Australian Government
Department of the Environment, Water, Heritage and the Arts

Marine and Tropical Sciences Research Facility Milestone Report, March 2009

Program 5(i): **Climate Change: Great Barrier Reef**

Project 2.5i.3: **Resilience to climate change**

Project Leader: **Professor Terry Hughes, James Cook University**

1. Summary of milestone report

Good progress has been made towards understanding the resilience of coral and reef fish assemblages to climate change on several fronts in the past eight months.

Paternity analyses were applied to a coral species for the first time to elucidate processes governing connectivity among populations of brooding corals and to determine the spatial extent of sperm dispersal. Preliminary results based on microsatellite genotyping of larval and adult populations of *Seriatopora hystrix* indicate exclusively sexually produced larvae, multiple paternity broods and limited sperm dispersal.

An oligonucleotide microarray has been developed for coral gene expression studies that includes 10,920 *A. millepora* ESTs and 1,269 *Symbiodinium* ESTs. Heat stressed and unstressed samples have been collected to test the array.

A new and efficient method for high-throughput gene expression analysis has been developed based on recently purchased software at the Australian Institute of Marine Science (AIMS). Preliminary trials indicate that the method was successful for 17 of the 18 genes tested and results are highly reproducible.

Annual summer surveys of coral disease prevalence have been completed in the northern and southern sectors and are nearing completion in the central sector for 2009. A modelling framework for investigating the relationship between coral disease abundance and thermal anomalies has been further refined. A regression model has been developed to fit the relationship between white syndrome abundance and both *Acropora* cover and heating rate, which explains greater than eighty percent of the variability seen in surveys. The model will form the basis for a predictive tool to forecast disease risk.

Preliminary results of regional studies evaluating the susceptibility of macroalgae to browsing by reef fishes indicate a highly conserved group of herbivores that exhibit high spatial variability at local scales.

Reef & Rainforest RESEARCH CENTRE

Microsatellite markers have been developed for two species of corallivorous butterfly fish and will provide tools to better understand population connectivity and limitations to recovery for these species following climate-induced coral loss.

2. Summary of project objectives

2.1. Resilience of coral assemblages to climate change

(a) Estimate genetic connectivity among GBR populations of coral and their algal endosymbionts to determine their potential for replenishment following disturbances associated with climate change.

Connectivity within and between coral populations is an important component of coral reef resilience. Exchange of larvae creates and maintains high levels of genetic diversity and buffers populations against disturbance. Migrants may carry new alleles that may be integrated into populations through reproduction, creating new gene combinations on which selection can potentially act. The spread of selectively advantageous alleles at DNA loci involved in physiological responses such as bleaching resistance is a potentially important consequence of migration. Furthermore, gene flow increases local effective population sizes, thereby enhancing the ability of populations to resist rapid random changes in allele frequencies from one generation to the next through drift. Larval-exporting or source reefs with diverse populations of healthy adult corals are essential to maintain the genetic diversity and resilience of larval-importing or sink reefs. Therefore, an assessment of larval transport in and out of reefs, i.e. the extent to which reefs are self-seeding or accumulate recruits from surrounding areas, as well as the direction of larval dispersal will improve our ability to forecast how reef corals are likely to respond to environmental change. Successful migrants leave a genetic signature of their movements and allow inference of connectivity using population genetic methods. We will estimate genetic connectivity among GBR populations of coral and their algal endosymbionts using analysis of DNA microsatellite loci, and link this information to hydrodynamic models to provide improved estimates of reef connectivity.

(b) Identify mechanisms of adaptation available to local coral populations to understand their potential for adaptation to climate change.

Observed differences in bleaching sensitivity between geographically distinct, conspecific coral populations may be caused by differential expression of genes involved in the bleaching response (as a consequence of either local adaptation or acclimatisation), and/or by the presence of distinct alleles at these loci (due to selection and local adaptation). We will identify fast diverging genes (that are therefore likely to be under selection) in a GBR coral species that is known to show a latitudinal gradient in thermal tolerance using DNA microarray technologies. Common garden experiments and microarray/quantitative real time PCR analyses will subsequently be used to examine whether or not genetically determined (i.e. a consequence of selection and hence reflecting adaptation) differences in gene expression levels exist between these latitudinal populations.

Theoretical models of the potential for corals to evolve greater bleaching resistance in response to climate change will be developed as part of this objective. Results from the empirical studies described above will be used to parameterise these models and model outputs will be used in turn to guide the design of further experiments.

(c) Identify links between thermal anomalies and coral disease dynamics to predict the response of coral assemblages to ocean warming associated with climate change.

Increases in the severity and frequency of wildlife disease epidemics over the past three decades are thought to be linked, in part, to increasing thermal stress associated with

climate change. Understanding the implications of increasing ocean temperatures for the spread of coral pathogens and for disease resistance of corals will significantly enhance current understanding of the resilience of GBR coral assemblages in relation to climate change. We will determine the linkages between seasonal thermal anomalies and the prevalence of coral disease. A modelling approach will be used to evaluate metrics of thermal anomalies based on NOAA satellite data that best explain spatial and temporal patterns in the prevalence of coral disease on the GBR. The relationship between peaks in disease prevalence and thermal anomalies will be analysed to determine thermal thresholds associated with outbreaks of coral disease. We will also identify interactions between bleaching and disease. We aim to produce algorithms to (a) relate temperature to past disease outbreaks, and (b) develop a product that provides predictive outlooks for outbreaks of key coral diseases, similar to the NOAA hotspot algorithm that predicts bleaching events.

2.2. Resilience of reef fish assemblages to climate change

(d) Quantify current levels of herbivory by reef fishes on the GBR and evaluate the extent to which reefs across the GBR shelf are vulnerable to ecosystem phase-shifts and domination by macroalgae as a result of climate change.

(e) Identify critical thresholds in macro-algal phase shifts and evaluate alternate management strategies in order to limit the impacts of climate change on the ability of fish assemblages to prevent ecosystem phase-shifts on coral reefs.

Climate change will influence the community structure of reef fish assemblages, however, it is unknown if these changes will affect ecosystem processes, and subsequently lead to a phase shift from coral to algal dominated reefs. Here, we will utilise and build on existing databases of the distribution and abundance of herbivorous fishes across the GBR to quantify current rates of herbivory. These data will be combined with direct experimental analyses of fish-algal interactions that will enable us to estimate the current capacity of GBR reef fish populations to maintain low macroalgal cover on mid and outer reefs.

The second part of this objective will use a modelling approach to permit direct estimation of critical thresholds in the coral – macroalgal phase shift and to evaluate alternative management strategies to respond to changes in macro-algal distributions. We will combine the results from part one with existing algal distribution data and algal growth trajectories to model fish-algal interactions and outcomes under a range of climate change scenarios. Furthermore, it will provide us with an indication of the relative resilience of different components of the GBR ecosystem. In particular, it will indicate to what extent the current stands of macroalgae on inshore reefs reflect a state of heightened vulnerability to climate change.

(f) Evaluate the long term recovery and resilience of reef fish communities to climate change induced habitat degradation.

The most immediate and substantial effects of climate change on coral reefs are severe episodes of climate-induced coral bleaching, which cause widespread mortality of reef corals. Extensive mortality of reef corals results in the loss of essential habitat for coral reef fishes, leading to reduced abundance and localised extinction of coral reef fishes. While many studies have documented sudden declines in the abundance of fishes immediately following extensive coral depletion, the degree to which fish communities are resilient and may eventually recover is currently unknown.

We will conduct a detailed analysis of the recovery and resilience of fish assemblages at Trunk Reef, central GBR, which were severely impacted by climate induced coral bleaching in 2001-02. Recovery of reef fish assemblages is likely to be contingent upon increases in coral cover and a return to pre-disturbance structure of benthic communities. Changes in the

structure and dynamics of fish communities will be monitored annually and directly compared to temporal changes in the physical and biological structure of benthic habitats.

3. Project Results

Objective	Targeted Activity for 2008/2009: 1 March 2009 Report	Completion Date
(a)-(f)	Participation in MTSRF wide meeting regarding climate change modeling, scenario development and mapping of deliverables across the MTSRF.	Before December 2009
(a)	Preliminary findings of microsatellite genotyping of larval and adult populations of the brooding coral *Seriatopora hystrix* from the Palm Islands [AIMS/JCU]	1 March 2009
(b)	Preliminary report on analyses of trait evolution and mutualism for modelling bleaching resistance evolution [AIMS]	1 March 2009
(b)	Report on development of an oligonucleotide microarray for coral gene expression studies [AIMS/JCU]	1 March 2009
(b)	Report on development of a higher throughput gene expression method [AIMS/JCU]	1 March 2009
(c)	Report on surveys of coral disease prevalence at key sites missing from JCU / AIMS LTMP coral disease surveys [JCU]	1 March 2009
(c)	Progress report on developing a modeling framework for investigating the relationship between coral disease abundance and ocean warming [JCU]	1 March 2009
(d), (e)	Report preliminary findings of regional scale variation in capacity of reefs to respond to increased macroalgae on the GBR, and delivery of outcomes to the Risk, Resilience and Response Atlas (Project 1.1.5) [(d/e) JCU] subject to external funding	1 March 2009
(f)	Preliminary findings of microsatellite development for key butterflyfishes as a potential tool for measuring population connectivity as a key factor in population resilience [JCU]	1 March 2009
(a-f)	Summary of any communication activities undertaken to date, including minutes of meetings / workshops if applicable [JCU/AIMS]	1 March 2009

Preliminary findings of microsatellite genotyping of larval and adult populations of the brooding coral *Seriatopora hystrix* from the Palm Islands [(a) AIMS/JCU]

Processes that govern the connectivity of brooding coral populations are likely to be different to those that govern connectivity in broadcast spawning species, because of differences in the frequency and scale of dispersal associated with internal versus external fertilisation. Limited sperm dispersal in brooding species is predicted to amplify signals of geographic subdivision, but current knowledge of the frequency and scale of dispersal of spermatozoa in brooding corals is scant. This study determines the spatial extent of spermatozoa dispersal in the brooding coral, *Seriatopora hystrix*, in Cattle Bay (Orpheus Island) using genetic paternity analysis. This type of genetic parental analysis has never been performed in corals. In addition, it determines small scale patterns of genetic structure in this coral species.

Sixty-two adult *S. hystrix* colonies within a 12 m x 12 m study quadrat in Cattle Bay, Orpheus Island were mapped (Figure 1) in November 2007 and genotyped. Two of ten microsatellite loci used for genotyping were found to be fixed (i.e. invariable) for the population. For the eight other loci typed, the mean allelic diversity was 3.00 (± 0.327) alleles per locus. Three

loci demonstrated significant departures from Hardy-Weinberg Equilibrium at p<0.05. Approximately 86% of the individuals had unique multilocus genotypes.

Fifteen colonies from within the quadrat were brought into the lab and larvae were collected from each of these (Figure 1). So far, DNA has been extracted from 150 larvae and one third of the 150 larvae were successfully typed at all ten loci; the genotyping success rate was not uniform across the fifteen "mother" colonies. The DNA extracted from the small tissue amounts yielded by individual larvae proved unstable and thus unsuitable for re-runs and further adjustments to the multiplex reactions. Appropriate extraction techniques and multiplex reactions for the larvae are being optimised.

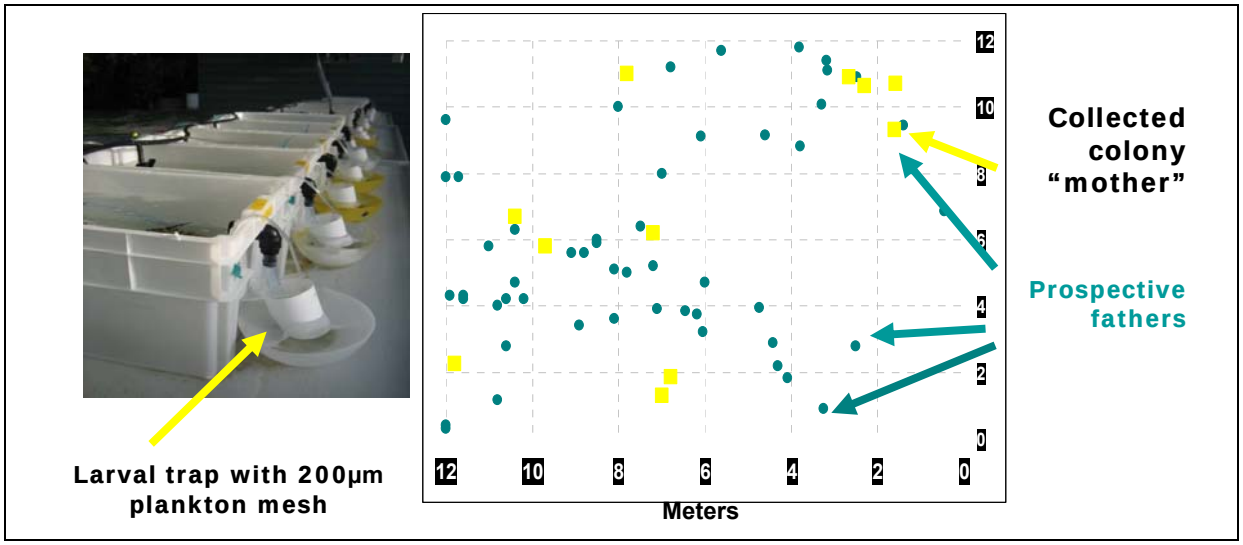

Figure 1: (*Right hand panel*) Map of the first quadrat showing the positions of 62 adult S. hystrix colonies. The yellow squares indicate the mother colonies brought into the laboratory for larval collection; the green circles represent potential father colonies. (*Photograph, left*) The tank set-up containing mother colonies and the traps to capture larvae.

The two loci that were fixed in the adults were also fixed in the larval sub-population, and mean allelic diversity was 2.75 (± 0.366) alleles per locus in the larvae. Only one locus demonstrated significant departures from Hardy-Weinberg Equilibrium at p<0.05.

The allelic diversity of this adult population is lower than that observed in samples collected over a slightly larger area in the same bay in 2003 (van Oppen *et al.* 2008). Given the very low allelic diversity and the presence of two fixed loci, more microsatellite loci need to be developed for satisfactory assignment of parentage in this population (which will take place in April 2009), however, preliminary results are discussed below.

Our analysis revealed exclusively sexually produced larvae, multiple paternity broods, and three instances of apparent self-fertilisation. Paternity assignments indicated limited sperm dispersal and no dominant direction of dispersal within the mapped area. We also assessed the gametogenic state (Figure 2) of twenty tagged colonies in the field over three months of biweekly histological monitoring (September to December) to compare the frequency and periodicity of reproductive events with those reported for southern GBR populations of *S. hystrix*. We found overlapping cycles of gametogenesis, which is consistent with multiple larval release events per year.

The study was repeated in October 2008 using a 10 m x 10 m study quadrat in Cattle Bay (OI), where 77 adult *S. hystrix* colonies were sampled for genetic analysis. Samples are still being processed for parentage analysis. Furthermore, four other reefs in the Palm Islands

are being sampled for an overall local population genetic survey of the region. The five reefs (Cattle Bay (sheltered), Pioneer Bay (s), SW Pelorus (s), SE Pelorus (exposed), NE Orpheus (e)) are each being sampled at two sites of fifty colonies.

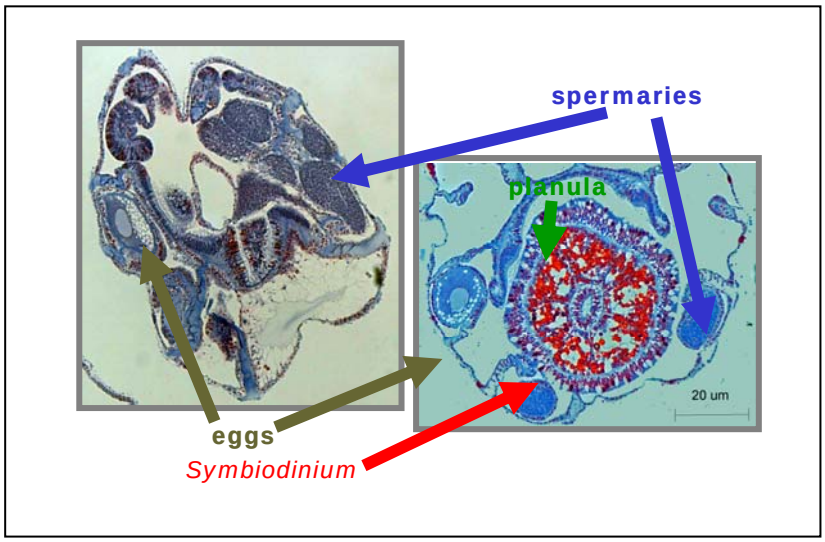

Figure 2: Photographs of histological slides showing eggs, spermaries and larvae within a single polyp of *Seriatopora hystrix* collected on 19 November 2007.

Preliminary report on analyses of trait evolution and mutualism for modelling bleaching resistance evolution [(b) AIMS]

This milestone was met in the June 2008 report and hence has been deleted.

Report on development of an oligonucleotide microarray for coral gene expression studies [(b) AIMS/JCU]

We have designed a dual genome oligonucleotide array using all publicly available *Acropora millepora* and *Symbiodinium* C2 ESTs. The array contains 10,920 *A. millepora* ESTs and 1,269 *Symbiodinium* ESTs, 100 *A. millepora* control probes, and 50 *Symbiodinium* control probes. All probes are 60 nucleotides long and three replicate probes have been designed for each EST. This results in an array with < 39,000 probes in total and allowed a 44K array to be printed (four arrays per slide). Samples to test the array have been collected. *A. millepora* containing C2 and D *Symbiodinium* respectively, and *A. tenuis* containing C1 *Symbiodinium* were heat shocked in the laboratory and stressed and unstressed samples were snap-frozen in Liquid Nitrogen. These samples will be used to verify the suitability of designed probes for 1) *A. millepora* host expression, 2) *Symbiodinium* C2 expression, and 3) cross species hybridization potential for *A. tenuis* host and *Symbiodinium* C2 and D gene expression. These hybridisation trials will be undertaken during March and April 2009.

Report on development of higher throughput gene expression method [(b) AIMS/JCU]

The Centre for Marine Microbiology and Genetics research (CMMG) at AIMS has recently purchased a software extension to existing equipment (Beckman Coulter CEQ8800 Sequencer) for multiplex quantitative, high-throughput gene expression analysis (GeXP). This method fills the gap between microarray (thousands to tens of thousands of genes) and real time quantitative PCR (up to ~10 genes), and allows multiplexing of up to thirty genes in a time and cost efficient manner. The method has not yet been widely used, but is explored here for corals. It is explained in Figures 3 and 4 below.

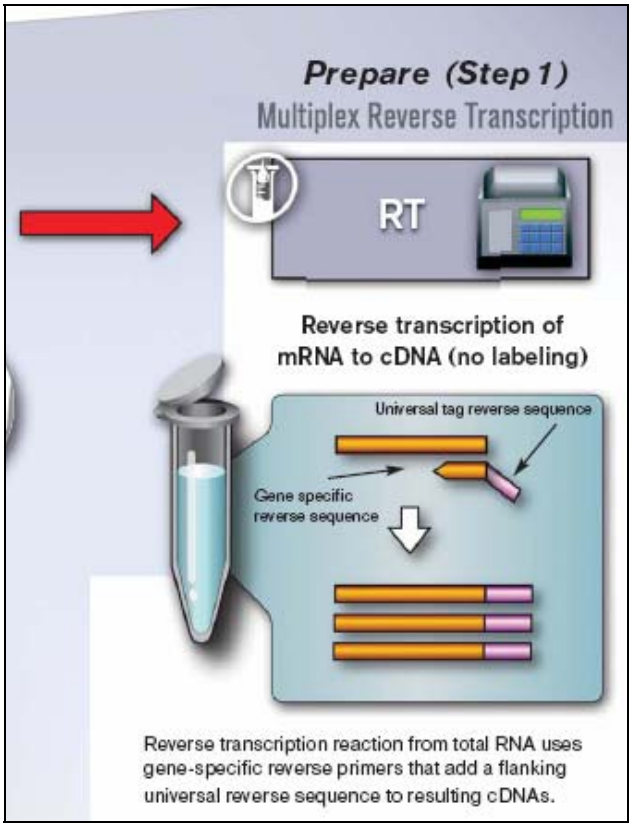

Figure 3: First step in GeXP method.

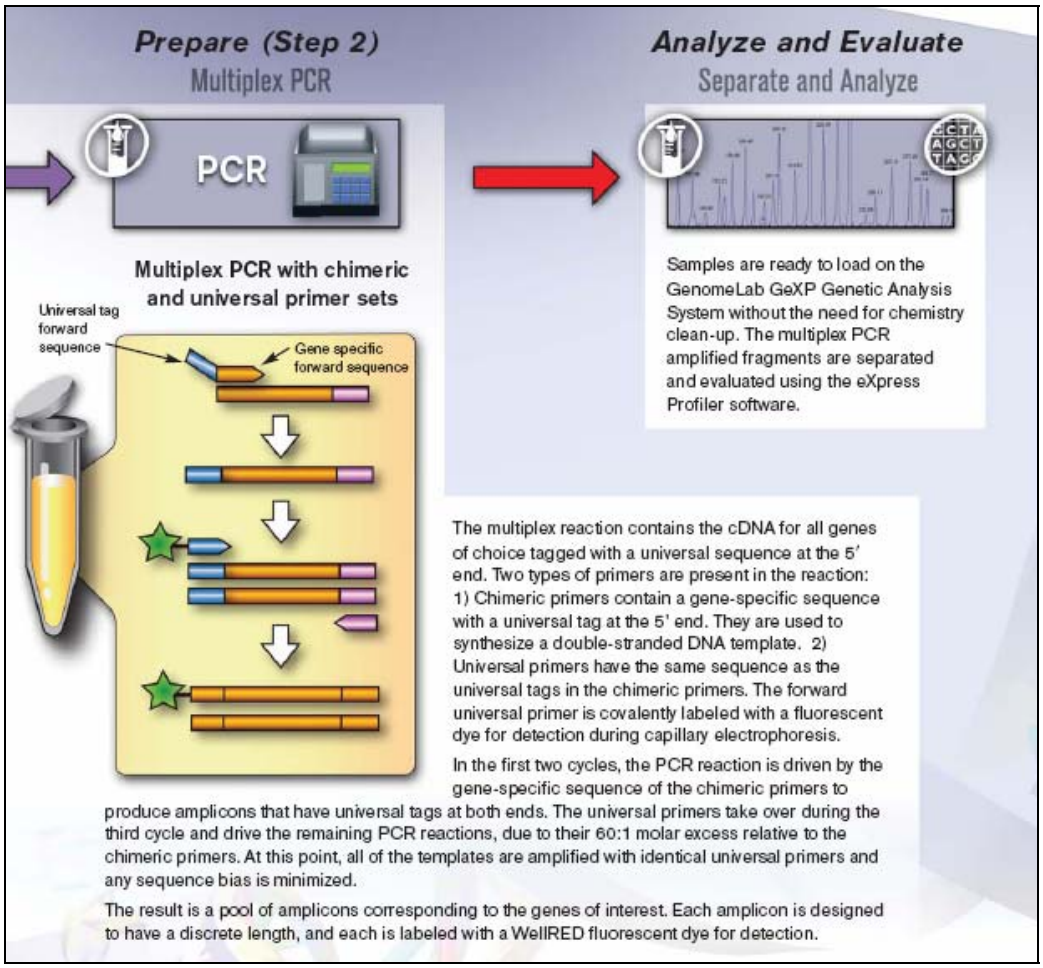

Figure 4: Second and analysis step in GeXP method.

A pilot experiment was designed using publically available sequence data from eighteen genes (Table 1) in *Acropora millepora*, which are combined in a multiplex GeXP assay. Genes were chosen for their known or suspected roles in the oxidative stress response or for their potential to serve as internal control genes (i.e. to normalise expression data against).

Table 1: Genes used in GeXP pilot assay. Those names followed by "CSAZSAR" are genes targeted in a real time quantitative assay performed by Nikolaus Császár (MTSRF Project 2.5i.2, heritability) and will be used to validate the GeXP method.

Gene Name
SOMA_FERRITIN
NFKB2
ZN_METALLOPROTEASE
THIOREDOXIN
Urokinase
GAPDH
RPS7
CATALASE
SOD_MN_CZAZSAR
PEROXIDASIN_HOMOLOG_PRECURSOR
HSP70A_CZAZSAR
SUPEROXIDE_DISMUTASE
CZAZSAR_FERRITIN
RIBOL9_CZAZSAR
CTG_1913_CZAZSAR
RPL9
Glutathione Peroxidase
NFKB1

PCR primer pairs were designed for the above genes and a human internal control gene (Kan(r)) with which the reagents are spiked. The primers were designed so that expected PCR products differed in size and can be separated by electrophoresis on the CEQ8800. Initial transcription used pooled messenger RNA isolated from heat stressed *A. millepora* samples from Orpheus Island (MTSRF project 2.5i.2, heritability) as a template, using one forward primer and all reverse primers in each reaction. Fig. 5 shows the internal control gene Kan(r) (the largest amplification product on the gel) co-amplified with individual genes (of different sizes) and a few faint non-specific products. Because the latter are of very different sizes compared to target genes, they will not interfere with down-stream analysis.

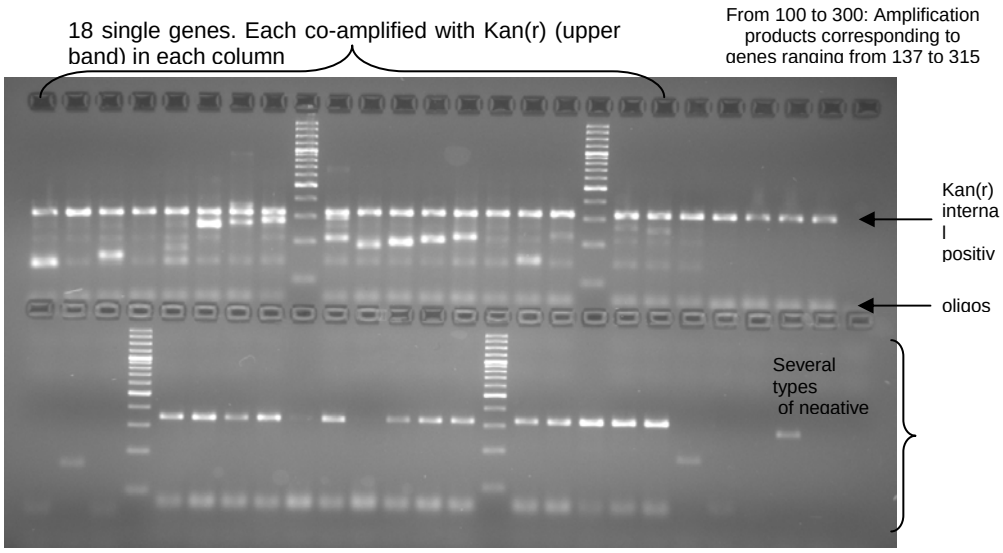

Figure 5: Gel picture showing the results of the PCR reactions using one forward primer and a mix of all reverse primers. Oligos = primers and primer dimers.

These single amplification products were run individually on the CEQ8800 (not shown) and this confirmed (by the size of the products) that these were the genes listed in Table 1.

Following this, all eighteen genes were co-amplified in a single reaction using messenger RNA isolated from a non-stressed and the same heat-stressed *A. millepora* colony from Orpheus Island as template. The amplification products were run on the CEQ8800. The results demonstrate (1) that the multiplex assay was successful for 17 of the 18 genes tested (Figure 6), and (2) that the method is highly reproducible (Figure 7). Four of the 17 genes included are known to show even expression levels throughout stress and non-stress conditions, which can therefore be used to normalise the expression levels of the other 13 genes. The GeXP data will be compared to real-time PCR data on the same samples for validation of the method. This data analysis is underway.

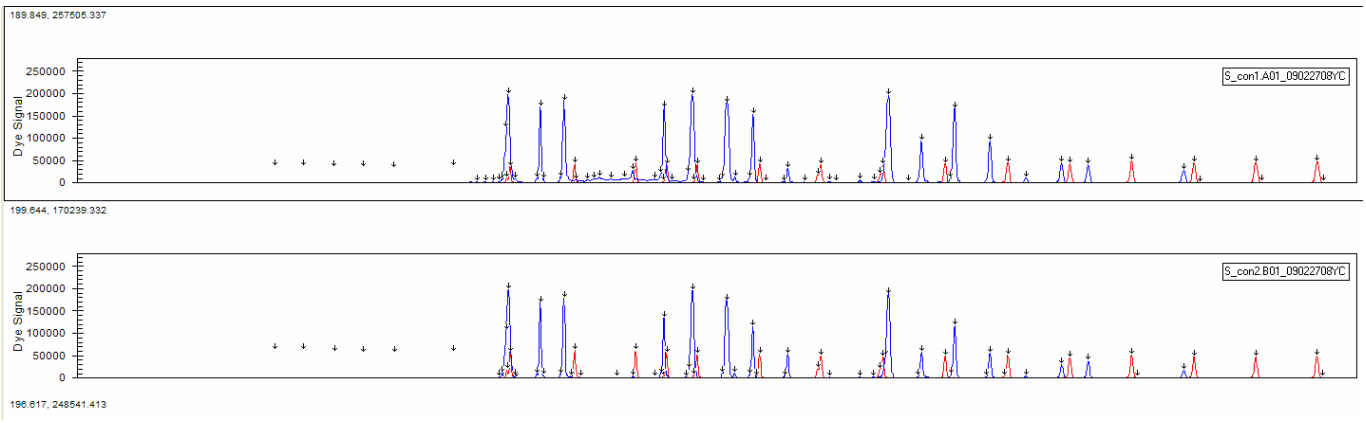

Figure 6: Multiplex electrophoresis profile of the seventeen coral genes and the Kan(r) (blue peaks) and internal size marker (red peaks). Top graph shows non-stressed profiles, bottom graph shows stressed profiles for the same coral colony.

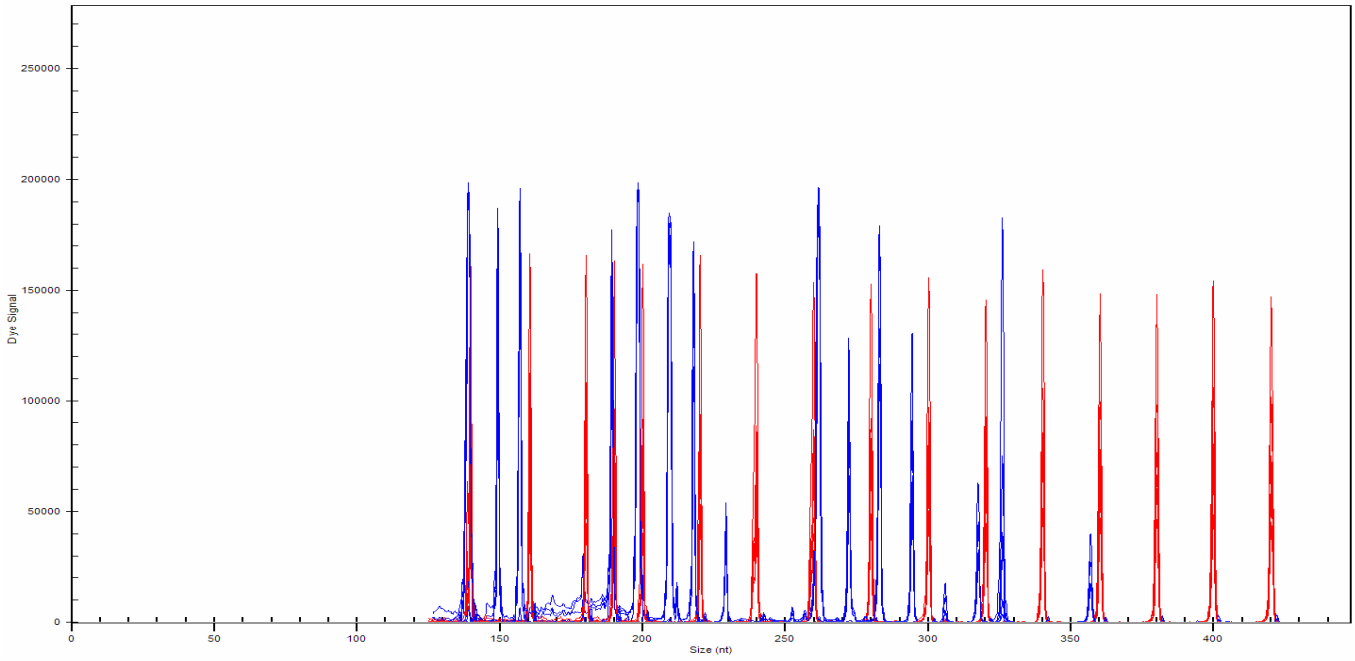

Figure 7: Overlayed multiplex electrophoresis profile from four independent runs, showing excellent reproducibility.

Report on surveys of coral disease prevalence at key sites missing from AIMS LTMP surveys [(c) JCU]

The fifth year of summer surveys of coral disease prevalence at inner, mid, and outer-shelf reefs in the northern, central and southern sectors of the GBR should be completed by mid March. Surveys of coral disease prevalence were completed for nine reefs (18 sites) in the northern Lizard Island sector, six reefs (12 sites) in the central Townsville Sector and three reefs (eight sites) in the southern Heron Island sector in January and February of 2009. Surveys at the remaining three inshore reefs in the central Townsville Sector will be completed as soon as weather permits. Surveys were funded primarily by the ARC Centre of Excellence for Coral Reef Studies, with supplementary funding by the MTSRF. Because the AIMS monitoring program is surveying the Long-term Monitoring Program (LTMP) sites in 2008-2009, additional surveys at missing LTMP sites were not required this year.

Progress report on developing a modelling framework for investigating the relationship between coral disease abundance and ocean warming [(c) JCU]

The work presented below and the proposed development of the predictive tool for white syndrome disease outbreaks on the GBR represent the outcomes of a close collaboration with Jeffrey Maynard from the University of Melbourne, who was appointed to work on this MTSRF project approximately six months ago and accordingly has been added to the project team.

1. Background: Value of predictive tools for coral disease

Since our last report, we have developed a model (see Section 2 below) that predicts outbreaks of white syndrome (WS), a coral disease that has amongst the highest impacts on GBR corals. The predictive tool we now aim to develop based on the model (see Section 3 below) will spatially display the presence of the sorts of temperature regimes that caused the white syndrome outbreaks which were observed by members of the Australian Institute of Marine Science's Long-term Monitoring Program (AIMS LTMP) in late 2002. Predictive tools will result in targeted, and hence cost-effective, monitoring of the onset and outbreak of WS at sites where temperature regimes suggest outbreaks are likely to occur. The project has met all milestones set at the project's inception and is now set to go several steps further through the release of predictive tools that we endeavour to make publicly available by mid to late April of this year. By being able to predict where WS outbreaks are likely to *have* occurred, as well as where they are likely *to* occur, we will be able to initiate research programs to address questions that have been difficult to impossible to address using available datasets. These include:

a) Is WS transmission density-dependent and hence most likely pathogen induced?
b) What percentage of WS cases result in mortality?
c) Does the percentage of WS cases that result in mortality vary spatially?
d) Does susceptibility to WS, whatever the cause, vary spatially and what can the variability be attributed to?

Importantly, shedding light on these questions will provide the basis to answer important management questions, like:

- Can susceptibility to and recovery from WS be influenced by actions that managers can take?
- Can WS outbreak risk be reliably modeled and displayed spatially based on proxy indicators of host cover and climate change-based projections of summer temperatures?

Given the applicability of the results of this project to management, the Great Barrier Reef Marine Park Authority (GBRMPA) has expressed interest in becoming a key partner in this project. The Climate Change Group from the GBRMPA has expressed interest in co-funding the production of a web-based predictive tool for coral diseases over the coming months.

2. Results: Predictive models for white syndrome

A growing body of evidence suggests that the highest abundance of coral diseases like white syndrome occurs in the months that follow higher-than-average summer seawater temperatures. Indeed, on the GBR, average white syndrome abundances were much higher following the exceptionally warm 2001 to 2002 summers than in any other year that reefs have been surveyed by the AIMS LTMP. We explored the links between temperature and coral disease abundance using high-resolution sea surface temperature data collected by environmental monitoring satellites. Recent advances in processing remotely sensed temperature data now ensure that data are closely correlated with the temperatures that corals experience on shallow reefs. White syndrome abundance varied widely in 2002, but our results indicate that most (>85%) of the variability can be explained by: 1) percent cover of corals in the genus, *Acropora,* which is the predominant host for white syndromes, and 2) the rate at which temperature stress accumulates, i.e. the 'heating rate'. Heating rate works to describe how anomalous a temperature regime is in a given year, in comparison to average temperatures experienced at that site in the preceding ten summers.

Our results also indicate that 100% of the sites monitored by the AIMS LTMP in the last ten years that had greater than 45% cover of *Acropora* and experienced a summer heating rate greater than 0.55, had a severe WS outbreak (>200 cases). Only four sites meet these criteria and WS outbreaks occurred at all of these sites in late 2002. This result comes from a regression model written to fit the relationships between *Acropora* cover and WS abundance and between heating rate and WS abundance (exponential for both factors in the model). Survey time was standardised to a three-month window and all sites were excluded that had less than 10% *Acropora* cover. For the resulting dataset of the 2002 outbreak, both heating rate and *Acropora* cover on their own explain greater than 80% of the variability seen. Use of the variables on their own, however, makes little sense biologically as we know that a high heating rate and high *Acropora* cover do not cause WS outbreaks independent of each other. The best-fit mathematical model produced supports that WS outbreaks require both a high heating rate and high *Acropora* cover (see Figure 8).

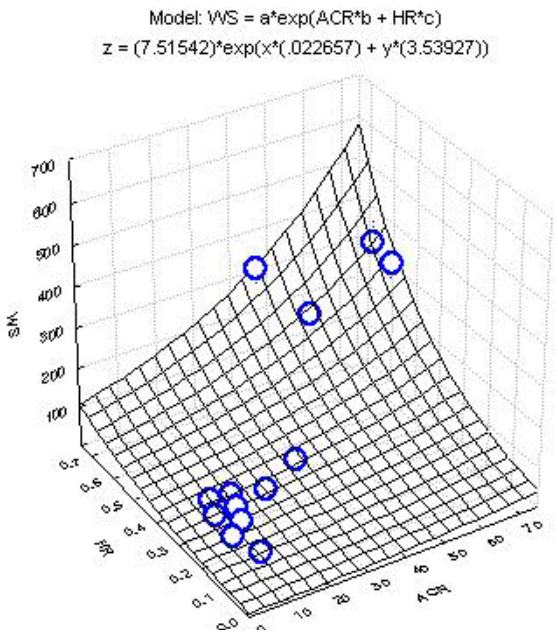

Figure 8: Significant (p=0.05) regression model depicting the relationships between Acropora cover, heating rate, and WS abundance.

A predictive tool based on the regression model described here will shed important light on a current debate on the causes of white syndromes on the GBR, specifically the role of 'programmed cell death', a natural cell remodeling process which gets out of control, versus a pathogen(s) as a causative agent. More importantly, white syndromes kill corals and the predictive tool we aim to develop in the coming months will aid managers in elucidating and, if justified, implementing management actions that: a) work to reduce susceptibility to WS and/or, b) reduce the timeframes over which colonies and reefs recover from WS.

3. Next steps: Predictive tool development and publication

In the coming two months a predictive tool based on the model described in the section above will be developed in close collaboration with managers, specifically those most likely to find it useful including, but not limited to, staff from the GBRMPA and the Department of Environment, Water Resources, Heritage and the Arts. Images will be produced that display, in red, locations where heating rates have exceeded the values known to cause the white syndrome outbreaks observed in 2002. These images, effectively maps of northern Australia, will have reef and island overlays that facilitate their interpretation and will be made viewable over the web as well as through the Google Earth™ platform. An image will be produced for each summer since the 2002 outbreak and all images as well as the Google Earth™ interface will be made available from the CSIRO Marine and Atmospheric Research homepage. Specialists in remote sensing from CSIRO will be collaborating on the project and will be helping to maintain the predictive tool and all supporting documentation over the coming years.

It is expected that at least two papers will be published in high-impact international journals on the research summarised briefly here. The first, with the same title as given to this report, will be submitted to *Global Change Biology* in the coming months, as soon as the predictive tool has been completed and just prior to its public release. The second paper will involve determining whether there are similarities between the temperature regimes that occurred at sites on the GBR where white syndrome outbreaks occurred and those that occurred at sites in the greater Pacific. This exercise will help our team determine whether there is scope to use the model described here to develop a predictive tool for white syndromes throughout the Pacific and potentially globally. The support of the MTSRF and the RRRC will be acknowledged in both cases.

Report preliminary findings of regional scale variation in capacity of reefs to respond to increased macroalgae on the GBR, and delivery of outcomes to the Risk, Resilience and Response Atlas (Project 1.1.5) [(d/e) JCU] subject to external funding

Recent research has highlighted a fundamental weakness in traditional visual methods of quantifying herbivory on coral reefs. This work has revealed the limitations of traditional methods in providing an understanding of removal of adult macroalgae by fishes. This discovery was based on the use of underwater video techniques. However, the application of these new video methods varied among studies and, in some cases, was impractical for extensive replication. We therefore undertook a series of dedicated trips to Orpheus Island to trial a standardised macroalgal bioassay that would permit spatial variation in the extent and nature of herbivory on macroalgae to be evaluated. The standardized method is based on a three-hour algal deployment with herbivore feeding activity recorded using remote digital videos. The trials were highly successful, with up to seventy percent removal of algae in the three-hour period.

1. An experimental evaluation of relative susceptibility of macroalgal species to browsing by reef fishes and island-scale variation in herbivory on macroalgae: site and local variation in browsing rates.

Following the above trials, our full scale deployment of algal bioassays revealed high among site (within bay) variation in algal removal but little variation among bays. There was more variation among sites a few hundred meters apart than sites a km apart. However the most striking result was the variation in the species responsible for algal removal. Despite similar rates of algal removal among bays (at a 1-2 km scale) the species responsible showed almost no overlap. In effect algal removal in each of the 3 bays was dominated by a single, and different, species in each bay.

The development of the protocol and experimental evaluation were both successful. Preliminary results were presented at the 11th ICRS in Florida and two publications have been submitted. One to the proceedings arising from the April 2008 MTSRF meeting (Cvitanovic *et al.*) in Cairns, the other in the international journal *Coral Reefs* (Cvitanovic and Bellwood, 2008).

2. Regional scale evaluation of inshore reef susceptibility to coral-algal phase-shifts

To date, research on herbivory has been restricted to two latitudes in the location of Lizard and Orpheus Islands (two key GBR Research Stations). At both sites spatial variation in herbivore activity was high. In order to obtain a broader view of inshore reef processes, detailed evaluations of inshore reef processes have begun. Preliminary reconnaissance trips to Low Islands, the Whitsunday and the Keppel Islands identified appropriate sites for experimental deployment of methods developed earlier in the programme. A full scale regional sampling programme has just been undertaken with over two hundred experimental assays deployed at the Low Islands, the Whitsunday and the Keppel Islands; all completed within a six-week period (to minimise seasonal effects). Analyses of video footage have only just begun, however, 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 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 (these local data will be uploaded on RRRA shortly). Removal rates are characterised 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. Data analysis will be completed in 2009 ready for submission of two manuscripts.

Preliminary report on long term recovery and resilience of reef fish communities to climate change induced habitat degradation [(f) JCU]

Microsatellite markers are currently being developed for two species *Chaetodon trifascialis* and *C. lunulatus*. These study species are both widespread and abundant corallivores, but differ in their degree of specialisation and susceptibility to disturbance. Microsatellites, once developed, will provide a useful tool to better understand the connectivity and limitations to recovery of these species following climate-induced coral loss. For both species, regions of DNA containing microsatellite repeats have already been isolated, cloned and sequenced. A total of 288 individual segments of DNA were sequenced for each species. Each sequence was edited, aligned and individually searched for microsatellite repeat segments. Primer pairs for microsatellite amplification were designed for 43 sequences for *C. lunulatus* and 47 sequences for *C. trifascialis*. Each primer was initially tested on 4 different individuals of each species; and of these 28 primers for *C. lunulatus* and 36 primers for *C. trifascialis* were selected for further testing, optimization and genotyping. Final analyses of the data are still to be conducted, but at this stage it appears that 14 primers will be useable for *C. lunulatus*, and a further 7 primers will be useable following further optimisation. For *C. trifascialis*, 13 primers are currently useable, and a further 8 primers will be useable following optimization. The final data analyses and optimisation process is expected to be completed by June 2009, leading to at least two methodological papers (primer notes).

4. Summary of communication activities undertaken to date

- (a) LK Bay, MJH van Oppen, BL Willis and E Howells: 11 November 2008, AIMS

  The progress of projects relating to coral gene expression and *Symbiodinium* microsatellites were discussed
- (a,b) LK Bay, MJH van Oppen, BL Willis and E Howells: 25 February 2009, JCU

  The progress of projects relating to coral gene expression and *Symbiodinium* microsatellites were discussed
- (b) LK Bay, MJH van Oppen, N Andreakis and P Souter: 4 February 2009, AIMS

  Genes for multiplex GeXP assay were selected.
- (b) LK Bay and R Beeden: 23 October 2008, GBRMPA

  An annual report outlining research findings of Bay and perceived management implications was presented and discussed.
- (b) LK Bay, MJH van Oppen, P Souter, D Souter, R Beeden, D Wachenfeldt, P Marshall and L Blackall: 5 November 2008, GBRMPA

  The management implications of MTSRF funded research were discussed.
- (b) MJH van Oppen, P Souter, D Souter, R Beeden, D Wachenfeldt, P Marshall, L Blackall and R Berkelmans: 29 January 2009, Townsville City

  The management implications of MTSRF funded research were discussed.

5. Summary of annual project achievements for 2007/2008

5.1 Resilience of coral assemblages to climate change

Milestones for the resilience of coral assemblage project area were delivered with good progress in 2007/2008. A publication on the genetic connectivity of algal endosymbionts of corals (*Symbiodinium*) on the GBR was submitted to the journal Coral Reefs and the paper is now published (Howells *et al.* 2009).

Population genetic data were obtained for five *Acropora* species from the Palm Islands. Only small sample sizes were available for *A. aspera*, and extensive data analysis on this species was therefore not conducted. A manuscript is in preparation.

Specific primers have been developed for population-level markers targeting additional strains of *Symbiodinium* commonly hosted by corals on the GBR. Over thirty potential loci work well across *Symbiodinium* strains and are currently being tested for levels of polymorphism.

A modelling framework for understanding the potential for corals to evolve greater resistance to bleaching under temperature stress was developed and published in *The American Naturalist* in early 2008 (Caley *et al.* 2008).

Annual surveys of coral disease have been completed in the northern and southern sectors and are underway in the central sector for the summer 2008 period. A framework for modelling disease abundance is currently being modified to include anomalously low and high winter temperatures (presented by Willis *et al.* at the 11th ICRS, Florida, July 2008).

Papers published:

Day T, Nagel L, van Oppen MJH and Caley MJ (2008) Factors affecting the bleaching resistance of corals. *American Naturalist* 171: E72-E88.

Howell EJ, van Oppen MJH and Willis BL (2009) High genetic differentiation and cross-shelf patterns of genetic diversity among Great Barrier Reef populations of the dinoflagellate, *Symbiodinium*. *Coral Reefs* 28: 215-225.

5.2. Resilience of reef fish assemblages to climate change

Preliminary results were obtained to quantify the response of critical functional groups of herbivorous fish to macroalgal increases. There results were presented at the Cairns MTSRF meeting by Cvitanovic and at the 11th ICRS in Florida by Bellwood. One publication has been published (*Coral Reefs*), one is in press (MTSRF proceedings), another submitted (JEMBE) and two more are in prep. An extensive regional sampling programme has just been completed and is currently being analysed.

Intensive field sampling of fish and coral assemblages was undertaken in January 2008 and data were analysed to assess recovery of coral communities and butterflyfish assemblages since the 2001-2002 bleaching event.

Papers published:

Cvitanovic C and DR Bellwood (2008) Local variation in herbivore feeding activity on an inshore reef of the Great Barrier Reef. *Coral Reefs* 28: 127-133.

Cvitanovic C, Bellwood DR and Hughes TP (In press) Developing a Technique to Quantify Spatial Variation in Herbivory on the Great Barrier Reef. MTSRF Conference Proceedings.