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Marine and Tropical Sciences Research Facility Milestone Report, June 2009

Program 7: Halting and Reversing the Decline of Water Quality

Project 3.7.1: Marine and estuarine indicators and thresholds of concern

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Summary

All milestones have been completed.

Biofilms: Work on all biofilm indicators is now well advanced. Several indicators have either been already applied (e.g. as part of the [Reef Rescue Marine Monitoring Program](#) – Reef Rescue MMP) or are now available for wider field testing as a first step towards application. Experiments exposing foraminifera in aquaria to different water quality conditions are ongoing. We have also tested if organic carbon and nitrogen content of foraminifera reflects water quality conditions. This has been achieved in three field exposure experiments, and initial data analysis suggests that C/N ratios may be good indicators for water quality conditions on coral reefs. Work on bacteria as indicators for water quality is ongoing with repeated sampling of sediments and biofilms in the field under way at the Whitsundays and Magnetic Island, and laboratory methods for bacterial

fingerprinting developed. Four field trips to the Whitsundays were conducted, for deployment and retrieval biofilms slides and foraminiferan experiments. Work on the effect of pesticides on foraminifera made large progress with dose response curved to diuron now established for thirteen species.

Corals: Four manuscripts on coral indicators and ecosystem resilience at changing water quality have been completed. All field work and much of the laboratory work to test the validity of the Whitsundays indicators in three other regions (Fitzroy, Burdekin, Wet Tropics) has been completed. A report on the response of bioeroders to changing water quality has been completed (based on literature review and field data) and submitted in March, and a draft of the results of the responses of these and other indicators are attached.

Seagrass: Preliminary analysis of seagrass plant morphometrics data (rhizome internode length and leaf width) collected from Reef Rescue MMP from Late-Dry 2006 to Late-Dry 2008 has revealed some patterns at spatial (>1km) and temporal scales (> 1 year). The most comprehensive datasets were from the Wet and Dry Tropics and although highly variable, some relationships at spatial (>1km) and temporal scales (> 1 year) were apparent. *Halophila ovalis* internode length was found to increase during the Late-Monsoon each year at reef habitats in the Dry Tropics. However internode lengths for both *H. ovalis* and *Halodule uninervis* increased during the Late-Dry at both coastal and reef habitats in the Wet Tropics. The leaf width of *H. uninervis* was found to have a strong association with habitat type, with narrow leaves found at coastal locations and the wide leaves at offshore locations. A significant amount of variation was also found for both internode length and leaf width for *H. ovalis* among all locations. Further investigation using multivariate analysis is yet to be conducted using site specific measures such as nutrient quantity and quality (sediment and tissue), temperature and light. This data may assist in indicating the state of a seagrass meadow and the surrounding environment.

Estuaries: Field work for the developmental stage of the project is complete. While some components await the availability of final laboratory (Stable Isotope) data, most analysis and modelling is complete, with models in their second or third phase of development. Fish assemblage structure has been developed as a monitoring tool, with a first round of model evaluation complete and a second round underway. Analysis and modelling of spatio-temporal patterns of change in fish biomass-abundance relationships are complete and testing of their value as indicators is underway. One manuscript is submitted and a second will be submitted by the end of June.

Agreed Project Outputs

Objective	Targeted Activity	Due Date
(a)	Field testing and analysis of marine biofilms (bacteria, diatoms, foraminifera) for their suitability to indicate changes in water quality. [AIMS]	June 2009
(b)	Field testing and analysis of coral reef organisms and physiological change tested for their suitability to indicate changes in water quality and ecosystem condition. [AIMS]	June 2009
(c)	Contribution to research on seagrass communities and their responses to changing environmental conditions along the Queensland coast. [QDPI&F]	June 2009
(d)	Conduct research towards identifying potentially useful ecological indicators of the condition of North Queensland's estuaries. [JCU, GU]	June 2009

Report 3 submission: • Complete Final Report on progress in the development of foraminifera and bacteria in biofilms as indicators for reef health (Objective a). [AIMS] • Complete laboratory analyses and data processing for the activity listed against Objective (b). [AIMS] • Final report on the use of coral physiology and reef ecosystem properties as indicators of changing water quality (Objective b). [AIMS] • Complete report on development of indicators (e.g. sediments, epiphytes and reproductive success) for seagrass health (Objective c). [QDPI] • Complete final report / publication on for Objective (d). [JCU, GU] • Final summary of liaison activities undertaken through course of Year 1 of project. [AIMS]	15 June 2009
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Project Results

Description of the results achieved for this milestone

a. Field testing and analysis of marine biofilms (bacteria, diatoms, foraminifera) for their suitability to indicate changes in water quality. [AIMS]

On track. A variety of experiments exposing foraminifera to different nutrient concentrations in aquaria is still ongoing, some of these testing interactive effects of light/temperature and nutrients. One Honours student (C. Altenrath) has submitted her thesis investigating the effects of nutrient addition and increased temperatures on two foraminiferan species. The two species tested showed stronger effects of temperature, with decreasing growth rates and decreasing photosynthetic yield at enhanced temperature. Studies on other species (Claire Reymond, PhD student) have also indicated distinct reductions in growth rates due to Nitrate or Phosphate additions. In total five large-scale transplant experiments (three Dry and two Wet seasons) have now been completed, each involving two field trips to the Whitsunday for deployment and retrieval. Data indicated that the species transplanted grow distinctly better in areas further from the mainland less impacted by runoff. We have now also developed methods to measure organic carbon and nitrogen in individual foraminifera of three species. Results suggest that C/N ratios in foraminifera can be used as indicators for nutrient status of reefs or health indicator for foraminifera. These values appear to decline with higher nitrogen availability (caused mainly by higher N-content in the foraminifera). Integration of foraminifera samples into the Reef Recue Plan is now well established, with four sets of sediment samples for foraminifera analysis received to date. A manuscript discussing relationships between water quality, coral- and foraminiferan communities on the Reef Rescue Plan reefs has been submitted. The effect of herbicides (diuron) has now been tested on thirteen foraminiferan species (PhD thesis J. van Dam) and threshold concentrations have been calculated. Work on threshold concentrations for diuron, atrazine and hexazinone in crustose coralline algae is ongoing. Initial experiments on the interactive effects of herbicides and temperature increase (global change) have been completed and are reported in MTSRF Extension Project 3.7.1a. Work on bacteria as indicators for water quality is also on track (PhD thesis V. Witt). Sediment samples and biofilms (exposed for four to five weeks) have now been repeatedly sampled along the Whitsunday water quality gradient and sampling stations at Magnetic Island established to investigate short term temporal variations. Development of a fast fingerprinting technique to describe bacterial diversity on biofilm and sediment communities is completed. A pilot study to establish the most suitable substrate (e.g. glass slides, dead coral material, terracotta tiles) to mimic 'natural' communities is near completion. A laboratory experiment testing the effects of elevated sea surface temperatures on crustose coralline algae and its associated microbial communities has been completed. This experiment

revealed a thermal threshold for bleaching in crustose algae, effects on photosynthesis and a distinct change in microbial community at 32°C.

b. Field testing and analysis of coral reef organisms and physiological change tested for their suitability to indicate changes in water quality and ecosystem condition. [AIMS]

In Year 3 we conducted two field trips in collaboration with the inshore Reef Plan MMP team to combine benthic and indicator data and to maximise the use of the inshore benthic and water quality monitoring data. We tested the consistency of the eight priority indicators across the four main inshore MMP regions (Fitzroy, Mackay-Whitsundays, Burdekin and Wet Tropics Regions), and related indicator values and other ecosystem measures to the MMP water quality data. We also sampled the MMP benthic and indicator data at two additional depths. We have also continued to work on identifying chlorophyll concentrations (chronic conditions and episodic floods) that are thresholds for the development of outbreaks of the crown-of-thorns starfish.

- (1) In order to test if five priority indicators for water quality identified from the Whitsundays are valid in other Natural Resource Management (NRM) inshore regions of the Great Barrier Reef (GBR), we examined its variation along water quality gradients in four regions of the inshore GBR (in collaboration with the Reef Plan monitors). This encompassed 26 ship days to collect coral indicator samples from twelve reefs along water quality gradients each within four of the Reef Plan monitoring regions. In each region, three reefs located at increasing distances to a river mouth were surveyed at two depths. The trip was conducted as a collaboration with the Reef Plan Monitoring Team (water quality and inshore monitoring teams), and became a Masters thesis of JCU student Helene LeGrand. Physiological and community indicators were sampled at two depths at each reef. We also sampled the MMP benthic and indicator data at two additional depths at each reef. The field indicator data included coral pigmentation, macro-bioeroder densities, surface complexity, partial mortality, and coral and octocoral juvenile densities and species richness. Laboratory samples for skeletal density, protein and pigmentation are completed. Initial results for three of the four regions (data on the Keppels region were too sparse for analysis due to bad weather and poor visibility, but we will collect more data in August) are presented in [Attachment 1](#). Data will undergo further statistical analysis, and other variables will be added, before submission as publication.
- (2) We have completed (and submitted to MTSRF in March 2009) the results of our field work and of a review of the existing literature on macrobioerosion response to high productivity. Our field work showed that on the GBR, bioeroder densities on reefs near the river mouths were twice those of the reefs at intermediate and far distances, whereas the latter two did not differ. Across regions, densities were related to the long-term mean turbidity at each reef. Macrobioeroder density increased by 19.8 and 11.6 bioeroders m⁻² per unit NTU at deep and shallow sites, respectively. In contrast, the relationship to mean water column chlorophyll was weak. Our study showed that internal macrobioeroder density, as quantified by the density of bivalvia boreholes on the upper surface of living massive *Porites*, can indeed be used as a bioindicator measure for changing water clarity on the GBR where turbidity data are unavailable. It also shows that internal macrobioerosion, which weakens coral and substrata and makes them more susceptible to storm damage, is intense on some inshore reefs, with densities averaging 2,500 internal macroborers per square meter of coral surface on Wet Tropics reefs near the Tully River. Most internal macrobioeroders (sponges, bivalves, polychaetes, sipunculans and barnacles) are filter feeders, and a number of previous studies have investigated whether their densities change with eutrophication. Our literature review showed that all studies conducted for long enough periods report an increase in total bioeroder densities with increasing nutrient levels. Especially bivalves and sponges tend to have higher densities in nutrient-enriched than

in oligotrophic waters, whereas polychaetes and sipunculans often do not vary in density but in their community composition.

- (3) Also completed and submitted as a book chapter has been the review:

Fabricius, K. E. (Submitted) Factors determining the resilience of coral reefs to eutrophication: A review and conceptual model. In: Z. Dubinski (ed.) *Corals and Coral Reefs*. Springer.

This chapter reviews the main impacts of eutrophication on the ecology of coral reefs, and the properties of reefs that determine their exposure, resistance and resilience to it. It shows that eutrophication affects coral reefs by way of nutrient enrichment, light loss from turbidity, and the smothering and alteration of surface properties from sedimentation. These changes lead to changes in trophic structures, reduced coral recruitment and diversity, the replacement of corals by macroalgae, and more frequent outbreaks of coral-eating crown-of-thorns starfish. The reefs and areas most susceptible to degradation from pollution are deeper reef slopes, reefs located in poorly flushed locations and surrounded by a shallow sea floor, frequently disturbed reefs, and reefs with low abundances of herbivorous fishes. The chapter concludes with a conceptual model of the main links between water quality and the condition of inshore coral reefs.

- (4) A review of a recommendation of eleven indicators for inclusion into monitoring programs has been published:

Cooper, T. F., Gilmour, J. P. and Fabricius, K. E. (2009) Bioindicators of changes in water quality on coral reefs: Review of recommendations for monitoring programmes. *Coral Reefs* 28(3): 589-606.

This paper reviews the suitability of a range of bioindicators for use in monitoring programmes linking changes in water quality to changes in the condition of coral reef ecosystems. From the literature, 21 candidate bioindicators were identified whose responses to changes in water quality varied spatially and temporally; responses ranged from rapid (hours) changes within individual corals to long-term (years) changes in community composition. From this list, the most suitable bioindicators were identified by determining whether responses were (i) specific, (ii) monotonic, (iii) variable, (iv) practical and (v) ecologically relevant to management goals. For long-term monitoring programmes that aim to quantify the effects of chronic changes in water quality, eleven bioindicators were selected: symbiont photophysiology, colony brightness, tissue thickness and surface rugosity of massive corals, skeletal chemistry, abundance of macro-bioeroders, micro- and meiobenthic organisms such as foraminifera, coral recruitment, macroalgal cover, taxonomic richness of corals and the maximal depth of coral reef development. For short-term monitoring programmes or environmental impact assessments that aim to quantify the effects of acute changes in water quality, a subset of seven of these bioindicators, plus partial mortality, were selected. The choice of a subset of these bioindicators will depend on the specific objectives and the timeframe available for each monitoring programme. An assessment framework is presented to assist in the selection of bioindicators to quantify the effects of changing water quality on coral reef ecosystems.

- (5) We have now published our study on the strong interactive effects of nutrients and sediments on coral fertilisation rates:

Humphrey, C., Weber, M., Lott, C., Cooper, T. and Fabricius, K. E. (2008) Effects of different types of sediment, dissolved inorganic nutrients and salinity on fertilisation and embryo development in the coral *Acropora millepora*. *Coral Reefs* 27: 837-850. (Previously reported as a MTSRF Milestone report)

- (6) We have published a study on the potential effects of the widely applied live bacterial mosquitocide on corals and sponges:

Negri, A. P., Soo, R. M., Flores, F. and Webster, N. S. (2009) *Bacillus insecticides* are not acutely harmful to corals and sponges. *Marine Ecology-Progress Series* 381: 157-165.

- (7) Finally, we have now completed and submitted to *Ecological Applications* the manuscript:

De'ath, G. and Fabricius, K. E. (In review) Water quality as a regional driver of coral biodiversity and macroalgal cover on the Great Barrier Reef. *Ecological Applications*.

This study models the relationships between large-scale data on water quality and measures of reef status along the whole of the GBR (GBR: 12° to 24°S). Large-scale data on water clarity and chlorophyll showed strong regional differences in GBR water quality, with water clarity increasing more than three-fold from inshore to offshore waters, and chlorophyll increasing roughly two-fold from inshore to offshore and two-fold from south to north. Four biotic groups comprising macroalgal cover and the taxonomic richness of hard corals, phototrophic and heterotrophic octocorals were predicted from water quality and spatial location. Richness of hard corals and phototrophic octocorals declined with increasing turbidity and chlorophyll, whereas macroalgae and the richness of heterotrophic octocorals increased. Macroalgal cover experienced the largest water quality effects, increasing five-fold with decreasing water clarity and 1.4-fold with increasing chlorophyll. For each of the four biota, ~45% of its variation was predictable, with water quality effects accounting for 18-46% of that variation. Effects were consistent with the trophic requirements of the biota, thereby suggesting control by energy supply limitation. Throughout the GBR, mean annual values of >10m Secchi disk depth (a measure of water clarity) and <0.45 g L⁻¹ chlorophyll were associated with low macroalgal cover and high coral richness. Our models suggest these values as useful water quality guidelines. Of the 2,833 gazetted reefs in the GBR, 22.8% exceeded these guideline values. The models also show that compliance with the guidelines, e.g. by minimising agricultural runoff, would likely reduce macroalgal cover by 39% and increase hard coral and octocoral richness by 16% and 33%, respectively, on the reefs where guidelines are presently exceeded.

c. Contribution to research on seagrass communities and their responses to changing environmental conditions along the Queensland coast. [QDPI]

The objective of this study was to explore seagrass meadow dynamics in relation to proximity of catchments, nutrient quantity and quality, to enhance the current understanding of anthropogenic impacts on the Great Barrier Reef World Heritage Area.

Although previous studies have found epiphyte loads closest to a discrete nutrient source had significantly greater epiphyte loads than sites downstream, preliminary analysis of qualitative data from the Seagrass-Watch program revealed no obvious relationship. This may be a consequence of a diffuse nutrient source. In an attempt to develop a quantitative measure, epiphyte cover was regressed against paired epiphyte biomass data collected from the field. Unfortunately the relationship was poor, primarily a consequence of the difficulty quantitatively sampling epiphyte biomass. Exploration of the data is still continuing with the possibility of developing guidelines which can be used as part of a seagrass health indicator 'toolbox'.

Previous studies suggest that seagrass plant morphometrics can indicated the state of the seagrass meadow and the surrounding environment. Seagrass morphometric data (rhizome internode length and leaf width) was collected for a range of species from Reef Rescue MMP sites seasonally from Late-Dry 2006 to Late-Dry 2008. The most comprehensive datasets were from the Wet and Dry Tropics and although highly variable, some relationships at spatial (>1km) and temporal scales (>1 year) were apparent. *Halophila ovalis* internode length was found to increase during the Late-Monsoon each year at reef habitats in the Dry Tropics. However internode lengths for both *H. ovalis* and *Halodule uninervis* increased during the Late-Dry at both coastal and reef habitats in the Wet Tropics. The leaf width of *H. uninervis* was found to have a strong association with habitat type, with narrow leaves found at coastal locations and the wide leaves at offshore locations. A significant amount of variation was also found for both internode length and leaf width for *H. ovalis* among all locations. Further investigation using multivariate analysis is yet to be conducted using site specific measures such as nutrient quantity and quality (sediment and tissue), temperature and light.

d. Estuarine indicators for ecosystem condition

Field work for the developmental stage of the project is complete. While some components await the availability of final laboratory (Stable Isotope) data, most analysis and modelling is complete, with models in their second or third phase of development. Fish assemblage structure has been developed as a monitoring tool, with a first round of model evaluation complete and a second round underway. Analysis and modelling of spatio-temporal patterns of change in fish biomass-abundance relationships are complete and testing of their value as indicators is underway. Trophic structure holds great promise as an indicator of estuary health but requires final Stable Isotope data for definitive results. Changes in taxonomic composition of the phyto-detritivore guild provide another potentially useful indicator of health. An apparent link between taxonomic composition and differential consumption of food sources has been identified and further studies to validate this initiated. Changes in the richness and species composition of benthic feeders have been identified, but, although these may useful indicators of major change, they are unlikely to provide sensitive indication of lower level effects. Nitrogen Stable Isotope values show characteristic changes in response to anthropogenic nutrient inputs making them useful indicators of such impacts. One manuscript is submitted and a second will be submitted by the end of June.

Communication, major activities and events

- K. Fabricius gave presentation on MTSRF water quality research and the [e-Atlas](#) at a James Cook University Minisymposium on “Ecosystem Services of the GBR”, 9 February 2009.
- K. Fabricius presented "Coral reefs and water quality: Physiological changes, bioindicators and the loss of biodiversity" at the University of Bremen, 4 June 2009.
- A. Negri presented “Herbicides and tropical marine organisms: Effects and thresholds. Minimising off-site movement of pesticides and impact on the Great Barrier Reef” at a Risk Based Approach Workshop, Cairns, 18-19 May 2009, followed by three media interviews after the workshop.
- Conference presentation: Uthicke, S., Schaffelke, B., Thompson, A. and Thomson, D. (2008) Water quality in nearshore areas of the Great Barrier Reef: A large scale monitoring program and an assessment of the use of benthic foraminifera as water quality indicators. 11th International Coral Reef Symposium, Fort Lauderdale, Florida, July 2008.
- Conference presentations by K. Fabricius, S. Uthicke, A. Negri and J. van Dam at the 2009 MTSRF Annual Conference:
 - van Dam, J., Roethig, T., Uthicke, S., Mueller, J., Flores, F. and Negri, A. (2009) *Testing effects of herbicide on a wider range of coral reef species and possible herbicide-climate interactions on tropical marine species.*
 - Uthicke, S., Schaffelke, B. and Thompson, A. (2009) *An assessment of the use of benthic foraminifera as water quality indicators in conjunction with the Reef Water Quality Marine Monitoring Program (MMP).*
 - Negri, A. and Mueller, J. (2009) *Herbicides and tropical organisms: effects and thresholds.*
- Completion of nine publications and three student theses:
 - Cooper, T. F., Gilmour, J. P. and Fabricius, K. E. (2009) Bioindicators of changes in water quality on coral reefs: Review of recommendations for monitoring programmes. *Coral Reefs* 28(3): 589-606.
 - Cooper, T. F. and Ulstrup, K. E. (2009) Mesoscale variation in the photophysiology of the reef building coral *Pocillopora damicornis* along an environmental gradient. *Estuarine, Coastal and Shelf Science* 83(2): 186-196.
 - De'ath, G. and Fabricius, K. E. (In review) Water quality as a regional driver of coral biodiversity and macroalgal cover on the Great Barrier Reef. *Ecological Applications*.
 - Fabricius, K. E. (Submitted) Factors determining the resilience of coral reefs to eutrophication: A review and conceptual model. In: Z. Dubinski (ed.) *Corals and Coral Reefs*. Springer.
 - Humphrey, C., Weber, M., Lott, C., Cooper, T. and Fabricius, K. E. (2008) Effects of different types of sediment, dissolved inorganic nutrients and salinity on fertilisation and embryo development in the coral *Acropora millepora*. *Coral Reefs* 27: 837-850.
 - Negri, A. P., Soo, R. M., Flores, F. and Webster, N. S. (2009) *Bacillus* insecticides are not acutely harmful to corals and sponges. *Marine Ecology Progress Series* 381: 157-165.
 - Sheaves, M. and Johnston, R. (In review) Implications of Spatial Variability of Fish Assemblages for Monitoring of Australia's Tropical Estuaries. *Aquatic Conservation Marine and Freshwater Systems*.
 - Uthicke, S., Thompson, A. and Schaffelke, B. (In review) Effectiveness of benthic foraminiferal and coral assemblages as water quality indicators on inshore reefs of the Great Barrier Reef, Australia. *Ecological Applications*.
 - Ziegler, M. and Uthicke, S. (In review) Photosynthetic plasticity of endosymbionts in larger benthic coral reef Foraminifera. *Marine Biology*.

- o Altenrath, C. (2009) *Growth and photophysiological response of benthic symbiotic foraminifera along a nutrient gradient on the Great Barrier Reef*. Diplomarbeit, University of Cologne, pp 31.
- o LeGrand, H. (2009) *Development of coral bioindicators to detect changes in water quality on inshore reefs of the Great Barrier Reef*. Masters Thesis, James Cook University.
- o Ziegler, M. (2009) *Photosynthetic plasticity of endosymbionts in larger benthic coral reef Foraminifera*. Diplomarbeit, University of Veterinary Medicine Hannover, pp 43.

Attachment 1

MTSRF Project 3.7.1(a) Final Report

Sven Uthicke, Verna Witt and Joost van Dam
Australian Institute of Marine Science

1. Foraminifera

(a) Transplant experiments: In 2008/2009 we have conducted four field trips to the Whitsunday area to conduct further experiments exposing foraminifera to different water quality conditions. In total, five tests have now been conducted and were highly successful. During three wet seasons and two dry season we transplanted a large number of foraminifera of three symbiont bearing species (*Amphistegina radiata*, *Heterostegina depressa*, *Marginopora vertebralis*) and recovered these in subsequent field trips (ca. four to six weeks later). Although we still measured growth and could consistently confirm that these species grow better in locations with larger distance to the mainland, experiments in Year 3 focussed on measuring organic carbon and nitrogen. These measurements were taken to investigate if these parameters can be used as integrative measures to describe amounts of nutrients available on coral reefs.

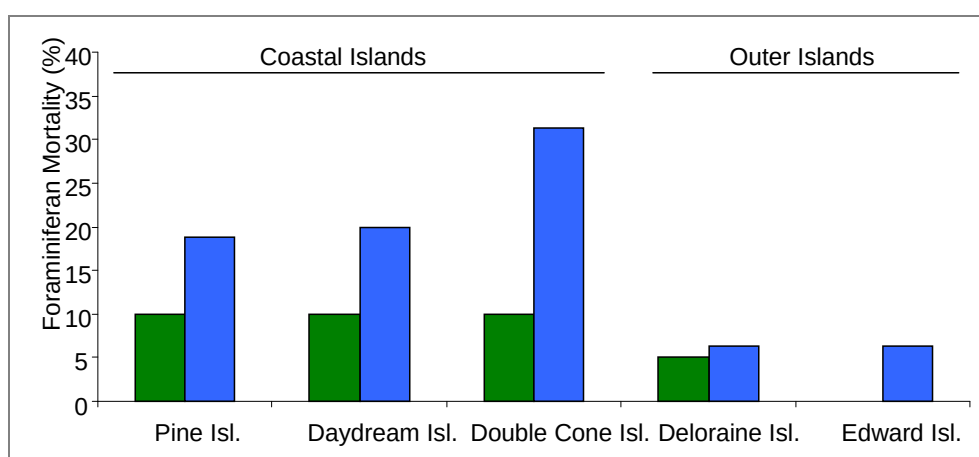


Figure 1: Mortality of benthic foraminifera in transplant experiments in the wet season of 2009. Mortality of *Amphistegina radiata* (green bars) and *Heterostegina depressa* (blue bars) was distinctly higher on islands closer to the shore subjected to distinct runoff plumes.

All experiments were successful and we could recover most foraminifera. Mortality in the experiments was generally low. However, relatively high mortality rates (~10-30%) were observed in the 2009 wet Season experiment in the islands close to the shore in the Whitsundays. Foraminiferan mortality was on a much lower level on islands further from the shore (Figure 1).

Extreme rainfall in January and February lead to very distinct sediment plumes much more distinct on the inner islands. Light loggers deployed during this period illustrated the effect of the sediment plumes on light availability on the benthic community (Figure 2). Already at three meters water depth the light over a two-week period was reduced to virtually zero. Although light was also reduced on Edward Island the effect is less pronounced. These flood plume conditions were also associated with distinctly reduced salinity values at the inner Islands and coral mortality was also observed.

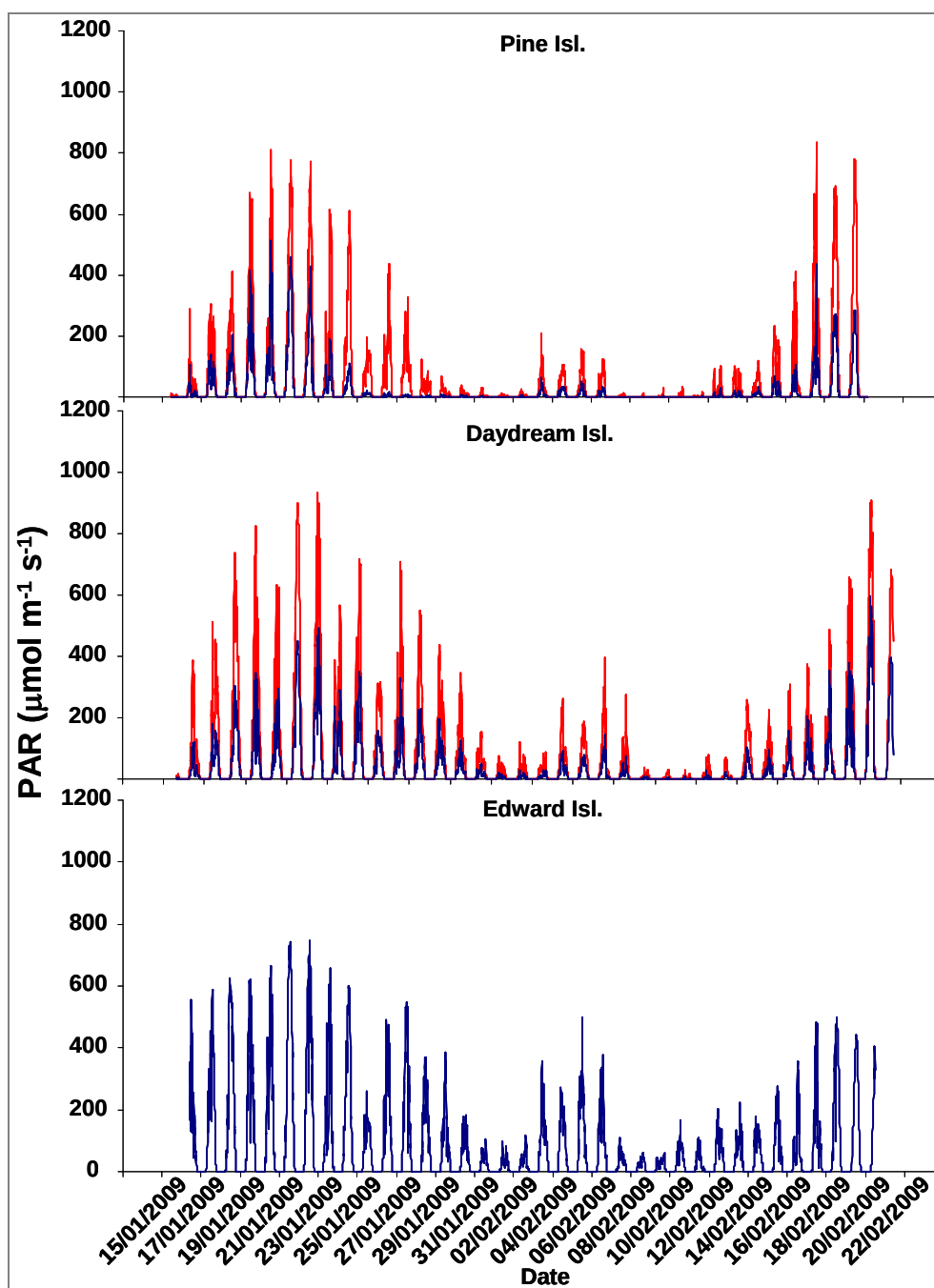


Figure 2: In situ light (PAR; red line: 3m water depth; blue line: 6m water depth) conditions during an extreme flood plume in the Whitsundays.

We have also finalised development of a method to measure carbon and nitrogen in individual foraminifera and have applied this method for measurements in *A. radiata*, *H. depressa* and *M. vertebralis* from our transplant experiments. Carbon and Nitrogen content of the foraminifera does vary with season and location. In the first dry season (August/September 2007) we conducted these experiments, C/N ratios for both species were ~20 on average and similar in reefs around the outer Island and those closer to the shore. In the following dry season (2008), C/N ratios in the outer reefs remained in that range whereas reduced values were measured inshore (Figure 3). In the only wet season (2009) investigated, values both inshore and offshore were reduced to values around 11-13. In each case where the C/N ratios were reduced this was mainly caused by an increase in N. Thus, it appears most likely that the nitrogen content in the foraminifera

and their symbionts do well reflect present nutrient status of the reef. Further analyses comparing these data to actual nutrient values are under way.

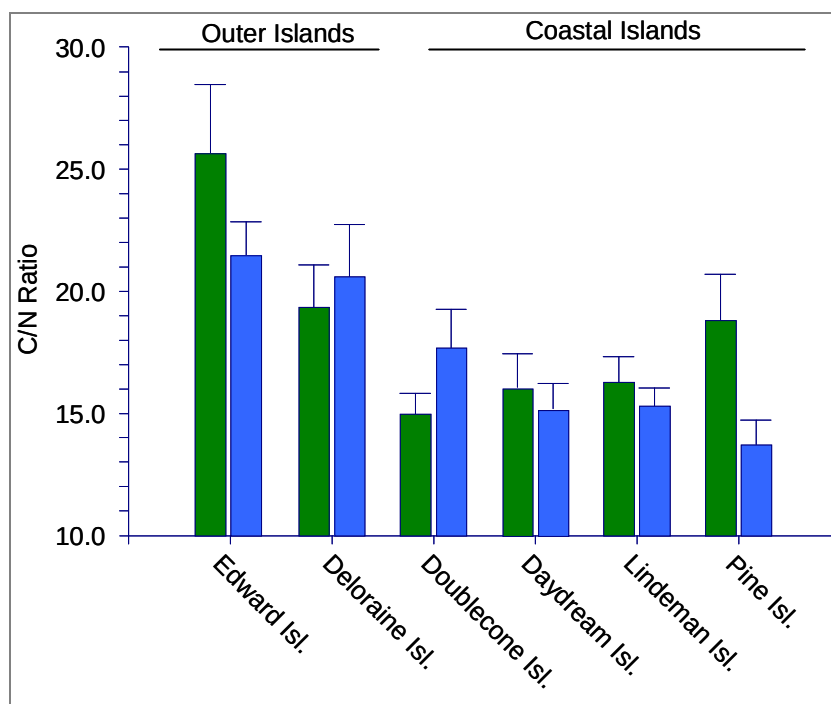


Figure 3: Carbon-Nitrogen ratios in benthic foraminifera used in transplant experiment in the dry season 2008 (August/September). C/N ratios in both *Amphistegina radiata* (green bars) and *Heterostegina depressa* (blue bars) was distinctly higher on islands further from the coastline. Error bars = 1 SD.

Thus, field experiments have provided further important information on the ecology of benthic foraminifera needed for the development of foraminiferal indicators for reef health. In addition, both growth measurements and measurements of C/N ratios have large potential as indicators for reef health and/or nutrient status

- (b) Aquarium experiments:** Several further experiments have been conducted at the Australian Institute of Marine Science (AIMS) aquarium system to study the effect of nutrients and light on the photophysiology, mortality and growth of symbiont bearing foraminifera. This work was supported by Honours student Maren Ziegler of the University of Hannover (see Abstract of submitted manuscript, [Attachment 2](#)) who finished her thesis with aquarium experiments at AIMS. Claire Reymond (University of Queensland) continued her work on temperature and nutrient effects on foraminiferal growth. Christine Altenrath (University of Cologne) has finished her thesis on "Growth and photophysiological response of benthic symbiotic foraminifera along a nutrient gradient on the Great Barrier Reef" (see Abstract of the thesis, [Attachment 3](#)). A new Honours student (Christiane Schmidt, University of Tuebingen) will conduct further work on the combined effects of nutrients and global warming on foraminifera and on isolated symbionts.
- (c) Hindcasting using sediment cores:** This activity (supported by AIMS appropriation funding) is well advancing after initial difficulties finding a laboratory to age the sediment cores. We collected thirteen sediment cores (up to 1.5m depth) from inshore and offshore location in the Whitsunday area and investigate foraminiferal communities in these. If the nutrient and light regime in near shore reefs has changed due to increased land runoff over the last 150 years, it is hypothesised that this change can be documented

in a change in the foraminiferal community. Foraminifera have already been picked and identified from ten of these cores and statistical analysis is imminent. The lead 210 (Pb210) content in the top six slices of eight cores has now been investigated by alpha spectrometry to estimate sediment accumulation rates. These data suggest values of 0.2-0.5 cm yr⁻¹ which is in the range found for other locations in the GBR. We have additionally aged foraminifera (*Elphidium* spp.) from the deepest horizon of four cores using C14 determined by accelerated mass spectrometry. These data revealed that communities in the longer cores are up to three thousand years old. Some additional cores now need to be aged and foraminiferan communities from some core slices still counted before final analysis.

- (d) Interaction of pesticides and global change:** A joint student (Joost van Dam) between S. Uthicke, A. Negri and J. Mueller (University of Queensland) commenced work on this topic in July 2007, supported by a MTSRF PhD scholarship. A brief summary of this work (provided by J. van Dam) is given below:

PhD Project Summary: Joost van Dam

Introduction

Recent reporting on water quality declines along inshore sites of the Great Barrier Reef World Heritage Area (GBRWHA) has called for a need to investigate a wide range of marine species and their reactions to poor water quality. Wet season flood events and associated plumes of fresh water carrying loads of suspended sediments, nutrients and agricultural pesticide residues into the GBRWHA can have adverse effects on marine communities. In this project we will focus on the negative effects calcifying and symbiont bearing marine species endure after exposure to different stressors. It is our goal to determine the susceptibility of corals, forams and their endosymbiotic algae to the combined effects of water quality and climate change factors. Synergistic inhibiting effects on photosynthesis might occur. Impairment can result in decreased fitness, calcification, expulsion of endosymbionts (bleaching) and mortality.

Multiple studies have shown elevated herbicide concentrations to be present in nearshore areas of the GBRWHA. These herbicides are often associated with terrestrial runoff during the wet season but can also have their origin in direct application into the marine environment, e.g. by the use of antifouling paints to prevent growth of unwanted flora on ships and other marine structures. Approximately 50% of commercially available herbicides find their mode of action through the inhibition of electron transport through Photosystem II (PSII) of the photosynthetic apparatus. As the fragile relationship between certain calcifying marine species and their endosymbiotic unicellular algae is based upon primary production of the symbionts, herbicide occurrence in the GBRWHA can have severe influence on these organisms and therefore implications for the ecosystem. In contrast to reef building corals bearing only one type of symbiotic algae, foraminifera can host a range of different species and can thus provide us with a wealth of information on the resilience of symbiotic algae to herbicide stress. In addition to being important species within reef communities for the extraction of carbonate from the water column, foraminifera are also important species for the fact they can make up the origin of over 50% of the calcareous sands in some reefs. In this study multiple species of foraminifera, bearing a multitude of symbiont types, were exposed to elevated herbicide levels to determine toxic threshold concentrations and to gain information on herbicide kinetics and effects.

Methods

Specimen collection

Foraminifera were collected at various inshore sites of GBRWHA between August 2008 and February 2009 (Table 1). Species were kept separated in 500mL clear plastic beakers while on board field vessels and immediately transferred into large-volume (20L)

aquaria under flow-through conditions at AIMS Townsville. Aquaria contained 0.5 µm filtered seawater and water was aerated throughout the time foraminifera were present in the aquaria. Aquaria were cleaned when algal build-up on the sides of the aquaria became evident. Rapid Light Curves (RLCs) using all different species were determined to establish ideal light conditions for keeping of specimens. Lights used consisted of high-energy blue aquarium bulbs with excitation peaks around 670 nm to promote most efficient photosynthesis. Light intensity was adjusted according to ideal conditions determined during RLC evaluation. A 10-14 hr diurnal light-dark cycle was introduced to mimic regular environmental conditions. A total of 13 different species of foraminifera were used in the toxicity tests, bearing at least four different symbiont types (Table 1) or retained plastids.

Experimental setup

Foraminifera were exposed to a solvent control and five different concentrations (0.3, 1, 3, 10 and 30 µg L⁻¹) of the PSII herbicide diuron in filtered seawater for 24 hours under static conditions. On the day the experiment was to start foraminifera were transferred from the holding aquaria to 6-well plates containing 10 mL per well of exposure medium, made up directly before the experiment was to start. Experiments were set up in a full orthogonal design. Wells containing exposure concentrations were randomly assigned within plates. Ideally, five specimens were assigned per well. Each separate toxicity test held 90 specimens per species divided randomly over three replicate six-well plates, bringing the total number of specimens per exposure concentration up to 15. For species lacking sufficient numbers because of difficulties collecting, the number of specimens per well was reduced according to the amount available. After transfer of foraminifera into exposure wells, plates were covered with lids and placed in similar light conditions as determined during RLC evaluation and occurred throughout keeping.

Table 1: Species used in dose response experiments; symbiont type, place, depth and date collected.

Species	Symbiont type	Location collected	Depth and date collected
<i>Heterostegina depressa</i> (macroscopic form)	Diatoms	Deloraine island, Whitsundays	9m, August 2008
<i>Amphistegina radiata</i>	Diatoms	Deloraine island, Whitsundays	9m, August 2008
<i>Operculina ammonoides</i>	Diatoms	various islands, Whitsundays	7m, August 2008
<i>Heterostegina depressa</i> (microscopic form)	Diatoms	Davies reef	12m, September 2008
<i>Calcarina mayorii</i>	Diatoms	Deloraine island, Whitsundays	5m, August 2008
<i>Alviolinella quoyi</i>	Diatoms	Orpheus island	7m, February 2009
<i>Amphisorous sp.</i>	Dinoflagellates	Lindeman island, Whitsundays	6m, August 2008
<i>Marginopora vertebralis</i>	Dinoflagellates	Davies reef	3-9m, September 2008
<i>Sorites orbiculus</i>	Dinoflagellates	Orpheus island	1m, September 2008
<i>Peneropolis planatus</i>	Rhodophytes	Orpheus island	7m, September 2008
<i>Peneropolis antillarum</i>	Rhodophytes	Orpheus island	7m, September 2008
<i>Parasorites marginalis</i>	Chlorophytes	Orpheus island	5m, September 2008
<i>Elphidium sp.</i>	Retained plastids	Magnetic island	9m, September 2009

Stress evaluation

To evaluate herbicide effects on symbiotic algae within the host tissue, Pulse-amplitude modulated (PAM) chlorophyll fluorescence techniques were used. In this study a MINI-

PAM fluorescence meter (Walz, Germany) was used to determine both effective quantum PSII yield ($\Delta F/F_m'$) in natural light and maximum quantum PSII yield (F_v/F_m) after dark adaptation. Both fluorescence parameters were recorded after specific exposure periods, $\Delta F/F_m'$ after six hours of light conditions and F_v/F_m after fourteen hours of dark. A reduction in the effective quantum yield ($\Delta F/F_m'$) provides us with information on the photosynthetic efficiency of PSII and is a direct result of herbicide binding to the electron acceptor. A reduction of maximum quantum PSII yield (F_v/F_m) after dark adaptation indicates reduced energy conversion in PSII and is the result of secondary damage to the photosystem, e.g. by the formation of reactive oxygen species.

After 24 hours exposure specimens were snap frozen in liquid nitrogen and transferred to a -80°C freezer for subsequent pigment extraction and HPLC analysis. Pigment analysis presents us with a way to determine total chlorophyll content. Also, ratios of particular pigments can provide evidence of oxidative stress. These analyses still need to be performed.

Results

All foraminiferan species were severely affected by elevated diuron concentrations after six and 24 hours exposure. The results show that the thirteen different species and associated symbiotic algae vary in their ability to manage sub-lethal stress caused by elevated herbicide concentrations. After six hours' exposure, inhibition of photosynthesis (reduction in effective quantum yield, $\Delta F/F_m'$) relative to control values ranged between 0% and 20% for the lower exposure concentrations ($1-10 \mu\text{g L}^{-1}$), while at higher concentrations ($3-30 \mu\text{g L}^{-1}$) inhibition of photosynthesis between 20% and 70% of control values was observed (Figure 4). Species associated with diatoms as endosymbionts appeared to be most sensitive to herbicide stress, while species bearing rhodophytes (red algae) demonstrated to be least receptive to the effects of elevated diuron concentrations. For most species, significant inhibition of photosynthetic efficiency was observed after exposure to $3 \mu\text{g L}^{-1}$ diuron.

After 24 hours exposure, damage to photosystem II (reduction of F_v/F_m , relative to control values) was evident for most species after exposure to $1 \mu\text{g L}^{-1}$. Exposure to concentrations of $1-3 \mu\text{g L}^{-1}$ of diuron inhibited F_v/F_m up to 35%, while higher diuron concentrations ($10-30 \mu\text{g L}^{-1}$) demonstrated extensive photodamage, illustrated by inhibitions of maximum quantum PSII yield (F_v/F_m) ranging from 15% to 70% (Figure 5). Again, species bearing diatom symbionts appeared most sensitive to photodamage caused by herbicide exposure, while rhodophyte bearing foraminifera displayed relative insensitivity.

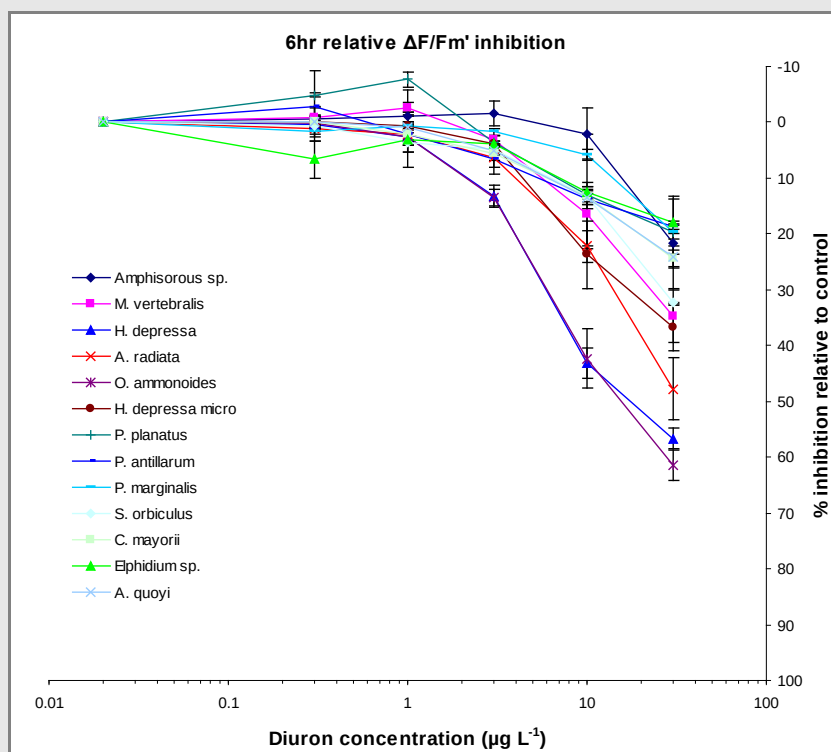


Figure 4: Inhibition of effective quantum PSII yield ($\Delta F/F_m'$) relative to control values after six hours' exposure to elevated diuron concentrations. Depicted are all fourteen species of foraminifera tested, bearing different symbiont species.

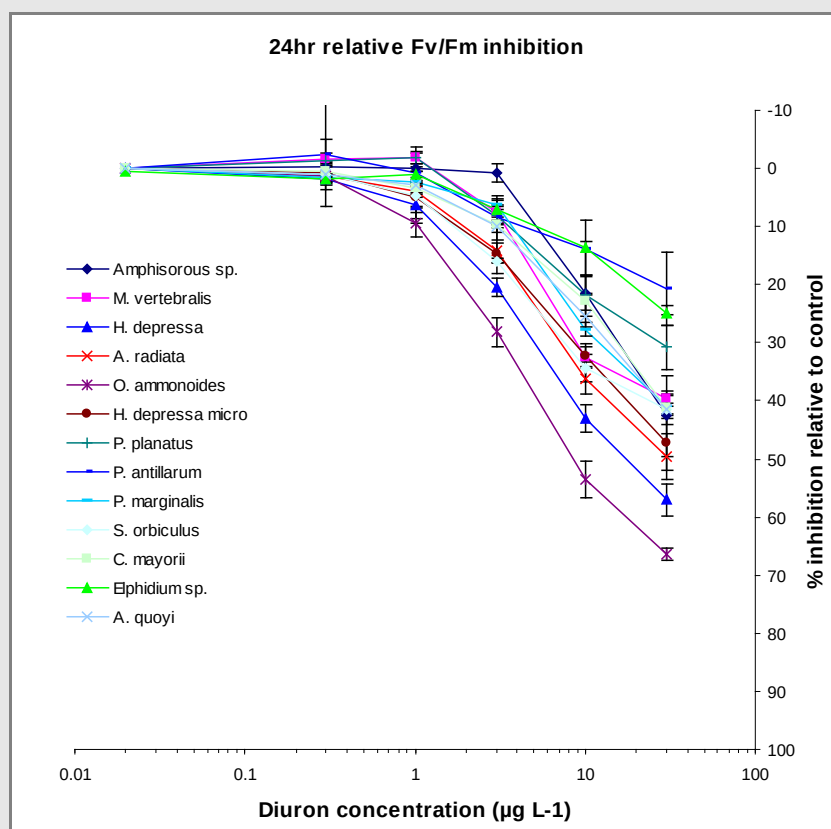


Figure 5: Inhibition of maximum quantum PSII yield (F_v/F_m) relative to control values after 24 hours exposure to elevated diuron concentrations. Depicted are all thirteen species of foraminifera tested, bearing different symbiont types.

(e) Road testing of the use of foraminifera as water quality indicators: In collaboration with the Reef Rescue Marine Monitoring Program we have obtained foraminiferan samples from nearly all reefs surveyed by this initiative over the last four years. These samples were used to investigate inter-annual variation in foraminiferan communities. In addition, we used these data to compare foraminiferan community composition to water quality and sediment quality data and coral community analysis. This work has been presented at the 11th International Coral Reef Symposium in Florida, USA and at the MTSRF Annual Conference in Townsville (2009). A manuscript on this comparison was recently submitted to *Ecological Applications* (see [Attachment 4](#)).

2. Bacteria

Initial microbial work on biofilms have been conducted by S. Uthicke and a Masters student (P. Kriwy). This work established distinct differences in microbial communities of inner and outer islands in the Whitsunday region. Since June 2008, a MTSRF-funded PhD student (Verena Witt) has continued these studies with Uthicke, co-supervised by C. Wild (University of Munich). A brief summary prepared by V. Witt of the first year of her PhD work is provided below.

PhD Project Summary: Verena Witt

Introduction

Microbial communities in biofilms are highly sensitive to changes in water quality due to their high species and functional diversity and therefore respond rapidly to changing environmental conditions. These features make biofilms potentially useful as biological indicators to changes in water quality.

The aim of this study is to investigate microbial community structure in biofilms developing on artificial and natural substrates and calcareous reef sediments along a previously studied and well-monitored water quality gradient in the Whitsunday Islands. This gradient comprises sites at near-shore islands, possibly adversely affected by terrestrial run-off and anthropogenic impact, and offshore islands of more pristine water quality. Particular emphasis will be placed on determining the effects of certain water quality parameters, such as light and nutrient conditions, on the community dynamics and to assess the potential of coastal marine biofilms as bioindicators of water quality.

Methods

During two field trips (August 2008 and January 2009) a variety of natural and artificial substrates, including terracotta tile, coral, glass, Teflon coated glass slides and sediment were deployed at the sites. After six weeks, the mature biofilm communities and sedimentary biofilm samples were collected.

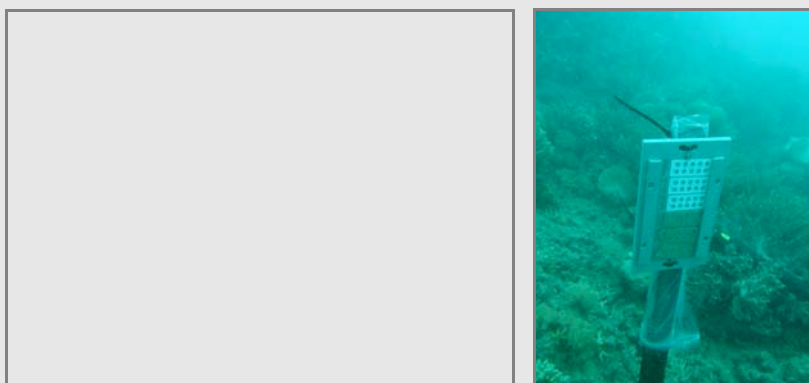


Figure 6: (Left) Substrates used for biofilm settlement (terracotta tiles, *Acropora* cores, glass slides and sediment coated glass slides). (Right) Substrates in situ.

Preliminary work included testing and optimising molecular methods (DNA extractions, PCR and the fingerprinting method terminal restriction fragment length polymorphism (T-RFLP) for molecular identification and phylogenetic analysis of these environmental samples to characterise microbial diversity.

Parallel to the Whitsundays study, a long-term monitoring station along a water quality gradient on Magnetic Island has been installed to monitor changes in diversity and activity of biofilms over a period of one year. Different substrates are deployed at three sites (Orchard Rocks, Geoffrey Bay and Middle Reef). Biofilms on substrates and sedimentary biofilms are being sampled regularly every six to eight weeks for fingerprinting analysis and activity of biofilms is investigated by focusing on oxygen fluxes.

A suitable method to measure oxygen production and respiration rates has been developed using optode measurements of oxygen. A pilot study on the effects of light manipulating experiments in situ has been conducted on Heron Island in May 2009 and will be continued on Magnetic Island.



Figure 7: Custom made glass vials for net oxygen production and respiration measurement of biofilms.

Results

Preliminary work shows that the T-RFLP method is reliable and reproducible and appears to be useful to determine differences in microbial community structures in biofilms from areas with different water quality.

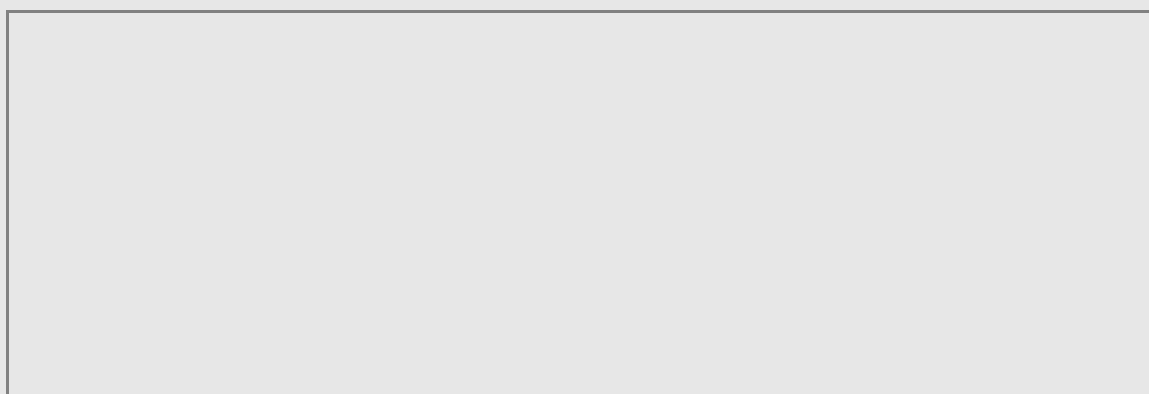


Figure 8: T-RFLP peak profiles showing differences in bacterial diversity in a biofilm community on different substrates: (*left*) tile; (*right*) coral.

Initial tests to measure respiration (in the dark) and oxygen production (under a standardised light intensity: $90 \mu\text{mol m}^{-2} \text{s}^{-1}$) in custom made glass containers (see Figure 7) on individual

biofilm slides were successful. Depending on age of the biofilms respiration could be measured after two hours in the dark and production after thirty minutes in the light. A pilot study on the development of respiration and production showed that these values could be measured after deploying a cleaned microscopic slide for 7d in AIMS aquarium water (Figure 10). After 14d, the autotrophic component (indicated by production values in the light) peaked and slightly declined to the end of the experiment after four weeks. The heterotrophic component showed a slight increase over time.

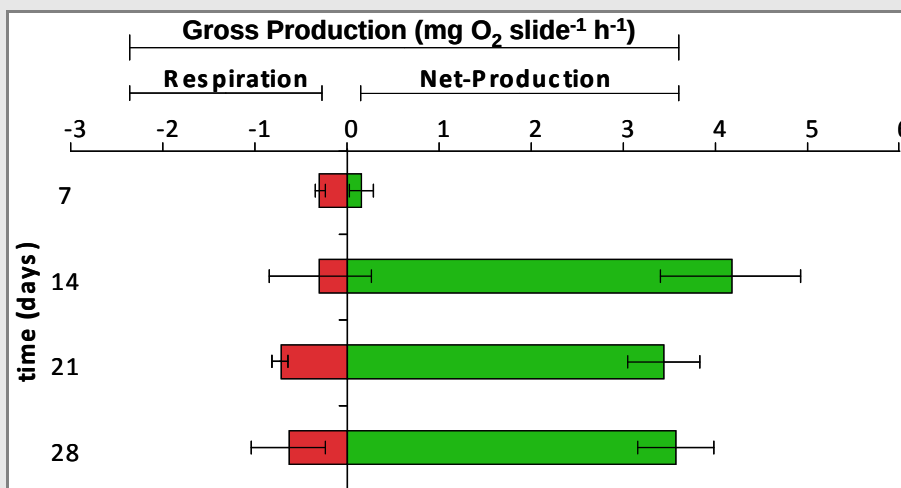


Figure 10: Net-Production at $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ (green bars) and respiration (red bars) of biofilms developing on glass slides in the AIMS aquarium. Error bars represent 1 SD.

Further respiration experiments have been conducted in 24-hour incubations of sediments in 300mL biological oxygen demand bottles under natural light and in darkness. The same parameters were measured on biofilm slides using 500mL glass jam jars. These measurements will be conducted in further field experiments and during the regular samples from Magnetic Island and the Whitsundays.

3. General outcomes of the biofilm work

Two further publications on benthic foraminifera as potential water quality indicators on the GBR have been submitted. In addition, two Honours theses have been finalised (for details see: [Communication, major activities and events](#)), and the web-based [e-Atlas](#) is now live. Thus, work on all biofilm indicators is now well advanced. Several indicators have either been already applied (e.g. as part of Reef Rescue Marine Monitoring Program) or are now available for wider field testing as a first step towards application.

Attachment 2

Manuscript submitted to 'Marine Biology': Abstract

Photosynthetic plasticity of endosymbionts in larger benthic coral reef Foraminifera

Maren Ziegler and Sven Uthicke

Larger benthic Foraminifera (LBF) are ecologically important coral reef protists that harbour a large diversity of symbionts from at least four algal phyla. In this study the photosynthetic plasticity of different endosymbiotic algae found within LBF was investigated using pulse amplitude modulated (PAM) fluorometry. Maximum quantum efficiencies (F_v/F_m) obtained from specimens directly after field collection indicated several distinct differences between endosymbiont groups and, to a lesser extent, also varied between species within the same group of endosymbionts. Species retaining functional chloroplasts and rhodophyte-bearing species had distinctly lower F_v/F_m , than LBF with dinoflagellates, diatoms or chlorophytes. A laboratory experiment was conducted over 48 hours exposing species occurring in high- (photophilic), medium- and low-light-adapted (sciaphilic) environments to manipulated light levels (10, 50 and 100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). Photophysiological responses were monitored by measuring F_v/F_m at regular intervals and the excitation pressure (Q) and rapid light curves (RLCs) at the end of the experiment. This experiment demonstrated diurnal oscillation of maximum quantum efficiencies according to the light-dark cycle. In sciaphilic species under high light the reduction of F_v/F_m was caused by an increase of minimum fluorescence (F_0). This decrease, in conjunction with increased Q values suggested light stress and resulting photoinhibition on photosystem II. Rapid light curves suggested that sciaphilic species have a reduced photosynthetic performance compared to the other species. Changes in the shape of the RLCs (e.g., higher α and lower E_k under low light conditions) indicated that photosynthetic plasticity allows LBF to acclimatise rapidly (< 48h) to different light conditions. However; this acclimatisation does have an impact on the photosynthetic performance of some species.

Attachment 3

Diplom thesis abstract

Growth and photophysiological response of benthic symbiotic foraminifera along a nutrient gradient on the Great Barrier Reef

Christine Altenrath

Coastal coral reefs are strongly affected by enhanced sediment and nutrient input, caused by major terrestrial runoff, and have experienced a decline in coral cover and diversity. Benthic symbiotic foraminifera are sensitive towards environmental and anthropologically induced disturbances and are therefore assumed to be potential bioindicators for changing water quality in coastal coral reef environments. The present study investigated the influence of enhanced nutrient levels on the growth rates and the photophysiological response of two abundant taxa of larger benthic foraminifera, *Heterostegina depressa* and *Amphistegina radiata*, along a water quality gradient in the Whitsunday Island Group, located in the Central Great Barrier Reef Marine Park World Heritage Area. Additionally, the influence of enhanced nutrient concentrations in combination with elevated temperatures have been observed in a laboratory based experiment. For both species, the differences in the growth rates revealed a noticeable trend towards increasing growth rates with increasing distance from the mainland (field study) and decreasing growth rates with increasing temperature (laboratory based experiment). Pulse-amplified modulated (PAM) fluorometry was used to measure the minimum (F0) and maximum (Fm) fluorescence of dark-adapted foraminifera to calculate the maximum quantum efficiency of photosystem II ((F0-Fm)/Fm), which gives information about algal health and the ability to use light. Decreasing maximum quantum efficiency with increasing temperature was observed under laboratory conditions for both species. Rapid light curves were obtained for the foraminifera kept in-situ to calculate the maximum light utilisation coefficient (α), the maximum electron transport rate (Pm) and the saturation irradiance (Ek). For both species, no statistically significant differences in these parameters were observed between the different water quality zones.

Attachment 4

Manuscript submitted to 'Ecological Applications': Abstract

Effectiveness of benthic foraminiferal and coral assemblages as water quality indicators on inshore reefs of the Great Barrier Reef, Australia

Sven Uthicke, Angus Thompson and Britta Schaffelke

Although the current debate about coral reef decline focuses on *global* disturbances (e.g. increasing sea temperatures and ocean acidification) *local* stressors such as nutrient runoff and overfishing continue to affect reef health and resilience. We assessed the effectiveness of benthic foraminiferal and hard coral assemblages as biological indicators for changes in water and sediment quality on 27 inshore reefs along the Great Barrier Reef (GBR). Environmental variables (several water quality and sediment parameters) and the composition of both foraminiferal and hard coral assemblages differed significantly between four regions. Grain size and organic carbon and nitrogen content of sediments, and a composite water column parameter (based on turbidity and concentrations of particulate matter) explained a significant amount of variation (tested by redundancy analysis) in the distribution of both assemblages. Foraminiferal assemblages differed in the relative abundance of heterotrophic species (dominant in sediments with high organic content and in low light availability) and symbiont-bearing species (preferring sediments with low organic content, and high light availability). A similar suite of parameters explained 89% of the variation in the FORAM index (a Caribbean coral reef health indicator) and 61% in foraminiferal species richness, but richness of corals did not relate to the environmental setting. Coral assemblages varied in response to environmental variables, but they are strongly shaped by acute disturbances such as cyclones, outbreaks of *A. planci*, coral bleaching and disease. Thus, due to differential recovery and recruitment, different coral assemblages may occur at sites with similar environmental conditions. Disturbances also affect foraminiferal assemblages but they are likely to recover over time scales of months to years, compared to decadal recovery times of coral assemblages. We propose that foraminiferal assemblages are effective bioindicators for assessments of turbidity/light regimes and organic enrichment of sediments on coral reefs. Hard coral abundance and diversity are important conservation targets and future assessments of coral population dynamics, especially recruitment and rate of recovery after disturbances under different environmental regimes, continue to be important. We suggest that the wider application of bioindicators to measure changes in water and sediment quality will support reaching these conservation targets.

Attachment 5

Final Report to MTSRF: Coral-based indicators to detect water changes in water quality on inshore reefs of the Great Barrier Reef

Katharina Fabricius, Helene Le Grand, Johnstone Davidson and Craig Humphrey

SUMMARY

This Report summarises 2008/2009 MTSRF Project 3.7.1(b) research aiming to identify bioindicators of changes in water quality that are consistently valid across many inshore regions of the GBR. The data are based on results from two field trips, investigating twelve inshore reefs in the four target regions of the inshore Great Barrier Reef (Keppels, Whitsundays, Burdekin and Wet Tropics). The three reefs in each of the regions are located along gradients of increasing distance away from river mouths, with contrasting long-term average turbidity and chlorophyll values. At each reef, biotic measures were investigated at two replicate sites and two depths per sites. The surveys assessed benthic cover and diversity based on photo transects, coral juvenile densities and diversity, and four physiological measures of massive *Porites* based on swim searches along the transects. Preliminary analyses of the complex data set are shown here, with the final analyses to be conducted in the coming months. The preliminary analyses show that a number of biotic measures responded consistently to changes in water quality across four regions of the GBR. Out of sixteen coral parameters tested, eleven showed graduated response to water quality and could potentially be used as bioindicators; colony brightness, the density of internal macrobioeroders and the depth of grooves in massive *Porites*; the density and diversity of soft coral juveniles; and the cover of macroalgae, *Acropora*, soft corals, and the soft coral families Xeniidae and Nephtheidae, and the ratio of *Acropora* cover to total coral cover. The methods used to measure these three parameters were cheap, rapid, non invasive and most of them are easily taught to non-specialists. Obviously, direct measurements of turbidity and chlorophyll a are the preferred means to assess changes in water quality. However, such loggers are expensive and long-term records are presently only available from less than twenty inshore reefs of the GBR. A final development of a robust and reliable indicator system may eventually allow us to assess changes in water quality on other inshore reefs of the GBR that are presently not instrumented with water quality loggers. Further analyses of the existing data, and some targeted studies to develop Standard Operational Procedures and field sampling protocols are now needed to complete the development of such indicator system.

1. INTRODUCTION

Reefs exposed to terrestrial runoff causing chronic sedimentation commonly have low coral cover and biodiversity, low coral recruitment, increased partial mortality and bioerosion, altered coral physiology, reduced skeletal density, and a transition of hard coral dominance to dominance by macroalgae (Montaggioni *et al.* 1993; West and Van Woesik 2001; Schaffelke *et al.* 2005). These responses are likely candidate measures to assess changes in water quality conditions. Here we investigated the consistency of relationships between water quality and a number of ecological indicators across four geographic regions in the GBR. Indicators were chosen based on measures that have previously been shown to be responsive to changing water quality in two GBR regions, namely the Wet Tropics and Whitsundays (Fabricius *et al.* 2007). The objectives of this study were:

1. To determine which parameters of corals vary consistently across regions with varying water quality (turbidity and chlorophyll a) on the GBR; and
2. To determine additional predictors other than water quality (such as water depth, colony size etc) of bioindicator values that need to be controlled for to detect responses of corals to changes in water quality.

The following three groups of indicators were selected.

1.1 Coral reef benthic cover and diversity

Reefs exposed to terrestrial runoff causing chronic sedimentation commonly show low coral cover and biodiversity, and a transition of hard coral dominance to dominance by macroalgae (Montaggionil *et al.* 1993; West and Van Woelk 2001; Schaffelke *et al.* 2005). These benthic responses, are likely candidate measures to assess changes in water quality conditions and particularly useful since many of them are being assessed during most coral reef monitoring programs. Here we investigated the relationships between water quality and a number of ecological attributes on cover and biodiversity of hard corals, octocorals and macroalgae along our water quality gradients. We focused on specific measures that allowed comparison with our results from the Whitsundays and Wet Tropics (Fabricius *et al.* 2007), and discuss the evidence for water quality effects on coral reef communities on these inshore reefs.

1.2 Coral juvenile density and diversity, and benthic composition

A number of studies have shown that coral recruits are more sensitive to changing water quality than adult corals (reviewed in Fabricius 2005). For example, sedimentation mortality thresholds for coral recruits are an order of magnitude lower than those for larger colonies (tens rather than hundreds of mg cm^{-2} ; Fabricius *et al.* 2003). Failing coral settlement and recruitment is therefore often considered the primary cause of reef deterioration related to terrestrial runoff. Indeed, where reef degradation has been associated with poor water quality, disturbances other than eutrophication were often the proximate causes of coral mortality, and runoff effects only became obvious when hard corals failed to re-establish. Coral recruitment is therefore commonly used as a proxy measure of the ability of reefs to recover from disturbance.

1.3 Measures of massive *Porites* colonies

1.3.1 The darkness of *Porites* pigmentation

Having wide physiological tolerance ranges, corals are able to adapt to changes in environmental factors such as increase in temperatures, elevated concentration of nutrients or decrease in light intensity by moderating the density and pigmentation of their endosymbionts (Pastorok and Bilyard 1985; Anthony and Hoegh-Guldberg 2003). Many studies have demonstrated that nutrient enrichment or decrease in light intensity increases the density of zooxanthellae and content of chlorophyll *a* per algal cell (Marubini and Davies 1996). Population density of zooxanthellae of the coral *Stylophora pistillata* doubled after fourteen days of exposure to 20 μM ammonium (Muscatine *et al.* 1989). Similarly corals adapted to shady environment have more chlorophyll *a* than light-adapted corals. In addition Marubini and Davies (1996) showed that the two coral species *Montastrea annularis* and *Porites porites* became darker after exposure to nitrate. As zooxanthellae density and chlorophyll *a* content per algal cell increase, coral colonies become darker; consequently colony darkness should increase with increasing distance away from river mouths (Falkowski and Dubinsky 1981; Fabricius 2006; Cooper *et al.* in press). As colony brightness is easily and non-destructively measured it has the potential to be a good indicator.

1.3.2. Internal macrobioeroder densities on massive *Porites*

Internal macrobioerosion is the colonisation of reef substrata by macro-organisms including sponges, bivalves, polychaetes and sipunculans Hutchings 1986. Internal macrobioeroders are mostly filter feeders and as a result of elevated concentration of nutritional resources such as nutrients and suspended particulate matter, they are likely to increase their densities (Pastorok and Bilyard 1985; Sammarco and Risk 1990). The relationship between eutrophication and internal macrobioerosion has been widely studied over the past two decades and the evidence indicates that internal macrobioerosion tends to be positively correlated to nutrient enrichment (Highsmith 1980; Rose and Risk 1985; ; Sammarco and Risk 1990; Kiene 1996; Edinger and Risk 1996; Chazottes *et al.* 2002b). For instance, on the GBR, macrobioeroder density of massive *Porites* did not vary seasonally but was higher in the inshore reef than in the two mid-shelf reefs (Cooper *et al.* 2008). Similarly, on three coral reefs of Costa Rica, the total density of internal macrobioeroders present on

Porites lobata (bivalves, sipunculans and polychaetes) was highest at the most polluted sites affected by high loads of terrestrial runoff than at the least polluted ones (Fonseca *et al.* 2006). Thus, density of internal macrobioeroders should increase with increasing distance away from river mouths and might be a potentially good bioindicator of water quality.

1.3.3. Depth of grooves of massive *Porites*

Scleractinian corals are phenotypically 'plastic' and a single coral species can have several growth forms (Barnes 1973). This variation of growth form can be controlled by genetic and environmental factors (Sanders and Baron-Szabo 2005). For instance, *Porites sillimaniani* develop branches in high light treatment whereas colonies remain flat in low light treatment (Muko, 2000). Similarly plates of *Turbinaria mesenteria* can adapt their morphology to optimise light capture as a result colonies from shallow water are vertical and narrow compared to colonies from deep water that are wide with a horizontal growth (Anthony *et al.* 2005). Furthermore, Darke 1991 studied the variation of massive *Porites* growth form on a cross-shelf gradient on the GBR and found that corals were bumpier on the inshore reef than on the mid-shelf and outer reefs. As food is more available on inshore reefs due to terrestrial runoff, tissue growth is higher and as skeletal growth and tissue growth are dependent, the skeleton develops bumps in order to accommodate this rapid tissue growth (Darke 1991). Therefore, the depth of grooves of massive *Porites* should increase with increasing distance away from river mouths.

1.3.4. Partial mortality of massive *Porites*

Being modular organisms, corals are able to survive when a part of their living tissue is dead. Sedimentation can cause partial mortality to corals directly through smothering (Wesseling *et al.* 2001). For instance, sediment exposure results in bleaching and necrosis on some species of corals after 24-36 hours of exposure (Philipp and Fabricius 2003). In addition corals produce high amounts of mucus in response to high sedimentation since this helps to remove sediment (Riegl and Branch 1995). Sedimentation being higher near the river mouth, partial mortality is expected to increase with increasing distance away from river mouths. Indeed, a number of studies have shown) showed that corals presented higher rate of partial mortality near the river mouth and concluded that partial mortality could be an indicator of sediment stress (Wesseling *et al.* 2001; Nugues and Roberts 2003).

2. MATERIALS AND METHODS

2.1 Study sites and sampling design

The study was conducted in four regions of the nearshore GBR: the Keppels, Whitsundays, Burdekin and Wet Tropic regions (Figure 1). Data were collected in July and August 2008. In each region, three reefs with decreasing distance away from river mouths (from south to north) were surveyed (location = 'near', 'mid' and 'far') (Figure 2). Two replicate sites located on the leeward sides of the reefs were sampled at each reef, and at each site, surveys were performed at two different depths ('shallow': 1-3 m, and 'deep' 7-11 m below lowest astronomical tide, LAT) (Table 1, Figure 1). All reefs were located less than twenty kilometres from the shore.

The Keppel Islands are located in the southern region of the GBR (23° S, 150-151°E) (Figures 1 and 2, Table 1). The three reefs surveyed in this region were Pelican Island (near), Humpy and Halfway Island (mid) and Barren Island (far). This region is influenced by one river system, the Fitzroy River, which provides a well-defined point source into Keppel Bay. Average annual rainfall in the Fitzroy catchment varies from 530 mm in the west to ~850 mm in central region, and ~2,000 mm in the northeast ranges near the coast (Packett *et al.* 2009). The amount of sediment discharged by the Fitzroy average 3.5 to 4 million tonnes per year, or ~33% of the modelled annual suspended sediment load from all GBR catchments (Packett *et al.* 2009). The dominant land use in the in the Fitzroy catchment is cattle grazing (~88% of area) and cropping and horticulture (~7%). Only four

percent are covered by national parks and other managed areas, and one percent are occupied by coal mining and other activities (Packett *et al.* 2009). Around sixty percent of the remnant vegetation in the Fitzroy catchment are substantially altered or cleared.

The Whitsunday Islands are located in the central region of the GBR (20°S; 148°E) (Figure 3, Table 1). The three reefs surveyed in this region were Pine Island (near), Daydream Island (mid) and Double Cone Island (far) (Figure 3). This region is influenced by two river systems, the Proserpine and O'Connell Rivers, that provide a well-defined point source in Repulse Bay (South-west of the Whitsunday Islands) (Figure 3). The catchment area for the two combined rivers is about 4,900km². Since 1991, water flow in the Proserpine River has been controlled by the Peter Faust Dam. Sediment from Repulse Bay is transported to the north directly affecting the three studied reefs (van Woesik *et al.* 1999). A total of 0.40 and 0.56 million tonnes of fine sediment, 906 and 1292 tonnes of nitrogen, 178 and 254 tonnes of phosphorus are discharged in the catchment area by the O'Connell and Proserpine rivers annually respectively (Furnas 2003). Annual rainfall is 1,600 mm (van Woesik *et al.* 1999). Having high rainfall and altered land-use, the Whitsundays region is considered at high risk of exposure to agriculture runoff (Brodie and Furnas 2001).

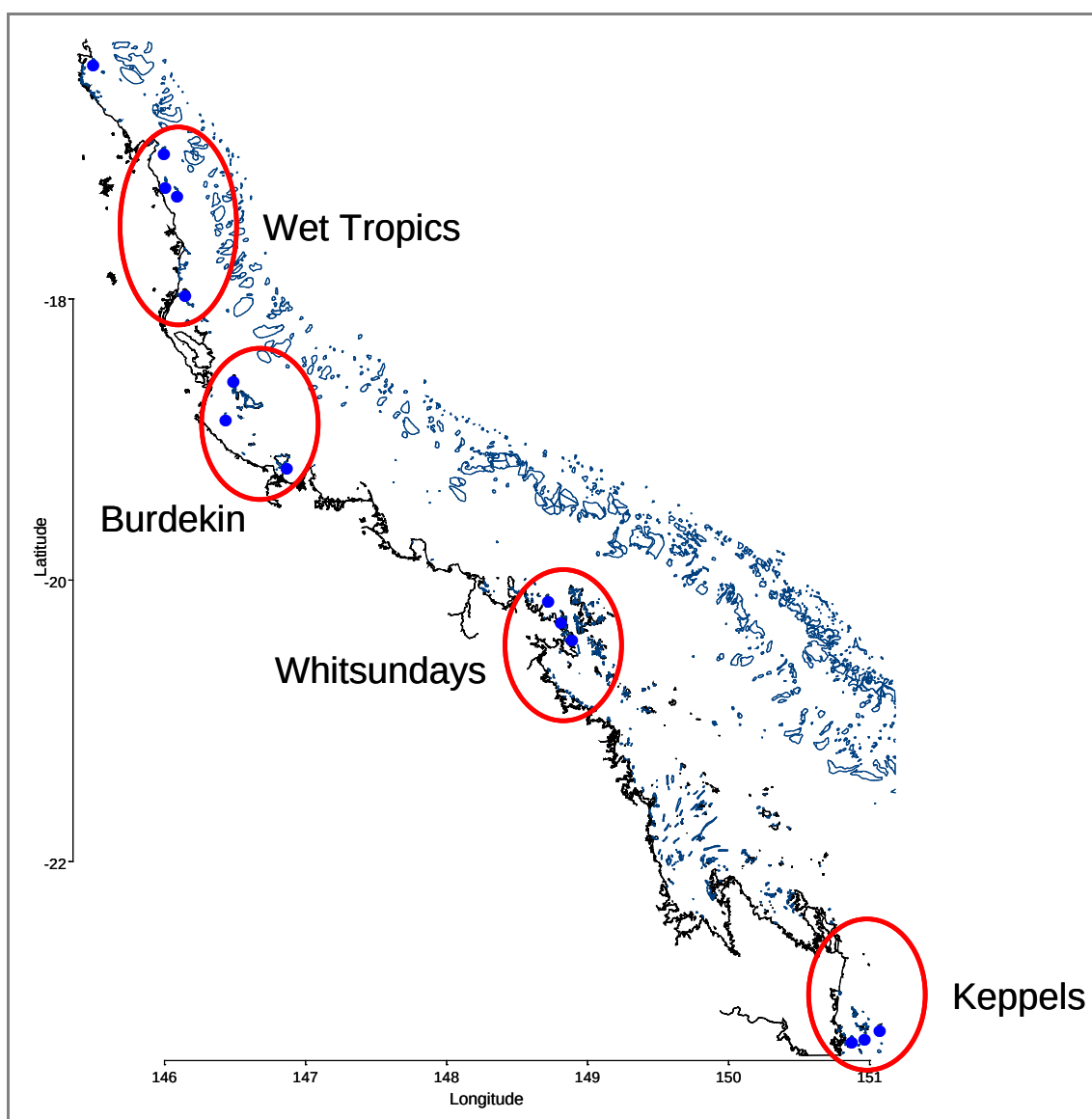


Figure 1: Location of the four studied regions: Wet Tropics (WT), Burdekin (BU), Whitsundays (WH), Keppels (KE).

The Burdekin region is located north of the Whitsundays (18°-19°S; 146°E) (Figure 4) (Table 1). The three reefs sampled in the Burdekin region were Geoffrey Bay of Magnetic Island (near), Pandora reef (mid) and Pelorus/Orpheus reefs (far) (Figure 4). The main river influence is from the Burdekin catchment which directly affects Geoffrey Bay (near), the Burdekin River (Figure 4). The Haughton and Ross Rivers also flow into this region but are smaller than the Burdekin River. The Burdekin catchment is about 130,126 km² and mainly used for grazing (Furnas 2003). The Burdekin river is the single largest source of sediment and nutrient inputs into the lagoons with runoff that could disperse up to the mid-shelf reef (McCulloch *et al.* 2003). A total of 3.77 million tonnes of fine sediment, 8633 and 1695 tonnes of nitrogen and phosphorus respectively are discharged per annum (Furnas 2003). There is often 10⁷ tonnes of suspended sediment during a single runoff event (McCulloch *et al.* 2003), but floods are often four to seven years apart. Generally these flood plumes flow northward along the GBR coastline affecting the three studied reefs.

The Wet Tropic region (16-17°S; 145-146°E) is located in the northern GBR (Figure 4) (Table 1). The three reefs surveyed were Dunk Island (near), High Island (mid) and Fitzroy Island (far). The Wet Tropics catchment comprises an area of approximately 12,660 km². Mean annual rainfall is 2,855 mm (Faithful and Finlayson 2005). Dunk Island is directly influenced by two rivers: the Tully River and to a lesser extent the Murray River. A total of 0.13 and 0.04 million tonnes of suspended sediment are exported by the Tully and Murray rivers into the catchment, respectively, each year (Faithful and Finlayson 2005). In addition, 1,303 and 420 tonnes of nitrogen and 138 and 44 tonnes of phosphorus are discharged from the Tully and Murray rivers respectively. High Island is directly affected by the Mulgrave-Russell Rivers and to a lesser extent the Johnstone River. Around 0.18 and 0.14 million tonnes of suspended sediment are exported by the Johnstone and Mulgrave-Russell rivers into the catchment, respectively, each year (Faithful and Finlayson 2005), with an additional 1,849 and 1,441 tonnes of nitrogen and 196 and 153 tonnes of phosphorus discharged from Johnstone and Mulgrave-Russell rivers per year respectively (Furnas 2003). Fitzroy Island is only affected by the Mulgrave-Russell Rivers. In this region, there are rivers all along the coastline, as a result the reefs are not considered to be located at increasing distance from the river. However Dunk Island is the most influenced by rivers and Fitzroy is the least influenced. High Island is considered to be at intermediate river influence. Having the highest local rainfall and erosion rates compared to other regions, the Wet Tropic catchment discharges the highest nutrient and sediment loads per unit of catchment area in nearshore reefs (Brodie and Furnas 2001). Major river flows occur every year in the Wet Tropics catchment during the moonsoonal wet season (December to May; Brodie and Furnas 2001). As a result this area, like the Whitsundays region, has been classified as having a high risk of exposure to agriculture runoff (Devlin and Brodie 2005).

Table 1: Distance between the reefs and the main river in each region.

Region	Reef	Approximate distance from river mouth	Coordinates
Keppels	Pelican Island	20 km	23°14.335'S 150°52.469'E
	Humpy Island	35 km	23°12.98'S 150°57.805'E
	Barren Island	44 km	23°9.449'S 151°4.228'E
Whitsundays	Pine Island	19 km	20°22'36.09"S 148°53'24.10"E
	Daydream	32 km	20°15'18.86"S 148°48'50.64"E
	Double Cone	52 km	20°06'07.16"S 148°43'09.69"E

Region	Reef	Approximate distance from river mouth	Coordinates
Burdekin	Geoffrey Bay	57 km	19°08'19.66"S 146°52'33.67"E
	Pandora Reef	117 km	18°48'49.98"S 146°25'55.16"E
	Orpheus/Pelorus	124 km	18°34'19.54"S 146°29'45.69"E
Wet Tropics	Dunk	10 km (from Tully R.)	17°56'16.75"S 146°08'36.90"E
	High	8 km (from Russell-Mulgrave R.) 70 km (Johnstone R.)	17°09'23.78"S 146°00'39.19"E
	Fitzroy	31 km (Russell-Mulgrave R.) 92 km (Jonstone R.) 122 km (Tully R.)	16°56'00.98"S 145°59'38.43"E

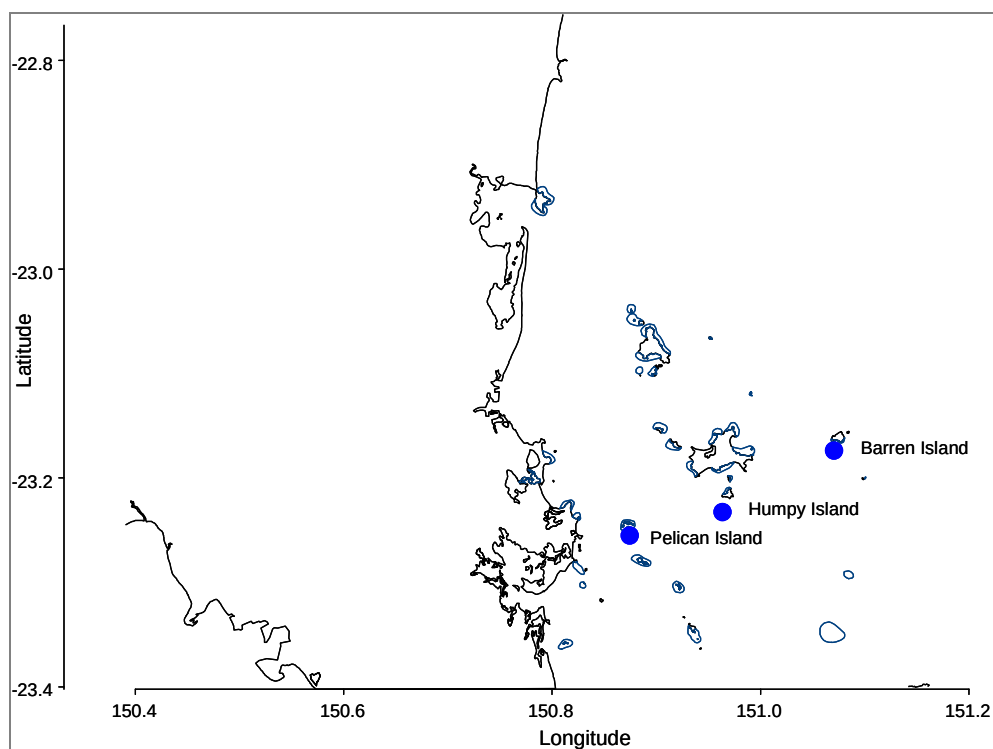


Figure 2: Location of the three studied reefs from the Keppels region (Pelican, Humpy-Halfway, and Barren Island).

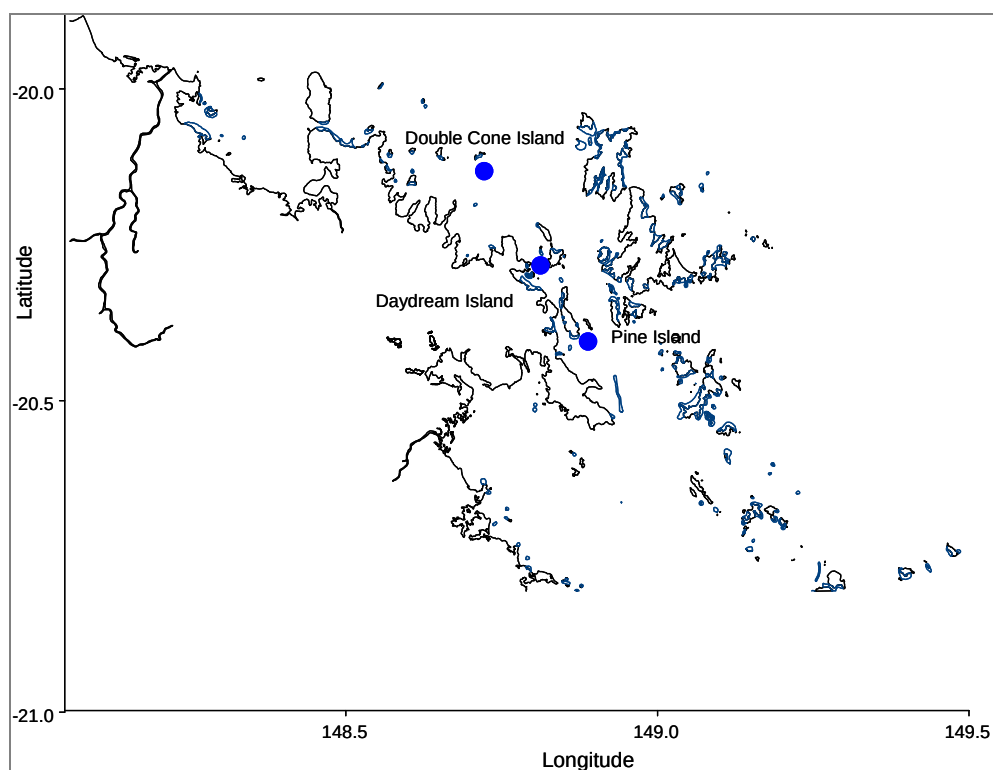


Figure 3: Location of the three studied reefs from the Whitsunday region (Pine, Daydream and Double Cone) and location of the two main rivers affecting these reefs (The Proserpine and O'Connell Rivers).

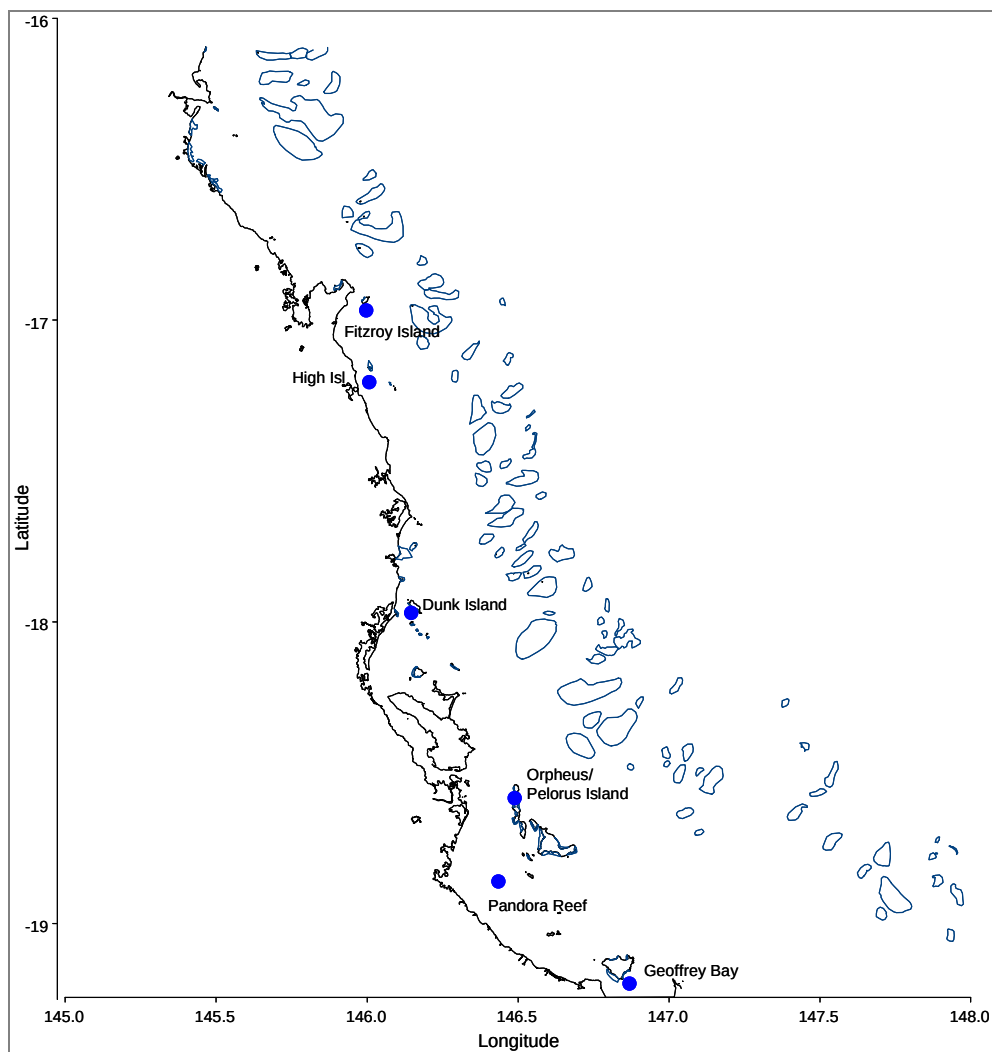


Figure 4: Location of the three studied reefs from the Wet Tropic region (Dunk, High and Fitzroy) and Burdekin region (Geoffrey, Pandora, and Orpheus/Pelorus).

2.2 Data collection

a) Benthic cover

The data collection and analysis was adapted from the protocol of the Reef Plan Marine Monitoring Program (Sweatman *et al.* 2007). In brief, a digital underwater photograph were taken every half meter along two 20m transects at each of two depths at each of two sites per reef. Thirty-two of the resulting images were selected at random and the organisms beneath five fixed points on each image identified to highest possible taxonomic resolution (governed by image quality). The depths sampled complemented that routinely collected by the Reef Water Quality Protection Plan Marine Monitoring group: while the latter sample at two and five metres below LAT, in this study we placed the 'shallow site' near the upper edge of the reef crest in very shallow water (~1 m below LAT), while the 'deep site' followed the lower edge of reef development typically at ~7-8 m below LAT. All data were collected in August 2008.

b) Juvenile densities

Demographic data were collected adapting the protocol described in Sweatman *et al.* 2007. In brief, all juvenile hard and soft corals smaller than ten centimetres in maximum diameter were recorded along each transect, within a belt 0.3 m wide. Surveys were conducted at very shallow (~1 m below LAT) and very deep (~7-8 m below LAT) transects (two transects per site, two sites per reef) in August 2008.

c) *Porites* indicators

Colonies of massive *Porites* were non-destructively sampled using scuba. At each site, the divers sampled the first ten colonies of massive *Porites* that were encountered at the deep sites (~7 m LAT), and then took the opposite direction to sample ten colonies at shallow sites (2 m LAT). Massive *Porites* are quite ubiquitous (DeVantier *et al.* 2006), however due to diving time constraints only one massive *Porites* was found and sampled in the deep mid reef (Pandora) of the Burdekin region; this site was excused from further analyses.

On each colony of massive *Porites*, five measures were taken:

- Darkness of pigmentation ('colour')
- Density of internal macrobioeroders
- Partial mortality
- Depth of grooves
- Surface area

The darkness of pigmentation (colour) of the colony was determined using the colour health monitoring chart (Siebeck *et al.* 2006; 'Coral Health Monitoring Chart', The University of Queensland, Australia, www.CoralWatch.org). The colour chart is composed of four hues corresponding to four different colour categories. Each hue comprises six colour graduations determining the level of brightness of a colony (1 = almost white, 6 = dark) (Siebeck *et al.* 2006). Only pigment changes associated with the symbiotic algae were monitored. Difference in hues was not taken into account. Only the numeric information corresponding to colony darkness was surveyed to the nearest half-score.

The density of internal macrobioeroders was estimated by counting the number of macrobioeroder apertures (e.g. bivalve siphonal opening) in a 14.9*14.9 cm quadrat placed on living colony surfaces. When the colony was smaller than the quadrat, the number of macrobioeroders was counted over the entire colony. When the colony was big enough, the density of macrobioeroders was subsampled within 1, 2 or 3 replicates. Massive *Porites* was considered as half a sphere. Their surface area was calculated using the formula: $2r^2\pi$. To calculate the density of internal macrobioeroders on massive *Porites*, the mean number of bioeroders counted within the quadrats or the total number of bioeroders counted over the whole colony was respectively divided by the surface area of the quadrat ($14.9 * 14.9 \text{ cm}^2$) or by the surface area of the colony. The mean bioeroders density ranged from 0.0 to >7340 boreholes m^{-2} .

The degree of rugosity of *Porites* was also measured. The 14.9 * 14.9 cm quadrat was held on the colony and the five deepest grooves within the quadrat were measured. The measures were taken with a plastic ruler placed in the groove (unit: cm). The mean depth of grooves varied from 0.0 cm on completely smooth colonies, to >5.6 cm on some larger colonies. When the colony was smaller than the quadrat, the five deepest grooves were measured from the entire colony. When the colony was big enough, three replicates were taken. The surface area of the colony was determined by measuring the biggest diameter of the colony and the one perpendicular to it. The surface area of the sampled *Porites* ranged from 0.007 to >70 m².

Partial mortality was visually estimated as a percentage of surface area. The partial mortality of the sampled *Porites* ranged from 0 to 80%; *Porites* with greater damage were excluded from the investigation.

d) Water quality analysis

Data provided by the Reef Plan Marine Monitoring Program were used for comparison with bioindicator variables (REF). Measures of turbidity and chlorophyll were taken at each studied reefs (12) from October 2007 to February 2008. In all studied reefs, loggers (FLNTU, Wetlabs) are continuously deployed at five metres below lowest astronomical tide, and take measurements of turbidity (Nephelometric turbidity unit, NTU) and chlorophyll (µg L⁻¹) every ten minutes; these values were averaged to obtain daily means. The deployment of FLNTU turbidity loggers ranged from 68 to 169 days. Long-term average means were used as predictor values against which the indicators were related.

2.3 Data analysis

Two-way ANOVA were performed to analyse the variation of the water quality data (turbidity and chlorophyll *a*) across stations (near, medium or far from the river mouth, and regions). Linear mixed model analyses were used to examine the variation of the bioindicators along water quality gradients, while accounting for between the four regions and two depths (as category, orthogonal). Data were transformed where necessary to improve normality and homogeneity of variances. Benthic cover and juvenile data were square root transformed, and turbidity and the density of internal macrobioeroders was log-(+1) transformed.

Non-sequential analyses of variance with single term deletions were used to determine the significance of the relationships to water quality (turbidity, chlorophyll *a* and km distance to the nearest river mouths), regions and depth. Turbidity and chlorophyll were highly correlated ($r^2 = 0.66$), and both were related to the distance of each reef to the river mouths. To avoid artefacts due to these correlations, the analyses were run including each of the three water quality variable separately in the models. The significance values of each factor, together with the slope and SE of slope of the relationship between the indicator and the water quality variable, were tabulated. Partial effects plots were produced to illustrate the relationships. Here, the outputs of the analyses with the three different water quality variables were combined for simplicity, and the relationship to region and depth were depicted only once (derived from the spatial model with distance to river). All analyses were conducted with the statistical software package 'R'.

3. RESULTS

3.1. Water quality analysis: chlorophyll a and turbidity

Chlorophyll a differed between regions and stations (Figure 5, Table 2a). Chlorophyll in the Whitsundays reefs were on average $0.15 \mu\text{g L}^{-1}$ higher, and in the northern two regions (Wet Tropics and Burdekin) $0.08\text{-}0.1 \mu\text{g L}^{-1}$ lower than the overall average value. Chlorophyll declined strongly away from the rivers in the Keppels and Burdekin region. In contrast, chlorophyll a at the near and far stations was higher than at the mid station in the Wet Tropics and the Whitsundays (Figure 5).

Figure 5: Mean chlorophyll a ($\mu\text{g/l}$) at each station (near, mid, far) from the four regions of the GBR. KE = Keppels, WH = Whitsundays, BU = Burdekin, WT = Wet Tropics. Data provided by the Reef Plan Marine Monitoring Program.

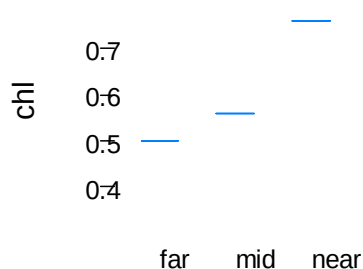


Table 2: Results of the two-way ANOVA on the variation of the water quality, turbidity (NTU) and chlorophyll ($\mu\text{g L}^{-1}$) between regions of the GBR and the three stations (near, mid and far distance from the river).

	df	MS	F	P
(a) Chlorophyll a ($\mu\text{g L}^{-1}$)				
Region	2	4.574	56.26	<0.0001
Station	2	2.111	25.97	<0.0001
Region* Station	4	1.435	17.65	<0.0001
Residuals	1234	0.08131		
(b) Turbidity (log NTU)				
Region	2	0.8440	0.2521	0.7772
Station	2	204.5	61.09	<0.0001
Region* Station	4	28.97	8.653	<0.0001
Residuals	1234	3.347		

3.2. Indicators based on massive *Porites*, juvenile density and diversity, and benthic cover

3.2.1 Benthic cover

Of the eight benthic indicators considered, seven were significantly related to one, two or all three water quality variables (Table 3, Figure 7). Macroalgal cover was particularly strongly related to all water quality measures, with steep increases with increasing chlorophyll, turbidity and proximity to the river mouth. The increase was an additional ~2.3% cover with every $0.1 \mu\text{g L}^{-1}$ increase in chlorophyll or with every additional 1 NTU of turbidity. *Acropora* cover, the ratio of *Acropora* to total hard coral cover, soft coral cover, and the cover of the soft coral family Xeniidae, all strongly declined with increasing values of chlorophyll and turbidity. The other common two soft coral families, Alcyoniidae and Nephtheidae, declined weakly along the same gradients (Figure 7). Only total hard coral cover was unrelated to the three water quality gradients.

Table 3: Results of the linear models on the variation of the benthic indicators to water quality. Results were controlled for the effects of potential differences between the four regions of the GBR, and the two depths (shallow and deep).

		Df	WQ = Chlorophyll ($\mu\text{g L}^{-1}$)			WQ = Turbidity (log NTU)			WQ = Distance River (km)		
			P	Slope	SE	P	Slope	SE	P	Slope	SE
Hard coral (% Cover)	WQ	1	0.201	8.854	2.264	0.129	-0.620	0.399	0.300	-0.017	0.016
	Depth	1	0.945			0.945			0.945		
	Region	3	0.050			0.058			0.606		
Acropora (% Cover)	WQ	1	0.0110	-9.9229	3.7131	0.0012	-1.3886	0.3970	0.2758	0.0199	0.0180
	Depth	1	0.0355			0.0270			0.0491		
	Region	3	0.0001			0.0001			0.0009		
Acropora/ Hard coral	WQ	1	0.0110	-1.1659	0.4360	0.0015	-0.1604	0.0469	0.0110	0.0047	0.0020
	Depth	1	0.0001			0.0001			0.0002		
	Region	3	<0.0001			<0.0001			0.0001		
MA cover (% Cover)	WQ	1	<0.0001	22.9685	2.8571	<0.0001	2.4300	0.3536	0.0002	-0.0718	0.0177
	Depth	1	0.0050			0.0098			0.0361		
	Region	3	<0.0001			0.0034			0.0002		
Soft coral (% Cover)	WQ	1	0.005	-9.544	3.208	0.042	-0.800	0.380	0.002	0.048	0.014
	Depth	1	0.118			0.136			0.109		
	Region	3	0.153			0.971			0.040		
Alcyoniidae (% Cover)	WQ	1	0.072	-5.693	3.078	0.276	-0.395	0.357	0.010	0.036	0.013
	Depth	1	0.345			0.358			0.323		
	Region	3	0.430			0.708			0.095		
Nephtheidae (% Cover)	WQ	1	0.048	-2.170	1.063	0.238	-0.149	0.124	0.055	0.010	0.005
	Depth	1	0.249			0.265			0.251		
	Region	3	0.091			0.368			0.487		
Xeniidae (% Cover)	WQ	1	0.006	-3.965	1.350	<0.0001	-0.558	0.142	0.142	0.010	0.007
	Depth	1	0.808			0.795			0.821		
	Region	3	<0.0001			0.001			0.004		

Figure 7 (continues to page 37): Partial effects plots of the variation of the benthic indicators with water quality, depth and regions. Each water quality variable was included separately and consecutively into the models. Results for the effects of depth and regions were derived from the spatial model (Distance from the river mouth), but the partial effects with the other water quality variables included were very similar. Solid lines show linear regression lines and their 2 SE. Dashed lines mark non-significant relationships ($p > 0.05$).

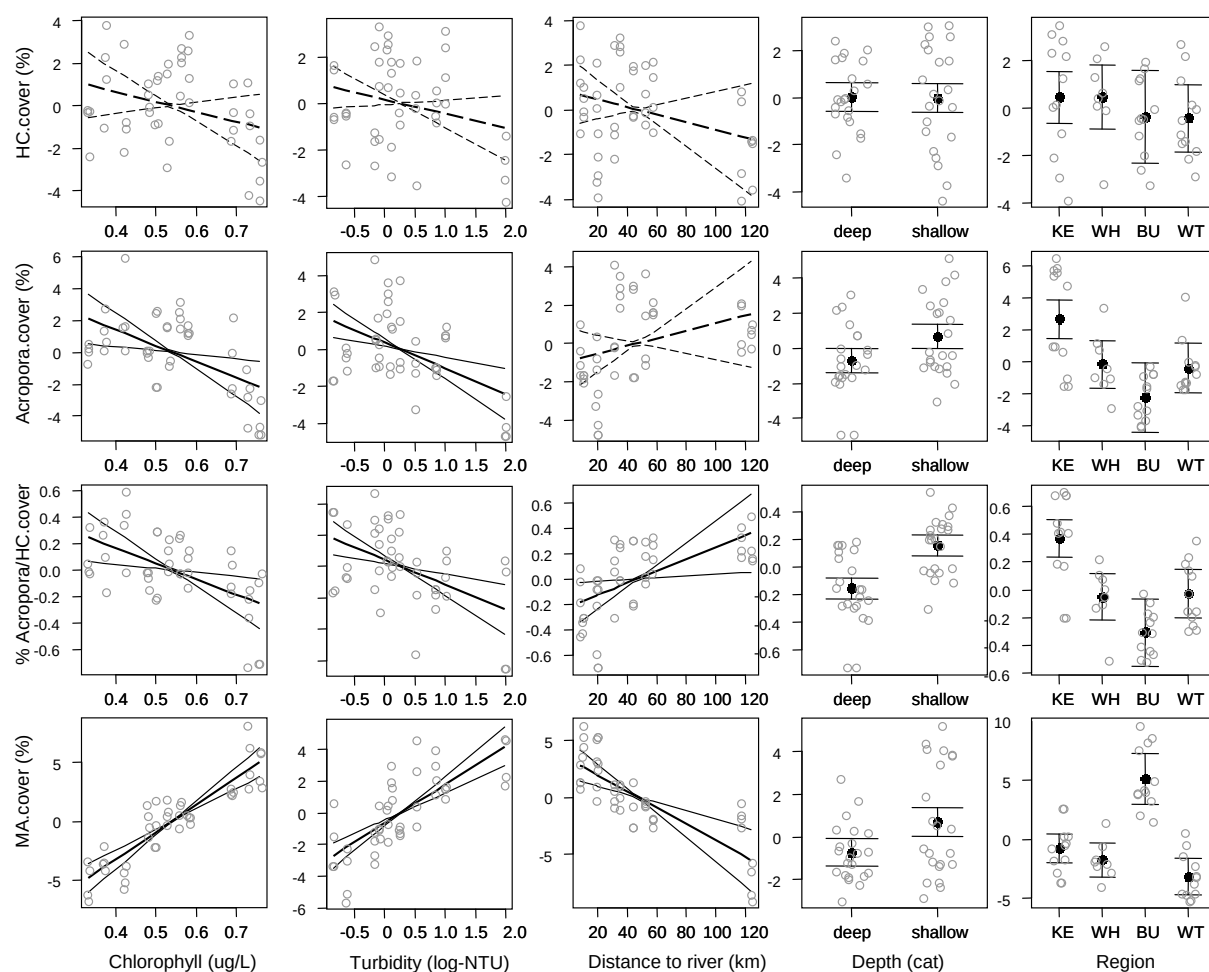
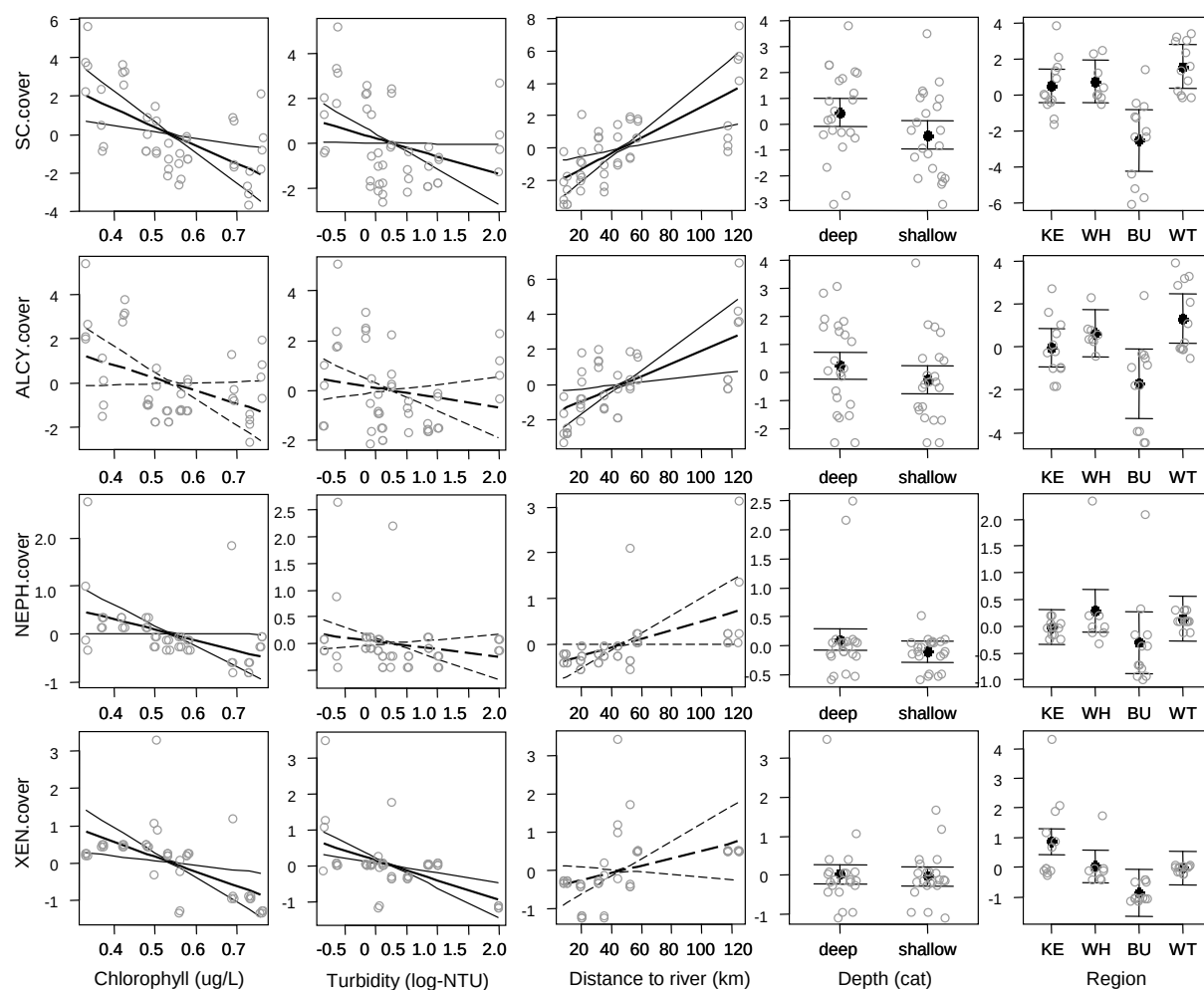


Figure 7 (continued from page 36): Partial effects plots of the variation of the benthic indicators with water quality, depth and regions. Each water quality variable was included separately and consecutively into the models. Results for the effects of depth and regions were derived from the spatial model (Distance from the river mouth), but the partial effects with the other water quality variables included were very similar. Solid lines show linear regression lines and their 2 SE. Dashed lines mark non-significant relationships ($p > 0.05$).



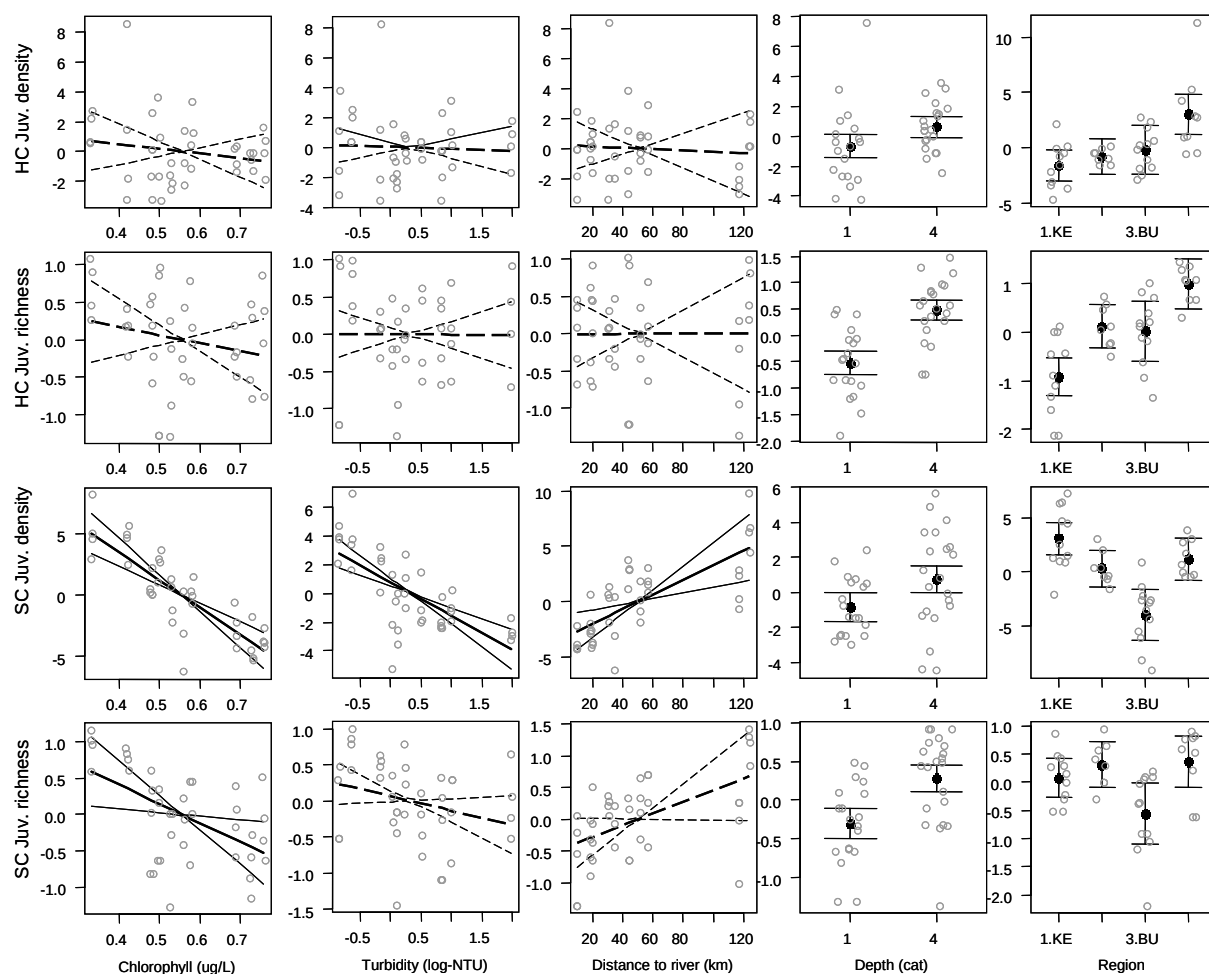
3.2.2 Coral juvenile indicators

Of the four coral juvenile indicators considered, soft coral juvenile density was significantly related to all three water quality variables, while the richness of soft coral juveniles was significantly related to chlorophyll (Table 4, Figure 8). Soft coral juvenile density steeply declined with increasing chlorophyll, turbidity and proximity to the river mouth. The decline was 2.2-2.3 juveniles with every 0.1 $\mu\text{g L}^{-1}$ increase in chlorophyll or with every 1 NTU addition of turbidity. The density and diversity of hard coral juveniles were unrelated to the three water quality gradients. There were also a number of significant differences across regions and depths in the density and diversity of both hard coral and soft coral juveniles (Table 4).

Table 4: Results of the linear models on the variation of the coral juvenile indicators to water quality. Results were controlled for the effects of potential differences between the four regions of the GBR, and the two depths (shallow and deep).

		Df	WQ = Chlorophyll ($\mu\text{g L}^{-1}$)			WQ = Turbidity (log NTU)			WQ = Distance River (km)		
			P	Slope	SE	P	Slope	SE	P	Slope	SE
HC Juv.dens	WQ	1	0.479	-3.100	4.335	0.787	-0.131	0.481	0.823	-0.004	0.019
	Depth	1	0.093			0.094			0.094		
	Region	3	0.005			0.001			0.001		
HC.Juv.rich	WQ	1	0.385	-1.056	1.199	0.973	-0.005	0.134	0.983	0.000	0.005
	Depth	1	<0.0001			<0.0001			<0.0001		
	Region	3	<0.0001			<0.0001			<0.0001		
SC.Juv.dens	WQ	1	<0.0001	-22.22	3.664	<0.0001	-2.354	0.421	0.003	0.066	0.020
	Depth	1	0.020			0.022			0.053		
	Region	3	0.000			0.003			0.002		
SC.Juv.dens	WQ	1	0.017	-2.610	1.045	0.107	-0.199	0.121	0.062	0.009	0.005
	Depth	1	0.003			0.004			0.004		
	Region	3	0.119			0.622			0.184		

Figure 8: Partial effects plots of the variation of the coral juvenile indicators with water quality, depth and regions. Each water quality variable was included separately and consecutively into the models. Results for the effects of depth and regions were derived from the spatial model (Distance from the river mouth), but the partial effects with the other water quality variables included were very similar. Solid lines show linear regression lines and their 2 SE. Dashed lines mark non-significant relationships ($p > 0.05$).



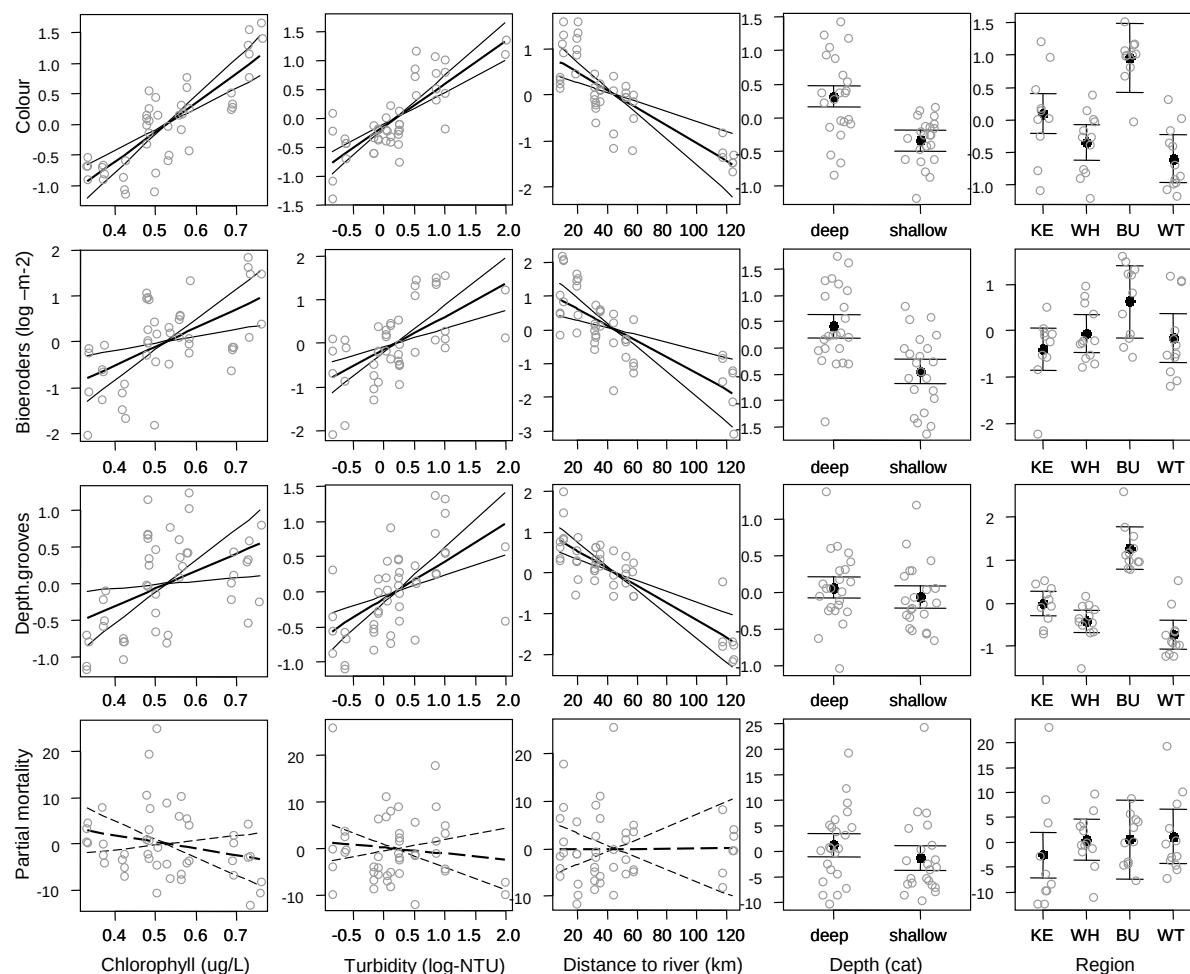
3.2.3 *Porites* Indicators

Of the four *Porites* indicators investigated, pigmentation, bioeroders density and the depth of grooves were significantly related to all three water quality variables, while partial mortality was unrelated to the three water quality gradients (Table 5, Figure 9). Bioeroder density steeply increased with increasing chlorophyll, turbidity and proximity to the river mouth. The increase was 6 additional bioeroders m^{-2} with every $0.1 \mu g L^{-1}$ increase in chlorophyll. There were also significant depth effects, with colonies being darker by 0.65 colour chart scores, and having 2.7 times as many bioeroders at the deep sites than at the shallow sites. There were also significant regional differences in *Porites* colour, bioeroder densities and depth of grooves, with higher values in the Burdekin region than in the other three regions (Figure 9)

Table 5: Results of the linear models on the variation of the *Porites* indicators to water quality. Results were controlled for the effects of potential differences between the four regions of the GBR, and the two depths (shallow and deep).

		Df	WQ = Chlorophyll ($\mu g L^{-1}$)			WQ = Turbidity (log NTU)			WQ = Distance River (km)		
			P	Slope	SE	P	Slope	SE	P	Slope	SE
Colour	WQ	1	<0.0001	4.756	0.693	<0.0001	0.739	0.094	<0.0001	-0.019	0.004
	Depth	1	<0.0001			<0.0001			<0.0001		
	Region	3	<0.0001			0.010			0.002		
Bioeroders (log m^{-2})	WQ	1	0.003	4.082	1.269	<0.0001	0.755	0.171	0.001	-0.024	0.006
	Depth	1	0.001			0.001			0.001		
	Region	3	0.001			0.007			0.248		
Depth of grooves (cm)	WQ	1	0.016	2.382	0.948	<0.0001	0.538	0.122	<0.0001	-0.021	0.004
	Depth	1	0.449			0.632			0.413		
	Region	3	0.098			0.092			<0.0001		
Partial mortality	WQ	1	0.243	-14.576	12.287	0.497	-1.248	1.821	0.977	0.002	0.065
	Depth	1	0.241			0.247			0.284		
	Region	3	0.725			0.666			0.708		

Figure 9: Partial effects plots of the variation of the *Porites* indicators with water quality, depth and regions. Each water quality variable was included separately and consecutively into the models. Results for the effects of depth and regions were derived from the spatial model (Distance from the river mouth), but the partial effects with the other water quality variables included were very similar. Solid lines show linear regression lines and their 2 SE. Dashed lines mark non-significant relationships ($p > 0.05$).



4. DISCUSSION

This study demonstrated that some coral reef indicators consistently change along water quality gradients in four regions of the GBR, and confirms that some of the indicators previously proposed for the Whitsundays (Fabricius *et al.* 2007) are indeed valid across the GBR. For reef benthos, macroalgal cover was particularly strongly related to all water quality measures, but also *Acropora* cover, the ratio of *Acropora* to total hard coral cover, soft coral cover, and the cover of the soft coral family Xeniidae, all strongly declined with increasing values of chlorophyll and turbidity. Only total hard coral cover was unrelated to the three water quality gradients, a pattern which deserves closer investigation. For coral juvenile indicators, soft coral juvenile density and diversity appeared to be related to water quality, while the preliminary analyses suggested that hard coral juvenile density and diversity were unrelated to water quality. This is contrary to many previous findings, and deserves closer inspection (e.g. inclusion of availability of substratum, A. Thompson, unpublished data). For the indicators based on massive *Porites*, colony brightness, internal macrobioeroder density and the depth of grooves of massive *Porites* changed strongly along all three water quality variables. In contrast, partial mortality of massive *Porites* (and skeletal density of *Pocillopora damicornis*, not shown here) did not differ in response to changing water quality, which again contrasts with some previous findings. However as many environmental conditions cause partial mortality, including bleaching or crown-of-thorns starfish, this result is not surprising and we suggest this measure should not be considered as a bioindicator of water quality.

The background to some of the indicators based on the benthic and coral recruitment metrics have been discussed previously (Fabricius *et al.* 2007), and will be refined once the final analyses will be completed. However the physiological indicators based on massive *Porites* will be discussed here in greater detail.

4.1. Colony brightness of massive *Porites*

Our results showed that the relationships between colony brightness of massive *Porites* and water quality measures, turbidity and chlorophyll a, were statistically significant at both depths. Our results matched our expectations since colonies of massive *Porites* located closer to the river mouth were darker by one colour chart score than colonies located further away from the river mouth. Elevated concentration of nutrients carrying into the inshore GBR by river waters might influence pigmentation of corals. Indeed, the two corals, *Montastrea annularis* and *Porites porites*, looked darker after exposure to different concentrations of nitrate (0, 1, 5, 20 μM) for thirty to forty days (Marubini and Davies 1996). The same trend was found between inshore and mid-shelf reefs with colonies of massive *Porites* being darker by two colour chart score in the inshore reefs (Cooper *et al.* 2008). Not all nutrients appear to result in increased pigmentation since zooxanthellae density and their chlorophyll a content were not affected by phosphate enrichment (Muscatine *et al.* 1989). Massive *Porites* respond within about twenty days to changes in water quality (Cooper 2008).

The difference of colour between colonies of massive *Porites* along the water quality gradient might be due not only due to variation in nutrients but also to the variation in light intensity. The difference that we found between deep and shallow colonies of massive *Porites* is probably due to variation of irradiance between shallow and deep sites, and turbidity (and hence light attenuation) increased towards the river mouths. At deep sites, colonies were darker by 0.5 colour chart score than at shallow ones. This pattern was consistent with Falkowski and Dubinsky (1981) who demonstrated that the shade-adapted corals *Stylophora pistillata* had 7.4 times more chlorophyll a than light-adapted corals from the same species and were therefore lighter than light-adapted corals. Seasonal variation in irradiance might also affect colony brightness since colonies of *Pocillopora damicornis* are lighter in winter than in summer (Stimson 1997).

Increased dissolved nutrients can however indirectly modify coral pigmentation by increasing phytoplankton production and consequently decreasing light intensity Tomascik and Sander 1987. Moreover dissolved inorganic nutrients can be taken up and converted into particulate organic matter within hours to days and consequently affecting turbidity (Furnas 2003). Thus light intensity might be the main direct factor affecting coral pigmentation on inshore reefs of the GBR, while nutrient addition may further contribute to changing pigmentation. Our results suggest that colony brightness seems to be an appropriate bioindicator of water quality. The colour chart created by Siebeck *et al.* (2006) is a simple method that is inexpensive, rapid, easily taught and non invasive, and therefore could be integrated in monitoring programs.

4.2. Internal macrobioeroder density on living surface of massive *Porites*

Our results showed that densities of internal macrobioeroders decreased with increasing distance away from river mouths. This pattern was consistent with other studies that have found higher densities of internal macrobioeroders in the most polluted sites compared to the least polluted sites (Holmes *et al.* 1997; Holmes 2000; Holmes *et al.* 2000; Hutchings and Peyrot-Clausade 2002; Fonseca *et al.* 2006). For example, internal macrobioerosion rate of live *Porites lobata* decreases with distance from the shore, from 11% (inshore) to 1.3% (offshore), across the central GBR (Sammarco and Risk 1990). Similarly, in French Polynesia, more boring individuals were found in high island sites (eutrophic sites) than in atoll sites (oligotrophic sites) (Hutchings and Peyrot-Clausade 2002). There have also been some studies that failed to show variation of internal bioeroder density with change in water quality (Kiene 1996; Chazottes *et al.* 2002a; Tribollet *et al.* 2002). These studies were conducted over less than a two years study period and used experimental blocks cut from massive *Porites*, a different method than the one used in the present study. Deployment times were not long enough to allow significant macrobioeroders such as sponges and bivalves to recruit and establish on these blocks (Kiene and Hutchings 1994; Chazottes *et al.* 1995). This is supported by the study of Tribollet and Golubic (2005) who showed that internal macrobioerosion intensity starts to display a significant pattern on experimental blocks after three years of exposure with sponges and bivalves dominating at inshore sites compared to mid-shelf and offshore reefs.

Nevertheless, internal macrobioerosion intensity is highly variable in space Hutchings *et al.* 1992, and appropriate sampling designs are required to detect the signal in amongst the noise. Since most macrobioeroders recruit as pelagic larvae, factors such as fine-scale currents might influence their recruitment and consequently their distribution across the reef Osorno *et al.* 2005. For instance, local pattern of recruitment of internal macrobioeroders via pelagic larvae differs between leeward and windward reef sites as well as between years Hutchings *et al.* 1992. Furthermore internal macrobioerosion is affected by various factors, biotic (e.g. productivity, structural characteristics, zonation of the reef, specific density of different bioeroders and solitary and colonial lifestyle of bioeroders) and abiotic factors (e.g. sedimentation rate, depth, wind and wave exposure; Peyrot-Clausade 1992; Perry 1998; Zubia and Peyrot-Clausade 2001; Londono-Cruz *et al.* 2003). Londono-Cruz *et al.* (2003) have demonstrated that bioerosion rates and abundances of lithophagids are higher in zones with strong hydrodynamics than at other sites Londono-Cruz *et al.* 2003. Such great variability might be the reason why there is a significant difference of internal macrobioerosion between the studied regions. Bioeroder density also varies with depth (Greenstein and Pandolfi 1997; Perry 1998; Londono-Cruz *et al.* 2003). In our study, bioeroder densities were also higher at deep sites than at shallow sites, in agreement with Perry (1998) but in contrast to some other studies which did not show any variation between depths (Greenstein and Pandolfi 1997; Londono-Cruz *et al.* 2003).

Some taxa are more sensitive to change in water quality than others (Zubia and Peyrot-Clausade 2001; Hutchings *et al.* 2005). Bivalves and sponges tend to be more abundant at the most polluted sites than at the least ones (Rose and Risk 1985; Cuet *et al.* 1988; Sammarco and Risk 1990; Holmes *et al.* 1997; Holmes 2000) whereas polychaetes and sipunculans do not seem to display variation in their density but in their community

composition (Sammarco and Risk 1990; Zubia and Peyrot-Clausade 2001; Hutchings and Peyrot-Clausade 2002; Osorno *et al.* 2005). Deposit feeding polychaetes, such as *Dodecaceria spp.*, are dominant at eutrophic sites while suspension feeding polychaetes, such as *Polydora spp.*, dominate at oligotrophic sites (Hutchings and Peyrot-Clausade 2002; Osorno *et al.* 2005). The survey of taxa, such as bivalves, that varies in abundance along the water quality gradient might be more sensitive to variation in water quality. The density of internal macrobioeroders could potentially be a good indicator of water quality but more studies are required to determine the other factors influencing internal macrobioeroder distribution.

4.3. Depth of grooves of massive *Porites*

Our results were as expected with the depth of grooves of massive *Porites* increasing towards the river mouth, with turbidity, and to a lesser extent with chlorophyll a. This trend can be explained since colony shape varies in response to light level, water energy, nutrient availability, sediment accumulation and substrate (Sanders and Baron-Szabo 2005). In corals, skeletal mass and tissue biomass are not independent since skeleton supports tissue and tissue deposit skeleton (Barnes 1973). The differentials between tissue growth and skeletal growth might be compensated by a change in polyp division and consequently a change in the geometry of growth (change of growth form). A bumpy growth form provides a larger surface area for tissue growth and new polyp formation (Darke, 1991). As there is more food available for corals near rivers, coral tissues grow faster Barnes 1973. The skeletal growth may not be rapid enough to sustain tissue growth and therefore the skeleton must change the growth form to accommodate to this rapid tissue growth Barnes 1973. The influence of environmental conditions on growth forms of massive *Porites* has also been demonstrated by Darke (1991), who found that corals were bumpier on the inshore reefs than on the mid-shelf and outer reefs of the GBR. The ratio tissue growth by skeletal growth was higher on the inshore reef where more food is likely to be available. Consequently tissue growth was more constrained by skeletal growth on this reef and to accommodate this constraint, corals became bumpier (Darke, 1991). Further, the depth of grooves and surface area were positively related (not shown), as also confirmed by Isdale (1977) who demonstrated that *Porites lobata* maintain a smooth growth for about five to eight years until they reach fifty to eighty centimetres in height and then their bumpy growth surface develops (Darkes, 1993; Isdale, 1977).

The method used to measure the depth of grooves has never been used before and showed interesting results however will require improvements to obtain more accurate measurements. In this study, the depth of grooves was measured with a single ruler placed in the groove. To get a better measure, a sliding bar should be adapted perpendicularly to the ruler. At each measurement, the ruler should be put in the groove and the bar should be slid until it touches the top of the bump. Thus the measure could be read more easily and more accurately. Overall, the depth of grooves in massive *Porites* seemed to be sensitive to change in water quality and could potentially be a good bioindicator of water quality.

4.4. Partial mortality

Our results showed that there was no significant difference in partial mortality of massive *Porites* with increasing river influence. They were consistent with Lewis (1997) who demonstrated that partial mortality of the coral *Siderastrea siderea* did not vary along an eutrophication gradient. However other studies found that coral partial mortality increased towards river mouths where sediment exposure was higher Nugues and Roberts 2003. Nugues and Roberts (2003) showed that the partial mortality of the two corals, *Siderastrea siderea* and *Montastrea annularis*, was higher when closer to the river mouth and suggested this parameter could be used as potential indicator of sediment stress. Another study conducted by Dikou and van Woesik (2006) exhibited similar results however water quality measurements were not taken and consequently was is not possible to know if this variation of partial mortality was due to increasing sedimentation or site effect.

Since partial mortality of corals can be caused by various factors (e.g. diseases, predation, wave exposure, hurricanes, scuba divers or senescence), it is difficult to distinguish which factors might influence partial mortality the most and consequently it is difficult to differentiate the effect of terrestrial runoffs among all the others (Nugues and Roberts, 2003; Wesseling *et al.* 2001; Wielgus, 2004). Wesseling *et al.* (2001) found that, in the Philippines, partial mortality of *Porites* can be due to variation in sedimentation, wave exposure, colony size and site effect that is probably caused by human exploitation. However as their model explained only a small proportion of the variation, they concluded that other factors might be linked to variation in partial mortality (Wesseling *et al.* 2001). On the other hand, susceptibility to partial mortality varies between species (Nugues and Roberts 2003). Some species have better capacity to clean off sediment and suffer less partial mortality (Riegl and Branch 1995; Wesseling *et al.* 2001). For instance, *Siderastrea siderea* and *Montastrea annularis* seem more sensitive to sedimentation stress and therefore more prone to partial mortality than *Porites astreoides* and *Colpophyllia natans* (Nugues and Roberts 2003). Massive *Porites* might be efficient at removing sediment and consequently might not be vulnerable enough to indicate variation of partial mortality. Our results disagree with those of Nugues and Robert (2003) who suggested that partial mortality of corals could be used as a potential indicator of sediment stress. Since it is affected by many factors, partial mortality would not make a good bioindicator of water quality.

5. CONCLUSION AND FURTHER STUDIES

These preliminary analyses of a complex data set have shown that a number of biotic measures respond consistently to changes in water quality across four regions of the GBR. Out of sixteen coral parameters tested, eleven showed graduated response to water quality and could potentially be used as bioindicators; colony brightness, the density of internal macrobioeroders and the depth of grooves in massive *Porites*; the density and diversity of soft coral juveniles; and the cover of macroalgae, *Acropora*, soft corals, and the soft coral families Xeniidae and Nephtheidae, and the ratio of *Acropora* cover to total coral cover. The methods used to measure these three parameters were cheap, rapid, non invasive and most of them are easily taught to non-specialists. The assessment of benthic cover is part of routine monitoring programs. The use of colour reference chart, developed to monitor bleaching events, is very useful to monitor colour response. The count of bioeroders on coral surface is done very easily and quickly with a quadrat placed on the coral. The method used to measure the depth of grooves will require improvements to obtain more accurate measurements. Only the assessment of juvenile densities requires a high level of expertise for taxonomic identification and a consistency in finding the I and often semi-cryptic coral juveniles in the field. Each bioindicator has different levels of sensitivity and timing in response of water quality. Coral colour changes within weeks to changing water quality, and seems appropriate to as early warning indicator of acute stress. In contrast, variation of community measures and bioeroder density occurs on time scale of months to years and might be a better indicator of chronic stress. The timing at which depth of grooves changes as a result of water quality needs to be determined.

Obviously, direct measurements of turbidity and chlorophyll a are the preferred means to assess changes in water quality. However, such loggers are expensive and presently only available from fourteen reefs. A final development of a robust and reliable indicator system may eventually allow us to assess changes in water quality along the other hundreds of inshore reefs of the GBR that are presently not supplied with water quality loggers. Further analyses of the existing data, and some targeted studies to develop Standard Operational Procedures and field sampling protocols are now needed to complete the development of such indicator system.

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Attachment 6

Final Report 2009: Estuarine Indicators of Ecosystem Condition

Objective (d): Conduct research towards identifying potentially useful ecological indicators of the condition of North Queensland's estuaries.

SUMMARY

- Studies have been conducted to develop appropriate approaches to the evaluation of estuarine ecosystem condition in a tropical Australian context.
- Sampling the eleven estuary locations provided a number of insights. These allowed considerable advances in the development of appropriate sampling and experimental strategies for evaluating estuarine ecosystem condition in the context of the unique ecological and physical attributes of north-eastern Australian estuaries.
- Except for interruptions due to adverse weather data collection for the main study has been completed and most primary analyses are complete or near completion.
- Field work for the developmental stage of the project is complete.
- While some components await the availability of final laboratory (Stable Isotope) data, most analysis and modelling complete, with models in their second or third phase of development.
- Fish assemblage structure has been developed as a monitoring tool, with a first round of model evaluation is complete and a second round underway.
- Analysis and modelling of spatio-temporal patterns of change in fish biomass-abundance relationships are complete and testing of their value as indicators is underway.
- Trophic structure holds great promise as an indicator of estuary health but requires final Stable Isotope data for definitive results.
- Changes in taxonomic composition of the phyto-detritivore guild provide another potentially useful indicator of health. An apparent link between taxonomic composition and differential consumption of food sources has been identified and further studies to validate this initiated.
- Changes in the richness and species composition of benthic feeders have been identified, but, although these may useful indicators of major change, they are unlikely to provide sensitive indication of lower level effects.
- Nitrogen Stable Isotope values show characteristic changes in response to anthropogenic nutrient inputs making them useful indicators of such impacts.
- One manuscript is submitted and a second will be submitted by the end of June.

REPORT

Field work for the developmental stage of the project is complete. While some components await the availability of final laboratory (Stable Isotope) data, most analysis and modelling is complete, with models in their second or third phase of development. Fish assemblage structure has been developed as a monitoring tool, with a first round of model evaluation complete and a second round underway. Analysis and modelling of spatio-temporal patterns of change in fish biomass-abundance relationships are complete and testing of their value as indicators is underway. Trophic structure holds great promise as an indicator of estuary health but requires final Stable Isotope data for definitive results. Changes in taxonomic composition of the phyto-detritivore guild provide another potentially useful indicator of health. An apparent link between taxonomic composition and differential consumption of food sources has been identified and further studies to validate this initiated. Changes in the richness and species composition of benthic feeders have been identified, but, although these may useful indicators of major change, they are unlikely to provide sensitive indication of lower level effects. Nitrogen Stable Isotope values show characteristic changes in response to anthropogenic nutrient inputs making them useful indicators of such impacts. One manuscript is submitted and a second will be submitted by the end of June.

Field Work

- **Sample Collection and Analysis:** All field data sampling and laboratory processing are now complete and data sets have been fully checked (double input) and errors rectified. Cast net sampling produced a total of 135,060 fish representing 52 Families, 82 Genera and 126 species. In addition, 169,865 crustaceans were recorded from 3 Families, 5 Genera and 11 species.
- **Weather Constraints:** Extensive flooding of estuaries from late December 2007 to February 2008, and again from January to March 2009 necessitated substantial modification to all aspects of the study, as follows:
 - Monthly cast net sampling aimed at collecting data for process level measures could not be collected from some sites in December 2007, any sites in January and February 2008 or February and March 2009, and some sites in January 2009. However, full samples were collected in November 2007, March, April, May, July, September and November 2008. Thus, although a full sequence of samples was not possible, this main sampling component for the collection of ecological process level ecological indicators has produced an extensive body of data.
 - The lack of a continuity of samples during wet seasons has meant that stable isotope studies were disrupted until an appropriate series of samples was available. Collection and initial preparation of those samples is now complete and final processing is underway. Further analysis cannot begin until results return from the Stable Isotope Laboratory. This has also held up final trophic analyses.
 - Similarly, studies of recruit persistence have been hampered by gaps in the data set but this is well underway.

Data Analyses, Results and Model Progress

Analysis of the major spatio-temporal data set is complete. This data set is the basis for ten potential approaches to assessing and monitoring estuary health that are being evaluated:

1. Spatio-temporal patterns of fish assemblage structure:

Analysis, modelling and initial testing are complete, development is continuing.

Results show clear estuary-to-estuary variation that is maintained over time, within-estuary faunal similarity once time of year is standardised and parallel patterns of temporal change among estuaries. Change in assemblage structure is principally driven by temporal patterns of juveniles of offshore spawners that use estuaries as nursery grounds. This has allowed the *development of an approach for monitoring tropical estuary health based on fish assemblage structure* that has been tested (manuscript in review, see below) and continues to be developed.

2. Spatio-temporal patterns of change in fish biomass-abundance relationships:

Analysis and modelling are complete, testing is underway.

Results show abundances are maximum and individual biomass minimum during high recruitment periods (late pre-wet season). There also appear to be influxes of sub-adults from offshore later in the season but specific Stable Isotope studies will be required to verify this. It seems likely that changes in the pattern of abundance Vs individual biomass over time could provide a useful indicator of health but further work will be needed to evaluate this.

3. Recruit persistence:

This measure involves detailed analysis of size and age structures over time, so requires additional laboratory work. This work is well advanced.

4. Trophic structure:

Initial modelling based on existing knowledge is complete but precise definition of trophic structure cannot be finalised until Stable Isotope results are available. These data will be analysed as soon as they are available from the Stable Isotope Laboratory.

Trophic structure holds great promise as an indicator of estuary health. In particular, extremely high planktivore abundances and biomasses seem to characterise systems with high levels of anthropogenic carbon inputs.

5. Phyto-detritivore dominance, species richness and species composition:

Analyses of phyto-detritivore dominance, species richness and species composition are complete, and indicate specific additional studies are required. These are underway.

Phyto-detritivores are abundant across the study sites but the taxonomic composition of this guild changes from place to place. Initial additional studies indicate that this taxonomic difference may reflect differences in the relative intake of living plants versus detritus. Consequently, it seems likely that phyto-detritivore taxonomic composition can respond sensitively to differences in the availability of these two food sources, providing obvious potential to indicate human-induced change.

6. Benthivore species richness and species composition

Analysis is complete but may need slight modifications when Stable Isotope data are available.

Except in the extreme case of the highly impacted Keyatta and Curralea Lakes, the distribution of benthic feeders do not seem to relate to apparent anthropogenic pressures. This suggests that although their richness or species composition may indicate major change it is unlikely to provide a sensitive indication of lower level effects.

7. Phyto-detritivore:benthivore (P-D:B) ratio:

Initial modelling based on existing knowledge is complete but as with other trophic components, definitive modelling requires final Stable Isotope results.

Preliminary modelling indicates differences in P-D:B ratio that line up with apparent differences in anthropogenic pressure but these are not consistent enough for any firm conclusions to be made before final Stable Isotope results are available.

8. Food web height:

Evaluation of food web height cannot be completed until final Stable Isotope results are available.

9. Food web complexity and extent of omnivory:

Again, evaluation of food web complexity & extent of omnivory cannot be completed until final Stable Isotope results are available.

10. Stable Isotope Anomalies:

Stable Isotope data already in hand show clear signatures of anthropogenic effects. Their further development and testing are underway.

$\delta^{15}\text{N}$ Stable Isotope values from sites with inputs of fertiliser and effluent treatment show very low (fertilizer) or high (effluent) values, validating that $\delta^{15}\text{N}$ is a valuable indicator of such impacts.

Outputs

One manuscript, "Implications of Spatial Variability of Fish Assemblages for Monitoring of Australia's Tropical Estuaries" is in review with *Aquatic Conservation: Marine and Freshwater Systems*, submitted in May 2009. The article proposes a simple index for monitoring and reporting of estuarine health based on shifts in the structure of fish assemblages within an estuary over time. A second manuscript, "Temporal Dynamics of Fish Assemblages of 11 Tropical Estuaries" is in final draft form with expectations it will be submitted to *Marine Ecology Progress Series* before the end of June 2009. This manuscript details the consistency of temporal change of fish assembly structure in the study estuaries, and shows that offshore spawned juveniles are the major contributors to temporal and spatial patterns in assemblage structure. This is key information needed for developing fish assemblage-based indicators because it defines the types of species that sampling and analysis need to concentrate on.

Project Inputs

Work inputs (excluding report writing, data analysis and planning): Cast net sampling totalled 2,066 person field hours, laboratory work-up of samples 4,458 person hours, data input and cross-matching 317 person hours, gill net and trap sampling 92 person hours; costs necessary to produce useful results. Input costs far exceeded the MTSRF budget with the difference made up from Coastal and Estuary Ecosystem Ecology Group (CEEEG) funds and by involving a MSc student (unfunded) in the project. The collection of additional data and the need to work cost effectively has added to both field and laboratory processing time, however, all field data are now complete and data sets have been fully checked (double input) and errors rectified. CEEEG funding is currently being used to fund additional studies stemming from the MTSRF project.