{o}S) to subtropical Tuléar (Toliara , SW Madagascar, 23.5^{o}S), as well as temperate coral communities at Fort Dauphin (Tolagnaro, SE Madagascar, 25^{o}S). Given the range of environmental conditions experienced by reef corals at these different sites, Madagascar represents an ideal location to study the distribution of algal symbionts (*Symbiodinium* spp.) in these coral hosts. To investigate the effect of latitudinal gradients in temperature on *Symbiodinium* distributions, 220 samples from 27 coral genera in 12 families were collected from these 4 sites in September 2001. To test the stability of these distributions over time, a further 337 samples were collected from the Nosy Bé and Tuléar regions in March 2007 and November 2009. *Symbiodinium* communities were screened using Denaturing Gradient Gel Electrophoresis (DGGE) to analyze the internal transcribed spacer-2 (ITS-2) region of *Symbiodinium* ribosomal DNA, with individual symbiont taxa identified by sequencing individual DGGE bands. Significant differences were found in the *Symbiodinium* cladal composition of reef corals at different sites, with corals at

northern sites containing a higher relative frequency of *Symbiodinium* in clade D (occurring as mixed clade C+D communities) than southern sampling sites. Nominal logistic analysis of the distribution of symbionts found a significant effect of coral taxa and site, but not of sea surface temperature metrics (environmental data obtained from NOAA's Coral Reef Watch satellite-derived data) in determining the distribution of different symbionts. Rarefaction analysis indicated there were no differences in *Symbiodinium* richness (at either the clade or the subtype level) between different sites, or between different sampling intervals. Differences existed in the subcladal composition of dominant ITS-2 types found in congeners at different latitudes, with corals in the genus *Acropora* being dominated by *Symbiodinium* C3 (specifically subtype C3z) in northern sites, and C1 in southern sites. Symbiont communities changed between 2001 and 2007/2009, with increases in mixed *Symbiodinium* C+D assemblages occurring at southern sites that had experienced temperature stress during the intervening period. Decreases in mixed *Symbiodinium* communities occurred at northern sites, which were not as severely affected by thermal stress. It is suggested that the latitudinal gradients in *Symbiodinium* found in Madagascar, and the environmental controls on community structure described here, provide important insight into how coral species in this understudied area can adapt or acclimatize to changing environmental conditions through shifts in the composition of their symbiont communities. This will help improve our understanding of how projected climate change in the SW Indian Ocean will affect survival trajectories for coral reefs in the region.

Acknowledgements

This thesis was completed thanks to the support, guidance and advice of multiple people at the University of Miami. I would first like to thank Dr. Andrew Baker, my primary advisor and mentor, for his constant support, attention, encouragement, guidance, and above all, patience. Andrew went out on the furthest limb of the molecular tree, and brought in someone who started as a fish biologist to work on *Symbiodinium* genetics. While my thesis was originally on a different subject, Andrew graciously passed the Madagascar samples he had collected on to me as project initially started as a lab technician and finished as a Masters thesis. Aside from thesis and employment-related subjects, Andrew Baker also funded and sponsored me on what became a recent flagship for international scientific exploration and effort. My three weeks aboard the Tara research vessel in the uninhabited and rarely visited St Brandon's Rocks archipelago north of Mauritius is a tale I will tell to whoever will listen, as well as a constant reminder that opportunity always lies around the next corner.

I would next like to thank the other two members of my thesis committee, Dr. Tim McClanahan and Dr. Diego Lirman. Dr. McClanahan selflessly advised me at all hours on statistical analyses and insights to the Western Indian Ocean, as well as providing half the sampling from Madagascar. Dr. Lirman helped to keep the big picture constantly in view, and was an invaluable guide in reminding me that, while I could always find new analyses to try, you need somewhere to start first.

Countless other people helped with this project through various forms of support, without whom my research would not have been as rounded and interdisciplinary as it became. At the university, my most sincere and heartfelt thanks goes to the Baker lab –

Paul Jones, Herman Wirshing, Rachel Silverstein, Xaymara Serrano, and Ross Cunning - who form the heart of my support group both in and out of the laboratory. Special thanks go to Stephen Manley, who dedicated numerous hours helping me with genetic analyses. As the lab manager, I always had multiple projects on my mind, and without laboratory volunteers such as Steve, research on my Master's thesis would inevitably have been delayed. Outside of the university, I am indebted to my family and Phil Kravitz, who supported me all through the rollercoaster that became my graduate degree.

Lastly, but certainly not least, this research would not have been possible without the funding from the Western Indian Ocean Marine Science Association (WIOMSA), the Wildlife Conservation Society and the Tiffany and Co. Foundation. The Rowland's Research award, graciously donated to the University of Miami by the Rowland family, also aided in funding a trip I made to Canada to study ancient DNA analytical techniques and protocol.

Table of Contents

List of Tables

List of Figures

Chapter One:

Symbiodinium richness and distribution on Malagasy coral reefs and the effect of latitudinal environmental gradients on regional diversity

---

Summary

The distribution and diversity of algal symbionts (dinoflagellates in the genus *Symbiodinium*) in reef corals were sampled over a latitudinal gradient of ~13^{o} in Madagascar (SW Indian Ocean). In total, 220 samples in 27 genera (12 families) of scleractinian coral were collected from Nosy Bé (NE), Vohemar (Volhmarina, NW), Tuléar (Toliara, SW) and Fort Dauphin (Tolagnaro, SE) in September 2001. *Symbiodinium* DNA was extracted, purified and amplified using primers specific to the internal transcribed spacer-2 (ITS-2) region of ribosomal DNA and amplified products analyzed using Denaturing Gradient Gel Electrophoresis (DGGE). Individual *Symbiodinium* types were identified by excising bands from DGGE gels and subsequent sequencing. While all sites contained *Symbiodinium* in clades A, C and D, (except for Ft. Dauphin which only contained clades C and D), it was hypothesized that there would be differences in symbiont communitie (both in composition and species richness) between the different sampling sites. There was a significant difference in symbiont richness, at the subcladal level, between the northern (Nosy Bé and Vohemar) and southern (Tuléar and Fort Dauphin) sites, with corals at northern sites hosting 25 symbiont types while those from southern reefs hosted only 13 symbiont subtypes. Corals at Nosy Bé were characterized by a higher incidence of mixed *Symbiodinium* communities (clades C and D) than other sites. Nominal logistic models revealted that mean temperatures, minimum

temperatures, temperature variability (SD) and degree heating weeks (DHWs) indicate increases of *Symbiodinium* clade D with increasing temperature and decreasing SD, but these trends were not significant. Site and taxa were found to be the most powerful (r^2>0.5) predictors of the symbiont community structure (frequency of clade D dominance, clade D incidence and mixed C+D assemblages) at each location.

Background

Tropical coral reefs dominated by hermatypic reef building corals are typically found between 25° N and 25° S (Veron 2000). The productivity and success of these corals is due to mutualistic associations with algae in the genus *Symbiodinium,* a genus of dinoflagellates colloquially referred to as "zooxanthellae" that translocate photosynthetic carbon to the coral host and supply metabolic needs. The genus *Symbiodinium* currently includes at least nine clades (A-I) (Pochon & Gates 2010), six of which (A-D, F, G) associate with scleractinian (stony) corals on tropical coral reefs (Rowan 1998, Baker 2003, LaJeunesse et al. 2010, Pochon and Gates 2010).

Studies have shown that different *Symbiodinium* can impart different physiological capabilities to their coral hosts (Iglesias-Prieto & Trench 1994, Baker 2001, Savage et al. 2002, Baker et al. 2004, Igelsias-Prieto et al. 2004, Little et al. 2004, Berkelmans & van Oppen 2006, Warner et al. 2006, Loram et al. 2007, Ulstrup et al. 2007, Sampayo et al. 2008, Stat et al. 2008, Cantin et al. 2009) and can potentially affect a coral's capacity to acclimatize or adapt to environmental changes (Baker 2004, Baker et al. 2004). For example, *Symbiodinium* in clade A are commonly found in shallow water (0-6m) in the Caribbean or at high latitudes in the Indo-Pacific, and produce UV-

protecting mycosporine-like amino acids (MAAs) (Banasak et al. 2000, Baker 2003). Consequently, corals hosting clade A in a particular environment (e.g., shallow depths) may be better suited to higher irradiance levels than those at deeper corals. In contrast, *Symbiodinium* in clade D has been found in corals that routinely experience high sea surface temperatures (such as those in the Arabian Gulf, Baker et al. 2005) and are often relatively abundant on reefs recovering from bleaching (Baker 2001, 2003, Baker et al. 2004, Jones et al. 2008, LaJeunesse et al. 2009).

Flexibility in host-symbiont associations and the role of environmental factors such as irradiance (depth), temperature and bleaching history may drive observed patterns of symbiont distribution. Symbiont flexibility has been extensively studied in recent years, in part due to their potential relevance in understanding how corals respond to climate change (Rowan & Knowlton 1995, Rowan et al. 1997, Rowan 1998, LaJeunesse 2001, Rodriguez-Lanetty et al. 2001, Baker 2003, Iglesias-Prieto et al. 2004, LaJeunesse et al.. 2004, van Oppen et al. 2005, Ulstrup et al. 2006, Warner et al. 2006, Winters et al. 2009). It is clear that, although many coral species usually host a particular symbiont type (Goulet 2006, 2007), many coral species are able to associate with multiple symbiont taxa (Baker & Romanski 2007), and the relative abundance of different symbionts in a coral can be driven byenvironmental factors such astemperature and irradiance (Baker 2003).

Field surveys show that symbiont community structure can respond to changes in light regime (e.g., Rowan and Knowlton 1995, Rowan et al. 1997, Baker 2001) and can often show predictable patterns of latitudinal distribution (e.g., Rodriguez-Lanetty et al. 2001, LaJeunesse et al. 2004, Berkelmans & van Oppen 2006). Corals transplanted from

deep to shallow depths on a Caribbean reef experienced changes in their dominant symbiont communities to match the symbionts found in conspecific hosts at the new depth (Baker 2001), while corals moved from cool sites on the southern Great Barrier Reef (GBR) to warmer sites on the central GBR experienced changes to favor heat tolerant symbionts (in *Symbiodinium* clade D) that were common in the warmer environment (Berkelmans & van Oppen 2006). Mid-latitude inshore reefs of the Great Barrier Reef (LaJeunesse 2004) are dominated by clade C subtype C3h, which is rarely found on higher latitude reefs of the inner and outer shelf reefs of the GBR. Baker (1999 and Rodriguez-Lanetty et al. (2001) showed that *Plesiastrea versipora* in tropical and subtropical latitudes of the Great Barrier Reef were dominated by symbiont clade C, while those of lower latitudes were dominated by clade B. Such studies (Tbls. 1, 2) reveal that the environmental conditions experienced by corals can influence their symbiont community composition, and that these communities can show dynamic changes in response to the environment.

Oceanography of Madagascar: a study site

Madagascar is an ideal site for studying symbiont distribution over latitudinal gradients. Sometimes dubbed the "eighth continent", it spans over 13.5^{o} of latitude in the SW Indian Ocean, covers more than 587,000 km² and has ~3500 km of coastline (Wells et al. 1998). Sea surface temperature (SST) regimes vary significantly across this island continent (Tbl. 3, Fig. 1). Four sites were selected that presented the latitudinal range of coral reefs along the western and eastern coast, and the thermal regimes of these environments characterized using satellite data from 1982 to 2009 collected by NOAA's Coral Reef Watch program. Continuous reefs around the island are largely interrupted by

freshwater inputs from large rivers that restrict the growth of corals; consequently the four sites investigated include the main centers of reef formation as well as covering the latitudinal and environmental ranges experienced by Malagasy corals (Wells et al. 1998).

The island of Madagascar causes wind stress disruption affecting the South Equatorial Current, which, without the presence of the island, would otherwise flow to the east coast of Africa and then south along the eastern continental coast until meeting the cooler Antarctic and Agulhas currents (Penven et al. 2006, Lutjeharms 2006). Instead, the South Equatorial Current runs both south along the east coast of Madagascar and also feeds the Mozambique Current, which is primarily a series of warm, southern moving, anti-cyclonic eddies (de Ruijter 2002, Schouter 2003) between Madagascar and the east African coast. These eddies eventually feed into the strong, warm Agulhas current that follows the coast of South Africa, providing the tropical warm environment that has maintained South African coral communities at lower latitudes than those at which corals are typically found (Sebastian et al. 2009). The Agulhas current eventually splits east and west at the cape, mixing with the cooler Antarctic circumpolar current, creating a temperate environment at the southern end of Madagascar. The only northern current of the region is the Madagascar Current, an irregular current that is found on the west coast of Madagascar as a result of the South Equatorial Current curving around the southern cape or as a countercurrent to the Mozambique Current (Wells et al. 1998). Local current patterns inshore are further complicated by large and irregular expanses of reefs (Fig. 1).

Due to these primarily warm currents, the tropical coasts are characterized by diverse coral reefs, some of which have been studied, such as Tuléar and Nosy Bé in the SW and NW respectively (Wells et al. 1998, McClanahan 2009). Reefs in the Tuléar

region are distinguished by high SST variation and highest cumulative degree heating weeks (McClanahan 2009). Reefs in Tuléar have experienced a major decline in coral cover and coral taxa, and an increase in erect macroalgae cover due to overfishing and environmental stresses (McClanahan et al. 2009). In contrast, reefs in Nosy Bé have the lowest cumulative degree heating weeks, lower temperature variability and higher average SSTs than their southern counterpart s(Tbl. 3), as well as higher coral richness typical of low-disturbance environments (McClanahan et al. 2009).

The eastern reefs of Madagascar are less well studied than the western reefs, and are characterized by extensive fringing reefs and islets which have been suggested as being more likely to survive future climate change (Wells et al. 1998, McClanahan et al. 2009). The NE reefs of Vohemar have similar SST patterns to the Tuléar area with respect to high temperature variability. Fort Dauphin, in the SE corner of the island, is more temperate in nature due to the pulses Antarctic water it receives. This site and is characterized by coral communities, rather than the reefs found in other parts of the coast (Tbl. 3).

Coral symbiont richness and latitudinal gradients

Significant intracladal diversity exists within the 9 different clades of *Symbiodinium* (LaJeunesse 2001, 2005, Baker 2003, Coffroth and Santos 2005, Pochon & Gates 2010). Clade C is the most diverse, and contains the globally most common subtypes, C1 and C3 (LaJeunesse 2005). Different clades, as well as different subtypes within these clades, can impart different physiological properties to their hosts (Little et al 2004, Warner et al. 2006, Stat et al. 2008). The common C1 subtype has been associated with faster coral growth through higher translocation of carbon to the coral

host, while clade D types have lower carbon translocation but higher stress tolerance (Cantin et al. 2008). Identifying symbionts to the subcladal level gives more insight to local variation in survivorship after stress events (Sampayo et al. 2008), and a locally abundant subtype may be better acclimatized to the local environment than other background types.

Symbiodinium community diversity may be influenced by the latitudinal gradient in coral diversity in Madagascar and may also reflect differences in environmental conditions. Reef corals in Fort Dauphin experience more temperate conditions due to the coldwater pulses they receive from Antarctic currents, but Tuléar regularly receives warmer water from the Mozambique current (Fig. 1) interspersed with seasonal and cyclone-induced cooler upwellings. Given the projected increase in warm water anomalies due to climate change Sheppard et al. 2003), both Fort Dauphin and Tuléar represent interesting study sites for symbiont community changes in response to environmental anomalies.

In the face of climate change and projected increases in sea surface temperatures, changes in symbiont communities at different latitudes may help understand how environmental parameters, particularly temperature and light, can influence the response of coral reefs to these global changes. Will symbiont communities of reef-building corals in temperate latitudes eventually change to resemble the symbiont communities of reef corals at lower latitudes? Furthermore, how will these cooler water reefs be impacted by thermal variability compared to more tropical locales?

Between the interactions of current systems, the large latitudinal distances involved and the projected increases in SST due to climate change (Sheppard 2003),

Malagasy reefs may offer insight into how *Symbiodinium* communities might be influenced by environmental gradients and variability. Symbiont communities at both the clade- and subtype-level are hypothesized to differ between the northern and southern regions of Madagascar as a reflection of the 13.5º differences in latitude between them.

Methods

Sample collections

Coral tissue samples were collected by A. C. Baker, T. P. Dunlop and F. Ratsifandriamanana in September 2001 from 4 sites around Madagascar: Nosy Bé (NW), Vohemar (NE), Tuléar (SW) and Fort Dauphin (SE). Samples were also taken in March 2007 and November 2009 by T. R. McClanahan from various sites in the Nosy Bé and Tuléar/Tuléar regions to assess the stability of the 2001 distributions over time. Coral samples, typically 1-3cm² in surface area, were collected using a hammer and hollow steel punch by scuba diving or snorkeling. Samples were preserved in 95% ethanol or saline DMSO (Seutin et al. 1991). Field sampling focused on the most common coral species – usually in the genera *Montipora*, *Acropora*, *Pavona*, *Pocillopora* and *Porites*, but other species were also sampled depending on abundance. Comparative analyses focused on corals in the families Acroporidae, Agariciidae, Pocilloporidae and Poritidae, so that results could be placed in the context of similar research in the greater Western Indian Ocean.

DNA extraction, amplification and analysis

From the initial coral sample, ~0.5 cm² fragments were lysed in 500µL DNAB containing 1% SDS for 30-60 minutes at 65°C (Rowan and Powers 1991) and digested *in*

situ with Proteinase-K at a final concentration of 10mg/mL for ~ 6h at 37°C. DNA was then extracted and purified from lysates following an organic extraction protocol (Baker et al. 1997). Extracted genomic DNA was suspended in TE and stored long-term at -80°C.

The internal transcribed spacer 2 (ITS-2) region of *Symbiodinium* ribosomal DNA (rDNA) was PCR-amplified for Deaturing Gradient Gel Electrophoresis (DGGE) analysis using the methods of LaJeunesse & Trench (2000), resulting in a 330-360 bp PCR product. The forward primer (5'-GAATTGCAGA ACTCCGTG-3') anneals to a conserved region of 5.8S rDNA, and the reverse primer (5'-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGGGATCCATA TGCTTAAGTTCAGCGGGT-3') is modified with a 39 bp GC clamp (underlined) (Sheffield et al. 1989). Using hotstart GoTaq (Promega), PCR amplifications were conducted using the following conditions: an initial denaturing step of 2 minutes at 92°C, followed by 35 cycles of 30 seconds at 92°C, 40 seconds at 55-58°C and 30 seconds at 72°C, and concluded with two cycles of 5 minutes at 72°C.

Reaction products were analyzed using DGGE, and band profiles representative of individual symbiont types were excised. Common DGGE profiles for the region were used as standards in later gels. Excised bands were used as templates for reamplification using primers as above (without the GC clamp). ITS-2 sequences (using BigDye Terminator Cycle Sequencing) were analyzed on a 16-capillary Applied Biosystems 3130xl Genetic Analyzer.

Sequencing analysis

Chromatograms were checked and edited using Geneious software 4.7.3, and default parameters of the Geneious Alignment option were used for all alignments (Drummond et al.2011). Initial cladal identifications were made using NCBI basic local alignment search tool (BLAST), and subsequent subtype identification were done by aligning sequences with established ITS2 sequences published in GenBank.

Community comparisons

The complete *Symbiodinium* dataset was tested for differences in symbiont richness and symbiont community structure at the 4 sampling sites. To compare richness at the different sites, *Symbiodinium* were identified to the lowest taxonomic level (ITS-2 type) and compared between sites. To test whether differences in the *Symbiodinium* richness between sites and years were influenced by the number of coral taxa sampled, rarefaction studies were undertaken using a richness estimator program EstimateS (Colwell 2009).

To examine community structure, *Symbiodinium* were grouped by clade and the following metrics calculated: 1) “Clade D dominance” where the frequency of colonies containing D-only was compared to the frequency of colonies containing C-only or C+D mixed communities: 2) “Clade D incidence”, where the frequency of colonies containing any D (either D-only or mixed C+D communities) was compared to the frequency of colonies containing C-only and 3) “mixed communities”, where the frequency of colonies containing C+D was compared to the frequency of colonies containing either C-only or D-only. Pairwise comparisons were done using Fisher’s exact test.

Chi-square tests of symbiont community structure were grouped by coral taxa commonly found on Malagasy reefs. The first group (an overall community comparison) consisted of the entire dataset (all samples collected from all four sites). The second group was limited to the commonly found genera *Acropora*, *Pocillopora*, *Porites* and *Pavona* as well as three genera in the family Pocilloporidae (*Pocillopora, Stylophora* and Seriatopora) to control for the effect of rarer species that were not present at all four sites.

Environmental methods

Sea surface temperature (SST) data for the NE, NW, SE and SW areas of Madagascar were acquired from the Advanced Very High Resolution Radiometer (AVHRR) sensor, which monitors daily global ocean temperatures. Interpretation of the fine scale (4km grid) satellite data was provided by Joseph Maina (Macquarie University, Sydney, Australia). Maximum, minimum and mean monthly SST, as well as standard deviation (SD) and standard error of the mean (SEM) were calculated from these data for the period 2000-2009. Degree heating weeks (DHWs) were also calculated for the period 1982-2009 as well as for specific sampling years (2001, 2007 and 2009).

The maximum monthly mean (MMM) was first found by calculating the mean monthly temperatures for each month during the baseline period (1990-2005) and then calculating the mean of the meaximum monthly mean values in each year. DHWs were then calculated in two different ways. The first DHW metric ("DHW") was calculated following standard NOAA methodology: whenever a weekly temperature exceeded the calculated mean weekly mean by >1 ^{o}C it contributed to the overall accumulated thermal stress (for example, a weekly temperature that was 1.5 ^{o}C above the MMM contributed 1.5DHWs, but a weekly temperature that 0.9 ^{o}C above the MMM contributed no DHWs).

A second DHW metric ("DHW-1") was also calculated which counted DHWs as soon as they exceeded the MMM, i.e. a 1 ^{o}C threshold was not required to accumulate thermal stress. The standard DHW metric only counts anomalies if they are fairly strong (>1 ^{o}C), whereas the DHW-1 metric allows weak anomalies to also contribute to the DHW metric. These weak anomalies can contribute significantly to the cumulative DHW calculation if they are sustained over several weeks.

In some cases excessive cloud cover prevented extraction of reliable weekly data. These weeks were coded as "missing data" and the temperature metrics above calculated from the remaining yearly data.

To test for an effect of temperature symbiont community structure, nominal logistic models were fit to each of the three symbiont community metrics (D dominance, D incidence and C+D mixed communities) with site, genus, year and temperature parameters (maximum SST, minimum SST, mean SST, SD, DHW and DHW-1) as predictive variables. All statistical tests were run using JMP statistical software from SAS software (Sall et al. 2001).

Limitations of methods

DGGE has limited power to resolve "background" symbionts because rare symbionts will not produce banding profiles that are detectable on denaturing gradient gels. In some cases, DGGE has failed to detect symbionts that may comprise 10-50% of the total symbiont community, depending on symbiont type and the type of molecular marker (LaJeunesse et al. 2008). For ITS-2 the detection range appears to vary from 5-20% depending on symbiont type (Thornhill et al. 2006, LaJeunesse et al. 2008). Due to these limitations, samples classified as C-only or D-only may actually contain significant

numbers of *Symbiodinium* belonging in other clades. Nevertheless, DGGE does allow accurate identification of dominant symbionts in a mixed community, and is a commonly accepted method for identifying symbionts in scleractinian corals and other hosts. Background or "cryptic" symbionts that are undetectable by conventional techniques are a topic of active research (Mieog et al. 2009, Correa et al. 2009, Silverstein et al. 2011).

Results

Symbiont richness in Malagasy corals

Rarefaction analysis indicated that *Symbiodinium* richness increased with sampling effort at both the clade- and subtype-level (Fig. 2). All had similar clade-level symbiont richness, except for Vohemar, which was the sole site where clade A was recorded (in one colony of *Acropora*). When this outlier was removed, the rarefaction analysis indicated that Vohemar closely resembles the other sites.

Overall, scleractinian corals of Madagascar were dominated by *Symbiodinium* in clades C and D with (rarely) some corals hosting detectable levels of *Symbiodinium* in clade A. Mixed assemblages (of C and D types) were relatively common. Corals at Nosy Bé were principally dominated by clade C-only (65% of colonies sampled), and by mixed C+D communities (29%). Very few colonies contained D-only (6%) and no colonies contained clade A. Corals at Tuléar and Vohemar were slightly more frequently dominated by C types (73% of colonies at both sites) and less commonly by C+D mixed communities (23% and 22%, respectively). D-only colonies were rare (4% of colonies at both sites) and A-type symbionts were only found at Vohemar (1% of colonies) (Fig. 3). Fort Dauphin was dominated by C-only (90% of colonies), and C+D mixed communities

were relatively rare (10%). However, the sample size at this site was relatively low (n=20).

At the subcladal level, 30 different subtypes were identified (Fig. 4). Most (26) were members of clade C, with multiple variants of C1 (8 types) and C3 (3 types). C15 was abundant in species of *Porites*, but other C subtypes varied in abundance depending on locale.

Figure 5 shows the six novel ITS-2 subtypes that were identified using DGGE (Genbank accession numbers: HQ232950, HQ232951, HQ232952, HQ232954, HQ232947, HQ232948). Each of these types varied by 2-3 bp from existing sequences. If a novel sequence was associated with another dominant band in the DGGE profile (such as C1), then it was defined as a subtype of the dominant type. Accordingly, two novel C1 subtypes were identified [C(HQ232950) and C(HQ232951)]. HQ232954 was specifically found in association with *Porites spp*, and is closely related to C15 as well as various other subtypes.

Figure 5 does not include all the symbiont subtypes that were identified in this study - a total of 30 symbiont subtypes were found on the reefs of Madagascar (Fig. 4). The most common types found were C1, C3 and C15 (included in Fig. 5), but C115 and D1/D1a were also very common. Other subtypes were more rare, isolated instances that were found in a specific coral host or region (Fig. 4).

Differences in symbionts communities between different sites

Symbiont community differences between sites

When considering all coral species, Tuléar, Vohemar and Nosy Bé had similar symbiont community structure: the majority of colonies are dominated by clade C (73%,

73% and 65%, respectively) (Fig. 3). Nosy Bé had 35.4% incidence of D, which is not significantly different (p=0.14, Chi-square test) from the other four sites (Tuléar 26.4%, Vohemar 25% and Fort Dauphin 10%). No significant difference in mixed colonies was found between the four sites for all coral taxa (Nosy Bé 41.3%, Tuléar 29.2%, Vohemar 28%, and Fort Dauphin 10%).

When considering the principal coral taxa only, there were significant differences in symbiont community structure between the four sites sampled in 2001. Nosy Bé had the highest incidence of D (36.7% compared to 11.5-12.5% for the other sites, p=0.049, Chi-square test) and the highest frequency of mixed communities at any of the four sites (36.7% compared to 7.3%-12.5% for the other sites, Fisher's exact test, p=0.007). There were no differences in symbiont community structure between the three other sites, although sampling size at Fort Dauphin was comparatively small.

Symbiont community differences between coral taxa

In addition to clade-level differences in overall symbiont community compositions of Malagasy reefs, there were considerable differences between cladal and subcladal symbiont communities between different coral taxa. In the coral family Pocilloporidae, there were strong significant differences between the symbiont communities of different coral taxa ($p<0.0001$, Fisher's exact test). *Seriatopora* and *Stylophora* were dominated by mixed assemblages of C+D while *Pocillopora* was almost solely associated with clade C (Fig. 6). In 2001 and 2007, *Acropora* species on Nosy Bé reefs associated predominantly with symbiont subtype C3z, while Tuléar reefs were co-dominated by C1 and C3 symbiont subtypes in 2001 ($p<0.0001$, Fisher's exact test) (Fig. 7). *Porites* species (Family Poritidae) associated specifically with symbiont type C15, a

commonly found association for this species throughout the Indo-Pacific region (Baker 2004, LaJeunesse 2010).

Temperature data

Mean temperatures at the four sites in Madagascar were very different, reflecting theie exposure to different current regimes (Tbl. 3, Fig. 1). The Nosy Bé area (NW Madagascar), perhaps due to its restricted patterns of current flow around the islands of this region, experiences higher mean and maximum temperatures than the other three sites, with a mean temperature of 28.5ºC (+/-1.4ºC SD) from 1981 to 2009. In contrast, Fort Dauphin (SE Madagascar) experiences regular exposure to cooler waters from the Antarctic currents, and had a mean temperature of 24.1ºC (+/-1.8ºC SD) during the same time period. Vohemar and Tuléar experienced intermediate mean temperatures of 26.6ºC (+/-1.9ºC SD) and 26.3ºC (+/-2.1ºC SD), respectively (Tbl. 3). In 2001, Tuléar and Vohemar experienced high temperature variability (SD= 2.4ºC and 1.9ºC, respectively), while Nosy Bé and Fort Dauphin had lower variability (SD= 1.4ºC and 2.1ºC, respectively).

Nominal logistic models

Coral taxon was the most powerful predictor of symbiont distribution, both for all coral taxa and for the four most commonly sampled taxa (Tbl. 4). Higher levels of taxonomic resolution increased the predictive power (R^2 values: 0.50-0.69 for species, 0.36-0.57 for genus, and 0.17-0.35 for family). Site and year were weak, but significant, predictors of symbiont distribution (R^2 values: 0.03-0.04 for site and 0.02 for year).

When considering the four most commonly sampled coral taxa, no single SST metric was significant in prediting symbiont distributions, except for standard deviation.

All temperature metrics showed consistent trends indicating that with increasing temperatures and decreasing standard deviation, there was a relative increase in clade D types, but these trends were not significant.

Discussion

Latitudinal gradients and environmental drivers

Madagascar's large size and the resulting differences in temperature and current patterns between the north and the south provide a model system for studying the effects of latitudinal gradients in coral-algal symbiosis. Nosy Bé is protected from direct currents by the main island of Madagascar to the east, as well as by the island archipelago in the area that interrupts current flow. This minimizes monthly temperature variability (SD=1.42ºC) and provides a protected environment that appears to promote rare coral species (Wells et al. 1998, McClanahan et al. 2009). Vohemar is directly exposed to the South Equatorial Current, creating a series of fringing reefs that experience greater temperature variability (SD=1.87ºC).

Despite its location near the latitudinal extreme for zooxanthellate corals, Tuléar experiences warm pulses from several currents, as well as cooler pulses from cyclones (Fig. 8). Seasonal fluctuations lead to high temperature variability (SD=2.06ºC). Coral reefs on the east coast of South Africa have survived under similar temperature regimes near the latitudinal extreme of reef formation (Sebastian et al. 2009). Consequently, these outlying reefs are now receiving scientific attention as to how they will respond to future climate change.

Fort Dauphin experiences significantly cooler water from the circumpolar Antarctic Current (SD=1.77ºC), resulting in coral communities, rather than extensive reef growth. Other coral communities at higher latitudes in the western Pacific (such as SW Australia) have symbiont distributions containing or dominated by clades A and B types (Silverstein et al. 2011). Clade A is also common to the higher latitude reefs of the northern hemisphere, such as in the Red Sea, the Arabian Gulf and the Gulf of Aqaba (Tbl. 4). On this basis, *Symbiodinium* clades A and B might have been expected at Fort Dauphin, since the area is at similar latitudes to reefs containing clades A and B elsewhere. However, corals at Ft. Dauphin were dominated by clades C and D, and the warm water pulses received at Ft. Dauphin may prevent other clades from becoming common. On the other hand, clades A and B have been recorded from Zanzibar in the equatorial western Indian Ocean (LaJeunesse et al. 2010), so it is possible that these symbionts are geographically restricted to particular areas of the Indian Ocean.

As shown by the nominal logistic models, the relative frequency of colonies containing clade D increases with temperature and decreasing temperature variability. High variability is associated with cooler seasonal temperatures, so it is perhaps not surprising that C types may be more optimal in these cooler instances. However, no single sea surface temperature metric appears to drive the distribution. Instead, coral taxonomy and site are more powerful predictors of symbiont distribution.

One reason for this is that satellite data are based on weekly data and lack the fine scale resolution that on-site temperature gauges can provide. Gauge data from sites near Tuléar suggest that on-site temperatures are consistently lower and slightly less variable than the satellite-derived data (Fig. 9, courtesy T.R. McClanahan). This may lead to an

overestimate of the accumulated heat stress (as a result of recording higher variability around a higher mean). The use of satellite data may therefore misrepresent the thermal environment on reefs and contribute to the lack of significance in the nominal logistics used here.

Coral host specificity and flexibility

High mean SSTs and low SST variability suggest that *Symbiodinium* in clade D should be relatively common on Nosy Bé reef systems (Iglesias-Prieto & Trench 1994, Baker 2001, Savage et al. 2002, Baker 2004, Baker et al.. 2004, Igelsias-Prieto et al. 2004, Little et al. 2004, Berkelmans & van Oppen 2006, Warner et al. 2006, Loram et al. 2007, Ulstrup et al. 2007, Sampayo et al. 2008, Stat et al. 2008, Cantin et al. 2009). In fact, although clade D is common on these reefs (shown by the high frequency of D incidence), clade D rarely dominates individual coral colonies (low D dominance). Many coral colonies in the region host mixed symbiont communities that contain members of both clades C and D. The mean maximum temperature may not be high enough to promote clade D only *Symbiodinium* communities.

Relatively low temperature variability in Nosy Bé might also predict fewer mixed symbiont communities compared to other sites with higher temperature variability, since fluctuation in temperature might prevent any one symbiont from becoming dominant. Yet Nosy Bé has a higher frequency of mixed communities than the other three sites with higher SST variability. While the mean maximum temperature may not be high enough to promote clade D monocultures, the maximum annual SST may still be high enough to promote the presence of clade D in many colonies. Nosy Bé may be a threshold site where there is a high incidence of clade D without a high amount of clade D dominance.

A potential confounding factor is the fact that more colonies of *Pavona, Seriatopora* and *Stylophora* were sampled in Nosy Bé in 2001 than in other sites. Since these genera commonly host mixed symbiont communities (Fig. 6), this may have influenced the frequency of mixed communities at this site. This hypothesis is supported by the fact that, when all coral samples are included, the incidence of D decreases (Fig. 3) and all sites have similar clade D frequencies and incidences (Fort Dauphin is only statistically different from Nosy Bé for clade D incidence).

This highlights the importance of considering differences in the relative specificity of different coral taxa in comparing the symbiont communities of different sites. One factor that may contribute to these differences is the life histories of different coral species and their method of symbiont transmission. Reef corals of the genus Pocilloporidae are generally brooding corals, which transmit symbionts to their larvae (except in the eastern Pacific where they are broadcast spawners). This may contribute to the relatively high incidence of clade D in *Seriatopora/Stylophora* compared to *Pocillopora* if symbionts can be more readily acquired by broadcasting species.

Differences in the relative frequency of *Symbiodinium* in clade D in *Pocillopora* and *Stylophora/Seriatopora* may also influence the survivorship of these coral taxa in response to thermal stress. Some members of *Symbiodinium* clade D (most notably D1 and D1a) are considered to be thermotolerant symbionts that are capable of maintaining a mutualistic relationship with the coral host in times of environmental stress (Baker 2003, Berkelmans & van Oppen 2006). The presence of members of this clade in Malagasy corals may indicate that *Stylophora* and *Seriatopora* will be more resilient to future climate change. Several other coral taxa, notable *Galaxea, Pavona* and various other

favid genera also show high incidence of clade D (Fig. 10), and these taxa may also rank highly in terms of their resilience to climate change.

Unusual symbionts in Madagascar and the greater western Indian Ocean

The northern coral reefs of Madagascar have a higher number of rare, "boutique" coral species that are not found in southern Malagasy sites. Similarly, a number of uncommon and locally abundant symbiont subtypes were found at the northern sites. Together, the northern sampling sites have 25 of the 30 total symbiont types found, while southern reefs have only 13 (Fig. 4).

For Nosy Bé, the presence of otherwise rare coral species may be due to the area's relatively low disturbance regime, since it is a comparatively protected and isolated coral reef system (Wells et al. 1998, McClanahan et al. 2009). In these cases, bleaching anomalies may be sufficiently rare that such maladaptive symbionts are not selected against and so remain in the host population. Or, such symbionts represent opportunistic subtypes that are found following bleaching events as the host symbiont community is being initially repopulated (Correa & Baker 2010).

Symbiont subtype C3z is locally abundant on northern reefs, but rare on southern reefs. Furthermore, species of *Acropora* in Nosy Bé are solely dominated by C3z while *Acropora* in Vohemar are dominated by C3. In contrast, *Acropora* in Tuléar are dominated by C1 and various C1 variants (Fig. 7). Since *Acropora* is not a brooding coral species, this symbiont is not transmitted maternally from the parent colony. Other corals of northern reefs also associate with C3z, but not as commonly as branching acroporid species. This indicates a longitudinal difference between sampling sites in the north of Madagascar, which may reflect differences in the local availability of symbionts.

Latitudinal differences in symbionts have been found in other regions, such as the GBR, where northern reefs are dominated by clade C and southern reefs by clade B (Rodriguez-Lanetty et al 2001). Similarly, at the subtype level, mid-latitude inshore reefs of the GBR are dominated by *Symbiodinium* subtype C3h, which is comparatively rare on higher latitude reefs (LaJeunesse et al. 2004).

One explanation for the latitudinal differences in the distribution of the C3z subtype is that it is a locally abundant on Nosy Bé reefs and comparatively rare on southern reefs and at Vohemar (NE). Subtype C3z has been found in *Acropora* and some faviids in Mozambique as well as in Tanzania and Thailand (LaJeunesse et al. 2010). Subtype C3 may become more prevalent in southern reefs as they experience future warming.

In addition to widespread symbionts, there are also potentially endemic symbionts in isolated areas. Clade D subtype D16 is unique to *Montipora circumvallata* species in Reunion Island (Guillaume et al. in prep), while Madagascar has multiple symbiont types not yet identified from other reefs. C(HQ232948) and C(HQ232952) are found in various faviids, while C(HQ232954) is only found in *Porites.* Both are >1bp different from other symbiont types identified to date, but require further phylogenetic analysis to be placed in relation to other established symbiont types. Various faviid species contain C(HQ232950) and C(HQ232951), which are both 1 bp different from C1, as well as C(HQ232947) which is 1 bp different from C15. Of these six symbiont subtypes, only C(HQ232948) is found in southern Malgasy reefs – the other five being found only on the northern reefs of Vohemar and Nosy Bé.

The future of Malagasy reefs

The symbiont communities of the northern and southern sites of Madagascar are superficially similar, but closer inspection reveals numerous differences in symbiont community composition and in subtype diversity, even when controlling for the effects of different coral taxa. These differences are likely due to the unique hydrodynamic and thermal characteristics of each site.

The different regions of Madagascar are already experiencing yearly increases in temperatures of ~0.016ºC per year (SW region, including Tuléar) and ~0.006ºC per year (NW region, including Nosy Bé) (McClanahan 2009). In the face of climate change, northern reefs may experience more frequent and severe episodes of thermal stress that exceed the current adaptive/acclimatization potential of corals in these areas. Temperature regimes on southern reefs, on the other hand, may resemble those currently experienced by northern reefs. The cyclones that affected the SE area of Madagascar also caused upwelling that may have mitigated what might otherwise have been a period of anomalous heating (Fig. 8). The regular occurrence of cyclones in these areas may play an important role in alleviating thermal stress, as a result of upwelling cooler water on reefs over an area of several hundred km (Eakin et al. 2010). Vohemar, however, which is also characterized by low cumulative degree heating weeks and moderate temperatures and temperature variation, may have the highest potential for resilience.

Climatological effects may cause southern Malagasy reefs to resemble the existing symbiont and coral communities and diversity of the northern reefs. Two dominant reef-building corals in shallow Caribbean waters, *Acropora cervicornis* and *A. palmata,* diminished their latitudinal range during the last climatic cooling event, only to

re-expand into these areas as climate warmed again (Precht & Aronson 2004). Symbiont communities on high-latitude reefs, such as those off the east coast of South Africa (Sebastian et al. 2009) may act as potential "transition zones" (MacDonald et al. 2008), and offer key insight to how symbiont communities along other latitudinal gradients (such as the Great Barrier Reef) may change with time (Baker 1999, Rodriguez-Lanettety et al. 2001, LaJeunesse et al. 2004, Berkelmans & van Oppen 2006, LaJeunesse et al. 2010).

Chapter Two:
The effect of thermal stress on algal symbiont communities (*Symbiodinium* spp.) of reef corals in Madagascar

---

Summary

Reef sites in Madagascar experienced episodes of high temperature stress in 2001-2009, but there was significant variability between sites. The Nosy Bé region (NW), which is comparatively remote and protected from outside currents and oceanic effects, saw fewer thermal anomalies while the Tuléar region (SW), which is exposed to strong oceanic influences and is more subject to secondary human impacts, such as fishing, experienced higher themal stress. I tested the hypothesis that thermal stress resulted in changes in the algal symbiont communities (*Symbiodinium* spp.) of reef corals at affected sites. Specifically, I tested whether sites that experienced thermal stress saw an increase in *Symbiodinium* in clade D at the expense of *Symbiodinium* in clade C. A total of 456 samples in 27 genera (12 families) of scleractinian coral were collected from Nosy Bé (NE) and Tuléar (Toliara, SW) in September 2001, March 2007 and November 2009. *Symbiodinium* DNA was extracted, purified and amplified using primers specific to the internal transcribed spacer-2 (ITS-2) region of ribosomal DNA and amplified products analyzed using Denaturing Gradient Gel Electrophoresis (DGGE). Individual *Symbiodinium* types were identified by sequencing excised bands from DGGE gels. The relative frequency of coral colonies containing *Symbiodinium* in clade D increased significantly in Tuléar between 2001 and 2009, while it decreased in Nosy Bé over the same time period. These changes occurred as a result of increases or decreases in the relative frequency of mixed communities of clade C+D, with reciprocal changes in the

frequency of colonies containing C-only. The incidence and dominance of clade D was highly dynamic at both sites, but changes were not directly correlated with sea surface temperature metrics derived from satellite data.

Background

The success of tropical coral reefs is based on the mutualistic association of stony corals (Order: Scleractinia) and members of the dinoflagellate algal genus *Symbiodinium*. The genus *Symbiodinium* comprises at least nine clades (A-I) (Pochon & Gates 2010), six of which (A-D, F & G) associate with stony corals found on tropical coral reefs (Rodriguez-Lanetty et al. 2001, LaJeunesse et al. 2010). These "zooxanthellae" can impart different physiological capabilities to their coral hosts (Iglesias-Prieto & Trench 1994, Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2001, Savage et al. 2002, Baker et al. 2004, Igelsias-Prieto et al. 2004, Little et al. 2004, Loram et al. 2007, Ulstrup et al. 2007, Warner et al. 2006, Stat et al. 2008, Cantin et al. 2009), and potentially affect a coral's capacity to acclimatize or adapt to environmental changes (Buddemeier & Fautin 1993, Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2004, Baker et al. 2004, Berkelmans & van Oppen 2006).

A variety of environmental stressors can disrupt coral-algal symbioses and threaten coral reefs, with rising sea surface temperatures due to climate change being the primary concern (Glynn 1993, Hoegh-Guldberg 1999, Hughes et al. 2003, Hoegh-Guldberg et al. 2007). Protracted increases in temperature result in physiological stress to corals, resulting in breakdown in coral-algal symbiosis, damage to coral hosts from the release of reactive oxygen species (ROS), expulsion of algal symbionts and loss of

autotrophic contributions to host metabolism (Asada 1996, Baker et al. 2008, Venn et al. 2008).

Without the presence of symbiotic algae, the white coral skeleton is visible through the translucent coral tissue, a condition termed "coral bleaching" (Glynn 1993). While bleached corals can still feed heterotrophically on particulate matter in the water (Grottoli et al. 2006), bleaching can reduce skeletal growth, causing an overall loss of colony health, an increase the incidence of disease, and affecting long-term reproductive output (Glynn et al. 1985, Porter et al. 1989, Szmant & Gassman 1990, Mendes & Woodley 2002, Baker et al. 2008). Severely bleached corals typically experience partial or complete mortality within days to weeks unless algal symbiont communities can recover (Glynn & D'Croz 1990, Berkelmans & Willis 1999), although survival time is variable and dependent on coral species (McClanahan et al. 2001).

Coral susceptibility to bleaching is highly variable between species and locales (Coles and Brown 2003), with reefs in different environmental regimes (e.g., deep reef slopes vs. shallow reef flats) and geographic locations (e.g., warm equatorial vs. cool temperate reefs) having different bleaching thresholds due to long-term adaptation and acclimatization to the local environmental conditions (Coles and Jokiel 1978, Shick et al. 1996, Hughes et al. 2003). Bleaching thresholds are dependent on exposure time of the coral host to stress, as well as the average environmental conditions to which the coral host is adapted or acclimatized (Berkelmans & Willis 1999, Hughes et al. 2003). For example, mean summer sea surface temperatures (SSTs) for reefs in the Caribbean or Great Barrier Reef are typically 29˚C, with a bleaching threshold of 30-33˚C, depending on the species. However, reefs in Abu Dhabi are accustomed to summer SSTs >33˚C,

with some individual colonies surviving to 40˚C (McClanahan et al. 2007, Baker et al. 2008).

Bleaching thresholds of individual coral hosts may also be affected by functional differences between different *Symbiodinium* (Little et al. 2004, Berkelmans & van Oppen 2006, Jones et al. 2008). Some clade C types have been found to optimize juvenile coral health and growth by incorporating higher amounts of photosynthetic carbon than conspecific corals that host clade D types, resulting in slower juvenile coral growth and accretion (Little et al. 2004, Cantin et al. 2009). However, associations with clade D may result in corals that are more resistant to environmental stresses, such as thermal anomalies (Rowan 2004) resulting in a tradeoff between growth rate and stress resistance. Prior to the 1997-1998 El Niño event, healthy corals in Panama associated mainly with clade C types, with clade D being less common. During the bleaching event, healthy corals were dominated by clade D types, while bleached corals contained residual populations of clade C. After the bleaching, surviving colonies more frequently hosted clade D than before bleaching (Baker et al. 2004), supporting the notion that clade D is a thermally tolerant symbiont type that can increase in abundance during times of thermal stress.

Studying how reef corals at different latitudes respond to thermal stress and coral bleaching may shed light on how these symbioses will adapt or acclimatize to climate change and other environmental disturbances (Savage et al. 2002, Wicks et al. 2010, Silverstein et al. 2011). For example, higher latitude reef corals may acclimatize to anomalous high temperature events by changing their symbiont communities to resemble those found at lower latitudes. This can be tested directly by substituting space for time to

see whether the symbiont communities of reefs at high latitudes more closely resemble those of low latitude reefs after bleaching compared to before bleaching.

Madagascar's reefs had experienced varying levels of prior thermal stress by the time of initial sampling in 2001. Reports suggest the thermal stress events in Tuléar (SW Madagascar) were more severe than in the Nosy Bé region in 2001-2002 and 2005 (Ahamada et al. 2008, ReefBase), which provides an opportunity to compare how thermal stress may have affected symbiont communities in these two areas. It is hypothesized that symbiont richness at the subclade level and community structure at the clade level will change between sampling intervals. Specifically, Nosy Bé may have experienced less pronounced changes in symbiont community structure compared to Tuléar as a result of differences in the thermal stress to which they were exposed. Given the subtropical-temperate nature of the reefs in southern Madagascar, changes in symbiont communities as a result of coral bleaching may provide insight as to how climate change will ultimately affect the acclimatization potential of reef corals.

Methods

Sample collections

Samples were collected in September 2001 from Nosy Bé (NW Madagascar) and Tuléar/Toliara (SW Madagascar) by A. C. Baker, T. P. Dunlop and F. Ratsifandriamanana. Additional collections were made in March 2007 (Nosy Bé and Tuléar) and November 2009 (Tuléar only) by T. R. McClanahan.. Coral samples, typically 1-3cm² in total surface area, were collected using a hammer and hollow steel punch by scuba diving or snorkeling. Samples were preserved in 95% ethanol or saline DMSO (Seutin et al. 1991).

Field sampling focused on the most common coral species – usually in the genera *Montipora*, *Acropora*, *Pavona*, *Pocillopora*, and *Porites* but other species were also sampled depending on abundance. Comparative analyses focused on corals in the families Acroporidae, Agariciidae, Pocilloporidae and Poritidae, so that results could be placed in the context of similar research in the greater Western Indian Ocean.

DNA extraction, amplification and analysis

From the initial coral sample, 0.5 cm^2 fragments were lysed in 1% SDS in DNAB and digested *in situ* with Proteinase-K at 10mg/mL. After following established organic extraction protocols (Baker & Rowan 1997), extracted genomic DNA was suspended in TE and stored at -80°C.

The internal transcribed spacer 2 (ITS-2) region of *Symbiodinium* ribosomal DNA (rDNA) was PCR-amplified for DGGE analysis using the methods of LaJeunesse & Trench (2000), resulting in a 330-360 bp PCR product that encompassed the ITS-2 region. The forward primer (5'-GAATTGCAGA ACTCCGTG-3') anneals to a conserved region of 5.8S rDNA, and the reverse primer (5'-CGCCCGCCGCGCCCCGCGCC CGTCCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAGTTCAGCGGGT-3') is modified with a 39 bp GC clamp (underlined) (Sheffield et al. 1989). Using hotstart GoTaq (Promega), amplifications using the polymerase chain reaction (PCR) were conducted under the following conditions: an initial denaturing step of 2 minutes at 92°C, followed by 35 cycles of 30 seconds at 92°C, 40 seconds at 55-58°C and 30 seconds at 72°C, and concluded with two cycles of 5 minutes at 72°C.

Reaction products were analysed using Denaturing Gradient Gel Electrophoresis (DGGE), and band profiles representative of individual symbiont types were excised. Common DGGE profiles were used a standards for later gels. The excised bands were used as templates for reamplification using primers as above, without the GC clamp. ITS-2 sequences (using BigDye Terminator Cycle Sequencing) were analyzed on a 16-capillary Applied Biosystems 3130xl Genetic Analyzer.

Sequencing analysis

Chromatograms were checked and edited using Geneious software 4.7.3, and default parameters of the Geneious Alignment option were used for all alignments (Drummond et al. 2011). Initial cladal identifications were made using NCBI's basic local alignment search tool (BLAST), and subsequent subtype identification were undertaken by aligning sequences with established ITS-2 sequences published in GenBank.

Community comparisons

Symbiont distributions were tested for differences in (1) symbiont richness and (2) symbiont community structure between the two sampling intervals at each site. To compare richness between Nosy Bé and Tuléar between the two sampling years, *Symbiodinium* were identified to the lowest taxonomic level (ITS-2 type). To examine community structure, *Symbiodinium* identifications were pooled by clade and the following metrics calculated: 1) "Dominance by D", where colonies containing D-only were compared to those containing C-only or C+D mixed communities; 2) "Incidence of D", where colonies containing any D (D-only or mixed C+D communities) were compared to the frequency of C-only colonies; and 3) "Mixed communities", where the

frequency of C+D mixed symbiont communities were compared to the frequency of colonies containing C-only and D-only.

The samples collected from Tulear in 2007 and 2009 were pooled to increase sample size. While initially the datasets were significantly different in the incidence of clade D ($p>0.05$, Fisher's exact test), larger amounts of *Porites* species were sampled in 2009 compared to 2007. When the datasets were considered without this genus, there were no significant difference between the 2007 and 2009 collections. There were no significant differences between the two sampling years in terms of the dominance of D.

Pairwise comparisons were used to test for differences among symbiont communities. The first group (an overall community comparison) consisted of the entire dataset (all samples collected from both sites). The second group was limited to the genera *Acropora*, *Pocillopora*, *Porites* and *Pavona* to control for the effect of rare species that were not present at both sites. Pairwise comparisons were done using chi squared tests or Fisher's exact test.

Environmental methods

Sea surface temperature (SST) data for the NE, NW, SE and SW areas of Madagascar were acquired from the Advanced Very High Resolution Radiometer (AVHRR) sensor, which monitors daily global ocean temperatures. Interpretation of the fine scale (4km grid) satellite data was provided by Joseph Maina (Macquarie University, Sydney, Australia). Maximum, minimum and mean monthly SST, as well as standard deviation (SD) and standard error of the mean (SEM) were calculated from these data for the period 2000-2009. Degree heating weeks (DHWs) were also calculated for the period 1982-2009 as well as for specific sampling years (2001, 2007 and 2009).

The maximum monthly mean (MMM) was first found by calculating the mean monthly temperatures for the each month during the baseline period (1990-2005) and then calculating the mean of the maximum monthly mean values in each year. DHWs were then calculated in two different ways. The first DHW metric ("DHW") was calculated following standard NOAA methodology: whenever a weekly temperature exceeded the calculated mean weekly mean by >1 ^{o}C it contributed to the overall accumulated thermal stress (for example, a weekly temperature that was 1.5 ^{o}C above the MMM contributed 1.5 DHWs, but a weekly temperature that was 0.9 ^{o}C above the MMM contributed no DHWs). A second DHW metric ("DHW-1") was also calculated which counted DHWs as soon as they exceeded the MMM, i.e. a 1 ^{o}C threshold was not required to accumulate thermal stress. The standard DHW metric only counts anomalies if they are fairly strong (>1 ^{o}C), whereas the DHW-1 metric allows weak anomalies to also contribute to the DHW metric. These weak anomalies can contribute significantly to the cumulative DHW calculation if they are sustained over several weeks.

Due to the fine temporal scale of the data, excessive cloud cover sometimes prevented the extraction of monthly data. These cases were coded as "missing data" and the temperature metrics above calculated from the remaining yearly data.

To analyze the environmental effect of SST on symbiont community distributions, a nominal logistic model was fit to each of the three symbiont community metrics (D dominance, D incidence and C+D mixed communities) with Site, Genus, Year and temperature parameters (above) included as variables in the model. All statistical tests were run using JMP statistical software from SAS software.

Limitations of methods

DGGE has limited power to resolve "background" symbionts because rare symbionts will not produce banding profiles that are detectable on denaturing gradient gels. In some cases, DGGE has failed to detect symbionts that may comprise 10-50% of the total symbiont community, depending on symbiont type and the molecular marker used (LaJeunesse et al. 2008). For ITS-2 the detection range appears to vary from 5-20% depending on symbiont type (Thornhill et al. 2006, LaJeunesse et al. 2008). Due to these limitations, samples classified as C-only or D-only may actually contain significant numbers of *Symbiodinium* belonging in other clades. Nevertheless, DGGE does allow accurate identification of the dominant symbionts in a mixed community, and is a commonly accepted method for identifying symbionts in scleractinian corals and other hosts. Background or "cryptic" symbionts that are undetectable by conventional techniques are a topic of active research (Mieog et al. 2009, Correa et al. 2009, Silverstein et al. 2011).

Results

Symbiont communities of Nosy Bé and Tuléar

When all samples were included in the analysis, coral colonies in Nosy Bé in 2001 contained C-only (65%), D-only (6%), or mixtures of C+D (29%). By 2007, the relative frequency of these symbionts had not changed significantly (62%, 25% and 13%, respectively). Corals in Tuléar in 2001 contained similar algal communities to those in Nosy Bé, with C-only (73%), D-only (4%) and mixed C+D (23%). By 2007, these communities had also not changed (62%, 29% and 9%, respectively).

However, when only the four most common coral taxa (*Acropora, Porites, Pavona,* and species in the family Pocilloporidae) were included in the analysis, Nosy Bé and Tuléar both experienced significant changes in their symbiont compositions from 2001-2007. Nosy Bé experienced a significant increase (Fisher's exact P=0.0057) in the D-only colonies at the expense of the mixed C-D colonies, while Tuléar experienced a significant increase (p=0.0059) in mixed C+D colonies at the expense of the C-only colonies (Fig. 11).

Changes in symbiont richness

Rarefaction analysis indicated that *Symbiodinium* richness increased with sampling effort at both the clade- and subtype-level (Fig. 2). However, there were no significant differences in richness at either taxonomic level between the two sites or between the sampling intervals.

Temperature data and nominal logistic models

Nominal logistic models revealed that all SST metrics (maximum SST, minimum SST, mean SST, SD or DHW) predicted an increase in clade D with increasing temperature and decreasing variability, though none were statistically significant. When using the less conservative DHW-1 metric (where any temperature that exceeds the maximum weekly mean contributes to a cumulative DHW score) Nosy Bé sometimes experienced anomalously higher mean temperatures for longer periods of time (e.g., 2007 had a DHW-1 of 3.03, compared to a DHW of 0.41 in 2007). In contrast, Tuléar experiences pulses of anomalous temperatures that were strong but short-lived (DHW-1=6.7, DHW=2.4 in 2007) (Figs. 12a, b). This may be due to the restricted current

patterns in the Nosy Bé area, while Tuléar was much more exposed to both the warm Mozambique current and the cooler southern currents..

Coral taxon was the most powerful predictor of symbiont distribution, both for all coral taxa and for the four most commonly sampled taxa (Tbl. 4). Higher levels of taxonomic resolution increased the predictive power (R^2 values: 0.50-0.69 for species,0.36-0.57 for genus, and 0.17-0.35 for family). Site and year were weak predictors of symbiont distribution (R^2 values: 0.03-0.04 for site and 0.02 for year).

Discussion

Changes in symbiont communities following bleaching

The most parsimonious explanation for the changes in Tulear and Nosy Be is that corals in Tuléar containing C-only in 2001 were replaced with colonies containing C+D mixed communities by 2007/09, with the frequency of D-only remaining constant. In contrast, colonies in Nosy Bé that contained C+D communities were replaced with colonies that contained D-only, with the frequency of colonies containing C-only remaining constant. In both cases, therefore, changes in symbiont communities favored *Symbiodinium* in clade D, but in neither site did it appear to involve shifts from C-only to D-only (Fig. 13).

The increase in C+D mixed assemblages in Tuléar may be a result of thermal stress events that occurred in the area prior to sampling in 2001 and thermal stress that occurred between sampling periods (Atewerbehan & McClanahan 2010). A higher incidence of mixed C+D colonies suggests that thermal stress favored an increase in the abundance of clade D symbionts in colonies that formerly were C-only. Given that

DGGE analysis only detects clade D symbionts when they represent at least 10-20% of the total symbiont community (LaJeunesse et al. 2009), it is possible that many of the C-only colonies actually contained low levels of clade D in 2001. These clade D symbionts may have increased in abundance following thermal stress and surpassed threshold levels for their detection by DGGE. On the other hand the changes in symbiont communities may have been the result of differential mortality of colonies containing C-only, and increased survivorship of colonies containing C+D. If this were the case, however, it would be expected that colonies containing D-only would also be favored. Consequently these data suggest that the symbiont community changes documented here are the result of "shuffling" symbiont communities within colonies (Baker 2003), rather than differential mortality of colonies.

In contrast, corals in Nosy Bé exhibited a decrease in mixed C+D communities ($p=0.0083$, Fisher's exact test) for an increase in D-only colonies C-only colonies. This difference in mixed communities may have been a result of the earlier 1998 bleaching in the region, or may have been due to more recent thermal stress. In fact, the mean temperature in Nosy Bé in 2001 was 28.8ºC, slightly higher than the 1981-2008 mean of 28.4 ºC. While not significant, this small temperature anomaly in an otherwise stable environment may have potentially affected the incidence of clade D in the area.

The mixed assemblages in Nosy Bé became C-dominant and D-dominant symbiont communities, although some mixed C+D assemblages did remain in 2007. Since mean environmental temperatures in Nosy Bé remained relatively stable from 2001-2007, the change to C or D-only communities may be a result of a lack of environmental pressure promoting natural selection of optimal symbiont associations.

Overlaid DHW data (using all cumulative, consecutive anomalies above the maximum weekly mean) support these hypotheses for the changes in symbiont communities from 2001-2009. Figure 14a shows that after prolonged anomalous temperatures in Nosy Bé, the frequency of D-only associations increases significantly in proportion to decreasing mixed and clade C-only associations. The same change may have occurred shortly after the 2001 DHW increase, but sampling at that time would have been necessary to show this. In Tuléar the opposite pattern occurs: mixed assemblages increase in response to intermittent anomalous temperatures (Fig. 14b).

Environmental drivers

The southern region of Madagascar has historically experienced higher cumulative DHWs (Fig. 12) and higher temperature variation than the northern region. However, there are few bleaching observations or reports, with the exception of 2001 and 2002. The northern regions also experience occasional high DHWs, however none exceed the bleaching threshold defined by the 1982-2009 baseline period. When modified to be less conservative (DHW-1), accumulated thermal stress on Nosy Be reefs increases in comparison to the other three sites, with the largest since anomaly occurring in 2002 in Nosy Be (exceeding the three years of DHW stress at Tuléar from 1999-2001).

With projected increases in climate change, the different regions of Madagascar are already experiencing annual increases in temperatures of ~0.016ºC per year (SW region, including Tuléar) and ~0.006ºC per year (NW region, including Nosy Bé) (McClanahan 2009). The SW is characterized by a complex set of environmental conditions as a result of the hydrodynamic regime in the area, and regular pulses of both cool and warm water may increase future reef resilience as a result of acclimatization to

extremes. However, overfishing heavily impacts Tuléar, and is believed to reduce resilience (McClanahan 2009). In fact, Nosy Bé may be more negatively impacted by future warm water anomalies, as it may receive more sustained anomalous temperatures with little likelihood of alleviation (compared to Tuléar with pulses of warming and cooling). Vohemar, however, which is also characterized by low cumulative DHWs and moderate temperatures and temperature variation, may have the highest potential for resilience.

Although the frequency of clade D dominated corals in Madagascar did not change overall from 2001-2007, closer analysis reveals that, in fact, there were significant changes occurring within sites that cancelled each other out. While Nosy Bé experienced increases in D-only at the expense of C+D, Tuléar experienced significant increases in C+D at the expense of C-only. The overall incidence of D, driven by an increase in mixed symbiont assemblages, increased significantly in SW coral reefs, while clade D dominance increased in NW reefs. The increased incidence of clade D in mixed assemblages in Tuléar may be a temporary, transitional post-bleaching event as the coral colony recovers and returns to its pre-bleaching symbiont community structure. This increase of the incidence or dominance of clade D at either site may provide the coral colony with higher resistance to future thermal stress that would otherwise result in bleaching (Baker et al. 2004).

However, increasing stress without sufficient recovery time for symbiont communities to show compensatory responses may result in initially maladaptive host-symbiont relationships, in which recovering corals establish an unusual association that

makes them more "stress prone" than corals containing other symbiont communities, impeding recovery (Jones et al. 2008, Correa et al. 2009).

Future of Malagasy reefs

Given the relatively strong ability to predict for symbiont communities when the identity of the coral host is known (Tbl. 4), it may be possible to predict the response of certain coral taxa to future thermal stress. Coral species that associate most commonly with clade D and may be the most resilient to thermal stress, and include the genera *Seriatopora*, *Stylophora* and *Pavona*, as well as various species of faviids (Fig. 9). Species of *Acropora* associating with *Symbiodinium* subtype C3z may also be resilient to future thermal stress, given their higher frequency at warmer sites.

With climate change forecasts suggesting an increased frequency and severity of thermal anomalies both regionally (Sheppard et al. 2003, McClanahan et al. 2009) and globally (Hoegh-Guldberg et al. 2007), coral algal-symbioses on coral reefs in Madagascar may have some capacity to adapt or acclimatize to these stresses by further shuffling their symbiont communities in favor of *Symbiodinium* in clade D. Once corals become dominated with clade D, however, further increases in thermal tolerance may be unlikely, and corals may also suffer from trade-offs that limit their success in other areas, such as growth (Little et al. 2004). Consequently, tropical reefs that already have high dominance of *Symbiodinium* clade D may be less flexible with regards to future climate change compared to higher latitude reefs where D is uncommon to begin with (Fig 13). Continued monitoring of symbiont communities in these, and other areas, is necessary to understand what the limits to symbiont community response are, and what the tradeoffs of these community changes might be.

Literature Cited

Ateweberhan M & McClanahan TR (2010) Relationship between historical sea-surface temperature variability and climate change-induced coral mortality in the western Indian Ocean. Marine Pollution Bulletin 60:964-970.

Baker AC (1999 The symbiosis ecology of reef-building corals. Ph.D. dissertation. University of Miami, Coral Gables, FL. 120pp.

Baker AC (2001) 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.* Annu. Rev. Ecol. Evol. Syst. 34:661-689.

Baker AC, Starger CJ, McClanahan T, and Glynn P (2004) Coral Reefs: Corals' adaptive response to climate change. Nature 430:741

Baker AC & Romanski AM (2007) Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: Comment on Goulet (2006). Marine Ecology Progress Series 335:237-242.

Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science 80: 435-471.

Berkelmans R & Willis BL (1999) Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs 18:219-228.

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 R Soc Lond B Biol Sci, 273:2305–2312

Buddemeier RW & Fautin DG (1993) Coral bleaching as an adaptive mechanism. American Institute of Biological Sciences 43(5):320-326.

Cantin NE, van Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. Coral Reefs 28:405-414.

Coles SL & Brown BE (2003) Coral bleaching – Capacity from acclimatization and adaptation. Advances in Marine Biology 46:183-223.

Coles SL & Jokiel PL (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Marine Biology 49:187-195.

Colwell, R. K. 2009. EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.2. User's Guide and application published at: http://purl.oclc.org/estimates.

Correa AMS & Baker AC (2009) Understanding diversity in coral-algal symbiosis: a cluster-based approach to interpreting fine-scale genetic variation in the genus *Symbiodinium*. Coral Reefs 28:81-93.

Correa AMS & Baker AC (2010). Disaster taxa in microbially mediated metazoans:how endosymbionts and environmental catastrophes influence the adaptive capacity of reef corals. Global Change Biology

Drummond AJ, Kearse M, Heled J, Moir R, Thierer T, Ashton B, Wilson A, Stones-Havas S (2006) Geneious v2.5, Available from http://www.geneious.com/

Fautin DG & Buddemeier RW (2004) Adaptive bleaching: a general phenomenon. Hydrobiologia 530/531:459-467

Glynn PW, Perez M, Gilchrist SL (1985) Lipid decline in stressed corals and their crustacean symbionts. Biological Bulletin 168:276-284.

Glynn PW & D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Niño-coincident coral mortality.

Glynn PW (1993) Coral reef bleaching: ecological perspectives. *Coral Reefs* 12: 1-17.

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186-1189.

Guillaume MMM, Boonstra RK, Guerin FJ, Denis V, Bruggemann JH, Baker AC (2010) New *Symbiodinium D*-type among dominance of D-types in *Montipora circumvallata*, a resilient coral species on a high-energy flat at Réunion Island. In press

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Marine & Freshwater Research 50(8):839-866.

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, Hatziolos ME (2007) Coral Reefs under rapid climate change and ocean acidification. Science 318:1737

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Heogh-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.

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. Royal Society Publishing 271(1549):1757-1763.

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc R Soc B 275:1359-1365

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. Journal of Phycology 37(5):866-880.

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Marine Biology 141:387-400.

LaJeunesse TC, Loh WKW, van Woesik R, Heogh-Guldberg O, Schmidt GW, Fitt WK (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnological Oceanography 48(5): 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

LaJeunesse TC, Bhagooli R, Hidaka M, deVantier L, Done T, Schmidt GW, Fitt WK, Hoegh-Guldberg O (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Marine Ecology Progress Series 284: 147-161.

LaJeunesse TC, Bonilla HR, Warner ME, Wills M, Schmidt GW, Fitt WK (2008) Specificity and stability in high latitude eastern Pacific coral-algal symbioses. Limnological Oceanography 53(2): 719-727.

Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304: 1492-1494.

Macdonald AHH, Sampayo EM, Ridgway T, Schleyer MH (2008) Latitudinal symbiont zonation in *Stylophora pistillata* from southeast Asia. Marine Biology 154:209-217.

McClanahan TR, Ateweberhan M, Muhando C, Maina J, Mohammed SM (2007) Effects of climate and seawater temperature variation on coral bleaching and mortality. Ecological Monographs 77(4):503-525.

McClanahan TR, Ateweberhan M, Graham NAH, Wilson SK, Sebastian CR, Guillaume MMM, Bruggemann JH (2007) Western Indian Ocean coral communities: bleaching responses and susceptibility to extinction. Marine Ecology Progress Series 337:1-13.

McClanahan TR, Ateweberhan M, Omukoto J, Pearson L (2009) Recent seawater temperature histories, status, and predictions for Madagascar's coral reefs. Marine Ecology Progress Series 380:117-128.

McClanahan, T. R., N. A. Muthiga, and S. Mangi. 2001. Coral and algal changes after the 1998 coral bleaching: Interaction with reef management and herbivores on Kenyan reefs. Coral Reefs 19:380-391.

Mendes JM & Woodley JD (2002). Effect of the 1995-1996 bleaching event on polyp tissue depth, growth, reproduction and skeletal band formation in *Montastrea annularis*. Marine Ecology Progress Series 235:93-102.

Penven P, Lutjeharms JRE, Florenchie P (2006) Madagascar: A pacemaker for the Agulhas Current system. Geophysical Research Letters 33:L17609

Pochon X & Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. Molecular Phylogenetics and Evolution 56:492-497.

Porter JW, Fitt WK, Spero HJ, Rogers CS, White MW (1989) Bleaching in reef corals: Physiological and stable isotopic responses. Proceedings of the National Academy of Sciences of the United States of America 86(23):9342-9346.

Rodriguez-Lanetty M, Loh W, Carter D, Hoegh-Guldberg O (2001) Latitudinal variability in symbiont specificity within the widespread scleractinian coral *Plesiastrea versipora*. Marine Biology 138:1175-1181.

Rodriguez-Lanetty M & Hoegh-Guldberg O (2003) Symbiont diversity within the widespread scleractinian coral *Plesiastrea versipora*, across the northwestern Pacific. Marine Biology 143:501-509.

Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. Journal of Phycology 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. Proceedings of the National Academy of Sciences of the United States of America 92(7):2850-2853.

Rowan R, Knowlton N, Baker AC & Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature 388:265–269

Rowan R & Powers DA (1991) Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Marine Ecology Progress Series 71:65-73.

de Ruijter WPM, Ridderinkhof H, Lutjeharms JRE, Schouten MW, Veth C (2002) Observations of the flow in the Mozambique Channel. Geophysical Research Letters 29(10):1502

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(30):10444-10449.

Santos SR, Taylor DJ, Kinzie RA, Hidaka M, Sakai K, Coffroth MA (2002) Evolution of length variation and heteroplasmy in the chloroplast rDNA of symbiotic dinoflagellates (*Symbiodinium,* Dinophyta) and a novel insertion in the universal core region of the large subunit rDNA. Phycologia 41(4):311-318

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. Molecular Phylogenetics and Evolution 23:86-111.

Santos SR, Gutierrez-Rodriguez C, Coffroth MA (2003) Phylogenetic Identification of symbiotic dinoflagellates via length heteroplasmy in Domain V of chloroplast large subunit (cp23S)-ribosomal DNA sequences. Marine Biotechnology 5:130-140.

Santos SR, Kinzie RA, Hidaka M, Sakai K, Coffroth MA (2003) Molecular characterization of nuclear small subunit (18S)-rDNA pseudogenes in a symbiotic dinoflagellate (*Symbiodinium*, Dinophyta). Journal of Eukaryotic Microbiology 50(6):417-421.

Sebastian CR, Sink KJ, McClanahan TR, Cowan DA (2009) Bleaching response of corals and their *Symbiodinium* communities in southern Africa. Marine Biology 156:2049-2062.

Schouten MW, de Ruijter WPM, van Leeuwen PJ, Ridderinkhof H (2003) Eddies and variability in the Mozambique Channel. Deep-Sea Research II 50:1987-2003.

Sheppard CRC, Harris A, Sheppard ALS (2009) Archipelago-wide coral recovery patterns since 1998 in the Chagos Archipelago, central Indian Ocean. Marine Ecology Profess Series 362:109-117.

Stat M, Morris E, Gates RD (2008) Functional diversity in coral-dinoflagellate symbiosis. PNAS 105(27):9256-9261.

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.

Thornhill DJ, Kemp DW, Sampayo EM, Schmidt GW (2010) Comparative analyses of amplicon migration behavior in differing denaturing gradient gel electrophoresis (DGGE) systems. Coral Reefs 29:83-91.

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.

Ulstrup KE, van Oppen MJH, Kuhl M, Ralph PJ (2007) Inter-polyp genetic and physiological characterization of *Symbiodinium* in and *Acropora valida* colony. Marine Biology 153:225-234.

van Oppen MJH, Palstra FP, Piquet AM-T, Miller DJ (2001) Patterns of coral-dinoflagellate associations in Acropora: Significance of local availability and physiology of *Symbiodinium* strains and host-symbiont specificity. Proceedings: Biological Sciences 268(1478):1759-1767.

Warner ME, LaJeunesse TC, Robison JD, Thur RM (2006) The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reefs corals in Belize: potential implications for coral bleaching. Limnology and Oceanography 51(4):1887-1897.

Wells S., Sheppard C., Jenkins M. Coral Reefs of the World: Indian Ocean, Red Sea and Gulf v.2. International Union for the Conservation of Nature & Natural Resources: October 1998.

Winters G, Beer S, Zvi BB, Brickner I, Loya Y (2009) Spatial and temporal photoacclimation of *Stylophora pistillata*: zooxanthella size, pigmentation, location and clade. Marine Ecology Progress Series 384:107.

Tables

Tbl.1) Summary of past published research on algal symbiont (*Symbiodinium* spp.) distributions in scleractinian corals from different latitudes.

Latitude (°)	Site	
0°-5°	Maldives (N)	McClanahan et al. (in prep.)
6°-10°	Malaysia (S), Zanzibar (S), Thailand (N)	Loh et al. 2001; LaJeunesse et al. 2010
11°-15°	North Madagascar (S)	This study
16°-20°	West /East Australia (S), Red Sea (N)	Rodriguez-Lanetty et al. 2001; van Oppen et al 2001; Silverstein et al. 2010
21°-25°	West/East Australia (S), Madagascar (S)	Rodriguez-Lanetty et al. 2001; van Oppen et al 2001; Silverstein et al. 2010; this study
26°-30°	West/East Australia (S), Mozambique (S), South Africa (S), Arabian Gulf (N), Red Sea (N), Gulf of Aqaba (N)	Rodriguez-Lanetty et al. 2001; MacDonald et al 2008; Sebastian et al. 2009; Silverstein et al. 2010; McClanahan et al. (in prep.)
31°-35°	West/East Australia (S), South Africa (S)	Silverstein et al. 2010; MacDonald et al. 2008; Sebastian et al. 2009

Tbl.2) Synopsis of algal symbiont (*Symbiodinium spp.)* distributions in scleractinian corals at different latitudes in the northern (N) and southern (S) hemispheres.

		Symbiodinium clade			
Latitude (°)	Site	A	B	C	D
0°-5°	Maldives (N)			X	X
6°-10°	Malaysia (S)			X	
	Zanzibar (S)	X	X	X	
	Thailand (N)			X	X
11°-15°	North Madagascar (S)			X	X
16°-20°	West/East Australia (S)			X	X
	Red Sea (N)	X		X	X
21°-25°	West/East Australia (S)	X		X	
	South Madagascar (S)			X	X
26°-30°	West/East Australia (S)			X	
	Mozambique (S)			X	X
	South Africa (S)			X	
	Arabian Gulf (N)	X		X	X
	Red Sea (N)	X		X	X
	Japan (N)			X	
	Gulf of Aqaba (N)	X		X	
31°-35°	West/East Australia (S)		X	X	
	South Africa (S)			X	

Tbl. 3) Sea surface temperature (SST) conditions at each site, calculated from NOAA's Coral Reef Watch (CRW) data accumulated from 1982-2009.

Site	Mean SST 1981-2008	Mean SST 2001	Standard deviation SST 1981-2008	Standard deviation SST 2001
Nosy Bé	28.51 °C	28.88 °C	+/- 1.42 °C	+/- 1.42 °C
Tuléar	26.33 °C	26.79 °C	+/- 2.06 °C	+/- 2.42 °C
Vohemar	26.57 °C	27.00 °C	+/- 1.87 °C	+/- 1.91 °C
Fort Dauphin	24.14 °C	24.64 °C	+/- 1.77 °C	+/- 2.13 °C

Tbl. 4) Nominal logistic regression results for *Symbiodinium* "D dominant", "D incidence" and "C+D" (mixed communities of clade C and D) for all corals taxa, as well as for the 4 most commonly sample coral taxa only. Coral taxon (at the species level) was the most powerful predictor of symbiont distributions. R^2 values in bold indicate those above 50%.

		Year	**Site**	**Family**	**Genus**	**Species**
All coral taxa	**D dominance**	p=0.0259* r^2=0.0157	--	p<0.0001* r^2=0.1848	p<0.0001* r^2=0.3805	p<0.0001* **r^2=0.5126**
	D incidence	--	--	p<0.0001* r^2=0.2470	p<0.0001* r^2=0.4404	p<0.0001* **r^2=0.5587**
	C+D	p=0.0225* r^2=0.0182	p=0.0488* r^2=0.0275	p<0.0001* r^2=0.3382	p<0.0001* r^2=0.4526	p<0.0001* **r^2=0.6070**
4 most common coral taxa only	**D dominance**	--	--	p<0.0001* r^2=0.1704	p<0.0001* r^2=0.4690	p<0.0001** **r^2=6729**
	D incidence	p=0.0076* r^2=0.0213	--	p<0.0001* r^2=0.3475	p<0.0001* **r^2=0.5679**	p<0.0001* **r^2=0.6912**
	C+D	p=0.0343* r^2=0.0186	p=0.0278* r^2=0.0378	p<0.0001* r^2=0.2221	p<0.0001* r^2=0.3568	p<0.0001* r^2=0.4950

Figures

Fig. 1) The four sites in Madagascar that are the focus of this study. Sites were sampled in 2001 (Tuléar, Nosy Bé, Vohemar, Fort Dauphin), 2007 (Tuléar, Nosy Bé) and 2009 (Tuléar).

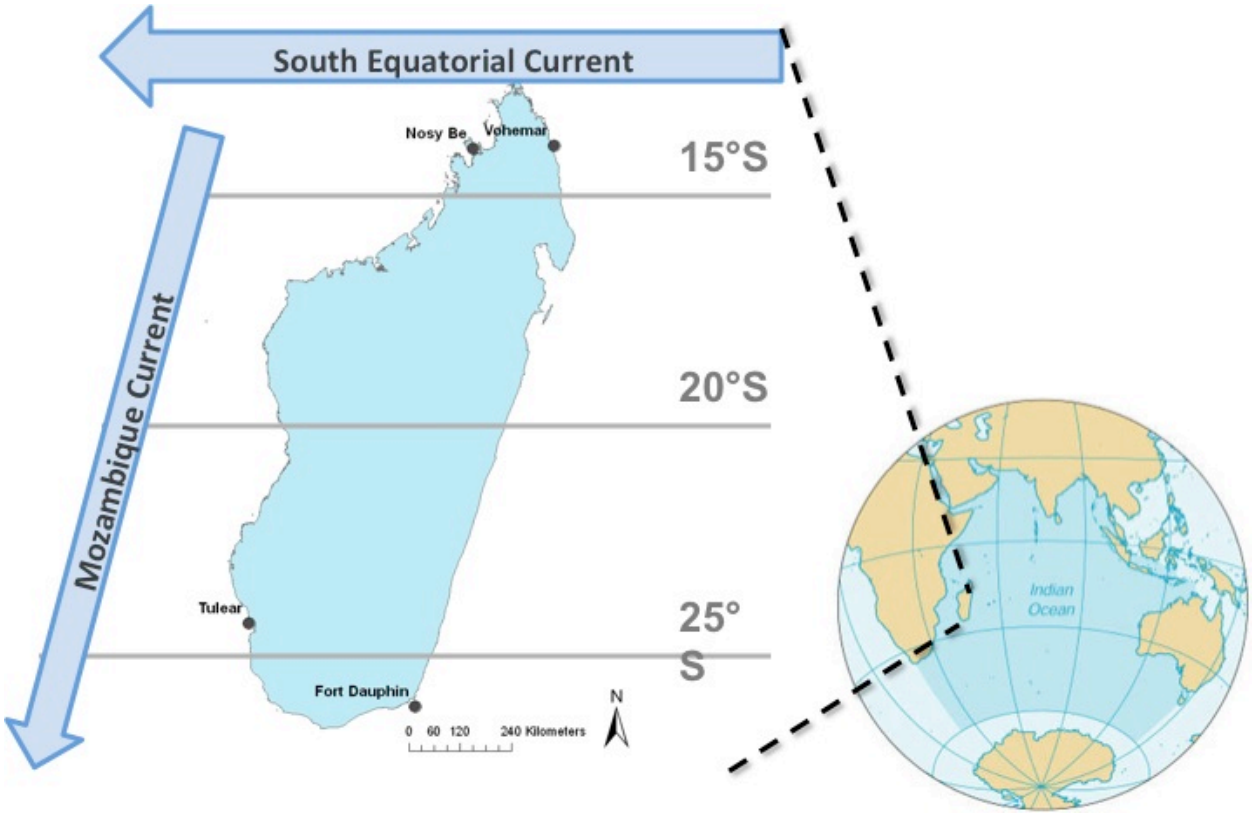


Fig. 2) Rarefaction curves of a) *Symbiodinium* clade against sampled coral genera, b) *Symbiodinium* clade against coral samples, c) *Symbiodinium* genera against sampled coral genera and d) *Symbiodinium* genera against coral samples. Samples (both corals and genera) are from Fort Dauphin in 2001 (FD2001), Nosy Bé in 2001 (NB2001), Nosy Bé in 2007 (NB2007), Tuléar in 2001 (T2001), Tuléar in 2007 and 2009 (T2007/09) and Vohemar in 2001 (V2001). The site with the highest number of *Symbiodinium* subtypes recorded (Vohemar in 2001, 12 subtypes) was also the only site where scleractinian corals were found containing members of Symbiodinium clade A (one colony of *Acropora*), leading to the unusual rarefaction curve for this site in panels (a) and (b). However, the upper confidence intervals for each curve in all panels (not shown) all overlap, indicating there is no evidence for differences in symbiont richness for each site/time interval.

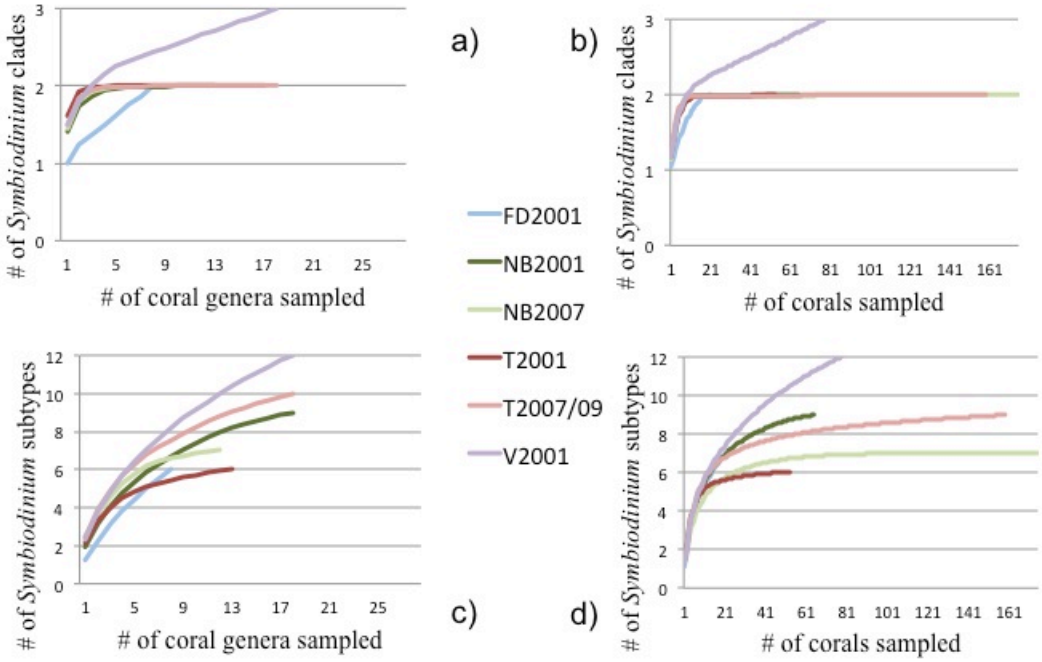


Fig. 3) Algal symbiont (*Symbiodinium* spp.) clade distributions for all a) the four most commonly sampled coral taxa (the genera *Pavona spp, Acropora spp*, *Porites spp* and members of the family Pocilloporidae) and b) all coral taxa sampled in Nosy Bé (NW), Vohemar (NE), Fort Dauphin (SE) and Tuléar (SW) in 2001.

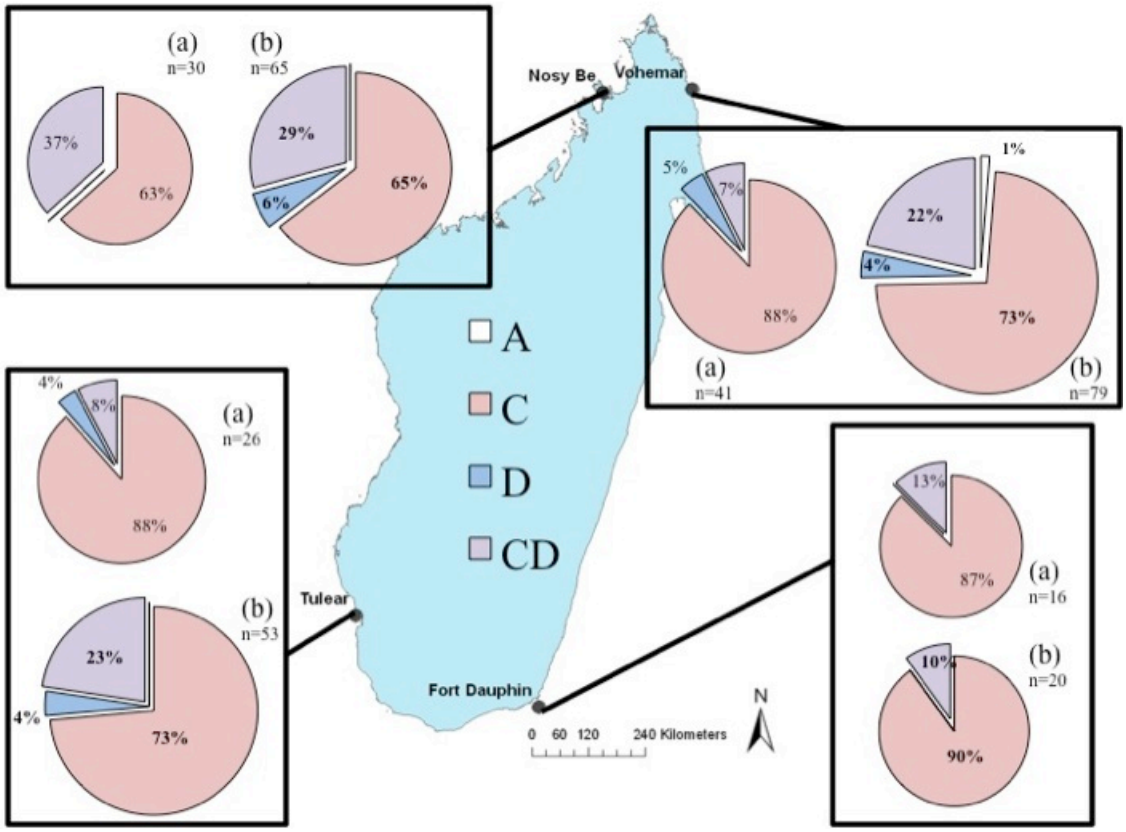


Fig. 4) *Symbiodinium* diversity found in all coral species sampled between the northern and southern reefs of Madagascar in 2001. Three clades (A, C and D) are represented here by the most common subtypes found during the 2001 sampling. Percentages are the relative proportions of samples containing the symbiont subtype, with samples containing mixed types split evenly between multiple categories from the 2001 collection. Northern reefs (blue bars) include the Nosy Bé and Vohemar samples while the southern reefs (orange bars) include the Tuléar and Fort Dauphin samples.

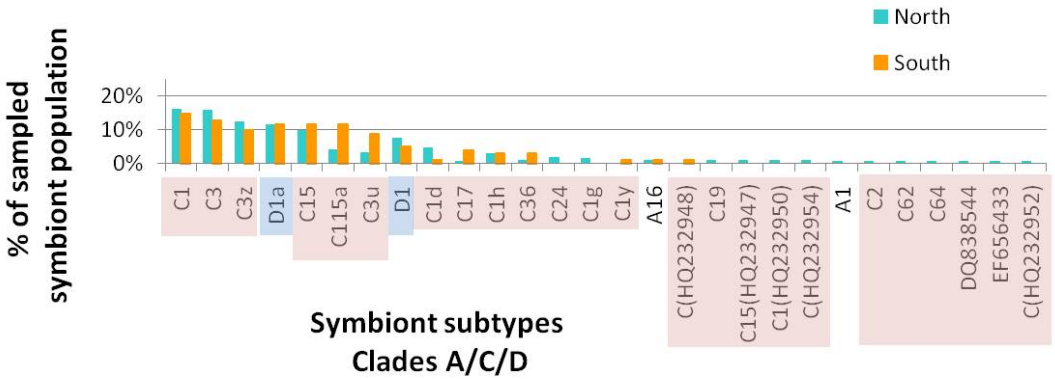


Fig.5) Denaturing gradient gel depicting common *Symbiodinium* spp types found in sampled corals from Madagascar. Designations beneath the image indicated the dominant symbiont types detected in each profile. Novel ITS-2 sequenes were submitted to GenBank (accession numbers with HQ prefixes used in designation). Arrows indicate the dominant band of the distinguishing subtype, with a-e identifying the novel sequence bands.

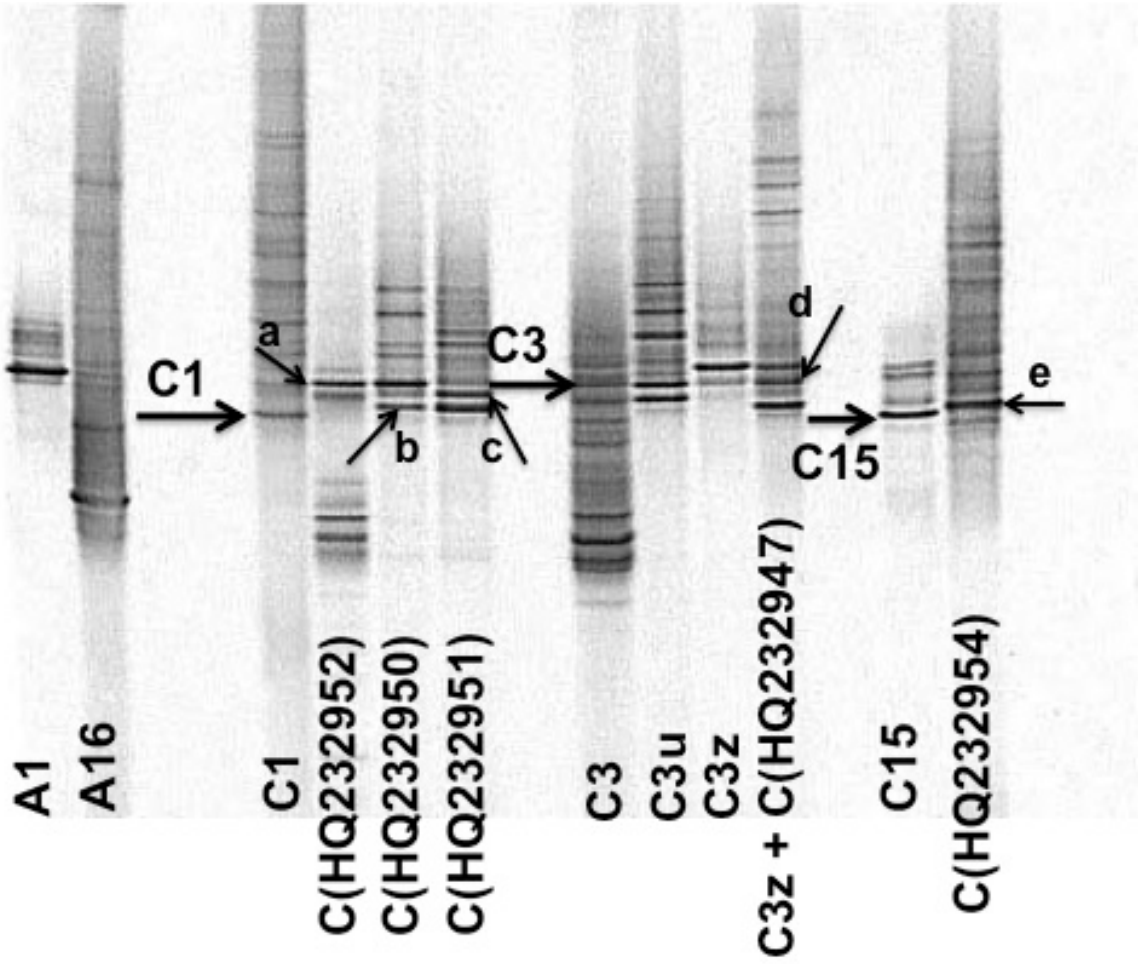


Fig. 6) Distribution of *Symbiodinium* in clades C and D in pocilloporid corals on Malagasy reefs. Percentages calculated as the proportion of each clade from the total symbiont community of sampled pocilloporid corals. N=52 for *Stylophora/Seriatopora* species samples and N=54 for *Pocillopora* species.

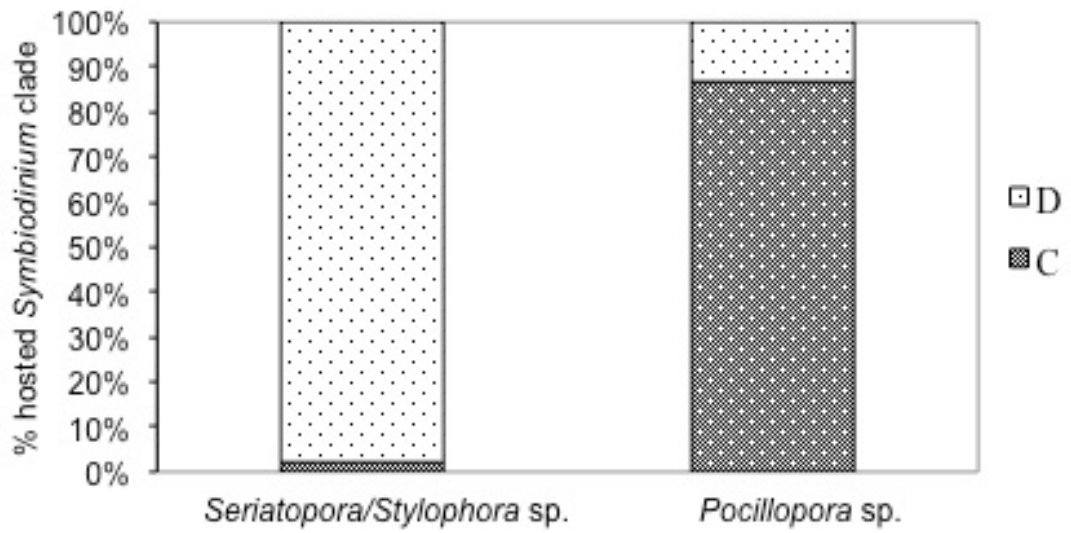


Fig. 7) *Symbiodinium* subtype diversity in acroporid species of corals between Nosy Bé (NW Madagascar, N=28) and Tuléar (SW Madagascar, N=26).

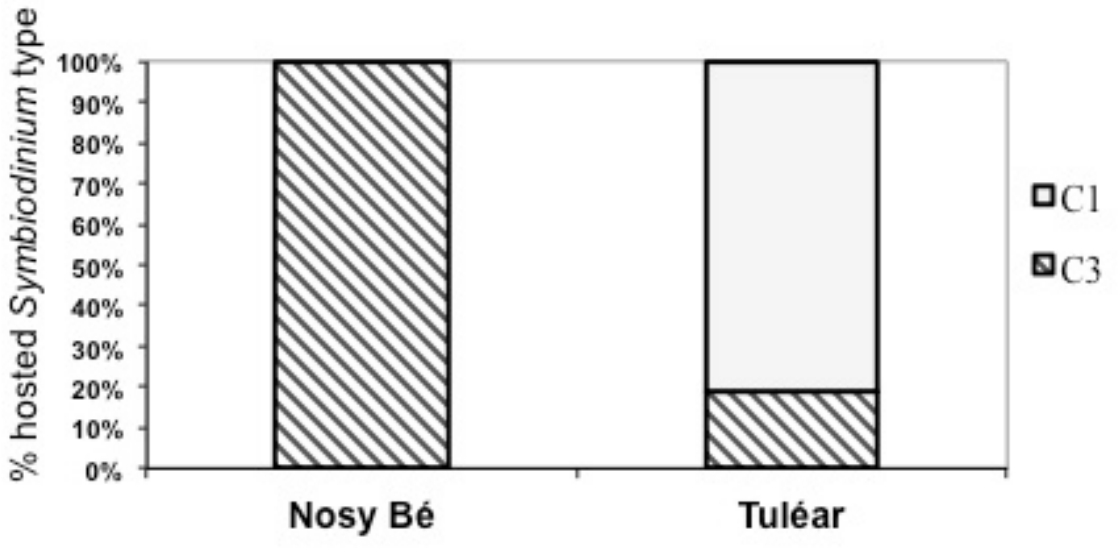


Fig. 8) Sea surface temperatures from July 2007 through January 2010 for Tulear overlaid with cyclones inducing cool water upwellings. Dates denoted as “month/year”. Image courtesy of Dr. Tim McClanahan.

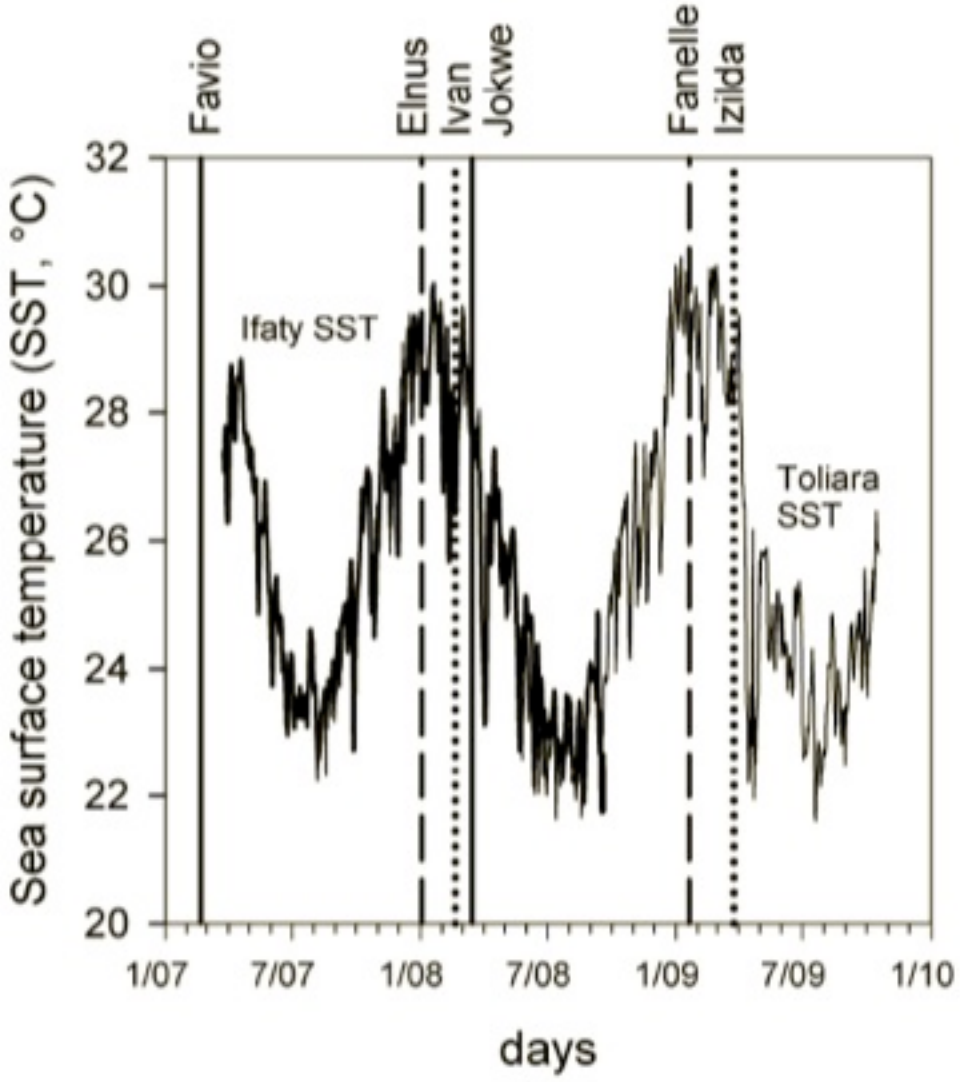


Fig. 9) Coral Reef Watch (CRW) satellite data taken weekly compared to on-site temperature gauge data recording temperature metrics recorded every three hours.

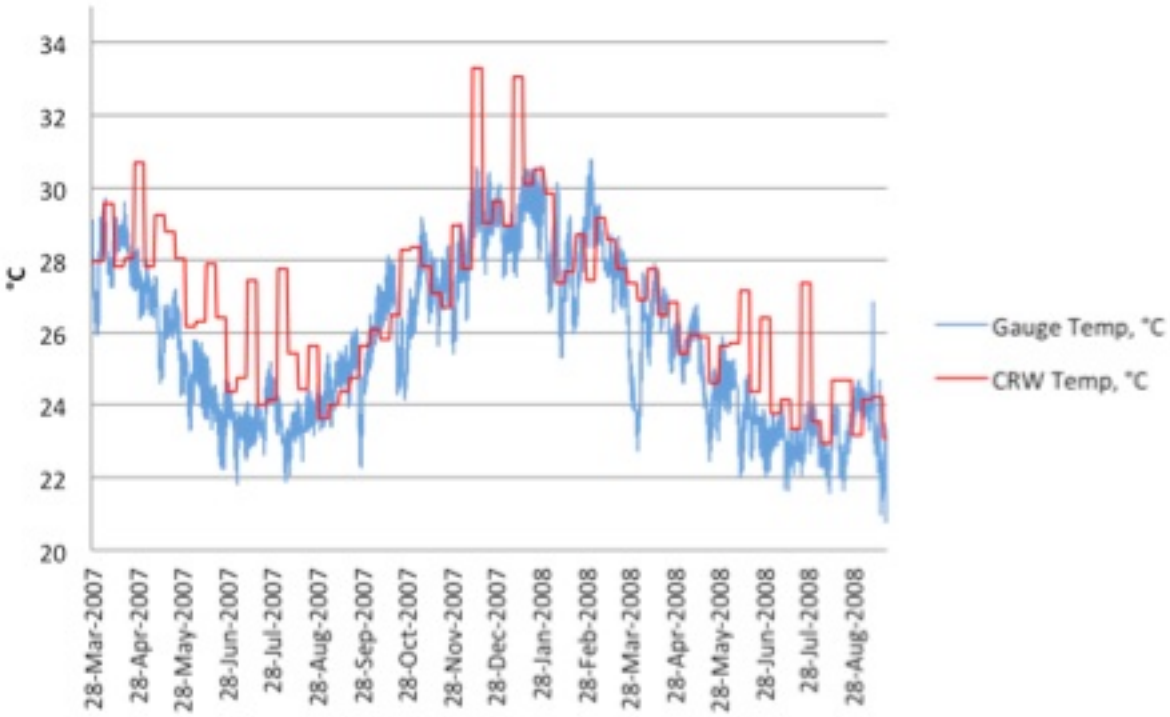


Fig. 10) Incidence of clade D (frequency of occurrence) in Malagasy corals. Coral taxa with a higher incidence of clade D may be more resilient to future climate change. Families abbreviated as following: Poc=Pocilloporidae; Ocu=Oculinidae; Mer=Merulinidae; Fav=Faviidae; Aga=Agariciidae; Acr=Acroporidae; Sid=Siderasteridae; Por=Poritidae. Species abbreviated as following: Ser=*Seriatopora*; Sty=*Stylophora*; Gal=*Galaxea*; Hyd=*Hydnophora*; Diplo=*Diploastrea*; Platy=*Platygyra*; Pav=*Pavona*; Cyph=*Cyphastrea*; Mont=*Montastrea*; Cosc=*Coscinaraea*; Poc=*Pocillopora*; Acr=*Acropora* and Por=*Porites*. "Misc" refers to miscellaneous species belonging to the family Faviidae.

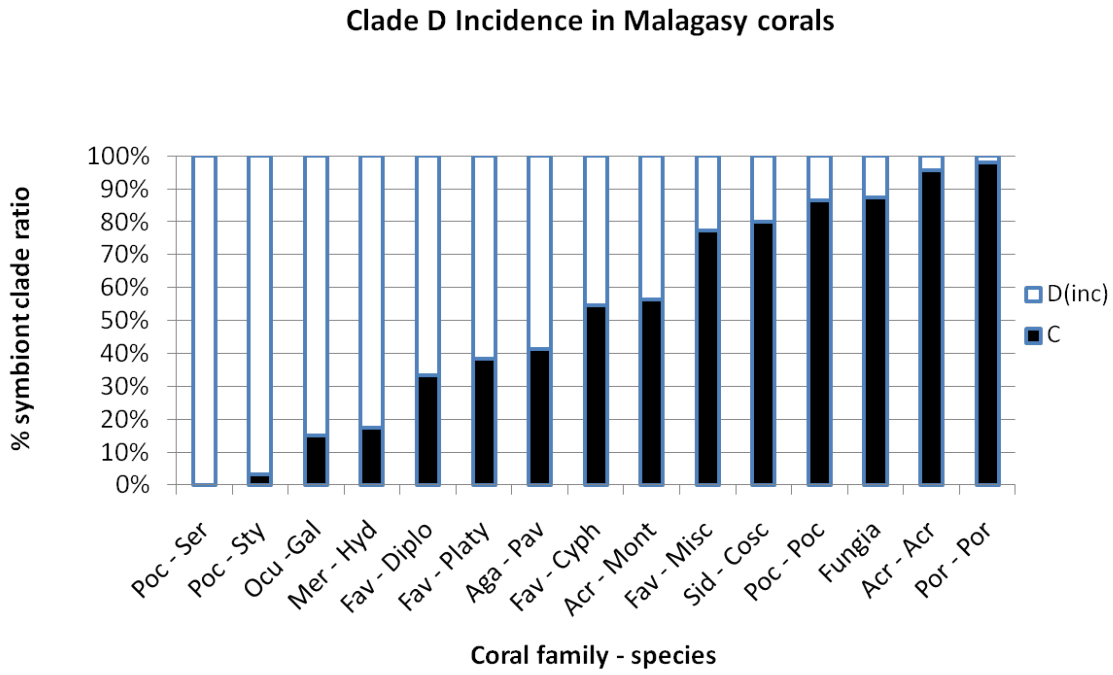


Fig. 11) Changes in cladal distributions of *Symbiodinium* in Nosy Bé and Tulear coral reefs. Size of chart proportional to number of samples taken at each site and time period. To control for differences in coral taxonomy sampled, a) are the four most common coral genera collected (Acropora, Pavona, Porites and species within the family Pocilloporidae) and b) is the symbiont distribution changes for all corals sampled.

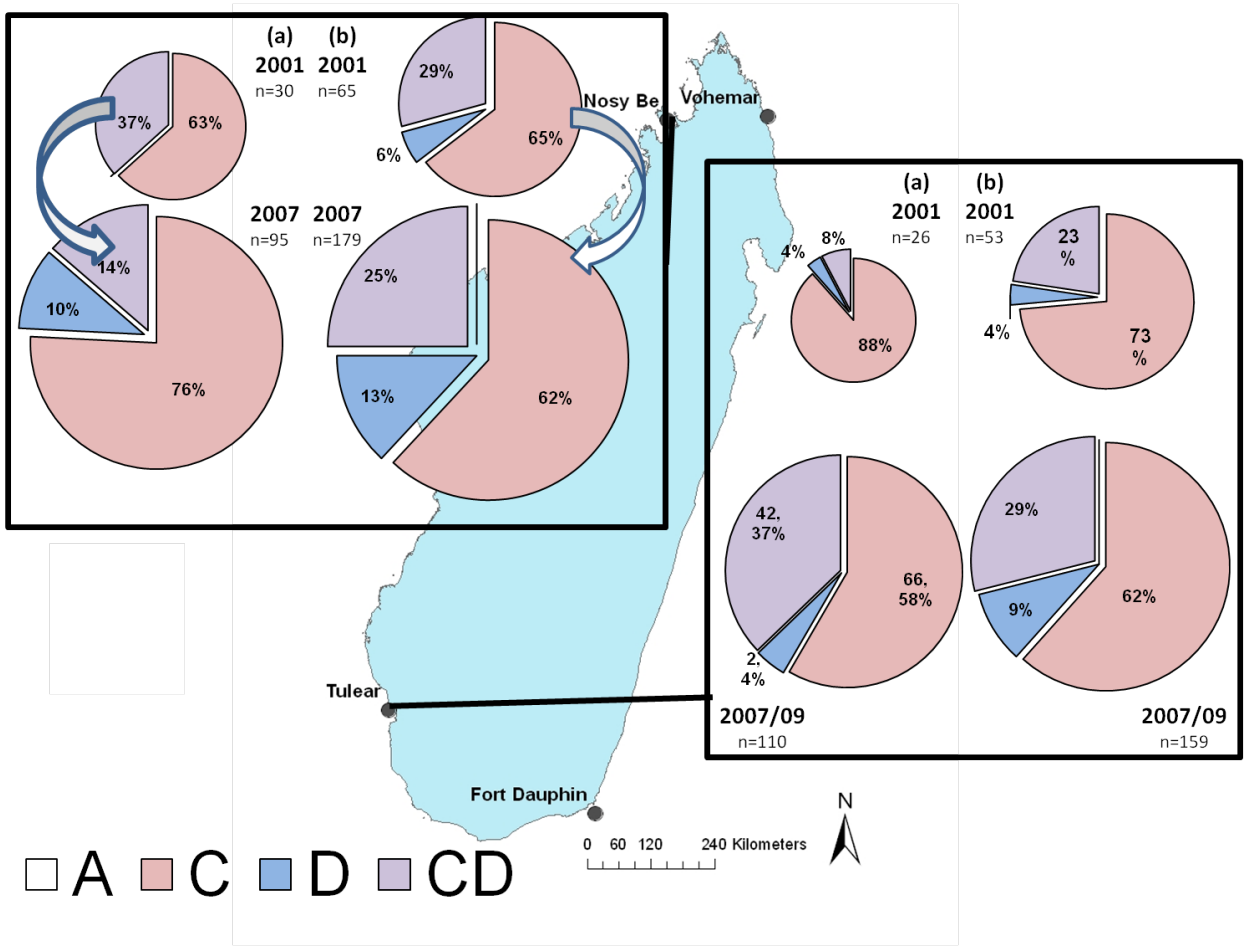
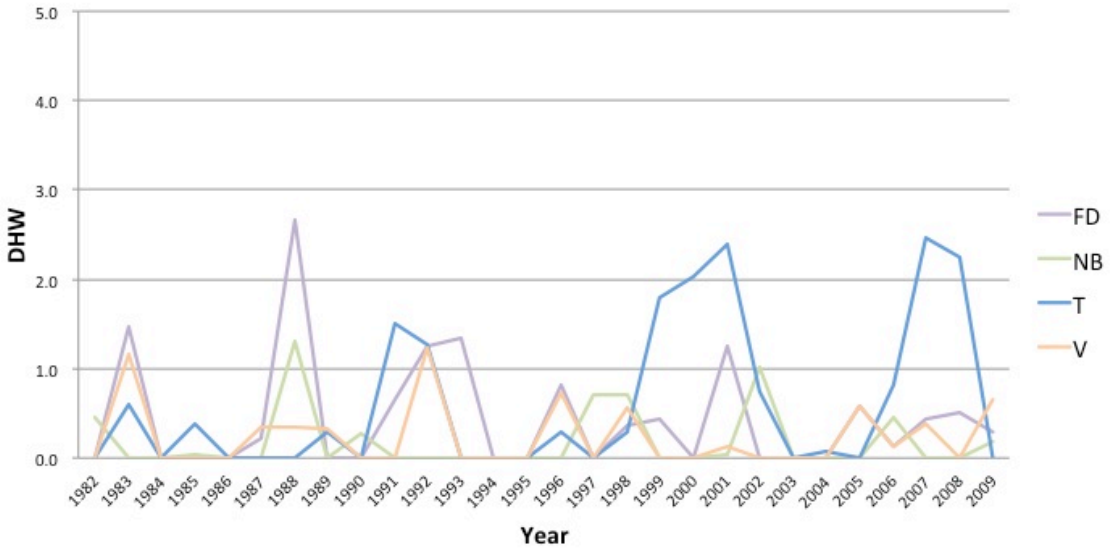


Fig. 12) Degree heating weeks (DHWs) from NOAA Coral Reef Watch database in degrees centigrade at the 4 sampling sites (FD=Fort Dauphin, NB+Nosy Bé, T=Tuléar and V=Vohemar) in Madagascar from 1982-2009. DHWs were calculated using cumulative and consecutive a) 1°C anomalies as a 1°C heating week and b) total anomalies above the maximum monthly mean (MMM). In a) DHW values of 2° heating weeks suggest potential bleaching and those above 4 predict more severe bleaching and mortality. In b) DHW values of 4 indicate the potential for mild bleaching, and those above 8 predict severe bleaching and mortality (NOAA Coral Reef Watch database).

a)



b)

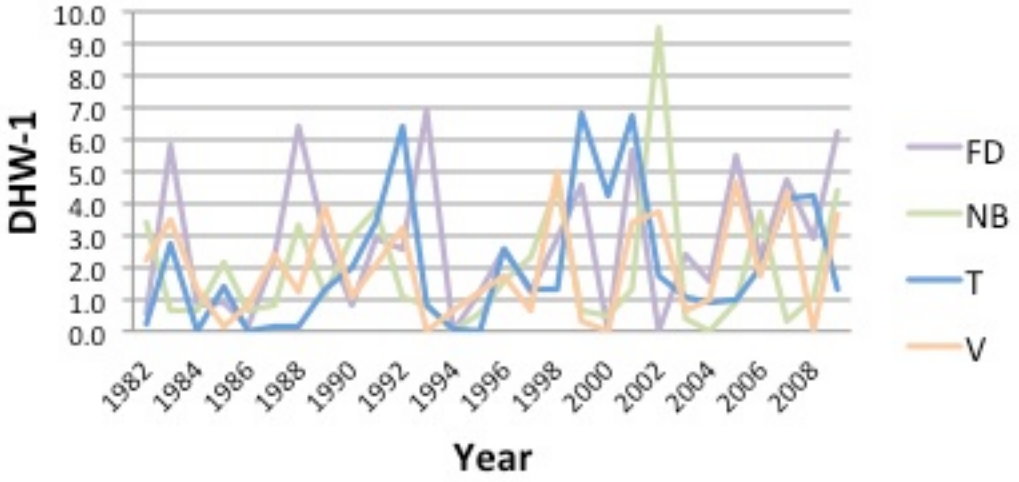

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
DHW-1
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
Year
FD
NB
T
V

Fig. 13) Example of changes in symbiont community structure between Nosy Bé (NW) and Tuléar (SW) with increasing thermal stress.

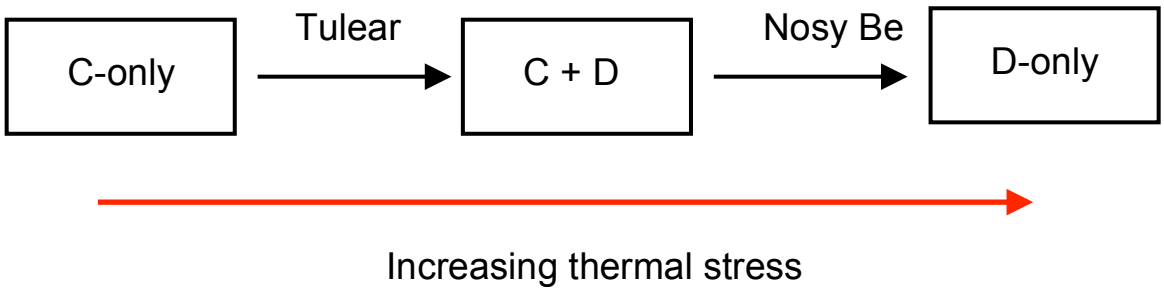
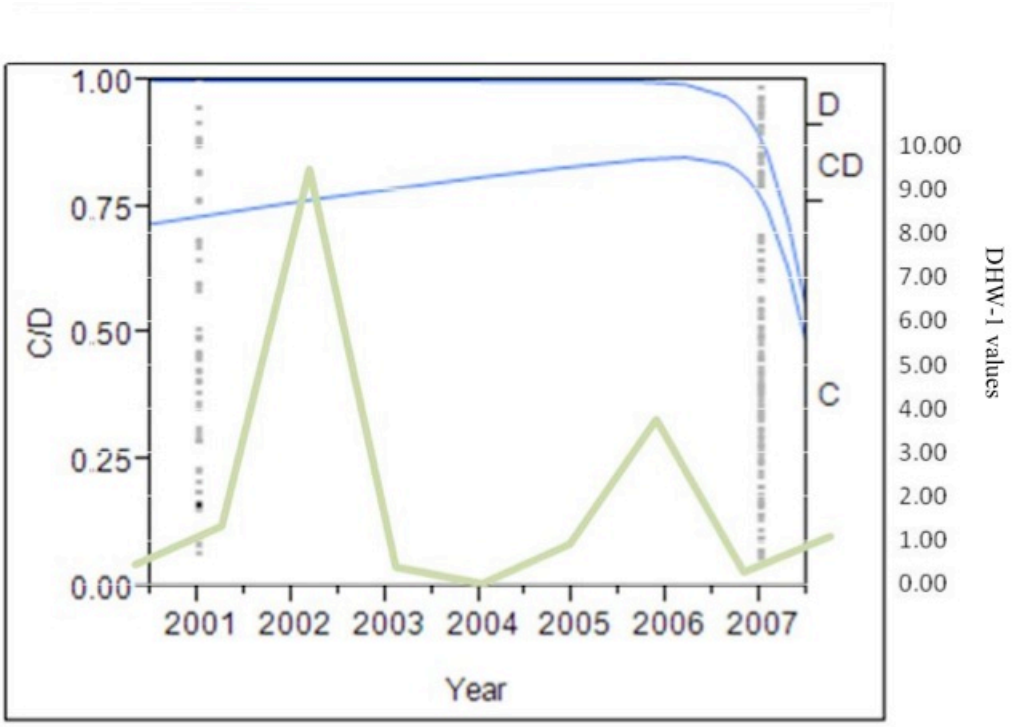
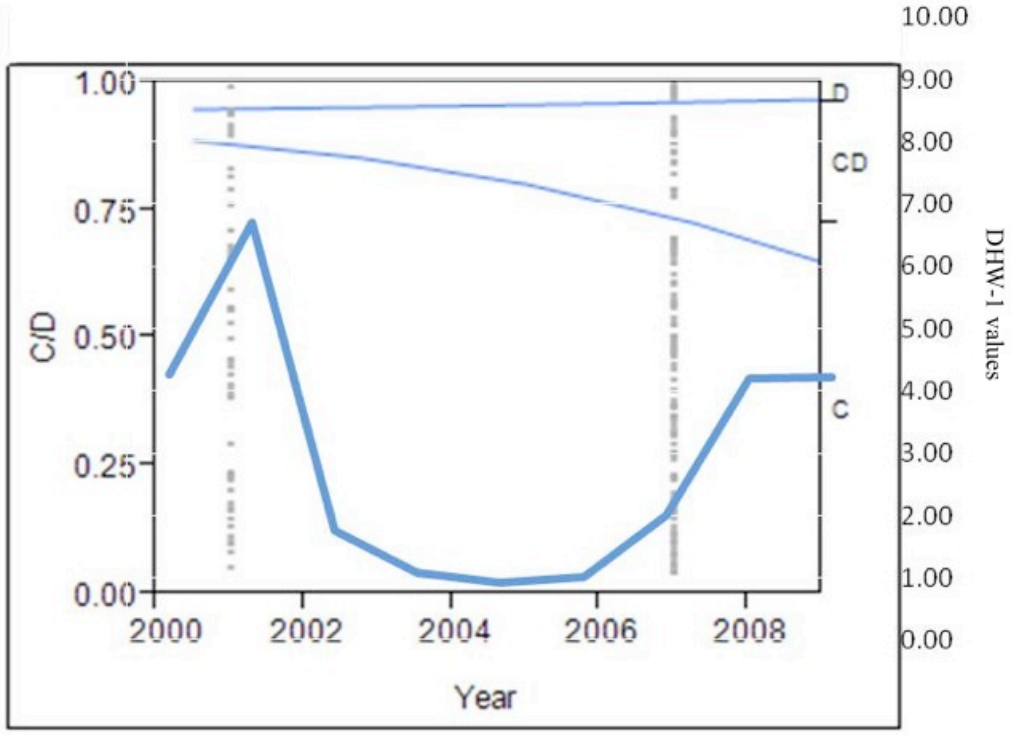


Fig. 14) Changes in *Symbiodinium* communities over time for a) Nosy Bé and b) Tuléar overlaid with cumulative anomalies above the monthly maximum mean (DHWs). While not significant ($p>0.05$), more sampling would be necessary between the 2001-2009 sampling periods to determine the dynamic response of symbiont communities change dynamically in response to thermal anomalies.

a)



b)


10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
DHW-1 values
1.00
0.75
0.50
0.25
0.00
C/D
D
CD
C
2000
2002
2004
2006
2008
Year

Appendix
Complete list of sampled corals of Madagascar

Fort Dauphin: 2001

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MDF-010	*Acropora* sp.	*Acropora*	Acroporidae		C1 + C3	
MDF-013	*Acropora* sp.	*Acropora*	Acroporidae		C1	
MDF-015	*Acropora* sp.	*Acropora*	Acroporidae		C1	
MDF-020	*Acropora* sp.	*Acropora*	Acroporidae		C3	
MDF-021	*Acropora* sp.	*Acropora*	Acroporidae		C3	
MDF-022	*Acropora* sp.	*Acropora*	Acroporidae		C3	
MDF-002	*Acropora nasuta*	*Acropora*	Acroporidae		C3z	
MDF-008	*Montipora* sp.	*Montipora*	Acroporidae		C17	
MDF-017	*Montipora* sp.	*Montipora*	Acroporidae		C17	
MDF-018	*Montipora* sp.	*Montipora*	Acroporidae		C17	
MDF-019	*Montipora* sp.	*Montipora*	Acroporidae		C17	
MDF-003	*Gyrosmilia interrupta*	*Gyrosmilia*	Eusmiliidae			D1a
MDF-006	*Favites pentagona*	*Favites*	Faviidae		C3z	
MDF-016	*Favites pentagona*	*Favites*	Faviidae		C3z	
MDF-007	*Goniastrea* sp.	*Goniastrea*	Faviidae		C(HQ232948)	
MDF-011	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae		C3	
MDF-004	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDF-001	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		C36	D1
MDF-005	*Stylophora subseriata*	*Stylophora*	Pocilloporidae		C36	
MDF-012	*Stylophora subseriata*	*Stylophora*	Pocilloporidae		C36	
MDF-009	*Psammocora* sp.	*Psammocora*	Siderastreidae		C1	

Nosy Bé: 2001

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MDN-020	*Acropora cuneata*	*Acropora*	Acroporidae		C3z	
MDN-015	*Acropora formosa*	*Acropora*	Acroporidae		C3z	
MDN-014	*Acropora gemmifera*	*Acropora*	Acroporidae		C3z	
MDN-073	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MDN-056	*Acropora millepora*	*Acropora*	Acroporidae		C3z	
MDN-070	*Acropora palifera*	*Acropora*	Acroporidae		C3z	
MDN-027	*Pavona cactus*	*Pavona*	Agariciidae		C1	D1a
MDN-072	*Pavona decussata*	*Pavona*	Agariciidae		C1	D1a
MDN-074	*Pavona decussata*	*Pavona*	Agariciidae		C1	D1a
MDN-029	*Pavona varians*	*Pavona*	Agariciidae		C1	D1a
MDN-045	*Pavona varians*	*Pavona*	Agariciidae		C3u	D1a
MDN-011	*Diploastrea heliopora*	*Diploastrea*	Faviidae			D1a
MDN-053	*Diploastrea heliopora*	*Diploastrea*	Faviidae			D1a
MDN-078	*Diploastrea heliopora*	*Diploastrea*	Faviidae		C3z	D1a
MDN-063	*Echinopora gemmacea*	*Echinopora*	Faviidae		C3	
MDN-004	*Echinopora lamellosa*	*Echinopora*	Faviidae		C1 + C24	
MDN-071	*Echinopora lamellosa*	*Echinopora*	Faviidae		C1	
MDN-054	*Favia* sp.	*Favia*	Faviidae		C3u	
MDN-068	*Favia annuligera*	*Favia*	Faviidae		C115a	
MDN-024	*Favia favus*	*Favia*	Faviidae		C3z	
MDN-030	*Favia favus*	*Favia*	Faviidae		C115a	
MDN-061	*Favia favus*	*Favia*	Faviidae		C3z	
MDN-075	*Favites* sp.	*Favites*	Faviidae		C1 + C24	
MDN-065	*Goniastrea edwardsi*	*Goniastrea*	Faviidae		C3z + C(HQ232947)	
MDN-009	*Goniastrea retiformis*	*Goniastrea*	Faviidae		C3u	
MDN-019	*Goniastrea retiformis*	*Goniastrea*	Faviidae			D1a
MDN-025	*Leptoria phrygia*	*Leptoria*	Faviidae		C3z	
MDN-040	*Leptoria phrygia*	*Leptoria*	Faviidae		C3z + C(HQ232947)	

MDN-028	*Platygyra crosslandi*	*Platygyra*	Faviidae		C3z	D1a
MDN-039	*Platygyra crosslandi*	*Platygyra*	Faviidae		C3z	
MDN-006	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDN-033	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDN-058	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDN-059	*Platygyra pini*	*Platygyra*	Faviidae		C3z	
MDN-035	*Platygyra* sp.	*Platygyra*	Faviidae		C2	
MDN-042	*Fungia* sp.	*Fungia*	Fungiidae		C(HQ232950)	D1a
MDN-052	*Hydnophora exesa*	*Hydnophora*	Merulinidae		C3z	
MDN-055	*Hydnophora exesa*	*Hydnophora*	Merulinidae		C3u + C115a	D1a
MDN-038	*Hydnophora microconos*	*Hydnophora*	Merulinidae		C3u + C115a	D1a
MDN-076	*Hydnophora microconos*	*Hydnophora*	Merulinidae		C3u + C115a	
MDN-010	*Merulina ampliata*	*Merilina*	Merulinidae		C3z	
MDN-021	*Millepora tenella*	*Millepora*	Milleporidae	A16		
MDN-047	*Millepora tenella*	*Millepora*	Milleporidae	A16		
MDN-062	*Echinophyllia aspera*	*Echinophyllia*	Pectiniidae			D1a
MDN-048	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae		C3	
MDN-080	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDN-082	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDN-013	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MDN-036	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	D1
MDN-049	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	D1
MDN-050	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	D1
MDN-077	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MDN-002	*Porites cylindrica*	*Porites*	Poritidae		C(HQ232954)	
MDN-034	*Porites cylindrica*	*Porites*	Poritidae		C3z	
MDN-081	*Porites cylindrica*	*Porites*	Poritidae		C15	
MDN-026	*Porites lobata*	*Porites*	Poritidae		C15	
MDN-043	*Porites lobata*	*Porites*	Poritidae		C15	
MDN-084	*Porites lobata*	*Porites*	Poritidae		C15	
MDN-066	*Porites lutea*	*Porites*	Poritidae		C15	
MDN-016	*Porites profundus*	*Porites*	Poritidae		C(HQ232954)	
MDN-007	*Porites rus*	*Porites*	Poritidae		C15	
MDN-051	*Porites rus*	*Porites*	Poritidae		C15	
MDN-008	*Porites solida*	*Porites*	Poritidae		C15	
MDN-037	*Psammocora obtusangula*	*Psammocora*	Siderasteridae		C1	
MDN-044	*Psammocora obtusangula*	*Psammocora*	Siderasteridae		C1	
MDN-023	*Palythoa* sp.	*Palythoa*	Zoanthidae		C62	
MDN-046	*Palythoa* sp.	*Palythoa*	Zoanthidae		C3	

Nosy Bé: 2007

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MD001	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD011	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD021	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD025	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD037	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD038	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD040	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD043	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD055	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD057	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD058	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD083	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD088	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD089	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD095	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD102	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD112	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD121	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD125	*Acropora humilis*	*Acropora*	Acroporidae		C3z	
MD134	*Acropora humilis*	*Acropora*	Acroporidae		C3z	

MD150	*Acropora humilis*	*Acropora*	Acroporidae	C3z	
MD172	*Acropora humilis*	*Acropora*	Acroporidae	C3z	
MD026	*Pavona decussata*	*Pavona*	Agariciidae	C1	D1a
MD167	*Pavona decussata*	*Pavona*	Agariciidae	C1	D1a
MD181	*Pavona decussata*	*Pavona*	Agariciidae	C1	D1a
MD003	*Pavona varians*	*Pavona*	Agariciidae	C1 + C3	D1a
MD046	*Pavona varians*	*Pavona*	Agariciidae	C3 + C115a	
MD103	*Pavona varians*	*Pavona*	Agariciidae	C1	
MD020	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3z + C3u	
MD042	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3z	
MD067	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3z + C115	D1a
MD079	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C1 + C3	
MD097	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3u	D1a
MD110	*Diploastrea heliopora*	*Diploastrea*	Faviidae		D1a
MD118	*Diploastrea heliopora*	*Diploastrea*	Faviidae		D1a
MD133	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3u + C115	D1a
MD137	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3u + C115	
MD143	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3z	D1a
MD153	*Diploastrea heliopora*	*Diploastrea*	Faviidae		D1a
MD165	*Diploastrea heliopora*	*Diploastrea*	Faviidae	C3z	D1a
MD028	*Favia* sp.	*Favia*	Faviidae	C3z	
MD051	*Favia stelligera*	*Favia*	Faviidae	C3z	
MD054	*Favia stelligera*	*Favia*	Faviidae	C3z	
MD065	*Favia stelligera*	*Favia*	Faviidae	C3z + C(HQ232947)	
MD066	*Favia stelligera*	*Favia*	Faviidae	C3z	
MD076	*Favia stelligera*	*Favia*	Faviidae	C3z	
MD080	*Favia stelligera*	*Favia*	Faviidae	C3z	
MD092	*Favia stelligera*	*Favia*	Faviidae	C3z + C(HQ232947)	
MD138	*Favia stelligera*	*Favia*	Faviidae	C3z	
MD139	*Favia stelligera*	*Favia*	Faviidae	C3z + C(HQ232947)	
MD022	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD030	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD033	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD034	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD036	*Goniastrea retiformis*	*Goniastrea*	Faviidae		D1a
MD048	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD068	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD090	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD100	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z + C(HQ232947)	
MD109	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3u + C115	
MD111	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD140	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z + C(HQ232947)	
MD163	*Goniastrea retiformis*	*Goniastrea*	Faviidae	C3z	
MD018	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD019	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD031	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD032	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD074	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD087	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD101	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z + C(HQ232947)	
MD119	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	D1a
MD120	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z + C(HQ232947)	D1a
MD141	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD145	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD149	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	D1a
MD168	*Platygyra daedalea*	*Platygyra*	Faviidae	C3z	
MD015	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD016	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD023	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3u	
MD059	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD071	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD073	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3u	
MD078	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3u	
MD082	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD113	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD184	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z + C3u	

MD185	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z + C3u	
MD208	*Hydnophora microconos*	*Hydnophora*	Merulinidae	C3z	
MD005	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD009	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD012	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C1	D1a
MD017	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C1	D1a
MD061	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD062	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C3z	
MD070	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C1	D1a
MD091	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C3z	
MD114	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C3z	
MD127	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C3z	D1
MD146	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C3	D1a
MD148	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD162	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C3u	
MD169	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD173	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD178	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD183	*Galaxea fascicularis*	*Galaxea*	Oculinidae		D1a
MD259	*Galaxea fascicularis*	*Galaxea*	Oculinidae	C1	D1a
MD002	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d	
MD013	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD024	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d	
MD039	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD041	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD081	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD105	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD106	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD174	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD175	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	
MD209	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1d + C3	D1
MD053	*Pocillopora eydouxi*	*Pocillopora*	Pocilloporidae	C1	
MD056	*Pocillopora eydouxi*	*Pocillopora*	Pocilloporidae	C1	
MD129	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C1	
MD164	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C3	
MD180	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C3	
MD182	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C3	
MD063	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	D1
MD077	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	D1
MD135	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	D1
MD152	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	D1
MD154	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	D1
MD158	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	
MD160	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C3	D1
MD010	*Stylophora madagascarensis*	*Stylophora*	Pocilloporidae		D1
MD014	*Stylophora madagascarensis*	*Stylophora*	Pocilloporidae		D1
MD072	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD075	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD085	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD093	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD098	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD128	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD170	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD171	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD179	*Stylophora pistillata*	*Stylophora*	Pocilloporidae		D1a
MD064	*Goniopora* sp.	*Goniopora*	Poritidae	C3u	
MD052	*Goniopora* sp.	*Goniopora*	Poritidae	C3u	
MD004	*Porites lutea*	*Porites*	Poritidae	C15	
MD007	*Porites lutea*	*Porites*	Poritidae	C15	
MD008	*Porites lutea*	*Porites*	Poritidae	C15	
MD027	*Porites lutea*	*Porites*	Poritidae	C15	
MD045	*Porites lutea*	*Porites*	Poritidae	C15	
MD049	*Porites lutea*	*Porites*	Poritidae	C15	
MD104	*Porites lutea*	*Porites*	Poritidae	C15	
MD107	*Porites lutea*	*Porites*	Poritidae	C15	
MD116	*Porites lutea*	*Porites*	Poritidae	C15	

MD122	*Porites lutea*	*Porites*	Poritidae	C15
MD123	*Porites lutea*	*Porites*	Poritidae	C15
MD132	*Porites lutea*	*Porites*	Poritidae	C15
MD147	*Porites lutea*	*Porites*	Poritidae	C15
MD155	*Porites lutea*	*Porites*	Poritidae	C15
MD161	*Porites lutea*	*Porites*	Poritidae	C3z
MD186	*Porites lutea*	*Porites*	Poritidae	C15
MD029	*Porites nigrescens*	*Porites*	Poritidae	C15
MD035	*Porites nigrescens*	*Porites*	Poritidae	C15
MD044	*Porites nigrescens*	*Porites*	Poritidae	C(HQ232954)
MD060	*Porites nigrescens*	*Porites*	Poritidae	C(HQ232954)
MD124	*Porites nigrescens*	*Porites*	Poritidae	C15
MD176	*Porites nigrescens*	*Porites*	Poritidae	C15
MD050	*Porites palmata*	*Porites*	Poritidae	C(HQ232954)
MD084	*Porites palmata*	*Porites*	Poritidae	C(HQ232954)
MD096	*Porites palmata*	*Porites*	Poritidae	C15 (HQ232954)
MD131	*Porites palmata*	*Porites*	Poritidae	C15
MD136	*Porites palmata*	*Porites*	Poritidae	C15 (HQ232954)
MD157	*Porites palmata*	*Porites*	Poritidae	C(HQ232954)
MD199	*Porites palmata*	*Porites*	Poritidae	C3
MD130	*Porites rus*	*Porites*	Poritidae	C15
MD156	*Porites rus*	*Porites*	Poritidae	C15
MD166	*Porites rus*	*Porites*	Poritidae	C15

Tuléar: 2001

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MDT-010	*Acropora* sp.	*Acropora*	Acroporidae		C3	D1a
MDT-023	*Acropora cuneata*	*Acropora*	Acroporidae		C3	
MDT-009	*Acropora palifera*	*Acropora*	Acroporidae		C1	
MDT-043	*Acropora palifera*	*Acropora*	Acroporidae		C1	
MDT-020	*Gardineroseris planulata*	*Gardineroseris*	Agariciidae		C3	
MDT-054	*Pavona decussata*	*Pavona*	Agariciidae		C1	D1a
MDT-045	*Pavona explanulata*	*Pavona*	Agariciidae		C1	
MDT-047	*Pavona laira*	*Pavona*	Agariciidae		C1	
MDT-034	*Pavona varians*	*Pavona*	Agariciidae		C3 + C115a	
MDT-028	*Cyphastrea serailia*	*Cyphastrea*	Faviidae		C3	D1a
MDT-019	*Favia* sp.	*Favia*	Faviidae		C3z + C115	
MDT-038	*Favia* sp.	*Favia*	Faviidae		C115a	
MDT-004	*Favia pallida*	*Favia*	Faviidae		C3 + C115a	
MDT-006	*Favia pallida*	*Favia*	Faviidae		C3 + C115a	
MDT-015	*Favia pallida*	*Favia*	Faviidae		C3 + C115a	
MDT-042	*Favia pallida*	*Favia*	Faviidae		C3 + C115a	
MDT-011	*Favia speciosa*	*Favia*	Faviidae		C3 + C115a	
MDT-012	*Favia speciosa*	*Favia*	Faviidae		C3 + C115a	
MDT-055	*Goniastrea retiformis*	*Goniastrea*	Faviidae			D1a
MDT-057	*Leptastrea inepcuelis*	*Leptastrea*	Faviidae		C1	D1
MDT-025	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDT-037	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDT-048	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDT-003	*Platygyra pini*	*Platygyra*	Faviidae		C1	
MDT-016	*Platygyra pini*	*Platygyra*	Faviidae		C3z	D1a
MDT-021	*Platygyra pini*	*Platygyra*	Faviidae		C3	
MDT-050	*Platygyra pini*	*Platygyra*	Faviidae		C3z	D1a
MDT-031	*Platygyra planae*	*Platygyra*	Faviidae		C3z	
MDT-033	*Platygyra planae*	*Platygyra*	Faviidae		C3	
MDT-035	*Plerogyra brucic*	*Platygyra*	Faviidae		C1	
MDT-014	*Hydnophora microconos*	*Hydnophora*	Merulinidae		C3z + C115a	D1a
MDT-036	*Hydnophora microconos*	*Hydnophora*	Merulinidae		C3z + C115a	D1a
MDT-044	*Hydnophora microconos*	*Hydnophora*	Merulinidae		C3u + C115a	D1a
MDT-030	*Millepora tenella*	*Millepora*	Milleporidae	A16		
MDT-024	*Galaxea fascicularis*	*Galaxea*	Oculinidae		C3	D1a
MDT-039	*Galaxea fascicularis*	*Galaxea*	Oculinidae		C3u	

MDT-013	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1	
MDT-017	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C1h	
MDT-022	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C1h	
MDT-018	*Stylophora* sp.	*Stylophora*	Pocilloporidae	C1	
MDT-026	*Stylophora* sp.	*Stylophora*	Pocilloporidae		D1
MDT-060	*Porites lichen*	*Porites*	Poritidae	C15	
MDT-061	*Porites lichen*	*Porites*	Poritidae	C15	
MDT-005	*Porites lobata*	*Porites*	Poritidae	C1	
MDT-040	*Porites lobata*	*Porites*	Poritidae	C15	
MDT-052	*Porites lobata*	*Porites*	Poritidae	C15	
MDT-053	*Porites lobata*	*Porites*	Poritidae	C15	
MDT-056	*Porites lobata*	*Porites*	Poritidae	C15	
MDT-001	*Porites lutea*	*Porites*	Poritidae	C15	
MDT-051	*Porites monticulosa*	*Porites*	Poritidae	C15	
MDT-058	*Porites monticulosa*	*Porites*	Poritidae	C15	
MDT-059	*Porites monticulosa*	*Porites*	Poritidae	C15	
MDT-062	*Porites monticulosa*	*Porites*	Poritidae	C15	
MDT-046	*Porites rus*	*Porites*	Poritidae	C15	

Tuléar: 2007

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MD187	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD188	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD192	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD195	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD202	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD205	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD211	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD223	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD253	*Acropora humilis*	*Acropora*	Acroporidae		C1	
MD193	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD212	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD217	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD219	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD224	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD238	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD239	*Montipora* sp.	*Montipora*	Acroporidae		C3	
MD190	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD197	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD218	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD226	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD231	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD240	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD244	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD247	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	D1a
MD257	*Pavona varians*	*Pavona*	Agariciidae		C1 + C2 + C3	D1a
MD236	*Favia* sp.	*Favia*	Faviidae			D1a
MD203	*Favia stelligera*	*Favia*	Faviidae			D1
MD229	*Favia stelligera*	*Favia*	Faviidae		C1g + C(HQ232951)	D1a
MD242	*Favia stelligera*	*Favia*	Faviidae		C1g	D1
MD200	*Goniastrea retiformis*	*Goniastrea*	Faviidae		?	
MD204	*Goniastrea retiformis*	*Goniastrea*	Faviidae		C3u + C115a	
MD222	*Goniastrea retiformis*	*Goniastrea*	Faviidae		C1	
MD248	*Goniastrea retiformis*	*Goniastrea*	Faviidae		C3u + C115a	D1a
MD234	*Galaxea fascicularis*	*Galaxea*	Oculinidae		C3	
MD249	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae		C3	
MD189	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MD206	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1	
MD214	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1	
MD215	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MD216	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1	

MD220	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1	
MD221	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1	
MD227	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1	
MD235	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MD198	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MD228	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MD241	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MD245	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MD251	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae		C3	
MD232	*Stylophora pistillata*	*Stylophora*	Pocilloporidae			D1
MD254	*Stylophora pistillata*	*Stylophora*	Pocilloporidae			D1a
MD255	*Stylophora pistillata*	*Stylophora*	Pocilloporidae			D1a
MD201	*Porites lutea*	*Porites*	Poritidae		C15	
MD207	*Porites lutea*	*Porites*	Poritidae		C15	
MD233	*Porites lutea*	*Porites*	Poritidae		C15	
MD237	*Porites lutea*	*Porites*	Poritidae		C15	
MD243	*Porites lutea*	*Porites*	Poritidae		C15	
MD246	*Porites lutea*	*Porites*	Poritidae		C15	
MD250	*Porites lutea*	*Porites*	Poritidae		C15	
MD256	*Porites lutea*	*Porites*	Poritidae		C15	

Tuléar: 2009

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MD-272	*Acropora humilis*	*Acropora*	Acroporidae		C1g	
MD-273	*Acropora humilis*	*Acropora*	Acroporidae		C1g	
MD-294	*Acropora humilis*	*Acropora*	Acroporidae		C1g	
MD-317	*Acropora humilis*	*Acropora*	Acroporidae		C1g	
MD-318	*Acropora humilis*	*Acropora*	Acroporidae		C3 + C115	
MD-321	*Acropora humilis*	*Acropora*	Acroporidae		C1g	
MD-368	*Acropora humilis*	*Acropora*	Acroporidae		C1g	
MD-274	*Acropora latistella*	*Acropora*	Acroporidae		C1g	
MD-275	*Acropora latistella*	*Acropora*	Acroporidae		C1g	
MD-295	*Acropora latistella*	*Acropora*	Acroporidae		C3 + C115	
MD-305	*Acropora latistella*	*Acropora*	Acroporidae		C3 + C115	
MD-281	*Acropora listeri*	*Acropora*	Acroporidae		C1g	
MD-282	*Acropora listeri*	*Acropora*	Acroporidae		C1g	
MD-311	*Acropora nobilis*	*Acropora*	Acroporidae		C3z + C(HQ232947)	
MD-319	*Acropora samoensis*	*Acropora*	Acroporidae		C1g	
MD-263	*Montipora aequituberculata*	*Montipora*	Acroporidae		C1g + C3	
MD-284	*Montipora aequituberculata*	*Montipora*	Acroporidae		C3	
MD-285	*Montipora aequituberculata*	*Montipora*	Acroporidae		C1g + C3	
MD-293	*Montipora aequituberculata*	*Montipora*	Acroporidae		C3	
MD-356	*Pavona decussata*	*Pavona*	Agariciidae		C1g	
MD-357	*Pavona decussata*	*Pavona*	Agariciidae		C1g	
MD-345	*Pavona venosa*	*Pavona*	Agariciidae			D1
MD-346	*Pavona venosa*	*Pavona*	Agariciidae		C1	
MD-324	*Cyphastrea serailia*	*Cyphastrea*	Faviidae		C1	
MD-326	*Cyphastrea serailia*	*Cyphastrea*	Faviidae			D1a
MD-330	*Cyphastrea serailia*	*Cyphastrea*	Faviidae		C1	
MD-334	*Cyphastrea serailia*	*Cyphastrea*	Faviidae		C1	
MD-338	*Cyphastrea serailia*	*Cyphastrea*	Faviidae			D1a
MD-283	*Echinopora gemmacea*	*Echinopora*	Faviidae		C3	
MD-355	*Favia matthaii*	*Favia*	Faviidae		C1	
MD-366	*Favia matthaii*	*Favia*	Faviidae			D1a
MD-296	*Goniastrea aspera*	*Goniastrea*	Faviidae		C3	
MD-287	*Leptoria phrygia*	*Leptoria*	Faviidae			D1a
MD-301	*Leptoria phrygia*	*Leptoria*	Faviidae			D1a
MD-264	*Fungia fungites*	*Fungia*	Fungiidae		C115a	
MD-265	*Fungia fungites*	*Fungia*	Fungiidae		C115a	
MD-266	*Fungia fungites*	*Fungia*	Fungiidae		C115a	
MD-291	*Fungia fungites*	*Fungia*	Fungiidae		C1g	

MD-308	*Fungia fungites*	*Fungia*	Fungiidae	C115a	
MD-309	*Fungia fungites*	*Fungia*	Fungiidae	C115a	
MD-310	*Fungia fungites*	*Fungia*	Fungiidae	C115a	
MD-353	*Hydnophora exesa*	*Hydnophora*	Merulinidae		D1a
MD-271	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1g	
MD-278	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1g	
MD-279	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1g	
MD-299	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1g	D1a
MD-302	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae	C1g	D1a
MD-300	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae	C1g + C(HQ232952)	
MD-261	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C1g	D1
MD-262	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C1g	D1
MD-270	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C1g	D1
MD-289	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C1g	D1
MD-290	*Seriatopora hystrix*	*Seriatopora*	Pocilloporidae	C1g	D1
MD-298	*Stylophora pistillata*	*Stylophora*	Pocilloporidae	C36	D1a
MD-320	*Stylophora pistillata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-322	*Stylophora pistillata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-323	*Stylophora pistillata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-325	*Stylophora pistillata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-343	*Stylophora pistillata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-269	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C36 + EF656433	D1
MD-276	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C36 + EF656433	D1
MD-277	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C36 + EF656433	D1
MD-306	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-307	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C36 + EF656433	D1
MD-312	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C36 + EF656433	D1
MD-313	*Stylophora subseriata*	*Stylophora*	Pocilloporidae	C1g	D1
MD-335	*Goniopora stokesi*	*Goniopora*	Poritidae	C2	D1a
MD-336	*Goniopora stokesi*	*Goniopora*	Poritidae	C1g	
MD-352	*Goniopora stokesi*	*Goniopora*	Poritidae	C2	
MD-260	*Porites lutea*	*Porites*	Poritidae	C15	
MD-267	*Porites lutea*	*Porites*	Poritidae	C15	
MD-268	*Porites lutea*	*Porites*	Poritidae	C15	
MD-288	*Porites lutea*	*Porites*	Poritidae	C15	
MD-303	*Porites lutea*	*Porites*	Poritidae	C15	
MD-304	*Porites lutea*	*Porites*	Poritidae	C15	
MD-327	*Porites lutea*	*Porites*	Poritidae	C15	
MD-328	*Porites lutea*	*Porites*	Poritidae	C15	
MD-329	*Porites lutea*	*Porites*	Poritidae	C15	
MD-344	*Porites lutea*	*Porites*	Poritidae	C15	
MD-347	*Porites lutea*	*Porites*	Poritidae	C15	
MD-360	*Porites lutea*	*Porites*	Poritidae	C15	
MD-361	*Porites lutea*	*Porites*	Poritidae	C15	
MD-362	*Porites lutea*	*Porites*	Poritidae	C15	
MD-342	*Porites palmata*	*Porites*	Poritidae	C15	
MD-348	*Porites palmata*	*Porites*	Poritidae	C15	
MD-349	*Porites palmata*	*Porites*	Poritidae	C15	
MD-351	*Porites palmata*	*Porites*	Poritidae	C15	
MD-280	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-292	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-314	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-315	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-331	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-332	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-333	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-341	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-365	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-367	*Synaraea rus*	*Synaraea*	Poritidae	C15	
MD-339	*Coscinarea monile*	*Coscinaraea*	Siderasteridae	C1g	
MD-340	*Coscinarea monile*	*Coscinaraea*	Siderasteridae	C1g	
MD-354	*Coscinarea monile*	*Coscinaraea*	Siderasteridae		D1

Vohemar: 2001

Sample	Coral species	Genus	Family	Clade A	Clade C	Clade D
MDV-051	*Acropora* sp.	*Acropora*	Acroporidae		C3	
MDV-047	*Acropora cuneata*	*Acropora*	Acroporidae			D1a
MDV-037	*Acropora formosa*	*Acropora*	Acroporidae		C3	
MDV-006	*Acropora gemmifera*	*Acropora*	Acroporidae		C3	
MDV-011	*Acropora gemmifera*	*Acropora*	Acroporidae		C3	
MDV-034	*Acropora humilis*	*Acropora*	Acroporidae	A1	C3	
MDV-039	*Acropora humilis*	*Acropora*	Acroporidae		C3	
MDV-058	*Acropora humilis*	*Acropora*	Acroporidae			D1a
MDV-057	*Montipora monasteriata*	*Montipora*	Acroporidae		C17	
MDV-012	*Pavona cactus*	*Pavona*	Agariciidae		C1	
MDV-038	*Pavona cactus*	*Pavona*	Agariciidae		C1	
MDV-072	*Pavona cactus*	*Pavona*	Agariciidae		C1	
MDV-041	*Pavona decussata*	*Pavona*	Agariciidae		C1	
MDV-045	*Pavona decussata*	*Pavona*	Agariciidae		C1	D1a
MDV-068	*Pavona decussata*	*Pavona*	Agariciidae		C1	
MDV-082	*Pavona varians*	*Pavona*	Agariciidae		C1 + C3	
MDV-070	*Pavona venosa*	*Pavona*	Agariciidae		C1 + C3	D1a
MDV-014	*Cyphastrea micropthalina*	*Cyphastrea*	Faviidae		C1g	D1a
MDV-017	*Cyphastrea micropthalina*	*Cyphastrea*	Faviidae		C1g	
MDV-025	*Cyphastrea ocellina*	*Cyphastrea*	Faviidae		C1g + C24/41	
MDV-055	*Cyphastrea ocellina*	*Cyphastrea*	Faviidae		C1g	
MDV-074	*Cyphastrea serailia*	*Cyphastrea*	Faviidae		C3	D1a
MDV-033	*Echinopora hirsutissima*	*Echinopora*	Faviidae		C1 + C3	
MDV-077	*Echinopora hirsutissima*	*Echinopora*	Faviidae		C1 + C24	
MDV-027	*Favia* sp.	*Favia*	Faviidae		C115a	
MDV-028	*Favia* sp.	*Favia*	Faviidae		C115a	
MDV-049	*Favia* sp.	*Favia*	Faviidae		C115a	
MDV-032	*Favites flexuosa*	*Favites*	Faviidae		C1	
MDV-056	*Favites goniastrea*	*Favites*	Faviidae		C3	D1a
MDV-004	*Favites pentagona*	*Favites*	Faviidae			D1
MDV-024	*Goniastrea retiformis*	*Goniastrea*	Faviidae		C3	
MDV-007	*Leptastrea purpurea*	*Leptastrea*	Faviidae		C1	D1
MDV-065	*Leptastrea transversa*	*Leptastrea*	Faviidae		C1	D1
MDV-001	*Leptoria phrygia*	*Leptoria*	Faviidae		C3	
MDV-023	*Leptoria phrygia*	*Leptoria*	Faviidae		C36	
MDV-043	*Leptoria phrygia*	*Leptoria*	Faviidae		DQ838544	
MDV-069	*Leptoria phrygia*	*Leptoria*	Faviidae		C(HQ232952)	D1a
MDV-059	*Montastrea serageldini*	*Montastrea*	Faviidae		C3	
MDV-073	*Montastrea* sp.	*Montastrea*	Faviidae		C(HQ232950)	
MDV-010	*Platygyra daedalea*	*Platygyra*	Faviidae		C3z	
MDV-018	*Platygyra daedalea*	*Platygyra*	Faviidae		C3	
MDV-022	*Platygyra daedalea*	*Platygyra*	Faviidae		C3	
MDV-029	*Platygyra daedalea*	*Platygyra*	Faviidae		C3	
MDV-083	*Platygyra daedalea*	*Platygyra*	Faviidae		C3	
MDV-016	*Hydnophora exesa*	*Hydnophora*	Merulinidae		C3z	D1a
MDV-026	*Hydnophora exesa*	*Hydnophora*	Merulinidae		C3z	D1a
MDV-081	*Hydnophora exesa*	*Hydnophora*	Merulinidae		C3z	D1a
MDV-002	*Hydnophora microconos*	*Hydnophora*	Merulinidae		C3u + C115a	D1a
MDV-019	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae		C1	
MDV-020	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae		C3	
MDV-040	*Pocillopora damicornis*	*Pocillopora*	Pocilloporidae		C3	
MDV-054	*Pocillopora eydouxi*	*Pocillopora*	Pocilloporidae		C1	
MDV-063	*Pocillopora meandrina*	*Pocillopora*	Pocilloporidae		C1	
MDV-008	*Pocillopora sp.*	*Pocillopora*	Pocilloporidae		C1	
MDV-013	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDV-030	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDV-036	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDV-052	*Pocillopora verrucosa*	*Pocillopora*	Pocilloporidae		C1h	
MDV-015	*Stylophora subsericita*	*Stylophora*	Pocilloporidae		C36 + EF656433	D1
MDV-080	*Porites* sp.	*Porites*	Poritidae		C15	
MDV-048	*Porites palmata*	*Porites*	Poritidae		C15	
MDV-066	*Porites cylindrica*	*Porites*	Poritidae		C15	

MDV-079	*Porites cylindrica*	*Porites*	Poritidae	C15
MDV-046	*Porites lobata*	*Porites*	Poritidae	C15
MDV-064	*Porites lobata*	*Porites*	Poritidae	C15
MDV-009	*Porites lutea*	*Porites*	Poritidae	C15
MDV-044	*Porites lutea*	*Porites*	Poritidae	C15
MDV-062	*Porites lutea*	*Porites*	Poritidae	C15
MDV-086	*Porites lutea*	*Porites*	Poritidae	C15
MDV-003	*Porites palmata*	*Porites*	Poritidae	C15
MDV-021	*Porites palmata*	*Porites*	Poritidae	C15
MDV-067	*Porites* sp.	*Porites*	Poritidae	C15
MDV-053	*Coscinaraea monile*	*Coscinaraea*	Siderasteridae	C3z
MDV-075	*Coscinaraea monile*	*Coscinaraea*	Siderasteridae	C3
MDV-076	*Psammocora haimeana*	*Psammocora*	Siderasteridae	C1
MDV-060	*Psammocora obtusangula*	*Psammocora*	Siderasteridae	C1
MDV-061	*Psammocora obtusangula*	*Psammocora*	Siderasteridae	C1
MDV-084	*Psammocora obtusangula*	*Psammocora*	Siderasteridae	C1