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Program 5(i): **Climate Change: Understanding the threat, ecosystem impacts and mitigation of the Great Barrier Reef**

Project 2.5i.3: Resilience to climate change

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1. Report Summary

To address objectives (a) and (b) of this project, significant progress was made in the technological development of genetic tools and markers for corals and their algal endosymbionts. Microsatellite markers for *Symbiodinium* developed in this project were used to assess patterns of diversity and connectivity of *Symbiodinium type* C2 within populations of the coral *Acropora millepora* in the Central and Southern regions of the Great Barrier Reef (GBR) (Objective a). Multiple *Symbiodinium* C2 clones were present within most coral colonies examined. Furthermore, levels of genetic differentiation of *Symbiodinium* differed significantly among all reefs investigated, suggesting that population connectivity is low. Limited *Symbiodinium* connectivity is due to both lack of active dispersal potential and low passive dispersal potential. Outside of the host, *Symbiodinium* do not have directional swimming ability, are negatively buoyant and spend most of their time in reef sediments (as opposed to the water column). Consequently, there is much less opportunity for *Symbiodinium* to be transported by water currents than positively or neutrally buoyant particles, such as invertebrate and fish larvae that have been the typical models of connectivity on the GBR. In a separate study of connectivity among populations of the brooding coral *Seriatopora hystrix,* additional coral host microsatellite markers are being developed for use with populations that have low genetic diversity.

To identify genes and examine their behaviour in a range of metabolic processes (e.g., oxidative stress and metabolism) (Objective b), we have developed an oxidative stress assay for the coral *Acropora millepora*, which allows for the rapid screening of expression patterns of 16 genes simultaneously. A paper was submitted to the journal *Molecular Ecology*

Resources[1]. We have also tested a high-density oligonucleotide microarray, which we had designed last year for *A. millepora* and its zooxanthellae. Lastly, we have published a paper in the journal *Marine Genomics* reporting on intra-specific variation in gene expression[2].

Increased prevalence of coral disease is one of the projected impacts of climate change on coral reefs. To develop an early warning system of potential coral disease outbreaks that could assist coral reef managers to plan for and mitigate impacts, as well as enable directed studies of the aetiology and impacts of coral disease, we developed a web-based tool that explains 93% of the variation in the abundance of white syndromes (WSs) observed during the 2002 outbreak year (Objective c). A website containing the predictive tool, which forecasts the likelihood of outbreaks of white syndromes (WSs) in northern Australia, is now available through the ReefTemp webpage at CSIRO. Currently, a media release is being drafted to publicly release the tool suite, which is planned to coincide with the updating of the website with this year's predictions in mid-March. A manuscript, which describes the tool, forecasts WS outbreak likelihood in 2009, and tests the efficacy of these forecasts, will be submitted concurrently with this milestone report to *Global Change Biology*. As a consequence of further funding contributions from the Great Barrier Reef Marine Park Authority (GBRMPA) and with the assistance of the Queensland Parks and Wildlife Services (QPWS) in the North Queensland Marine Region, ground-truthing surveys to test the model were conducted late last year on reefs off Port Douglas, where outbreak likelihood was predicted to be high. Surveys showed that: 1) the abundance of white syndromes was greatest at sites where outbreaks were predicted, and 2) that the required density of hosts for an outbreak of WS probably exceeds 45%. The forecasting tool is contributing to the development of GBRMPA's new coral disease response plan, and a manuscript on this project is in preparation.

One of the greatest challenges facing coral reefs is the threat of phase-shifts, particularly an increase in macroalgae. On the GBR today, inner shelf reefs are often dominated by macroalgae. The primary goal of this study was to evaluate the extent to which herbivorous fishes are able to respond to an increase in macroalgae that may arise as a result of human activity or climate change (Objective d). Following the development of a macro-algal bioassay to quantify browsing pressure, a full scale regional sampling programme was undertaken with over 200 experimental assays deployed at the Low Islands, the Whitsunday and the Keppel Islands. Initial results point strongly to a highly restricted suite of fishes capable of removing macroalgae. These species appear to play a similar role on inshore reefs along the entire length of the Great Barrier Reef. The emergent pattern appears to be supporting earlier findings of high local heterogeneity in algal removal rates with just one or two species filling the role in a given location. Removal rates are characterized by high among-site and among-location variation. The capacity of inshore reefs to respond to an increase in macroalgae is likely to be spatially highly variable. It appears to depend, at all scales, on the behaviour and densities of a few key fish species. The primary goal now is to identify potential thresholds and a range of appropriate management options.

To date this work has resulted in a review of herbivory on the GBR (MTSRF internal report), while results have been presented at the 2010 MTSRF Annual Conference, the 11th International Coral Reef Symposium in Florida and at several meetings in Townsville. Three publications have arisen from this work to date: a conference paper (Cvitanovic *et al.* in 2009 MTSRF Conference Proceedings) and two in international journals *Coral Reefs* (Cvitanovic &

[1] Souter, P., Bay, L. K., Andreakis, N., Császár, N., Seneca, F. O. and van Oppen, M. J. H. (Submitted, 2010) A multi-locus, temperature stress related gene expression profile assay in Acropora millepora, a dominant reef-building coral. Submitted to *Molecular Ecology Resources*.

[2] Bay, L. K., Bjorn Nielsen, H., Jarmer, H., Seneca, F. and van Oppen, M. J. H. (2009) Transcriptomic variation in a coral reveals pathway of clonal organisation. *Marine Genomics* 2: 119-125 [doi:10.1016/j.margen.2009.07.004]

Bellwood, 2008, *Coral Reefs* 28:127-133[3]; Bennett *et al.*, *Coral Reefs*, in press). Another is in review and two more publications are in preparation. In terms of outcomes, this work, and meetings with end users, has laid the foundations for further development of coral reef management in order to ensure protection of critical functional groups. Specific herbivorous fishes have been identified as a key ecological feature in the East Marine Region and are likely to be considered a priority for protection when planning conservation policies.

Linked to objective (d), Objective (e) has been developed in the last year and will become the major focus in the final year. Most approaches to date have assumed that visual censuses can give an indication of herbivore activity. Unfortunately visual counts do not equate to fish presence and presence does not reflect removal rates. There is marked regional variation in ecosystem processes, with multiple thresholds based on herbivore presence and realised effect. Based on direct assays and video-based censuses of fish activity, some southern reefs (Keppel Islands) recorded only 5% of the browsing activity of reefs in the northern GBR. Southern reefs have fewer fishes that are less likely to eat macroalgae. These reefs are highly vulnerable to algal overgrowth.

To evaluate the long term recovery and resilience of reef fish communities in the aftermath of the 2001-02 coral bleaching on the central Great Barrier (Objective f), final field surveys were conducted in January 2010. Despite adverse weather conditions (Tropical Cyclone *Olga*), visual censuses were completed at 8 (out of 9) field sites to assess changes in coral cover and composition, as well as abundance and composition of reef fishes. It was not possible however, to progress collection of butterflyfishes, which were intended to provide insights on levels of genetic connectivity and thereby scales of dispersal, for fishes recovering from the 2001-02 coral bleaching and associated habitat degradation. Detailed findings will be presented in a report to be finalised by June 2010, and are expected to be published shortly thereafter. Initial publications arising from this research also include Pratchett *et al.* 2009 (11th ICRS) and Lawton *et al.* 2010 (*Conservation Genetics Resources*).

2. Science Summary

Significant progress was made in the technological development of genetic tools and markers for corals and their algal endosymbionts (Objectives a/b). These will be used to identify genes and examine their behaviour in a range of metabolic processes (e.g. oxidative stress, metabolism), and to assess connectivity among coral reefs in relation to recovery following major disturbances. For gene expression studies, we have developed an oxidative stress assay for the coral *Acropora millepora*, which allows for the rapid screening of expression patterns of 16 genes simultaneously. Further, we have tested a high-density oligonucleotide microarray which we had designed last year for *A. millepora* and its zooxanthellae. Additional microsatellite markers are being developed for the coral *Seriatopora hystrix*.

Microsatellite markers previously developed in this project for *Symbiodinium* were used to assess patterns of diversity and connectivity of *Symbiodinium type* C2 within *Acropora millepora* coral populations in the Central and Southern regions of the GBR. Multiple Symbiodinium C2 clones were present within most coral colonies examined. Further, genetic differentiation of *Symbiodinium* was significantly different between all reefs investigated, suggesting connectivity is low. Limited *Symbiodinium* connectivity is due to a lack of active dispersal potential and low passive dispersal potential. Outside of the host, *Symbiodinium* do not have directional swimming ability, are negatively buoyant and spend most of their time in reef sediments as opposed to the water column. Consequently, there is much less

[3] Cvitanovic, C. and Bellwood, D. R. (2009) Local variation in herbivore feeding activity on an inshore reef of the Great Barrier Reef. *Coral Reefs* 28: 127-133 [doi:10.1007/s00338-008-0433-0]

opportunity for *Symbiodinium* to be transported by water currents than positively or neutrally buoyant particles, such as invertebrate and fish larvae, which have been the typical models of connectivity on the GBR.

In accord with projected increases in coral disease with climate change, links have been made between anomalously high summer temperatures and outbreaks of the group of coral diseases known as white syndromes (WSs) on Indo-Pacific reefs (Objective c). However, further advances in understanding their aetiologies and in developing management actions to mitigate their impacts are hampered by not knowing where or when outbreaks will occur. Before 2009, the only known outbreaks of WSs on the Great Barrier Reef (GBR) were documented in 2002. These sites experienced high values of heating rate; a metric developed as a measure of thermal stress, and also had high cover of corals within the genus *Acropora*, the primary hosts. We developed an empirical regression model that explains 93% of the variation in the abundance of WSs observed during 2002 and suggests that abundance of WSs increases exponentially as heating rate and host cover increase. We used the model to hindcast the likelihood of WS outbreaks on Australian reefs for 2002 and each following summer. The regression model, which combines data from 45 sites surveyed from 2003 to 2008, confirmed that the model produced no false negative or false positive predictions during this period. The model identified reefs with high heating rates in 2009 and forecast high outbreak likelihood in both the north-central and southern GBR. Targeted surveys to evaluate the efficacy of the model detected greatest abundance of WSs at a site with high heating rate but medium host cover. Although a severe outbreak was not documented, abundance of WSs approached outbreak thresholds. The imperfect fit of the forecast with survey data highlights the need to consider host density threshold requirements for outbreaks. Forecasting disease outbreaks in an era of changing climate requires integration of biological and physical data, and can be accomplished for selected disease syndromes. The tools presented here are already helping managers of Australian reefs to target research and monitoring to enable informed responses to disease outbreaks.

We describe the potential vulnerability of marginal coral reef systems to algal overgrowth (Objective d/e). One particularly widespread algal type *Lobophora* (a brown leathery alga) appears to be highly resistant to grazing or browsing by fishes. This species may have the capacity to dominate inshore reefs regardless of herbivore presence. It is this species that has driven localized algal outbreaks in the Keppels Islands in the past. What is particularly striking is that this algal species appears to use corals for cover, as a means of avoiding herbivore activity. This algae shelters under the corals but is ever ready to take over if the corals decline. It appears that herbivorous fishes on some southern GBR reefs may not be able to prevent the proliferation of this algal species.

Given inevitable increases in the scale and severity of climatic disturbances, the future of coral reef ecosystems is contingent on recovery from, and resilience to, episodic disturbances. Ongoing monitoring of fish and coral assemblages in the aftermath of severe coral bleaching (2001-02) in the central GBR has revealed important insights on when, why and how reef populations recover (Objective f). In the eight years since the 2001-02 bleaching there has been notable coral recovery at some (but not all) study locations. Spatial variation in current coral cover (which ranges from 5 to 87% among sites) is strongly linked to differences in larval supply. For fish communities, recovery is conditional upon both larval supply as well as availability of appropriate settlement habitat. Hence recovery is only just beginning to become apparent. Significant progress has also been made towards studying patterns of connectivity in recovering populations for two species of coral-feeding butterflyfishes (*Chaetodon trifascialis* and *Chaetodon lunulatus*), following isolation and characterization of 29 microsatellite loci (Lawton *et al.* 2010). However, adverse weather conditions throughout January 2010 prevented collections of genetic samples from the 9 study locations and surrounding habitats, intended to reveal likely scales over which fishes

are dispersing in order to contribute to slow recovery of fish populations following coral bleaching and habitat degradation in 2001-02.

For reference: Milestones extracted from Project Schedule

Project 2.5i.3, March 2010

a) Genotyping of S*ymbiodinium* C2 within *Acropora millepora* coral populations on the Great Barrier Reef using developed *Symbiodinium* microsatellites to infer the spatial connectivity of populations on reefs within and between the Central and Southern regions of the GBR [(a) AIMS/JCU]

b) Development of new microsatellite markers for *Seriatopora hystrix* on the GBR for parentage analysis in low diversity populations [(a) AIMS/JCU]

c) Submission of a paper (with appropriate attribution of MTSRF funding and copy delivered to the RRC) on high through-put multiplex oxidative stress gene expression method for corals [(b) AIMS/JCU]

d) A report describing the results of Agilent oligonucleotide array testing [(b) AIMS/JCU]

e) Submission of a paper (with appropriate attribution of MTSRF funding and a copy delivered to the RRRC) on intra-colony variation in gene expression levels [(b) AIMS/JCU]

f) Completion of coral disease prevalence surveys at key sites that experienced thermal stress, consistent with models predictions of likely white syndrome outbreaks, during summer 2008-09 [(c) AIMS/JCU]

g) Submission of a paper (with appropriate attribution of MTSRF funding and a copy delivered to the RRRC) that describes the disease-temperature model to predict the likelihood of white syndrome outbreaks in response to SST anomalies on the GBR. Outputs of model delivered to the e-Atlas [(c) AIMS/JCU]

h) An initial report on regional scale variation in inshore reef susceptibility to coral-algal phase-shifts and ecosystem collapse [(d,e) AIMS/JCU]

i) Initiation of quantitative evaluation of herbivore abundances and ecosystem thresholds [(d,e) AIMS/JCU]

j) Collection of genetic material from fish populations using hierarchical sampling across multiple spatial scales to establish relevant scales of population connectivity [(f) AIMS/JCU]

k) A report on status and trends in recovery of fish communities following ongoing recovery of coral habitats in the Central GBR [(f) AIMS/JCU]

3. Project Results

3.1 Resilience of coral assemblages to climate change

a) Genotyping of Symbiodinium C2 within *Acropora millepora* coral populations on the Great Barrier Reef using developed *Symbiodinium* microsatellites to infer the spatial connectivity of populations on reefs within and between the central and southern regions of the GBR [Objective a, AIMS/JCU]

Very little is known about the population dynamics of *Symbiodinium* within both host and free living habitats, as well as the ability for *Symbiodinium* to disperse and recruit to neighbouring or distant reefs (= population connectivity). To date, there has been one previous investigation of *Symbiodinium* population connectivity on the Great Barrier Reef (GBR),

which demonstrated that populations of *Symbiodinium* type (C1:3a sensu La Jeunesse) known only to associate with soft corals were disconnected at distances greater than ~15 km (Howells *et al.* 2009). The objective of this study was to expand on this information by investigating connectivity for one of the most common *Symbiodinium* strains on the GBR.

Genetic variation of *Symbiodinium* type C2 (sensu van Oppen = C3 sensu La Jeunesse) was investigated within samples of the scleractinian coral *Acropora millepora* from three sites at the Palm Island group within the Central GBR and an additional two sites within the Southern GBR (n = 34-50 colony samples per site; Figure 1). Each sample was genotyped with six microsatellite loci described in Bay *et al.* (2009) and an additional two microsatellite loci described in Howells *et al.* (2009). Microsatellite loci were amplified using PCR at the Australian Institute of Marine Science and allele size differences were determined using capillary electrophoresis at the Genetic Analysis Facility at James Cook University. The presence/absence of alleles within samples was scored and population genetic differentiation was evaluated using analysis of molecular variance (AMOVA; performed in GENALEX v6, Peakall and Smouse 2006) and Bayesian cluster analysis of sample allelic profiles (performed in STRUCTURE v2.3.2, Pritchard *et al.* 2000).

The composition of *Symbiodinium* C2 within colonies of *Acropora millepora* was genetically diverse, with an average of 2.5 alleles per locus detected per colony (range = 1-5) and 4.4 alleles per locus per reef (range = 2-8). Population genetic structure of *Symbiodinium* was significantly different between all reefs investigated (AMOVA, Φ_{ST} = 0.421, P = 0.001; Table 1). Both of the Southern GBR reefs showed the greatest differentiation to each other as well as to all reefs in the Central GBR, which were more closely related to one another (Table 1; Figure 2). The two most similar reefs to one another were those on either side of the Orpheus-Pelorus channel, ~1 km apart, yet their genetic composition was still significantly different from one another.

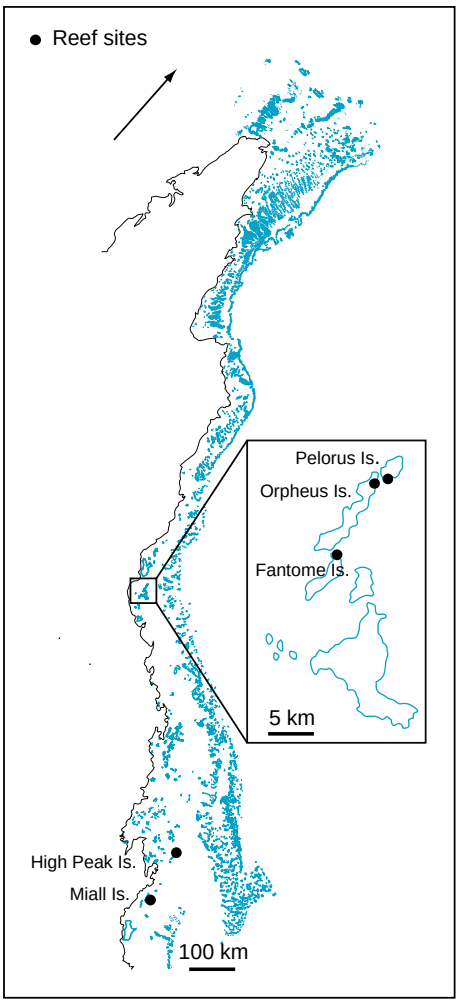

Figure 1: Location of five reef populations of *Symbiodinium* type C2 hosted by *Acropora millepora* on the Great Barrier Reef.

High genetic structure of populations of the generalist *Symbiodinium* type C2 is consistent both with the population structure of the specialist *Symbiodinium* type C1:3a on the Great Barrier Reef (Howells *et al.* 2009) and with *Symbiodinium* type B1 in the Caribbean (Santos *et al.* 2003, Kirk *et al.* 2009, Thornhill *et al.* 2009). Limited *Symbiodinium* connectivity is due to a lack of active dispersal potential and low passive dispersal potential. Outside of the host, *Symbiodinium* do not have directional swimming ability, are negatively buoyant and spend most of their time in reef sediments as opposed to the water column. Consequently, there is much less opportunity for *Symbiodinium* to be transported by water currents than positively or neutrally buoyant particles, such as invertebrate and fish larvae, which have been the typical models of connectivity on the GBR.

A potential benefit of limited connectivity is that *Symbiodinium* populations may in fact be well adapted to local environmental conditions as selective processes will be relatively uninhibited by recruitment of new genotypes to a population. However, a potential cost of limited

connectivity is that *Symbiodinium* populations could be vulnerable to disturbance because any reduction of population size and/or genetic diversity cannot be readily replenished from external population sources.

(Acknowledgements: B.L. Willis, M.J.H. van Oppen, A.M. Jones & R. Berkelmans collected coral samples analysed in this project; Muirhead & A. Jones contributed to DNA extractions and *Symbiodinium* type I.D.)

Table 1: Differences in genetic structure among reef populations of *Symbiodinium* type C2 hosted by *Acropora millepora* from the central and southern Great Barrier Reef (GBR). Results of analysis of molecular variance: population pairwise genetic differences (Φ_{ST}) are given below the diagonal and significance of comparisons (*P*) are given above the diagonal.

	Central GBR			Southern GBR	
	Pelorus Is.	**Orpheus Is.**	**Fantome Is.**	**High Peak Is.**	**Miall Is.**
Pelorus Is.		0.001	0.001	0.001	0.001
Orpheus Is.	0.080		0.001	0.001	0.001
Fantome Is.	0.292	0.160		0.001	0.001
High Peak Is.	0.351	0.396	0.521		0.001
Miall Is.	0.464	0.515	0.585	0.604	

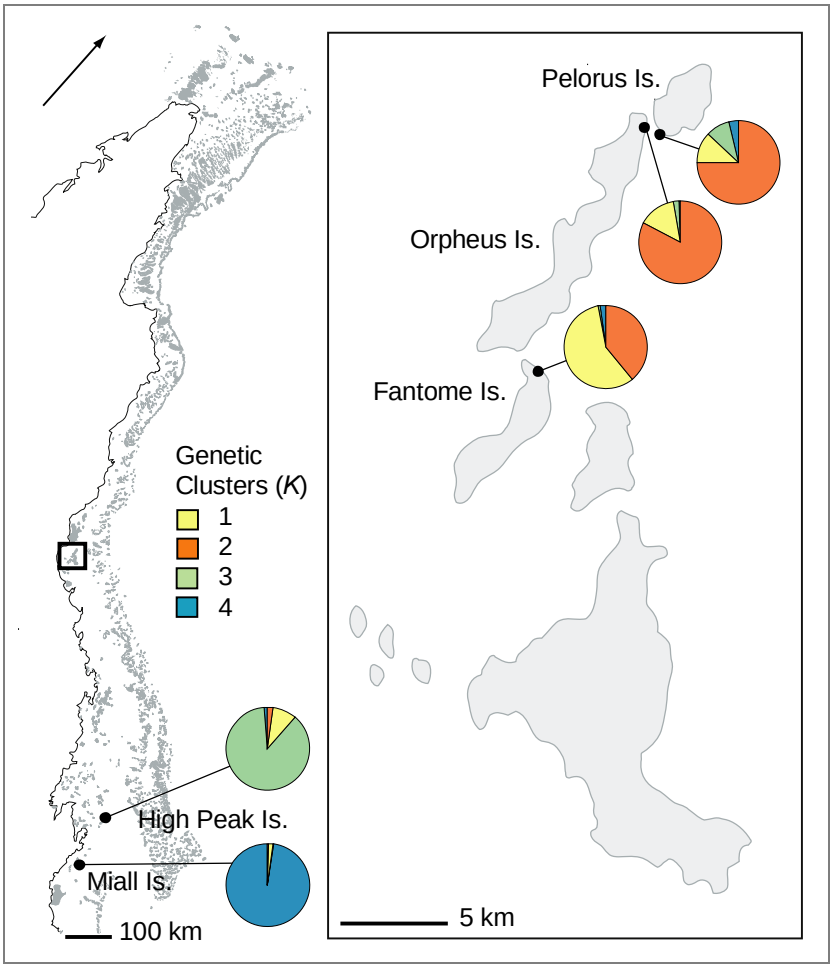

Figure 2: Genetic structure of reef populations of *Symbiodinium* type C2 hosted by *Acropora millepora* on the Great Barrier Reef. Results of Bayesian cluster analysis in the program STRUCTURE, where different colours in pie charts represent the genetic composition of samples within a population.

b) Development of new microsatellite markers for *Seriatopora hystrix* on the GBR for parentage analysis in low diversity populations [Objective a, AIMS/JCU]

Previous MTSRF-funded work has indicated that the reef at Cattle Bay, a sheltered area along northwest Orpheus Island, supports a highly dense yet genetically depauperate population of the abundant brooding coral, *Seriatopora hystrix*. Of the populations of this species that have been genotyped across the Northern GBR, Cattle Bay remains one of the least genetically diverse locations of more than 20 sampled (van Oppen *et al.* 2008). In order to investigate low-diversity populations of *S. hystrix*, the 10 previously developed microsatellite markers developed by Underwood *et al.* (2006) were assessed but do not provide enough information to satisfactorily identify and assess relatedness. Therefore in 2009, live coral samples were collected from Cattle Bay at the northwestern area of Orpheus Island. These coral fragments were gradually bleached with the herbicide Diuron in order to minimize the contamination of genetic material from *Symbiodinium* symbionts. Following bleaching, one individual from the population was selected to develop a microsatellite-enriched genomic library by methods described in Glenn and Schable (2005).

We originally screened 192 bacterial colonies with positive microsatellite inserts, and found that approximately 68% (130 colonies) contained inserts of the targeted size range (600-1200bp). Ninety-six promising colonies were sent to an external service provider (MACROGEN) for DNA sequencing. The resultant sequences were screened for microsatellite repeat motifs, with preference to long repeats of di-, tri-, and tetra-nucleotide repeating units with the web-based software WebSat. From the 96 sequences, eleven potential microsatellite regions were identified. Primer pairs were designed and commercially ordered from Sigma.

Further testing of the eleven potential markers on a small sample of individuals (n=10) from both high and low-diversity populations, indicated that three of the eleven were good candidates for further testing on larger sample sizes (i.e. showing polymorphism and limited technical artifacts, such as stuttering and multiple binding sites). These three primer pairs are currently being tested on thirty individuals from Cattle Bay to assess the allelic diversity and usefulness for studies of low-diversity populations. Subsequently, these same primers can be tested on individuals from a range of populations to assess whether any might enhance the power of currently available markers for most populations.

Finally, the remaining bacterial colonies from the original cultures were screened for additional microsatellite inserts. Between those previously identified, but not sequenced, and those newly identified, we recently sent 84 additional microsatellite inserts to be sequenced. The resulting sequences will be screened as before, and further testing will ensue to identify additional potentially informative markers.

Overall, we identified and will have sequenced 180 colonies (~80% of cultured colonies) with microsatellite inserts of the targeted size range. To date, approximately 10% of returned sequences have yielded potential markers with 30% of those (3% of the total) yielding promising candidates for informative markers in low-diversity populations. For comparison, the previous work by Underwood *et al.* (2006) using the same protocol on *S. hystrix* yielded the same number of positive insert colonies (n=180) for sequencing. Overall, 89 microsatellites were identified, of which 36 loci were selected for primer pair design. Work by Underwood *et al.* (2006) resulted in 10 loci (ranging from 3 to 22 alleles per locus), which is approximately 5% of the sequenced colonies.

Although the 5% development rate of Underwood *et al.* (2006) is comparable to our 3% potential success rate, it is clear that isolating regions from an individual in a low diversity population (Cattle Bay) yields a more limited selection of regions of nucleotide repeats (10%

or 11/96 in this study compared to 50% or 89/180 in Underwood *et al*. (2006)). However, if we can expect similar success with the remaining 84 samples, there is a possibility of developing at least 2-3 additional microsatellite markers for a potential total of 5-6 microsatellite markers for low-diversity populations.

c) Submission of a paper (with appropriate attribution of MTSRF funding and copy delivered to the RRRC) on high through-put multiplex oxidative stress gene expression method for corals [Objective b, AIMS/JCU]

The following paper was submitted to the journal *Molecular Ecology Resources* on 3 February 2010: Souter, P., Bay, L. K., Andreakis, N., Császár, N., Seneca, F. O. and van Oppen, M. J. H. (Submitted, 2010) A multi-locus, temperature stress related gene expression profile assay in Acropora millepora, a dominant reef-building coral. Submitted to *Molecular Ecology Resources*.

Abstract:

We report an accurate multiplex PCR-based assay, capable of reproducing gene-expression profiles from 16 target genes (12 genes of interest [GOIs] and four reference genes [RGs]) in *Acropora millepora,* a common reef-building model coral species. The 12 GOIs are known or suspected to be involved in the coral bleaching response, but the method is not restricted to this particular assay and gene set. The procedure is based on the Beckman Coulter (Fullerton, CA, USA) GenomeLab™ GeXP Genetic Analysis System and bridges the gap between quantitative real-time PCR (qPCR) expression analysis of a single or a small number of genes and microarray gene expression surveys of thousands of genes. Despite large variation among biological replicates, the majority of GOIs were up-regulated (up to 4000%) in most colonies during a laboratory-based thermal stress experiment. Two genes, *Nf-kβ2* and *MnSod*, were consistently up-regulated in all colonies tested and we therefore propose these as candidate markers useful for population-level evaluations of thermal stress. Our assay provides an important new tool for coral bleaching studies; due to the lower cost, labour and amount of cDNA required compared to single-plex qPCR, population level studies with large biological replication are feasible.

d) A report describing the results of Agilent oligonucleotide array testing [Objective b, AIMS/JCU]

The Agilent oligonucleotide array designed using *Acropora millepora* and *Symbiodinium* clade C sequence data was successfully tested in 2009. We achieved this by labelling one heat stressed and one control (unstressed) sample with one dye each (Cy3 or Cy5) and then hybridizing each of four pairs of two samples to one of four arrays comprised of three pairs of *A. millepora* and one pair of *A. tenuis* (Table 2).

The accuracy of signal intensities was examined using MA plots. Briefly, MA plots are used to compare signal intensities of two samples labelled with different dyes on the same array. $M = \log_2 Cy5 - \log_2 Cy3$ and $A = ½ \times (\log_2 Cy5 + \log_2 Cy3)$. The expectation (and underlying assumption of microarray data analysis) is that most spots will be located in a data cloud centred on M = 0 (i.e., most genes are not differentially expressed) regardless of expression intensity (A). We obtained good results using only 50 ng of mRNA starting material in most of our comparisons. *Acropora tenuis* did not hybridise well and this was particularly evident in the red channel (Cy5). This result can be seen in Figures 3 and 4 as the upward projecting data cloud on the left hand side of the plots. This species therefore appears unsuitable for use with our *A. millepora* array although further tests will have to be conducted before this can be conclusively established. The distribution of spike-in control spots indicated successful hybridization. As expected, probes with expected equal intensity between the Cy3 and Cy5 channels occurred in the middle of the data cloud (Figure 3 – yellow dots). The

control spots, where the Cy5 signal was expected to be three times (red) and ten times (pink) greater than the Cy3 signal, fell above the yellow probes. Conversely, the probes with three times (green) and ten times (blue) greater Cy3 compared to Cy5 signal intensity fell below the equal intensity yellow probes (Figure 3). Both coral and *Symbiodinium* probes showed a range of signal intensities, as indicated by their spread along the x-axis (Figure 4). Negative control probes and dark corners predictably displayed low intensities. Approximately 80% of coral probes displayed signal intensities above background (negative controls) when hybridized with *A. millepora* RNA. This indicates that our array provides useful signal intensities for most coral probes; however it is unclear if the 20% of probes for which signal intensities fell below detection was because of poor probe fit or because of generally low expression. Approximately 95% of the *Symbiodinium* probes displayed signal intensities above background when hybridized with C type RNA. This indicates that our array is suitable for measuring gene expression in almost all included *Symbiodinium* probes. Approximately 40% of *Symbiodinium* probes displayed signal intensities above background when hybridized with clade D RNA, indicating lower probe fit across *Symbiodinium* types. A study to examine acclimatization in gene expression in *A. millepora* and its *Symbiodinium* populations is currently being undertaken using our oligonucleotide array.

Table 2: The coral host and *Symbiodinium* strain hybridised to four test microarray slides.

Array number	Coral species	*Symbiodinium* type
1	*A. millepora*	C2
2	*A. millepora*	C2
3	*A. millepora*	D
4	*A. tenuis*	C1

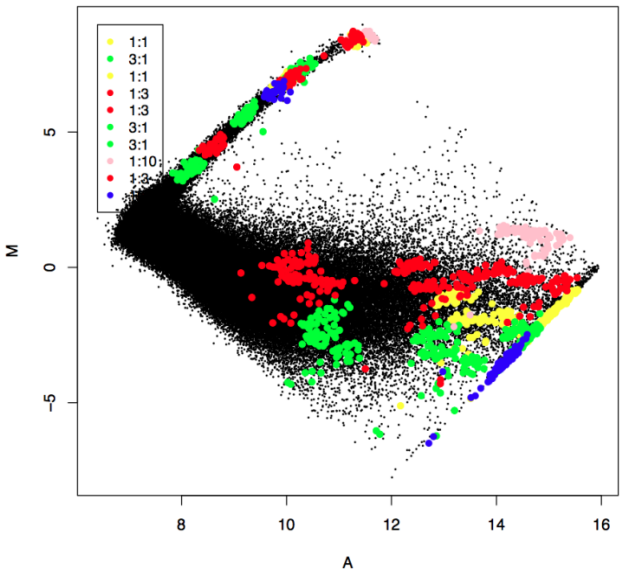

Figure 3: MA plot of the four arrays combined with spike-in controls highlighted in colour.

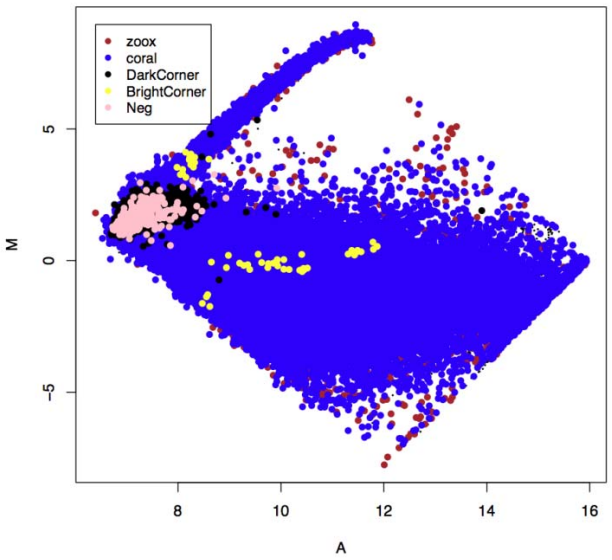

Figure 4: MA plot of the four arrays combined with coral and *Symbiodinium* probes indicated in blue and red respectively. Please note that the *Symbiodinium* probes are hidden below the coral probes.

e) Submission of a paper (with appropriate attribution of MTSRF funding and a copy delivered to the RRRC) on intra-colony variation in gene expression levels [Objective b, AIMS/JCU]

The following paper was submitted and accepted: Bay, L. K., Bjorn Nielsen, H., Jarmer, H., Seneca, F. and van Oppen, M. J. H. (2009) Transcriptomic variation in a coral reveals pathway of clonal organisation. *Marine Genomics* 2: 119-125 [doi:10.1016/ j.margen.2009.07.004]

f) Completion of coral disease prevalence surveys at key sites that experienced thermal stress, consistent with models predictions of likely white syndrome outbreaks, during summer 2008-09 [Objective c, AIMS/JCU]

Our white syndrome (WS) outbreak likelihood model was run in forecast mode for the 2009 summer and areas of high outbreak likelihood were identified on reefs in the north-central (Port Douglas region) and southern GBR (Capricorn Bunkers region). As a consequence of further funding contributions from the Great Barrier Reef Marine Park Authority (GBRMPA) and with assistance from the Queensland Parks and Wildlife Service (QPWS in the North Queensland Marine Region, targeted surveys were completed at twenty sites on ten reefs in the Port Douglas region in October 2009. Outbreak likelihood was predicted to be high on ten of these reefs and low on the remaining ten reefs based on our metric of thermal stress (MPSA, the mean positive summer anomaly). Surveys comprised three replicate manta tows covering an approximate area of 500m^2, within which all cases of WSs were recorded. To further verify manta tow observations, replicate 20 m x 2 m belt transects were completed on SCUBA, within which all corals were classified as healthy or diseased, with specific details of diseases recorded.

In the Port Douglas region, survey data from the Australian Institute of Marine Science Long-term Monitoring Program indicated that few of the reefs with high MPSA values in 2009 had host covers greater than 50%, which was the minimum cover documented at sites with severe WS outbreaks in previous surveys. Unsurprisingly, no outbreaks were documented at eight low-cover (<30%) sites that had high MPSA values (>0.35°C) in 2009, and no outbreaks were documented at six low-cover sites that had low MPSA values. Importantly,

no sites with medium host cover (30-50%) are known to have experienced high MPSA values from 2002 to 2008, highlighting that the host threshold density for an outbreak of WS remains uncertain. Six sites with medium host cover were also surveyed in the north-central GBR in 2009. Outbreak likelihood was predicted to be low at four of these sites and, in accordance with predictions, no outbreaks were documented. Thus, as with all other years since 2002, there were no known false negative predictions in 2009. Notably though, host cover was patchy but between 30% and 40% on all transects at two sites surveyed in 2009 that were predicted to have high outbreak likelihood. Given the interaction between high host cover and high MPSA values suggested by the regression model, the likelihood of a WS outbreak in 2009 was higher at these two sites – Mackay and Opal Reefs in the north-central GBR – than anywhere else surveyed in the GBR. Although WS abundance corresponding to a severe outbreak (>100 cases /1500 m^2) was not documented at either site, more cases of WS were observed at Opal Reef (abundance equated to 45 cases/1500 m^2) than on average (4.03 $\pm$ 0.95) or at any of the other sites surveyed in 2009.

g) Submission of a paper (with appropriate attribution of MTSRF funding and a copy delivered to the RRRC) that describes the disease-temperature model to predict the likelihood of white syndrome outbreaks in response to SST anomalies on the GBR. Outputs of model delivered to the e-Atlas [Objective c, AIMS/JCU]

The following paper was submitted to the journal Global Change Biology and accepted:

Maynard, J.A., Anthony, K.R.N., Harvell, C.D., Burgman, M.A., Beeden, R., Heron, S.F., Lamb, J.B. and Willis, B.L. (Submitted, 2010) Forecasting climate-driven coral disease outbreaks. *Global Change Biology.*

Abstract:

Coral reefs are already highly endangered by warming events associated with climate change. Links between anomalously high temperatures and outbreaks of coral diseases known as white syndromes (WS) in the Indo-Pacific represent a further threat, but further advances in understanding aetiologies of disease and in developing management actions to mitigate their impacts are hampered by not knowing where or when outbreaks will occur. Before 2009, the only known outbreaks of WS on the Great Barrier Reef (GBR) were documented in 2002. Outbreak sites experienced high values of temperature stress, as measured by the mean positive summer anomaly (MPSA), and had high cover of corals within the genus *Acropora*, the primary hosts. Here, we use an empirical regression model based on the MPSA and *Acropora* abundance at outbreak sites to hindcast the likelihood of WS outbreaks on Australian reefs for 2002 and subsequent summers. Data from 45 sites surveyed from 2003 to 2008 confirmed that the model never predicted outbreaks when there were none, nor failed to predict an event that occurred. The model identified reefs with high MPSA values in 2009 and forecast high outbreak likelihood in both the north-central and southern GBR. Targeted surveys to validate the model detected greatest WS abundance at a site in the north-central GBR with a high MPSA value but medium host cover. Although a severe outbreak was not documented, WS abundance approached outbreak thresholds. This discrepancy between model predictions and survey data highlights the need to further consider host density effects in the triggering of WS outbreaks. Forecasting disease outbreaks in an era of changing climate requires integration of biological and physical data, and can be accomplished for selected disease syndromes. The approach and forecasting model for coral disease outbreaks developed here can be widely adapted for use in other ecosystems.

The predictive tools (model outputs for the years 2002-2009) are now available through the ReefTemp webpage at CSIRO. Currently, a media release is being drafted through the ARC Centre of Excellence for Coral Reef Studies that will be reviewed by the GBRMPA in the coming weeks to publicly release the tool suite, which is planned to coincide with the updating of the website with this year's predictions in late March. Datasets were submitted in KML format to the e-Atlas for review.

3.2 Resilience of reef fish assemblages to climate change

h) An initial report on regional scale variation in inshore reef susceptibility to coral-algal phase-shifts and ecosystem collapse [Objective d, e, AIMS/JCU]

i) Initiation of quantitative evaluation of herbivore abundances and ecosystem thresholds [Objective d, e, AIMS/JCU]

Major findings to date:

1. **The vulnerability of marginal systems:** One algal species, *Lobophora* (a brown leathery alga), appears to be highly resistant to grazing. This species may have the capacity to dominate inshore reefs regardless of herbivore presence. It is this species that drove algal outbreaks in the Keppels and elsewhere on inshore reefs. This is one algal species that herbivores may struggle to control (details in Bennett ***et al.***, *Coral Reefs* in press).

   Positive news: We know that outbreaks of algae are highly likely in the Keppels (due to extensive algal reservoirs and low browsing rates) but that grazing scarids are the most useful agents in limiting the extent of subsequent macroalgal cover.

2. **Extensive spatial variation in the magnitude of algal removal:** We found huge variation in the capacity of reef herbivores to remove algae on inshore reefs. In the north (Cairns Low Isles region) algal removal was strong with good prospects for continued coral development. In contrast the Keppel Islands had just five percent of the effective herbivore activity, due to low herbivore numbers and low feeding rates. Thresholds are going to depend on both fish abundance and behaviour. This suggests that southern reefs and the Keppels in particular, are more likely to be dominated by macro algae and once in place the algae are likely to remain.

   Positive news: Some northern reefs have healthy herbivore communities where browsing surgeonfish and rudderfish can help keep areas clear of algae. The Keppels, however, are more vulnerable to prolonged domination by algae (details in Bennett & Bellwood, manuscript in review).

To date:

1. All major field work, video and statistical analyses of initial field data are completed.
2. We are currently exploring thresholds on inshore reefs, incorporating additional data sets to explore why inshore reefs have such low grazing rates and high algal cover.
3. Three papers have been published, one is in review and two more are in prep.
4. All field data are currently being delivered to the e-Atlas.

j) Collection of genetic material from fish populations using hierarchical sampling across multiple spatial scales to establish relevant scales of population connectivity [Objective f, AIMS/JCU]

The primary aim of Objective f was to evaluate the long term recovery and resilience of reef fish communities to climate change induced habitat degradation. A key component of resilience among coral reef fishes is the scale of dispersal relative to the spatial scale of most major disturbances, whereby larvae from nearby unaffected population are required to re-

establish local populations devastated by episodic disturbances. Recent recovery (appearance of larval recruits) among coral-feeding butterflyfishes that were devastated during the 2001-02 coral bleaching in the central GBR (Pratchett *et al.* 2006), provided an ideal opportunity to establish relevant scales of dispersal among these fishes based on collections of new larvae as well as potential parental individuals at a hierarchy of spatial scales.

To progress this study, two study species were identified; i) *Chaetodon trifascialis*, which went locally extinct following the 2001-02 coral bleaching at Trunk Reef, and ii) *Chaetodon lunulatus*, which was formally the most abundant butterflyfish at Trunk Reef and exhibited the largest absolute declines in abundance of all species (Pratchett *et al.* 2006). Genetic material for both these species was obtained from the northern GBR (outside the area affected during the 2001-02 bleaching) to develop independent microsatellite markers for studies of population connectivity. A total of 13 polymorphic microsatellite loci were identified and described that are suitable for quantification of population connectivity for *C. trifascialis*, and 16 polymorphic microsatellite loci developed for *C. trifascialis* (Lawton *et al.* 2010).

Following development of appropriate microsatellite markers (described above), collection of genetic material from *Chaetodon trifascialis* and *Chaetodon lunulatus* were planned for January 2010. It was not possible however, to progress collections of butterflyfishes from within the affected area for two reasons: i) rates of replenishment (especially for *C. trifascialis*) are still very low, such that any significant collections for genetic analyses would further inhibit rates of recovery, and ii) adverse weather conditions have thus far, prohibited extensive collections of potential parental individuals across a wide range of locations beyond the major study locations. As such, results of this study, which was a recent addition (2009) to the milestones for Project 2.5i.3(f), will not be available for at least twelve months. It is hoped however, that additional funding can be sort to progress the project as planned.

k) A report on status and trends in recovery of fish communities following ongoing recovery of coral habitats in the central GBR.

The purpose of this study was measure rates of recovery in butterflyfish and coral assemblages at Trunk reef, Great Barrier Reef (GBR), Australia, following climate-induced coral bleaching in 2001-02. Coral depletion was further exacerbated by localised outbreaks of *Acanthaster planci* causing extensive and widespread coral loss throughout the region (Pratchett *et al.* 2009a). Periodic monitoring of butterflyfish and coral assemblages have been undertaken at Trunk Reef since May 2000 (before the bleaching), testing for long-term changes in abundance of both fish and corals. Until 2005, both coral cover and abundance of most butterflyfishes had exhibited significant declines (Pratchett *et al.* 2006). It was expected however, that coral cover would have increased significantly from 2005, and that coral recovery would initiate increases in the abundance of coral-dependant butterflyfishes. Annual monitoring of coral communities and fishes (including butterflyfishes, as well as six other major families of coral reef fsihes) was conducted at Trunk Reef, as well as Rib and Bramble in January 2008, 2009 and 2010. Three sites were surveyed at each reef, giving a total of nine study locations. The recovery and replenishment of fish and coral assemblages was further related spatial variation in topographic complexity at each of the nine locations.

Up until 2008, coral cover had increased to 6.5% (±1.0 SE) mainly due to increased cover of *Acropora* species. However, there has not been any concomitant increase in the abundance of butterflyfishes (Figure 5). Mean densities of butterflyfishes declined from 19.5 (±1.2SE) fishes per 200m^2 (per transect) in March 2000 down to 3.7 (±0.4SE) fishes per 200m^2 in January 2008. The greatest decline occurred between 2002 and 2005, but there had been

further (albeit not significant) declines between 2005 and 2008 (Figure 5).The group of butterflyfishes that exhibited the most rapid and dramatic declines in abundance following extensive coral depletion were the obligate coral feeders (comprising *Chaetodon aureofasciatus, C. baronessa, C. lunulatus, C. plebeius, C. rainfordi,* and *C. trifascialis*). Facultative corallivores (e.g., *C. citrinellus* and *C. melannotus*) and non-coral feeding butterflyfishes (e.g. *C. auriga* and *C. vagabundus*) did not exhibit any significant variation in abundance to 2005, but there have been significant recent declines in abundance (Figure 5, *right*).

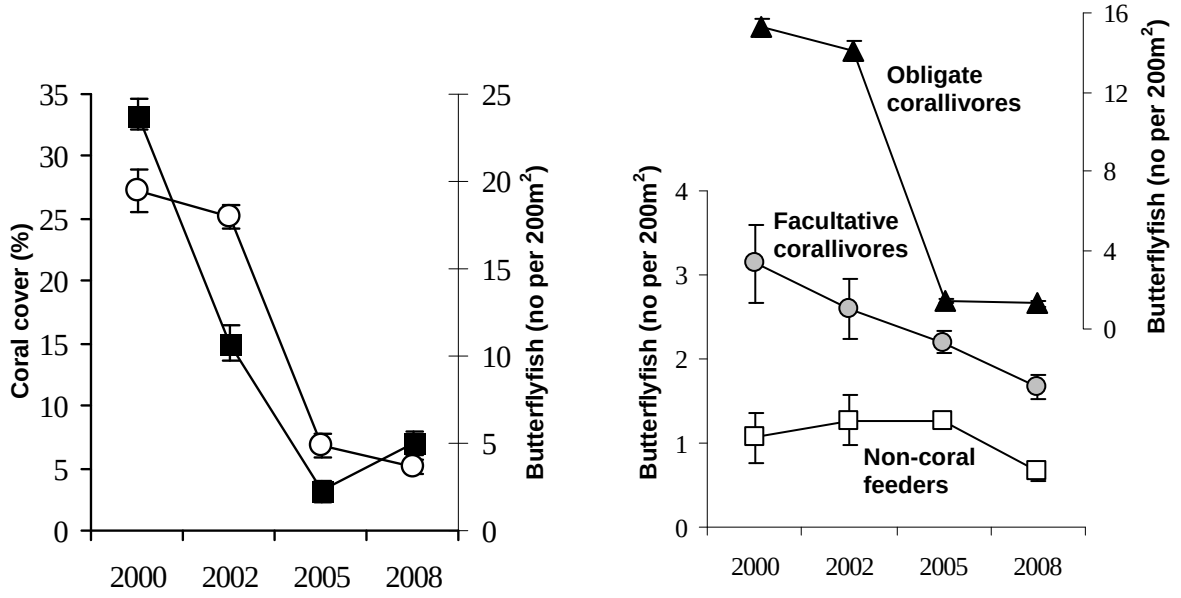

Figure 5: (***Left***) Mean ± SE percentage cover of scleractinian corals (■) and densities of butterflyfishes (O) at Trunk Reef in May 2000 (before bleaching), March 2002 (during bleaching), March 2005, and January 2008 (post-bleaching). (***Right***) Temporal variation in mean ± SE densities of butterflyfishes within each trophic group (i. obligate corallivores, ii. facultative corallivores, and iii. non-coral feeders) on the reef crest at Trunk Reef. Figures redrawn form Pratchett *et al.* (2009).

From 2008 to 2010, coral cover has continued to increase across the central GBR, but recovery has been very patchy. Currently (in January 2010), coral cover ranges from <5% to >80% on the reef crest among the nine study sites. To understand these differences in coral recovery, counts of juvenile corals (reflective of population replenishment) were conducted across the nine different study locations, revealing that sites with rapid recovery of coral cover are those that have the greatest density of juvenile corals. This suggests that population replenishment is limiting coral recovery; through it is not known whether variability in replenishment relates to differences in larval supply, differential suitability of substrates for coral settlement, or spatial variation in post-settlement survival of scleractinian corals. To better understand the role of post-settlement processes in driving recovery of coral cover, we also measured the size structure of *Acropora hyacinthus*, which is the dominant coral on the reef crest in the central GBR, in each year from 2008 to 2010. Comprehensive analyses of this data, testing for variation in coral growth and mortality, will be reported in the final report to be presented in June 2010. In addition, data on coral composition (rather than coral cover) will be presented, which is critical in assessing resilience of coral assemblages. This data is not yet available as photo transects from the field trip in January 2010 are not yet processed.

In terms of coral reef fishes, local densities of adult butterflyfishes are steadily increasing at some, but not all study sites. More importantly, significant number of new recruits (<1 year

old) of many butterflyfishes, including many obligate coral-feeding species, were recorded in 2009 and 2010. Notably however, there has been no apparent recovery of fish populations, nor any significant settlement of coral dependent fishes at sites with limited coral cover. Overall, there are two sites with very low coral cover (<10%), four sites with moderate cover (20-40% cover), and three sites with high coral cover (>50% cover). Based on comprehensive sampling of coral reef fish assemblages at each location, it will be possible to relate patterns of abundance for different reef fishes to the recovery of corals and/ or the structural complexity of reef habitats in each location. Coral cover and topographic complexity are critical and distinct components of coral-reef habitats (Pratchett *et al.* 2008), but few studies have been able to separate these factors and test their independent effects on the structure and abundance of coral reef fishes (Pratchett *et al.* 2009b). Comprehensive analyses are awaiting input of coral data from the most recent field trip (January 2010), but will be presented at the 2010 MTSRF Annual Conference and will be clearly outlined in the final report (June 2010).

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