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No-take marine protected areas: abundance, biomass, batch fecundity and genetic connectivity of target species on the Great Barrier Reef

Thesis submitted by
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in February 2009

For the degree of Doctor of Philosophy
in the School of Marine and Tropical Biology
James Cook University

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A1130).

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Statement of contribution of others

I declare that this thesis is my own work, and has been supported by the following oraginsations and people. The research budget was supported by a number of organisations. They include, the ARC Centre of Excellence for Coral Reef Studies, the Australian Government's Marine and Tropical Scientific Research Facility (MTSRF), the Queensland Government's Growing the Smart State PhD Funding, Australian Coral Reef Society Terry Walker Prize 2007, JCU Merit Research Grants, JCU Graduate Research Scheme funding, the Institute of Marine Engineering Research and Technology (Imarest), the Great Barrier Reef Marine Park Authority and the CRC Reef. The research was also funded by Australian Research Council grant and a Merit Research Grant to Garry Russ.

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List of publications arising from this thesis

Chapter 2

- Evans RD, Russ GR (2004). Larger biomass of targeted reef fish in no-take marine reserves on the Great Barrier Reef, Australia. Aquatic Conservation: Mar. Freshw. Ecosyst. 14: 505-519.

- Davis KLF, Russ GR, Williamson DH, Evans RD (2004). Surveillance and poaching on inshore reefs of the Great Barrier Reef Marine Park. Coastal Management 32:373-387.

- Graham NAJ, Evans RD, Russ GR (2003). The effects of marine reserve protection on the trophic relationships of the Great Barrier Reef Marine Park, Australia. Environmental Conservation 30(2): 200-208.

Chapter 3

- Russ GR, Cheal AJ, Dolman AM, Emslie MJ, Evans RD, Miller I, Sweatman H, Williamson DH (2008). Rapid Increase in Fish Numbers Follows Creation of World's Largest Marine Reserve Network. Current Biology 18(12): 514-515.

- Diaz-Pulido G, McCook LJ, Dove S, Berkelmans R, Roff G, Kline DI, Weeks S, Evans RD, Williamson DH, Hoegh-Guldberg O (In review). Doom and Boom on a Resilient Reef: Climate Change, Algal Overgrowth and Coral Recovery. PLoS ONE.

Chapter 4

- Evans RD, Kritzer JP, Russ GR (2008). Batch fecundity of *Lutjanus carponotatus* (Lutjanidae) and implications of no-take marine reserves on the Great Barrier Reef, Australia. Coral Reefs 27: 179-189.

Chapter 5

- Evans RD (2008). Assessment of an underwater fish biopsy probe for collecting teleost fish tissue samples. Marine Ecology Progress Series. 368: 305-308.

Chapter 6

- Evans RD, van Herwerden, L, Frisch AJ, Russ GR (In review.). Strong genetic but not spatial subdivision of two reef fish species on the Great Barrier Reef. Fisheries Research.

Publications in preparation

- Almany GR, Evans RD, Hamilton RJ, Jones GP, Matawai M, Potuku T, Rhodes KL, Russ GR, Sawynok B, Williamson DH (In prep.) Getting fishers involved in marine protected area research: two case studies from Papua New Guinea and Australia.

- Berumen ML, Evans RD, Fauvelot C, Heredia P, Hogan D, Moland E, Williamson DH (In prep.) Understanding Larval Connectivity in Coral Reef Systems: Questions and Ways to Answers.

- Evans RD, Williamson DH, Russ GR (In prep.). Temporal investigation of the nature of predator prey relationships.

Proposed publications

- Evans RD, Williamson DH, Russ GR. Effect of marine reserve protection on smaller serranid species relative to the major serranid, *Plectropomus* spp.

- Evans RD, Williamson DH, Russ GR. Effect of coral bleaching on the fish community inside and outside no-take marine protected areas.

- Harrison H, Evans RD, van Herwerden L, Jones GP, Williamson DH. Assessing the temporal genetic variation in the recruitment of target species

- Williamson DH, Evans RD, Russ GR. BACIP sampling design studying the density patterns of newly protected species, *Cheilinus undulatus* and *Cromileptes altivelis*.

- Williamson DH, Evans RD, Russ GR. Comparison of three techniques to assess populations of species targeted by hook and line fisheries.

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Abstract

The aim of this thesis was to understand the effects of no-take marine protected areas (MPAs) on fish and corals on the inshore reefs of the Great Barrier Reef (GBR), Australia. The study focused on the principal fishery target species, the coral trout (*Plectropomus* spp.) and a secondary target, the stripey snapper (*Lutjanus carponotatus*). The investigation of effects of no-take marine protected areas (MPAs) on the GBR was of particular significance to management during the course of this study, since the amount of no-take protection in the GBR Marine Park (GBRMP) increased from 4.5% to 33.4% in 2004. This study investigated both the effect of the original (established mid 1980's) and the new (established 2004) MPAs on the density, biomass and reproductive potential of species targeted by fisheries. It explored in detail the effect of the 2004 zoning plan on a representative sample of the reef fish community. It also investigated genetic connectivity of both *Plectropomus* spp. and *L. carponotatus* between inshore island groups separated by over 800km, including (and extending) the study area for the preceding ecological and biological studies of these species.

During the planning stages of the new (2004) zoning plan for the GBRMP, there was limited evidence that MPAs on the GBR had increased abundance of reef fish targeted by fisheries. Chapter 2 provided such evidence from the inshore reefs of the GBR. Underwater visual surveys were used to estimate the effect of MPAs on abundance of species targeted by hook-and-line-fisheries around the Palm, Whitsunday and Keppel Islands, spanning 600 km of the length of the GBR. The MPAs in the original zoning plan had been protected for 14-18 years. Densities of *Plectropomus* spp. and *Lutjanus carponotatus*, both targeted by fisheries, were much higher in MPAs than fished areas in two of the three island groups. The biomass of both *Plectropomus* spp. and *L. carponotatus* were significantly greater (3.9 and 2.6 times, respectively) in the MPAs than fished areas at all three island groups. There were significantly

higher densities and biomass of legal-sized *Plectropomus* spp. >35cm Total Length (TL) (density- 3.8 times, biomass- 5.1 times) and legal-sized *L. carponotatus* >25cm TL (density- 4.2 times, biomass 5.3 times) in MPAs than fished areas at all three island groups. No significant difference in abundance between MPAs and fished areas was found for two species not captured by line fisheries (*Siganus doliatus* and *Chaetodon aureofasciatus*), and there were no significant differences in benthic characteristics between MPAs and fished areas. Results suggest that no-take marine protected areas have increased biomass of targeted fish species on inshore GBR reefs.

In Chapter 3, the implementation of the new (2004) zoning plan enabled a Before-After-Control-Impact-Pair (BACIP) design to investigate the effects of the MPAs on the major fish groups and benthos on the inshore coral reefs at three island groups of the Great Barrier Reef (Palms, Whitsundays and Keppels). After three years of no-take protection, the new zoning plan had affected the density and biomass only of the major target of the hook-and-line fishery, the coral trout (*Plectropomus* spp.). The density of *Plectropomus* spp. increased from 11.1 to 15 fish $1000m^{-2}$, and the biomass increased from 7.2 to 17.2 kg $1000m^{-2}$ after three years of protection. No other species, fish group, family or trophic group, displayed any significant change over time attributable to the establishment of the no-take marine reserves. Regression analysis demonstrated some temporal changes in a predator-prey relationship that may, in time, indicate a secondary effect of zoning due to the increase of *Plectropomus* spp. density in the no-take areas. Benthic variables, hard coral cover, macro-algal cover and structural complexity were not affected by the rezoning. This study also demonstrated that a reduction in live coral cover due to a coral bleaching event in one region had a larger impact on the fish community structure than the implementation of no-take status.

With evidence that no-take protection increased biomass of *Lutjanus carponotatus* (Chapter 2), Chapter 4 investigated body size to fecundity relationships and examined the potential benefits of increased batch fecundity in MPAs compared to fished areas around the Palm, Whitsunday and Keppel Island Groups, Great Barrier Reef, Australia. *Lutjanus carponotatus* batch fecundity increased with fork length in a non-linear relationship that was best described by a power function. Batch fecundity differed by more than one hundredfold among individuals, with a range from 7,074 to 748,957 eggs in fish ranging from 184 to 305mm fork length. Furthermore, egg diameter increased with fish size. Based on underwater visual census, the potential batch fecundity per unit area in all three island groups ranged from 1.0 to 4.2 times greater in the MPAs than in the fished areas from 2001 - 2004. In 2002, a mean 2.3 fold difference in biomass between MPAs and fished areas converted to a mean 2.5 fold difference in batch fecundity per unit area. Greater batch fecundity, longer spawning seasons and potentially greater larval survival due to larger egg size from bigger individuals may enhance the potential benefits of MPAs on the Great Barrier Reef significantly.

Increased density, biomass and egg production per unit area of the focal species within no-take marine protected areas on inshore reefs of the Great Barrier Reef (GBR) lead to the question: are no-take MPAs connected via larval transport to each other and/or to fished areas? The phylogenetic and population genetic study in this thesis (Chapter 6) is a broad scale analysis of the genetic connectivity of *Plectropomus maculatus* and *Lutjanus carponotatus* within and between inshore islands of the GBR. DNA sequences from the mitochondrial (mt) control region were analysed to determine whether there was any genetic partitioning between populations from four island groups (Palms, Whitsundays, Keppels and Capricorn Bunkers) spanning a latitudinal gradient of approximately 800 km. Tissue samples for part of this study were collected by a new *in situ* biopsy probe (Chapter 5). Analysis of molecular variance (AMOVA) indicated high levels of gene exchange between locations within and between the

island groups. Phylogenetic analysis showed no geographic partitioning but identified two distinct lineages for both species that were distributed throughout the sampled range, suggesting that both *L. carponotatus* and *P. maculatus* were admixtures of differentiated lineages, rather than stable populations. Coalescence analysis showed that *P. maculatus* may be up to four times younger than *L. carponotatus* on the GBR and lineages may be either: i) refugial expansions between glacial maximums during the Holocene and Pleistocene periods; and/or ii) one lineage in each species may represent migrants from outside the GBR. Sampling from further afield will help to answer this question. The study showed that populations of both species within the sampling range were panmictic. Under current conditions they may be managed as a single stock across the sampled range of the GBR. It also showed that the co-existence of two genetically distinct lineages throughout the sampled area increases genetic diversity up to fourfold for both species.

Overall, no-take MPAs on the inshore reefs of the GBR have been effective at increasing density and biomass of two species targeted by fishers, *Plectropomus* spp. and *Lutjanus carponotatus*, and egg production per unit area (*L. carponotatus* only). With current knowledge of larval dispersal and such high levels of gene flow over large expanses from north to south along the GBR, one would expect that there would be some larval export from no-take marine protected areas to fished areas. More detailed larval marking or parentage analysis will be required to demonstrate unequivocal larval links.

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Chapter 1: General Introduction

Capture fisheries are struggling on a global scale to supply the growing demand for marine resources from the rapidly expanding human population (Pauly *et al*. 2002; Myers & Worm 2003, Sale et al. 2005). Increasing fishing technologies and techniques have vastly increased the ability of fishers to extract huge amounts of fishery products (FAO 2007). However, this has come at the expense of many fishery stocks and has eventually caused the collapse of some fisheries, for example the Atlantic Cod off Nova Scotia (Myers *et al*. 1997). Many fisheries the world over are displaying obvious signs of overexploitation (Roberts 1997; Pauly *et al*. 1998; Hutchings 2000; Myers and Worm 2003; Worm et al. 2006). In 2005, 23% of marine fisheries were under exploited, 52% were considered fully exploited, 17% were overexploited, 7% were depleted and 1% were recovering from depletion (FAO 2007). Overexploitation is rapidly increasing in developing nations, with a growing human population often relying on coastal coral reefs for their primary source of protein (Newton et al. 2007). Recently, the Asian demand for the live fish trade has increased interest in large predatory species on coral reefs throughout the world. The live reef fish fishery has expanded greatly on the Great Barrier Reef (GBR) in the past decade (Mapstone et al 2001; QDPI&F 2007; Welch et al 2008). At present the GBR reefs are not overexploited but may become so if fishing pressures continue to increase at their current rate (Bellwood *et al.* 2004).

Conventional methods of fishery management (catch and effort controls, bag limits, size limits, closed seasons, gear and licence restrictions etc.) have proved unsuccessful in many fisheries, especially on coral reefs with multi-specific fisheries (Russ 2002). Fishery managers recently have begun to show interest in marine protected areas as fishery tools (Russ and Zeller 2003; Sale et al. 2005; McClanahan et al. 2006; Mora et al. 2006; Russ et al. 2008). Marine protected areas are also known as no-take marine protected areas (MPAs) or marine reserves and are

established for several reasons (Sale et al. 2005). These include protecting: 1) particular endangered species, 2) specific habitats, 3) biodiversity or 4) certain areas from extractive activities such as mining (Roberts and Polunin 1993; Bohnsack 1998). As a fishery tool, MPAs are established to protect a portion of the spawning stock from exploitation (PDT 1990; Roberts and Polunin 1991; Dugan and Davis 1993; Bohnsack 1998; Russ and Alcala 1996a; Russ 2002). The benefits of MPAs have been discussed at length in the literature, they are best summarised by Russ (2002). There are five proposed benefits within the reserve for fishery target species: 1) reduced or no fishing mortality, 2) increased abundance, 3) increased average size, 4) increased biomass, and 5) increased propagule production per unit area (Russ 2002). However, fisheries will only benefit from MPAs if they provide benefits to surrounding fished areas. On the basis of the five benefits inside the reserve, conventional theory predicts that MPAs may enhance fisheries via two methods: 1) Density dependence may encourage movement of individuals from higher density to lower density areas (Abesamis & Russ 2005, Abesamis et al. 2006) for food or shelter (known as 'the spill-over effect'), and 2) greater propagule production per unit area within the MPAs should equate to net export of larval products to fished areas (known as 'the recruitment effect') (Russ 2002).

Throughout the literature there is growing evidence of increased abundance, density and biomass of target species in MPAs compared to fished areas (Roberts & Polunin 1991, Dugan & Davis 1993, Russ 2002, Halpern 2003). However, many of the early studies are spatial comparisons focused on a single MPA and fished area at one point in time and are confounded by habitat, history and larval supply differences between fished and protected areas (Roberts & Polunin 1991, Dugan & Davis 1993, Russ 2002; Shears and Babcock 2003; Willis et al. 2003; Barrett et al. 2007). These early studies led fishery managers to avoid using MPAs as fishery management tools, as the evidence for benefits beyond MPA boundaries was highly equivocal (Roberts and Hawkins 2000; Russ 2002; Gell and Roberts 2003; Roberts et al. 2005; Sale et al.

2005). More studies using monitoring data are appearing in the literature, and the knowledge gained from these studies is invaluable (e.g. Barrett et al. 2007). However many of these studies are still equivocal since they often do not have information on the MPA before it was protected. Recent Before-After-Control-Impact-Pair (BACIP) sampling designs published since the beginning of this study (Russ and Alcala 2003, Shears et al. 2006, Samoilys et al. 2007, Francini-Filho and Moura 2008) unequivocally demonstrate that MPAs affect target species positively. However, long term BACIP studies of MPA effects are still not available for GBR reefs.

The fifth benefit inside marine protected areas is based largely on the assumption that larger female fish produce more eggs (Berkeley *et al.*, 2004). This is supported by the premise that reef fish fecundity increases exponentially with body size (Thresher 1984). For example PDT (1990) suggested that one 61cm *Lutjanus campechanus* produced the same amount of eggs as 212 42cm *L. campechanus*. Therefore, if there is a greater abundance of larger fish in MPAs, then naturally the propagule production per unit area should be higher than in fished populations. The numbers of empirical studies investigating whether MPAs produce greater propagule production per unit area are still relatively few. A limited number of studies estimating batch fecundity per unit area of fish suggest that MPAs have greater reproductive potential than surrounding areas (Bohnsack and Ault 1996, Ault et al. 1997, Paddack and Estes 2000, Kamukuru and Mgaya 2004), but this has never been studied for any species on the GBR.

Increased abundance, density and biomass have been proposed to cause net adult export near marine protected areas. Net adult export can be demonstrated by abundance gradients from reserve boundaries (Rakitin & Kramer 1996, Russ & Alcala 1996b), higher CPUE in fished waters close to marine reserve boundaries (Alcala & Russ 1990, McClanahan & Kaunda-Arara 1996; Abesamis et al. 2006), tagging and movement (see review Russ 2002), and modelling

studies (Polacheck 1990, Attwood & Bennet 1995; Hilborn et al. 2006). However, modelling does not provide unequivocal evidence and should only be used as a guide to reality. The results of most of these adult export studies suggest that most teleost fish are relatively site-attached, and if there is any movement it is typically on a very local scale. Thus, any individuals living near a boundary between fished and protected areas may cross the border on a daily basis (Zeller and Russ 1998; Zeller et al. 2003) but the evidence for the spill-over effect is still highly equivocal and requires more detailed research to show the extent and spatial scale of net adult export of target fish species from protected to fished areas.

It is important to stress that evidence for net larval export from MPAs to fished areas is essentially non-existent, despite the general belief that it should provide the greatest benefits to fisheries (Carr & Reed 1993, Sladek-Nowlis & Roberts 1999, Russ 2002). The sheer scale and budget of an experiment to test net larval export has led most scientists to believe that an adaptive management, large-scale modelling approach is the best way to investigate net export of larvae from reserves (Russ 2002). However, in the past decade, several empirical studies have shown that self recruitment (larvae returning to their natal reef or even close to the original spawning site) may occur at greater rates than previously expected. Jones et al. (1999) were able to mark the otoliths of pre-hatched *Pomacentrus amboinensis* larvae with tetracycline at Lizard Island, GBR, and showed that larvae spawned on that reef could actually return and settle on their natal reef. Jones et al. (2005), using similar methods, demonstrated empirically that 30% of *Amphiprion polymnus* settle to a 2 hectare natal area, sometimes <100m from their birth site after a 9-12 day larval duration. The most recent study using trans-generational markers and genetics found up to 60% self recruitment for coral reef fish species, one with a 10d larval duration (*A. percula*) and another with a 30d larval duration (*Chaetodon vagabundus*) (Almany et al. 2007). However, the juvenile and adult stages of these two species are relatively easy to locate and capture, compared to larger commercially important species. It

is now vital to demonstrate the larval dispersal capacity of fishery target species to and from MPAs. Until such methods are developed, basic inferences of batch fecundity per unit area and genetic connectivity are the next best thing, for we know from the studies by Jones and colleagues that 40-70% of reproductive products may be exported to other reefs if they survive.

Connectivity of MPAs can be inferred by the population genetics of a species. Population genetic studies of species targeted by fishers have shown no genetic structure along the length of the GBR, apart from one isolated *P. maculatus* clade south of the GBR (van Herwerden et al. 2006). High genetic connectivity has been shown for *Lutjanus argentimaculatus* (Ovenden and Street, 2003), *Lethrinus miniatus* (van Herwerden et al. 2003), *P. maculatus* and *P. leopardus* (van Herwerden et al 2006). The inshore stocks of reef fish have received relatively little genetic stock assessment compared to the mid- and outer-shelf reefs. Commercial reef fin-fish fishers have low to no impact on the inshore reefs but recent evidence shows that recreational fishers have significant effects on inshore fish stocks (Evans and Russ 2004; Williamson et al. 2004; Russ et al. 2008) and thus a genetic assessment of their population status and connectivity should be conducted.

There has been no unequivocal evidence on the GBR to suggest that marine protected areas provide the fishery benefits of MPAs reported from other countries. Reasons for this may be low levels of fishing due to low human population numbers and large distances of reefs from the coastline (approx. 50km) in comparison to developing nations with large populations in close proximity to coastal reefs. The distance to the reefs on the GBR may also aid in illegal poaching, as surveillance is costly and time consuming. Evidence of poaching has been demonstrated on the inshore reefs of the GBR; Davis et al. (2004) showed that poaching decreased with increased surveillance around the Palm and Magnetic Islands, GBR. The best evidence of MPA benefits on the GBR is from an opportunistic BACIP sampling design

assessing fish stocks on the near shore reefs of the GBR, where two researchers sampled similar areas of the same island groups for the same species, greater than a decade apart, and using slightly different methods. Williamson *et al.* (2004) showed that coral trout biomass, around the Palm and Whitsunday Islands, GBR, was 4-6 times higher in the MPAs after 12-13 years of protection. They also demonstrated that coral trout biomass in fished areas had not changed greatly during the same period.

1.1 Thesis aims and outline

This thesis is not an attempt to answer all the outstanding issues of MPA research but to bridge some of the knowledge gaps in the literature, particularly on the Great Barrier Reef of Australia. The specific objectives of this thesis are:

1. Assess the effect of the no-take marine protected areas on the abundance and biomass of species targeted by fishers in three island groups spanning 700km of the Queensland coastline after 14 years of protection.

2. Investigate short term temporal effects of the new (2004) zoning plan on the fish and benthic communities of the inshore reefs of the GBR using a Before-After-Control-Impact-Pair sampling design.

3. Explore the effects of no-take marine protected areas on the reproductive output of a fishery target species.

4. Examine the genetic connectivity of the MPAs and island groups for two focal species.

The focal species' of this thesis are *Plectropomus* spp. and *Lutjanus carponotatus*. *Plectropomus* spp. are the primary target of both recreational and commercial hook and line fishers on the GBR. *Lutjanus carponotatus* are considered to be a secondary target of

commercial fishers and are regularly caught and kept by recreational fishers. **Chapter 2** provides a spatial comparison to determine the effect of the original (mid 1980's) zoning plan on these two species. Two non-target groups are used as control species and benthic variables were also compared to control for habitat differences.

With the implementation of the 2004 zoning plan on the GBR, baseline data was collected for a unique BACIP design to assess the effects of MPAs on approximately 160 species of reef fish. **Chapter 3** is a temporal study of the focal species in the same three island groups studied in Chapter 2, but also includes a large suite of secondary target and non-target species divided into Family or trophic groups. In this study, predator-prey relationships were analysed and the influence of climate change was investigated as well.

Chapter 4 tests the theory that MPAs of the near shore reefs of the GBR provide greater batch fecundity per unit area of target fishery species. It compares the biomass and propagule production per unit area of a gonochoristic species (*Lutjanus carponotatus*) between MPAs and fished areas in three island groups for up to 3 years of sampling. The implication of body size, age and frequency of spawning in relation to increased biomass within MPAs compared to fished areas is discussed.

With evidence of greater density, biomass and batch fecundity in MPAs, the next step was to assess larval export. This thesis uses genetics to assess connectivity of populations on the inshore reefs of the GBR. Originally, the plan was to investigate broad-scale connectivity between Islands and fine-scale connectivity between MPAs and fished areas within one of the island groups. However, research permitting issues took too much time and thus only broad-scale connectivity was assessed in this thesis. **Chapter 5** is a methods paper for **chapter 6**. **Chapter 5** describes a new tool designed for collecting tissue samples of small to medium

sized teleost reef fish for genetic analysis. A biopsy probe was designed by RE that could be fitted to a spear gun and fired at fish collecting a small tissue sample with minimum impact. **Chapter 5** evaluates the efficacy of this new tool on the focal species, *Plectropomus* spp. and *L. carponotatus*, considering their behaviour, and physical attributes in regard to the success of the biopsy probe. **Chapter 6** assessed the genetic connectivity of the focal species, *Plectropomus* spp. and *L. carponotatus* between the islands in this study. The Hyper variable region (HVR) mitochondrial D-loop was used to assess the population connectivity of both species.

Chapter 7 concludes the thesis with a general discussion summarizing the key findings and suggesting further work that will increase our knowledge and provide a better understanding of the potential benefits of no-take marine protected areas.

Chapter 2: Larger Biomass of Targeted Reef Fish in No-take Marine Protected Areas on the Great Barrier Reef, Australia.

2.1 Introduction

Evidence to support the benefits of no-take marine protected areas (MPAs) is developing rapidly (Roberts and Hawkins 2000; Gell and Roberts 2002). MPAs should reduce fishing pressure on target species, resulting in greater density, individual size, biomass, and fecundity of species targeted by fisheries (Bohnsack 1998; Roberts and Hawkins 2000; Gell and Roberts 2002; Russ 2002; Halpern 2003). Adjacent fisheries may benefit from MPAs due to spill-over (net export) of adult individuals (Russ and Alcala 1996; Zeller and Russ 1998; McClanahan and Mangi 2000; Roberts et al. 2001; Galal et al. 2002) and net export of propagules via larval dispersal (Stoner and Ray 1996; Roberts 1997; Gell and Roberts 2002).

The Great Barrier Reef (GBR), on Australia's east coast, is the largest managed tropical reef marine park in the world. The total area of the World Heritage site is approximately 348,000km^2, equal to the total land area of Japan. The benefits of MPAs on the GBR have come under intense scrutiny during the recent implantation of the Representative Areas Program (RAP). RAP was designed to protect at least 20% of each of 70 different bioregions in the Great Barrier Reef Marine Park (GBRMP) (30 reefal and 40 non-reefal) (Day et al. 2003). To achieve this, the Great Barrier Reef Marine Park Authority (GBRMPA), with input from scientists, commercial and recreational fishers, tourist operators and the broader community, expanded the current level of no-take protection on the GBR from 4.6% to 33.4% of the park area. Opposition to the expansion, by fishing lobbies, was very strong. To win the support of these groups evidence was required that suggested the current MPAs, implemented mostly in

the mid 1980's, were effectively sustaining, and perhaps even increasing, fish stocks targeted by fishers.

A substantial recreational fishery for reef fish occurs on the GBR (Goggin et al. 2002). *Plectropomus* spp. (Serranidae: Epinephelinae), known locally as coral trout, are a primary target of this recreational fishery on GBR coral reefs (Goggin et al. 2002). In 1997 and 1999 the annual recreational harvest of coral trout in Queensland was estimated from surveys of recreational fishers at 315 tonnes (Williams 2002). This recreational fishery is largely concentrated on near shore areas, due to the small size of most of the vessels involved (4-6m) (Goggin et al. 2002). The present study was conducted in these near shore areas. A much larger commercial hook and line fishery exists on offshore, rather than near shore, reefs of the GBR (Goggin et al. 2002). The total landing of this commercial fishery in 2000 was approximately 4,600 tonnes (Williams 2002). *Plectropomus* spp. are the primary target of this commercial fishery, comprising 40-45% of the fishery catch by weight. Commercial line fishing effort has doubled over the past decade, due to the increased demand of the live reef fish markets throughout Asia (Goggin et al. 2002).

Russ (2002) reviewed 22 studies of spatial comparisons of density, biomass and mean size of large predatory reef fish between MPAs and fished locations. He reported that 36% of studies had significantly higher density, 60% a greater biomass, and 73% a higher mean size in MPAs than in fished locations. More recently Halpern (2003) reviewed the literature on 80 different MPAs. He concluded that most of the MPAs had greater density (66% of studies), biomass (84% of studies) and average size (83% of studies) of carnivorous fish.

Studies of the effects of MPAs on abundance of *Plectropomus* spp. on the GBR are somewhat contradictory. Craik (1981) and Goeden (1978) demonstrated higher density and average size

of *Plectropomus* spp. in MPAs than fished areas around Heron Island, which was protected in 1975. However, several other early studies found no significant differences in coral trout densities between MPAs and fished areas (e.g. Ayling and Ayling 1984, 1986; Ayling et al. 1993). Williams and Russ (1994) summarised the results of 24 separate studies of the effects of MPAs on reef fish populations on the GBR. Most of these studies used underwater visual census (UVC). Closures to fishing were of only 3 to 4 year's duration in most studies. While the evidence for increased average size was good, Williams and Russ (1994) concluded that the effect of MPAs on density of *Plectropomus* spp. was "equivocal". A good example of such results was the study of Mapstone et al. (1997). They used visual census to compare 10 no-take reefs with 14 fished reefs in the Cairns section of the GBRMP and concluded that, after 8 years of no-take zoning, there was virtually no difference in the density of *Plectropomus* spp. between MPAs and fished areas. Ayling et al. (2000) concluded that there were no significant differences in densities of *Plectropomus* spp. between MPAs and fished areas along much of the GBR. Mapstone et al. (2003) used UVC to compare density of both legal-sized (>=38 cm TL) and below legal sized (<38cm TL) *Plectropomus* spp. in no-take and fished areas offshore, spread across four regions of the GBR (spanning 7° of latitude) over a 6 year period (1995-2000). Density of legal-sized *Plectropomus* spp. was significantly higher in no-take than fished areas in only 7 of the 24 combinations of region (4) and years (6). The mean difference in density was ~50% higher in MPAs in the one region with the most consistent difference over the 6 years (Mapstone et al. 2003). Counter to this result, sub-legal *Plectropomus* spp. had significantly (by ~10%) higher density in fished than MPAs across all regions and years (Mapstone et al. 2003).

Recently, Williamson et al. (2004) used UVC to show that inshore reefs of the GBR, which have high recreational fishing pressure and relatively effective surveillance (Davis et al. 2004), have almost 5 times the biomass of *Plectropomus* spp. in MPAs than fished areas after 12-13

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years of protection (1999/2000). This difference has remained consistent from 1999 to 2008 (Williamson et al. unpublished data). Furthermore, such large differences in biomass of *Plectropomus* spp. between MPAs and fished areas on these inshore reefs results in detectable differences in the community structure of prey fish of *Plectropomus* spp. (Graham et al. 2003).

Although UVC comparisons of density of *Plectropomus* spp. between no-take and fished areas are inconclusive, one pattern that is consistent is higher catch rates of experimental hook and line fishing in no-take than fished areas (Ferreira and Russ 1995; Zeller and Russ 1998; Mapstone et al. 2003). Mapstone et al. (2003) reported 2-2.5 times higher catch rates in no-take than fished areas over 6 years in 3 of the 4 regions of the GBR that they studied. A second consistent result is higher than average size of *Plectropomus* spp. in MPAs than fished areas (e.g. Williams and Russ 1994; Ayling et al. 2000; Mapstone et al. 2003). Evidence that MPAs increase average age of *Plectropomus* spp. are however, inconclusive, with no difference (Ferreira and Russ 1995), or small but spatially inconsistent differences, reported (Adams et al. 2000; Mapstone et al. 2003).

The type and structure of the benthic habitat can influence the distribution and abundance of coral reef fish (Rakitin and Kramer 1996; McClanahan and Arthur 2001). However, McClanahan and Arthur (2001) found that protection from fishing on Kenyan reefs had a larger effect on abundance of reef fish than did benthic habitat. Clearly, fishing can have a direct effect on abundance of targeted species, but can also affect abundance indirectly if such fishing alters benthic habitat. To determine effects of protection from fishing by MPAs unequivocally, habitat differences between MPAs and fished sites should be accounted for.

The aims of this study were to compare the density and biomass of *Plectropomus* spp. (coral trout), *Lutjanus carponotatus* (Lutjanidae) (both targeted by fishing on the GBR), and two non-

target (control) species (*Siganus doliatus*: Siganidae and *Chaetodon aureofasciatus*: Chaetodontidae), between MPAs and fished areas of three inshore island groups of the Great Barrier Reef Marine Park.

2.2 Study Areas

The Great Barrier Reef Marine Park Authority (GBRMPA) in Australia was established in 1975, and Marine Park zoning was first implemented formally in 1981 in the Capricornia (southern) section of the park. The original multiple-use zoning plan for the entire GBR had been in place since 1988 (Williams and Russ 1994). Zoning within the GBRMP allows different user groups varying levels of use, ranging from fully preserved ("Preservation zone"), to open access (all fishing, including trawling, allowed). Under the original zoning plan 23.3% of nearly 3000 reefs were in the 'green' zones or "no- take" marine protected areas. However, only ~ 4.6% of the total area of the Marine Park had complete no-take protection under the original zoning plan. Thus, whilst many reefs were no-take, they accounted for a very small percentage of the marine park. Furthermore, most of the no-take area was either well off the coast (50-90km), or concentrated in the sparsely populated far north of the GBRMP. The inshore area, where recreational fishing is concentrated, had relatively few MPAs.

 The three island groups in this study- Palm, Whitsunday and Keppel (Figure 1), all have international resorts and cater for many other tourist activities, such as diving, snorkeling, yacht charters, fishing and sightseeing. Orpheus Island, in the Palm Group, also has a research station in operation year-round. Accessibility to all three island groups by recreational users makes them very popular, thus placing them under more regular and intensive recreational fishing activities than the mid- and outer-reefs (Higgs and McInnes 2003). The close proximity of these island groups to the coast, and the presence of tourist resorts, ensures that surveillance of

the MPAs by Queensland Parks and Wildlife Service (QPWS) vessels is likely to be better than

that on offshore reefs (Davis et al. 2004).
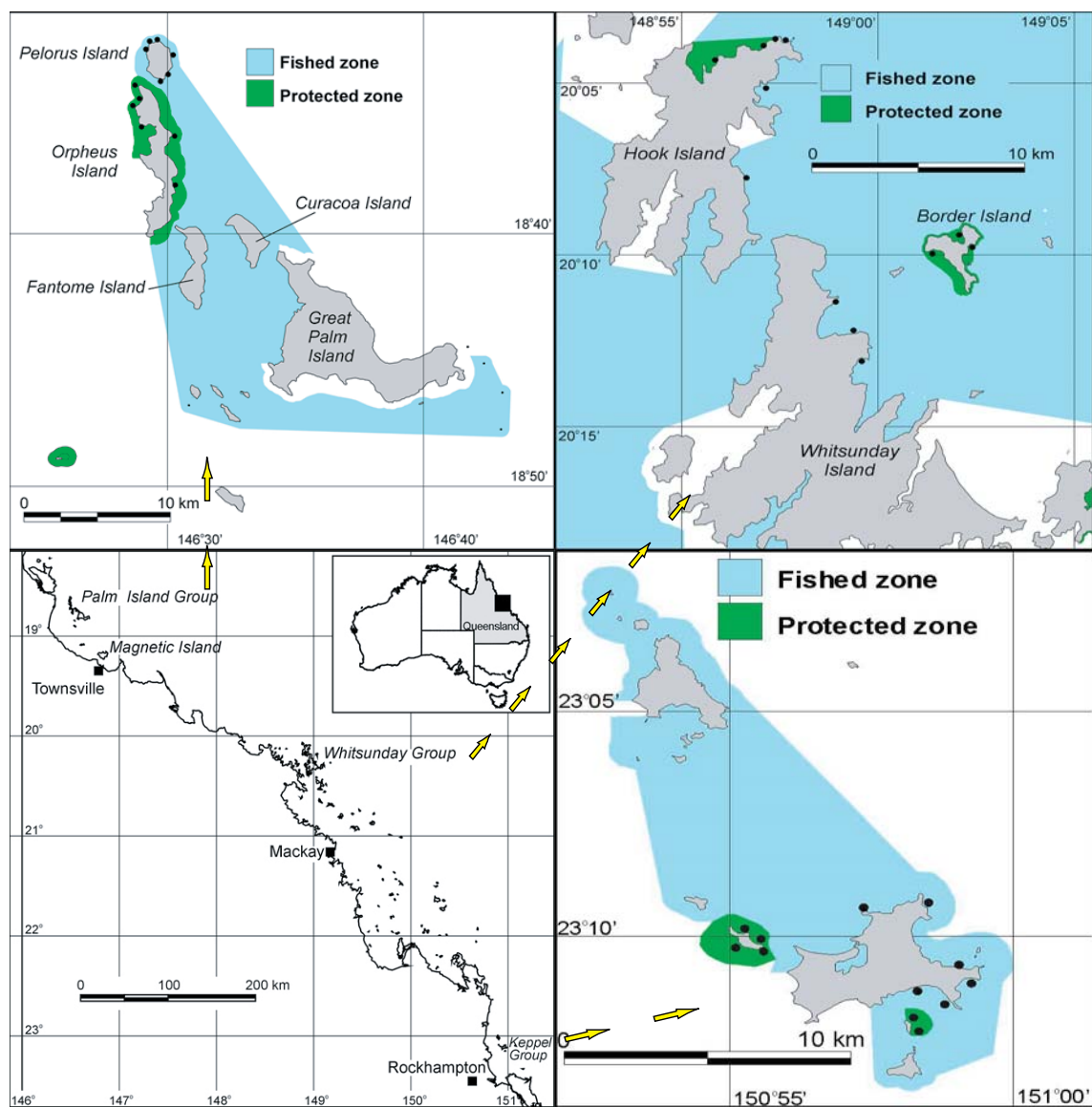
Figure 2.1: Map of the Queensland Coast and the three Island Groups-Palm, Whitsunday and Keppel Islands. No-take marine protected areas and fished areas are shown. Black dots indicate sampling sites.

2.3 Methods

Sampling was carried out in the Whitsunday Islands (20°08'S, 146°56'E) in December 2001, in the Palm Islands (18°34'S, 146°29'E) in April 2002, and in the Keppel Islands (23°10'S, 150°57'E) in October 2002 (Figure 1). The differences in sampling times confound the study somewhat. For example, *Plectropomus* spp. generally settle onto reefs of the GBR around January/February (Ferreira and Russ 1994; Mapstone et al. 2003), but may not be detected by visual census methods designed to sample adult fish until much later that year. Thus we may have been able to detect 2002 recruitment of *Plectropomus* spp at the Keppels, but not at the Palms and Whitsundays. Note also that *Plectropomus* spp. can potentially live for up to 14 years or more (Ferreira and Russ 1994). In 2001/2002 the MPAs in these island groups had been 'zoned' MPAs for approximately 14 years. Sampling in the Whitsunday Islands in December was carried out on full moons to reduce the chances of sampling of *Plectropomus* spp. during their new moon spawning aggregations (Samoilys 1997). The reef flats at all three island groups are exposed during astronomically low tides, with a reef slope that ranges from gentle to vertical walls, with high structural complexity. The bottom of the reef slope varies in depth from 5m to 20m. Data on the abundance of fish and benthic organisms were collected by underwater visual census (UVC) along the reef slope at a depth of 5-10m.

Plectropomus leopardus and *P. maculatus* (Serranidae) are the most common coral trout found in the sampling area. The line fishery targets both species equally, so the two species were grouped together as *Plectropomus* spp. in this study. *Plectropomus* spp. are commonly referred to as 'coral trout'. The most abundant secondary target on inshore islands is *Lutjanus carponotatus* (Lutjanidae), commonly known as stripey. This species was chosen to test whether fishing on inshore reefs has an effect on a secondary target species. Two non-target species were also chosen as controls, *Siganus doliatus* (Siganidae) and *Chaetodon*

aureofasciatus (Chaetodontidae). These species feed on algae and hard coral, respectively, and are never caught by hook and line or speared on the GBR.

2.3.1 Data Collection

Data was collected at six randomly selected sites in each MPA and fished areas in the Keppel Islands. We sampled 12 randomly selected sites per MPAs and fished areas in the Palm and Whitsunday Islands. The available area of MPAs was smaller in the Keppels than at the other two island groups (Fig. 1). To maintain a balanced sampling design, the six sites used in this study were chosen randomly from the original twelve per zone at both the Palm and Whitsunday Island groups. Within each of the sites, Underwater Visual Census (UVC) was conducted on scuba along five transects of 50m*6m (300m^2). The same observer (RE) made all fish counts and size estimates. Total length was estimated by placing target species of fish into 5cm size classes. Training in size estimation of fish was carried out at the start of each day, using wooden fish models of known length. Biomass was estimated from published biomass-length relationships from Fishbase (Froese and Pauly 2002) for *L. carponotatus*, and from Ferreira and Russ (1994) for *Plectropomus* spp. The surveys did not proceed if visibility was less than 4m, and typically ranged from 4-15 metres.

On the GBR, the minimum length of *Plectropomus leopardus* and *P. maculatus* that can be caught and retained legally on hook and line is greater than or equal to 38cm Total Length (TL). For this study, we considered *Plectropomus* spp. above and including the 36-40cm TL size class to be close to "legal-sized". Although this size class includes fish 2cm below the legal size, it gives a more appropriate estimate than using the 41-45 cm size class. The latter would exclude fish up to 3cm above legal size in the density and biomass estimates. The legal

minimum size for *L. carponotatus* is 25cm TL. In this study the 26-30cm size class was legal size.

Benthic habitat data were collected from the transects used to make the fish counts. Firstly, structural complexity of the substratum was estimated whilst swimming the transects. A pre-determined structural complexity index (from 1-5) was assigned to each transect (Table 1). Secondly, a line intercept technique was used to estimate cover of benthic categories. These categories were recorded every two metres along the 50m transect tape. Benthic categories included several hard coral morphologies (branching, massive, digitate, plate, foliose, encrusting), soft coral, algae, rubble, sand, and 'other'. The 'other' category included sponges, zoanthids, anemones, clams and seagrass. Categories were recorded as either dead or alive.

Table 2.1: Categories of structural complexity of the benthic substratum.

1	Flat, sandy, rubble with a few bommies (coral heads).
2	Bommies amongst mostly rubble. <45° reef slope.
3	Rubble amongst mostly coral bommies. ~45° reef slope.
4	Good coral structure with some overhangs. >45° reef slope.
5	High coral complexity, large holes and caves. Vertical wall.

2.3.2 Data Analysis

The fish transect data contained many zero estimates for some species, and often did not conform to the assumptions of ANOVA. Thus, all fish transect data were pooled to site level (5 transects per site). Since the focus of this study was on variation between MPAs and fished areas and island groups, rather than within sites, pooling did not affect the comparisons of major interest. Thus all data were analysed with a two-factor orthogonal design, using two zones (MPAs and fished), three island groups (Palm, Whitsunday and Keppel Island groups),

and 6 sites within each combination of zone and island group as replicates. Included in this analysis were three benthic covariates, live hard coral, live soft and hard coral, and structural complexity.

Plectropomus spp. (density and "legal-sized" density), *S. doliatus* density, live hard coral and live hard and soft coral, conformed to all assumptions of ANOVA. *Lutjanus carponotatus* (density, legal-sized density and legal-sized biomass), *Chaetodon aureofasciatus* density, and the structural complexity index were $Log_{10}(x)$ transformed. The remaining variates were square root transformed. Quantile-quantile plots were used to test the assumption of normality. Levene's test was used to test for homogeneity of variances.

2.4 Results

2.4.1 Density of <u>Plectropomus</u> spp.

Two of the island groups had greater densities in the MPAs than fished areas (Fig. 2) (Palm Islands- 3.6 times higher; Whitsunday Islands- 2.3 times higher). However, the Keppel Islands had a higher density of *Plectropomus* spp. in the fished areas (9.3 fish/1000m^2) than the MPAs (6.3 fish/1000m^2) (Figure 2). This latter result was due to a large number of new recruits (<20cm TL) of *Plectropomus* spp. being recorded in two of the six fished sites in the Keppel islands. The lack of consistency in the effect of the MPAs between island groups resulted in the analysis concluding that there were no significant differences in density of *Plectropomus* spp. between island groups (Table 2). The lack of a significant Zone * Island interaction in the ANCOVA appears to be due to effects of the benthic co-variates.

2.4.2 Biomass of <u>Plectropomus</u> spp.

The biomass of coral trout was significantly higher in MPAs than fished areas (Table 2). Coral trout biomass was 3.9 times higher in MPAs ($8.3kg/1000m^2$) than in fished ($2.1kg/1000m^2$) areas (Figure 2). The pattern was consistent at all three island groups (Figure 2, Table 2).

2.4.3 Density and Biomass of "Legal-Sized" <u>Plectropomus</u> spp.

There were 3.8 times more *Plectropomus* spp. > 35 cm TL in the MPAs (3 fish/$1000m^2$) than the fished (0.8 fish/$1000m^2$) areas (Figure 3). The biomass of such fish was 5.1 times higher in the MPAs ($6.6kg/1000m^2$) than the fished ($1.3kg/1000m^2$) areas (Figure 3). Both results were statistically significant (Table 3).

2.4.4 Density of <u>Lutjanus carponotatus</u>

MPAs in two of the three island groups (Whitsunday and Keppel Islands) had more than double the density of *L. carponotatus* than the fished areas (Fig. 4). However, the Palm Islands had greater densities in the fished than in the MPAs (Figure 4). The lack of consistency in the effect of the MPAs between island groups resulted in the analysis concluding that there were no significant differences in density of *L. carponotatus* between zones or island groups (Table 2). The lack of a significant Zone*Island interaction in the ANCOVA appears to again be due to effects of benthic co-variates and limited power of the analysis. For example, the covariate "Hard+soft coral" had a significant influence on the density of *L. carponotatus* (Table 2).

2.4.5 Biomass of <u>L. carponotatus</u>

The biomass of *L. carponotatus* was significantly greater in the MPAs ($5.3kg/1000m^2$) than the fished ($2.0kg/1000m^2$) areas at all three island groups (Figure 4, Table 2). The covariate

"Hard+soft coral" affected the biomass of *L. carponotatus* significantly in a positive way (Table 2). Larger numbers of *L. carponotatus* were found in areas of high soft coral cover (see section above). However, there was no significant effect of any other benthic variable (including live coral cover) on biomass (or density) of *L. carponotatus* (Table 2).

2.4.6 Density and Biomass of Legal-Sized L. carponotatus

There were 4.2 times more legal-sized *L. carponotatus* in MPAs (5.0 fish/1000m^2) than fished (1.2 fish/1000m^2) areas (Figure 3). The biomass of legal-sized *L. carponotatus* was 5.3 times greater in the MPAs (3.2kg/1000m^2) than fished (0.6kg/1000m^2) areas (Figure 3). Both results were statistically significant (Table 3).

Table 2.2: Results of univariate two-factor ANCOVA on the density and biomass of *Plectropomus* spp., *Lutjanus carponotatus*, and the density of *Chaetodon aureofasciatus* and *Siganus doliatus* in the Palm, Whitsunday and Keppel Island Groups. Covariates were Live Hard Coral, Live Hard and Soft coral, and Structural complexity Index. ***P<0.001; **P<0.01; *P<0.05; ns: not significant; NA: not applicable.

Source of Variation	Live Hard Coral (1,27df)	Live Hard+Soft Coral (1,27df)	Structural Index (1,27df)	Zone*Island Group (2,27df)	Island Group (2,27df)	Zone (1,27df)
Plectropomus spp. density	0.486 (ns)	2.250 (ns)	0.934 (ns)	2.301 (ns)	0.339 (ns)	0.882 (ns)
Plectropomus spp. biomass	0.843 (ns)	2.833 (ns)	0.000 (ns)	0.392 (ns)	0.189 (ns)	18.077 (***)
Lutjanus carponotatus density	4.205 (ns)	7.175 (*)	0.154 (ns)	2.114 (ns)	1.671 (ns)	2.187 (ns)
L. carponotatus biomass	3.099 (ns)	4.834 (*)	0.017 (ns)	2.550 (ns)	0.705 (ns)	14.606 (**)
Siganus doliatus density	0.725 (ns)	0.622 (ns)	0.951 (ns)	0.683 (ns)	4.490 (*)	0.095 (ns)
Chaetodon aureofasciatus density	3.169 (ns)	0.395 (ns)	1.190 (ns)	0.651 (ns)	4.516 (*)	0.009 (ns)
Live Hard Coral Cover	NA	NA	NA	0.887 (ns) (2,30df)	6.819 (**) (2,30df)	0.390 (ns) (1,30df)
Live Coral Cover (Hard & soft)	NA	NA	NA	0.167 (ns) (2,30df)	0.506 (ns) (2,30df)	0.983 (ns) (1,30df)
Structural Complexity Index	NA	NA	NA	1.321 (ns) (2,30df)	3.043 (ns) (2,30df)	1.761 (ns) (1,30df)

Table 2.3: Results of univariate two-factor ANCOVA on the density and biomass *Plectropomus* **spp. (>35cm TL) and of legal sized** *Lutjanus carponotatus* **(>25cm TL) in the Palm, Whitsunday and Keppel Island Groups. Note that the minimum legal size of** *Plectropomus* **spp. is >=38 cm TL. ***P<0.001; **P<0.01; *P<0.05; ns: not significant.**

Source of Variation	Hard coral (1,27df)	Hard+soft coral (1,27df)	Structural Index (1,27df)	Zone*Island Group (2,27df)	Island Group (2,27df)	Zone (1,27df)
Plectropomus spp. density	1.969 (ns)	4.063 (ns)	0.028 (ns)	0.243 (ns)	0.116 (ns)	19.484 (***)
Plectropomus spp. biomass	0.777 (ns)	2.282 (ns)	0.009 (ns)	0.930 (ns)	0.691 (ns)	27.604 (***)
Lutjanus carponotatus density	1.909 (ns)	1.823 (ns)	0.037 (ns)	0.246 (ns)	1.519 (ns)	15.166 (**)
L. carponotatus biomass	2.408 (ns)	2.548 (ns)	0.148 (ns)	0.343 (ns)	1.870 (ns)	19.604 (***)

2.4.7 Density of Non-Target Species

Density of *Siganus doliatus* also did not differ significantly between zones, but did differ significantly between island groups (Table 2, Tukeys HSD: Whitsunday Islands = Palm Islands > Keppel Islands; Figure 5). The density of *Chaetodon aureofasciatus* did not differ significantly between MPAs and fished areas (Figure 5) but did differ significantly between Island Groups (Table 2, Tukeys HSD: Keppel Islands>Whitsunday Islands>Palm Islands). The latter result was likely related to differences in hard coral cover between the island groups (Fig. 5, Table 2)

2.4.8 Benthic Variables

There were no significant effects of MPAs on live hard coral cover, total live coral cover, or the index of structural complexity (Table 2). There was, however, a significant difference in live hard coral cover between the Island Groups (Figure 5, Tukeys HSD: Keppel Islands=Whitsunday Islands>Palm Islands). Live hard coral cover was slightly higher in the

MPAs of the Whitsunday and the Keppel Islands. However, the ANCOVA indicated that live hard coral cover did not affect the abundance of target species significantly (Table 2). Total live coral cover was also slightly higher in the MPAs at all three Island Groups (Figure 5). This may have influenced the abundance of *L. carponotatus* (Table 2). It did not, however, affect the abundance of legal sized *L. carponotatus* significantly (Table 3). The structural complexity index (SI) was slightly higher in the fished areas than in the MPAs at all three island groups (Figure 5). This result was not statistically significant (Table 2). The ANCOVA indicated that the structural complexity index did not influence the abundance or biomass of any target species significantly (Table 2).
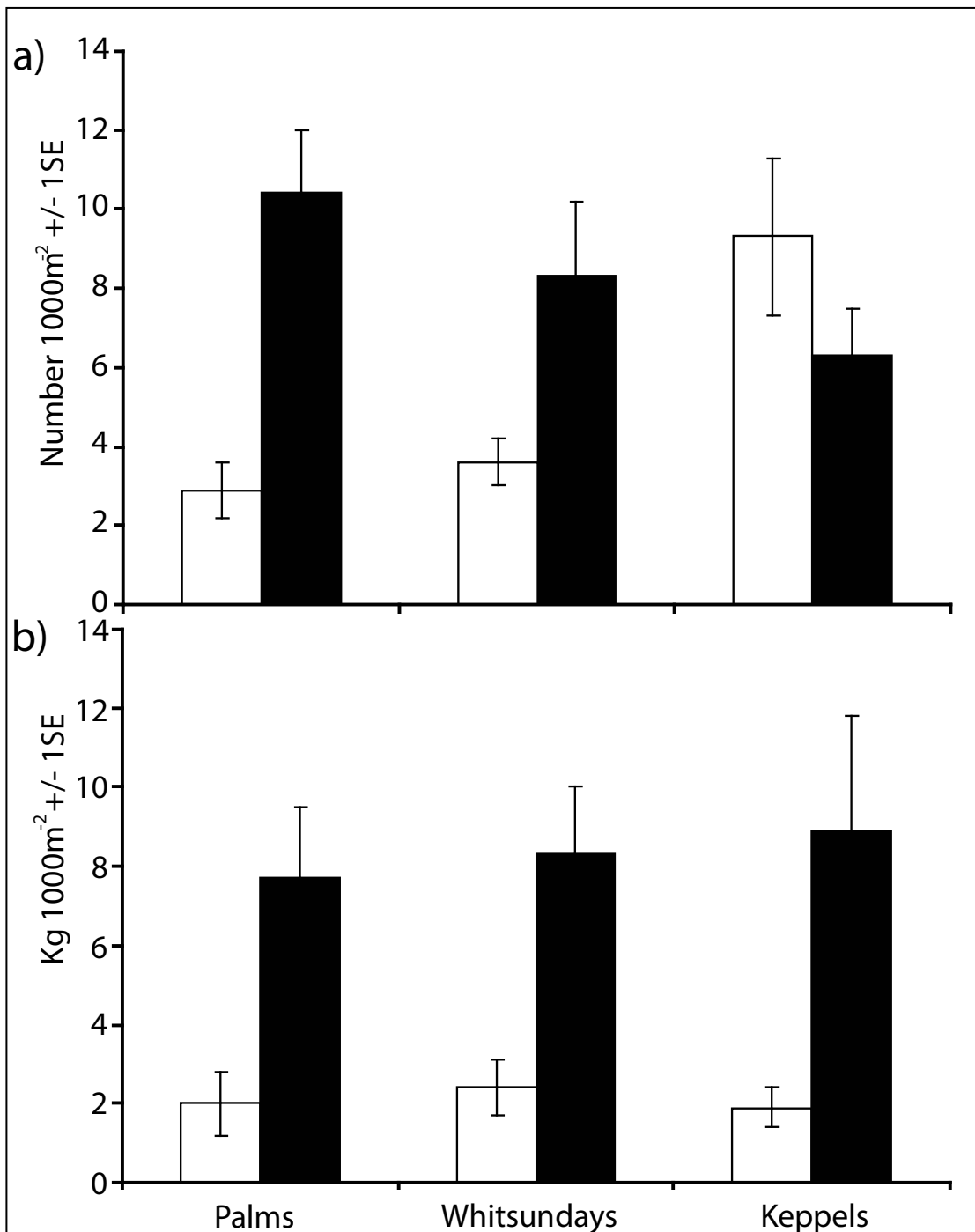
Figure 2.2: The a) density and b) biomass of *Plectropomus* spp. in protected and fished areas at three island groups of the GBR. Black bars represent no-take MPAs and white bars are fished areas.
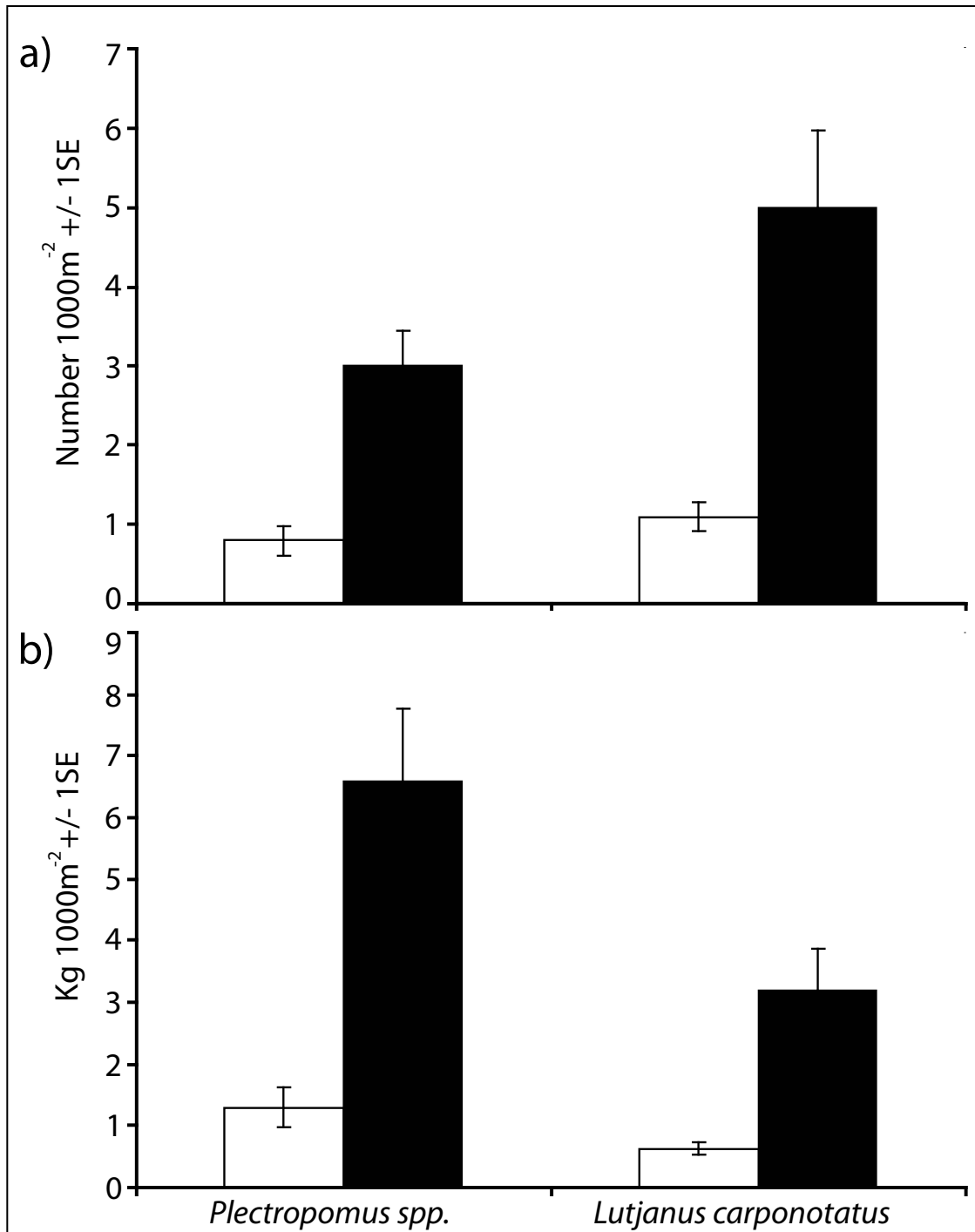
Figure 2.3: The average a) density and b) biomass of *Plectropomus* spp. (>35cm TL) and legal-sized *Lutjanus carponotatus* (>25cm TL) in no-take MPAs (black bars) and fished areas (white bars) of the Palm, Whitsunday and Keppel Island Groups combined.
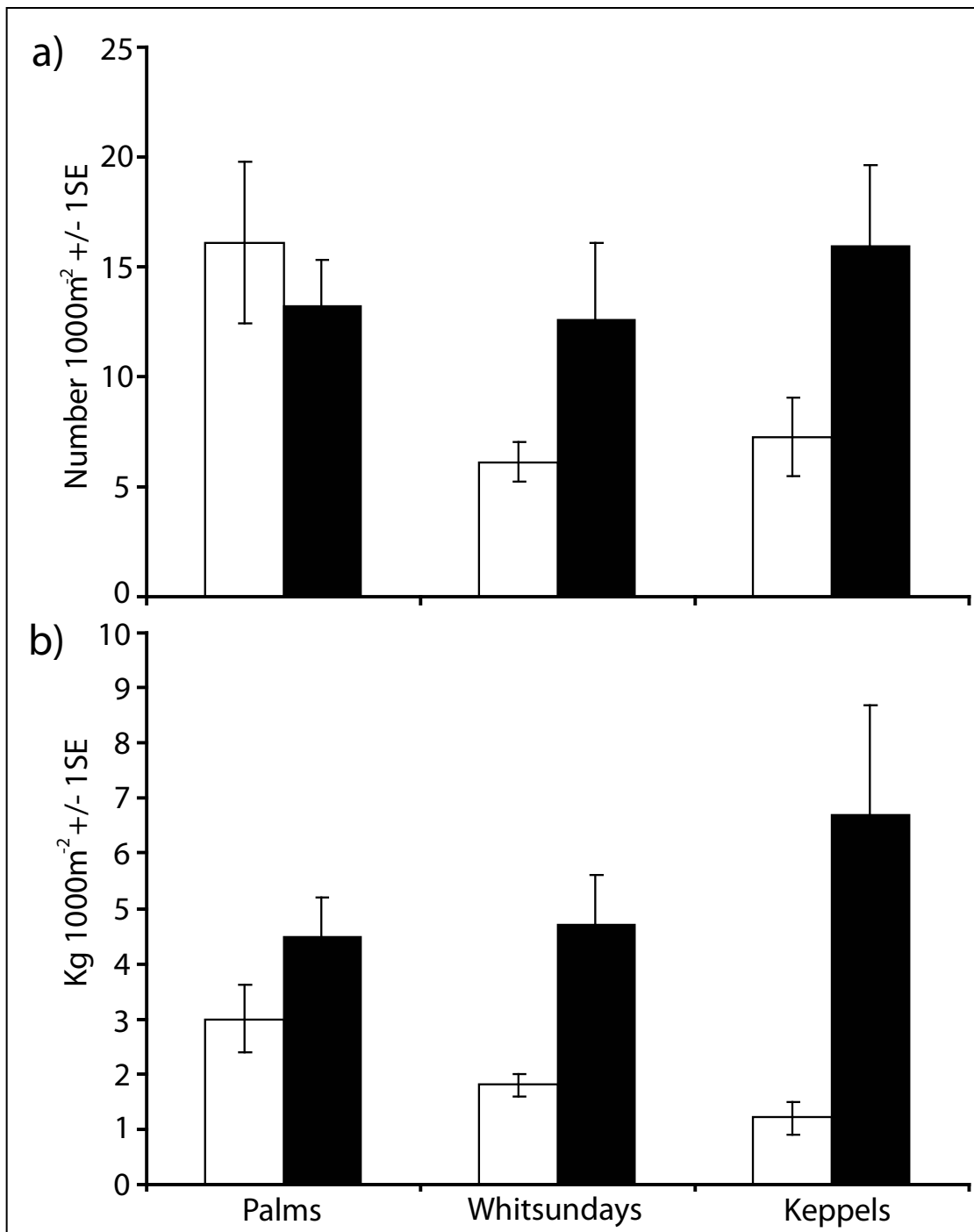
Figure 2.4: The a) density and b) biomass of *Lutjanus carponotatus* in protected and fished areas at three island groups of the GBR. Black bars represent no-take MPAs and white bars are fished areas.
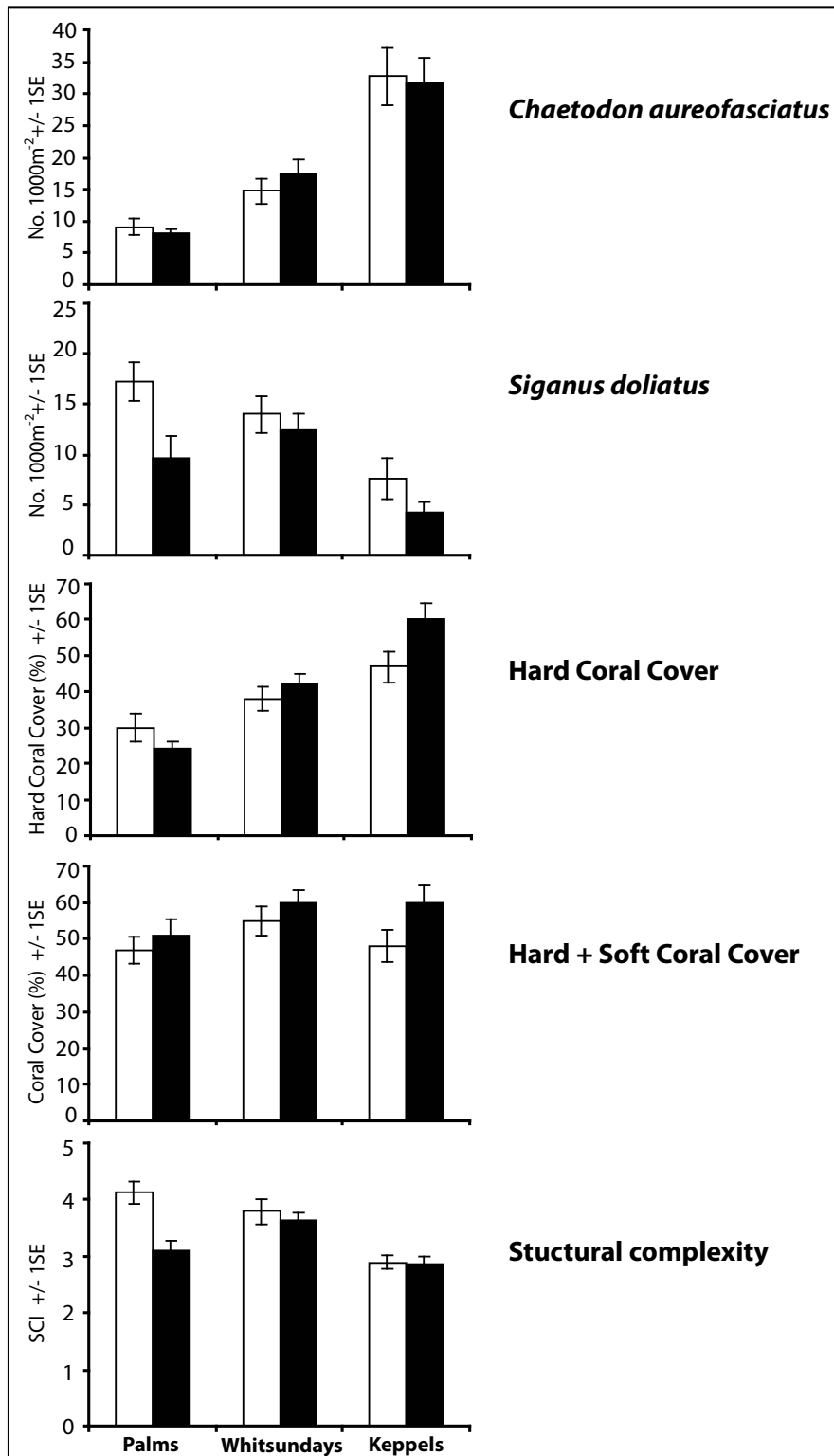
Figure 2.5: Mean density of *Siganus doliatus*, *Chaetodon aureofasciatus*, % of live hard coral cover; % live coral cover (hard & soft coral); and indices of benthic structural complexity for no-take MPAs (black bars) and fished (white bars) areas at three different island groups of the GBR.

2.5 Discussion

The Great Barrier Reef Marine Park (GBRMP) is the largest marine park in the world. Most of the coral reefs of the GBR occur on the mid- and outer continental shelf, 40-90 km from the coast. These reefs are also often great distances from the nearest centres of human population. An expansion of MPAs of the GBRMP under the Representative Areas Program (RAP) from 4.6% to 33.4% of the area of the park was implemented in 2004. This clearly represented a considerable challenge for future surveillance and enforcement of the GBRMP (Davis et al. 2004). A more immediate concern was that such an expansion required justification, especially to one of the major users of the park, the fishers. Many fishers claim they have no impact on target fish stocks, and that they are losing recreational fishing grounds. It is important to note that the objective of RAP was to protect (by placing in MPAs) 20% of each of 70 different bioregions in the GBRMP (Day et al. 2003). It was not a fisheries management plan, but a plan to protect biodiversity. The rationale for the present study was to determine if existing MPAs do have higher abundance and biomass of targeted fish than fished areas on inshore reefs of the Great Barrier Reef.

There are four key findings of this study. Firstly, our results contrast with many other studies of MPAs and fished areas on the GBR. Most of these other studies were carried out on offshore coral reefs. Secondly, that biomass, but not necessarily density, of reef fish targeted by hook and line fisheries is significantly higher in some MPAs than fished areas on inshore GBR reefs. Thirdly, such differences in biomass between MPAs and fished areas cannot be explained by differences in characteristics of the benthic habitat between these zones. Fourthly, that the results are consistent across a large expanse of the GBRMP (600 km).

Plectropomus spp. are the primary target of recreational and commercial fishers on coral reefs of the Great Barrier Reef (GBR). However, commercial fishers rarely fish the inshore island

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groups sampled in this study (Williams 2002). Thus the differences in biomass of target species between MPAs and fished areas in this study are probably attributable to the effects of recreational fishing. Furthermore, the surveillance and enforcement of MPAs is most likely better on inshore than offshore reefs, simply due to greater access of the inshore reefs to enforcement agencies like the Queensland Parks and Wildlife Service (Davis et al. 2004). Aerial surveillance of offshore reefs has, in the past, been relatively limited, simply because of the sheer size of the marine park, and perhaps due to limited funds for such surveillance. Thus the unique aspect of our results is that we have demonstrated, for one of the first times, clear differences in biomass of fish targeted by line fisheries between MPAs and fished areas of the GBRMP. We argue that such results are likely caused by reasonably high recreational fishing pressure (Higgs and McInnes 2003) and relatively effective surveillance and enforcement (Davis et al. 2004) on the inshore reefs that we studied.

The effect of the MPAs on density of *Plectropomus* spp. clearly varied with island group. This resulted in an overall non-significant difference in density of *Plectropomus* spp. between MPAs and fished areas. Such a non-significant effect of zoning on *Plectropomus* spp. density is similar to those reported in many other studies on the GBR (e.g. Ayling and Ayling 1984, 1986; Ayling et al. 1993, 2000, Mapstone et al. 2003). In some cases, these studies also reported spatially variable effects of zoning. However, all of these studies were carried out on coral reefs well off the coast (40-90 km), where surveillance and enforcement may be less effective than in inshore areas (Davis et al. 2004). Williamson et al. (2004) demonstrated a significant difference in density of *Plectropomus* spp. between MPAs and fished areas at the Palm and Whitsunday Islands in 1999/2000. Our results are similar for these two island groups. However, in the Keppel Islands this pattern was reversed. However, the biomass in MPAs at all three island groups ranged from 3.5-4.6 times greater than in the fished areas, and this difference was highly significant. In fact the greatest observed difference in biomass between MPAs and fished areas

was at the Keppel Islands (Fig. 2). This result was clearly caused by a larger average size of fish targeted by fisheries in the MPAs than fished areas (Table 3 and see also Williamson et al. 2004). It illustrates the need for studies to include comparisons of biomass of target species between no-take and fished areas.

Williamson et al. (2004) reported marginally larger differences in biomass between MPAs and fished areas than this study, 5.0 and 3.9 times more coral trout biomass in MPAs than fished areas, respectively. The difference between the results of these studies is probably due to observer differences, combined with temporal variation in environmental conditions (e.g. underwater visibility). Annual variations in fish movements, and the inclusion of a third island group in our study, could also have contributed to the small differences in results of the two studies. However, both studies observed significantly more biomass of *Plectropomus* spp. overall in the MPAs than the fished areas.

The much higher density of *Plectropomus* spp. in fished areas than MPAs in the Keppel Islands was caused mainly by two fished sites at these islands having large numbers of *Plectropomus* spp. recruits (5-20cm TL; 0+ age). Furthermore, the density of *Plectropomus* spp. >35 cm TL were significantly greater in the MPAs (Table 3). Thus, fish, on average, were larger in the MPAs. This was likely due to two processes, each affecting a different side of the length frequency distribution in each zone - higher recruitment of *Plectropomus* spp. in fished areas, and higher survival rates of large *Plectropomus* spp. in MPAs. The different patterns of *Plectropomus* spp. density between MPAs and fished areas at the three island groups could have been caused by differences in recruitment on large spatial scales (e.g. Doherty 2002). Settlement of *Plectropomus* spp. onto GBR reefs occurs around January to February (Ferreira and Russ 1994; Mapstone et al. 2003). Alternatively, the Keppel islands, the only islands sampled in October, may have provided us with the opportunity to detect the recruitment of

these fish (especially in the size ranges 15-20 cm) with a visual census technique designed to sample adults.

Much higher densities of recruits of *Plectropomus* spp. in fished areas than MPAs have been observed consistently on the GBR (Ayling et al. 1993; Mapstone et al. 2003). The higher density of recruits in the fished areas than MPAs may be due to increased rates of cannibalism caused by higher densities of large *Plectropomus* spp. in the MPAs (Ayling et al. 1993). Furthermore, Graham et al. (2003) demonstrated a negative relationship between biomass of *Plectropomus* spp. and biomass of their prey fish species in the Palm and Whitsunday Island groups in 2001-2002.

The effect of the MPAs on density of *L. carponotatus* clearly also varied with island group. There was a much higher density of *L. carponotatus* in MPAs than fished areas in the Whitsundays and Keppels, but a slightly higher density in fished areas than MPAs in the Palm Islands (Fig. 4.). This resulted in an overall non-significant difference in density of *L. carponotatus* between MPAs and fished areas. However, the biomass of *L. carponotatus* was consistently higher in the MPAs than the fished areas (Fig. 4). Fish were, on average, larger in MPAs than fished areas at all island groups. Legal-sized *L. carponotatus* had significantly higher density and biomass in MPAs than fished areas (Table 3). In addition, there was a higher density of small *L. carponotatus* in the fished areas than the MPAs in the Palm Islands.

Density comparisons of *L. carponotatus* between MPAs and fished areas on the GBR have been made in only one other study. Kritzer (2001) reported density of *L. carponotatus* in the Palm Islands (MPAs- 10 fish/1000m^2; fished areas- 14 fish/1000m^2) to be slightly lower than those of the density estimates in this study in 2002 (MPAs – 13.2 fish/1000m^2; fished areas -

16.1 fish/1000m^2). However, he did not provide biomass estimates for MPAs and fished areas in the Palm Islands.

Differences in biomass of target species between MPAs and fished areas cannot be explained by differences in characteristics of the benthic habitat between these zones in this study. When an ANOVA was applied to density of *Plectropomus* spp. and *L. carponotatus* without the benthic covariates, the interaction between zones and island group was statistically significant. This suggests that some of the interactions between zone and island groups are accounted for by variations in benthic variables, not zoning *per se*, in the ANCOVA. A substantial proportion of the observed variation in abundance of demersal fish is often determined by habitat structure (Friedlander and Parish 1998; Garcia-Charton and Perez-Ruzafa 1999). Several marine reserve studies have found that habitat differences between fished areas and MPAs confounded estimates of reserve effects (Garcia-Charton and Perez-Ruzafa 1999; Clark 2001). Others have found that fishing influenced abundance of fish more than benthic habitat (Polunin and Roberts 1993; McClanahan and Arthur 2001; Valles et al. 2001; Graham et al. 2003).

In our study, fish density and biomass were significantly associated with some habitat variables. For example, the significant differences in density of the coral feeding *Chaetodon aureofasciatus* among island groups was likely related to similar differences in hard coral cover among these island groups (Fig. 5). However, there were no clear differences in benthos between the MPAs and fished areas (Table 2, Fig. 5). The significant effect of the covariate 'Hard+soft coral' on *L. carponotatus* density and biomass may be due to the large number of juveniles settling into areas of high soft coral cover (pers. obs.). This association, however, does not affect the overall result of the zoning comparison, since there was no significant difference in total coral cover between MPAs and fished areas. The lack of an effect by any of the benthic covariates on the abundance of legal-size *L. carponotatus* also suggests that an

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effect of benthos on settlement patterns of this species may exist. Thus, our results are likely to be due to an effect of marine reserve protection from fishing, not an effect of habitat variability. Furthermore, the lack of difference in density of non-target species, *Siganus doliatus* and *Chaetodon aureofasciatus*, between MPAs and fished areas, suggests that hook and line fishing is the most likely reason for the patterns observed in this study.

Unequivocal demonstration that MPAs are the cause of higher abundance of fish targeted by fisheries requires a Before-After-Control-Impact-Pair experimental design (Russ 2002). Like most other studies of MPAs, our study lacks data on abundance of fish before protection. Therefore, we have not shown unequivocally that protection by zoning caused the higher biomass of target fish in MPAs. However, variations in the benthos do not account for differences in abundance of target species between fished areas and MPAs. Furthermore, Williamson et al. (2004) examined pre-zoning (1983/1984) data in the Palm and Whitsunday Islands, and concluded that protection from fishing was the likely cause of the larger biomass of target groups in MPAs than fished areas.

2.5.1 Conclusion

Our study of no-take marine protected areas and fished areas of inshore GBR reefs contrast with many other such studies on offshore GBR reefs. Inshore it is likely that recreational fishing pressure is high, and surveillance and enforcement is relatively good. The clear differences in biomass between MPAs and fished areas of inshore reefs for two species of reef fish targeted by hook and line fishing on the GBR are not due to habitat differences between the zones. Furthermore, our results are consistent across three island groups spanning 600km. Thus, it is likely that MPAs, if effectively protected, can maintain higher biomass of targeted fish than fished areas of inshore GBR reefs. Thus the expansion of the no-take protection of the Great

Barrier Reef Marine Park could likely increase spawning stock biomass of species targeted by fisheries inshore.

Chapter 3: The effects of the 2004 Representative Areas Program zoning plan on inshore coral reefs of the Great Barrier Reef: the first three years of protection.

3.1 Introduction

The use of no-take marine protected areas (MPAs), to protect spawning stocks of fish targeted by fisheries and to protect biodiversity, is becoming widespread throughout the world. New MPAs were introduced to coral reefs at a rate of ~40 per year from 1996-2006 (Mora et al. 2006) and continue to be established. However, coral reefs are still in decline despite increased awareness of the pressures of climate change and over population of human coastal communities (Bellwood et al. 2004; Worm et al. 2006; Bruno and Selig 2007). Calls have been made to establish a global network of MPAs protecting from 20-30% of coral reefs (Mora et al. 2006). However, by 2006 less than 20% of coral reefs were protected and, due to poor management and enforcement, only 0.01% of those were defined as no-take, had no poaching and were at low risk from external influences (Mora et al. 2006). Thus, the world is far from establishing a well coordinated global network of marine reserves, but there are some examples of extensive regional networks, such as in the Philippines (Alcala and Russ 2006), in the North West Hawaiian Islands (Sale et al. 2005) and on the Great Barrier Reef (Day et al. 2003).

Despite reports of poor management and enforcement, MPA research is well established and evidence is mounting for increases of abundance, biomass, size and reproductive output of species targeted by fisheries within many MPAs (Russ 2002; Halpern and Warner 2003). Limited evidence is available for adult spill-over (Russ and Alcala 1996; Zeller and Russ 1998; McClanahan and Mangi 2000; Zeller et al. 2003; Russ et al. 2004; Abesamis and Russ 2005;

Abesamis et al. 2006; Guidetti 2007; Francini-Filho and Moura 2008) and evidence of larval export from MPAs is still in its infancy (Jones et al. 2005; Almany et al. 2007). Researchers are now turning their attention to downstream ecological effects of MPAs on the rest of the fish community (Mumby et al. 2006; Samoilys et al. 2007; Watson et al. 2007), the benthos (Mumby et al. 2007), and the overall resilience of coral reefs to external impacts such as nutrient input (Sandin et al. 2008), bleaching and climate change (Hughes et al. 2007; Pratchett et al. 2008). In the case of climate change, MPAs seem unable to mitigate such large scale impacts (Jones et al. 2004; Graham et al. 2007). Several studies have demonstrated increases in predatory species in MPAs, often targeted by fisheries, which have then affected the fish community composition (Graham et al. 2003; Mumby et al. 2006; Watson et al. 2007), and may have facilitated coral recruitment (Mumby et al. 2007). Other studies, often long-term ones, have found little or no down stream effects from increased predators in the MPAs (Russ and Alcala 1998a,b; Barrett et al. 2007; Samoilys et al. 2007). Determining the downstream effects of MPAs depends on the amount of time since protection, the strength of predator-prey interactions, fishing pressure on different trophic levels before and during protection, and the varying states of the habitat and the changing environment (Russ and Alcala 1998a,b; Mumby et al. 2006; Samoilys et al. 2007; Pratchett et al. 2008).

A growing number of BACIP (Before-After-Control-Impact-Pair) design and long-term studies of MPAs are appearing in the literature. Such studies highlight the limitations of spatial comparisons of MPAs and control sites at one point in time, and increase our understanding of how the ecology of communities within the MPAs are influenced (Babcock et al. 1999; Russ and Alcala 2003; Shears et al. 2006; Barrett et al. 2007; Francini-Filho and Moura 2008). The benefits of MPAs for target species are not always noticeable. For example, a recent 10 year study, demonstrated that the target of a reef fishery, the teleost *Latridopsis forsteri*, resided within coastal Tasmanian MPAs for only five years before ontogenetic movement out of the

MPAs to deeper reefs, a period of time much shorter than the time between episodic large recruitment pulses (7-10yrs) (Barrett et al. 2007). If a spatial comparison was made in the period between the residence and recruitment time then no benefit of the reserve would be recorded. Therefore, spatial comparisons of MPAs with reference areas should be interpreted carefully (Russ et al. 2005), and duration and sampling frequency will affect the degree to which changes are observed (Edgar et al. 2004; Barrett et al. 2007).

Evidence of the effects of MPAs on biota in the GBRMP remain limited, usually because little is known about conditions before zoning was implemented (Evans and Russ 2004). Only one study has used a semi-BACIP design on the GBR (Williamson et al. 2004). They demonstrated six times greater biomass in the MPAs of the Palm and Whitsunday Islands 12-13 years after the original zoning plan was implemented in 1987 (Williamson et al. 2004). However the "Before" data in their study was collected by another scientist using slightly different sampling methods. The re-zoning of the Great Barrier Reef Marine Park (GBRMP), Australia, presented a unique opportunity to explore the temporal effects of MPAs on fishery resources and coral reef ecology using a BACIP sampling protocol. A unique pre-zoning sampling was implemented by the authors, specifically as a baseline for future assessments of the Representative Areas Program (RAP) rezoning plan of the Great Barrier Reef (GBR) (Day et al. 2003; Fernandes et al. 2005). Baseline Underwater Visual Census (UVC) surveys were conducted in the Palm, Whitsunday and Keppel Island groups prior to the implementation of the RAP rezoning plan in July 2004 and post implementation in 2006 and 2007.

The specific objective of this study was to investigate the early (first 3 years) effects of the Great Barrier Reef Marine Park 'Representative Area Program' rezoning plan on fish (including target and non-target species) and benthic assemblages in areas open and recently closed to fishing, on inshore fringing reefs of the GBRMP. Data presented here provide the first

quantitative assessment of the effects of the RAP rezoning plan on the fish community anywhere within the GBRMP.

3.2 Methods

3.2.1 Study Sites

The Great Barrier Reef Marine Park (GBRMP) Act was passed in 1975, and the original multiple-use zoning plan for the entire GBR was in place from 1988 (Williams and Russ 1994). Zoning within the GBRMP allows varying levels of use, ranging from fully preserved ("Preservation zone"), to open access (all fishing, including demersal trawling, permitted). Approximately 23.3% of nearly 3000 reefs were in the original (mid 1980's) MPAs. However, only ~ 4.6% of the total area of the Marine Park had complete no-take protection under this original zoning plan. In 1998 the GBRMPA embarked upon a rezoning process for the GBRMP called the Representative Areas Program (RAP). The RAP aimed to protect at least 20% of the area of each of seventy defined bioregions (30 reefal, 40 non-reefal) within the GBRMP (Day et al. 2003). After two rounds of public consultation over three years, the RAP achieved this goal. On July 1st 2004, the Great Barrier Reef Marine Park Authority (GBRMPA) implemented an updated multiple-use zoning plan in which 33.4% of the marine park was allocated into highly protected no-take marine reserves (Day et al. 2003). The network of MPAs implemented under the RAP was the world's largest spatial fishery closure (Day et al. 2003). Assessing the effectiveness of the RAP was a top priority of the GBRMPA and the Australian Government.

3.2.2 Data Collection

Baseline data were collected using Underwater Visual Census (UVC) in 2003/2004 at 32 sites open to fishing in the Palm (6), Whitsunday (18) and Keppel (8) Island groups. Sites were

randomly selected in areas with at least 300m of contiguous fringing reef. Each site, a total of 1500 m^2, consisted of five spatially independent transects of 50m by 6m wide separated by 10-15m. On July 1 2004, half of the fished sites at each island group changed to MPAs. Post-protection surveys were conducted in 2006 and 2007.

Approximately 160 species of coral reef fish from twelve families were surveyed (Appendix 1), including species in the families Acanthuridae, Chaetodontidae, Haemulidae, Labridae, Lethrinidae, Lutjanidae, Mullidae, Nemipteridae, Pomacentridae, Scaridae, Serranidae, and Siganidae. This study investigated the effects of no-take protection on fishery target and non-target species. The target species were divided into primary and secondary targets of the hook and line fishery. Two target species are analysed separately as their relative abundance and commercial/recreational importance warranted a more detailed investigation. The first was the primary target species of the hook and line fishery, *Plectropomus* spp., and the other was a secondary target species, *Lutjanus carponotatus*. Other secondary target groups analysed in this study were 'Other Serranids' (excl. *Plectropomus* spp.), 'Other Lutjanids' (excl. *Lutjanus carponotatus*) and a group of the 'Benthic predators' (See Appendix 1).

Non-target taxa included Family groups of Chaetodontidae, Scaridae, Acanthuridae, Siganidae, 'Small Labridae' and Pomacentridae, and groups of Corallivores and Roving herbivores (See Appendix 1). This study also recognized specific species as prey fish of the predators. Three groups were included in the prey analysis; they were Pomacentridae, 'Pomacentridae minus *Chromis nitida*' and 'Prey minus *C. nitida*'. High densities of *C. nitida* in the Keppel region masked patterns for other Pomacentridae, so this species was excluded from the Prey group and the Pomacentridae group. Two species of scarid, *Chlorurus sordidus* and *Scarus rivulatus*, were included in the 'Prey minus *C. nitida*' group to make the results comparable to another predator-prey study performed in the same regions (Graham et al. 2003). Fish total lengths of

primary and secondary fishery target species were estimated to within 5cm categories. To ensure accuracy of counts, and size estimation, two fish observers divided the species list (predatory species and non-predatory) for surveys and another person swam behind these two observers to measure the transect length and conduct the benthic survey on the return swim.

Two methods were used to assess the sessile benthic community. A predetermined structural complexity index (Table 1) was developed to estimate the slope and rugosity of the benthos every 10m along each transect (5 estimates for both slope and rugosity per transect). The second technique was a line intercept method used to record a point sample every metre along the transect tape (50 samples per transect). Categories sampled at each point were live/dead hard coral (branching, plate, solitary, tabular, massive, foliose, and encrusting), soft coral, sponge, clams (*Tridacna* spp.), other invertebrates (such as ascidians and anemones), macro-algae and turf algae, rock, rubble or sand. All transects were carried out within a depth range of 4-12m with an average depth of 6m. Visibility was recorded on each transect and typically ranged from 5 to 12m. The surveys did not proceed if visibility was less than 5m.

Table 3.1: Definitions of the categories used for visual estimation of rugosity and slope to estimate structural complexity of the reef habitats on inshore reefs of the GBR.

Category	Rugosity	Slope
1	Flat, expanses of rubble or sand with small scattered bommies (coral heads).	0°
2	Coral bommies amongst mostly rubble.	22.5^o
3	Rubble amongst mostly coral bommies.	45°
4	Good reef structure with some overhangs and holes.	67.5^o
5	High reef complexity. Many overhangs, holes and caves. Large bommies.	90°

3.2.3 Analysis

Three methods of analysis were used to explore the data in this study. Multivariate Repeated Measures Analysis of Covariance with three Years (2004, 2006, 2007), two Zones (Fished and No-take Protected) and three Regions (Palm, Whitsunday and Keppel Island groups) with three covariates (% Algal Cover, % Hard Coral Cover and Structural Complexity) were performed to investigate the general patterns of change over time in the fish taxa and benthos in response to the implementation of the new zoning plan in 2004. All variates which showed no interaction with the co-variates were then analysed without co-variates to increase the power of the Multivariate Repeated Measures Analysis. *Plectropomus* spp. density was square root transformed; *Plectropomus* spp. biomass, Roving herbivore density, SCI and 'Pomacentridae minus *C. nitida*' density were all log(x) transformed and *L. carponotatus* biomass was log (x+1) transformed to meet the assumptions of repeated measures ANOVAs.

Certain patterns in the data were clarified by the use of Univariate Regression Trees. Trees are only presented in this study if they met the scree plot assumptions. One hundred replicates of the tree were run and the tree size with the highest frequency was chosen as the most representative tree. All trees were run with Year, Zone and Region as the explanatory variables. However, Hard Coral Cover was added as an explanatory variable to investigate its influence on corallivore distributions.

Regression analysis was conducted to investigate the effect of the rezoning on the nature of predator-prey relationships through time after half of the sites studied became no-take. The abundance of the major predator in this study, *Plectropomus* spp., was plotted against abundance of three groups of non-target taxa that were likely prey, Pomacentridae,

'Pomacentridae minus *Chromis nitida*' and 'Prey minus *C. nitida*'. See Appendix 1 for the full list of prey species included in these groups.

3.4 Results

There were numerous changes over time in the density and biomass of the fish groups and benthos observed in this study (Table 2). Many of these changes are likely due to random or sampling variation. However, several key results are also likely due to anthropogenic (re-zoning) effects, others may be natural changes, or related indirectly to anthropogenic activities (i.e. caused by coral bleaching). Although determining whether a change is natural or due to direct or indirect anthropogenic influence is very difficult. The results presented here focus primarily on: 1) the zoning effects, with less emphasis placed on explaining regional (between island groups) differences unless necessary; and 2) the effects of natural or indirect anthropogenic processes and how these may confound the effects of the rezoning, with a focus on the 2006 bleaching event in the Keppel region.

The relative changes in abundance for the fish and benthos through time are shown in Table 2. The purpose of this table is to show the overall percentage change from pre-protection (2004) to three years after protection in 2007, and most importantly, the changes through time in the protected relative to the fished areas. If both fished and no-take protected areas increase at the same rate then increases in the MPAs were most likely not caused by the rezoning, but if fished areas remain stable and protected areas increase, it suggests an effect of the rezoning if all other influences remain equal. This is expressed by the net increase in the protected versus the fished areas (Table 2, shown as a percentage). A positive percentage net increase suggests a greater increase in the protected relative to the fished areas and the greater the percentage the more obvious was the difference between the MPAs and fished areas.

Table 3.2: Mean numbers per 1000m^2 (unless kg specified) and the percentage increase between 2004 and 2007 for each group in fished and no-take marine protected areas of the Palm, Whitsunday and Keppel Islands. Last column is the net increase within the no-take protected relative to the fished areas. HCC is hard coral cover, SCI is structural Complexity Index. SE is 1 standard error.

Taxa	Fished				No-take Protected				% Net Increase
	2004 (SE)	2006 (SE)	2007 (SE)	% Increase 2004:2007	2004 (SE)	2006 (SE)	2007 (SE)	% Increase 2004:2007	
Plectropomus spp. density	8.1 (0.8)	7.2 (0.7)	7.3 (0.9)	-12.4	11.1 (1.6)	12.6 (1.4)	15.0 (1.5)	+35.1	+47.5
Plectropomus spp. biomass (kg)	7.7 (1.0)	4.8 (0.7)	4.3 (0.6)	-44.2	7.2 (1.1)	10.8 (1.3)	17.2 (2.2)	+138.9	+183.1
Lutjanus carponotatus density	9.9 (1.0)	9.1 (0.9)	12.1 (1.4)	+22.2	8.7 (1.2)	12.5 (2.0)	10.8 (1.0)	+24.1	+1.9
L. carponotatus biomass (kg)	2.6 (0.3)	2.5 (0.3)	4.0 (0.5)	+53.8	2.7 (0.4)	4.5 (0.7)	4.2 (0.3)	+55.6	+1.9
Other Serranids	8.0 (0.7)	8.8 (0.8)	10.2 (0.8)	+27.5	11.3 (1.0)	12.5 (1.2)	14.2 (1.4)	+25.7	-1.8
Other Lutjanids	24.0 (11.5)	16.5 (5.4)	29.1 (8.5)	+21.3	28.6 (11.0)	68.7 (27.9)	46.6 (14.1)	+62.9	+41.6
Benthic predators	40.2 (3.2)	30.4 (2.3)	37.9 (2.6)	-5.7	39.6 (2.8)	28.7 (2.1)	35.0 (1.9)	-11.2	-5.5
Corallivores	36.2 (3.1)	20.0 (1.9)	28.7 (2.4)	-20.7	25.0 (2.1)	16.0 (1.3)	21.2 (1.5)	-15.2	+5.5
Chaetodontids	51.3 (5.4)	32.9 (4.3)	43.9 (5.2)	-14.5	40.5 (4.2)	28.0 (3.2)	35.9 (4.6)	-11.4	+3.1
Roving herbivores	123.5 (13.3)	89.8 (11.4)	90.6 (14.6)	-26.6	57.1 (4.3)	53.0 (5.9)	81.5 (8.4)	+42.7	+69.3
Scarids	101 (13.2)	69.0 (10.1)	63.5 (11.0)	-37.2	40.5 (4.0)	33.8 (3.7)	59.7 (6.8)	+47.4	+84.6
Siganids	11.5 (1.4)	15.5 (3.5)	27.2 (8.7)	+136.5	10.3 (1.2)	16.7 (4.2)	22.9 (4.3)	+122.3	-14.2

Table 3.2 (cont.)

Taxa	Fished				No-take Protected				% Net Increase
	2004 (SE)	2006 (SE)	2007 (SE)	% Increase 2004:2007	2004 (SE)	2006 (SE)	2007 (SE)	% Increase 2004:2007	
Acanthurids	10.9 (3.2)	5.6 (2.6)	6.8 (1.8)	-37.7	6.2 (1.6)	3.0 (0.7)	5.3 (1.5)	-14.6	+23.1
Small Labrids	34.7 (2.6)	23.2 (1.5)	19.0 (1.6)	-45.2	32.1 (2.1)	19.2 (1.5)	19.9 (2.3)	-38.0	+7.2
Pomacentrids	2539.3 (583.1)	734.6 (130.7)	848.3 (166.9)	-66.6	1537.0 (243.8)	1059.5 (172.1)	854.3 (109.2)	-44.4	+22.2
Pomacentrids minus *C. nitida*	198.7 (15.7)	398.2 (32.0)	382.6 (30.7)	+92.6	270.8 (29.2)	435.2 (30.2)	368.1 (23.8)	+35.9	-56.7
Prey minus *C. nitida*	503.3 (30.9)	452.2 (31.7)	435.7 (31.4)	-13.4	531.2 (31.6)	466.0 (31.3)	414.9 (22.9)	-21.9	-8.5
% Algal cover	0.3 (0.1)	5.7 (1.7)	3.5 (1.4)	+1066.7	0.5 (0.2)	13.3 (2.8)	15.8 (3.1)	+3160	+2093.3
% HCC	33.3 (2.4)	37.6 (2.6)	45.2 (2.4)	+35.7	34.7 (2.7)	33.4 (1.9)	37.8 (1.7)	+8.9	-26.8
SCI	10.0 (0.7)	11.8 (0.8)	12.1 (0.7)	+21	9.0 (0.6)	11.2 (0.6)	12.0 (0.6)	+33.3	+12.3

3.4.1 Primary target and secondary target species of the hook and line fishery

The only change through time that may be attributed to the implementation of new MPAs was biomass of *Plectropomus* spp. (Tables 2, 3, Fig. 2). The pre-protection results show that *Plectropomus* spp. biomass was very similar between sites earmarked for protection and those intended to remain open to fishing in 2004 (Fig. 2,4B, Table 2). In less than two years the density and biomass increased dramatically (up to 50%), and 3 years after protection the biomass of *Plectropomus* spp. in the MPAs (17.2 kg $1000m^{-2}$) was significantly greater (Tables 3, 4) than in the fished areas (4.3 kg $1000m^{-2}$) (Fig. 2). This was a net increase in *Plectropomus* spp. biomass in the MPAs of +183% over three years (Table 2). Univariate regression tree analysis showed the major difference between MPAs and fished areas developed between 2006 and 2007 (Fig. 3).

Plectropomus spp. density increased in the MPAs and not the fished areas through time (net increase 47.5%), but not as dramatically as biomass (Table 2). The large difference between MPAs and fished sites in 2006 was also partially due to a decrease in the fished areas by 12% (density) and 44% (biomass). However, both density and biomass in the fished areas levelled out between 2006 and 2007 (Table 2) due to increases in the Palm and Keppel Island groups, whereas the fished areas of the Whitsunday region continued to decrease throughout the study (2004 to 2007) from a density of 6 to 2 fish $1000m^{-2}$ and a biomass of 7.7kg to 2.2kg $1000m^{-2}$ (Fig. 4A).

The most abundant secondary target species, *Lutjanus carponotatus*, also increased substantially in density (45%) and biomass (67%) in the MPAs and not the fished areas within two years of protection (Fig. 2), but levelled out in the third year, resulting in no significant

difference between MPAs and fished areas (Protected area net increase = 1.9%) by 2007

(Tables 2, 3, 4; Fig. 2).

Table 3.3: Results of Multivariate (Pillai's trace) Repeated Measures ANCOVA for density or biomass of several target species or trophic or Family groups and three benthic variates: Percentage Hard Coral Cover (%HCC), Percentage Algal Cover and Structural Complexity Index in fished and no-take protected areas in the Palm, Whitsunday and Keppel Island Groups between 2004 and 2007. ***P<0.001; **P<0.01; *P<0.05; ns: not significant. Covariates are listed across the first row along with the variates.

Source of Variation	Algae '04 (df)	Algae '06 (df)	Algae '07 (df)	HC '04 (df)	HC '06 (df)	HC '07 (df)	SCI '04 (df)	SCI '06 (df)	SCI '07 (df)	Year*Zone *region (df)	Year* region (df)	Year* Zone (df)	Year (df)
Plectropomus spp. Density	-	-	-	3.17 ns (2,19)	0.12 ns (2,19)	0.13 ns (2,19)	0.95 ns (2,19)	0.07 ns (2,19)	0.51 ns (2,19)	1.01 ns (4,40)	2.56 ns (4,40)	1.02 ns (2,19)	**4.83 * (2,19)**
Plectropomus spp. Biomass	-	-	-	1.58 ns (2,19)	0.08 ns (2,19)	0.22 ns (2,19)	2.54 ns (2,19)	1.00 ns (2,19)	1.71 ns (2,19)	0.54 ns (4,40)	1.69 ns (4,40)	**4.81 * (2,19)**	**6.08 * (2,19)**
L. carponotatus Density	-	-	-	1.74 ns (2,19)	0.55 ns (2,19)	2.66 ns (2,19)	0.56 ns (2,19)	0.36 ns (2,19)	0.47 ns (2,19)	0.59 ns (4,40)	1.50 ns (4,40)	0.20 ns (2,19)	1.83 ns (2,19)
L. carponotatus Biomass	-	-	-	0.03 ns (2,19)	1.11 ns (2,19)	2.83 ns (2,19)	2.32 ns (2,19)	0.03 ns (2,19)	0.09 ns (2,19)	0.55 ns (4,40)	0.58 ns (4,40)	0.58 ns (2,19)	2.26 ns (2,19)
Other Serranids Density	-	-	-	1.16 ns (2,19)	0.65 ns (2,19)	0.34 ns (2,19)	0.25 ns (2,19)	0.56 ns (2,19)	0.92 ns (2,19)	1.38 ns (4,40)	1.13 ns (4,40)	0.34 ns (2,19)	3.05 ns (2,19)
Other Lutjanids Density	-	-	-	0.32 ns (2,19)	0.17 ns (2,19)	0.16 ns (2,19)	0.19 ns (2,19)	0.72 ns (2,19)	0.09 ns (2,19)	0.64 ns (4,40)	1.01 ns (4,40)	0.59 ns (2,19)	1.72 ns (2,19)
Benthic Predators Density	-	-	-	0.17 ns (2,19)	0.64 ns (2,19)	0.68 ns (2,19)	1.96 ns (2,19)	0.66 ns (2,19)	0.70 ns (2,19)	0.38 ns (4,40)	0.79 ns (4,40)	0.004 ns (2,19)	0.10 ns (2,19)
Corallivore Density	-	-	-	**4.51 * (2,19)**	0.87 ns (2,19)	0.50 ns (2,19)	0.08 ns (2,19)	0.26 ns (2,19)	1.26 ns (2,19)	1.81 ns (4,40)	1.01 ns (4,40)	1.66 ns (2,19)	0.54 ns (2,19)
Chaetodontid Density	-	-	-	2.07 ns (2,19)	0.38 ns (2,19)	0.13 ns (2,19)	0.04 ns (2,19)	0.86 ns (2,19)	0.90 ns (2,19)	1.72 ns (4,40)	0.98 ns (4,40)	1.17 ns (2,19)	0.28 ns (2,19)
Roving herbivores	0.39 ns (2,16)	1.45 ns (2,16)	1.21 ns (2,16)	0.74 ns (2,16)	0.28 ns (2,16)	0.18 ns (2,16)	0.94 ns (2,16)	0.13 ns (2,16)	0.34 ns (2,16)	1.49 ns (4,34)	1.12 ns (4,34)	0.60 ns (2,16)	0.42 ns (2,16)

Table 3(Cont.)

Source of Variation	Algae '04 (df)	Algae '06 (df)	Algae '07 (df)	HC '04 (df)	HC '06 (df)	HC '07 (df)	SCI '04 (df)	SCI '06 (df)	SCI '07 (df)	Year*Zone*Island (df)	Year*Island (df)	Year*Zone (df)	Year (df)
Scarid Density	0.24 ns (2,16)	0.41 ns (2,16)	**3.96 *** (2,16)	0.41 ns (2,16)	0.35 ns (2,16)	0.41 ns (2,16)	0.76 ns (2,16)	0.16 ns (2,16)	0.12 ns (2,16)	2.11 ns (4,34)	1.43 ns (4,34)	0.70 ns (2,16)	0.77 ns (2,16)
Siganidae Density	1.64 ns (2,16)	**5.80 *** (2,16)	**7.60**** (2,16)	1.21 ns (2,16)	1.29 ns (2,16)	1.63 ns (2,16)	1.28 ns (2,16)	0.46 ns (2,16)	0.24 ns (2,16)	1.25 ns (4,34)	0.94 ns (4,34)	0.08 ns (2,16)	1.41 ns (2,16)
Acanthurid density	1.11 ns (2,16)	1.47 ns (2,16)	2.83 ns (2,16)	1.10 ns (2,16)	**5.01 *** (2,16)	1.94 ns (2,16)	1.67 ns (2,16)	0.67 ns (2,16)	1.00 ns (2,16)	1.36 ns (4,34)	1.60 ns (4,34)	0.77 ns (2,16)	0.76 ns (2,16)
Small Labrid Density	**5.18 *** **(2,16)**	2.45 ns (2,16)	0.50 ns (2,16)	0.47 ns (2,16)	1.41 ns (2,16)	0.73 ns (2,16)	0.10 ns (2,16)	0.01 ns (2,16)	0.11 ns (2,16)	0.26 ns (4,34)	1.11 ns (4,34)	0.06 ns (2,16)	0.07 (2,16)
Pomacentrid Density	0.86 ns (2,16)	0.68 ns (2,16)	**73.25 *** (2,16)	0.87 ns (2,16)	1.74 ns (2,16)	2.19 ns (2,16)	0.42 ns (2,16)	0.22 ns (2,16)	0.23 ns (2,16)	**7.61 *** (4,34)	**5.13 *** (4,34)	**112.42 *** (2,16)	2.18 ns (2,16)
Pomacentrid (minus *C. nitida*) density	1.34 ns (2,16)	1.12 ns (2,16)	1.01 ns (2,16)	2.55 ns (2,16)	0.51 ns (2,16)	3.22 ns (2,16)	3.01 ns (2,16)	0.27 ns (2,16)	0.27 ns (2,16)	1.03 ns (4,34)	2.62 ns (4,34)	0.91 ns (2,16)	0.40 ns (2,16)
Prey (minus *C. nitida*) Density	0.99 ns (2,16)	0.22 ns (2,16)	0.42 ns (2,16)	0.83 ns (2,16)	1.68 ns (2,16)	**4.63 *** (2,16)	2.39 ns (2,16)	0.36 ns (2,16)	0.14 ns (2,16)	098 ns (4,34)	1.47 ns (4,34)	0.85 ns (2,16)	0.73 ns (2,16)
% Macro-algae	-	-	-	**8.37 ** ** (2,19)	**5.01 *** (2,19)	**5.28 *** (2,19)	0.96 ns (2,19)	0.81 ns (2,19)	1.76 ns (2,19)	**7.01 *** (4,40)	**12.34 *** (4,40)	**16.03 *** (2,19)	**25.29 *** (2,19)
% HCC	1.65 ns (2,19)	**6.10 ** ** (2,19)	**4.80 *** (2,19)	-	-	-	1.43 ns (2,19)	1.43 ns (2,19)	1.21 ns (2,19)	1.47 ns (4,40)	**9.06 *** (4,40)	1.98 ns (2,19)	**4.13 *** (2,19)
SCI	0.51 ns (2,19)	0.63 ns (2,19)	0.41 ns (2,19)	1.56 ns (2,19)	1.38 ns (2,19)	2.75 ns (2,19)	-	-	-	0.67 ns (4,40)	1.13 ns (4,40)	0.25 ns (2,19)	1.55 ns (2,19)

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'Other Serranids' showed no change through time at any level (year, year x zone, year x region or year x zone x region) when using covariates in the Repeated Measures Analysis of covariance (Table 3). However when the non-significant covariates were removed from the analysis, there was a significant result for year and year x region (Table 4). Part of the year x region variation is illustrated in the univariate regression tree indicating that the Palm and Whitsunday Islands differed from the Keppel islands, and that within the Keppel Islands there was an effect of zoning (Fig. 5). The serranid primarily responsible for this result was *Epinephelus quoyanus*, which were always more abundant in the MPAs than fished areas in the Keppel region (Fig. 5) and were always relatively rare in the Palm and Whitsunday regions (unpublished data).

'Other Lutjanids' showed a year and a year x region effect (Table 4) due to the patchy distribution of species within this group, particularly *Lutjanus vitta, L. fulviflamma, L. lutjanus*. In this study, if a school of Lutjanids move slightly off or on a transect at the time of the survey the numbers recorded could vary dramatically. This is indicated by the large standard error bars in the 'Other Lutjanid' results (Fig. 4F). There was no effect of year, zoning or region on the 'Benthic predators' (Tables 2, 3, 4).

3.4.2 Non-target taxa

Non-target taxa did not show any significant effects of the re-zoning. This includes Pomacentridae, which showed significant year x zone, year x region and year x zone x region effects (Table 3, Fig. 6F). The latter result was due to extremely high densities of *Chromis nitida* (8600 fish 1000m^{-2}) in fished areas of the Keppel region, which decreased due to coral bleaching and subsequent algal growth in 2006, thus decreasing available habitat for shelter for *C. nitida*. This is supported by the significant effect of the 'Algae 2007' covariate for

Pomacentridae (Table 3). Sites that were to become protected had half as many *C. nitida* (4200 fish $1000m^{-2}$) in 2004 and declined to approximately the same density as the fished areas in 2006 (2000 fish $1000m^{-2}$), but the impact was less because the density was lower to begin with. Therefore, the 22% net increase of Pomacentrid density in the MPAs relative to the fished areas was due to greater declines from higher original densities within the fished areas (Table 2, Fig. 6F). Furthermore, 'Pomacentrid minus *C. nitida*', a variate analysed to reduce the swamping-effect of the high densities of *C. nitida*, actually increased more, but not significantly, in the fished areas (93%) than in the MPAs (36%) from 2004 to 2007 (Tables 2, 3).

'Roving herbivores' were dominated by the 'Scarid' densities. Thus the results for both variates are similar (Table 2, 3). Positive net increases in the MPAs relative to fished areas for both groups was caused by decreasing densities in the fished areas (even though these fish are not fished), and also statistically insignificant increases in the MPAs (Tables 2, 3, Fig 6B). 'Siganids' displayed greater increases in density in the MPAs of the Whitsunday islands and in the fished areas of the Keppel Islands (Fig. 6C). Thus no net increase was demonstrated overall (Table 2). 'Small Labridae' and 'Prey minus *C. nitida*' densities were variable with slight declines in both MPAs and fished areas (Table 2, Fig. 6E, H), but no significant patterns of change were observed through time (Table 3).

The family Chaetodontidae was analysed in two separate ways, firstly as a Family, and secondly as a subset of primary corallivores. As corallivores are the most abundant species of Chaetodontidae (predominately *C. aureofasciatus*) on the inshore reefs, the results are very similar. Both groups showed statistically non-significant declines in both MPAs and fished areas (Table 3). The corallivores showed some covariate interaction with 'Hard Coral Cover' (Table 3) and this variate was analysed using a univariate regression tree with 'Hard coral cover' as an explanatory variable for Year, Zone and Region (Fig. 7). The results suggest that

below 62.8% coral cover the mean density of corallivores on the inshore island reefs was approximately 21 per 1000m^{-2}. Above 62.8% hard coral cover, the mean corallivore density in the Palm and Whitsunday regions (23 fish 1000m^{-2}) was much less than in the Keppel region (58.5 fish 1000m^{-2}) (Fig. 7).

3.4.3 Prey Fish

Changes to densities of 'Prey minus *C. nitida*', Pomacentridae and 'Pomacentridae minus *C. nitida*', due to the rezoning of the GBRMP and the consequent increase of *Plectropomus* spp. density and biomass in MPAs, were not detected by MANCOVA (Table 2, 3; Fig. 6H, F, G). However, when the density of 'Pomacentridae minus *C. nitida*' was plotted against density of *Plectropomus* spp. for 2004, 2006 and 2007, in three independent regression analyses, the coefficient (slope of the line) gradually became more negative over time (Fig. 8B) and eventually in 2007 the regression analysis approached significance (p = 0.053). In contrast, Pomacentridae (including *C. nitida*) began in 2004 with a highly significant positive relationship (p < 0.01). However, through time the coefficient of the slope decreased (Fig. 8A). 'Prey minus *C. nitida*' in 2004 had a highly significant negative relationship (p < 0.01), but over time the coefficient of the slope became less negative (Fig. 8C) and the relationship was not significant in 2006 (p = 0.16).

Table 3.4: Results of Multivariate (Pillai's trace) Repeated Measures Anovas, for variates that demonstrated no relationship with covariates in Table 3, in fished and no-take protected areas in the Palm, Whitsunday and Keppel Island Groups between 2004 and 2007.; ***P<0.001; **P<0.01; *P<0.05; ns: not significant.

Source of Variation	Year* zone*Region (4,52df)	Year * Region (4,52df)	Year * Zone (2,25df)	*Year (2,25df)*
Plectropomus spp. Density	1.081 ns	4.695 **	0.581 ns	4.427 *
Plectropomus spp. Biomass	1.757 ns	1.560 ns	10.060 ***	1.979 ns
L. carponotatus Density	0.7331 ns	5.391 **	0.7457 ns	5.603 **
L. carponotatus Biomass	1.347 ns	1.895 ns	1.864 ns	0.741 ns
Other Serranids Density	2.213 ns	3.376 *	0.354 ns	6.903 **
Other Lutjanids Density	1.023 ns	3.687 *	0.241 ns	5.216 *
Benthic Predators Density	0.906 ns	0.290 ns	0.382 ns	12.366 ***
Roving herbivores	0.993 ns	2.724 *	0.537 ns	9.248 ***
Pomacentrid (minus *C. nitida*) density	1.742 NS	6.868 ***	0.465 ns	3.130 ns
Chaetodontid Density	2.362 ns	1.360 ns	1.800 ns	13.930 ***
SCI	1.226 ns	6.429 ***	0.144 ns	19.018 ***

3.4.4 Benthos

Structural complexity varied through time but there were no significant patterns clearly related to the rezoning of the GBRMP (Table 2, 3, Fig. 9C). Significant changes at all levels of analysis were observed for Macro Algal Cover, and significant differences were observed for Hard Coral Cover for Year and Island x Year (Table 3). These results were caused by two coral bleaching events in the Keppel region in 2006 that led to significant decreases in coral cover at most sites and subsequent increase in algal cover, which eventually affected fish densities. In

the Keppel region coral cover declined from approximately 60% to 32% (Fig. 9B) and algal cover increased from 1 to 62.6% in the protected areas from 2004 to 2007 (Fig. 9A). In the fished areas of the Keppel region hard coral cover decreased from 60 to 39% cover in 2006 and increased slightly to 43% in 2007 (Fig. 9B). Concomitantly, algal cover increased from 0.4 to 22.9% in 2006 and then declined to 13.7% in 2007 in fished areas of the Keppels (Fig. 9A).

The majority of the fish groups analysed with Algae as a covariate were affected by this increased cover of algae (Table 3). In the Keppel region, nine of the 14 fish groups in this study had reductions in density, in both MPAs and in fished areas, from 2004 to 2006 (Fig. 4, 6). These declines included the density of the primary targets of the hook and line fishery, *Plectropomus* spp., in MPAs, but the biomass of this group remained stable at between 9.5 - 10kg 1000m^{-2}, and then increased substantially to 17.8kg 1000m^{-2} in 2007. Interestingly, the density of *Plectropomus* spp. has continued to increase in the MPAs in both the other regions of this study where algae have not increased dramatically. In the Keppel region, the majority of the fish groups increased in density from 2006 to 2007, with greater improvement in the fished areas for Acanthuridae, Siganidae, Chaetodontidae and Pomacentridae (Fig. 6D, C, A, F), where the algal cover declined. Greater increases were observed for *Plectropomus* spp. and Small Labridae in the MPAs than in the fished areas (Fig. 4A, 6E) and Scarids showed the greatest increases in the MPAs where the algae did not decline (Fig. 6B).

The hard coral cover in both the fished areas and MPAs in the Palm region decreased by 50% in 2006 (from 20% to 10% in fished; and from 40% to 20% in MPAs). These likely affected declines in densities of fish tightly associated with hard coral cover. This however, was likely due to sampling variation caused by the deployment of the transect tape, since coral cover and fish densities returned to original levels and higher in 2007 (Fig. 9B). For example, density of coral dependent Families, Chaetodontidae and Pomacentridae, tracked the "decline" and

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subsequent "recovery", but large predatory, species targeted by the fishers, such as the Lutjanids and Serranids, showed no declines and actually continued to increase through time. Previous experience with this coral transect technique suggests that this sampling error is a rare occurrence. However, without the temporal collection of data, the data would be misrepresented. Thus, this rare occurrence demonstrates the advantages of collecting temporal data rather than spatial data.
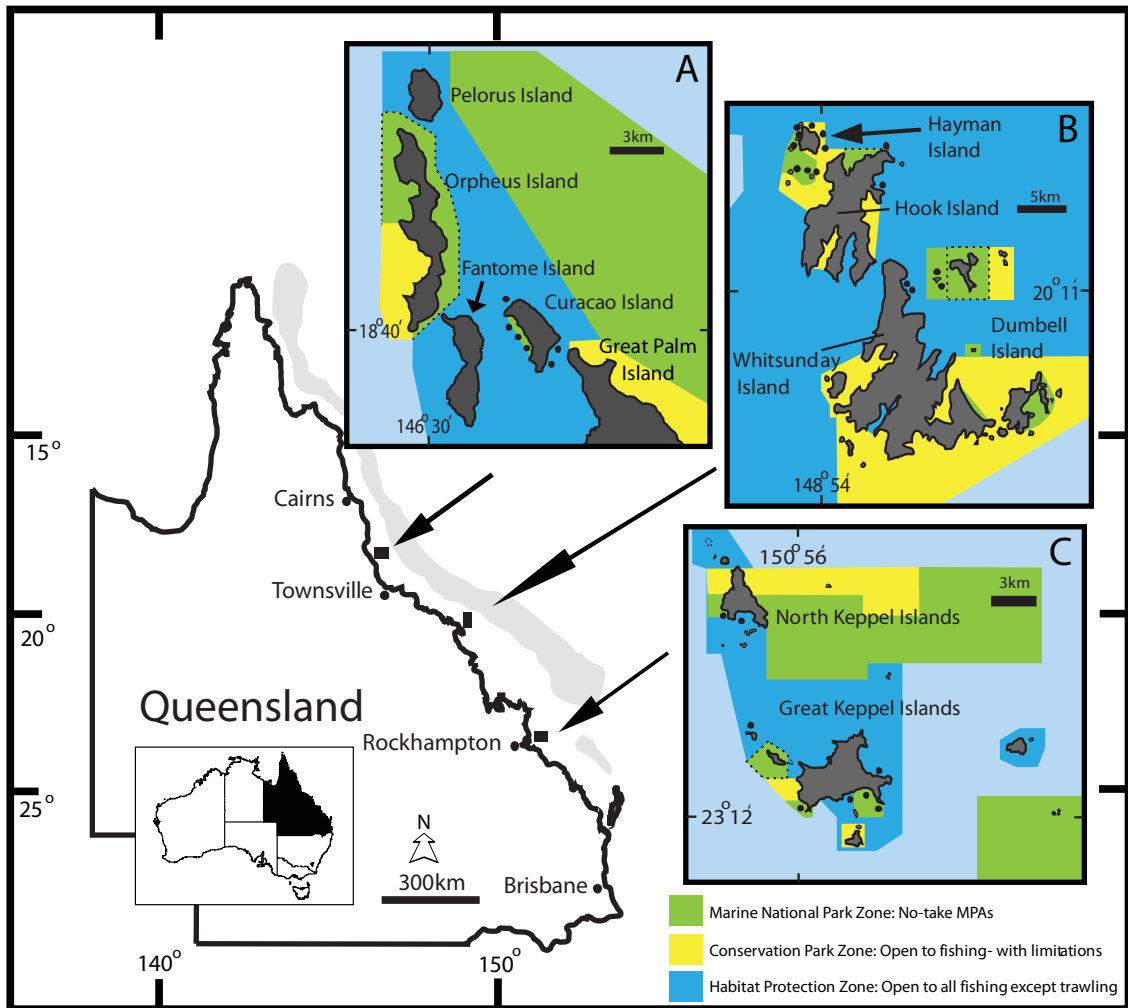
Figure 3.1: Map of Sites in the A) Palm, B) Whitsunday and C) Keppel Islands along the Queensland Coast, Australia. Green areas are MPAs, those with black dots around are pre-2004 MPAs; Yellow are areas where only recreational fishers can catch fish but with one hook and one line or go spear fishing (except in the Whitsundays); Dark blue is open to all fishing except trawling; Light blue is open to all uses.
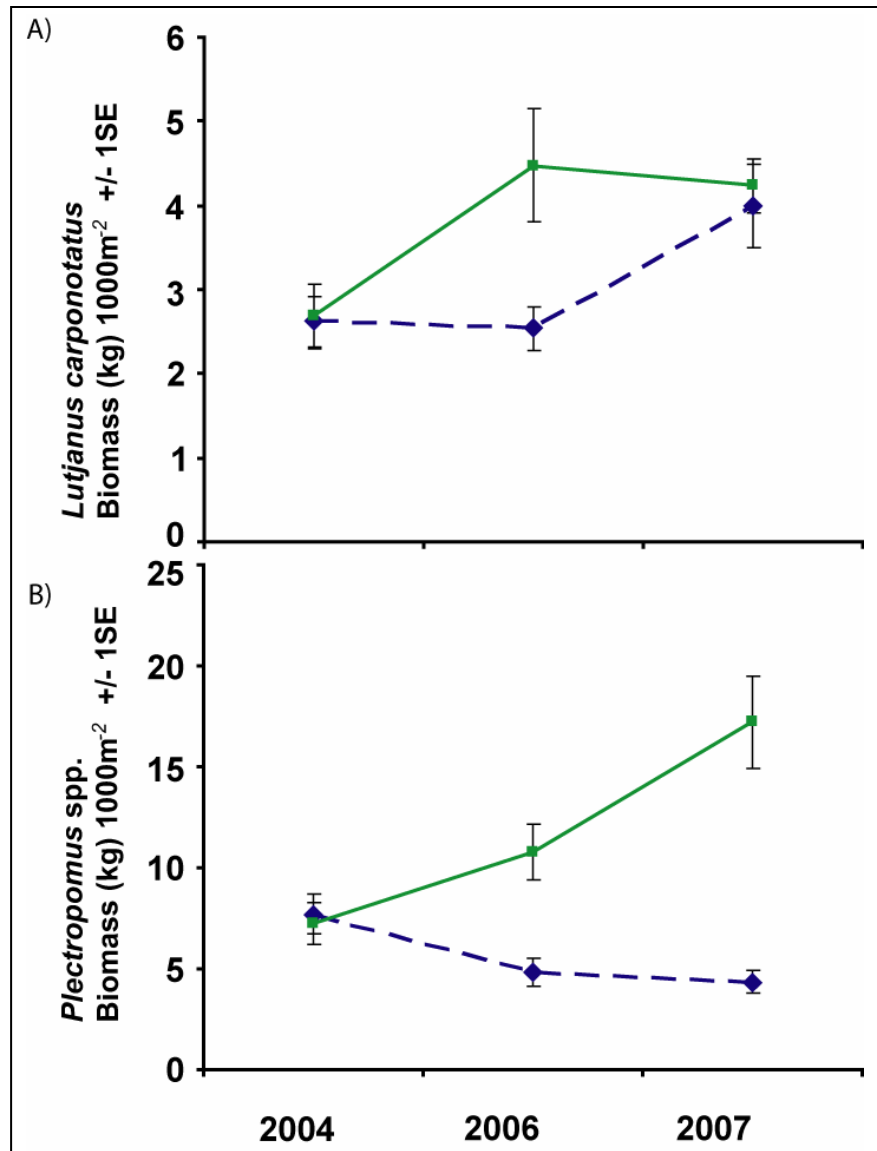
Figure 3.2: Biomass of *Lutjanus carponotatus* and *Plectropomus* spp. in fished (dash line) and no-take protected (solid line) areas of the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and at two resurveys in 2006 and 2007.
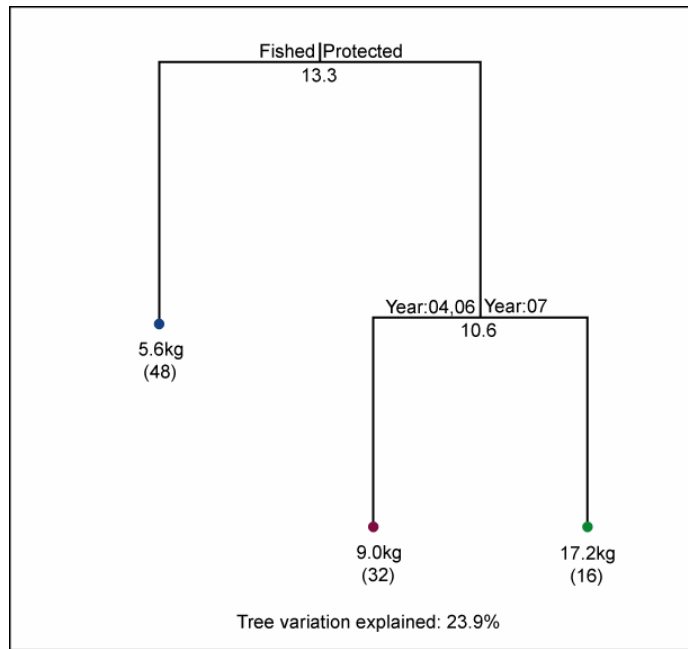
Figure 3.3: Univariate regression tree of *Plectropomus* spp. biomass in fished and no-take protected areas of the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and at two resurveys in 2006 and 2007. Numbers below forks in the tree represent the percentage of the tree explained at that split. Figures below each dot are *Plectropomus* spp. biomass (kg per 1000m-2) at the location/time. Figures in brackets are number the sites.
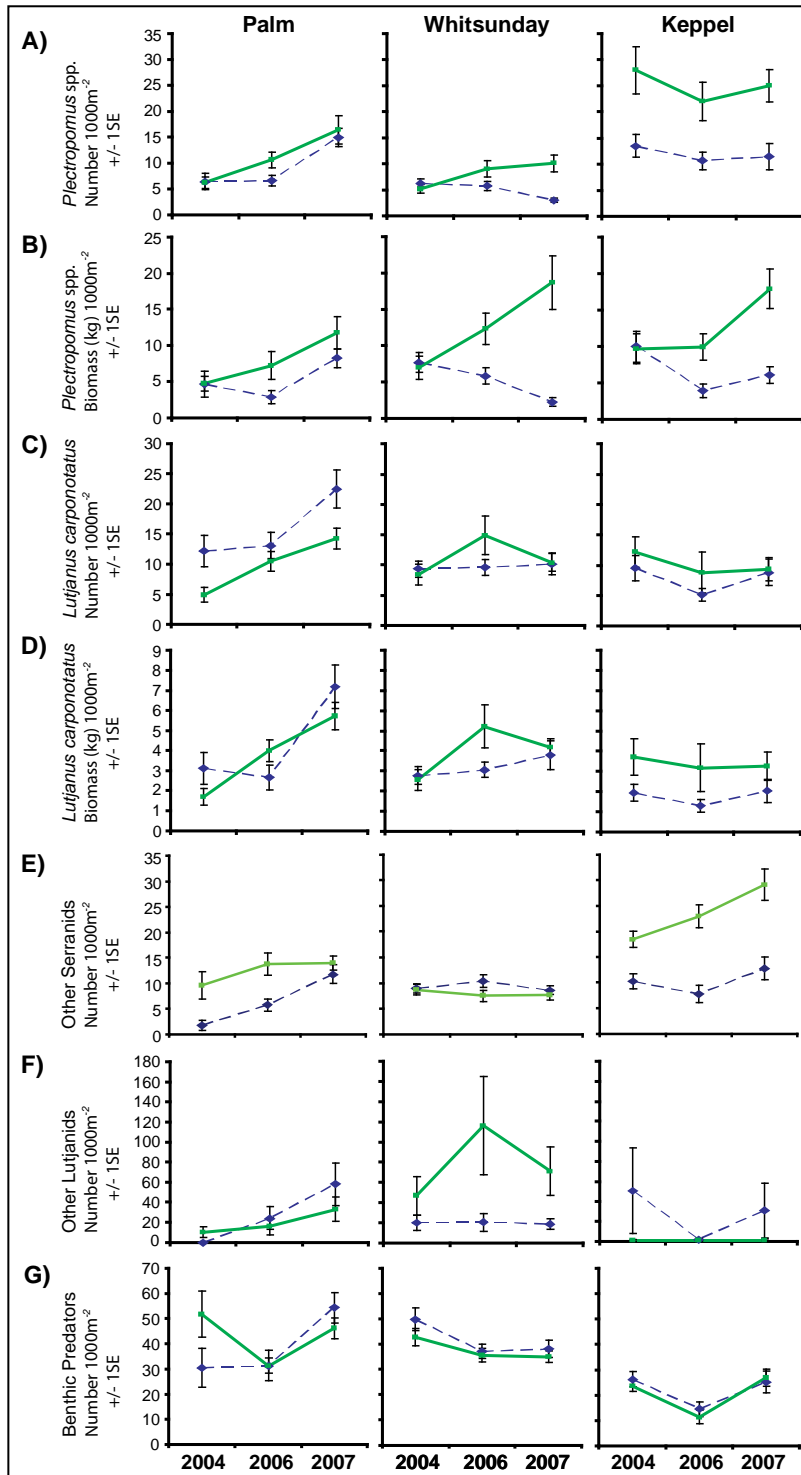
Figure 3.4: Density and biomass (+/- 1 SE) of primary and secondary target fishery species in fished (dash line) and no-take protected areas (solid line) in the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and two resurveys in 2006 and 2007.
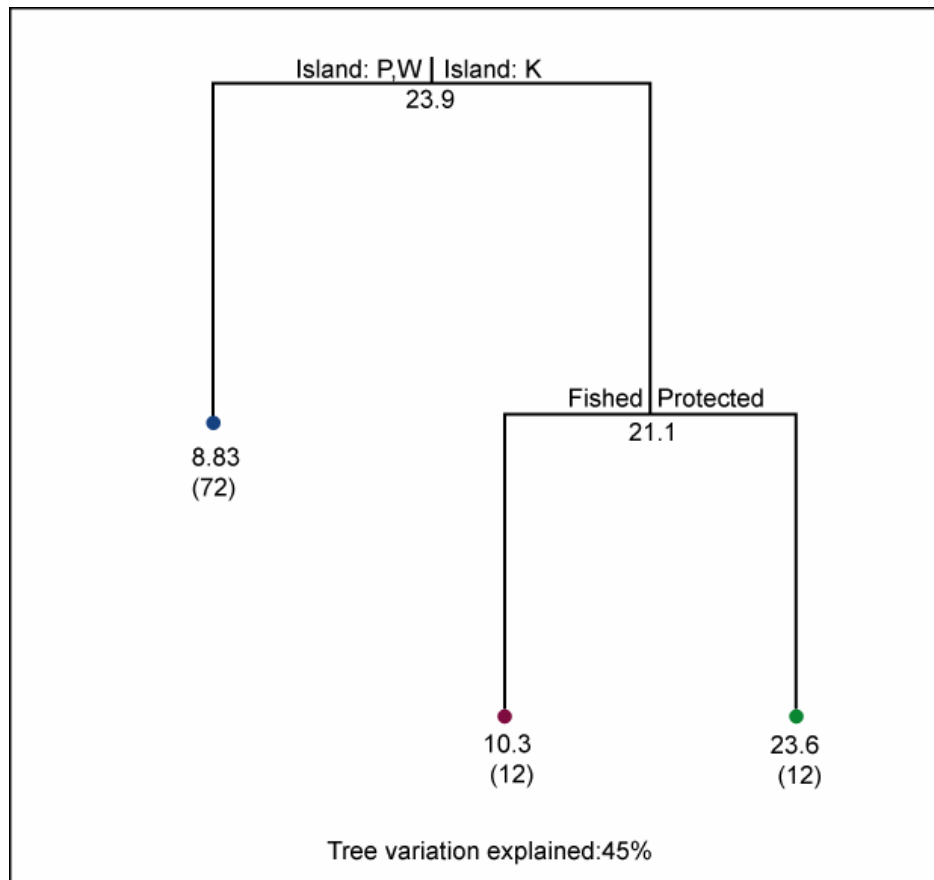
Figure 3.5: Univariate regression tree of 'Other Serranids' density in fished and no-take protected areas of the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and two resurveys in 2006 and 2007. Numbers below forks represent the percentage of the tree explained at that split. Figures below each dot are 'Other Serranids' density 1000m-2 at the location/time. Figures in brackets are number the sites.
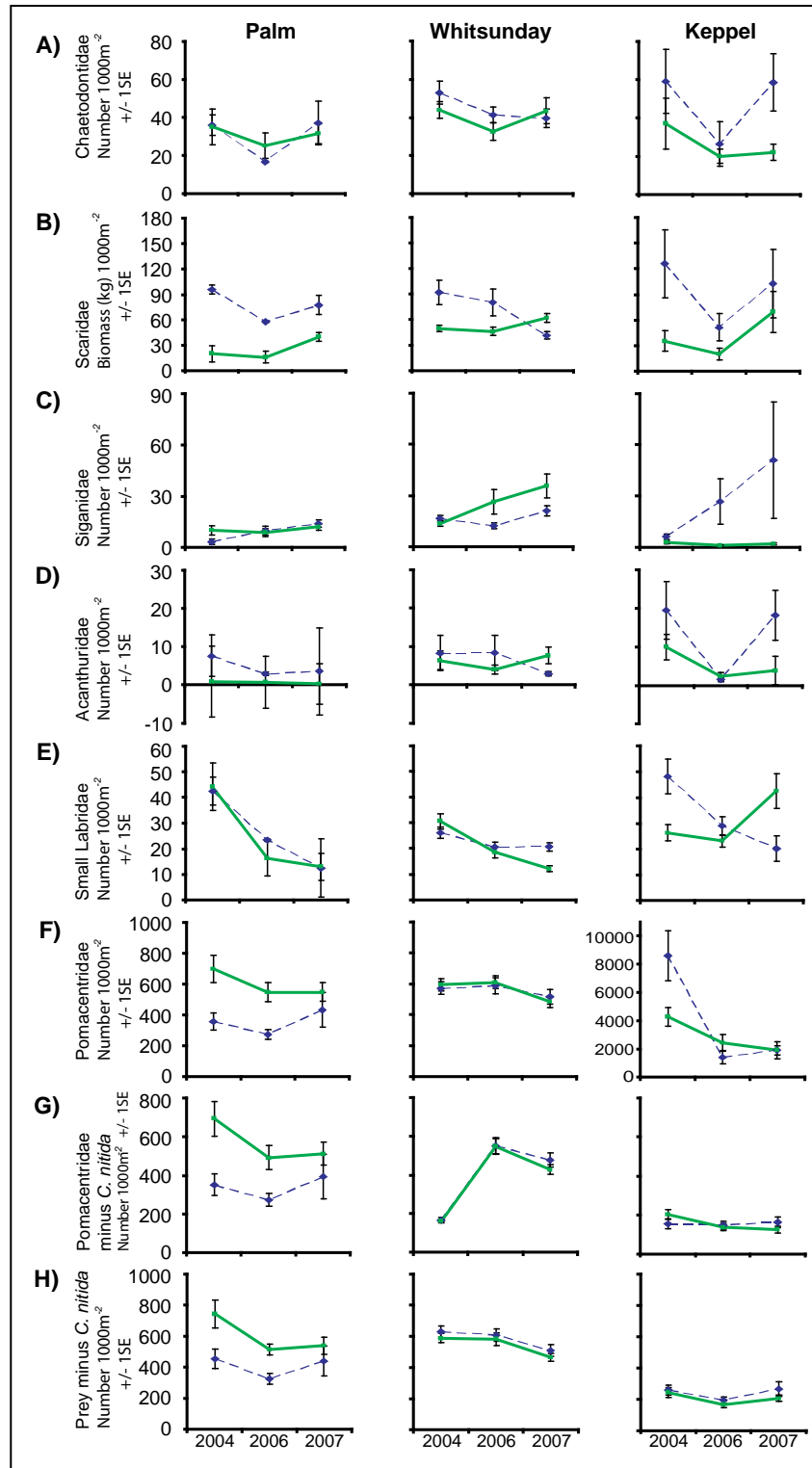
Figure 3.6: Density (+/- 1 SE) of non-target species in fished areas (dash line) and no-take protected areas (solid line) in the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and at two resurveys in 2006 and 2007. Note different y-axis scale for Pomacentridae in the Keppel region.
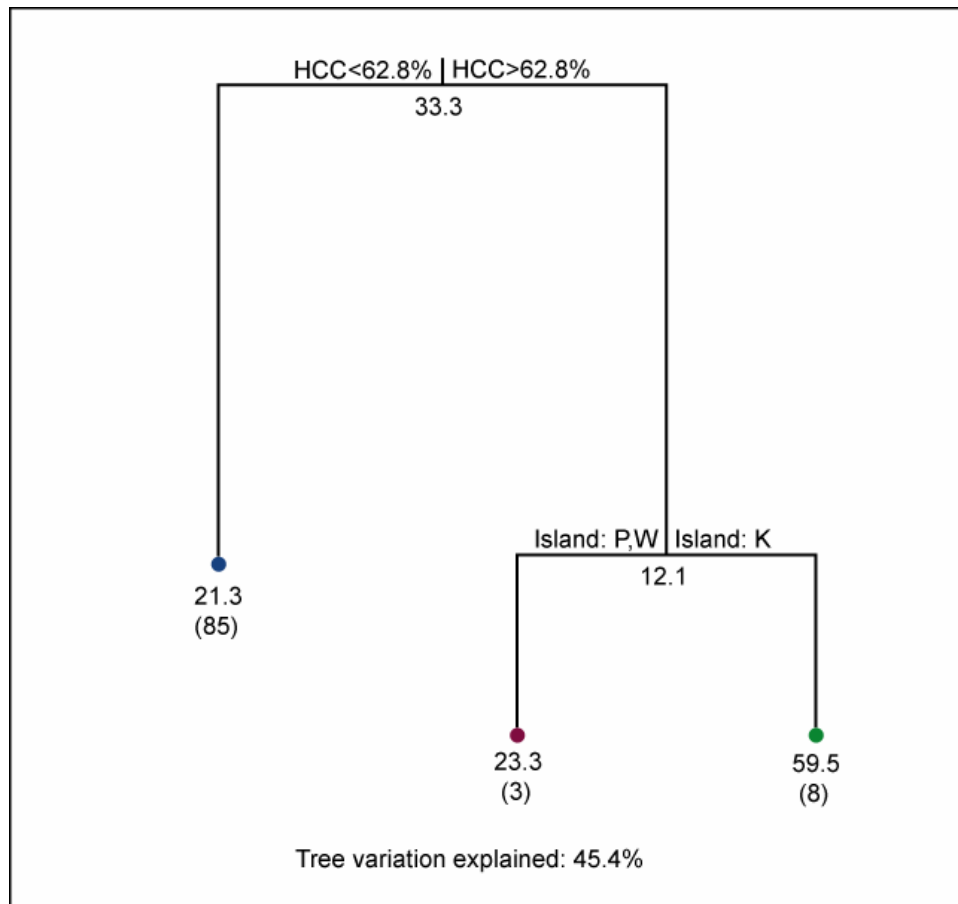
Figure 3.7: Univariate regression tree of Corallivores with Hard Coral Cover as an explanatory variable in fished and no-take protected areas of the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and at two resurveys in 2006 and 2007. Numbers below forks represent the percentage of the tree explained at that split. Figures below each dot are 'Corallivore' density 1000m-2 at the location/time. Figures in brackets are number of sites.
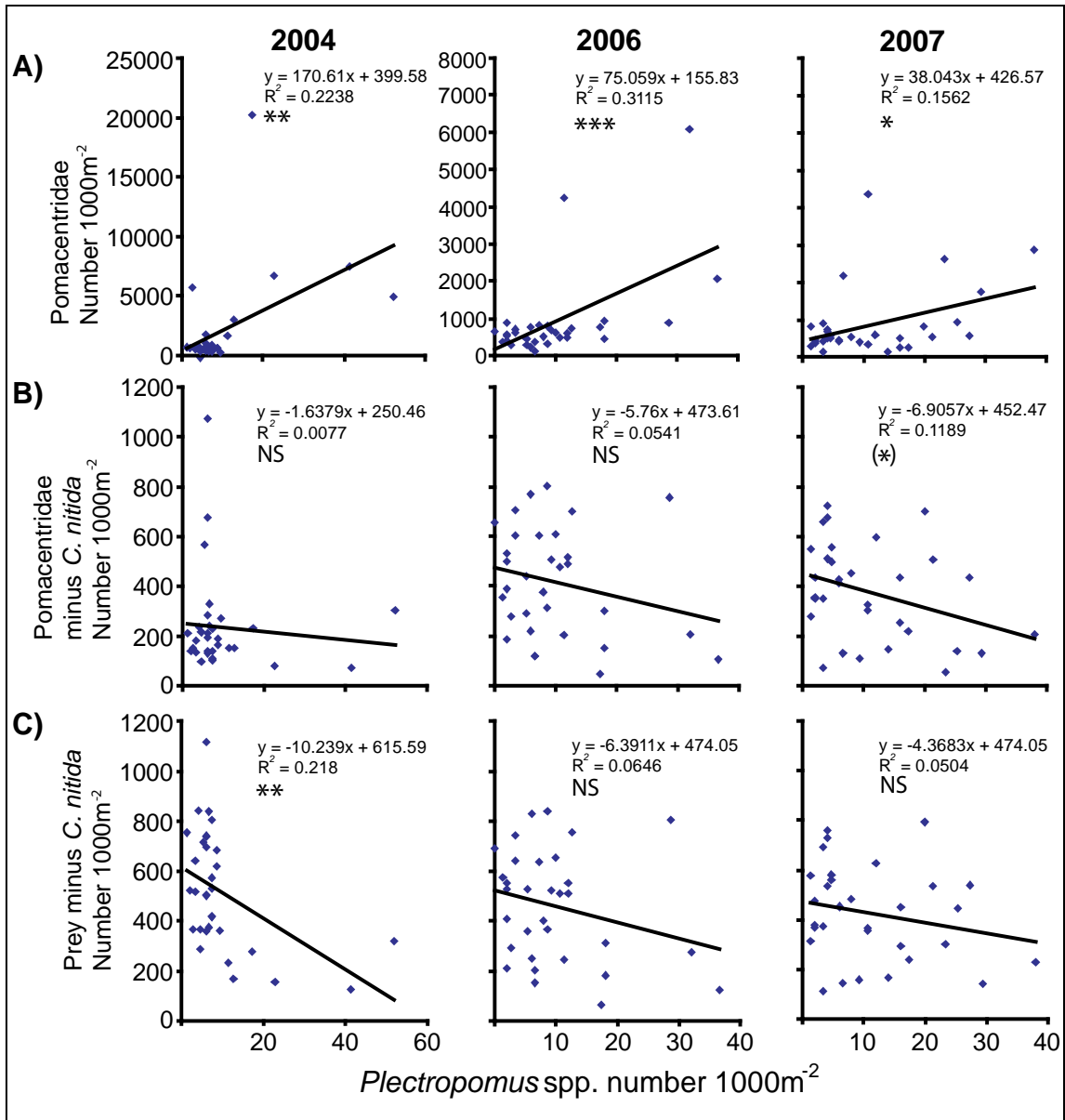
Figure 3.8: Regression plots of *Plectropomus* spp. density versus Pomacentridae density, Pomacentridae density minus *C. nitida* and Prey density minus *C. nitida* in the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and at two resurveys in 2006 and 2007. Note the different y-axis for Pomacentridae in 2004 compared to 2006 and 2007 in the top three plots. NS: not significant; (*): p =0.05; *: p<0.05; **: p<0.01; ***: p<001.
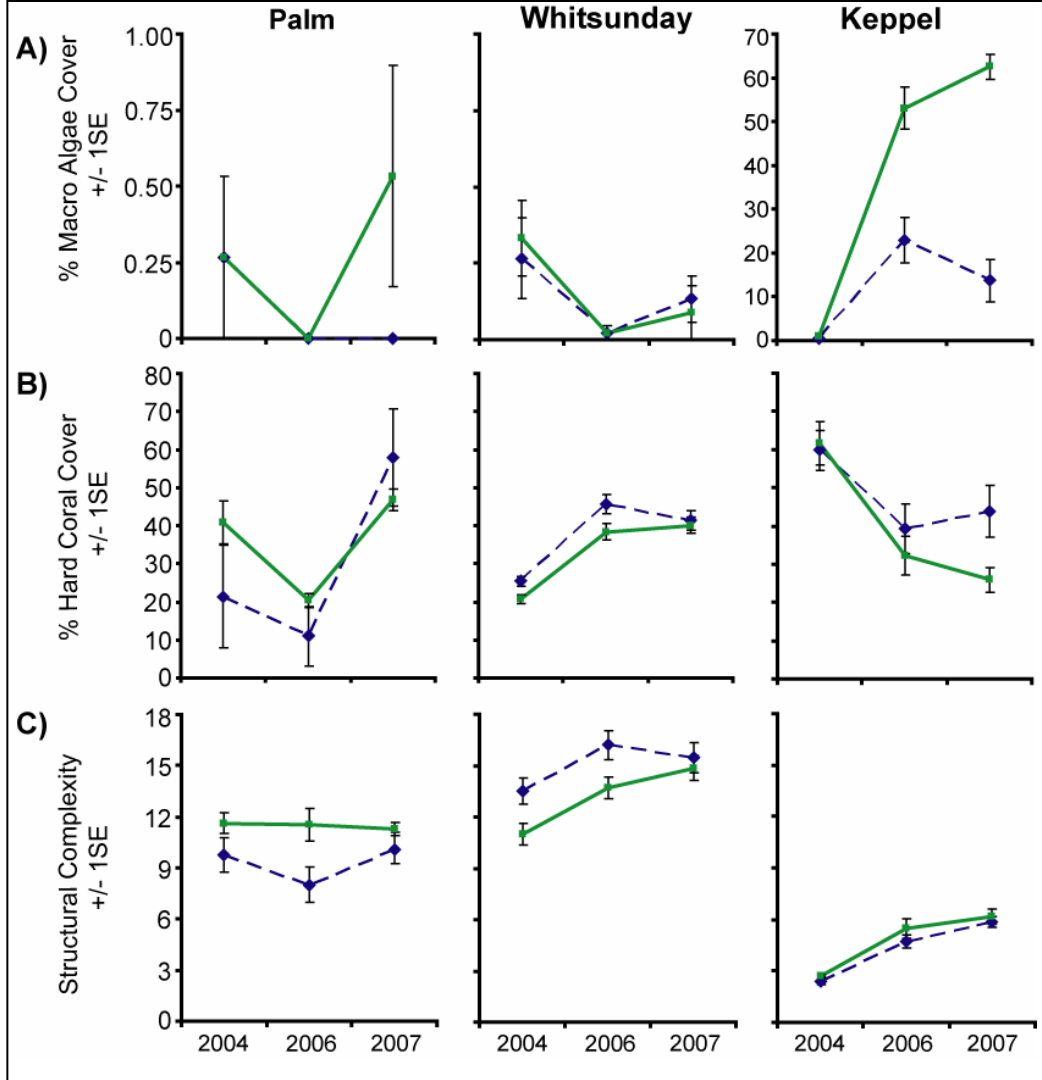
Figure 3.9: Benthic variables measured in fished areas (dash line) and no-take protected areas (solid line) of the Palm, Whitsunday and Keppel Islands from before the re-zoning of the GBRMP in 2004 and at two resurveys in 2006 and 2007. Note the different y-axis for Macro-algae in the Keppel region.

3.5 Discussion

The most important result from this study was the rapid increase of the species targeted by the hook and line fishery, *Plectropomus* spp., within the new MPAs. The results of this study form part of the data reported by Russ et al. (2008) documenting rapid increases in the density of *Plectropomus* spp. in the first two years of the rezoning of the GBR on both inshore and offshore reefs. Such rapid increases of target species, within 1-3 years, have been reported before (Halpern and Warner 2002; Halpern 2003), but never over such large spatial scales, over 700km (inshore) and 1000km (offshore) from north to south along the GBR.

Initial decreases of *Plectropomus* spp. in the fished areas in 2006 may be due to effort relocation in to these areas due to reduction of fishing area (Bohnsack 2000), especially in the Whitsunday Islands where very high numbers of tourists visit everyday. However recovery in two of the three regions suggests the initial spatial change in effort had minimal effect on fish abundances (Fig. 4A, B). This may also be related to the introduction of new fishery management rules implemented at the same time as the rezoning, which decreased recreational bag limits of *Plectropomus* spp. from 10 to 7 fish per fisher (QLD Reef Fish Management Plan 2003). This may have had an important role in dampening the transferred effort when the protected areas were increased from 4.5% to 33% of the GBRMP. Marine protected areas are an important tool for conserving a portion of spawning stock of target species, but for successful management of marine resources, they must be used in conjunction with sound fishery management practices (Bohnsack 2000; Hilborn et al. 2006).

There were many temporal changes for all the secondary target taxa in this study, but none were consistent with, or could be attributed to the rezoning. Of note, *Lutjanus carponotatus* demonstrated rapid increases in the protected areas and not the fished areas within two years of

the rezoning. This rapid increase is consistent with previous findings of higher densities and biomass of *L. carponotatus* in MPAs than in fished areas at these island groups prior to the 2004 rezoning (Evans and Russ 2004; Williamson et al. 2004; Evans et al. 2008). However, in 2007 their densities increased in the fished areas and remained stable in the MPAs (Fig. 2). These results are not easily interpreted and may be due to random movements and patchy distributions of this species. More time is required to determine if any effects of the rezoning are noticeable for secondary target species with patchy distributions.

3.5.1 Non-target species

Previous studies of these inshore GBR islands have found no significant differences between fished and protected areas for a small subset of non-target species: chaetodontids and two species of siganid (*Siganus doliatus* and *S. lineatus*) (Evans and Russ 2004; Williamson et al. 2004). This study is the first to investigate the effects of zoning on a large suite of species from several families. The results for all the non-target fish groups suggested little to no effect of the rezoning, which would be expected given the short time frame (3 years maximum). Similar conclusions were also reached in longer term studies of six (Samoilys et al. 2007) and ten years of protection (Russ and Alcala 1998a, b; Barrett et al. 2007). In contrast, weak trophic signals were reported in one-off spatial comparisons of MPAs and surrounding fished areas after 14 years of protection in the Palm and Whitsunday Islands, Great Barrier Reef (Graham et al. 2003), and after 18 years in the Exuma Cays, Caribbean (Mumby et al. 2006). The effects of protection on non-target species reported in the latter studies may or may not reflect the amount of time lapsed (14-18 years). These two studies are equivocal in nature in the sense that they were both spatial comparisons at one time, and lacked temporal data.

3.5.2 Benthos

The results reported here are similar to studies that have shown no effect of no-take zoning on the habitat of inshore GBR coral reefs (Evans and Russ 2004; Williamson et al. 2004). However, some changes to the habitat have occurred recently. These changes have had a substantial effect on the distribution and abundance of some fish families, far more influence than the implementation of no-take status. Pomacentridae demonstrated a statistical effect of zoning through time, which was driven by declining pomacentrid densities in the Keppels since 2004. This was largely due to the circumstantial differences of *Chromis nitida* densities between the sites prior to rezoning, and the effects of the coral bleaching event in 2006 in the Keppel Islands, which reduced coral cover and led to decreases in the densities of *C. nitida* over time. Therefore, there was no clear MPA effect on Pomacentridae and the population was not buffered from environmental stresses within the reserves. Whilst marine reserves are important for conserving species targeted by fisheries, it is evident that global climate change will have a greater and more spatially extensive impact on coral reef fish communities through reduction of coral cover and structural complexity of the benthos, and must be addressed immediately to ensure long term persistence of coral reefs (Jones et al. 2004; Graham et al. 2007; Munday et al. 2008; Pratchett et al. 2008).

The two coral bleaching events and a subsequent algal bloom, mostly an increase in abundance of the macro-alga *Lobophora variegata*, in the Keppel Islands in 2006, reduced the density of many of the fish families in this study, including the density of the primary target species, *Plectropomus* spp. However, several key fish groups (scarids, acanthurids, siganids and chaetodontids) recovered rapidly in the fished areas within one year but not in the MPAs. The speed of the declines and recovery of fish abundance observed in this study, suggests that rather than fish mortality, some of the fish moved to more suitable habitat following the coral

bleaching event, and then returned to (1) graze on the new macro-algae (herbivores); (2) after macro algae was reduced, returned to feed on new coral growth (Chaetodontids) or shelter (Pomacentrids); and (3) prey on the returning fish (*Plectropomus* spp.). It is acknowledged that the time frame for all of these to occur was remarkably brief (1 yr maximum). However, a concurrent study in the Keppels by Diaz-Pulida et al. (2009), shows corals in this region have rapid regeneration ability. If the fish groups did relocate to more suitable habitat during the coral bleaching and macro-algal bloom, then the subsequent movement of fish groups to rapidly regenerated corals is also plausible.

Continued macro-algal growth in the MPAs in the Keppel Islands (Fig. 9A), may be due to lower densities of herbivores before and after the rezoning. Of note, Scarids have increased in abundance in the MPAs, but the Acanthurids and Siganids have not, suggesting that Scarids may not be having an effect on the macro-algal dominance of the MPAs in the Keppel Islands. This result is consistent with other studies that have shown that Siganids are the primary remover of brown and red macro algae on inshore coral reefs of the GBR, while Scarids mostly graze the Epilithic Algal Matrix (EAM) and calcified macro algae such as *Halimeda* sp. (Mantyka and Bellwood 2007a; Mantyka and Bellwood 2007b; Bonaldo and Bellwood 2008; Fox and Bellwood 2008). Two possible explanations for why herbivore densities are low in MPAs, that may not be exclusive of each other, are: 1) the levels are natural caused by low levels of larval supply and poor recruitment; and/or 2) high baseline densities of the top predator, *Plectropomus* spp., in the sites earmarked for protection were limiting recruitment of herbivorous fishes to the protected reefs through predation on their juveniles. Therefore, ecology of the fishes, physical oceanography and variation in the benthos, some due to climate change, may have more influence on the fish community than the implementation of no-take marine protected areas.

3.5.3 Implied predator - prey interactions

Plectropomus spp. is the primary target of the hook and line fishery and is the most abundant large predator on inshore coral reefs of the GBR. Furthermore, this genus may influence the abundance of its prey noticeably on the inshore reefs of the GBR (Graham et al. 2003). This study suggests that the response of prey species to the abundance of a key predator, *Plectropomus* spp., depends on the identity of the prey group and time since the predator – prey manipulation was made (MPAs implementation). At the time of the baseline study (2004) all sites were open to fishing, and *Plectropomus* spp. abundance showed natural variation due to site differences and relative fishing pressure. Protecting half of the sites from fishing caused an increase in the density and biomass of *Plectropomus* spp. in the MPAs. This affected the nature of the relationship between *Plectropomus* spp. and their fish prey. Three prey groups were defined in this study, and each had a different baseline relationship with the predator, which led to different changes through time. It is important to note from the outset that the results herein provide evidence of changes that are very preliminary (3yr maximum protection), and more surveys through time are required to produce conclusive statements regarding the effect of MPAs on predator prey relationships.

Pomacentridae are the main prey item of *Plectropomus* spp. (Kingsford 1992; St John et al. 2001), due to their small body size and high natural abundance. Very abundant species (e.g. *Neopomacentrus azyzron*) tend to swamp the Family-level results and often have to be excluded to find any worthwhile family patterns (Graham et al. 2003). *Chromis nitida* formed large schools, but were included in 'Pomacentridae' in this study because the numbers of Pomacentrids (other than *C. nitida*) in the Keppel region were relatively low compared to the other two regions, and they may be a major food source for *Plectropomus* spp. in that region. This is supported by the significant positive relationship between Pomacentridae and

Plectropomus spp. in the baseline study in 2004. The positive regressions (Fig. 8A) suggest that predators tend to cluster where prey is abundant. The large decline of *C. nitida* after the coral bleaching in 2006 increased the coefficient of the slope and made the predator - prey relationship even more significant, but the coefficient of the slope decreased again in 2007. As the bleaching was a natural (or indirect anthropogenic) event, it will be informative to monitor the relationship changes within this group through time.

With *C. nitida* removed from the prey group, the 'Pomacentridae minus *C. nitida*' baseline predator – prey relationship was slightly negative, but not significantly so (Fig. 8B). However, there was an obvious pattern over time: when there were large densities of *Plectropomus* spp. there are low densities of Pomacentrids and vice versa (Fig 8B). During the course of this study, this relationship became more negative and within three years became significant. This suggests some weak secondary effect of the rezoning on the common Pomacentrids, a major prey item of *Plectropomus* spp.

In contrast to both of these prey groups, the 'Prey minus *C. nitida*' already had a significantly negative relationship with *Plectropomus* spp. density at the time of the baseline surveys (Fig. 8C), and changes to this relationship became less negative, and not significant, over the three years to 2007. This prey group includes Scarids, which attain large adult sizes (>25cm), and are not preyed upon by *Plectropomus* spp. above this size (Kingsford 1992; St John et al. 2001). Similar prey-size exclusions due to predator gape relationships were found in the Exuma Cays Land and Sea Park in the Caribbean (Mumby et al. 2006). Adult Scarids were included in this prey group because predation on their juveniles and initial phases should eventually affect the size of the adult population. If there is a measurable predator-prey relationship, as the adults die and the smaller sized individuals are preyed upon, the relationship should have a propensity to be more negative over time.

Changes to all three relationships in 2006 and 2007 are largely the result of *Plectropomus* spp. densities increasing in MPAs and not in the fished areas (Fig. 2B), due to the rezoning. Meanwhile, prey group changes remained similar in relation to MPAs and fished areas (Fig. 6F, G, H). Therefore, observed changes to prey species are likely due to a combination of 'typical' predation, environmental factors and natural variation, not increased predation as yet, despite the statistical changes in the predator – prey relationships observed herein. The interesting question is: When, if at all, will the prey groups be affected by the increases in the predator so that we begin to see a difference in prey densities between MPAs and fished areas? After 10 years of protection in Special Protection Areas in the Florida Keys National Marine Sanctuary, some observable effects of increased predator abundances may have influenced prey species abundances and sizes (Kramer and Heck Jr. 2007). Graham et al (2003) also showed that after 14 years of protection, *Plectropomus* spp. had a significant negative relationship with a similar selection of prey species (mostly pomacentrids) in the Palm and Whitsunday Islands. However, without a baseline or temporal study, neither of these studies could address the rate of development of the predator-prey relationship. Continued monitoring of predator-prey relationships in newly established MPAs will provide insight into how long it takes before the downstream effects on the wider fish community are noticeable.

Focusing on just the baseline results, the variation in fish abundances suggest contrasting driving forces of the fish community composition, which are difficult to interpret. In summary, positive predator – prey relationships (Fig. 8A) suggest the abundance of Pomacentridae may drive predator abundance. As noted in this study and others, changes in coral cover can have substantial effects on Pomacentridae abundances (Jones et al. 2004), suggesting that the benthic habitat processes is a major factor determining fish communities and subsequently abundances of large predators. Alternatively, the negative baseline predator-prey relationship of the specific

Plectropomus prey group, 'Prey minus *C. nitida*', suggests that abundances of large predatory fish, which are influenced by fishing pressure, can affect abundances of the prey fish community (Fig. 8C). If the prey species consist of key trophic groups, such as herbivores, then predation of these groups in an unstable system (that is a system in a state of nutrification and/or climate change) may lead to algal increases and coral declines (Bellwood et al. 2004). Whether it is a 'top-down' or 'bottom-up' process determining the fish community structure, the most important fact is the maintenance of healthy coral reefs. Without coral there can be no coral reefs or coral reef fisheries. Therefore, on the GBR with relatively low fishing pressure and an established network of MPAs, managers need to focus on maintaining and improving coral habitat. No-take marine protected area networks are important as fishery management tools to protect a portion of spawning stock biomass, but unless humans address the bigger issues of climate change and nutrification, then MPAs will not be enough.

3.5.4 Conclusion

This study of the first three years of RAP zoning on the GBR had four key results. 1) The major species of fish targeted by the hook and line fishery, *Plectropomus* spp., had developed greater density and significantly greater biomass in the newly protected than fished areas around two of three inshore islands spanning 700km. 2) No other fish group or benthic variable showed any direct response to the new zoning plan. 3) There was some indirect evidence that *Plectropomus* spp. density may influence the density of likely prey species, demonstrating potential secondary effects of marine protected areas on the broader fish community. More time is required to determine the importance and validity of these relationships. 4) Variations to the benthos had stronger impacts on the non-target fish community than did implementation of MPAs. Therefore the results of this study suggest that after three years the new MPAs are successful at protecting and increasing the biomass of primary target fishery species and have no short-term

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effect on the broader fish community. Stronger measures will be needed to counteract other larger issues, such as water quality and climate change, that impact the overall ecology of these coral reefs.

Chapter 4: Batch fecundity of *Lutjanus carponotatus* (Lutjanidae) and implications of no-take marine protected areas on the Great Barrier Reef, Australia

4.1 Introduction

Increasing size of human populations and frequent decreases in the size of stocks of numerous marine fishes have led many fishery scientists to consider the benefits of no-take marine protected areas (MPAs) as fisheries management tools (Pauly et al. 2002). Some fishery managers have begun to support the implementation of such MPAs (Russ and Zeller 2003; Sale et al. 2005; McClanahan et al. 2006; Mora et al. 2006; Russ et al. 2008). These MPAs are established for many reasons. The most notable are conservation of species, ecosystems and bioregions, and in a fishery context, protecting a portion of the spawning stocks of target fishery species (Roberts and Polunin 1993; Bohnsack 1998). The expected benefits of no-take marine reserves for target fishery species are decreased fishing mortality, increased density, increased average age and size, increased biomass and greater propagule production per unit area (Russ 2002). Numerous empirical studies have provided considerable information on the positive effects of reserves for the first four of these expectations (Russ 2002; Halpern 2003). However, comparisons of propagule production per unit area of target species between fished and MPAs are rare, and have never been estimated in the world's largest network of no-take marine reserves, Australia's Great Barrier Reef (GBR).

Demonstrating greater egg production per unit area of target species in MPAs compared to fished areas is an important prerequisite for reserves to eventually become net exporters of propagules, a major expectation for fishery enhancement. Several studies suggest that marine

reserves provide great benefits to the reproductive output of marine invertebrates. Abalone (*Haliotis kamschatkama*) had up to 20 times higher reproductive potential in three reserve sites compared to fished areas in British Colombia, Canada (Wallace 1999). In New Zealand, annual egg production of spiny lobster (*Jasus edwardsii*) increased, by 4.8% in shallow sites and 9.1% in the deep sites, in reserves that had been protected for up to 20 years compared to fished areas (Kelly et al. 2000). The reproductive products of Chilean gastropod (*Concholepas concholepas)* were up to 3 orders of magnitude higher in marine reserves than fished areas at Las Cruces, central Chile (Manriquez and Castilla 2001). Male Limpets (*Cymbula oculus*) in Dwesa marine reserve, South Africa produced 113 times more sperm and females produced 182 times more eggs than outside the reserve (Branch and Odenal 2003).

Such results are substantially greater than those quantified to date for most teleost fishes. The reproductive output of rockfish (*Sebastes* spp.) in no-take and fished areas was compared using length-specific fecundity to determine that two of three no-take marine reserves in the kelp forests in Central California (Hopkins and Pt. Lobos) had larger individuals of *Sebastes atrovirens* and *Sebastes chrysomelas* (Paddack and Estes 2000). Using length-specific fecundity relationships, Paddack and Estes (2000) demonstrated *S. atrovirens* and *S. chrysomelas* had greater batch fecundity in reserves than in nearby fished areas that lacked larger individuals (~2.8 times and ~4.5 times, respectively). Lingcod (*Ophiodon elongatus*) had from 3.1 to 4.5 times greater reproductive potential in three MPAs compared to three areas open to fishing in the San Juan Islands, USA (Eisenhardt 2001). In a recent attempt to compare the reproductive output of *Lutjanus fulviflamma* in fished and MPAs around Mafia Island, Tanzania (Kamukuru and Mgaya 2004) estimated batch fecundity in the protected area ranged from 45,200 – 430,200 oocytes per female for individuals between 207 and 293 mm (total length [TL]). However, they were unable to compare batch fecundities between protected and fished areas because they could not find any females in a breeding condition in the fished areas. Length fecundity relationships

were used to show that daily batch fecundity of the temperate snapper *Pagrus auratus* was 11 –
18 times higher in the Poor Knights no-take reserve than at nearby reference locations after 4
years of full protection in New Zealand (Denny et al. 2004).

The size-fecundity relationship in teleost fishes is generally represented by a power function
($y=ax^b$), and the exponent (*b*) can be as high as 5 (Jennings et al. 2001). Therefore, fecundity
increases rapidly with length. Using Grimes (1987) estimates of the largest and smallest batch
fecundity from a population of *Lutjanus campechanus*, Plan Development Team (1990)
suggested that one 60.5cm fork length (FL) *L. campechanus* could produce the same number of
eggs as 212 42cm FL individuals. Based on this observation, a protected population with many
larger individuals should have a greater reproductive output per unit area than a fished
population with proportionally more, smaller individuals.

Few studies directly demonstrate substantially higher fish egg production per unit area in MPAs.
There are at least four reasons for this: 1) few people measure egg production, often assuming
that the egg production rises sharply with fish size; 2) estimating true egg production from a no-
take reserve would require removal of individuals from that protected area, imposing negative
implications for that no-take area; 3) estimating total egg production of a serial pelagic spawner
is very difficult because a) estimating batch fecundity requires capture of samples at time of
spawning; b) spawning frequency is difficult to estimate; c) not all mature females reproduce
every year; and d) social interactions can affect the number of spawns and eggs produced within
a species (See review Sadovy 2001); and 4) the number of MPAs effectively protected for long
periods of time (e.g., decades) remains limited.

On the inshore coral reefs of the Great Barrier Reef (GBR) the stripey sea perch (*Lutjanus
carponotatus*) is a secondary target of the commercial fishery, but is commonly caught by

recreational fishers. It has significantly greater biomass in the MPAs than in fished areas on the inshore coral reefs of the GBR (Evans and Russ 2004; Williamson et al. 2004). On the GBR, *L. carponotatus* is a gonochoristic serial spawner, with a peak spawning period from October to December, with some larger individuals probably spawning over a longer period (Kritzer 2004). This study aimed to estimate batch fecundity of *L. carponotatus* and compare estimates of batch fecundity per unit area (BFUA) for fished and protected populations on the inshore reefs of the GBR after 14 years of fisheries protection. Batch fecundity per unit area was estimated assuming that all mature fish per $1,000m^2$ would spawn once. In the absence of information about annual spawning frequency, this study only estimated the BFUA of *L. carponotatus*, and did not extrapolate batch fecundity estimates to total egg production.

4.2 Materials and methods

4.2.1 Brief History of Zoning

The Great Barrier Reef Marine Park (GBRMP) in Australia was established in 1975, and Marine Park zoning was first formally implemented by the GBRMP Authority in 1981 in the Capricornia (southern) section of the park. No-take protection of reefs in this study was implemented by 1987 (Williams and Russ 1994). Thus at the time of the field surveys (see below) these reefs had been protected for 14-17 years. The multiple-use zoning plan for the entire GBRMP changed on 1 July 2004. This re-zoning entailed an increase of no-take marine protected areas from 4.5% of the marine park to 33.4%. In terms of the actual number of coral reefs, the protection increased from approximately 21% to 30% of nearly 3000 individual coral reefs in the GBRMP.
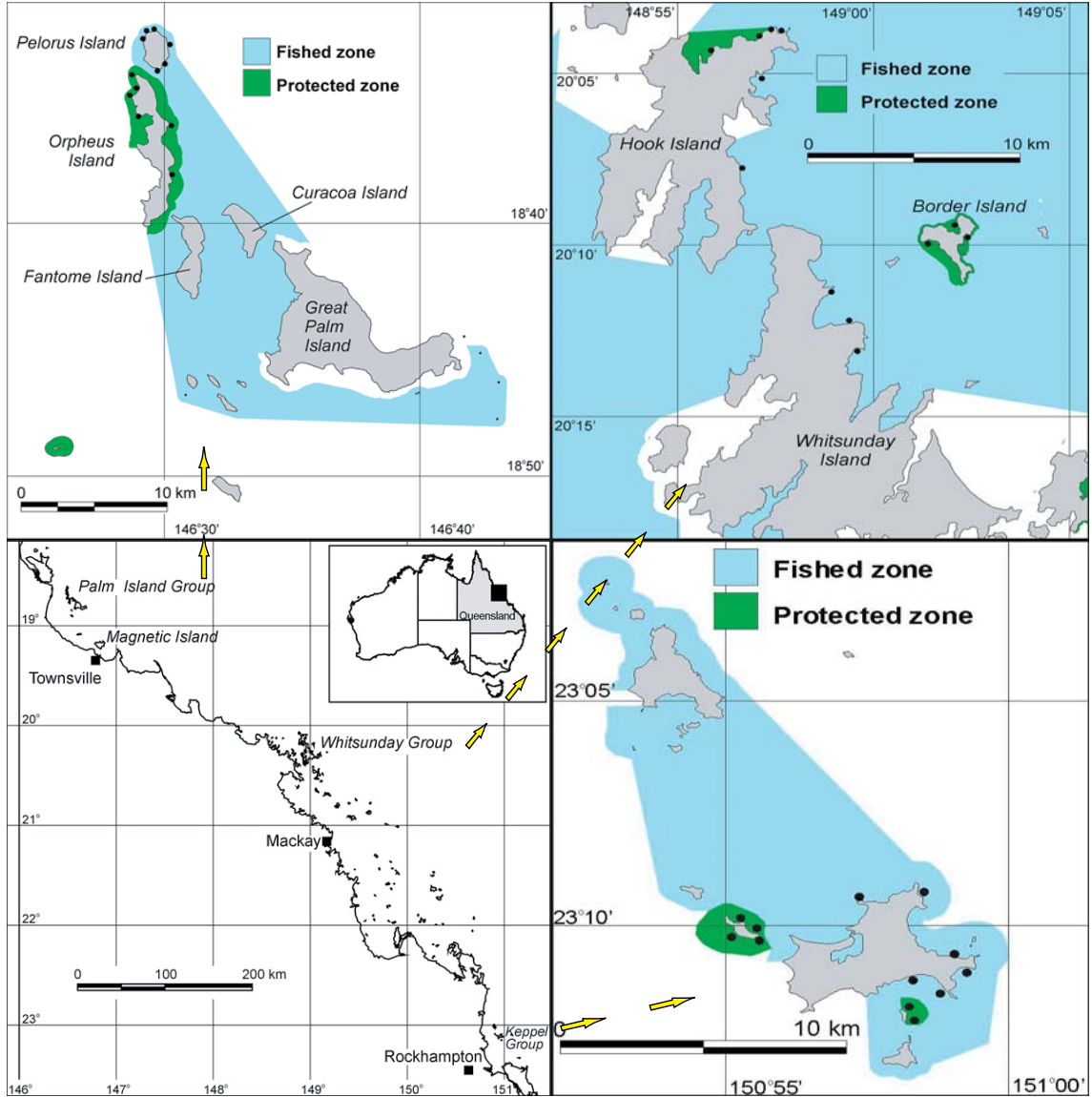
Figure 4.1: Map of the Queensland coast and the three island groups: Palm, Whitsunday and Keppel Islands. Green/ Protected Zone = No-take marine protected areas; Blue/ Fished zone = fished area. Black dots indicate sampling sites.

4.2.2 Surveys

Underwater visual census (UVC) was conducted at the Palm Islands during March-April from 2001 to 2003, the Whitsunday Islands during November- December from 2001 to 2003, and in the Keppel Islands during October in 2002 and May in 2004 (Fig. 1). The Keppel Island group was not surveyed in 2001 and 2003 due to bad weather. Fish and benthic data were collected at all three island groups in 2002. Thus, this was the only year for which formal (statistical) spatial comparisons of *L. carponotatus* populations from all three island groups were made. This study was part of a larger long-term project surveying 12 sites each in fished and reserves at all island groups. However, only 6 sites each were surveyed in fished and reserve areas in the Keppel Islands in 2002. Therefore, to balance the data sets, 6 sites each were randomly removed from the 12 fished and reserve sites in the Palm and Whitsunday Islands. The reef flats at all three island groups are exposed at lowest astronomical tide, and the reef slope, which ranges from gentle to vertical walls, has high structural complexity. The bottom of the reef slope varied in depth from 5m to 20m. Data on the abundance of fish and benthic organisms were collected by underwater visual census (UVC) along the reef slope at a depth of 4-9m.

The abundance and individual size of *L. carponotatus* were estimated at each site on 5 replicate transects measuring 50m by 6m (300m²). Fish were assigned to 5cm size classes (i.e., 0-5, 5.1-10, 10.1-15cm Fork Length (FL) etc., up to 35.1 – 40cm). For ease of presentation, size classes are presented as the largest length in each size class (5, 10, 15, 20cm). Target species biomass did not correlate with measured habitat variables (Evans and Russ 2004), so habitat variables were not included in the present study. A spatial and temporal comparison of the biomass of *L. carponotatus* in the no-take and fished areas is presented to illustrate effects of zoning on the target species in this study.

4.2.3 Batch fecundity

Lutjanus carponotatus samples were collected from Pelorus Island (Palm Islands) by divers using scuba and spear guns in the last quarter moon phase of October 1997 and from the first quarter to the full moon in October 2001. To date, no unequivocal evidence has shown that *L. carponotatus* spawn at any particular time of the month during the peak spawning season. Preliminary evidence suggests that fish sampled over the new moon at Lizard Island had larger gonads than fish captured in the last quarter at Pelorus Island (Kritzer 2004). However, this may have been a regional difference, as it was also noted that samples collected in the same study at Lizard Island had higher proportions in the larger size classes (Kritzer 2002).

The individual ripe females were divided into 4 size classes: 15.1 -20cm FL (n=13), 20.1 – 25cm FL (n=26), 25.1 – 30cm FL (n=15), and 30.1 - 35cm FL (n=2). Gonads were weighed (g wet weight) immediately after removal and placed in the gonad fixative FAACC (Formaldehyde, Acetic Acid, Calcium Chloride). The sex of *L. carponotatus* cannot be determined by external features underwater so all individuals above 16cm (approximate size of first maturity determined by Kritzer (2004)) were targeted for collection. This random sampling of mature individuals (>15cm) provided the necessary estimate of female to male ratio per size class required to estimate batch fecundity per unit area (BFUA) of the females in the population.

L. carponotatus gonads can be sexed and staged macroscopically, except for determination between ripe (stage IV) and running ripe (stage V) gonads (Kritzer 2004). Stage IV and V gonads were sectioned at 5 microns, stained with haematoxylin and eosin and histologically examined to determine the exact stage of development of the gonad.

Histological staging was based on Ganias et al. (2004) to ensure the individuals with the correct yolk globule stages were chosen for batch fecundity estimates. Ganias et al. (2004) found that the oocyte spawning batch of *Sardina pilchardus sardina* begins to separate in size from the smaller oocytes at the secondary yolk globule stage, and a well-developed size-difference occurs at the tertiary yolk-globule stage. This size difference at the secondary yolk globule stage was observed in *L. carponotatus* gonads (Fig. 2). To allow for cutting artefacts, multiple measurements were made of oocytes on all the histological sections (n = 56) to determine which size oocytes to include in the batch fecundity counts. The maximum size of primary yolk globule stage oocytes (which had a nucleus) was less than 0.30mm diameter. Therefore, all oocytes greater than 0.30mm diameter were in the secondary yolk globule stage and were included in the study. Macroscopically, these oocytes had a very distinct colour difference from less developed oocytes. The oocytes more mature than and including secondary yolk globule stages (SY/TY and HO) (Fig. 2b) were dark yellow compared with the whitish/pale yellow of the inactive oocytes (YV and PO) (Fig. 2b).

Few females had hydrated oocytes, and approximately 40% had tertiary yolk development. To determine if batch fecundity estimates could be determined using secondary yolk stage gonads the batch fecundity of 3 to 23 randomly selected females with secondary and tertiary yolk staged gonads was estimated and compared in an ANOVA. Batch fecundity estimates for females, with secondary or tertiary yolk development, in the three size classes analysed had no significant difference. No analysis was performed on the fourth size class (greater than 30cm) as both samples had secondary yolk development. Therefore, all samples at the secondary yolk-globule stage and above were included in the study (n=56 fish).

Batch fecundity was determined using gravimetric techniques (Hunter et al. 1985). Batch fecundity estimates have not previously been made for *L. carponotatus*. Thus, the amount of

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gonad tissue examined and where in the gonad to remove the tissue, needed to be determined. To account for the gonad wall during weighing of the entire gonad, a small tissue sample from the gonad wall was included in sections of two sizes (0.005, 0.010g). These were removed from the anterior, median and posterior regions of both lobes of three gonads in the 20, 25 and 30cm FL size classes and from only two gonads of the 35cm size class (n=2). There was a significant difference in batch fecundity among size classes ($F_{3,\,84}$ = 146; $p<0.0001$). However, there was no significant difference in batch fecundity based on the size of section, the location of the lobe, and between gonad lobes. For consistency, estimates were only taken from the left lobe sections weighing 0.005g. Once the oocytes were separated from the surrounding tissue, they were photographed using a photomicroscope. The resulting images were loaded into the software package Image Tool to count and measure all of the oocytes in the sample. The batch fecundity per individual per size class, the average number of eggs per gram of gonad, and the egg diameter per size class were determined.

4.2.4 Batch fecundity per unit area

The population was divided into 4 size classes: ≤ 20cm (FL) (n=13); 20.1 –25cm (FL) (n=26); 25.1 – 30cm (FL) (n=15); and >30cm (FL) (n=2). The overall female to male sex ratios of the samples collected in 2001 from the Palm Islands was 1.06:1. On the GBR, Kritzer (2004) observed the same female to male ratios at Lizard Island, but recorded a female biased ratio of 1.3:1 in the Palm Islands from 1997 to 1998. Kritzer (2004) concluded that the results for the Palms in 1997-98 were not representative as they did not conform to what would be expected for a gonochore lacking complex mating interactions such as defence of females or territories. The sex ratios (female: male) in the four size classes were: ≤ 20cm (FL) (1:1.4), 20.1 –25cm (FL) (1.4:1), 25.1 – 30cm (FL) (1.2:1), and >30cm (FL) (1:1). The batch fecundity/ individual/

size class was multiplied by the size specific sex ratios and density data collected between 2001 and 2004 in the Palm, Whitsunday and Keppel Islands.

4.2.5 Assumptions

A number of assumptions were made to generate the results. These assumptions include: (1) the sex ratios of *L. carponotatus* at Pelorus Island (Palm Islands) were the same as those at Orpheus Island (no-take protected area in the Palm Islands), whereas sex ratios of 1:1 were used in the Whitsunday and Keppel Island Groups, as suggested by Kritzer (2004); (2) all individuals above minimum reproductive size spawn (in the absence of any estimate of annual spawning frequencies for *L. carponotatus* we do not extrapolate beyond the BFUA); (3) all of the secondary yolk-globule oocytes were spawned.

4.2.6 Analysis

Due to the different sampling years for the Keppel Islands (compared to the Palm and Whitsunday Islands), the temporal data were analysed in two sets, the Palms and Whitsunday Islands (2001-2003) and the Keppel Islands individually (2002 and 2004). The biomass data contained many zero estimates at the transect level, and often did not conform to the assumptions of ANOVA. Thus, all data were pooled to site level (five transects per site). Since the focus of this study was on variation between fished and MPAs and between island groups, rather than between or within sites, pooling did not affect the comparisons of major interest. Thus, the spatial data were analysed with a two-factor orthogonal design ANOVA, using two zones (no-take and fished), three island groups (Palm, Whitsunday and Keppel Island groups), and six nested sites as replicates within each combination of zone and island group.

To meet the assumptions of ANOVA in the spatial comparison, the *L. carponotatus* biomass data had to be square root transformed. The temporal Whitsunday and Palm Island data were log10(x) transformed to meet the assumptions of repeated measures ANOVA (normal distribution, homogeneity of variance, sphericity). Comparisons between batch fecundity per size class (square root transformed to meet Levene's test of homogeneity of variance) and egg diameter per size class were analysed using a fixed factor one-way ANOVA using four fish size classes and either batch fecundity or egg diameter as the replicates.

BFUA was analysed in a two-factor orthogonal ANOVA using the data from all three island groups in 2002. In addition, BFUA was subjected to a repeated measures ANOVA across time. The analyses were divided into two groups to allow for the different sampling times in the Keppel Islands, as indicated above. The spatial data (2002) for *L. carponotatus* was log10(x) transformed. The temporal Palm and Whitsunday Island data for *L. carponotatus* were log10(x) transformed to pass the homogeneity of variance assumption of ANOVA.

4.5 Results

4.5.1 Biomass per unit area

In all three island groups combined in 2002, the overall mean biomass per unit area of *L. carponotatus* in the reserves (4.9kg 1,000m^{-2}) was approximately 2.3 times greater than that in fished areas (2.2kg 1,000m^{-2}) (Fig. 3a). Although there was some variability in biomass per unit area within each zone at each island group among years (Fig. 3b), there was no significant effect of year for the combined Whitsunday and Palm group analysis or the Keppel Island group analysis (Table 1). The only significant factor in any analysis was zone (Table 1).

Table 4.1: Results of ANOVA and Repeated Measure ANOVAs of biomass/ unit area for *Lutjanus carponotatus* in the Palm, Whitsunday and Keppel Island Groups between 2001 and 2004. W&P = Whitsunday & Palm Islands; KI = Keppel Islands; ***P<0.001; **P<0.01; *P<0.05; NS: not significant; df: degrees of freedom.

Source of Variation	Year*zone *Island (df)	Year * Island (df)	Year * Zone (df)	Year (df)	Zone* Island (df)	Island (df)	Zone (df)
L. carponotatus 2002	-	-	-	-	2.923 (1.30) NS	0.107 (1,30) NS	12.258 (1,30) ***
L. carponotatus W&P	0.86 (2,40) NS	1.72 (2,40) NS	0.14 (2,40) NS	2.93 (2,40) NS	0.21 (1,20) NS	0.15 (1,20) NS	4.62 (1,20) *
L. carponotatus KI	-	-	1.627 (1,10) NS	0.450 (1,10) NS	-	-	8.16 (1,10) *

4.5.2 Batch fecundity

Batch fecundity of *L. carponotatus* increased with fork length, best described by a power function:

$$F = 0.0054 \times FL^{5.28} \ (r^2 = 0.64),$$

where F is batch fecundity and FL is fork length (cm). For statistical analysis of the regression, the raw data was transformed to logs ($F_{1, 56} = 110.8$; $p < 0.001$), but the raw data was plotted to demonstrate the length: fecundity relationship. The large exponent in this relationship may be influenced substantially by the two largest fish in the study (Fig. 4a). However, if these two data points are removed, the exponent was still greater than 5.

The highest individual batch fecundity recorded was 748,957 eggs (FL = 305mm) and the lowest was 7,074 (FL = 184mm). This represents a more than one hundredfold difference (Fig. 4a). The average batch fecundity per individual in each size class ranged from 33,621 eggs in the 20cm size class up to 698,394 eggs in the 35cm size class (Fig. 4b). Thus, fish in the largest

size class produced on average 20 times more eggs than fish in the smallest size class. Batch fecundity significantly increased with size classes ($F_{3, 52} = 32.9$; p <0.001) (Fig. 4b). The 35cm size class had significantly greater batch fecundity than all other size classes (Tukeys HSD p<0.0001 in all cases), while the 20cm size class had significantly less batch fecundity than the three larger size classes (Tukeys HSD p=0.002 and p<0.001). Batch fecundity did not statistically differ between the two middle size classes. For statistical analysis of the regression, the raw data was logged ($F_{1, 2} = 48.3$; p = 0.02), but the raw data was plotted to demonstrate the size class: fecundity relationship.

There was no overall relationship for egg diameter (Fig. 4c), however, the largest size class (35cm) had significantly greater egg diameter than the 25cm size class (Tukeys HSD, p = 0.03) (Fig. 4c). The other size classes recorded no statistical differences between them.

4.5.3 Batch fecundity per unit area

In all three island groups combined in 2002, the overall mean batch fecundity per unit area BFUA of *L. carponotatus* in the reserves (1,077,130 eggs 1,000m^{-2}) was approximately 2.5 times greater than that in fished areas (429,379 eggs 1,000m^{-2}) (Fig. 5a).   The inter-annual variability in BFUA within each zone at each island group among years was comparable to the variability in biomass (Fig. 3b, 5b). There was no significant effect of year for the combined Whitsunday and Palm group analysis or the Keppel group analysis (Table 2).   The only significant factor in any analysis was zone (Table 2).

Table 4.2: The no-take marine reserves/ fished areas ratios biomass and batch fecundity/ unit area (BFUA) in the Palm and Whitsunday Islands in 2001, 2002, 2003 and Keppel Island in 2002 & 2004.

	Palms			Whitsundays			Keppels	
	2001	**2002**	**2003**	**2001**	**2002**	**2003**	**2002**	**2004**
L. carponotatus biomass	1.0	1.4	1.2	2.4	1.6	1.6	5.4	1.4
L. carponotatus BFUA	1.1	2.1	1.0	2.8	1.6	1.7	4.2	1.8

4.5.4 Comparing biomass per unit area to BFUA

An average 2.3-fold difference in biomass between MPAs and fished areas converted to an average 2.5-fold difference in batch fecundity per unit area. Therefore, the difference in batch fecundity per unit area between zones was 9% greater than the difference estimated for biomass per unit area. However, this result is surprisingly small considering the difference between the exponents for the length: body weight conversions ($b\sim3$) and the length: fecundity relationships ($b\sim5$). Three of the eight surveys conducted in the three Island groups produced estimates of BFUA that were equal to or less than biomass per unit area differences when comparing no-take to fished areas (Table 3). The minimal increase from biomass per unit area to BFUA is put into perspective by examining the relative contributions of the different size classes in the no-take and fished areas. The 25cm size class in the fished areas, with relatively high abundance and medium size, produced the highest proportional contribution (45%) to the BFUA in the fished areas (193,000 eggs $1,000m^{-2}$ from the total 429,000 eggs $1,000m^{-2}$) (Fig. 6). However, the least abundant size class (35cm) produced the highest proportional contribution (47%) to the BFUA in the MPAs (504,000 eggs $1,000m^{-2}$ from the total 1,077,000 eggs $1,000m^{-2}$) (Fig. 6). In fact, the BFUA of the relatively few 35cm size class individuals in the MPAs (504,000 eggs $1,000m^{-2}$) was more than the total mean estimate of all size classes in the fished areas (429,000 eggs $1,000m^{-2}$) (Fig. 6).

Table 4.3: Results of the ANOVA and Repeated measure ANOVAs of the batch fecundity/ unit area of *Lutjanus carponotatus* in the Palm, Whitsunday and Keppel Island Groups. W&P = Whitsunday & Palm Islands; KI = Keppel Islands; ***P<0.001; **P<0.01; *P<0.05; NS: not significant; df: degrees of freedom.

Source of Variation	Year*zone *Island (df)	Year* Island (df)	Year* Zone (df)	Year (df)	Zone* Island (df)	Island (df)	Zone (df)
L .carponotatus 2002	-	-	-	-	2.44 (2,30) NS	0.20 (2,30) NS	13.32 (1,30) ***
L. carponotatus W&P	0.97 (2,40) NS	0.66 (2,40) NS	0.36 (2,40) NS	1.95 (2,40) NS	0.16 (1,20) NS	0.01 (1,20) NS	7.87 (1,20) *
L. carponotatus KI	-	-	0.86 (1,10) NS	0.08 (1,10) NS	-	-	8.33 (1,10) *
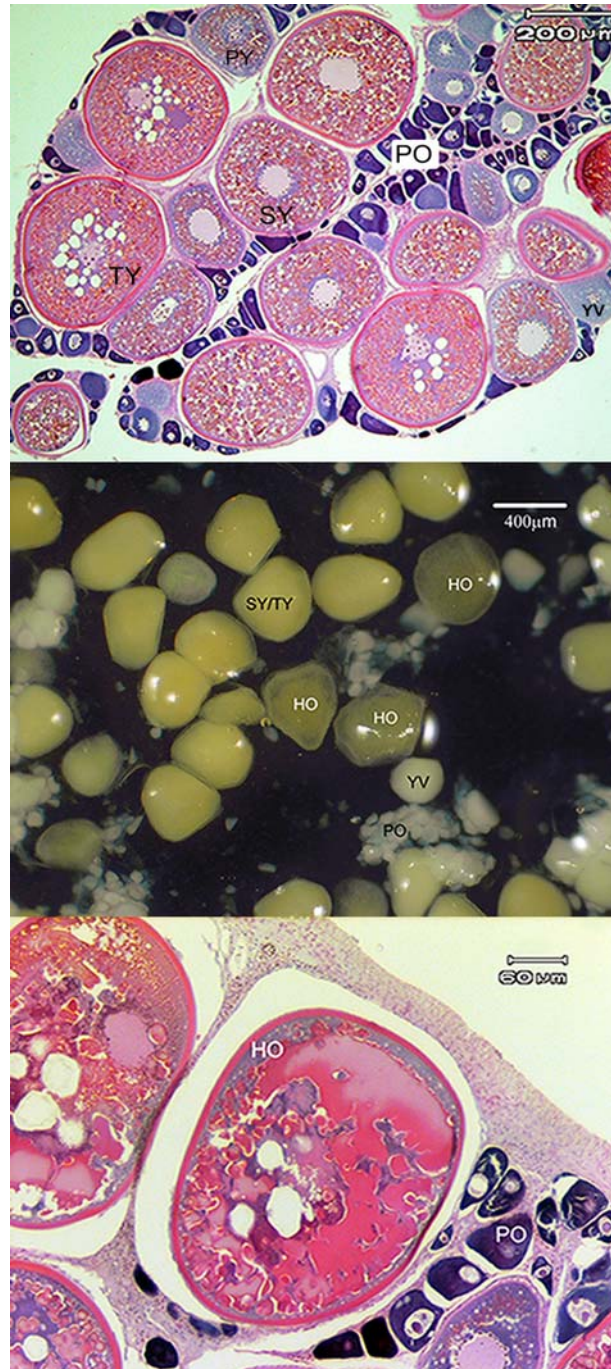
Figure 4.2: Photographs of the oocytes of *Lutjanus carponotatus*. a) Histological photograph (4x) of the tertiary yolk globule stage indicating the group-synchronous pattern of oocyte development. b) Macroscopic photo of Hydrated Oocyte stage and c) Histological photo (10X) of hydrated Oocyte stage. YV= Yolk Vesicle stage; PY = Primary Yolk globule stage; SY= Secondary yolk globule stage; TY= Tertiary Yolk Globule Stage; PO= Primary oocytes.
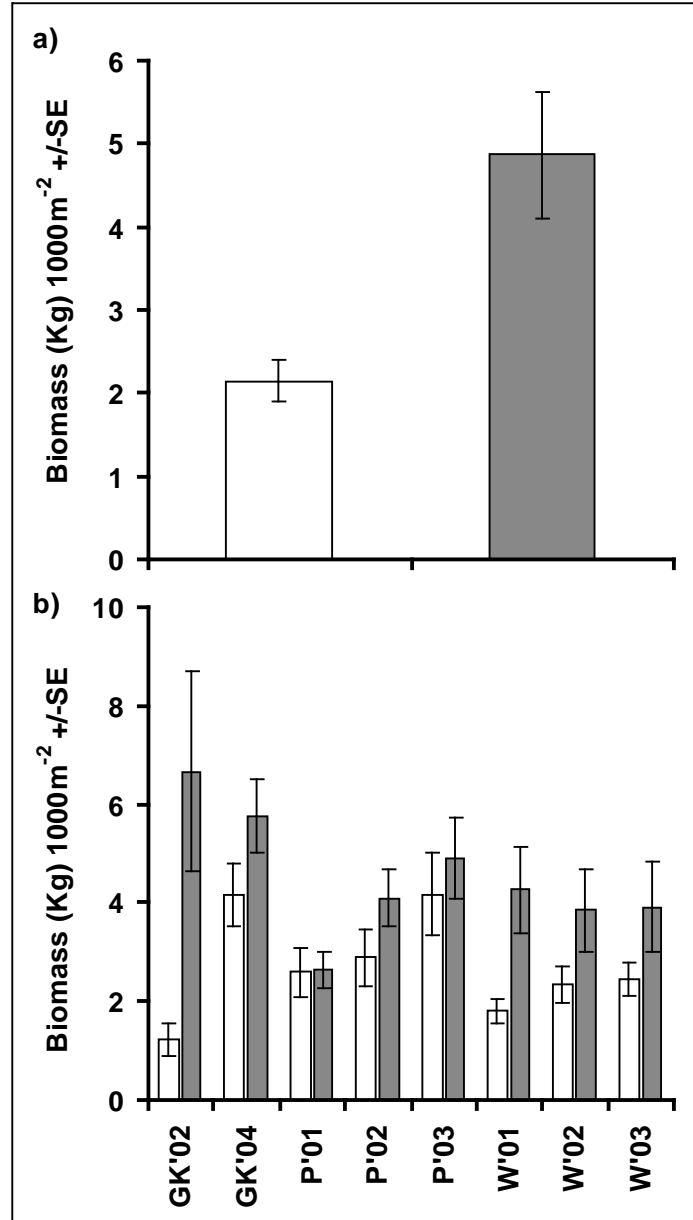
Figure 4.3: a) Spatial and b) Temporal comparisons of *Lutjanus carponotatus* biomass in no-take and fished areas in the Palm, Whitsunday and Keppel Islands from 2001 – 2004. GK = Great Keppel Islands, P = Palm Islands, W = Whitsunday Islands, White bars = Fished, Grey bars = Protected. The year is designated by '01, etc.
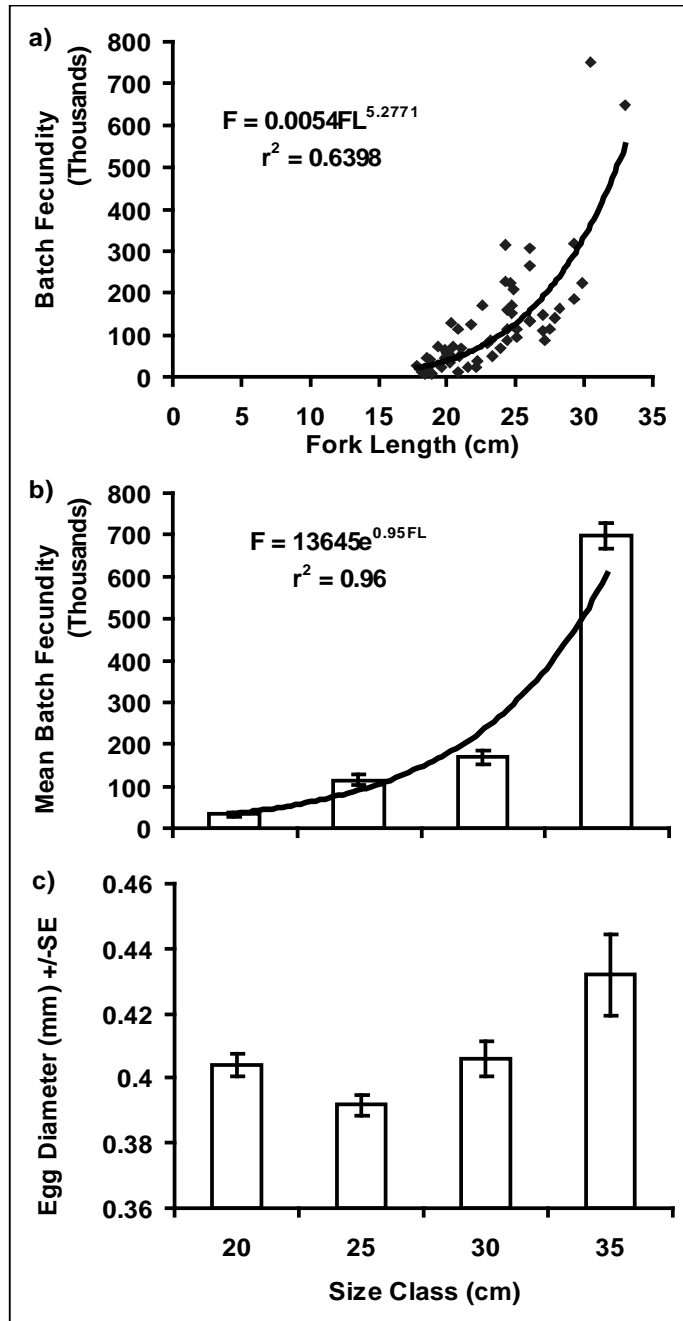
Figure 4.4: Batch fecundity data from *Lutjanus carponotatus*. a)
Fork Length vs batch fecundity; b) Average batch fecundity/
individual/ size class with a power curve fitted, and c) Average egg
diameter/ size class. ED = Egg diameter, F = Fecundity, FL = Fork
Length, SC = Size Class.
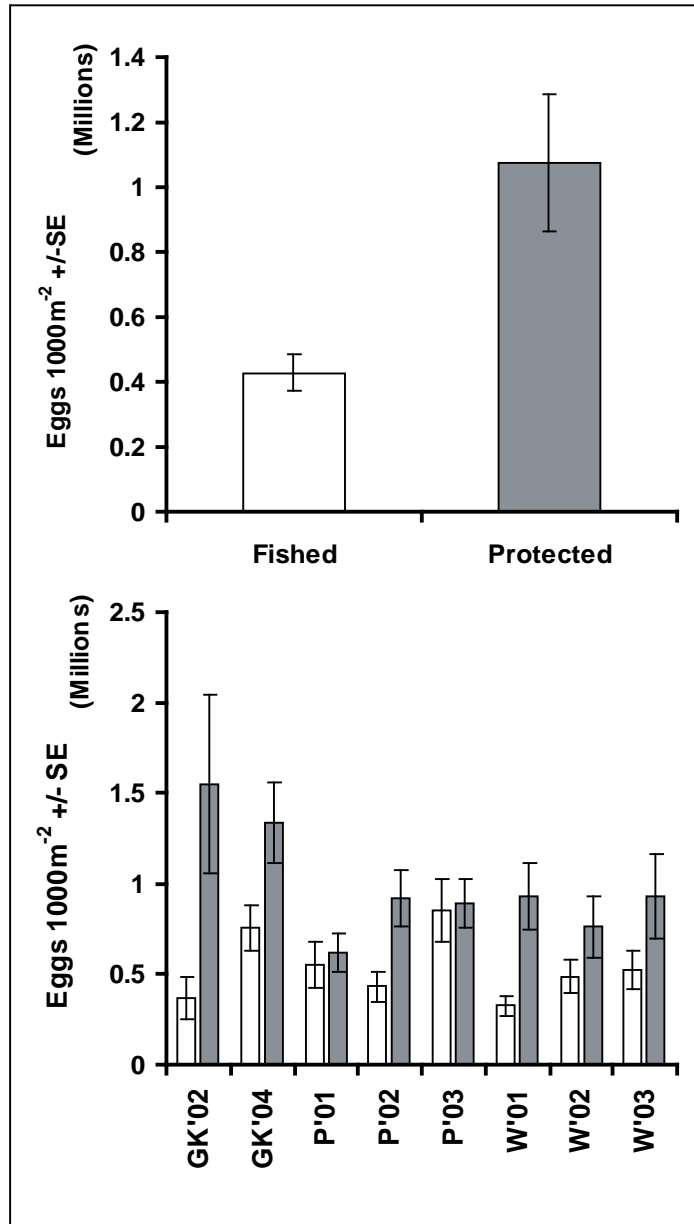
Figure 4.5: a) Spatial and b) temporal comparison of batch fecundity per unit Area of *Lutjanus carponotatus* in the no-take and fished areas of the Palm, Whitsunday and Keppel Islands from 2001 – 2004. GK = Great Keppel Islands, P = Palm Islands, W = Whitsunday Islands, White bars = Fished, Grey bars = Protected. The year is designated by '01, etc.
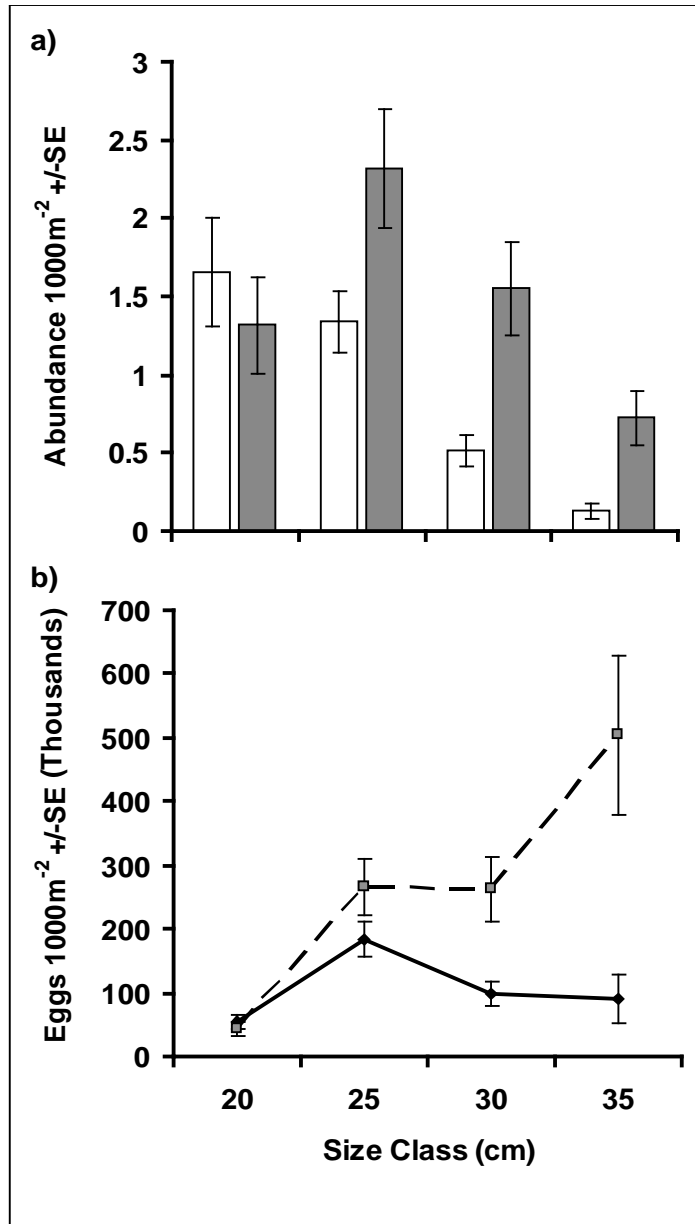
Figure 4.6: Density of *Lutjanus carponotatus* females and the mean batch fecundity per size class in each zone in the Palm, Whitsunday and Keppel Islands 2002. White bars = Fished, Grey bars = No-take; Dash line = Protected; Solid line = Fished.

4.6 Discussion

The aim of this study was to compare the Batch Fecundity per unit Area (BFUA) between no-take and fished areas of inshore GBR coral reefs based on underwater visual census of fished and no-take marine reserves. This study provides preliminary evidence that a fishery target species in MPAs has a greater BFUA than fished areas on the inshore reefs of the GBR. However, the BFUA estimates are lower than might be expected given that fecundity increases with length more rapidly than biomass. In other words, fecundity of larger fish is a greater proportion of body size than smaller fish, which corroborates Kritzer's (2004) finding that the gonadosomatic index increases with body size in *L. carponotatus*. Despite this disproportionately greater fecundity of large fish, the relative difference in BFUA between protected and fished areas was only slightly higher than the relative difference in biomass.

Larger fish generally have greater egg production per spawning event (Berkeley et al. 2004). The frequently cited comparison of individual fish fecundity for red snapper (*L. campechanus*; Plan Development Team 1990) has often been used to justify the expectation that, since MPAs produce more fish and bigger fish, they will enhance egg production per unit area even more than they enhance biomass (Palumbi 2004a). Given this expectation, one might expect larger differences in BFUA between no-take and fished areas in this study, such as the 11- to 18-fold differences reported for *Pagrus auratus* in New Zealand by Denny et al. (2004), but this was not the case. Relative differences in BFUA were more modest, and were similar to those estimated for *Sebastes* in California by Paddack and Estes (2000). Individuals in larger size classes that would have the greatest effect on the relative increases in BFUA were comparatively less abundant in some MPAs, due to natural mortality schedules, lack of recruitment in previous decades, or potential poaching within these MPAs (Davis et al. 2004).

Larger individuals, as there are typically so few, often have relatively little effect on reproductive output, and only become important when there is a shift in population structure toward older and larger age and size classes (Kritzer and Davies 2005). The results of this study tend to support their claims. After 15 years of protection, there was consistently greater abundance of larger fish in all legal size classes (individuals > 25cm) (Fig. 6). In the MPAs in this study, the largest size class contributed nearly 50% of the BFUA with relatively few individuals. In contrast, nearly 50% of the BFUA in the fished areas was provided by the greater numbers of mid-sized fish (<25cm), not yet recruited to the fishery (Fig. 6). Furthermore, the mean BFUA for all size classes in the fished areas was approximately 429,000 eggs 1,000m^{-2}. The mean BFUA for the largest size class in the MPAs was approximately 504,000 eggs 1,000m^{-2}. Therefore, the contribution to BFUA, by only the largest size-class in the MPAs, is greater than the mean of all the size classes in the fished areas of this study. This in itself demonstrates the value of long term protection to enable the build up of larger individuals.

Batch fecundity alone does not determine the relative reproductive output of individuals within a population. Age and size of a fish may determine the amount of times an individual spawns, as well as the quality of eggs produced (Berkeley et al. 2004). Kritzer (2004) determined that larger *L. carponotatus* have longer spawning seasons and therefore may also spawn more times in a year than smaller individuals. Older and larger rockfishes (*Sebastes* spp.) produced larger eggs that may result in faster larval growth, higher survival rate in the plankton and greater recruitment success (Berkeley et al. 2004). Therefore, larger fish tend to produce more eggs that are bigger, and presumably may be more viable (Ojanguren et al. 1996; Pepin et al. 1997; McCormick 1998). The present study detected a pattern of increased egg diameter from smaller to larger individuals of *L. carponotatus*. If there are a greater number of larger individuals

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producing more viable offspring in the MPAs, the increased BFUA should provide greater benefits than the present results suggest. That is, recruitment potential may also be higher from these protected populations than from a fished population with smaller and younger fish. However, more investigation is required as these issues are still unresolved due to the ambiguity of the results in the smaller size classes of this study. Furthermore, information on spawning frequency of different age and size classes is required for more accurate estimates of egg production per unit area.

Recent advances have enabled direct estimates of self-recruitment within local populations of coral reef fishes. By tagging the demersal eggs of *Amphiprion polymnus* with tetracycline, Jones et al. (2005) found 30% self recruitment to one population of anemones in Kimbe Bay, Papua New Guinea (PNG). Furthermore, they used microsatellite markers to determine that some individuals actually settled to within 100m of their natal anemone after a ten day pelagic period. Almany et al. (2007) demonstrated up to 60% self recruitment of *Chaetodon vagabundus* (30-40 day pelagic period) and *Amphiprion percula* (~11 day pelagic period) to Kimbe Island in Kimbe Bay, PNG. Based on these figures, more than 40% of larvae settling in that area originated from reefs up to 10km away. Thus, greater BFUA in the MPAs on the inshore reefs of the GBR has the potential to effectively replenish nearby no-take and fished areas. Just how far the larval fish on the GBR disperse requires a multi-disciplinary approach incorporating larval tagging, genetics and biophysical modelling.

Increased egg production from MPAs may be irrelevant if recruitment is already at saturation levels and post-recruitment processes such as food availability and predation determine adult populations. On the GBR where fish stocks are exploited, but not overly so, increased levels of egg production from MPAs may have slightly less benefits than on heavily depleted reefs elsewhere in the world. In such over-fished areas, increased density of larger size classes in a

network of MPAs could provide huge benefits to surrounding fished and protected reefs (Russ 2002). Potential recruitment benefits from increased export from reserves should be assessed on a regional basis depending on reef fish stock status, exploitation levels and connectivity of populations.

Greater biomass of *L. carponotatus* in the MPAs compared to fished areas around the same inshore island groups of the GBR has been recorded in other years of sampling (Williamson et al. 2004). The temporal consistency reported in the present study strengthens the conclusions of Williamson et al. (2004) that there is a significant influence of reef zoning on the biomass of species targeted by fishing. In contrast, Kritzer (2002) found that density and biomass of *L. carponotatus* were greater in the fished area in the Palm Island group than the no-take area in 1999. He speculated that fishing pressure might not be high enough to affect differences, or that release from competition with, or predation by, preferred target species (larger serranids and lutjanids) might be advantageous to *L. carponotatus* in fished areas. Another possibility is that these studies are detecting a transition in the effects of protection at the Palm Island group. At the time of Kritzer's (2002) sampling in 1999, the site had been protected for 12 years. By the end of the sampling in the present study, the site had been protected for 16 years, approximately the maximum longevity of individuals in the species at the Palm Island group (Kritzer 2002, 2004). Therefore, these studies may have occurred during a progressive accumulation of reserve effects and are capturing the state of the system at different points along that increasing trajectory. It is notable that Kritzer's (2002) estimates of biomass per km^2 for the fished site (3,139 and 3,890, using different approaches, for Pelorus Island) is intermediate among those estimated in this study, but his estimates for the no-take site (1,966 and 2,235 for Orpheus Island) are below any estimated herein. Furthermore, biomass at the no-take site shows a continual increase from Kritzer's (2002) data through the final year of this study that is not seen for the fished site.

In conclusion, this study and several others (Graham et al. 2003; Evans and Russ 2004; Williamson et al. 2004), show that no-take marine protected areas in the inshore regions of the Great Barrier Reef had a greater biomass of species targeted by fisheries than nearby fished areas. This study also demonstrated greater batch fecundity per unit area for *L. carponotatus* in MPAs compared to the fished areas. Recent research on the connectivity of fish populations within the Great Barrier Reef (e.g., Jones et al. 1999) suggests that the greater batch fecundity per unit area in MPAs should benefit the no-take area itself, but should also be of benefit to surrounding fished areas. Two key areas of research require immediate attention to improve our understanding of marine reserve connectivity: 1) measurement of reef fish larval dispersal to determine connectivity regimes to ground truth the predictions from models; and 2) research on spawning frequency of synchronous-batch pelagic spawners, such as lutjanids, to determine the benefits of increased mean body size and to establish annual egg production estimates for no-take and fished areas. This study is the first on the Great Barrier Reef to attempt the estimation of BFUA of reef fish stocks in MPAs. The results have shown that no-take marine protected areas are effectively protecting fish stocks on the inshore reefs of the GBR, allowing for greater biomass and potentially more egg production than in surrounding fished areas.

Chapter 5: Assessment of an underwater biopsy probe for collecting teleost fish tissue samples

5.1 Introduction

Collecting tissue samples from mobile marine organisms without capturing or killing them is difficult. Until recently, most tissue sample collections required the capture of an organism to remove a sample of skin or flesh. Spear guns or poisons are commonly used to kill smaller, manageable animals. Traps, nets or fishing lines are used to capture animals of all sizes to remove a tissue sample. The latter process is intended to be non-fatal, although barometric pressure changes and stresses on the organism may lead to fatality (Bartholomew and Bohnsack 2005). New techniques of collecting tissue samples that decrease or eliminate fatalities of mobile marine species, such as teleost fish, are required.

Various non-fatal techniques are used to target large mega fauna such as cetaceans and reptiles that surface to breathe, including crossbow, poles, above-water spear guns or modified rifles (Krutzen et al. 2002; Borrell et al. 2004; Dalebout et al. 2006; Spinsanti et al. 2006). Equivalent underwater techniques have only recently been developed for sharks using a biopsy probe fitted to a spear gun (Robbins 2006). The diver fires the probe attached to a spear at sharks underwater to remove a small core of tissue for molecular analysis, and the sharks survive with only a minor plug-hole. Opportunistic use of the shark biopsy probe on two larger teleost fish, *Cheilinus undulatus* (Ruppell, 1835) and *Bulbometapon muricatum* (Valenciennes, 1840), also proved successful (Robbins 2006). However, a technique has not been trialled for small- to medium-sized teleost fish (15 – 70cm).

Several collection methods are used for teleost fish depending on their size and the information required. Spear guns are typically used to kill fish and collect genetic samples in conjunction with collection of gonads and otoliths for reproductive, age determination and growth studies. Hook-and-line fishing techniques and nets are employed to ensure survival of the targeted species. However, this process may be fatal, via barotrauma or physical injury (Diggles and Ernst 1997). A biopsy probe for small- to medium-sized fish may alleviate any fishing induced mortality and enable selectivity of individuals or size-classes in a population. Potential uses for the biopsy probe may include fishery independent genetic stock assessments; population and phylogenetic studies of rare endangered species or species within marine protected areas; and parentage analysis from spawning aggregation sites. The success of such a tool would be dependent on the researchers' ability to approach the target species. A tool of this nature may not be efficient for fast moving, pelagic species. However, suitably-sized fish that are demersally attached or home-ranging would be suitable target species.

A probe designed to collect tissue samples for molecular analysis, without capturing or killing the fish, is tested on two coral reef fish species, *Plectropomus maculatus* (Bloch, 1790) (Serranidae) and *Lutjanus carponotatus* (Richardson, 1842) (Lutjanidae). These two species differ in certain aspects of their behaviour and morphology. *P. maculatus* grows to 75cm with very small scales and is a curious fish that will often approach a non-aggressive diver. In contrast, *L. carponotatus* grows to 45cm, has relatively large scales and is very nervous around divers. This study documents the efficiency and the inherent problems of using a particular biopsy probe on two small- to mid-sized teleost fish species with different behaviours and morphological characteristics.

5.2 Methods

The biopsy probe tested in this study (Fig.1) used a similar configuration to the two probes (Type I and II) developed by Robbins (2006) with three major design changes. Firstly, the design of the two types (I, II) were combined, using the barrel of Type I and the dental Brooches of Type II (Fig.1). In this study, the two dental brooches were twisted around each other, inserted into the barrel of the probe and both were secured in a stainless steel spear-shaft adaptor with a split pin (Fig. 1). Secondly, all dimensions were reduced to minimize penetration into the smaller fish. Thirdly, notch orientation was inverted from rearward-facing to forward-facing (Fig. 1) to account for size and morphology differences between sharks and teleost fish. Shark flesh is much firmer than teleost fish and the relatively large size of sharks allows for a direct entry core to be removed. In contrast, the impact with a small teleost fish is more of a scraping motion through the soft flesh, so forward-facing notches increased tissue retention.

The adaptor was screwed onto a spear-shaft that was fired by a rubber-propelled 3ft (76cm) spear gun, with standard 18mm diameter spear rubbers. The reduced power and range (2m max. reach from diver) of such a small spear gun helped to ensure the accuracy and impact of the biopsy probe. The power of the spear guns was not measured in absolute terms. Initially the guns were powered at a minimal strength (lower power) to reduce the chance of injury to the fish, which provided mixed results for the two species. For greater consistency the spear guns were adjusted to a greater power (higher power). Therefore, the terms 'lower power' and 'higher power' are relative, not absolute.

Samples were collected in the Whitsunday Islands (S 20°05.600; E 148°56.992), Great Barrier Reef, Australia. Scuba divers hunted the fish or baited the water with pilchards to attract them. Divers targeted the dorsal posterior section of the fish to avoid serious injury to the head and

vital organs. Tissue collection success rate was recorded based on the number of tissue samples collected versus the number of hits for each species. After a successful tissue extraction, the probe adaptors with probe were unscrewed underwater and placed in specimen jars and labelled. Divers typically carried up to 15 probes on a dive. Post dive, the samples were extracted from the probes and placed directly in 80% ethanol. To reduce the chances of cross infection between individuals, and contamination of samples, all probes were soaked in 42 g l^{-1} solution of sodium hypochlorite (household bleach) for 30 min before rinsing in fresh water.

All tissue samples were weighed to 4 decimal places (wet weight in grams) and DNA was subsequently extracted from the tissues using the method developed by Elphinstone et al. (2003). Using this method, DNA was successfully extracted from a wet tissue weight of 0.0007g of flesh and/or skin. However, DNA could not be extracted from the scales of these two species using this particular method. Therefore, all samples greater than 0.0007g of flesh/skin were considered successful regarding the use of biopsy probes.

5.2.1 Survival Rate

In situ, the fish were observed for any adverse effects of the impact of the biopsy probe. Observation time was minimal as all but a few fish departed immediately. Therefore a tank experiment was set up to observe the fish for two weeks after probing. *L. carponotatus* were used to test post-impact effects of the biopsy probe because they are the smaller of the two species, and impact from the probe would obviously create a greater wound relative to body size. Nine *Lutjanus carponotatus* individuals were caught from Pelorus Island, Palm Island Group, Great Barrier Reef, using Hook-and-line. Three individuals were placed in three separate 600 litre tanks to test mortality rates caused by the probe. After a period of 4 days acclimatization, all fish were measured (mm) and injected with a T-bar tag for identification.

One fish from each tank was scraped with a probe in the upper half of the caudal region, one scraped near the dorsal fin and the third fish, the control, was only measured and tagged. Fish were subsequently held in the tanks for a period of 15 days. Daily observations of feeding rate and general health were recorded throughout the experiment. At the end of 15 days the fish were measured and the scratches were observed for signs of infection, partial or complete healing. All fish were released back to the reef.

5.3 Results

The success of the biopsy probe was species-specific, particularly with regards to differences in scale size of the species and also depended upon the power of the spear gun. Behaviour may also have played a role in the ability to hit and retain a tissue sample from the two species. *P. maculatus* were often curious towards the diver. This made it easy to ensure an accurate shot. In contrast, it was difficult to aim at the fast moving, cautious *L. carponotatus*, and thus achieve a successful hit. Overall, the probe was only 63% successful in collecting a tissue sample from *Lutjanus carponotatus*, but 80% successful for *Plectropomus maculatus* (Table 1)*.*

At lower power the relatively larger scales of *L. carponotatus* reduced the ability of the probe to extract and retain fish flesh (Table 1). The lower powered probe successfully collected *L. carponotatus* tissue for DNA processing in 9 out of 20 hits (45%). Seven of the unsuccessful *L. carponotatus* tissue sample extractions consisted only of scales. At high power the probe collected ample *L. carponotatus* tissue samples 28 times from 38 hits (73%). *P. maculatus* has relatively smaller scales that did not impede the biopsy probe. Successful DNA tissue collection was achieved for the probe at the lower (81%) and higher power (84%) settings of the spear gun (Table 1). Average wet weight of tissue samples collected from *L. carponotatus* was 0.014g (±0.002 S.E) and from *P. maculatus* was 0.02g (±0.003 S.E.). There was no significant

difference in the average wet weight of tissue collected from both species (Single factor ANOVA: p= 0.06; 1,84df). All three mortalities in situ occurred using the higher power setting on the spear gun (Table 1).

Table 5.1: Success rates of tissue extraction by the biopsy probe from *Lutjanus carponotatus* and *Plectropomus maculatus*.

Species	No. Hits	No. Tissue	% tissue Success	DNA success (>0.0007g)	% DNA Success	Mortality
Lutjanus carponotatus	58	46	79	37	63	2
Lower Power	20	17	85	9	45	0
Higher Power	38	29	76	28	73	2
Plectropomus maculatus	47	39	83	39	83	1
Lower Power	22	18	81	18	81	0
Higher Power	25	21	84	21	84	1

In situ observations of the fish after probing were insufficient to fully understand the effects of probe impact on individual fish. Of the 105 fish probed only three mortalities were recorded (2.8%) due to the spearing process. In these cases, the probe was miss-fired and struck the head or the ventral nerve of the fish. The vast majority of fish swam away rapidly, but in cases where pilchards were used as bait to attract the target species, several individuals swam back into the feeding frenzy and continued their normal feeding behaviour.

In the tank experiment all *L. carponotatus* individuals were eating approximately two small pieces of pilchard per day before the probe trial. Post impact, the fish returned to their normal 'tank' feeding behaviour the following day. After 9 days the fish were consuming up to 3 pieces each per day. At the end of 15 days all lesions but one had healed. No scales were present at all the lesions, but more importantly, no infections occurred during the experiment. All nine fish were released back to the reef successfully.
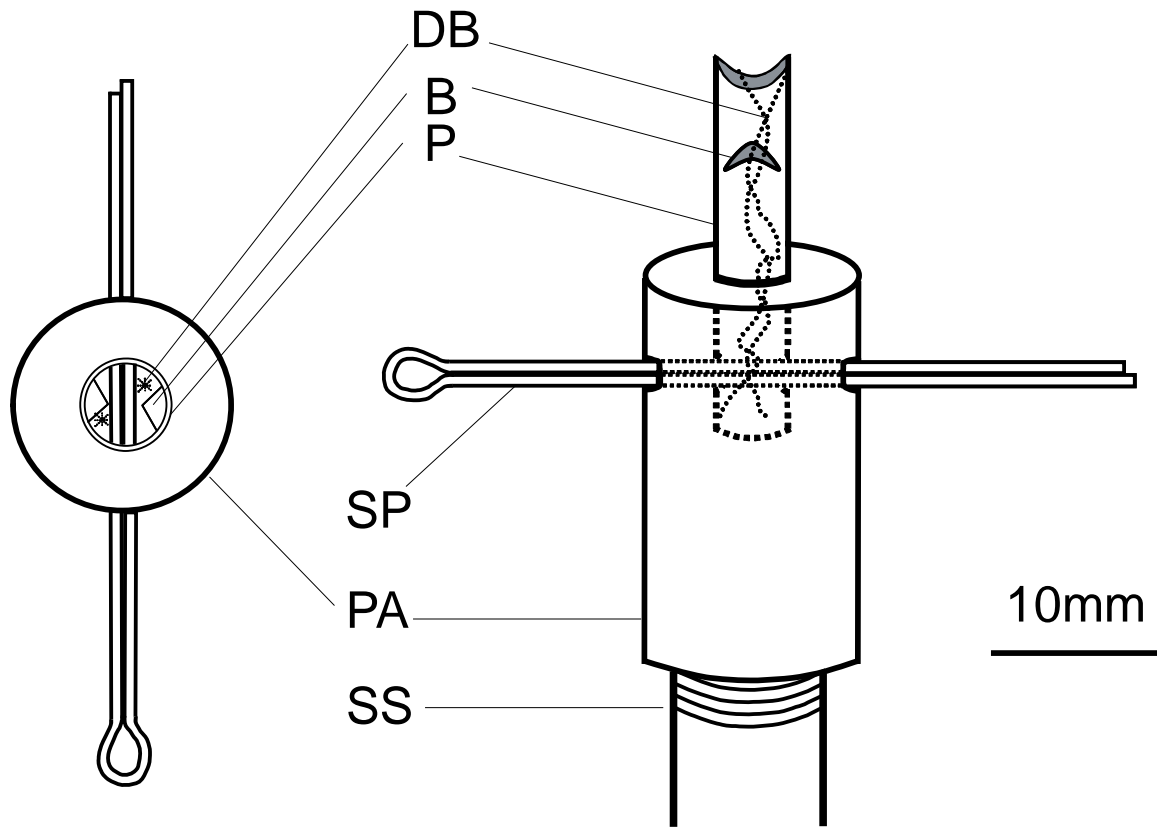
Figure 5.1: Underwater biopsy probe, for small- to medium-sized teleost fish. DB: Dental brooches; B: Forward-facing barb; P: Probe; SP: Split pin; PA: Probe adaptor; SS: Spear shaft.

5.4 Discussion

The use of the fish biopsy probe to remove small samples of tissue from highly mobile species of marine teleost fish was successful, with results varying depending upon the scale-size of the target species. High tissue retention rates and low mortality levels demonstrate the utility of the biopsy probe for endangered species of fish or on species within marine protected areas. Conventional tissue collection methods for marine teleost fish are expensive and often detrimental to the health of the sampled species. Although the technique used here is useful only for genetic population studies, rather than for age and/or reproductive studies, it does provide a highly selective and relatively fast method of tissue collection for molecular studies. For example, tissue samples from this trial have been used in population and phylogenetic studies of both *Plectropomus maculatus* and *Lutjanus carponotatus* on the inner-shelf of the Great Barrier Reef (Evans et al. in review).

It should be noted that the small size of the tissue sample collected requires extreme care at all stages from actual impact to DNA extraction. Care must be taken to ensure enough tissue is removed and preserved to enable successful DNA extraction. Furthermore, if the entire sample of a rare individual has to be used in the DNA extraction, there is no margin for error. DNA was extracted from most samples, but not all. Unsuccessful hits usually resulted in scales drifting in the water column. If these could be used, the success rate would increase. DNA has been extracted from *Epinephelus daemelii* scales (van Herwerden pers comm.), but it is unsure if it was the technique used or just the sheer size of the scale and the amount of skin still attached to the scale. Smaller species of fish have much smaller scales and inherently less or no skin attached to the scale. Further work is needed in this area.

The biopsy probe can be used on any size/ power spear gun but the results show that the correct power and range of the apparatus is very important for success rate. Understanding the morphology and behaviour of the target species will help to determine the size of the probe and the spear gun power, to increase tissue retention and minimize fish injury. The fish in this study were resilient to small scratches, *Lutjanus carponotatus* did not suffer infections in the laboratory trial, and in situ, some individuals of both species returned to feeding within minutes of impact from the biopsy probe. However, sub-lethal effects on long term growth or reproduction were not ascertained, which is important to know, particularly if fish are targeted on spawning aggregations.

In conclusion, this biopsy probe will enable researchers to collect valuable genetic information from rare or endangered fish species, fish in spawning aggregations sites, and in no-take marine protected areas, without removing or killing any individuals. The small- to medium-sized fish biopsy probe tested in this study is a successful tool for future population genetic studies.

Chapter 6: Strong genetic but not spatial subdivision of two reef fish species on the Great Barrier Reef

6.1 Introduction

Identifying connectivity regimes of marine organisms is vital for managing populations of marine resources (Palumbi 2004b). Most coral reef fish have a bipartite life cycle consisting of a pelagic larval phase and benthic adult phase (Doherty et al. 1995). In marine organisms, the pelagic larval phase can potentially disperse great distances and thus reduce genetic structure over large spatial scales (Edmands et al. 1990). Many studies support this idea and show very little or no genetic structuring over scales of hundreds to thousands of kilometres, even across ocean basins (Dudgeon et al. 2000; Lessios and Robertson 2006; Klanten et al. 2007). However, some studies demonstrate genetic structure within reef systems or within the dispersal capabilities of the species without the presence of any obvious physical barriers (Planes et al. 1998; Taylor and Hellberg 2003; Munday et al. 2004; Rocha et al. 2005; van Herwerden and Doherty 2006). Previous studies on the Great Barrier Reef (GBR) concluded that there was no correlation between genetic diversity and pelagic larval duration (PLD), a measure of potential dispersal distance (Stobutzki and Bellwood 1997), for several species of reef fish (Doherty et al. 1995; Shulman 1998; Bay et al. 2006).

Knowledge of larval dispersal has progressed substantially in the past decade. Previous predictive models were often based on observed movements of particles that floated passively on ocean currents (Caley et al. 1996; Roberts 1997; Levin 2006). However, recent behavioural research has shown that larvae of coral reef fish have considerable swimming and sensory abilities that can aid or limit their dispersal from natal reefs (Stobutzki and Bellwood 1997;

Leis et al. 2007). In addition, recent ecological studies have demonstrated that up to 60% of larval *Amphiprion, Pomacentrus* and *Chaetodon* remained at or returned to their natal reef after a pelagic larval duration of up to 30 d (Jones et al. 1999; Jones et al. 2005; Almany et al. 2007). Therefore, connectivity in open populations on the scale of ocean basins or coral reef networks may be very limited; but at least the remaining 40% may disperse great distances. Although contemporary predictive models now incorporate larval behaviour (Paris and Cowen 2004; Cowen et al. 2006; Leis 2007), such models still require field validation.

The Great Barrier Reef Marine Park (GBRMP) is a coral reef network consisting of over 2900 reefs in an area of 344000 km^2 (Day et al. 2003). However, very little is known about the levels of ecological connectivity between reefs. The GBRMP is managed by the Great Barrier Reef Marine Park Authority (GBRMPA) and their primary management tool is a multiple-use zoning plan which allows for different levels of exploitation in certain areas ranging from 'no entry' to 'open' fishing areas. From 1987 to 2004, 'no-take' marine protected areas (MPAs) comprised 4.5% of the GBRMP. On July 1 2004, the GBRMPA increased the amount of MPAs to 33% of the GBRMP under the Representative Areas Program (RAP). RAP was designed to protect a network of 70 different key bioregions (Fernandes et al. 2005). The RAP was not designed specifically to protect fishery stocks. However, it has, as one would expect based on the literature, increased the density and biomass of the target fish species, *Plectropomus* spp. (Russ et al. 2008).

Commercial and recreational fisheries operate within the GBRMP and are regulated by state and federal legislation. Commercial reef fish fishers typically operate on mid- to outer-shelf reefs. Recreational fishers often use smaller vessels than commercial fishers and thus tend to operate on inner-shelf reefs. Recreational fishers are typically opportunistic and target a large range of serranids, lutjanids, and lethrinids. Although the coral trout, *Plectropomus* spp., is

considered a 'trophy fish' for recreational fishers, other smaller and more abundant species are often also harvested.

Over the past decade, underwater visual surveys have revealed that a target group and a secondary target species, *Plectropomus* spp. and *Lutjanus carponotatus* respectively, have consistently demonstrated greater abundance and biomass in the MPAs around the inshore islands of the GBR (Evans and Russ 2004; Williamson et al. 2004; Evans et al. 2008), and since the implementation of the RAP in 2004, the density of the target species *Plectropomus* spp., has nearly doubled in new MPAs (Russ et al. 2008). *Plectropomus maculatus* is the inshore coral trout which is the focus of this study, along with *L. carponotatus*. Both species are predominantly found on inshore reefs (Ferreira 1993; Newman et al. 2000), but both have significant populations in the Capricorn Bunkers, a mid-shelf cluster of reefs in the southern-most GBR. *L. carponotatus* also have moderate distributions on the mid-shelf reefs from south to north along the GBR (Newman and Williams 1996).

This study is the preliminary phase of a large scale investigation of the connectivity of target fishery species between MPAs and fished areas, and between MPAs on the inshore reefs of the GBR. The broad scale aim is to determine the amount of genetic partitioning and evolutionary processes between the near shore islands of the GBR, focusing on two fishery target species, *P. maculatus* and *L. carponotatus*. Given the relatively long pelagic larval phase (*P. maculatus*- 28 d; *L. carponotatus*- 34 d [Evans unpublished data]) and the wide distribution of both species throughout the Indo-West Pacific (Allen et al. 2003), the null hypothesis of this study is that there is no genetic structuring of *P. maculatus* and *L. carponotatus* populations on widely separated reefs of the GBR between the inshore island groups and one mid-shelf cluster of reefs.

6.2 Methods

6.2.1 Collection and sampling design

Tissue samples were collected from individuals of *Plectropomus maculatus* and *Lutjanus carponotatus* from two reef locations at each of three inshore regions (Palm, Whitsunday and Keppel Islands) and one mid-shelf region (Capricorn Bunker Islands) of the Great Barrier Reef (GBR) in 2006 and 2007 (Fig. 1). The sampling was designed to allow the use of a hierarchical analysis (Analysis of Molecular Variance, AMOVA) between the different regions and within each region. In most locations samples were collected using lethal spear fishing techniques. However, in the Whitsunday Islands a new tissue collection method was trialled successfully using an underwater biopsy probe to collect tissue samples (Evans 2008).
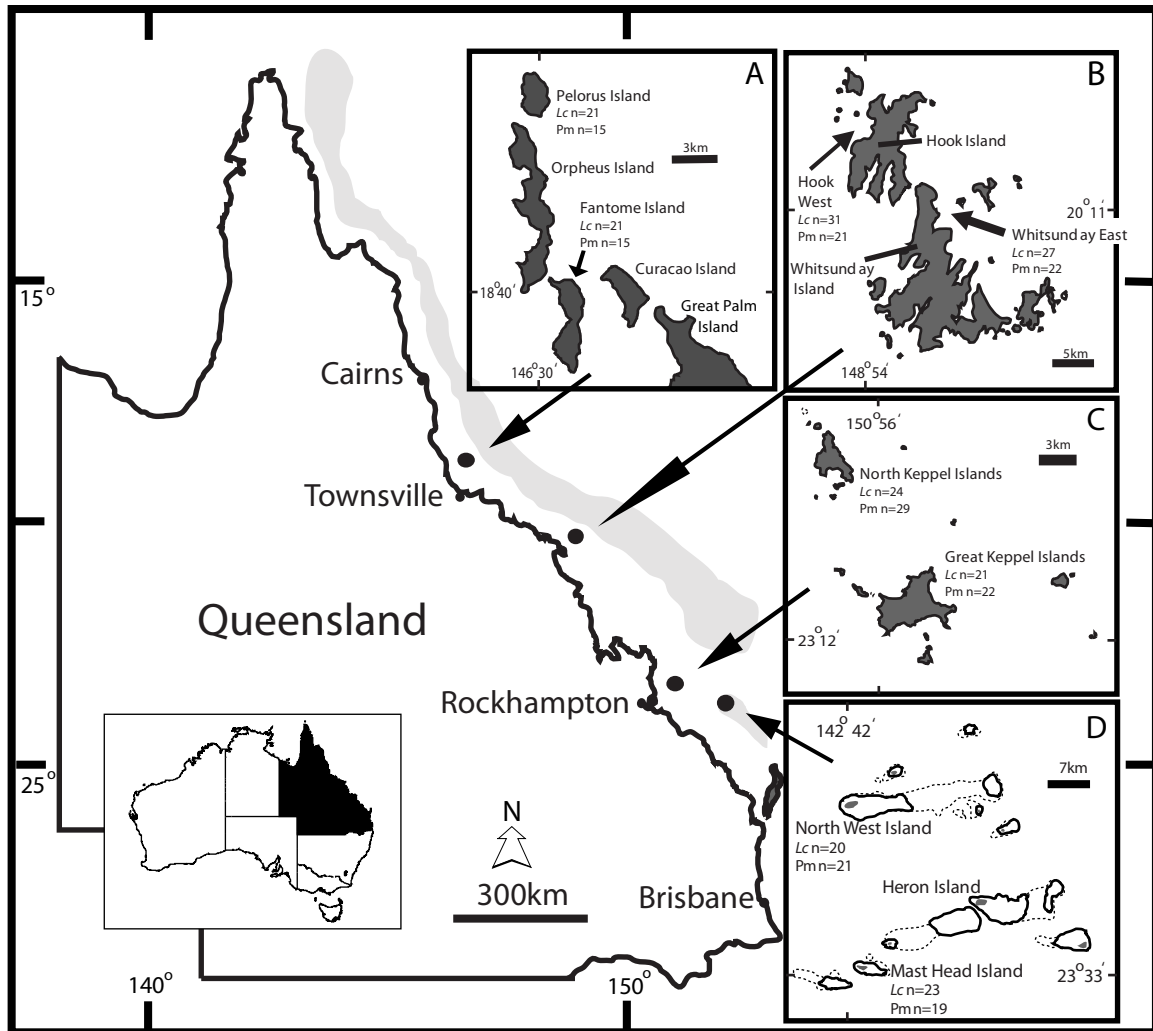
Figure 6.1: Map of the Great Barrier Reef (grey shade) along the Queensland Coast, Australia. Black dots represent the four insets showing the locations and number (n) of *Plectropomus maculatus* (*Pm*) and *Lutjanus carponotatus* (*Lc*) sampled in each island group. A) Palm Islands; B) Whitsunday Islands; C) Keppel Islands; and D) Capricorn Bunker Islands. Dotted lines in the Capricorn Bunker region (D) represent submerged reef and the solid lines around the islands (dark gray) are reefs that are exposed at tidal datum.

6.2.2 DNA analysis

Genomic DNA was extracted from 164 *P. maculatus*, 188 *L. carponotatus* and 3 *L. vitta* (out-group) individuals. This was done using a Chelex (silica) extraction method (Elphinstone et al. 2003). The hyper variable region I (HVRI) of the mitochondrial DNA (mtDNA) of both species were amplified using the universal D-loop primers

L15995 (5'-AACTCTCACCCCTAGCTCCCAAAG-3') and

H16498 (5'-CCTGAAGTAGGAACCAGATG-3) (Lee et al. 1995).

Polymerase chain reactions (PCR) were conducted in 20μl reactions containing 1x PCR buffer, 2.5 mM MgCl$_2$, 500 μM dNTP, 0.025 units/μl of Taq polymerase (Bioline), 0.25 μM of each primer, and 1-10 ng template DNA (DNA was not quantified for each sample) in a MJ Research Tetrad Thermocycler PCR engine (Biorad). Thermocycler programs for all PCR began with an initial denaturation step for 3 mins at 94°C followed by 35 cycles of 94°C for 45s, 50°C for 30 sec and 72°C for 45 sec, then a final extension step at 72°C for 5 mins. PCR products were 'cleaned up' prior to sequencing via centrifugation through Sephadex columns (GE Healthcare) to remove excess salts and primers. Sequences of all three species were submitted to GenBank (Accession numbers: *P. maculatus* FJ468014-FJ468177; *L. carponotatus* FJ423773-FJ423960; *L. vitta* FJ416857-FJ416859). *Plectropomus maculatus* out group individuals from Western Australia and Hervey Bay (see appendix 1) already had accession numbers: DQ643607-DQ643609 (WA), DQ643594-DQ643600 (HB) (van Herwerden et al. 2006).

6.2.3 Statistical analysis

Nucleotide sequences were aligned using Clustal W Multiple Alignment (Thompson et al. 1994) and manual adjustments were made by eye in BIOEDIT Version 7.0.9 (Hall 2007). The evolutionary relationships between samples were demonstrated using Maximum Parsimony

(MP) and distance (neighbour joining, NJ with 500 bootstrap replicates, Tamura-Nei Model with transitions and transversions) in MEGA (Molecular Evolutionary Genetics Analysis, Version 4.0, Tamura et al. 2007) and in a Maximum Likelihood (ML) analysis using the best tree from ten independent runs of 10000 generations in GARLI (Zwickl 2006). Trees were rooted using an out-group method. For out-group rooting of the two study species, three West Australian (WA) *P. maculatus* sequences were obtained from van Herwerden et al. (2006) and *L. vitta* was used for the *L. carponotatus* analysis. Gaps were treated as a fifth character. A Consensus tree of the ML bootstrap replicates was compiled in PAUP 4.0b.10 and 50% majority rule consensus support values from these replicates, and from the MP and NJ analyses, were presented on the best of ten independent ML trees obtained from GARLI.

6.2.4 Population genetic analysis

Fixation indices (ϕ_{CT}, ϕ_{SC}, ϕ_{ST}) which measure genetic differentiation at various hierarchical levels and nucleotide diversities were determined using Arlequin 2.000 (Schneider et al. 2000). Haplotype diversities were calculated using the formula: $h = n \, (1- \sum x_i^2) \, / \, (n\text{-}1)$ (Nei 1987) where x_i = population of the ith haplotype and n = number of individual samples. Haplotype networks are displayed using Minimum Spanning Trees (MST) based on calculations by Arlequin 2.000 as per Rohlf (1973). Hierarchical population subdivision between island groups (ϕ_{ST}), between reefs within island groups (ϕ_{SC}) and among individuals in the population (ϕ_{CT}) were examined using analysis of molecular variance (AMOVA) implemented in Arlequin Version 2.000 (Schneider et al. 2000). The significance of ϕ-statistics and variance were tested using 1023 permutations.

6.2.5 Coalescence Analysis

Coalescence analyses were performed in Arlequin Version 2.000 (Schneider et al. 2000) for *P. maculatus* and *L. carponotatus* to estimate whether genetic bottleneck/expansion events occurred (as measured by the mtDNA). For the coalescence analysis, *P. maculatus* had two indels of 10 and 18 characters each respectively, replaced by a single indel to reflect the single insertion-deletion event, rather than being interpreted as multiple independent indel events. Mismatch distribution analysis was used to demonstrate the historical demography of both species based on the observed number of differences among all pairs of haplotypes. A least squares procedure was used to fit pair-wise mismatch distributions to a sudden expansion model. Monte Carlo Markov Chain (1000 steps) simulations were performed to test the validity of a stepwise expansion model for both *P. maculatus* and *L. carponotatus*. If $p > 0.05$, the stepwise expansion model was accepted. A more stable population history was inferred if the stepwise model was rejected. The age of expansion events were estimated for both clades and the whole population of both species using the formula $t = 2\mu t_1$, where t_1 is the expansion time in generations and μ is the mutation rate, expressed as % substitutions MY^{-1}. The divergence rate used in this study is based on divergence rate estimates for slower conserved sections (1.1%) and the hyper-variable sections (12.9%) present in the HVR I sequences for both species (Alvarado et al. 1995). Female generation times for both species were estimated approximately as per (Pianka 1978), using the formula $t_2 = (\alpha + \omega)/2$, where α = female age at maturation and ω = age at last female reproduction. For *L. carponotatus* ($\alpha = 2$ and $\omega = 18$), the generation time is 10 yr. For *P. maculatus*, which are protogynous hermaphrodites, females become males at approximately 8 yr (Ferreira 1993; Adams et al. 2000) and maturation is at 2 yr. Therefore, the generation time of female *P. maculatus* is 5 yr, as per (van Herwerden et al. 2006).

6.3 Results

6.3.1 Genetic variation

A 595 bp fragment of the HVR-I region was sequenced for 164 *Plectropomus maculatus* individuals with 171 variable sites; and a smaller, 392 bp fragment of the HVR-I region was sequenced for 188 *L. carponotatus* individuals with only 48 variable sites. *Lutjanus carponotatus* out-group (*L. vitta*) had the same number of base pairs as the in-group. For *P. maculatus*, an 18 bp insertion was present at one GBR sample from position 233 – 250, and another 11 bp insertion was present in nine other GBR samples from position 280 – 290. The WA *P. maculatus* out-group had a 73 bp deletion from position 205 – 277. The transition: transversion substitution ratio for the HVR I region was 4.6:1 for *P. maculatus* and 5.8:1 for *L. carponotatus*. The percentage nucleotide composition was AT biased and was similar for both *P. maculatus* (A: 36.56%, T: 31.28%, C: 18.17%, G: 14.00%) and *L. carponotatus* (A: 33.12%, T: 30.40%, C: 20.56%, G: 15.92%), which is consistent with other assessments of vertebrate mtDNA HVR-I variability (McMillan and Palumbi 1997).

6.3.2 Phylogenetic analysis

Neighbour joining, maximum parsimony and maximum likelihood analyses all produced similar phylogenetic trees. Ignoring the out-group, two sister clades and a cluster of older (remnant) individuals without phylogenetic structure were identified for *P. maculatus*. Likewise, two clades exist for *L. carponotatus* (Fig. 2). The best tree did not differ from the bootstrap consensus tree in the assignment of individuals to each of the clades presented in this study and there were at least 59 and 64% support for the division of clades based on ML and NJ analyses (Fig. 2). MP analyses for both species were not informative or consistent with ML and NJ trees, and failed to identify the out-group as a separate clade. The main feature of

phylogenetic trees of both species is the lack of geographical partitioning, despite the presence of two phylogenetically distinct clades. For both species, all four locations are well represented in both clades and there is no north-south difference (Fig. 2).

6.3.3 Population genetic analysis

Sequence variation of *P. maculatus* consisted of 86 haplotypes with 171 variable sites, of which 60 were parsimony informative with 50 discrete indels. For *L. carponotatus*, there were 43 haplotypes with only 48 variable sites, 20 that were parsimony informative and 17 independent indels. Haplotype and genetic diversities for the individual locations and the whole population for *P. maculatus* were high (Table 1) and are comparable to other GBR fish species (Dudgeon et al. 2000; Ovenden and Street 2003; Messmer et al. 2005; Bay et al. 2006). *Lutjanus carponotatus* haplotype and nucleotide diversities (Table 1) were relatively low for the GBR, but still high for marine fish species (Grant and Bowen 1998). In contrast, when samples were separated into the two phylogenetic clades identified, the nucleotide diversity for both *P. maculatus* and *L. carponotatus* was low (up to fourfold lower than in the non-separated analyses) compared to other marine fishes (Grant and Bowen 1998). However, both two-clade analyses demonstrated high haplotype diversity. For both *P. maculatus* and *L. carponotatus* there was high genetic diversity spatially, but this dissipated when samples were partitioned into the two phylogenetic clades.

The relationships between haplotypes were represented in a single (non-unique) minimum spanning tree (MST) for each species (Fig. 3). Consistent with the phylogenetic analysis, the MST identified the division between two sister clades for *P. maculatus* (with a group of remnant individuals) and two clades for *L. carponotatus*. Both MSTs, have a single large cluster of individuals sharing the most common single haplotype (*P. maculatus* - Clade B, n =

39; *L. carponotatus* - Clade B, n = 93) with the majority of haplotypes linked by one or a few mutations. In the case of *P. maculatus*, there is a sister clade (A) to Clade B branching from the older remnant individuals (Fig. 3). As with the phylogenetic trees, there was no obvious haplotype partitioning by geographic location (Fig. 3). Pairwise F_{ST} values were not significant between locations for either species, but when samples were grouped according to the division identified in the phylogenetic analyses, inter-clade pairwise F_{ST} values were highly significant (Fst > 0.85, p < 0.001) for both species (Table 2, Fig. 2). This confirms the existence of at least two clades within the sampled range of the GBR that are spatially intermingled (sympatric), for both *P. maculatus* and *L. carponotatus*.

Table 6.1: Genetic diversity estimates for *Plectropomus maculatus* and *Lutjanus carponotatus*: samples size (*n*), number of haplotypes (*n_h*), haplotype diversity (*h*), and nucleotide diversity +/- SE (*π*).

Location	*n*	*n_h*	*h*	*π*
P. maculatus				
Palm	30	21	0.948	0.012 +/- 0.007
Whitsunday	43	36	0.984	0.016 +/- 0.008
Keppel	51	37	0.967	0.017 +/- 0.009
Capricorn	40	33	0.982	0.014 +/- 0.007
Total	164	86	0.941	0.014 +/- 0.007
Clade A	9	6	0.889	0.003 +/- 0.002
Clade B	145	80	0.944	0.008 +/- 0.004
L. carponotatus				
Palm	42	14	0.804	0.013 +/- 0.007
Whitsunday	58	19	0.794	0.009 +/- 0.005
Keppel	45	13	0.662	0.012 +/- 0.007
Capricorn	43	18	0.780	0.012 +/- 0.006
Total	188	43	0.742	0.011 +/- 0.006
Clade A	26	10	0.881	0.004 +/- 0.003
Clade B	162	33	0.658	0.003 +/- 0.002

Table 6.2: Population pairwise Fst values (left of the diagonal) and corresponding *p* values (right of the diagonal) of *Plectropomus maculatus* and *Lutjanus carponotatus*, as estimated by a distance method assuming the Tamura substitution model.

Variables	Palm	Whitsunday	Keppel	Capricorn	Clade A	Clade B
P. maculatus						
Palm	-	0.496	0.342	0.937		
Whitsunday	-0.001	-	0.991	0.910		
Keppel	0.003	-0.017	-	0.631		
Capricorn	-0.014	-0.014	-0.007	-		
Clade A					-	<0.001
Clade B					0.849	-
L. carponotatus						
Palm	-	0.153	0.649	0.550		
Whitsunday	0.024	-	0.054	0.153		
Keppel	-0.013	0.028	-	0.622		
Capricorn	-0.007	0.008	-0.012	-		
Clade A					-	<0.001
Clade B					0.90675	-

6.3.4 AMOVA

To test the genetic connectivity of each species between the four regions, this study defined two locations within each region *a priori* as independent populations (Fig. 1).

Analyses of molecular variance (AMOVA) based on haplotype divergence were used to test variance components and fixation indices (ø-statistics). All ø-statistics were very low and non-significant, demonstrating a lack of genetic structure (Table 3). The greatest amount of genetic variance (>99%) was in the local populations. *Plectropomus maculatus* (among regions) had negative correlation coefficients, which implies greater differences between two randomly selected individuals from the same locality compared to two individuals from different localities (Dudgeon et al. 2000). AMOVA was not performed on the two clades of either species, since there is no hierarchical component when samples are partitioned into only two clades (but see pairwise Fst results noted above) (Table 2).

Table 6.3: Hierarchical analysis of molecular variance (AMOVA) within and among three inshore island groups, Palm, Whitsunday, Keppel, and one mid shelf reef cluster, Capricorn Bunkers, of the Great Barrier Reef for *Plectropomus maculatus* and *Lutjanus carponotatus*.

Comparison	Variance Component	df	Observed Partition		Ø-statistics (*p*)
			Variance	% Total	
P. maculatus	Among Island groups	3	-0.04556	-1.06	øCT = -0.011 (ns)
	Among locations within Island groups	4	0.01307	0.31	øSC = 0.003 (ns)
	Among individuals within Island groups	156	4.31497	100.76	øST = -0.008 (ns)
L. carponotatus	Among Island groups	3	0.00987	0.45	øCT = 0.004 (ns)
	Among locations within Island groups	4	0.00667	0.30	øSC = 0.003 (ns)
	Among individuals within Island groups	180	2.18453	99.25	øST = 0.008 (ns)

6.3.5 Coalescence

Mismatch distributions were plotted for all sequences (excluding the out groups) of both species (Fig. 4). The presence of at least two clades is clear in the distributions. Harpending's raggedness index, for the entire population and the represented clades, ranged from 0.008 to 0.111 for *P. maculatus* and from 0.035 to 0.05 for *L. carponotatus* (Table 4) with no significant *P* values. Low index values are indicative of a stepwise expanding population. The significant SSD result for the *L. carponotatus* population suggests that it is a stable population as a whole but each of the clades is undergoing an expansion. The estimated rate of *P. maculatus* mitochondrial control region substitutions for the combined variable (29% of sites mutating at a rate of 12.9% MY^{-1}) and conserved (71% of sites mutating at a rate of 1.1% MY^{-1}) portions was 5.34% MY^{-1} bp^{-1}. The estimated rate of *L. carponotatus* mitochondrial control region substitutions for the combined variable (12% of sites mutating at a rate of 12.9% MY^{-1}) and conserved (88% of sites mutating at a rate of 1.1% MY^{-1}) portions was 2.0% MY^{-1} bp^{-1}. Using a

female generation time of 5 yr with the calculated *P. maculatus* substitution rate, and the female generation time of 10 yr and the calculated *L. carponotatus* substitution rate, estimates of mtDNA expansion times for both species were calculated assuming neutrality and a molecular clock operates within the entire sampled population and each of the constituent clades. These ages span a large range of possible times since expansion (Table 4), and should not be considered absolute.

Table 6.4: *Plectropomus maculatus* **and** *Lutjanus carponotatus* **coalescence analysis parameters for the whole population and for the two clades defined by phylogenetic and population genetic analyses. SSD = Sums squared Differences, R = Raggedness Index, t = time (1000's yrs).**

	Mean # diff.	Tau (95% CI)	$Theta_0$	$Theta_1$	SSD	R	t divergence (95% CI)
P. maculatus							
Clade A	1.874	1.72 (0.0-3.64)	0.34	4375.43	0.013 ns	0.111 ns	80.2 (0.0-161.9)
Clade B	4.814	3.59 (0.78-7.69)	1.90	1350.63	0.000 ns	0.008 ns	167.6 (36.6-359.3)
Population	6.236	2.24 (0.37-5.41)	4.79	4415.44	0.002 ns	0.005 ns	104.8 (17.6–252.8)
L. carponotatus							
Clade A	1.420	1.44 (0.31-2.59)	0.14	2635.95	0.003 ns	0.045 ns	360.9 (77.9-649.5)
Clade B	1.198	1.15 (0.70-1.55)	0.12	2289.04	0.001 ns	0.050 ns	287.2 (174.2-388.9)
Population	1.568	1.84 (0.42-3.79)	0.09	1294.48	0.028*	0.035 ns	461.3 (106.0-950.5)

The entire *P. maculatus* population appears to have started expanding sometime during the period of 18 kY to 252 kY ago (kya). However, the age of appearance of the most recent common ancestor for each of the two clades shows that clade B is older than the entire population (37 to 359 kya), followed by the younger, sister clade A that expanded approximately 80 to 161 kya (Table 4). As there is consistent overlap in the ranges these results are not conclusive. The *L. carponotatus* population appears to have expanded approximately 106 to 950 kya. Clade A was oldest ranging from 78 to 650 kya, and was basal to an apparently younger clade B (174 to 389 kya) (Table 4). Similar to *P. maculatus*, the ranges for *L. carponotatus* overlap considerably so the results can only be inferred rather than providing conclusive evidence. Taken together, it appears that *L. carponotatus* is substantially older than *P. maculatus* on the GBR and that all four clades (two clades per species) expanded at different times during the late Pleistocene (Quaternary).
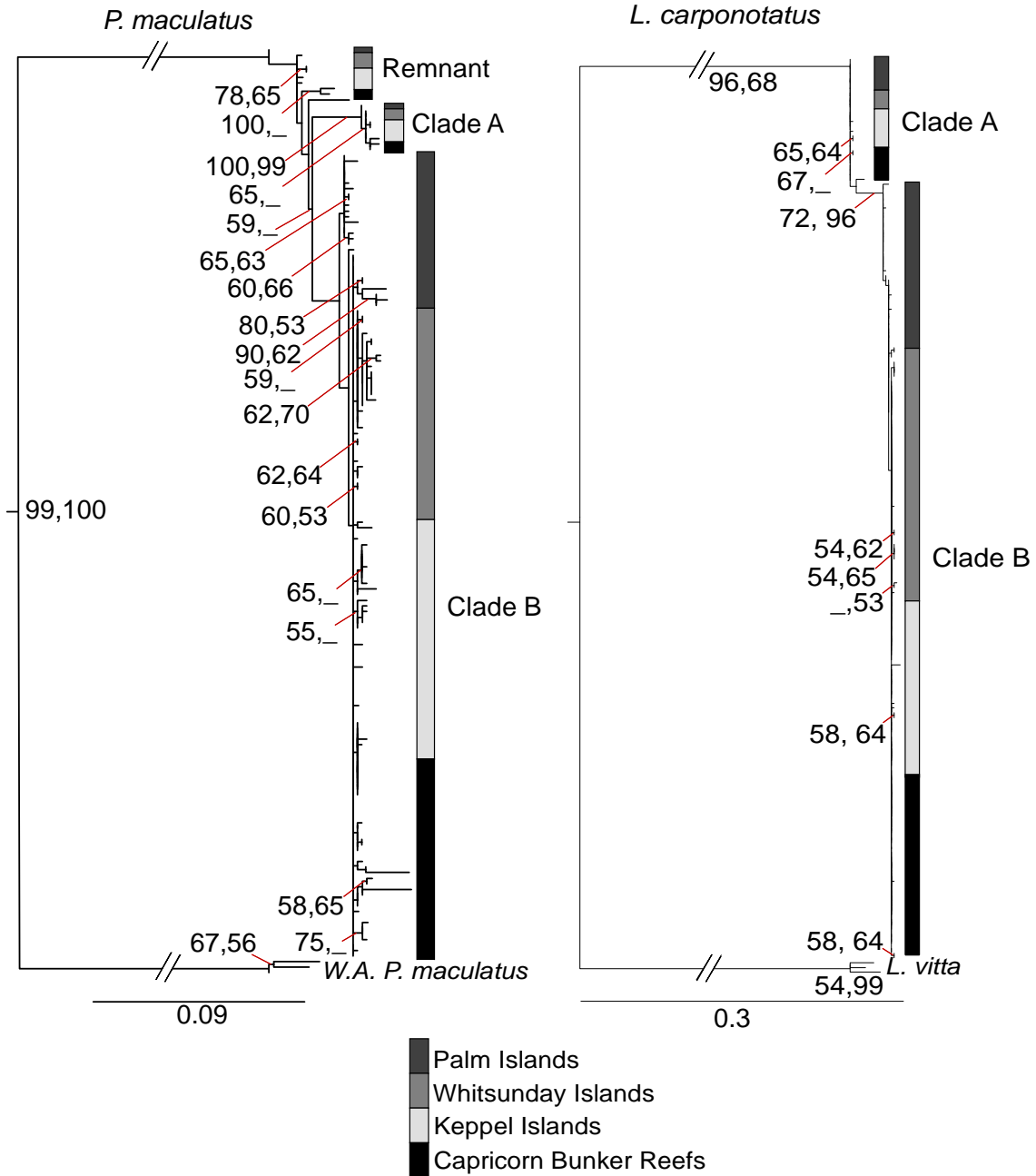
Figure 6.2: Best out-group rooted maximum likelihood (ML) trees of mitochondrial control region from 164 individuals of *Plectropomus maculatus* (three individuals of *P. maculatus* from West Australia as the out-group) and 188 individuals of *Lutjanus carponotatus* (three individuals of *L. vitta* as out-group). Numbers on inter nodes indicate bootstrap support values obtained from 100 ML and 500 NJ bootstrap replicates. Remnant *P. maculatus* samples are those which do not belong to either sister clade A or B. Shaded bars indicate proportional representation of individuals from each island group, as identified by the embedded key to the Figure.

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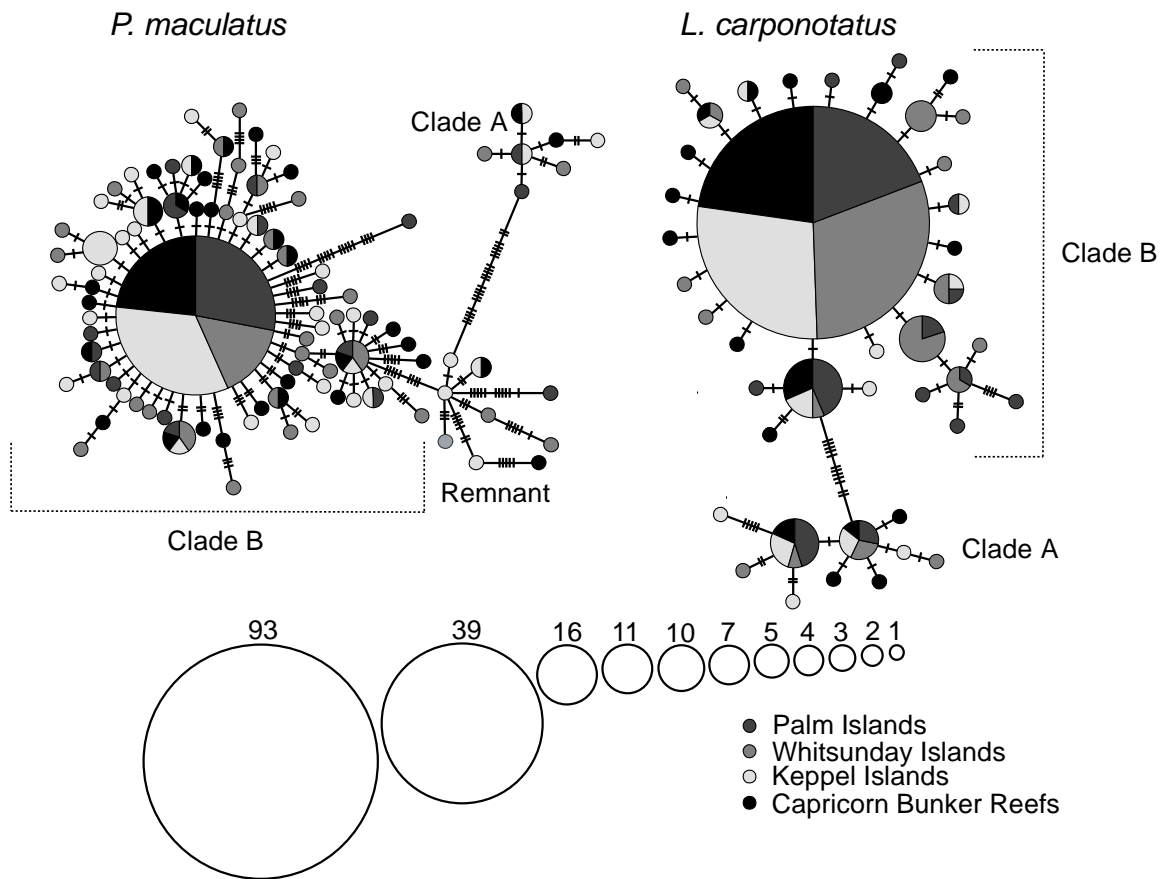
Figure 6.3: Haplotype minimum spanning tree based on the mitochondrial control region of *Plectropomus maculatus* and *Lutjanus carponotatus* from three near shore islands (Palm, Whitsunday, Keppel) and one mid-shelf cluster of reefs (Capricorn Bunkers) on the Great Barrier Reef. Remnant *P. maculatus* samples are those which do not belong to either clade A or B. Shaded portions in haplotypes indicate proportional representation of individuals from each Island group, as identified by the embedded key to the Figure. Circle sizes are proportional to the number of individuals sharing each haplotype, as indicated.
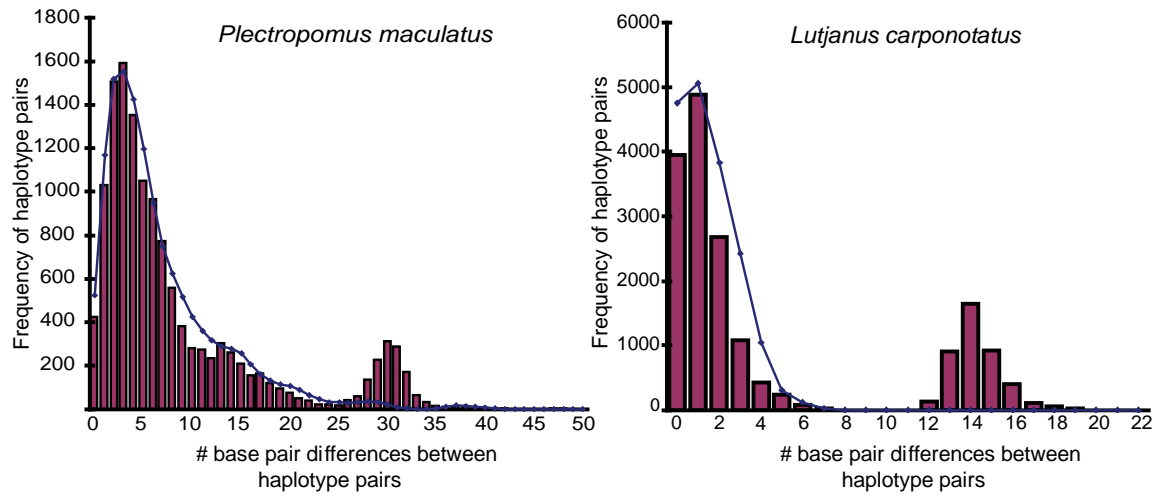
Figure 6.4: Mismatch distribution of pairwise sequence differences for *Plectropomus maculatus* and *Lutjanus carponotatus*. Histograms indicate observed numbers of pairwise haplotypes that differ by the given number of base pairs. Curves indicate the expected number of pairwise haplotypes that differ by the given number of base pairs under the expansion model.

6.4 Discussion

The purpose of this study was to investigate the connectivity of two of the most abundant targeted reef fish species on the inshore reefs of the GBR, at a large and fine spatial scale. It provides a framework for future finer spatial-scale studies (e.g. spanning 10's km), to enable an understanding of where populations may be receiving recruits from outside their local area. It is a first attempt at understanding connectivity of exploited reef fish species on the near shore GBR Reefs, and thus progress toward improved spatial management of these species.

Plectropomus maculatus had higher haplotype and nucleotide diversity than *Lutjanus carponotatus*. The haplotype diversities of *P. maculatus* (h = 0.94) were relatively high and similar to those of *Scarus frenatus* and *Chlorurus sordidus* (Dudgeon et al. 2000) on the GBR, which were considered among the highest at that time. Although, recent studies have found extremely high haplotype diversities in *Lutjanus campechanus* in the Atlantic (h = 1.0 in some locations) (Garber et al. 2004) and in three *Naso* species in the Indo and Pacific basins, *N. vlamingii* (h = 1) (Klanten et al. 2007), *N. brevirostris* (h = 1.0 in some locations) and *N. unicornis* (h = 1.0 in some locations) (Horne et al. 2008). In contrast, *L. carponotatus* haplotype diversity (h = 0.74) was much lower than for *P. maculatus* and was similar to that for A*canthochromis polyacanthus* on the GBR (van Herwerden and Doherty 2006), which is a brooding species. Compared to congeners, *L. carponotatus* haplotype diversities on the GBR were much lower than for *L. campechanus* in the Atlantic (h = 1) (Garber et al. 2004) and *L. erythropterus* in East Asia (h = 0.99) (Smith et al. 2006), but these are not considered low (<0.5) by marine fish standards (Grant and Bowen 1998). The nucleotide diversities of both species were similar to that of other species on the GBR (Dudgeon et al. 2000; van Herwerden and Doherty 2006).

The clade analysis of both species shows low nucleotide diversity but relatively high haplotype diversity in each clade of both species. This suggests genetic bottleneck events, followed by population expansion (Grant and Bowen 1998; Alves et al. 2001). The analysis of both species among and within island groups show high haplotype and nucleotide diversities. This indicates either relatively stable populations or an admixture between previously differentiated populations (Grant and Bowen 1998). Identification of two clearly distinct lineages of both species, despite the lack of spatial genetic structuring, suggests that at least two external source populations have successfully recruited to the GBR. For both species, this has resulted in wide expansions (within the study areas at least), so that both clades co-occur throughout the sampled ranges of both species. This suggests that both *L. carponotatus* and *P. maculatus* are admixtures of differentiated, expanding lineages rather than stable populations.

Phylogenetic and population genetic analyses clearly showed support for two clades in *P. maculatus* and *L. carponotatus*. However, no geographic genetic structure was evident for either of the species or for their respective clades. Both species have a typical "shallow" evolutionary pattern (Grant and Bowen 1998), dominated by one large clade (*P. maculatus* 88.4%, *L. carponotatus* 86.2% of individuals) with one prevalent haplotype (*P. maculatus* 22%, *L. carponotatus* 49% of individuals) surrounded by numerous rare haplotypes differentiated by few mutations. Interestingly, *P. maculatus* also has an unresolved older group of individuals, the 'remnants' that do not fit into either of the two supported clades. These are likely to be individuals from presently unsampled source locations originating from outside the GBR. Extended spatial sampling would be required to clarify this.

The lack of phylogeographic structure combined with low and insignificant F_{ST} values and the high 'within sample' differences dominating the molecular variance, suggests that populations of both species lack any geographical structuring and probably constitute single panmictic

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populations. Geographical clades have previously been demonstrated for two species on the GBR. *Acanthochromis polyacanthus* is a brooding damselfish that lacks a larval phase but has a wide distribution throughout the Indo-Pacific (Allen et al. 2003). With such a short dispersal range, it is not surprising that *A. polyacanthus* has a phenotypic separation of clades from south to north along the GBR (van Herwerden and Doherty 2006).

More relevant to this study, *P. maculatus* has a geographically separated subclade just south of the GBR in Hervey Bay (van Herwerden et al. 2006). van Herwerden et al (2006) also found one Hervey Bay individual which grouped with the Western Australian clade of *P. maculatus,* albeit with a number of autapomorphic substitutions. To determine whether *P. maculatus* in either clade A or B of the present study align with these Hervey Bay samples, another ML analysis, incorporating the Hervey Bay samples (Genbank numbers DQ643594-DQ643600) was run in GARLI. The best tree with bootstrap consensus support shows that the Hervey Bay individuals are a subclade within Clade B, but excludes the one Hervey Bay individual which grouped with the WA out-group as per van Herwerden et al (2006) (Appendix 1). Overall, this suggests that *P. maculatus* on the GBR consists of at least four lineages with independent ancestry (not including the "remnants"). These are clade A, clade B, the Hervey Bay subclade to clade B and the representative from Hervey Bay that is more like the WA *P. maculatus* than are the rest of the GBR *P. maculatus* samples. Only the Hervey Bay subclade is geographically defined.

Marine organisms with high levels of dispersal (via pelagic eggs or larvae), and continuous distributions in space are expected to have extensive and recent historical gene flow interconnections, resulting in limited genetic structure (Avise et al. 1987) . The GBR consists of more than 2000 km of highly inter-connected reefs, with the southward flowing East Australian Current (EAC) permitting extensive connectivity between populations stretching along the

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length of the GBR. In addition, the PLD of ~1 month for both study species helps to explain the lack of spatial genetic partitioning observed for these and numerous other reef fish species, e.g. the closely related coral trout, *P. leopardus* (van Herwerden et al. 2006), Dottybacks (Messmer et al. 2005), parrotfish (Dudgeon et al. 2000; Bay et al. 2004) and a lethrinid, red throat emperor (van Herwerden et al. 2000). Therefore, the lack of genetic structuring identified in this study is no surprise, and supports the null hypothesis that populations of *P. maculatus* and *L. carponotatus* on the near shore reefs are evolutionarily connected.

The genetic (nucleotide and haplotype) diversities in this study suggest that *P. maculatus* is older than *L. carponotatus* due to the greater genetic diversities of *P. maculatus* from each site and overall, but coalescence analyses show that on average *L. carponotatus* (mean = 460 kya) may be more than four times older than *P. maculatus* (mean = 100 kya). When comparing the genetic diversity and coalescent rates of these two very different species, one must take into account their different life history characteristics, as faster generation times and reproduction usually amounts to faster genetic change (Mitton and Lewis 1989). Both species sexually mature at 2 yr and live up to 18 yr (Ferreira 1993; Newman et al. 2000; Kritzer 2002), but on the GBR *P. maculatus* grow to approximately 80 cm (authors' personal observation) and *L. carponotatus* only reach 45 cm (authors' personal observation). During that time, 50% of *P. maculatus* females change sex to male at age 5 yr (45-50 cm) and 100% at age 8 yr (60 cm) (Ferreira 1993). Therefore, *P. maculatus* must grow faster to reach a size twice that of *L. carponotatus* in the same time frame, and females must also take advantage of their reproductive effort over a shorter time period than *L. carponotatus*. The mtDNA control region mutation rate of *P. maculatus* (5.35%), which is 2.7 times faster than that of *L. carponotatus* (2.0%), combined with the shorter estimated generation time of a protogynous hermaphrodite may be the reason why *P. maculatus has* greater genetic diversity yet is the younger of the two species according to coalescence analysis.

The temporal phylogentic variation, as evidenced by the presence of the two clades in both species, demonstrates bottleneck and expansion events in the history of both species. Quaternary interglacial periods around 130 kya, 200 kya, 330 kya and 400 kya all occur within the potential GBR-lifespan of *L. carponotatus* (Galewsky et al. 1996; Bard et al. 2002; Lambeck et al. 2002). *P. maculatus*, with its remnant group, may represent three expansion periods due to sea level rise and fall during three of the most recent inter-glacial periods. The youngest clade, clade A (0 – 161kya), may have expanded during the most recent inter-glacial period in the Holocene only 10 kya (Flood 1983) or in the earlier interglacial period 130kya. The distinct clades may represent either different GBR refugial populations or immigrants from genetically differentiated locations outside the GBR or a combination of the two, as was also suggested for the two deeply divergent *Acanthochromis polyacanthus* lineages along the length of the GBR (van Herwerden and Doherty 2006). We have no evidence to favour either hypothesis over the others, but note that fossil reefs on the GBR would have been likely candidates for refugial populations to persist at times when the GBR was emergent (Webster et al. 2008). Further analysis of other individuals from locations further to the north (e.g. Taiwan) may help determine whether one of the clades is refugial or consists of immigrants from elsewhere.

6.4.1 Implications for management

At present populations of both *P. maculatus* and *L. carponotatus* on the GBR are managed spatially as single stocks. Despite the evidence for temporal (as opposed to spatial) clades of *P. maculatus* and *L. carponotatus*, the geographical distribution of individuals from both clades in all locations suggests that both species are panmictic and are interconnected on an evolutionary time-scale. Although GBR fisheries are healthy relative to global standards, studies of no-take

MPA show that stocks of *Plectropomus* spp. are typically lower in open reefs than in no-take MPA (Williamson et al. 2004; Evans 2008; Evans et al. 2008; Russ et al. 2008). The pressure on inshore fish stocks is increasing due to Queensland's human population growth, and increased recreational fishing effort.

Exchange of a limited number of migrants between sub-populations is sufficient to maintain apparent panmixia (Ovenden 1990), but may not provide ample connectivity to maintain a fishery in exploited areas. Therefore, refined ecological testing is required to determine actual ecological limits of dispersal to realize contemporary population connectivity and to test the present level of protection within the current distribution of the no-take MPA network on the GBR. New trans-generational isotope markers are an excellent physical tag and are improving our understanding of larval dispersal but are expensive and time consuming and still require genetic parentage analyses or assignment tests to determine actual parents in fine scale studies (Jones et al. 2005; Almany et al. 2007). Physical stable isotope markers provide the necessary validation of larval dispersal, but may become obsolete as quicker and less expensive techniques are developed, such as parentage and assignment methods (Manel et al. 2005) which use microsatellite data and assign individuals to locations if possible or else exclude them. These techniques coupled with modern three-dimensional oceanographic modelling, are likely to revolutionise connectivity studies in the future.

Chapter 7. General Discussion

There were four major objectives of this study. The first was to investigate the effects of 14 years of protection on species targeted by fisheries in three near shore island groups separated by 700km. The second objective was to use a Before-After-Control-Impact-Pair sampling design to examine the short-term effect of the 2004 zoning plan on fish and benthic communities in the same near shore islands. The third objective was to determine the effects of at least 14 years of protection on the batch fecundity per unit area of *Lutjanus carponotatus*. This latter study also allowed the investigation of how fish size affects egg size and batch fecundity. The fourth objective was to use fast mutating mitochondrial DNA to examine the rates of genetic exchange within and between the island groups in this study.

Overall, no-take marine protected areas (MPAs) around three near shore islands of the Great Barrier Reef have been successful at protecting species targeted by fishers. The results show that density and biomass of the primary target species, *Plectropomus* spp., increased in MPAs while fished areas remained relatively stable. Furthermore, this research showed that the biomass and batch fecundity per unit area of the secondary target species, *Lutjanus carponotatus*, was also significantly higher in MPAs than in fished areas after 14 years of protection. Genetic connectivity between the island groups was extensive at an evolutionary time-scale with no geographical distinction for either of the focal species. Benthic variables and fish species not targeted by fishers were not affected by the original or the new zoning plans.

7.1 Comparing original zoning results to 2004 zoning results

Chapter 2 lacked temporal data, an attribute common to most MPA studies, so there was no way to know how long it took for populations of targeted species to increase in the protected areas. The results of chapter 3 from the BACIP sampling design for the new (2004) zoning plan

suggest that it might have occurred rapidly, within a few years. However, this is purely speculative. Community compliance within MPAs in the mid 1980's may not have been the same as it was in 2004 and beyond. There was very little evidence of the benefits of MPAs during those earlier times and illegal fishing in MPAs (poaching) may have been more prevalent then than now. A recent study around the near shore islands shows that poaching levels decreased at times of increased surveillance (Davis et al. 2004). With growing awareness of the evidence of MPA benefits, increased public awareness campaigns and surveillance after the implementation of the 2004 zoning plan, compliance levels may have increased, thus allowing the target species in the MPAs better growth and survival than may have been achieved pre-2004.

Different sites obviously had to be used to assess the effects of the new zoning plan, so direct comparison of the results between chapters 2 and 3 was not possible. However, comparing the rate of increase in target fish biomass in the new zones relative to the results of the old zoning raises an interesting issue. In the MPAs of the Palm and Whitsunday Islands, where there was no negative impact of coral bleaching (as was the case in the Keppel Islands), the density and biomass of both *Lutjanus carponotatus* and *Plectropomus* spp. had reached or exceeded the results recorded for 14 yrs of protection (old zoning) after just three years from 2004 to 2007. Significant increases in density and biomass of species targeted by fisheries have been found in the Philippines within 3-4yrs (Russ et al. 2005) and a meta-analysis of 89 MPAs suggested a significant increase in fish density and biomass within 1-3 yrs (Halpern 2003). However, this thesis suggests that within 3 yrs the density and biomass of both species had exceeded the results obtained for the same species in associated areas that were protected for 14 yrs. This is much faster than rates of increase recorded in the Philippines, where target species biomass was still increasing 9-18 yrs after protection (Russ et al. 2004). This raises several questions that are yet to be answered: 1) Are the newly protected sites on the GBR going to continue to increase

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in target fish biomass? 2) If so why would these sites produce higher biomass than the old sites? 3) Were the original protected sites receiving the total compliance of all fishers (i.e. could potentially higher biomass have been reached if poaching was insignificant)? Questions 1 and 2 are tractable, requiring further study. Question 3 is difficult to answer, as are so many historical questions.

7.2 Reproductive connectivity

Protecting a species targeted by fisheries in a no-take marine protected area is a positive result. However, for fishers to finally accept MPAs as a tool that enhances their fishery, scientists need to demonstrate net larval and/or adult export (Russ 2002). Chapters 4 and 6 of this thesis do not demonstrate larval dispersal from MPAs but they advance knowledge on the way to achieving this goal. The results of this study show that larger individuals produce more eggs of larger size. Furthermore, other studies have shown that larger individuals reproduce for longer (Kritzer 2004) and therefore produce greater quantities of larger and more viable eggs that could potentially disperse within or away from their natal reef. The latest studies show that some reef fish have 60% self-recruitment and 40% go elsewhere or die (Jones et al. 2005, Almany et al. 2007). Movement away from the natal reef could be potentially up to tens to hundreds of kilometres away. Based on the results of chapter 4 the eggs of the larger fish in the MPAs have the potential to survive for a longer period and therefore may survive to disperse over longer distances. Many MPAs are surrounded by fished areas on contiguous reefs or if the MPA covers the whole reef, it is typically not far from a fished reef, as is the case on the GBR. Considering these relatively short distances, then the larvae do not have to travel far to ensure larval export form MPAs.

The genetic analysis in chapter 6 demonstrated evolutionary connectivity within and between island groups. If this level of connectivity was also occurring at ecological time scales, it would clearly indicate that MPAs are exporting recruits to fished areas adjacent to MPAs (and probably further afield). However, evolutionary and ecological time scales may not have congruent levels of larval export. This chapter showed that using the hypervariable control region of the mitochondrial DNA is too coarse a tool to determine ecological connectivity for the two study species. If this study had shown high haplotype diversity and geographic partitioning then this may have been an appropriate tool for further investigation at a fine-scale using genetic assignment methods. This does not mean that the study did not contribute to this area of science. Two interesting results and one new methodological advance were made during this study. 1) The use of mitochondrial DNA demonstrated some level of inter-connectedness within and between the island groups in this study. This implies that MPAs within this study definitely have some level of larval connection. 2) Within the sampled area of the GBR populations there are at least two genetically distinct lineages for both species which may provide some resilience in the current environment of climate change. 3) A new *in-situ* biopsy probe was developed for collecting tissue samples for molecular studies (Evans 2008). This technique could be useful for a number of studies where fish mortality is undesirable or would hinder the results of the study. Examples include collection of fish tissue samples at spawning aggregations, collection inside MPAs or from endangered species.

7.3 Management Implications

This study was very timely in relation to the introduction of the new zoning plan in 2004 on the GBR. There was very little previous evidence of benefits from MPAs on the Great Barrier Reef (e.g. Williams and Russ 1994). Chapter 2 of this thesis provided evidence of the benefits for species targeted by fishers within MPAs during the public consultation process for the

Representative Area Program (RAP). Recreational fishers were among the most vocal groups opposing the RAP, and their main arguments were that they (recreational fishers) did not have an impact on fish stocks, and that MPAs would not increase fish stocks. Evidence from Chapter 2 contradicts their arguments with some of the best evidence of zoning effects on the GBR collected where recreational fishers are the only group fishing. At present the stocks are probably not overly exploited, but management should be aware of the issues that may arise as coastal human populations expand. The results from chapter 3, along with publications and reports from the continued monitoring of the sites through until 2012, will provide valuable information to all stakeholders to better understand and appreciate the management implications of MPAs on the fish and coral communities around the near shore islands of the GBR.

A network of no-take marine protected areas is very likely to be of benefit to all marine systems. However, such networks have to be used in conjunction with other fishery regulations to reduce over fishing in the areas left open for fishing (Bohnsack 2000; Hilborn et al. 2006). All three island groups showed declines in density of targeted fish species in the fished area after the 2004 zoning plan. However, in 2 of the 3 island groups there was recovery in the fished areas through time. New, stronger fishery regulations were introduced at the same time as the new zoning plan in 2004 (QLD Reef Fish Management Plan 2003). These regulations reduced the bag limit for many key species and may have helped to reduce the potential increased effort in the remaining fished areas. More time is required to resolve this but it is an important fact that cannot be ignored. MPAs alone are not the best management practice to ensure continued coral reef fisheries. There needs to be well understood scientifically based fishery regulations set in place in conjunction with MPAs containing a reservoir of spawning stock biomass, to ensure coral reef fisheries into the future (Bohnsack 2000; Hilborn et al. 2006; Mapstone et al. 2008). On the GBR this management process is already in place and

seems to be working (QDPI&F 2007; Mapstone et al. 2008). However, this management will have to be adaptive in the face of rapidly growing human populations along the Queensland coast and climate change.

The changes to the benthos and the subsequent fish declines in the Keppel Islands in 2006 that are shown in chapter 3 of this thesis illustrated the inability of MPAs to counteract the negative impacts of climate change and coral bleaching (Jones et al. 2004). Coral reefs are at the fragile edge of their survival range regarding ocean temperatures and ocean acidification (Hoegh-Guldberg et al. 2008). Unfortunately, these serious issues are due to global warming, which extend well outside the range of control of many coral reef managers and Governments all over the world. In the mean time, coral reef managers need to focus on helping their local coral reefs by ensuring water quality and good fishery practices (i.e. maintenance of adequate spawning stock biomass of targeted species).

7.4 Ideas arising from the thesis and Future research

Recovery of a target reef species depends on the degree of depletion before protection is applied and also the state of the reef over time (Russ et al. 2005). The Great Barrier Reef has a long history of good fishery management and the fish stocks are generally not exploited as much as those of other nations (Mapstone et al. 2008). In fact the baseline densities and biomass for *Plectropomus* spp. in the new sites intended for protection were already higher than the old zoning fished areas when surveyed in mid-2004. This suggests that either there were lower levels of fishing in these areas, which is highly unlikely, or the conditions in these sites were more favourable than in the fished areas. Benthic variables and non-target species abundance were not significantly different, ruling out habitat effects (chapters 2 and 3). Additionally, prey densities did not differ between MPAs and fished sites, rejecting theories of

prey limitation (chapter 3). Adult natural survival and movement, as well as recruitment success and/or survival are important aspects to investigate in the future to potentially explain the rapid increases in *Plectropomus* spp. abundance reported in chapter 3.

With so much positive evidence from within MPAs, more effort is required to demonstrate adult and larval export. With the development of stable isotope trans-generational markers and microsatellite genetic markers, self-recruitment levels of 60% have been demonstrated for two small reef fish species, one with demersal eggs (*Amphiprion percula*) and the other a broadcast spawner (*Chaetodon vagabundus*) (Almany et al. 2007). This research now needs to be scaled up to study fishery target species. Williamson et al (in press) tested the toxicity of such markers in the common coral trout and found levels were within safe levels for the fish and for human consumption. Such research opens the way for a large larval tagging experiment in the Keppel islands, GBR (Williamson et al. unpublished)

To carry out such a large-scale fishing experiment successfully inside and outside MPAs, a large scale collaboration of ecologists, geneticists, oceanographic modellers, and fishermen will be required. They would have to capture, physically tag externally and internally (to avoid re-injection and to investigate adult movement), remove a tissue sample for genetics, inject the trans-generational marker and release fish with as little distress as possible. Ideally an acoustic array would be set up to track fish movements over time. At the same time, a series of current meters would need to be deployed to collect water temperatures and current regimes in the local area. After all this is achieved, a large-scale collection of recruits is required. To complete this phase of the experiment, detailed knowledge of spawning times, pelagic larval durations, juvenile growth rates (to maximize catch per unit effort) and recruitment habitats are essential for success. Depending on the results from such an experiment, scientists may be able to say unequivocally that MPAs are exporting either adults and/or larvae to fished areas or vice versa.

The use of genetic markers for connectivity studies is growing rapidly. Mitochondrial markers did not provide enough resolution at a large-scale so it would not be adequate for a fine-scale study of the two focal species in this study. However, there is growing evidence that microsatellite markers are suitable for such studies (Jones et al. 2005). If this is the case, the biopsy probe developed in this thesis will be a valuable tool for collecting large numbers of tissue samples in a cost effective way without imposing mortality on fish populations. Although the results of chapter 5 were successful, there remains room for improvement. Further adjustments are required to increase the success rate (number of samples collected: number of fish hit with the biopsy probe) from 70-80% to 100%.

To summarize, no-take marine protected areas are effective conservation tools. The information in this thesis, and the broader literature, lends one to believe that MPAs would have some fishery benefits via larval and adult export to fished areas. There is still an absence of unequivocal evidence to show this but new methods are developing and such evidence should not be too far away. However, most research shows that MPAs are not resilient to climate change. At the scale of reef management, managers must do everything possible to keep reef systems locally resilient. Therefore coral reefs require a dual approach of effective networks of no-take MPAs combined with rigorous conservation and fisheries management outside the MPAs.

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Appendix 1: List of surveyed fish species (2004 - 2007), the GBR commercial/recreational fishery status of each species and the analysis group to which each species was assigned. NT = Non-target species; ST = Secondary Target species; T = Target species; P = Protected species; * = Species surveyed at level of genus; # = Species not included in biomass calculations.

FAMILY / SPECIES	FISHERY STATUS	ANALYSIS GROUPS
Acanthuridae		
Acanthurus blochii	NT	Acanthurid
Acanthurus dussumieri	NT	Acanthurid
Acanthurus grammoptilus	NT	Acanthurid
Acanthurus lineatus	NT	Acanthurid
Acanthurus nigracauda	NT	Acanthurid
Acanthurus nirgrofuscus	NT	Acanthurid
Acanthurus xanthopterus	NT	Acanthurid
Ctenochaetus binotatus	NT	Acanthurid
Ctenochaetus striatus	NT	Acanthurid
Naso annulatus	NT	Acanthurid
Naso brevirostris	NT	Acanthurid
Naso lituratus	NT	Acanthurid
Naso tuberosus	NT	Acanthurid
Naso unicornis	NT	Acanthurid
Prionurus microlepidotus	NT	Acanthurid
Zebrasoma scopas	NT	Acanthurid
Zebrasoma veliferum	NT	Acanthurid
Chaetodontidae		
Chaetodon auriga	NT	Chaetodontid
Chaetodon aureofasciatus	NT	Chaetodontid
Chaetodon baronessa	NT	Chaetodontid
Chaetodon citrinellus	NT	Chaetodontid
Chaetodon ephippium	NT	Chaetodontid
Chaetodon flavirostris	NT	Chaetodontid
Chaetodon kleinii	NT	Chaetodontid
Chaetodon lineolatus	NT	Chaetodontid
Chaetodon lunula	NT	Chaetodontid
Chaetodon lunulatus	NT	Chaetodontid
Chaetodon melannotus	NT	Chaetodontid
Chaetodon plebius	NT	Chaetodontid
Chaetodon rainfordi	NT	Chaetodontid
Chaetodon speculum	NT	Chaetodontid
Chaetodon trifascialis	NT	Chaetodontid
Chaetodon unimaculatus	NT	Chaetodontid
Chelmon rostratus	NT	Chaetodontid
Coradion altivelis	NT	Chaetodontid
Coradion chrysozonus	NT	Chaetodontid
Forcipiger flavissimus	NT	Chaetodontid
Heniochus acuminatus	NT	Chaetodontid

Heniochus varius	NT	Chaetodontid
Heniochus monoceros	NT	Chaetodontid
Haemulidae		
Diagramma pictum	ST	Benthic Predator
Plectorhinchus chrysotaenia	ST	Benthic Predator
Plectorhinchus chaetodonoides	ST	Benthic Predator
Plectorhinchus flavomaculatus	ST	Benthic Predator
Plectorhinchus gibbosus	ST	Benthic Predator
Plectorhinchus lessoni	ST	Benthic Predator
Plectorhinchus lineatus	ST	Benthic Predator
Plectorhinchus unicolor	ST	Benthic Predator
Labridae		
Anampses caeruleopunctatus*	NT	Small Labrid
Anampses geographicus*	NT	Small Labrid
Anampses neoguinaicus*	NT	Small Labrid
Bodianus axillaris*	NT	Benthic Predator
Bodianus mesothorax*	NT	Benthic Predator
Cheilinus chlorurus	NT	Benthic Predator
Cheilinus fasciatus	NT	Benthic Predator
Cheilinus trilobites	NT	Benthic Predator
Cheilinus undulatus	P	Benthic Predator
Choerodon anchorago	ST	Benthic Predator
Choerodon cephalotes	ST	Benthic Predator
Choerodon cyanodus	ST	Benthic Predator
Choerodon fasciatus	NT	Benthic Predator
Choerodon graphicus	ST	Benthic Predator
Choerodon monostigma	ST	Benthic Predator
Choerodon schoenleinii	ST	Benthic Predator
Choerodon vitta	ST	Benthic Predator
Epibulus insidiator	NT	Benthic Predator
Gomphosus varius	NT	Benthic Predator
Halichoeres chloropterus *	NT	Small Labrid; Prey
Halichoeres margaritaceus *	NT	Small Labrid; Prey
Halichoeres marginatus *	NT	Small Labrid; Prey
Halichoeres melanurus *	NT	Small Labrid; Prey
Halichoeres nigrescens *	NT	Small Labrid; Prey
Halichoeres prosepeion *	NT	Small Labrid; Prey
Halichoeres trimaculatus *	NT	Small Labrid; Prey
Hemigymnus melapterus	NT	Benthic Predator
Hemigymnus fasciatus	NT	Benthic Predator
Labroides bicolor *	NT	Small Labrid
Labroides dimidiatus *	NT	Small Labrid
Oxychelinus diagrammus	NT	Benthic Predator
Stethojulis bandanensis *	NT	Small Labrid
Stethojulis strigiventer *	NT	Small Labrid
Thalassoma amblycephalum *	NT	Small Labrid; Prey
Thalassoma hardwicke *	NT	Small Labrid; Prey

*Thalassoma lunare *	NT	Small Labrid; Prey

Lethrinidae

*Gymnocranius euanus *	ST	Benthic Predator
*Gymnocranius sp. *	ST	Benthic Predator
Lethrinus atkinsoni	ST	Benthic Predator
Lethrinus harak	ST	Benthic Predator
Lethrinus laticaudus	ST	Benthic Predator
Lethrinus lentjan	ST	Benthic Predator
Lethrinus miniatus	T	Benthic Predator
Lethrinus nebulosus	T	Benthic Predator
Lethrinus obsoletus	ST	Benthic Predator
Lethrinus olivaceus	ST	Benthic Predator
Lethrinus ornatus	ST	Benthic Predator
Monotaxis grandoculis	ST	Benthic Predator

Lutjanidae

Lutjanus argentimaculatus	T	Other Lutjanid
Lutjanus carponotatus	ST	*Lutjanus carponotatus*
Lutjanus fulviflamma	ST	Other Lutjanid
Lutjanus fulvus	ST	Other Lutjanid
Lutjanus kasmira	ST	Other Lutjanid
Lutjanus lemniscatus	ST	Other Lutjanid
Lutjanus lutjanus	ST	Other Lutjanid
Lutjanus monostigma	ST	Other Lutjanid
Lutjanus quinquelineatus	ST	Other Lutjanid
Lutjanus russelli	ST	Other Lutjanid
Lutjanus sebae	T	Other Lutjanid
Lutjanus vitta	ST	Other Lutjanid

Mullidae

Parupeneus barberinoides	NT	Benthic Predator
Parupeneus barberinus	ST	Benthic Predator
Parupeneus ciliatus	NT	Benthic Predator
Parupeneus indicus	ST	Benthic Predator

Nemipteridae

Scolopsis bilineatus	NT	Benthic Predator
Scolopsis margaritifer	NT	Benthic Predator
Scolopsis monogramma	NT	Benthic Predator

Pomacentridae

|		
Abudefduf sexfasciatus	NT	Pomacentrid; Prey
Abudefduf whitleyi	NT	Pomacentrid; Prey
Acanthochromis polyacanthus	NT	Pomacentrid; Prey
Amblyglyphidodon aureus	NT	Pomacentrid; Prey
Amblyglyphidodon curacao	NT	Pomacentrid; Prey
Amblyglyphidodon leucogaster	NT	Pomacentrid; Prey
Amphiprion akindynos	NT	Pomacentrid; Prey

*Amphiprion clarkii**	NT	Pomacentrid; Prey
*Amphiprion melanopus**	NT	Pomacentrid; Prey
*Amphiprion percula**	NT	Pomacentrid; Prey
*Amphiprion perideraion**	NT	Pomacentrid; Prey
*Chromis nitida**	NT	Pomacentrid
*Dascyllus aruanus**	NT	Pomacentrid; Prey
*Dascyllus reticulatus**	NT	Pomacentrid; Prey
*Dascyllus trimaculatus**	NT	Pomacentrid; Prey
*Dischistodus melanotus**	NT	Pomacentrid; Prey
*Dischistodus perspicillatus**	NT	Pomacentrid; Prey
*Dischistodus prosopotaenia**	NT	Pomacentrid; Prey
*Dischistodus pseudochrysopoecilus**	NT	Pomacentrid; Prey
Neoglyphidodon melas	NT	Pomacentrid; Prey
Neoglyphidodon nigroris	NT	Pomacentrid; Prey
Pomacentrus amboinensis	NT	Pomacentrid; Prey
Pomacentrus moluccensis	NT	Pomacentrid; Prey
Stegastes apicalis	NT	Pomacentrid; Prey

Scaridae

Bulbometopon muricatum	NT	Scarid
Cetoscarus bicolor	NT	Scarid
Chlorurus bleekeri	NT	Scarid
Chlorurus microrhinos	NT	Scarid
Chlorurus sordidus	NT	Scarid; Prey
Hipposcarus longiceps	NT	Scarid
Scarus altipinnis	NT	Scarid
Scarus chameleon	NT	Scarid
Scarus dimidiatus	NT	Scarid
Scarus flavipectoralis	NT	Scarid
Scarus forsteni	NT	Scarid
Scarus frenatus	NT	Scarid
Scarus ghobban	NT	Scarid
Scarus globiceps	NT	Scarid
Scarus niger	NT	Scarid
Scarus psittacus	NT	Scarid
Scarus rivulatus	NT	Scarid; Prey
Scarus rubroviolaceus	NT	Scarid
Scarus schlegeli	NT	Scarid
Scarus spinus	NT	Scarid

Serranidae

Anyperodon leucogrammicus	ST	Other Serranid
Cephalopholis argus	ST	Other Serranid
Cephalopholis boenak	ST	Other Serranid
Cephalopholis cyanostigma	ST	Other Serranid
Cephalopholis miniata	ST	Other Serranid
Cephalopholis microprion	ST	Other Serranid
Cromileptes altivelis	P	Other Serranid
Epinephelus caeruleopunctatus	ST	Other Serranid

Epinephelus coioides	ST	Other Serranid
Epinephelus cyanopodus	ST	Other Serranid
Epinephelus fuscoguttatus	ST	Other Serranid
Epinephelus lanceolatus	ST	Other Serranid
Epinephelus maculatus	ST	Other Serranid
Epinephelus merra	ST	Other Serranid
Epinephelus ongus	ST	Other Serranid
Epinephelus polyphekadion	ST	Other Serranid
Epinephelus quoyanus	ST	Other Serranid
Plectropomus laevis	T	*Plectropomus* spp.
Plectropomus leopardus	T	*Plectropomus* spp.
Plectropomus maculatus	T	*Plectropomus* spp.
Siganidae		
Siganus argenteus	NT	Siganid
Siganus corallinus	NT	Siganid
Siganus doliatus	NT	Siganid
Siganus fuscescens	NT	Siganid
Siganus lineatus	NT	Siganid
Siganus puellus	NT	Siganid
Siganus punctatus	NT	Siganid
Siganus spinus	NT	Siganid
Siganus vulpinus	NT	Siganid

Appendix 2

Plectropomus maculatus
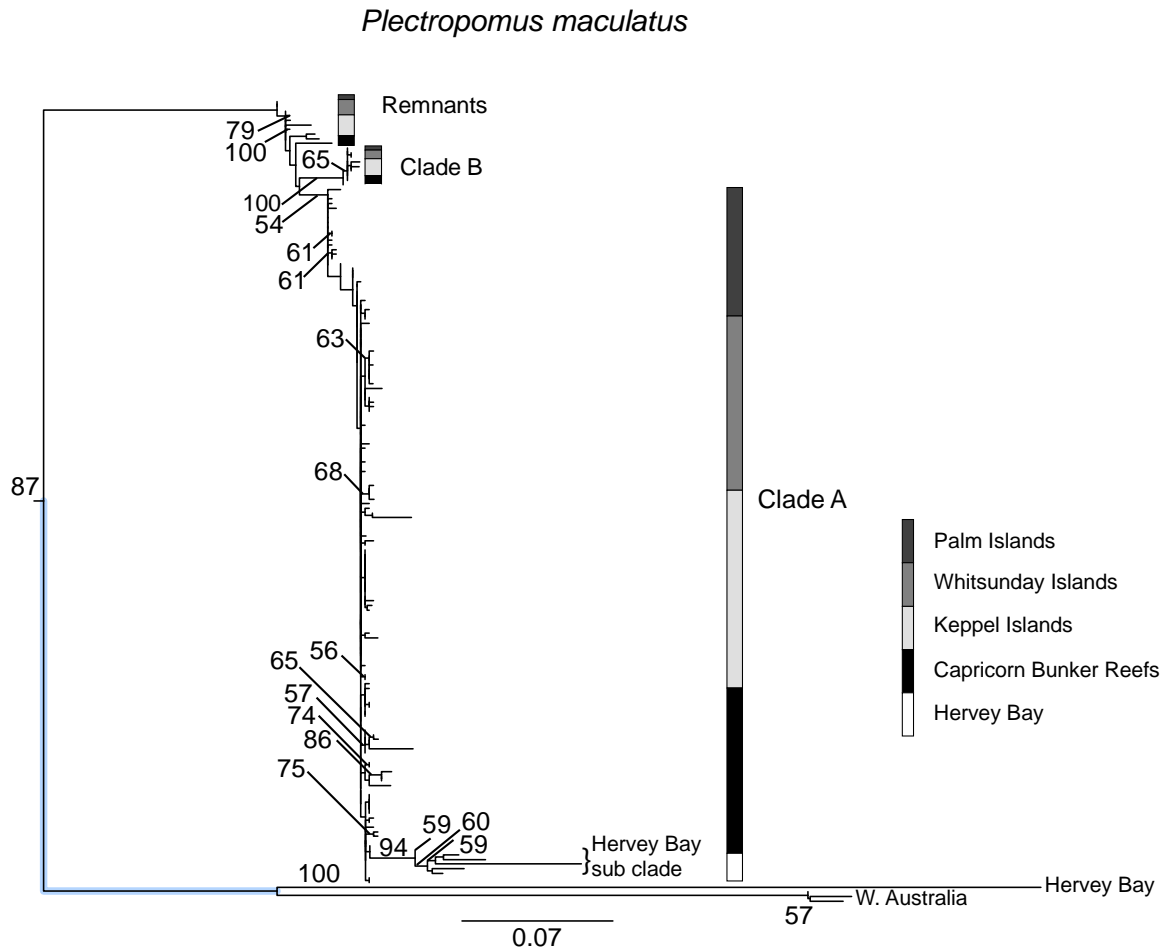
Appendix 2: Best out-group rooted maximum likelihood (ML) tree of mitochondrial control region from 171 individuals of *Plectropomus maculatus* (three individuals of *P. maculatus* from West Australia as the out-group) Numbers on inter-nodes indicate bootstrap support values obtained from 100 bootstrap replicates. Remnant *P. maculatus* samples are those which do not belong to either sister clade A or B. Shaded bars indicate proportional representation of individuals from each Island group, as identified by the embedded key to the Figure. Hervey Bay samples were obtained from van Herwerden et al. (2006) to determine the evolutionary relationship between *P. maculatus* from this location and the from GBR sites investigated here.