Fishery effects and benefits of marine protected areas within the Great Barrier Reef Marine Park.

Thesis submitted by

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DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in 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 A1001).

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CONTRIBUTION OF OTHERS TO THIS THESIS

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- Contributed baseline visual survey data for coral trout (Chapter 3)

Richard Evans (School of Marine & Tropical Biology, James Cook University):

- Assistance with field trip logistics and data collection (Chapters 5 & 8)

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THESIS ABSTRACT

No-take marine reserves (NTRs) are areas of the marine environment in which fishing and all other extractive activities are prohibited. In many regions of the world, NTRs are now relatively widespread and they are considered to be a fundamental tool in achieving sustainable management of marine habitats, communities and ecosystems. There is a wealth of evidence that networks of adequately protected NTRs can potentially buffer the negative effects of marine resource exploitation, protect or restore natural states of biodiversity and ecosystem function, and deal with many fishery problems that are not effectively addressed by more traditional management measures.

It has been well documented that the abundance, average size, age and per-capita fecundity of fish species which are targeted by fisheries often increase significantly within adequately protected NTRs. It is expected that NTRs may also function to maintain and perhaps enhance fishery yields via recruitment subsidies to fished areas from enhanced populations within NTRs ('recruitment subsidy') and via net-emigration of post-settlement fish from reserves to surrounding fished areas ('spill-over effect'). However, due to the difficulties associated with tracking the dispersal of microscopic pelagic larvae in the marine environment, empirical demonstrations of the potential contribution of NTRs to enhancing fish populations in surrounding areas via recruitment subsidy remain elusive.

This thesis builds on the body of evidence that adequately protected NTRs can generate benefits for exploited species by presenting fishery independent data on the effects of long term NTR protection (12 – 20 years) on fish populations within Australia's iconic Great Barrier Reef Marine Park (GBRMP). Data presented in Chapters 3, 4, and 5 was generated using standard underwater

visual census (UVC) methodologies which have been widely used to assess patterns of distribution and abundance in reef fishes and to assess the structure of fish and benthic communities. The research presented here also extends to the development and testing of new tools for tracking fish larval dispersal and defining the demographic connectivity of fish populations. Chapters 6 and 7 of this thesis present the findings of two experiments which assessed the safety and effectiveness of transgenerational isotope labeling (TRAIL) of reef fish larvae using enriched stable isotopes of barium. The final data chapter of this thesis (Chapter 8) provides an overview of research currently underway which is attempting to demonstrate both NTR recruitment subsidies and spill-over effects for target fish species within the GBRMP.

Chapter 3 of this thesis presents quantitative estimates, UVC data, of density and biomass of coral trout (*Plectropomus* spp.), the major target of the hook and line fisheries on the Great Barrier Reef (GBR), Australia. Data was collected from inshore fringing reefs of the Palm and Whitsunday Island groups, 3-4 years before (1983-1984), and 12-13 years after (1999-2000) the establishment of NTRs in 1987. Density and biomass of coral trout increased significantly (by factors of 5.9 and 6.3 in the Palm Islands and 4.0 and 6.2 in the Whitsunday Islands) in the NTR sites, but not the fished sites, between 1983-1984 and 1999-2000. In 1999-2000, density and biomass of coral trout, and a secondary target of the fisheries, the stripey snapper (*Lutjanus carponotatus*), were significantly higher in NTR zones than in the fished zones at both island groups. The density and biomass of non-target fish species (Labridae, Siganidae and Chaetodontidae) did not differ significantly between NTR and fished zones at either island group. Results are also presented for a range of other fish species and groups

Chapter 4 examines the short-term (bi-annual samples over a 3 year period) temporal dynamics of populations of *Plectropomus* spp., *Lutjanus carponotatus*, Siganidae and Chaetodontidae within

NTR and fished zones of the Palm Island group. Although considerable temporal variation in mean density and biomass was detected for all fish groups, persistent and significant effects of NTR protection were evident for the target fish groups (*Plectropomus* spp. and *L. carponotatus*), while as expected, no clear effects of NTR protection were detected for the control (non-target) fish groups (Siganidiae and Chaetodontidae). It was determined that when examining the effects of NTR protection on fish populations, bi-annual UVC sampling of these reefs provided little, if any, benefit over once-yearly sampling. Quantitative estimates of fishing effort near NTRs and rates of zoning infringements (poaching) within the Palm Island group are also provided within chapter 4. It was calculated that the total fishing effort on the fringing reefs surrounding Pelorus Island (fished zone) was approximately 1164 vessels per year, while approximately 91 vessels per year were illegally fishing within the Orpheus Island NTR.

Chapter 5 presents long-term (5 – 9 years) temporal UVC monitoring data for fish populations and the sessile benthic (coral) community on fringing reefs of the Palm, Whitsunday and Keppel Island groups. As in chapter 4, significant temporal variability in mean density and biomass was detected in all fish groups (*Plectropomus* spp., *L. carponotatus*, Siganidae, Chaetodontidae) and in the mean cover of live coral. However, the strong effects of NTR protection on target fish species persisted throughout the monitoring period in all three island groups. As previously shown, there were no detectable effects of NTR protection on non-target fish groups or on the benthic community. Data presented in chapters 3, 4 and 5 of this thesis have provided some of the most convincing evidence available that the management zoning of the GBRMP has been effective in protecting target fish species. Enhanced populations of exploited fish species within the NTRs examined here should lead to recruitment subsidy and spill-over benefits for surrounding fished areas. In order to begin examining export effects of NTRs it was necessary to develop and test techniques for tracking larvae of pelagic spawning fish in the marine environment.

Injection of an enriched stable isotope barium chloride ($BaCl_2$) solution into female marine fish has been shown to provide an effective chemical marker that is transmitted to developing eggs and is subsequently detectable in the otoliths of larvae and juveniles. The technique provides a new means of mass-marking larval fish and facilitates investigations of larval dispersal patterns, demographic population connectivity and export effects of NTRs. However, successful field applications must be preceded by trials of the technique on target species within controlled conditions.

Chapter 6 of this thesis examines the toxicological and physiological responses of the common coral trout (*Plectropomus leopardus*), to injection of enriched stable isotope $BaCl_2$ solution. Thirty adult *P. leopardus* were subject to one of two $^{138}BaCl_2$ injection treatment groups (corresponding to dosage rates of 2 mg and 4 mg ^{138}Ba / kg body weight) and a control group in which fish were injected with 0.9% sodium chloride solution. Fish from each group were sampled at post-injection intervals of 48 hours, 1 week, 3 weeks, 5 weeks and 8 weeks, at which time blood and tissue samples were removed from each fish. Residual concentrations of barium and ^{138}Ba:^{137}Ba ratios were measured in muscle, gonad, liver and bone tissues of each experimental fish. Elevated barium concentrations were detected in all treatment fish tissue samples within 48 hours post-injection. Within muscle tissue, the highest residual barium concentration recorded was 0.29 mg Ba / kg wet weight, 1 week post-injection in the 4 mg ^{138}Ba / kg treatment group. Residual barium concentrations decreased throughout the remainder of the 8 week experimental period in all tissues except bone. The $BaCl_2$ injection had no significant effects on measured whole blood parameters or on the plasma concentrations of steroid hormones. It was concluded that enriched barium stable isotopes can be used at low dosages to mark larvae of commercially important marine fish, without adverse effects on the health of the fish or on humans who may consume them.

Chapter 7 provides details of a trial conducted to validate that injection of enriched stable barium isotopes (^{135}Ba and ^{137}Ba) produces unequivocal geochemical tags on the otoliths of offspring of the brown-marbled grouper (*Epinephelus fuscoguttatus*). The study also assessed potential negative effects on reproductive performance, egg size, condition and larval growth due to injection of adult female fish. The injection of enriched stable barium isotopes at 0.5 mg and 2.0 mg Ba / kg fish weight into the body cavities of gravid females were both 100% successful in the geochemical tagging of the otoliths of larvae from the first spawning after injection. The low dose rate had no negative effects on eggs or larvae. However, the higher dose rate of 2 mg Ba / kg body weight produced small reductions in yolk sac area, oil globule area, standard length and head depth of pre feeding larvae. Given the success of the 0.5 mg / kg dose rate, it is clearly possible to get a reliable mark and keep the concentration below any level that could affect larval growth or survival. The findings presented in chapters 6 and 7 demonstrate that enriched isotope barium injections provide an effective and safe means of mass-marking grouper larvae.

The next development for this project involves the utilisation of our existing monitoring data and these new larval marking technologies to begin examining larval dispersal, connectivity and export effects of NTRs in the field. Although this project is currently still in progress, chapter 8 provides an overview of the outcomes achieved to date. The study was carried out in the Keppel Islands. The specific objectives of the study were to; 1. Utilise the enriched stable isotope marking technique to track larvae of three target fish species (*Plectropomus maculatus, Lutjanus carponotatus, Epinephelus quoyanus*) from their natal reef of origin within NTRs to their settlement locations; 2. Measure demographic population connectivity, larval dispersal patterns and rates of self-recruitment within a network of marine reserves; 3. Track movements of adult fish within NTRs and from reserves to surrounding fished areas.

Of the recruit fish samples collected in May 2008 and February 2009, only 10 – 20% have currently been analysed for barium isotope markers. One barium tagged *P. maculatus* recruit (of 63 analysed) and one tagged *L. carponotatus* recruit (of 35 analysed) have been detected thus far. Of 23 *E. quoyanus* recruits analysed to date, no barium tagged individuals have been detected. Both of the tagged recruits obtained thus far were recaptured on reefs which are in very close proximity (less than 2km) to the natal reef on which they were spawned. The tagged *P. maculatus* recruit dispersed from its natal NTR reef and settled into an adjoining fished zone reef. The tagged *L. carponotatus* recruit was retained (self-recruited) within its natal NTR reef. The number of barium tagged recruits is likely to increase as further otolith samples are analysed, at which stage, an in depth analysis of dispersal patterns will be conducted.

To date, 12 adult *E. quoyanus* and 3 *L. carponotatus* have been recaptured in close proximity to the marine reserve boundary which runs between the western end of Clam Bay and the northern end of Halfway Island, Keppel Island group. In these 15 cases, the total distance moved from tagging to recapture location is less than 1 km. Three *P. maculatus* have also been captured outside of marine reserve boundaries. Two of these fish moved a distance of approximately 2 km, from within the Clam Bay NTR, to areas outside the reserve. One individual *P. maculatus* which was tagged at the Middle Island reserve (23°10.066´ S, 150°55. 042´ E) in January 2008 was recaptured at Middle Rock (23°59.799´ S, 151°46.531´ E) in November 2008. This fish was 430 mm in total length, and the movement undertaken represents a straight line distance of approximately 125 km.

Although the results of the study reported in Chapter 8 are currently incomplete, a significant amount of valuable information has already been obtained. Of fundamental importance, is the demonstration that the trans-generational larval marking technique which was trialed and reported

in chapters 6 and 7 of this thesis, can be successfully applied to large, pelagic spawning reef fish species in the field.

The findings presented in this thesis have provided a significant contribution to the understanding of the effects of NTRs on fish populations within the GBR Marine Park. It is my hope that utility will be found in the data presented here and that it can be treated as a robust baseline for continued monitoring of fish and benthic communities on these inshore fringing reefs. Furthermore, I encourage researchers to continue to push the boundaries of what has traditionally been seen as possible, form multi-skilled collaborative teams and tackle the formidable issues currently facing coral reefs and other marine ecosystems.

Chapter 1: General Introduction

1.1. The global status of marine ecosystems and fish stocks

During the last century the human population quadrupled and the demand for basic commodities has increased at an exponential rate. Marine environments have been under increasing pressure to provide food, employment and recreation for millions of coastal peoples. Unfortunately, increased use has brought user conflicts and many marine habitats have been severely degraded (Worm *et al.* 2006; Halpern *et al.* 2008). Many of the worlds' fisheries have been over-exploited and several have collapsed completely (Pauly & Christensen 1995; Pauly *et al.* 2002; Worm *et al*. 2005; SOFIA 2007). The historical perception that marine resources are inexhaustible has been proven false (Roberts 2007). Ocean resources are finite, and human activities can be devastating to marine ecosystems and processes (Jennings *et al.* 1995; Jennings & Polunin 1996; Pauly *et al*. 1998; Jackson *et al*. 2001; Myers & Worm 2003; Myers *et al*. 2007; Roberts 2007; Halpern *et al*. 2008).

On a global scale fisheries are in trouble (Pauly *et al.* 2002; SOFIA 2007). Declining yields, stock collapse, decreasing catch per unit effort in spite of improved fishery technologies, reduced fish abundance, reduced average size and reproductive output of fish, loss of genetic variation, replacement of high value species by lower value species, increased by-catch mortality, recruitment failures and habitat destruction are all commonplace (Roberts 1997a, 2007; Pauly *et al.* 1998, 2002, 2005; Myers & Worm 2003, Zeller & Russ 2004; Myers *et al*. 2007). The situation is particularly serious on coral reefs, where fish stocks have been subject to unsustainable levels of exploitation due to growing human populations and the advent of lucrative live reef fish exports to Asian markets (Jennings & Polunin 1996; Polunin & Roberts 1996; Roberts 1997a; Sadovy & Vincent

2002). Furthermore, the impacts of climate change and the increased frequency of coral bleaching events is severely degrading coral reef ecosystems, altering benthic and fish communities and undermining many of the ecosystem goods and services which reefs have traditionally provided (Hughes *et al.* 2003, 2005; Bellwood *et al*. 2004; Munday *et al.* 2007, 2008).

Selective targeting and heavy exploitation of species of tropical reef fish at high trophic levels (eg. Serranidae: Epinephelinae, Lutjanidae and Lethrinidae) is of major concern to fisheries biologists and managers (Polunin & Roberts 1996; Bohnsack 1998; Jennings & Kaiser 1998; Russ 2002; Myers & Worm 2003; Birkeland & Dayton 2005). The life history characteristics of such species, and the formation of seasonal spawning aggregations, make them particularly vulnerable to overexploitation (Johannes 2000; Russell 2001; Sadovy & Vincent 2002; Sadovy & Domeier 2005; Sadovy De Mitcheson *et al.* 2008). Furthermore, depletion of large predatory reef fishes can cause significant impacts upon prey species, and the structure of coral reef communities (Bohnsack 1982; McClanahan and Muthiga 1988; Hughes 1994; Jennings *et al*. 1995; Roberts 1995; Jennings & Kaiser 1998; Graham *et al.* 2003). It has become critical that the ability of humans to catch fish be tempered with new approaches to preventing overfishing, resource depletion and habitat destruction (Bohnsack 1993; Roberts 1997a; Sale 2002; Sale *et al*. 1994, 2005; Mumby & Steneck 2008).

1.2. Development and use of no-take marine reserves

No-take marine reserves (NTRs), sometimes referred to as no-take marine protected areas (MPAs) or marine harvest refugia, are areas of the marine environment in which fishing and all other extractive activities are prohibited (Roberts & Polunin 1991; Jones *et al.* 1992; Dugan & Davis 1993; Russ & Alcala 1996a). In many regions of the world, NTRs are now relatively widespread

and are considered to be a fundamental tool in achieving sustainable management of marine habitats, communities and ecosystems (Bohnsack 1993; 1998; Roberts 1997a; Roberts & Hawkins 2000; Russ 2002; Sale *et al*. 2005). The increasing use of NTRs stems partly from the growing need to protect the marine environment from human impact and partly from the increasing evidence that reserve networks could be a relatively simple and effective means of managing coral reef fisheries (Russ 2002; Gell & Roberts 2003; Roberts *et al*. 2001, 2005). There is now much evidence that networks of adequately protected no-take marine reserves could buffer the negative effects of resource exploitation and address many fishery problems that are not effectively addressed by more traditional management measures (Plan Development Team (PDT) 1990; Roberts & Polunin 1991, 1993; Dugan & Davis 1993; Roberts 1995, 1997a; Russ & Alcala 1996a; Bohnsack 1998; Roberts *et al.* 2001, 2005; Russ 2002; Gell & Roberts 2003; Lubchenco *et al.* 2003).

Since 1984, the rate of establishment of new MPAs has increased by approximately 4.6% annually. However, only a proportion of those MPAs are designated as no-take and fewer still receive adequate protection and levels of compliance (Mora *et al*. 2006; Wood *et al*. 2008). Only 0.2% of the total marine area within exclusive economic zones and 1.4% of the world's coral reefs are currently protected within NTRs. Furthermore, most existing NTRs are small (less than a few km^2), and only about half form part of a coherent and representative network (Mora *et al.* 2006; Roberts 2007; Wood *et al*. 2008).

1.3. Coral Reef Fisheries and the use of NTRs

Fishing intensity on coral reefs has increased rapidly and in many areas this has occurred with little or no control and monitoring of catch and effort (Jennings *et al.* 1995; Jennings & Polunin 1996;

Polunin & Roberts 1996). Intense and sustained fishing pressure on coral reefs has been shown to lead to breakdowns in community and ecosystem structure, functioning and productivity. Some demonstrated examples of this come from the coral reefs of Jamaica and the Caribbean (Hughes 1994; Jackson *et al*. 2001) and the Seychelles (Jennings *et al.* 1995; Graham *et al*. 2008). Species of large predatory reef fish (serranids, lutjanids, lethrinids, carangids, haemulids and labrids) are often the primary targets of tropical reef fisheries and are the first to suffer from intense fishing pressure on reefs (Munro 1996; Russ & Alcala 1996b). Predatory fish species have all but disappeared from many heavily fished areas, local extinctions have occurred and the possibility of global extinction for some species is now being considered (Morris *et al*. 2000; Sadovy 2005).

Coral reef fisheries most commonly target many species of fish and with effort typically spread over a wide variety of gears (eg. hook & line, trap, spear, gill net etc.). Many of the typical fishing gears are relatively unselective, imposing mortality on a wide range of species (Munro & Williams 1985; Russ 1991; Bohnsack 1993; Munro 1996; Jennings & Kaiser 1998). Coral reef fisheries often display an uneven spatial distribution of fishing effort and in many regions, a large number of municipal (artisanal and subsistence) fishers land their catch at a large number of sites spread over a wide geographical area (Munro & Williams 1985; Russ 1991; Polunin & Roberts 1996). Accurate catch and effort information for these fisheries is extremely difficult to obtain (Munro & Williams 1985; Polunin & Roberts 1996). Thus, coral reef fisheries are notoriously difficult to manage with conventional fishery management methods (Munro & Williams 1985; PDT 1990; Polunin 1990; Roberts & Polunin 1991, 1993; Russ 1991).

Networks of NTRs have several inherent advantages over more conventional fishing controls in management of coral reef fisheries. Marine reserves can be implemented without the need for thorough information on population parameters and biological characteristics of every species and of the interactions between species (PDT 1990; Roberts & Polunin 1991, 1993; Bohnsack 1998).

Furthermore, the surveillance and enforcement of NTRs is relatively simple in comparison to enforcement of traditional fishing controls (Alcala & Russ 1990; PDT 1990; Polunin & Roberts 1996). The effectiveness of NTRs in sustaining marine resources is largely dependent on the level of local compliance, community awareness and support that exists for reserves (e.g. Alcala & Russ 2006). This is especially the case in developing countries where levels of surveillance and enforcement are generally low due to the remoteness of reefs, limited facilities and limited funding (White 1988; Alcala & Russ 2006). Initially, the general perception surrounding NTR establishment is that a portion of the fishing area will be lost and yields will decrease. It is essential that local people be made aware of the potential longer-term benefits of sustained marine reserve protection (Alcala & Russ 1990, 2006). Furthermore, it is important that these effects be studied and demonstrated to local communities (Alcala & Russ 2006). Despite the potential benefits, NTR networks cannot be considered a panacea for the world's threatened coral reef ecosystems (Sale *et al*. 2005; Mumby & Steneck 2008). Most proponents of NTR networks stress from the outset the essential need for effective fishery and ecosystem management outside the no-take areas (e.g. Russ 2002, Roberts *et al*. 2005; Hilborn *et al*. 2006; Steneck *et al.* 2009). It is widely recognised however, that adequately designed and representative networks of NTRs can provide considerable benefits for both exploited fish stocks and the ecosystem as a whole (Roberts & Hawkins 2000; Pauly *et al*. 2002; Lubchenco *et al*. 2003; Hilborn *et al*. 2006).

1.4. Fishery effects of NTRs

When protected from fishing mortality, fish may build up in numbers, live longer, grow larger and produce an exponentially larger number of eggs (PDT 1990; Roberts 1997a). Numerous studies of NTRs have documented increased population density, fish biomass and mean size of target species within the boundaries of adequately protected reserves (Roberts & Polunin 1991; Dugan & Davis

1993; Russ 2002; Halpern 2003; Williamson *et al*. 2004; Russ *et al*. 2008). However, empirical demonstrations of the potential contribution of NTRs to achieving sustainability of fish stocks in surrounding areas remain elusive (Sale *et al*. 2005; Jones *et al*. 2007).

NTRs may function to maintain and perhaps even enhance fishery yields via recruitment subsidies to fished areas from enhanced populations within NTRs ('recruitment subsidy'), or via net-emigration of post-settlement fish from NTRs to surrounding fished areas ('spill-over ') (PDT 1990; McClanahan & Kaunda-Arara 1996; Russ & Alcala 1996a; Bohnsack 1998; Russ 2002; Gell & Roberts 2003; Sale *et al*. 2005; Jones *et al*. 2007). Whilst empirical evidence for spill-over is increasing rapidly (Roberts *et al*. 2001; Russ 2002; Gell & Roberts 2003; Russ *et al.* 2003, 2004; Zeller *et al*. 2003; Alcala *et al*. 2005, Abesamis & Russ 2005), evidence of recruitment subsidies remains very rare (Roberts 1997b; Russ 2002; Gell & Roberts 2003; Sale *et al*. 2005).

The duration of the planktonic larval phase of marine species varies considerably, but it may be as long as several months (PDT 1990; Sale 1991). Therefore, larvae may potentially disperse over very large geographical distances and non-reserve areas well away from the 'source' NTR may benefit from enhanced levels of larval recruitment (Roberts 1997b; Russ 2002). However, definitive empirical estimates of larval dispersal, rates of self-recruitment and demographic connectivity of populations are rare, due to the difficulties associated with tracking microscopic larvae from their natal origins, through the pelagic environment to their eventual settlement locations (Thorrold *et al.* 2002, 2006; Sale & Ludsin 2003; Sale & Kritzer 2003; Jones *et al.* 2007). This lack of empirical data remains an impediment to the wider uptake of NTR networks into marine resource management strategies (Sale *et al.* 2005; Mumby & Steneck 2008). A substantial amount of research is required to assess 'net export' of eggs and larvae from NTRs and the potential recruitment benefits for non-reserve areas (Roberts 1997b; Russ 2002; Sale *et al*. 2005).

Furthermore, the integration of empirical measurements of larval connectivity into coupled biophysical models of larval dispersal is a fundamental step in achieving optimal design and functioning of marine reserve networks (Cowen *et al.* 2000, 2006; Sale *et al.* 2005; Jones *et al.* 2007; Almany *et al*. 2009).

Many post settlement juvenile and adult reef fish are capable of swimming considerable distances (1-10 km at least) and are therefore capable of moving across boundaries of NTRs. Spill-over occurs when there is a net emigration of fish from reserve areas to surrounding non-reserve areas (Russ 2002). Conceptually, spill-over is driven by a build up of fish population density and biomass and thus increased competition for space and resources within NTR boundaries (Alcala & Russ 1990; Roberts & Polunin 1991, DeMartini 1993, Russ & Alcala 1996a). Alcala & Russ (1990), Russ & Alcala (1996a), Abesamis & Russ, (2005) and Alcala *et al*. (2005) have documented spill-over from fringing reef marine reserves in the central Philippines. In comparison to broad scale recruitment subsidies from NTRs, spill-over of juvenile and adult fishes from reserves is likely to have relatively localised benefits for fisheries (Russ & Alcala 1996a; Abesamis & Russ.2005; Alcala *et al*. 2005).

1.5. Non-fishery benefits of NTR protection

No-take marine reserves can provide many non-fishery benefits while still allowing fisheries to operate in surrounding areas (Bohnsack 1993; Bohnsack & Ault 1996; Roberts & Hawkins 2000; Palumbi 2002; Lubchenco *et al.* 2003; Sobel & Dahlgren 2004). Non-fishery benefits include: improving conservation through the protection of biodiversity and ecosystem structure, function and integrity; increasing the knowledge, understanding and appreciation of marine ecosystems; and creating opportunities for non-consumptive human activities such as eco-tourism (Bohnsack 1998;

Roberts & Hawkins 2000; Sobel & Dahlgren 2004). Many of these benefits, such as protecting marine biodiversity, are incompatible with full exploitation or are simply not addressed by traditional management measures (Roberts 1997a; Bohnsack 1998; Sobel & Dahlgren 2004).

Permanent NTRs provide essential reference areas to evaluate the impacts of fishing and other human activities on marine ecosystems and to facilitate a better understanding of ecosystem structure, function and performance (Roberts & Hawkins 2000; Sobel & Dahlgren 2004). Established NTRs can also provide undisturbed areas for studying natural rates of mortality, growth, behaviour, recruitment variability, trophic interactions and ecosystem function (Roberts & Hawkins 2000; Sobel & Dahlgren 2004). Increased knowledge and understanding of these parameters will lead to improved scientific information and greater precision in estimates of the effects of fishing and other ecosystem-scale disturbances.

1.6. The Great Barrier Reef Marine Park

The Great Barrier Reef Marine Park (GBRMP) adjoins the North-Eastern coast of Australia and encompasses an area of approximately 350,000 km^2. The GBRMP Act was passed in 1975 and multiple-use zoning management plans were first introduced in the Capricornia Section (Southern region) of the marine park in July 1981. By September 1987 the entire GBRMP was under zoning management. After 1987, approximately 23% of nearly 3000 individual coral reefs (~ 4.7% of the total area of the Marine Park) were zoned as NTRs (Day *et al*. 2003). The Great Barrier Reef Marine Park Authority (GBRMPA) is responsible for ensuring conservation of the GBR and "wise use" of the park, including planning of activities, issuing permits for commercial and research activities, and day to day management of the GBRMP.

Williams and Russ (1994) reviewed the available evidence of the effectiveness of NTRs of the GBRMP (after 3-10 years of zoning) in protecting fish stocks. They found that the evidence for significantly increased densities of the major target of reef line fisheries, the coral trout (*Plectropomus leopardus*), was equivocal. However, evidence that NTRs increased mean sizes and hook and line catch rates was more convincing (Williams & Russ 1994, Mapstone *et al*. 2003). More recent evidence also suggests that NTRs may not have been as successful as expected in increasing density and average age of coral trout on mid and outer-shelf reefs of the GBRMP (Zeller & Russ 1998; Adams *et al.* 2000; Ayling *et al.* 2000; Mapstone *et al.* 2003, 2008).

The paucity of clear effects of NTRs in the GBRMP could result from natural variability in the productivity of different reefs, or from relatively low fishing pressure on 'open' reefs (Williams & Russ 1994). However, measures of total mortality rates of the inshore coral trout (*Plectropomus maculatus*) (Ferreira & Russ 1992) and natural mortality rates of the common coral trout (*P. leopardus*) (Russ *et al.* 1998), suggest that this scenario is unlikely for many areas of the GBR. A third possibility is that movement of fish between NTRs and fished reefs 'swamps' any effect of no-take protection. Within the GBRMP the majority of NTRs protect entire reefs or clusters of reefs. Although coral trout have been shown to move considerable distances within reefs to reach spawning aggregation sites (Samoilys 1997a; Zeller 1998; Davies 2000), Davies (2000) showed that movements of coral trout between reefs, across expanses of open sandy substrate, are rare. Movement of coral trout between NTRs and fished reefs may occur in certain areas, but it is dependent upon factors such as distance between reefs and substrate composition of inter-reefal areas (Samoilys 1997; Zeller 1998; Zeller & Russ 1998).

Another potential explanation for the lack of strong effects of NTRs in the GBRMP is poaching (illegal fishing) on reserve reefs. Poaching can potentially mask the effects of NTRs by selectively

removing large fish from the population and rapidly reducing fish biomass (Russ & Alcala 2003). Some evidence suggests that poaching in no-take zones by both commercial and recreational fishers occurs in the GBRMP (Gribble & Robertson 1998; Davis *et al*. 2004). Most of the reefs on the GBR are a long distance (40-100km) from the coast and are often hundreds of kilometers from centres of human population. This makes effective surveillance and enforcement of no-take zones difficult (Russ 2002). Data presented in this thesis was collected on inshore reefs of the GBRMP (10-30 km from the coast), where the vast majority of fishing pressure is recreational, not commercial (Higgs & McInnes 2003), surveillance is relatively effective and compliance with zoning regulations is generally high (Davis *et al*. 2004; Williamson *et al.* 2004).

The inshore reefs of the GBR Marine Park are high use recreational areas which are exposed to significant levels of fishing effort (Higgs & McInnes 2003). Furthermore, these reefs have been subjected to relatively frequent mass coral bleaching events in the past decade (Berkelmans *et al.* 2004). They are also periodically exposed to low salinity levels and high sediment and nutrient loads following wet season floods (Haynes & Schaffelke 2004; Fabricius *et al*. 2005).

In July 2004, a new zoning management plan was introduced to the entire GBRMP under the Representative Areas Program (RAP) (Day *et al*. 2004; Fernandes *et al.* 2005). This was the first broad-scale rezoning of the GBRMP since 1987. Under the RAP zoning plan, the marine park area assigned with 'no-take' protection status (NTR) was increased from about 4.7% to 33.4%. The focus of the RAP was on increasing protection of biodiversity and representative habitats in 70 distinct bio-regions (30 reefal & 40 non-reefal) within the GBRMP. Reef areas under NTR status increased from approximately 23% to over 33% of the total reef habitat within the GBRMP. The RAP was the largest marine park zoning management plan, and the largest spatial closure to fishing, to be implemented in Australia or internationally (Fernandes *et al.* 2005).

During the development of the RAP zoning plan, the GBRMPA undertook a nationwide public consultation process which included obtaining a large amount of data and general information from the scientific community, the tourism industry, GBR fishing industries and the wider community. Over 31,000 submissions were received by GBRMPA in 2003/2004, and the overwhelming majority of Australians were supportive of increased protection for the Marine Park (Fernandes *et al.* 2005). However, as with any major management intervention, there was a degree of opposition to the RAP by sectors of the commercial and recreational fishing lobbies throughout the rezoning process (Fernandes *et al.* 2005). This opposition continued after the implementation of the zoning plan. Lack of demonstrated positive conservation and fishery effects of NTRs, loss of fishing grounds, displacement of effort, job losses, and inadequate government compensation to displaced commercial fishers, remain as the primary issues of discontent for some sectors of the commercial and recreational fishing industries.

Although not specifically designed to sustain fisheries, the RAP program may generate many benefits for GBR reef fisheries through long term protection of portions of fish populations and protection of critical fish habitats. The effectiveness of any GBR zoning plan in sustaining reef fisheries and ensuring habitat and ecosystem stability may be limited by factors such as inadequate surveillance, enforcement and compliance, poaching and a lack of wider community awareness of the benefits of maintaining NTRs. It is essential that rigorous scientific studies be undertaken to monitor the performance of the GBRMP marine reserve network and to convey the outcomes to the national and international community.

The broad objective of the work presented in this thesis was to assess the ecological effects of NTRs on the inshore reefs of the GBRMP. Specifically, the study set out to examine the effects of

no-take protection on populations of target and non-target species of reef fish, by employing a spatially and temporally replicated underwater visual census (UVC) monitoring program. A further objective was to examine temporal dynamics of fish and benthic (coral) communities within NTRs and in fished areas and to assess persistence of NTR effects through time.

The study also extends to the development and testing of a new transgenerational larval marking technique for fishes. The objective of this component of the study was to assess the safety and effectiveness of the larval marking technique for use on large commercially important species of reef fish. This mass-marking technique has facilitated studies which aim to track larval fish dispersal from natal reefs to settlement locations and has thus provided the means by which the demographic connectivity of populations and larval export from NTRs can be measured. Although the results are preliminary at this stage, the final component of this study involved an experiment undertaken to test larval subsidies and spill-over of target reef fish species from NTRs to surrounding fished areas of the GBRMP.

Information presented in this thesis provides considerable insight into the effects of NTR protection on the inshore reefs of the GBRMP. It is my hope that this thesis will make a valuable contribution to our understanding of the effects of implementing NTRs and provide further evidence of the benefits of integrating NTR networks into marine resource management strategies.

Chapter 2: General Methods

Note: The methods detailed in this chapter apply to data chapters 3, 4 and 5.

2.1. Study locations

This project was conducted on fringing coral reefs of the Palm, Whitsunday and Keppel Island groups, within the Great Barrier Reef Marine Park, Queensland, Australia. The spatial component of the project (Chapter 3), was conducted on fringing coral reefs at Orpheus and Pelorus Islands in the Palm Island group, and at Hook, Whitsunday and Border Islands within the Whitsunday Island group. The short-term temporal monitoring component of this project (Chapter 4), was conducted on fringing reefs of Orpheus Island and Pelorus Island. The long-term temporal component of the study (Chapter 5), was conducted on the above mentioned reefs of the Palm and Whitsunday Island groups, and at Great Keppel Island, Middle Island and Halfway Island within the Keppel Island group (Figure 2.1).

The Palm Island group (18°34'S, 146°29'E) is located approximately 15 km offshore from the Queensland coast and is made up of 10 granite-based continental islands. Great Palm Island is the largest in the archipelago, and has a resident Aboriginal community of around 3000 people. Other islands in the group are uninhabited national parks and Aboriginal land areas. A tourist resort and the James Cook University Research Station are located on the western side of Orpheus Island. Except for these leases, the remainder of Orpheus Island is a national park. Pelorus Island is owned by the local mainland council. There is a small private lease on the south-western corner of Pelorus Island which is permanently maintained by a caretaker, the remainder of the island is uninhabited. Orpheus and Pelorus Islands are separated by a channel, which is approximately 1km wide and

reaches a depth of approximately 25m. The fringing reef surrounding Pelorus Island has remained open to fishing. The majority of the Orpheus Island reef area has been zoned as a protected no-take marine reserve since 1987 (Figure 2.1).

The Whitsunday Island group (20°08'S, 148°56'E) includes approximately 55 continental islands, stretching between 1 km and 38 km from the Queensland coast. Several of the islands within the Whitsunday group have large tourist resorts. However, the three islands included in this study are national parks. The islands are primarily uninhabited, with the exception of a small tourist resort at the southern end of Hook Island. The fringing reefs surrounding Whitsunday Island and the Eastern side of Hook Island have remained open to fishing. Border Island and the northern end of Hook Island have been zoned as protected no-take marine reserves since 1987 (Figure 2.1).

The Keppel Island group (23°10' S, 150°57' E) is comprised of 16 continental islands and several isolated rocky outcrops that are located between 5km and 30km from the coast. The majority of the islands within the group are uninhabited National Parks, however a resort, airstrip, several guest houses and some private dwellings are located on Great Keppel Island. Middle Island and the Eastern side of Halfway Island have been protected no-take reserves since 1987, at the time of the present study, all fringing reefs surrounding Great Keppel Island were open to fishing (Figure 2.1).

The Palm, Whitsunday and Keppel Island groups are high-use recreational areas and are popular locations for boating, fishing and diving. There is a significant level of recreational fishing pressure (hook and line, and spear) on the fringing reefs of these island groups (Blamey & Hundloe 1991; Higgs & McInnes 2003). In comparison to more remote areas of the GBRMP, there is a relatively high level of formal surveillance of fishing activities in these island groups. Furthermore, general compliance with Marine Park zoning and fishery regulations is generally high (Davis *et al*. 2004).

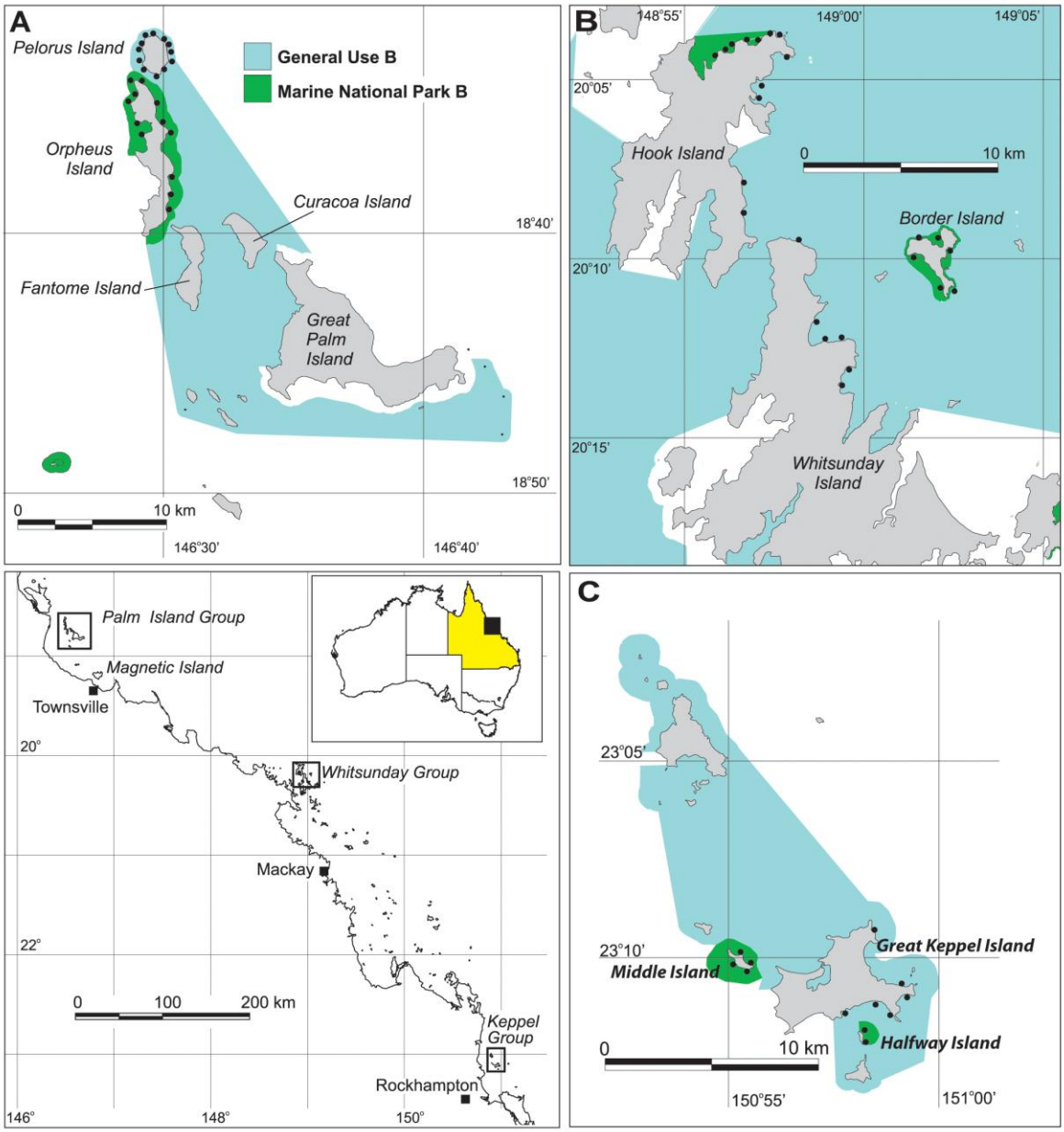


Figure 2.1: Regional map showing the position of each island group and management zoning (1987 – 2004) and site position maps for; **A**. the Palm Island group, **B**. the Whitsunday Island group, **C**. the Keppel Island group. Areas shaded green are protected Marine National Park (no-take) zones; blue and white shaded areas are Habitat Protection and General Use zones which are open to fishing. Black markers indicate the approximate position of monitoring sites at each island group.

The day-to-day management, surveillance and enforcement of the GBRMP is undertaken by government agencies including; the Queensland Parks and Wildlife Service (QPWS), the Queensland Boating and Fisheries Patrol (QBFP), the Queensland Water Police, the Australian Federal Police (AFP) and the Australian Customs Service (ACS) vessels and aircraft (Davis *et al.* 2004). Passive surveillance is carried out on a daily basis by local tourism operators, research station and resort staff, and by members of the community.

The previous (prior to July 2004) multiple-use zoning management plan for the Great Barrier Reef Marine Park (GBRMP) was introduced in 1987. At the commencement of the present study (1998), the no-take marine reserve areas of these fringing reefs had been formally protected for 11 years.

2.2. Site Characteristics

The fringing reefs of the Palm, Whitsunday and Keppel Island groups consist of a reef flat, crest and slope. The reef flat typically has a patchy cover of live coral (hard and soft), as well as expanses of dead coral, coral rubble and algal-covered rock. In most sites, the reef crest is at a depth of between 1 and 2 metres at mean tidal level. Beyond the crest, the reef slope drops steeply to a depth of between 5m and 20m (depending on the site), where it levels out to a flat sandy bottom.

At the majority of sites in the Palm and Whitsunday Islands, reef slope topography is complex, with many overhanging ledges and holes. Numerous bommies (distinct coral or rock outcrops) of varying sizes project from the reef slope, and also rise out of deeper water beyond the base of the reef slope. Reef slope topography is less complex in the Keppel Island group, where reef slopes in the majority of sites are not as steep or as structurally complex as those in the Palm and

Whitsunday Island groups. Keppel Island group reef slopes are however dominated by expanses of branching and tabulate *Acropora* sp. corals.

2.3. Visual census of reef fishes

Fifty-six species of diurnally active reef fish from within eight families (Lutjanidae, Lethrinidae, Serranidae, Labridae, Haemulidae, Centropomidae, Siganidae and Chaetodontidae) were surveyed using a modified version of the underwater visual census (UVC) technique developed by Ayling and Ayling (1983). Using SCUBA, a single observer (D.W.) would slowly swim a 50m transect line counting numbers and estimating the size (in 5cm categories) of fishes within 3m either side of the observer ($300m^2$ survey area). A second diver would run the transect tape out behind the fish observer to measure the distance covered. This method reduced disturbance to the fish community and minimised diver-negative or diver-attractive behaviour of several of the surveyed fish species (see McCormick & Choat 1987; Cheal & Thompson 1997; Kulbicki 1998). All transects were conducted on the reef slope, parallel to the reef crest, at 4 - 12m depth. In order to reduce the level of error associated with underwater fish size estimation, observer calibration was undertaken at the start of each survey trip using wooden fish models (*see* Thompson & Mapstone 1997).

2.4. Visual census of the benthic community and reef structural complexity

The sessile benthic community was surveyed using a line-intercept method, which was conducted as each transect tape was reeled in. A point sample was taken every 1m along each transect tape (50 samples per transect). Categories sampled were live hard coral (eg. branching, digitate, tabular, massive, foliose, encrusting), soft coral, sponge, giant clams (*Tridacna* spp.), other invertebrates (eg. ascidians, anemones), macro-algae, bleached or dead coral, rock, rubble or sand.

In order to provide a measure of the structural complexity of the reef slope for each site, five visual estimates of reef slope angle and rugosity were recorded by the observer on each transect according to a five point categorical scaling system (Table 2.1). Weather conditions and underwater visibility were recorded for each site. Surveys were not conducted if the underwater visibility was less than five metres.

Category	Description of Reef Slope & Rugosity
1	Reef slope 0 - 10°. Expanses of rubble and sand with some small scattered bommies.
2	Reef slope < 45°. Bommies dispersed amongst mostly rubble and sand.
3	Reef slope ~ 45°. Small rubble and sand patches amongst bommies and /or coral structure.
4	Reef slope > 45°. Good coral structure, bommies, some small over-hangs, holes and caves.
5	Reef slope ~ 90°. High reef complexity, large over-hangs, holes, caves and bommies.

Table 2.1: Description of categories of reef slope and rugosity, estimated visually on each transect conducted in the Palm, Whitsunday and Keppel Island groups.

2.5. Data handling and treatment

Of the fifty-six species surveyed, ten were classified as 'primary target' species of GBR recreational and commercial hook and line, and spear fisheries. Thirty- eight species were classified as 'secondary target' species, which are not directly sought after, but are often captured incidentally and commonly retained by fishermen if they are above the minimum legal size. Eight species were classified as 'non-target' species, which were not sought after and are rarely captured within GBR recreational or commercial fisheries (Williams & Russ 1994). A list of surveyed

species, the fishery status of each species, and the analysis group/s to which each species was assigned is presented in ***Appendix 1***.

Biomass estimates were calculated for all individual species using published length-weight relationships (Ferreira & Russ 1992; Kulbicki *et al*. 2005; Froese & Pauly 2008). Density and biomass estimates for individual fish species were pooled into family groups for analysis (Serranidae, Lutjanidae, Labridae, Haemulidae, Chaetodontidae and Siganidae). The Lethrinidae and Centropomidae were not analysed as groups since the counts of these species were low and there was a high level of variability in the data.

The three species of coral trout (*Plectropomus leopardus*, *P. maculatus* and *P. laevis*) were pooled into one analysis group, as they are equally vulnerable and equally targeted by hook and line, and spear fishing. *Plectropomus* spp. are the primary target species of the GBR hook and line fishery. All *Plectropomus* spp. were included in the Serranidae analysis group.

Lutjanus carponotatus (stripey sea perch) was analysed as an individual species as it is common and is often captured and retained by hook and line fishers. Furthermore, *L. carponotatus* are relatively uniformly distributed on the reefs surveyed and the variability associated with the counts was low.

The 'predator' group was a broad grouping of forty predatory reef fish species (piscivores and benthic carnivores), containing six families (Serranidae, Lutjanidae, Lethrinidae, Labridae, Haemulidae, Centropomidae). The three *Plectropomus* spp. were excluded from the predator group to eliminate a swamping effect on the group which tended to obscure patterns in the distribution and abundance of other predatory fish.

Three species of Lutjanidae (*Lutjanus fulviflamma*, *L. lutjanus* and *L. vitta*) were excluded from the analysis as they are small mass schooling species with patchy distributions on the fringing reefs surveyed. The high variability in the counts of these species made data analysis problematic. *Chelinus undulatus* (Labridae) and *Epinephelus fuscoguttatus* (Serranidae) were included in density calculations, but excluded from the biomass calculations for their respective family groups and for the predator group. The population density of *C. undulatus* and *E. fuscoguttatus* is generally low and their distribution is highly variable. Furthermore, the average size of these species is large (> 80cm TL and over 20 kg in weight) and if they had been included in biomass calculations, each individual fish sighted would have made a disproportionate contribution of weight to the estimates. This would have skewed the data and potentially masked or exacerbated differences in mean biomass between sites and zones.

Two labrid species (*Cheilinus fasciatus* and *Choerodon fasciatus*), the two siganid species (*Siganus doliatus* and *S. lineatus*), and the four species of chaetodontids (*Chaetodon aureofasciatus*, *C. melannotus*, *C. rainfordi* and *Chelmon rostratus*) were pooled to provide the 'non-target' group of fish which are not targeted and are rarely captured by fishing gears currently in use on the GBR.

2.6. Statistical analysis of data

The statistical analysis of data was specific to each data chapter. Further details of data treatment procedures and the methods of statistical analyses for each data chapter are described in chapters 3, 4 and 5.

Chapter 3: Spatial patterns in the effects of marine reserve protection on inshore GBR fringing reefs.

3.1. INTRODUCTION

No-take marine reserves (NTRs) are popular for their dual potential as conservation and fishery management tools (Gell & Roberts 2002, 2003; Roberts *et al.* 2001, 2005; Russ 2002; Willis *et al* 2003b). They are perceived as a means to protect marine habitats and communities, separate conflicting uses of marine resources, enhance tourism opportunities, and act as reference areas for investigating ecological process and the effects of fishing (Bohnsack & Ault 1996; Roberts 1997a; Gell & Roberts 2002). Furthermore, networks of NTRs have been widely advocated as a relatively simple and effective means of managing multi-species reef fisheries (PDT 1990; Roberts & Polunin1991; Roberts 1997; Gell & Roberts 2002; Russ 2002).

Within NTRs, species targeted by fisheries are expected to increase in abundance and mean size. Networks of adequately protected marine reserves may maintain or even enhance fisheries operating outside them by becoming net exporters of biomass through the 'spill-over' of post settlement fish and via the supply of larval recruits from reserves to surrounding fished areas (recruitment subsidy) (*see reviews by*; Roberts & Hawkins 2000; Russ 2002; Gell & Roberts 2003; Sale *et al*. 2005).

The majority of studies examining the effects of NTRs have involved spatial comparisons at one time of sites with and without reserve protection. Few studies have data on the abundance and size structure of species targeted by fisheries in an area prior to marine reserve status being applied

(Jones *et al.* 1993; Russ 2002; Willis *et al.* 2003b). This study is one of the few conducted within the GBRMP, or elsewhere, to provide reliable data on abundance and size structure collected before establishment of no-take marine reserve status, and then collected after a substantial period of protection, to infer reserve effects. Furthermore, those studies which have been conducted within the GBRMP have often yielded equivocal results or have only detected weak reserve effects (Williams & Russ 1994; Zeller & Russ 1998; Adams *et al.* 2000; Ayling *et al.* 2000; Mapstone *et al.* 2003).

For this chapter, the principal aim was to measure the potential effects of 12-13 years of no-take management zoning (1987 to 1999-2000) on primary target, secondary target and non-target reef fish species on fringing coral reefs of near-shore island groups within the Great Barrier Reef Marine Park (GBRMP). A secondary objective was to examine evidence of reef fish community interactions and potential trophic effects within protected reserves and fished zones of these island groups.

3.2. METHODS

3.2.1. Study Locations

Study locations and site descriptions are described in Chapter 2 (General Methods).

3.2.2. Data collection

The method of data collection is described in Chapter 2 (General Methods).

3.2.3. Sampling design

Very few data exist on the status of fish and coral populations on fringing reefs in the Palm and Whitsunday Islands prior to the implementation of management zoning in 1987. A few previously unpublished data were available (collected by Dr Tony Ayling in 1983 and 1984). These data provide the only reliable UVC estimates of density and size structure for coral trout (*Plectropomus* spp.) in the Palm and Whitsunday Island groups prior to the establishment of the no-take protected areas in 1987. Coral trout (*Plectropomus* spp.) are the primary target species of the recreational and commercial hook and line fisheries on the Great Barrier Reef (Williams & Russ 1994). Three treatments were used to assess the effects of the no-take reserves on coral trout abundance: pre-protected, protected (reserve) and fished zones (non-reserve) (Table 3.1).

Pre-protected estimates of coral trout density and size structure were collected from the back reef slopes of Havannah and Curacoa Islands in the Palm Island group, and Border and Hook Islands in the Whitsunday Island Group. Five replicate 50m x 20m (1000m^2) transects were conducted once in 1984 at two sites at each of the Palm Island group locations, and once in both 1983 and 1984 at two sites at each of the Whitsunday Island group locations (Table 3.1).

For sampling of the protected and fished zones in 1999-2000, six sites were randomly positioned within each of four locations in each island group (Figure 3.1). Five replicate 50m x 6m (300m^2) transects were sampled at each site in November 1999 (Whitsunday Islands) and March and June 2000 (Palm Islands). The only pre-protected (1983-1984) data available were for coral trout. Comparisons of density and biomass for species and groups other than coral trout are thus restricted to two treatments; protected and fished, both sampled in 1999-2000.

Island Group	Pre-protection 1983-1984	Protected 1999-2000	Fished 1999-2000
Palm Islands	Havannah Island (n = 2)	Orpheus Island (East) (n = 6)	Pelorus Island (East) (n = 6)
	Curacoa Island (n = 2)	Orpheus Island (West) (n = 6)	Pelorus Island (West) (n = 6)
Whitsunday Islands	Hook Island (North) (n = 4)	Hook Island (North) (n = 6)	Hook Island (East) (n = 6)
	Border Island (n = 4)	Border Island (n = 6)	Whitsunday Island (n = 6)

Table 3.1: Locations and numbers of sites used to sample coral trout density and size structure in pre-protected (1983-1984) and in the protected and fished zones (1999-2000) of the Palm and Whitsunday Island groups.

Two weaknesses of this sampling design are acknowledged from the outset. Firstly, the "before" data for the Palm Islands were not collected at the same islands within the Palm Island group as the "after" data. Thus, the effect of no-take reserve protection is potentially confounded by any spatial variations between sites sampled at Curacao / Havannah and sites sampled at Orpheus / Pelorus (Table 3.1). The fringing reefs surrounding Curacao, Havannah, Orpheus and Pelorus Islands are similar however, the islands are all relatively close together and the same habitat (reef slope) was sampled at all locations. In the Whitsunday Islands UVC sampling during 1999 was conducted in the same locations as in 1983/84. The only way this weakness in sampling design at the Palm Islands could have been avoided was if baseline data had been collected at Orpheus and Pelorus islands in 1983-84. A second weakness in the sampling design is that at the time of sampling, Orpheus Island had the only protected NTR in the Palm Island group. Thus, the location of all the protected sites on Orpheus, whilst unavoidable for the Palm Island group, could be considered pseudo-replication. The same criticism applies to the location of all the fished sites at Pelorus Island in 2000.

It was not until 2001 that DW became aware of the existence of the pre-protection (1983-84) data for *Plectropomus* spp. after the completion of sampling in 1999-2000. Thus the sampling design utilised in collection of pre-protection data in 1983-84 was not replicated in 1999-2000. Furthermore, the transect size used in 1999-2000 (50m x 6m) has been shown to provide the most precise estimates of coral trout population density (Mapstone and Ayling 1993).

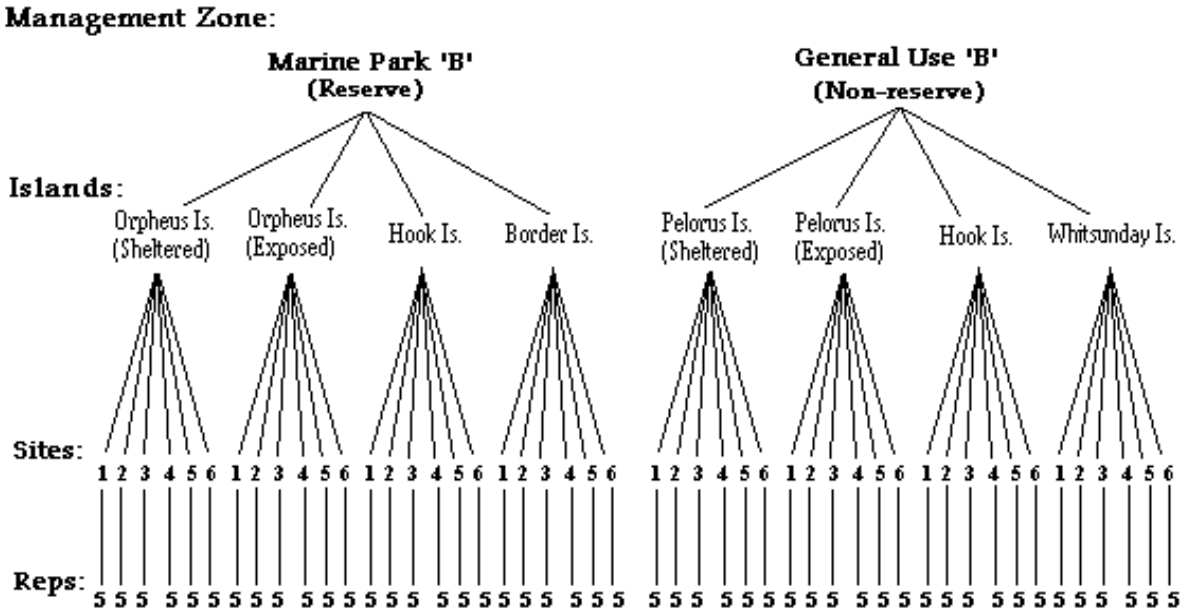


Figure 3.1: Sampling design for the spatial component of the project (All species other than *Plectropomus* spp.).

3.2.4. Treatment of Data

The methods of data handling and treatment are described in Chapter 2 (General Methods).

3.2.5. Analysis of data

Univariate two-factor analysis of variance (ANOVA) was used to test for differences in *Plectropomus spp.* density and biomass between pre-protected, protected and fished zones. Factors in the analysis were zone (3 levels) and island group (2 levels). Due to large between-transect variation within sites, assumptions of homogeneity of variance for ANOVA could not be met with any data transformations when attempting to analyse data at the transect level. Thus, the transect data were pooled at the site level; making the 12 randomly selected sites in each zone at each island group the replicates. All variates (density and biomass of fish groups, live coral cover, structural complexity and underwater visibility) were analysed by orthogonal, two-factor univariate ANOVAs (fixed factors: zones, island groups). Cochran's test and a quantile-quantile normal plot were used to assess homogeneity of variances and normality, respectively.

Analyses of covariance (ANCOVA) were performed on density and biomass of fish surveyed in 1999-2000, with live hard coral cover, live hard and soft coral cover, structural complexity and underwater visibility used as co-variates. Interactions between variates and co-variates in the ANCOVA were tested by examining the B-weights and beta weights. Following ANOVAs, means were compared using Tukey's HSD tests.

Density estimates of *Plectropomus* spp., *Lutjanus carponotatus* and the Lujanidae group were log (x + 1) transformed, and biomass estimates were square root (x + 1) transformed to satisfy ANOVA assumptions of normality and homogeneity of variances. Similarly, biomass estimates for the Serranidae and the Siganidae groups were square root (x + 1) transformed to conform to ANOVA assumptions. Density and biomass estimates of the non-target fish group were log (x + 1) transformed in order to conform to the assumptions of ANOVA. Density and biomass estimates for all other fish groups were analysed using untransformed data.

Length-frequency distributions of *Plectropomus* spp. in protected reserves and fished zones of the Palm and Whitsunday Island groups were compared statistically using 2-sample Kolmogorov-Smirnov tests.

3.3. RESULTS

3.3.1. Effects of no-take reserve protection on fish species and groups

Plectropomus spp. (Coral Trout)

In both the Palm and Whitsunday Island groups, density and biomass of *Plectropomus* spp. were significantly higher in the protected no-take reserves (1999-2000) than in pre-protection zones (1983-1984) and fished zones (1999-2000) (Figure 3.2; Tables 3.2 & 3.3); (Tukey's tests: protected > fished = pre-protection, $p < 0.001$ for density and biomass at both island groups). Density and biomass estimates of *Plectropomus* spp. in fished zones (1999-2000) were slightly higher, but not significantly higher, than estimates obtained from pre-protection zones (1983-1984) at both the Palm and Whitsunday Island groups (Figure 3.2; Tables 3.2 & 3.3).

In 1999-2000, the density, but not biomass, of *Plectropomus* spp. was significantly higher in protected zones of the Palm Islands than in protected zones of the Whitsunday Islands (Figure 3.2; Table 3.3 & 3.4). Neither density nor biomass of *Plectropomus* spp. differed significantly between open 'fished' zones of the Palm and Whitsunday Island groups in 1999-2000 (Figure 3.2; Table 3.3 & 3.4). No significant interactions between zone and island group were detected for either density

or biomass of *Plectropomus* spp. (Tables 3.3 & 3.4). There were no significant effects of coral cover or habitat structural complexity on coral trout density or biomass at either the Palm or Whitsunday Island groups (Table 3.4).

Island Group	Comparison	Density ratio	Biomass ratio
Palm Islands	PP : P	1 : 5.9	1 : 6.3
	F : P	1 : 3.6	1 : 6.1
	PP : F	1 : 1.6	1 : 1.0
Whitsunday Islands	PP : P	1 : 4.0	1 : 6.2
	F : P	1 : 2.7	1 : 4.1
	PP : F	1 : 1.4	1 : 1.5

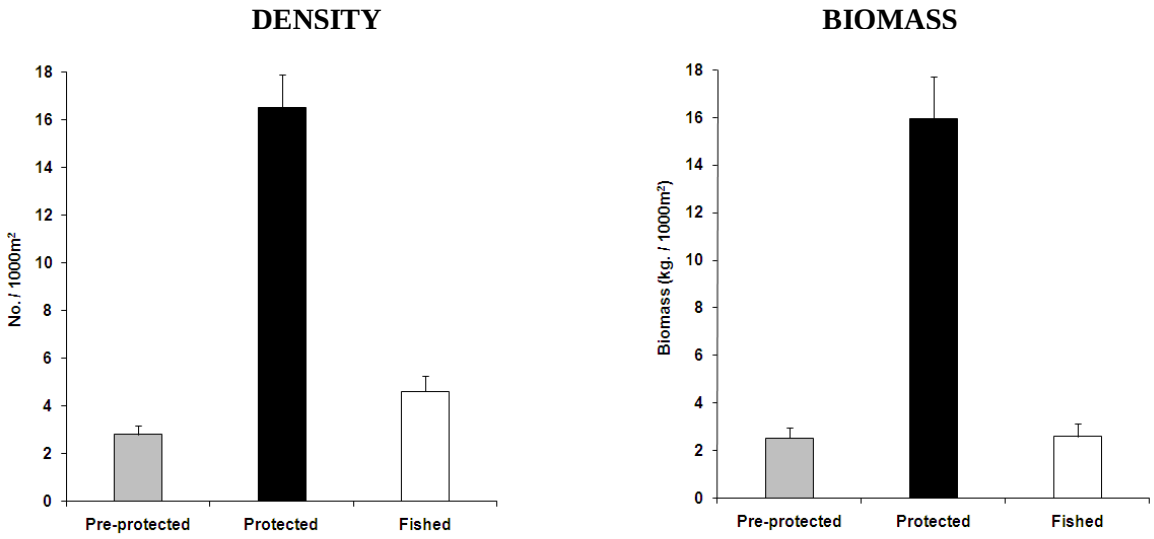
Table 3.2: Mean density and biomass ratios for *Plectropomus* spp. in pre-protected (PP: 1983-1984), protected (P: 1999-2000) and fished (F: 1999-2000) zones of the Palm and Whitsunday Island groups.

Source of Variation	Island Group * Zone (2, 54 df)	Island Group (1, 54 df)	Zone (2, 54 df)
Plectropomus spp. density	2.31 (ns)	13.98 (***)	41.09 (***)
Plectropomus spp. biomass	1.44 (ns)	3.59 (ns)	49.55 (***)

Table 3.3: Results of two-factor univariate ANOVA on the density and biomass of *Plectropomus* spp. in the Palm and Whitsunday Island groups, in pre-protected, protected and fished zones. Numerical figures are F values. Symbols in brackets are significance levels of tests; ******* = < 0.001; **ns** = non significant.

***Plectropomus* spp.**

Palm Island Group



Whitsunday Island Group

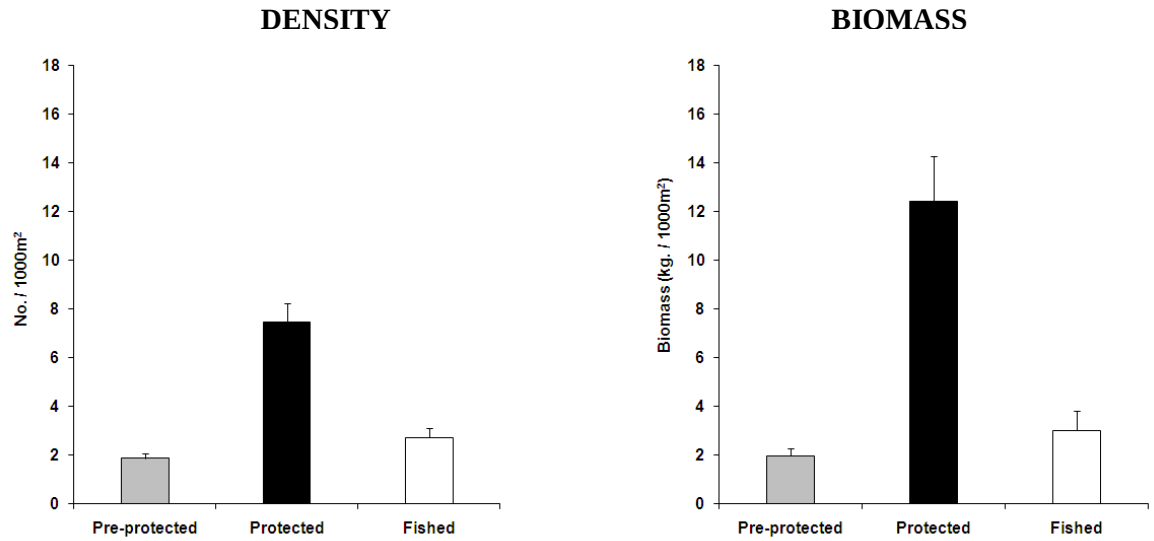


Figure 3.2: Mean (± 1SE) density (number / 1000m^2) and biomass (kg / 1000m^2) of *Plectropomus* spp. within pre-protected (1983-1984), protected (1999-2000) and fished (1999-2000) zones of the Palm and Whitsunday Island groups.

Table 3.4: Results of two-factor univariate ANCOVA on the density and biomass of fish species and groups within protected and fished treatments of the Palm and Whitsunday Islands. Covariates were live hard coral cover, live coral cover (hard and soft coral combined) and the structural complexity index. Univariate ANOVA (1, 44 df) results for benthic variables and under water visibility are also shown. Numerical figures are F values. Symbols in brackets are significance levels of tests; * = < 0.05; ** = < 0.01; *** = < 0.001; ns = non significant.

Source of Variation	Hard coral (1,41 *df*)	Hard + soft coral (1,41 *df*)	Structural index (1,41 *df*)	Island Group * Zone (1,41 *df*)	Island group (1,41 *df*)	Zone (1,41 *df*)
Plectropomus spp. density	1.30 (ns)	0.04 (ns)	0.81 (ns)	0.82 (ns)	19.81 (***)	42.66 (***)
Plectropomus spp. biomass	0.53 (ns)	0.05 (ns)	1.22 (ns)	1.17 (ns)	1.28 (ns)	43.69 (***)
Serranid density	1.10 (ns)	0.07 (ns)	2.52 (ns)	1.70 (ns)	15.55 (***)	5.54 (*)
Serranid biomass	0.08 (ns)	0.00 (ns)	0.19 (ns)	0.24 (ns)	1.78 (ns)	32.79 (***)
Lutjanus carponotatus density	0.01 (ns)	0.52 (ns)	0.16 (ns)	3.34 (ns)	4.46 (*)	10.68 (**)
Lutjanus carponotatus biomass	0.01 (ns)	0.28 (ns)	0.09 (ns)	2.26 (ns)	0.03 (ns)	12.48 (**)
Lutjanid density	0.03 (ns)	1.30 (ns)	0.28 (ns)	1.54 (ns)	9.30 (**)	0.70 (ns)
Lutjanid biomass	0.22 (ns)	1.28 (ns)	0.13 (ns)	0.19 (ns)	1.42 (ns)	2.89 (ns)
Labrid density	0.02 (ns)	3.03 (ns)	0.08 (ns)	0.00 (ns)	6.45 (*)	4.24 (*)
Labrid biomass	0.79 (ns)	0.29 (ns)	0.33 (ns)	0.59 (ns)	0.03 (ns)	0.03 (ns)
Haemulid density	0.25 (ns)	4.14 (*)	0.05 (ns)	0.00 (ns)	0.00 (ns)	2.38 (ns)
Haemulid biomass	1.98 (ns)	0.00 (ns)	0.07 (ns)	0.18 (ns)	0.14 (ns)	0.76 (ns)
Predator density	1.63 (ns)	1.06 (ns)	0.85 (ns)	0.00 (ns)	3.17 (ns)	0.65 (ns)
Predator biomass	0.20 (ns)	0.34 (ns)	0.12 (ns)	0.38 (ns)	0.18 (ns)	2.24 (ns)

Table 3.4 (*Continued*):

Source of Variation	Hard coral (1,41 *df*)	Hard + soft coral (1,41 *df*)	Structural index (1,41 *df*)	Island Group * Zone (1,41 *df*)	Island group (1,41 *df*)	Zone (1,41 *df*)
Chaetodontid density	7.06 (*)	4.65 (*)	0.71 (ns)	3.69 (ns)	5.08 (*)	6.89 (*)
Chaetodontid biomass	7.26 (*)	1.02 (ns)	1.28 (ns)	4.21 (*)	0.56 (ns)	3.84 (ns)
Siganid density	0.81 (ns)	5.05 (*)	3.80 (ns)	0.31 (ns)	25.18 (***)	1.48 (ns)
Siganid biomass	0.52 (ns)	6.54 (*)	4.50 (*)	0.29 (ns)	27.27 (***)	2.23 (ns)
Non-target fish density	3.24 (ns)	3.49 (ns)	4.65 (*)	0.02 (ns)	19.42 (***)	2.47 (ns)
Non-target fish biomass	0.88 (ns)	6.14 (*)	7.05 (*)	0.00 (ns)	37.03 (***)	3.75 (ns)
Live hard coral cover	-	-	-	6.86 (*)	0.39 (ns)	0.60 (ns)
Live coral cover (hard & soft)	-	-	-	1.99 (ns)	1.03 (ns)	7.51 (**)
Structural complexity index	-	-	-	2.07 (ns)	0.33 (ns)	4.66 (*)
Under water visibility	-	-	-	7.10 (**)	0.79 (ns)	3.16 (ns)

In 1999-2000, length-frequency distributions of *Plectropomus* spp. differed significantly between protected and fished zones of the Palm Islands (2-sample Kolmogorov-Smirnov test critical value = 0.17, $p < 0.05$) but not of the Whitsunday Islands (2-sample Kolmogorov-Smirnov test critical value = 0.23, $p > 0.05$).

In the Palm Islands, the modal length of fish in protected zones was 40cm, whereas in the fished zones it was 30cm. 77% of individuals of *Plectropomus* spp. in the fished zones were 35cm or less in length, 58% were 35cm or less in the protected zones. 18% of individuals of *Plectropomus* spp. were greater than 45cm in length in the protected zones, 6% were greater than 45cm in the fished zones (Figure 3.3). In the Whitsunday Islands, the modal length was 40cm in both protected and fished zones. 59% of individuals of *Plectropomus* spp. in the fished zones were 35cm or less in length, 42% were 35cm or less in the protected zones. 31% of *Plectropomus* spp. were greater than 45cm in the protected zones, 18% were greater than 45cm in the fished zones (Figure 3.3).

Overall mean percent compositions (across both zones and island groups) of the three coral trout species pooled to form the *Plectropomus* spp. group were; *P. leopardus* 63.1%, *P. maculatus* 32.73% and *P. laevis* 4.17%. In the Palm Islands, the relative proportion of *P. leopardus* to *P. maculatus* was not significantly different between protected and fished zones. In the Whitsunday Islands, over 92% of coral trout sighted in protected zones were *P. leopardus* and approximately 6% were *P. maculatus*. In fished zones approximately 43% of fish sighted were *P. leopardus* and 51% were *P. maculatus* (Table 3.5).

***Plectropomus* spp.**

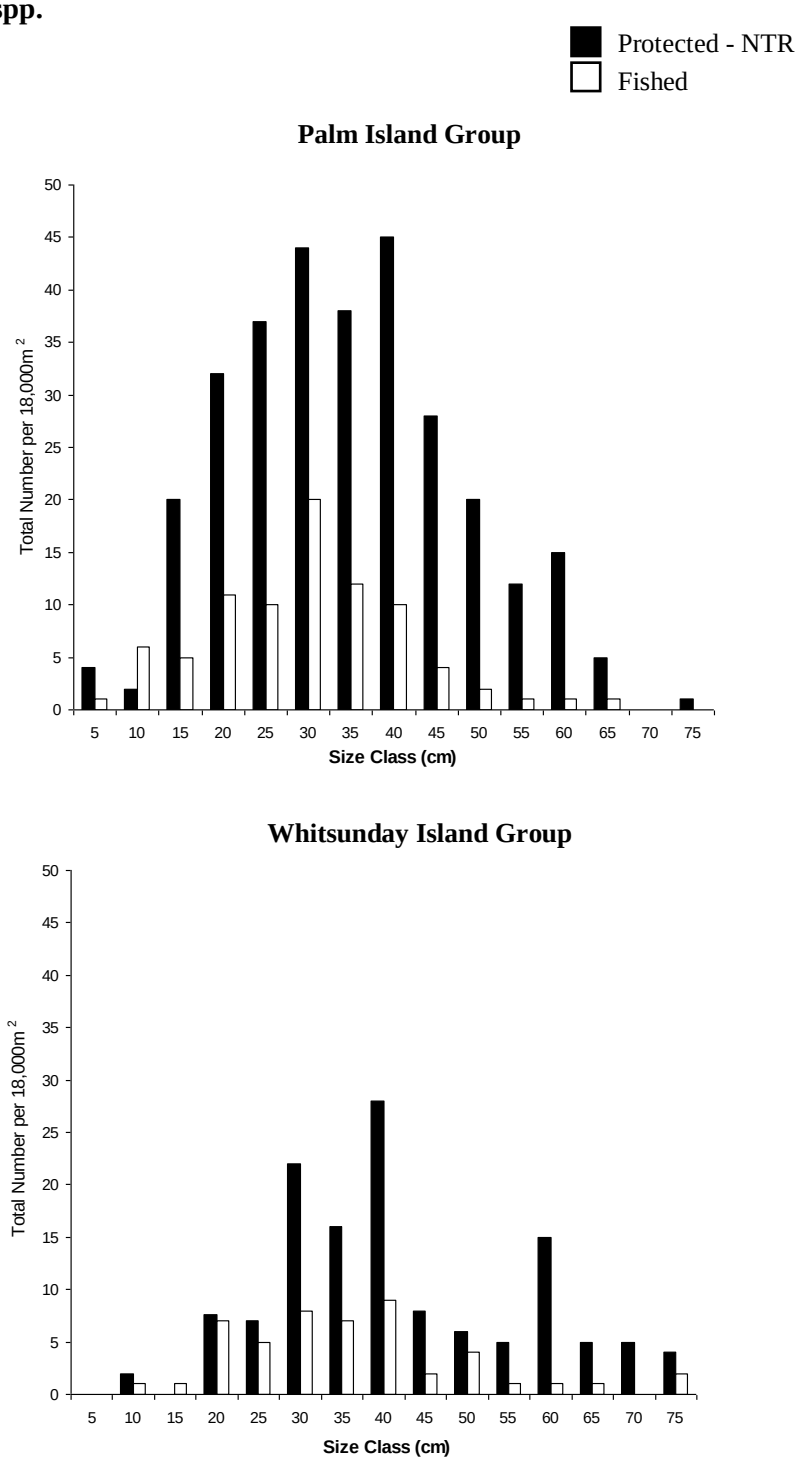


Figure 3.3: Length-frequency distributions for *Plectropomus* spp. within protected and fished zones of the Palm and Whitsunday Island groups (1999-2000).

Island group	Zone	*P. laevis*	*P. leopardus*	*P. maculatus*
Palm Islands	Protected	4.71 %	55.56 %	39.73 %
	Fished	3.61 %	61.45 %	34.94 %
Whitsunday Islands	Protected	2.24 %	92.54 %	5.22 %
	Fished	6.12 %	42.86 %	51.02 %

Table 3.5: Relative percent compositions of the three species of *Plectropomus* (coral trout) as surveyed in protected and fished zones of the Palm and Whitsunday Island groups during 1999 and 2000.

Serranidae (Cods and Groupers)

Significantly higher densities ($p < 0.05$) and biomass ($p < 0.001$) of the Serranidae group were detected in protected zones than in fished zones of the Palm Islands (P : F = 1.5 and 3.31 for density and biomass respectively) and the Whitsunday Islands (P : F = 1.32 and 3.03 for density and biomass respectively). Mean population density (protected and fished zones combined within each island group) of Serranids' was significantly higher ($p < 0.001$) in the Palm Island Islands than in the Whitsunday Islands. No significant difference in serranid biomass was detected between the two island groups. There were no significant interactions between zone and island group (Figure 3.4; Table 3.4). There were no significant effects of benthic habitat variates on density or biomass of the Serranidae at either the Palm or Whitsunday Island groups (Table 3.4).

Serranidae

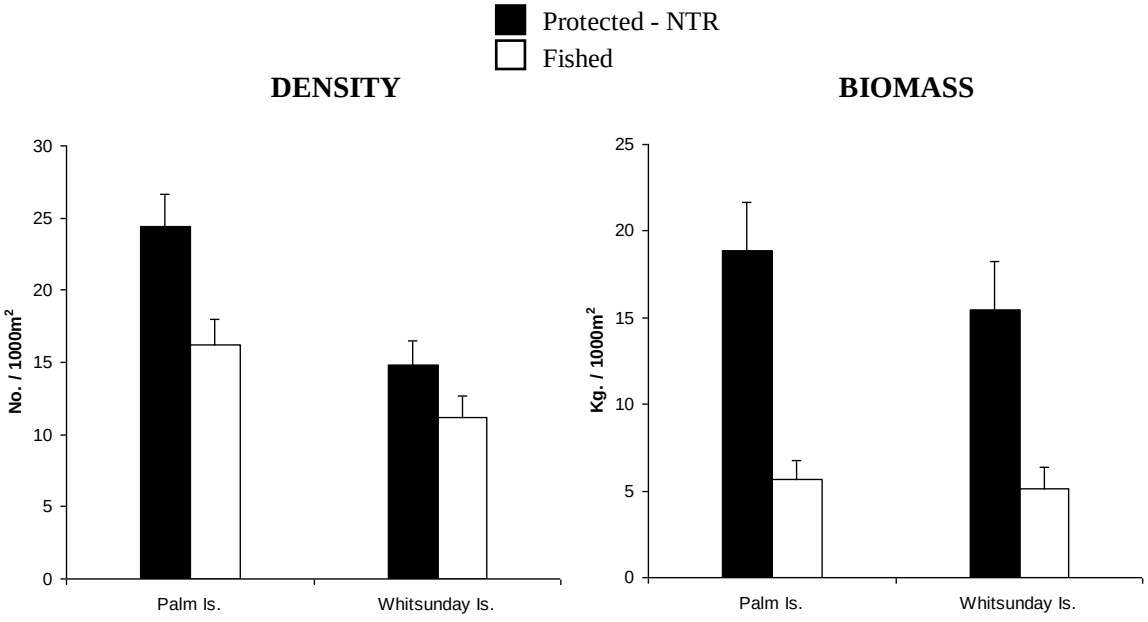


Figure 3.4: Mean (± 1 SE) density and biomass of the Serranidae group in protected and fished zones of the Palm and Whitsunday Island groups.

***Lutjanus carponotatus* (Stripey Sea Perch)**

In 1999-2000, density and biomass of *L. carponotatus* were significantly higher ($p < 0.01$) in protected zones than in fished zones of the Palm Islands (P : F = 3.1 for both density and biomass), and the Whitsunday Islands (P : F = 1.7 and 1.9 for density and biomass respectively) (Figure 3.5; Table 3.4). Density of *L. carponotatus* was significantly higher ($p < 0.05$) in the Palm Islands than in the Whitsunday Islands, with no significant interaction between zone and island group (Table 3.4). There was no significant difference in biomass of *L. carponotatus* between island groups, and no significant interaction between zone and island group (Table 3.4). There were no significant effects of benthic habitat variates on density or biomass of *L. carponotatus* at either the Palm or Whitsunday Island groups (Table 3.4).

Lutjanus carponotatus

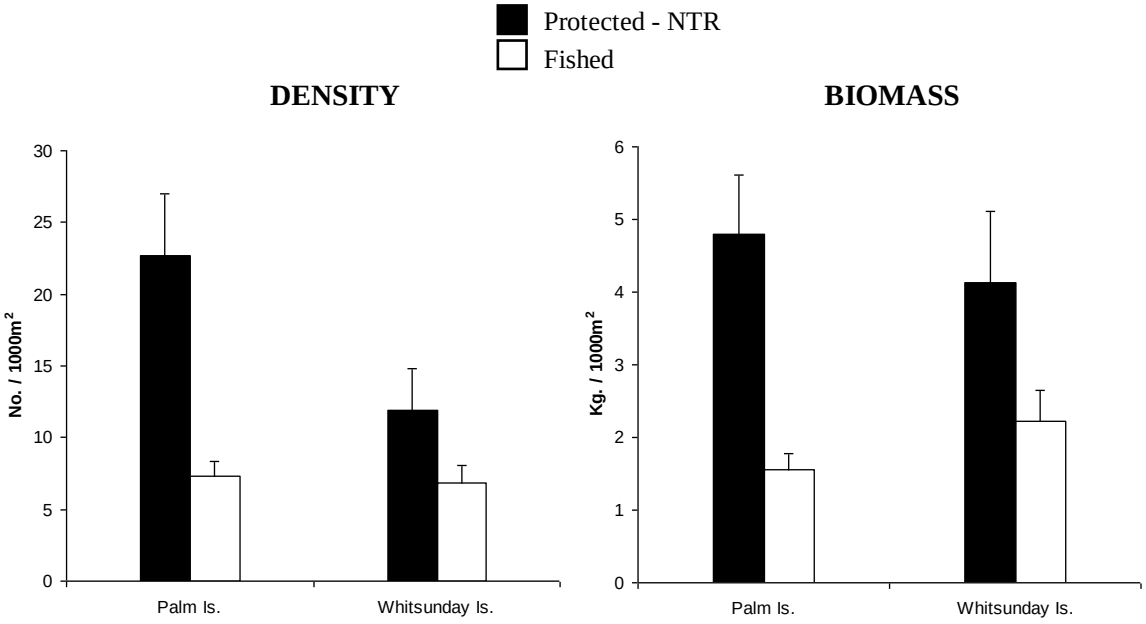


Figure 3.5: Mean (± 1 SE) density and biomass of *Lutjanus carponotatus* in protected and fished zones of the Palm and Whitsunday Island groups.

Lutjanidae (Tropical Snappers)

No significant differences in lutjanid density or biomass were detected between protected and fished zones of either the Palm or the Whitsunday Island groups in 1999-2000. Lutjanid density, but not biomass, was significantly higher ($p < 0.01$) in the Palm Islands than in the Whitsunday Islands (Figure 3.6; Table 3.4). There were no significant interactions between zone and island group (Table 3.4). There were no significant effects of benthic habitat variates on density or biomass of the Lutjanidae group at either the Palm or Whitsunday Island groups (Table 3.4).

Lutjanidae

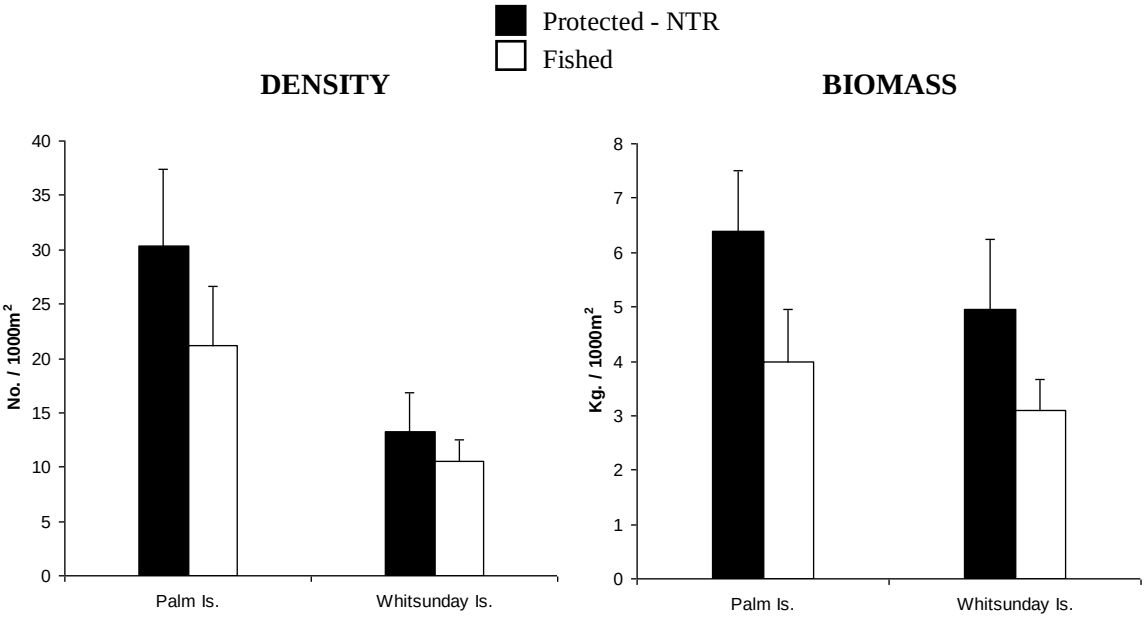


Figure 3.6: Mean (± 1 SE) density and biomass of the Lutjanidae group in protected and fished zones of the Palm and Whitsunday Island groups.

Labridae (Wrasses)

Mean density of the Labridae group was significantly higher ($p < 0.05$) in fished zones than in protected reserves of both the Palm and Whitsunday Island groups (F : P = 1.26). Labridae biomass was higher, but not significantly, in fished than protected zones of both island groups (Figure 3.7; Table 3.4). Mean population density, but not biomass, of Labrids' was significantly higher ($p < 0.05$) in the Palm Islands than in the Whitsunday Islands (Figure 3.7; Table 3.4). There was no significant interaction detected between zone and island group (Table 3.4). There were no significant effects of benthic habitat variates on density or biomass of the Labridae group at either the Palm or Whitsunday Island groups (Table 3.4).

Labridae

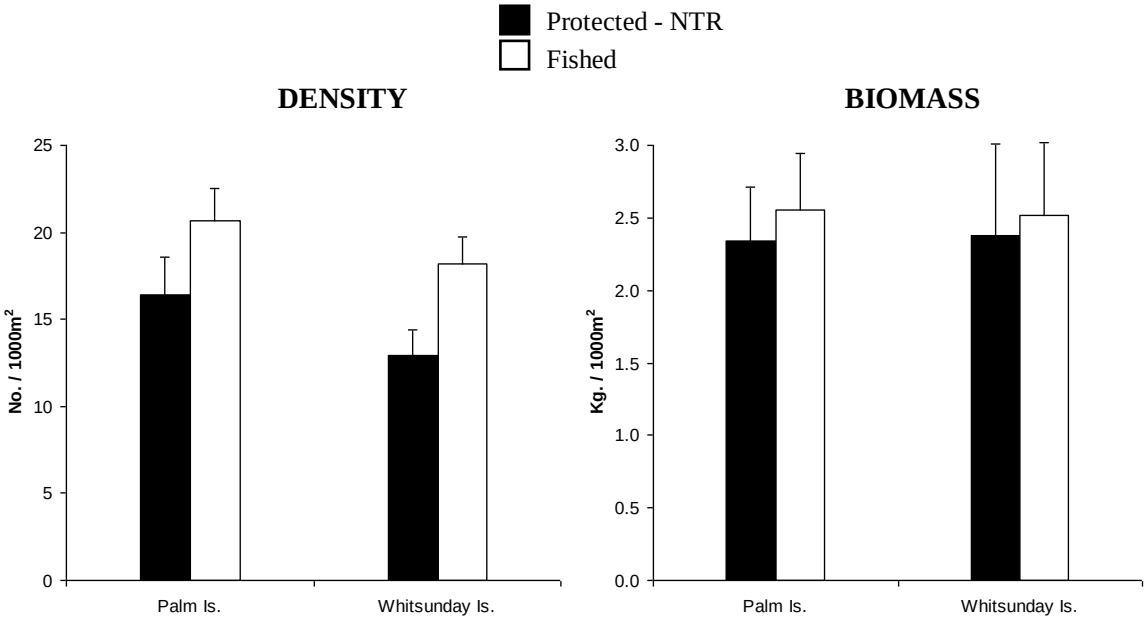


Figure 3.7: Mean (± 1 SE) density and biomass of the Labridae group in protected and fished zones of the Palm and Whitsunday Island groups.

Haemulidae (Sweetlips)

Haemulid density and biomass was higher, but not significantly, in protected zones than in fished zones of both the Palm and the Whitsunday Island groups (Figure 3.8; Table 3.4). No significant differences in haemulid density or biomass were detected between the two island groups (Figure 3.8; Table 3.4). There were no significant interactions between zone and island group (Table 3.4). Live coral cover was found to have a significant effect ($p < 0.05$) on the population density of the Haemulidae group (Table 3.4).

Haemulidae

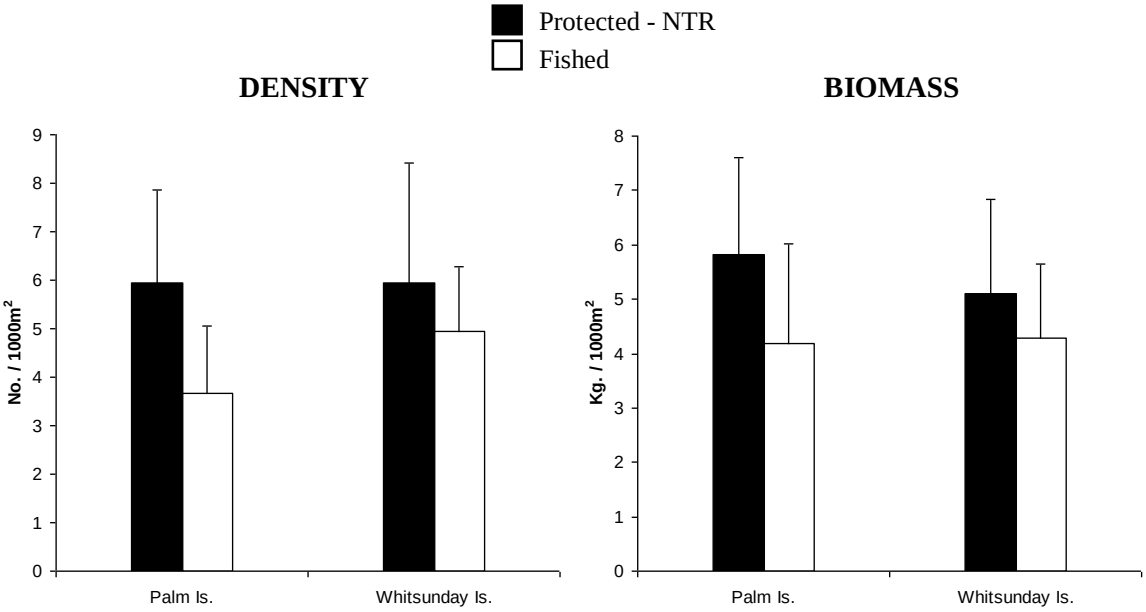


Figure 3.8: Mean (± 1 SE) density and biomass of the Haemulidae group in protected and fished zones of the Palm and Whitsunday Island groups.

Predatory fish (Piscivores & Benthic Carnivores)

In 1999-2000, the density of the predator group was higher, but not significantly, in fished zones than in protected zones of both the Palm and Whitsunday Island groups. Predator group biomass was higher, but not significantly, in protected zones than in fished zones of both island groups (Figure 3.9; Table 3.4). No significant differences in mean density or biomass were detected between island groups (Figure 3.9; Table 3.4). No significant interactions between zone and island group were detected (Table 3.4). There were no significant effects of benthic habitat variates on density or biomass of the predator group at either the Palm or Whitsunday Island groups (Table 3.4).

Predators

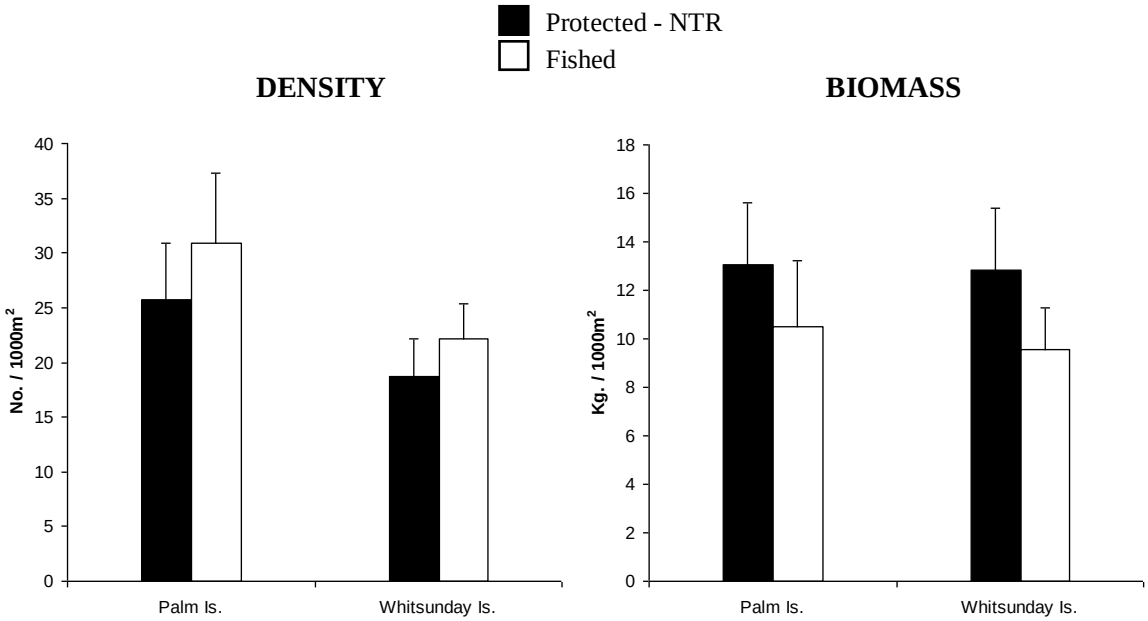


Figure 3.9: Mean (± 1 SE) density and biomass of the Predator group in protected and fished zones of the Palm and Whitsunday Island groups.

Chaetodontidae (Butterfly fishes)

Chaetodontid density was significantly higher ($p < 0.05$) in protected reserves than in fished zones of the Palm Islands (P : F = 1.37) and higher, but not significantly so, in protected than fished zones of the Whitsunday Islands (Figure 3.10 & Table 3.4). Biomass estimates for Chaetodontidae were higher, but not significantly so, in protected zones than in fished zones of both the Palm and Whitsunday Island groups (Figure 3.10; Table 3.4). In 1999-2000, chaetodontid density was significantly higher ($p < 0.05$) in the Palm Islands than in the Whitsunday Islands (Figure 3.10; Table 3.4). A significant island group by zone interaction ($p < 0.05$) was detected for chaetodontid biomass, but not for density (Table 3.4)

A significant positive relationship ($p < 0.05$) was detected between chaetodontid density and biomass, and live hard coral cover in both the Palm and Whitsunday Islands. Similarly, the density, but not the biomass of chaetodontids was significantly positively influenced ($p < 0.05$) by total live coral cover (hard and soft coral combined) (Table 3.4).

Chaetodontidae

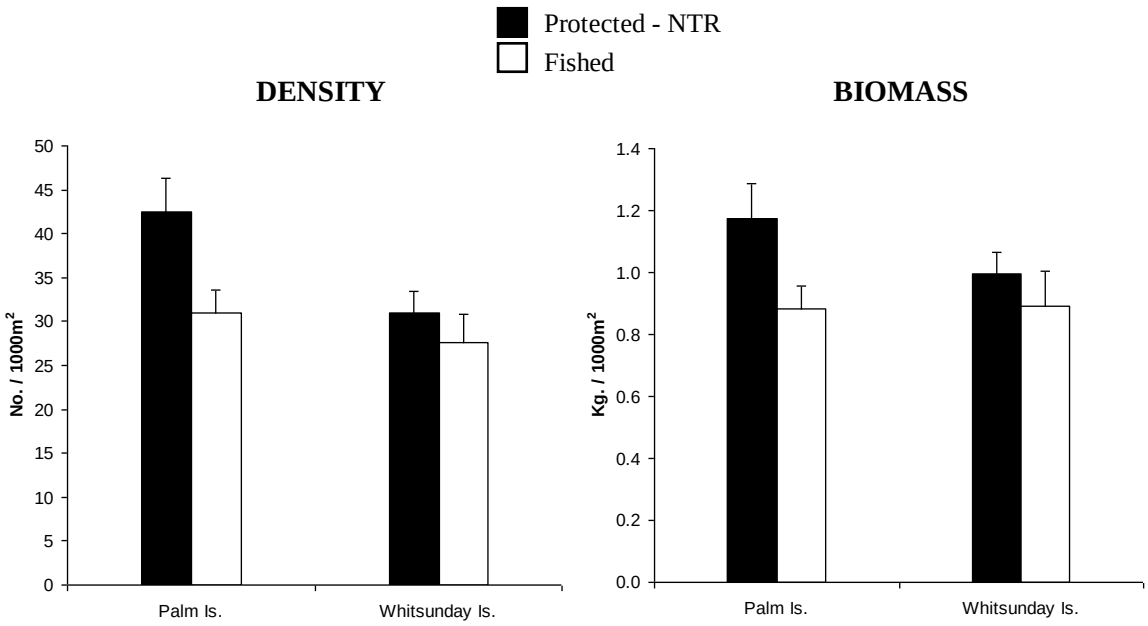


Figure 3.10: Mean (± 1 SE) density and biomass of the Chaetodontidae group in protected and fished zones of the Palm and Whitsunday Island groups.

Siganidae (Rabbit fishes)

Siganid density and biomass did not differ significantly between protected and fished zones of either the Palm or the Whitsunday Island groups (Figure 3.11; Table 3.4). In 1999-2000, both the

mean density and biomass of Siganidae were significantly higher ($p < 0.05$) in the Palm Islands than in the Whitsunday Islands (Figure 3.11; Table 3.6). No significant interactions between zone and island group were detected for Siganidae (Table 3.4).

Significant interactions ($p < 0.05$) between the density and biomass of the Siganidae group and live coral cover (hard & soft coral combined) were detected (Table 3.4). Structural complexity of the habitat had a significant effect on the biomass ($p < 0.05$), but not the density of siganids (Table 3.4).

Siganidae

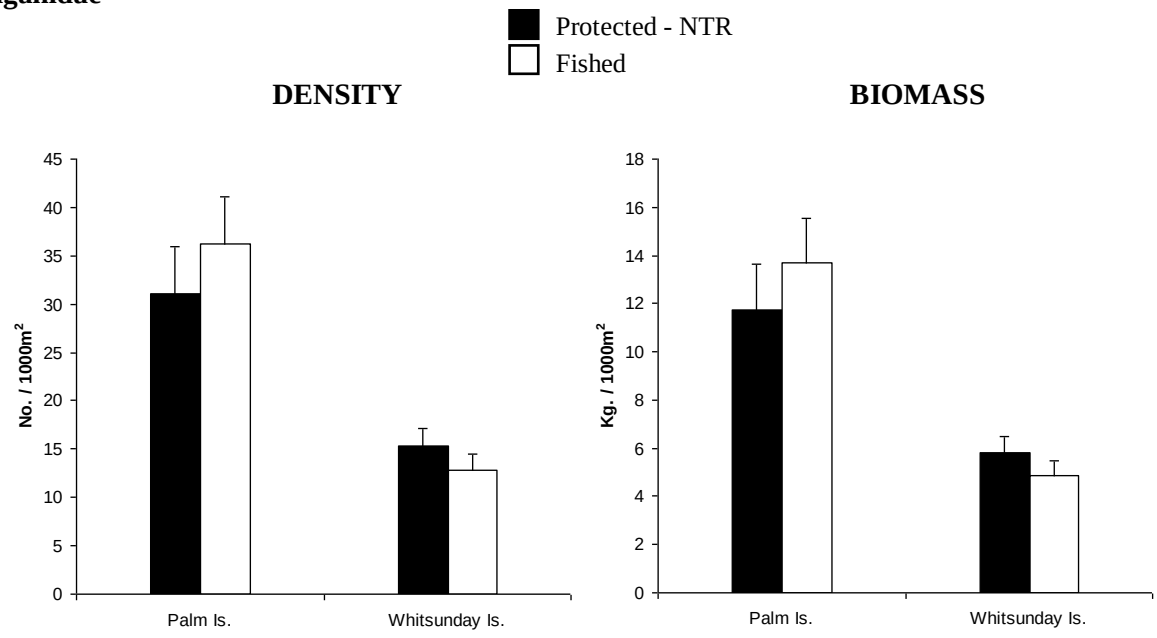


Figure 3.11: Mean (± 1 SE) density and biomass of the Siganidae group in protected and fished zones of the Palm and Whitsunday Island groups.

Non-target fish (herbivores & small benthic invertebrate feeders)

Density and biomass of non-target fish species did not differ significantly between protected and fished zones of either the Palm or the Whitsunday Island groups (Figure 3.12; Table 3.4). Density and biomass of non-target fish was significantly higher at the Palm Islands than at the Whitsunday Islands. There were no significant interactions between zone and island group (Table 3.4). Significant interactions ($p < 0.05$) were detected between structural complexity of the substratum and density and biomass of non-target fish (Table 3.4). Live coral cover (hard and soft coral combined) had a significant positive effect ($p < 0.05$) on biomass, but not density of non-target fish (Table 3.4).

Non-target fish

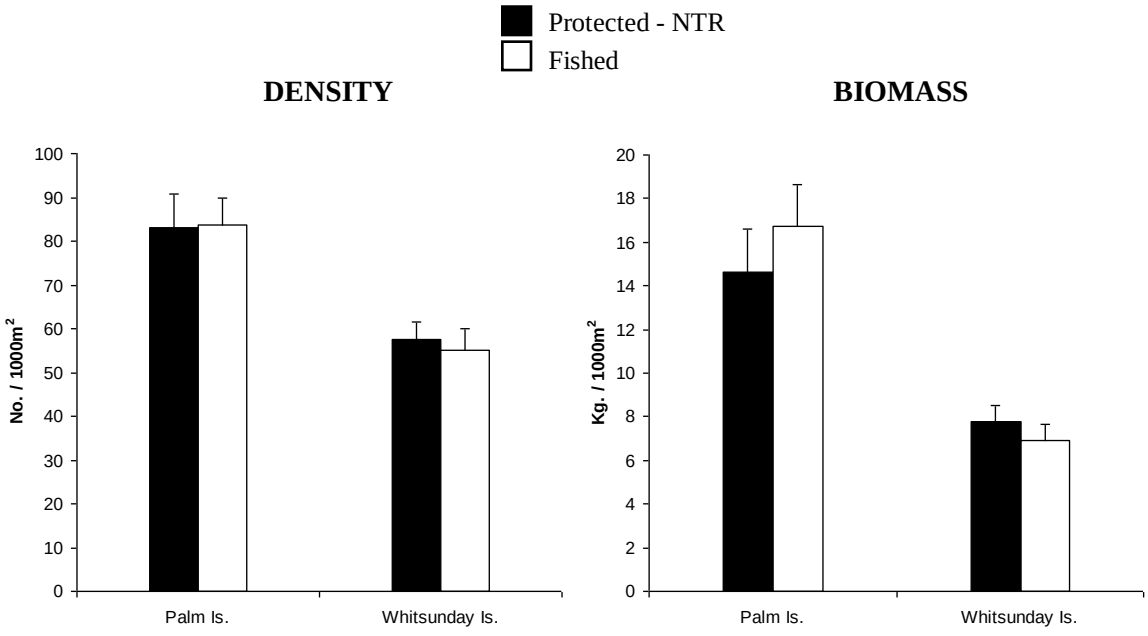


Figure 3.12: Mean (± 1 SE) density and biomass of the non-target fish group in protected and fished zones of the Palm and Whitsunday Island groups.

3.3.2. Variations in benthic cover and structural complexity between zones and regions

In 1999-2000, live coral cover (hard and soft coral combined - LCC) was significantly higher ($p < 0.05$) in protected reserves than in fished zones of the Whitsunday Islands, and higher, but not significantly so, in protected zones of the Palm Islands (Figure 3.13a; Table 3.4). There were no significant differences in LCC between the Palm and Whitsunday Island groups (Table 3.4).

Structural complexity of the fringing reef habitats was significantly higher ($p < 0.05$) in fished zones than in protected reserves of the Palm Islands, and higher, but not significantly so, in fished zones of the Whitsunday Islands (Fig. 3.13c; Table 3.4). There were no significant differences in the structural complexity of the reef slopes between the Palm and Whitsunday Island groups (Table 3.4).

3.3.3. Variations in underwater visibility

There were no significant differences in underwater visibility between zones or island groups during the 1999-2000 sampling periods (Figure 3.13d; Table 3.4).

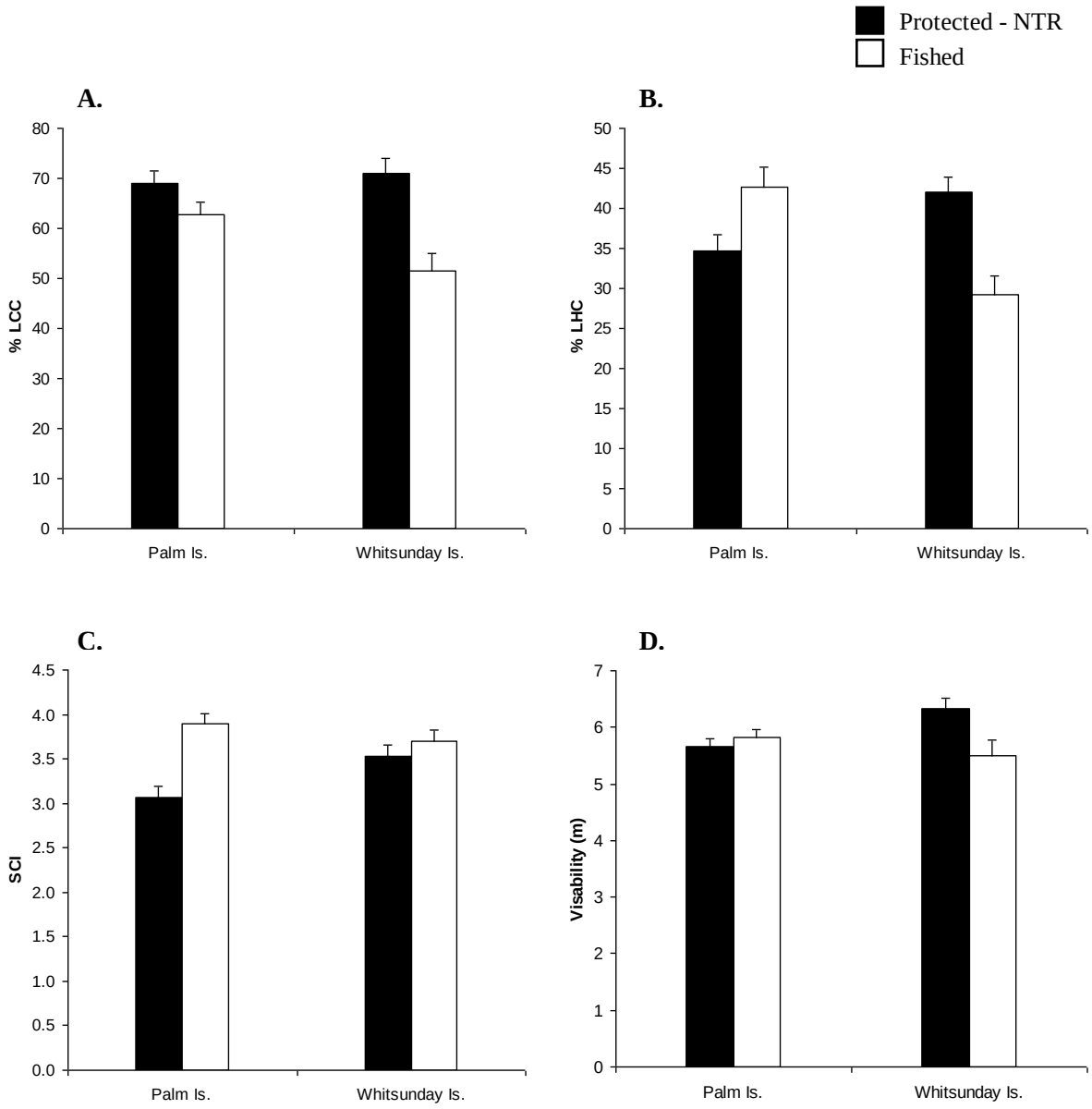


Figure 3.13: Mean (± 1SE) benthic cover and structural complexity of the habitat, in protected and fished zones of the Palm and Whitsunday Island groups (1999-2000). **A**. Mean (± 1SE) percent total live coral cover (% LCC - hard and soft coral combined). **B**. Mean (± 1SE) percent live hard coral cover (% LHC). **C**. Mean (± 1SE) structural complexity indices (SCI). **D**. Mean (± 1SE) underwater visibility.

3.4. DISCUSSION

This study has demonstrated strong effects of no-take marine reserve protection on reef fish populations of near-shore coral reefs in Australia's Great Barrier Reef Marine Park (GBRMP). It did so by comparing abundance of the major target of the hook and line fisheries on the GBR, *Plectropomus* spp., at sites before, and 12-13 years after, the application of NTR status. Few studies of the effects of NTRs present data on abundance of target species before application of reserve status (Jones *et al.* 1993; Russ 2002; Willis *et al.* 2003b). The few studies in the marine reserve literature that do draw on pre-reserve data include White (1988) in the Philippines, Clark *et al.* (1989) in Florida, McClananhan & Kaunda-Arara (1996) in Kenya, Russ and Alcala (1996) in the Philippines, Lincoln-smith *et al.* (2006) in the Solomon Islands; Nardi *et al.* (2004) in Western Australia, Roberts *et al.* (2001) and Hawkins *et al.* (2006) in St. Lucia and Russ *et al.* (2008) on the Great Barrier Reef. In most of these cases the duration of protection of the reserves was less than a decade.

Studies examining NTR effects within the GBRMP are surprisingly few (*e.g.* Craik 1981; Ayling & Ayling 1983, 1992; Ferriera & Russ 1995; Gribble & Robertson 1998; Zeller & Russ 1998; Adams *et al.* 2000; Ayling *et al.* 2000; Mapstone *et al.* 2003; Graham *et al.* 2003; Evans & Russ 2004; Williamson *et al.* 2004; Russ *et al.* 2008). Furthermore, until recently, the results of the few studies examining NTR effects on population densities of the major targets of the hook and line fisheries in this region have often been equivocal (Williams & Russ 1994; Ayling *et al.* 2000; Mapstone *et al.* 2003). The most consistent differences in population characteristics of the main target of the fisheries, coral trout, are larger average size and higher experimental hook and line catch rates in protected NTRs than in fished zones (Ferriera & Russ 1995; Mapstone *et al.* 2003).

It has been often documented that the abundance and size of large predatory reef fishes (e.g. serranids, lutjanids and lethrinids) are good indicators of the effects of fishing and NTR protection on coral reefs (Russ 1991; Jennings & Kaiser 1998; Russ 2002; Russ & Alcala 2003; Dulvy *et al.* 2004). This study has demonstrated significantly higher abundances of coral trout (*Plectropomus* spp.) within NTRs than in areas which have remained open to fishing. In addition, coral trout were, on average, larger and heavier inside the reserves than in the surrounding fished areas in 1999/2000. Furthermore, significant increases in mean density and biomass of coral trout were detected within NTRs of the Palm and Whitsunday Island groups between the baseline pre-protected (1983/84) sampling period and the 1999/2000 sampling period. Although not statistically significant, density and biomass estimates of coral trout were consistently higher by 2 – 65% (Table 4.2) in fished (1999-2000) than pre-protection (1983-1984) treatments. These differences may be due to the difference in the size of the sampling unit used in the 1983-1984 surveys. Pre-protection data was collected using 50m x 20m transects (by Ayling), while data collected for protected and fished zones in 1999-2000 utilised 50m x 6m transects (by Williamson). Mapstone and Ayling (1993) showed that the wider transect can underestimate coral trout density by 50% compared to the narrower transect.

In light of this, the data presented here suggests little change in coral trout abundance in areas open to fishing in the Palm and Whitsunday Islands between 1983-1984 and 1999-2000. Given that the abundance of coral trout has increased considerably over this period in the NTRs, the data suggests that coral trout abundance was reduced from 'natural' levels by fishing on inshore reefs as early as 1983-1984, and prior to the establishment of the GBR Marine Park. This is not consistent with suggestions that line fishing, particularly by recreational fishers, has had little effect on reef fish populations of the GBR.

The significant regional (island group) effect on *Plectropomus* spp. density shows that the protected reserves of the Palm Islands were supporting a higher number of coral trout than the reserves of the Whitsunday Islands in 1999-2000. The mean size of coral trout was larger however, in Whitsunday Island group reserves and mean biomass was not significantly different between protected reserve areas of the two island groups. This effect was primarily driven by the higher number of large, presumably male coral trout (70 – 75cm. TL) recorded in protected reserves of the Whitsunday Islands than in the Palm Islands.

Density and biomass estimates obtained here for coral trout in no-take reserves of the Palm and Whitsunday Islands demonstrate that given time and adequate protection, target fish stocks can build up considerably within NTRs. At some point, ecological constraints such as intra- and inter-specific competition, prey availability, and niche space will govern the population carrying capacity (Jennings 2000). It cannot be concluded from this study that coral trout abundance in protected zones had reached the population carrying capacity for these reefs. Recent empirical evidence suggests that the duration to full recovery of predatory reef fish biomass inside NTRs may often require several decades or more (Russ & Alcala 2004).

In both the Palm and Whitsunday Islands, the exposed (windward) sections of fringing reef are ecologically similar in many respects to the inner mid-shelf reefs in the central section of the marine park. Offshore water is constantly being flushed onto the exposed fringing reefs, water turbidity is lower and live coral cover is generally higher than in sheltered (leeward) areas of fringing reef (*Pers. obs.* 1996-2008). The relative abundance of the three coral trout species observed here suggest that *Plectropomus leopardus* were more abundant than *Plectropomus maculatus* in exposed fringing reef habitats, and *P. maculatus* were generally more abundant in sheltered fringing reef habitats where water turbidity and silt loading on the reef were often higher.

In the Palm Islands, there was an equal number of exposed and sheltered sites in both protected reserves and fished zones. Thus, for the Palm Island group, no significant difference in the relative compositions of *P. leopardus* and *P. maculatus* were detected between protected and fished zones. In the Whitsunday Islands however, the two protected reserves (Border Island & Northern Hook Island) are relatively exposed compared to the two fished zones, water clarity is also generally better and live coral cover was higher. Differences in these fringing reef habitats were likely to be driving the strong disparity in the relative abundances of *P. leopardus* and *P. maculatus* between protected reserves and fished zones of the Whitsunday Island group. These observed patterns are consistent with the findings of previous studies examining the distribution and abundance of coral trout species within the GBRMP (Hoese *et al*. 1981; Ayling & Ayling 1983a,b, 1985; Williams & Russ 1994; Mapstone *et al.* 1998; Ayling *et al*. 2000).

The Serranidae group comprised fifteen grouper species in this study, including the three species of coral trout (*Plectropomus* spp.). The most abundant serranids in the surveys were the smaller species including: *Cephalopholis cyanostigma* (blue-spotted rock cod), *Cephalopholis boenak* (brown-barred rock cod), *Epinephelus merra* (dwarf spotted rock cod) and *Epinephelus quoyanus* (long-fin rock cod). *Plectropomus* spp. were the only large serranids which were frequently sighted. Other larger serranid species, including *Cromileptes altivelis* (barramundi cod) and *Epinephelus fuscoguttatus* (flowery cod), were only rarely sighted in the surveys. As such, the smaller serranid species made a large contribution to mean density estimates, but a comparatively small contribution to mean biomass estimates. The strong influence of coral trout on the Serranidae group has clearly contributed substantially to the highly significant zone effect detected for serranid biomass. Although not presented in the results due to very low counts and high variability, the incidence of sightings of larger serranid species, including the highly sought after barramundi cod,

were higher in protected reserves than in fished zones of both island groups in 1999-2000. *Cromileptes altivelis* was declared as a no-take protected species in Queensland waters in 2004.

The stripey sea perch (*Lutjanus carponotatus*) is common on inshore reefs of the GBRMP and is considered a secondary target species of line fishing in the GBR region (Williams & Russ 1994). Most fish captured incidentally that are above minimum size limits (25cm TL) are retained by fishers. Although not as pronounced as for *Plectropomus* spp., significant effects of NTR protection were detected for *L. carponotatus*. This suggests that the benefits of reserve protection extend to a range of species, beyond those most favoured and sought after by fishers. Regional differences in the abundance of *L. carponotatus* were detected in 1999-2000. The mean population density of *L. carponotatus* was significantly higher in protected reserves of the Palm Islands than in reserves of the Whitsunday Islands in 1999-2000. Mean biomass of *L. carponotatus* was not significantly different between reserve zones of the two island groups. As with the regional patterns in coral trout abundance discussed above, the average size of stripey sea perch was higher in Whitsunday Island group reserves than in the reserves of the Palm Islands.

Density and biomass of the Lutjanidae group were higher, but not significantly so, in NTR than in fished zones of both island groups. Although the density of *Lutjanus carponotatus* was significantly higher in NTRs, the density of other common lutjanids, which made up a substantial proportion of the Lutjanidae group, was not significantly different between NTRs and fished zones of either island group. Other than *L. carponotatus* and *L. argentimaculatus*, most of the commonly sighted lutjanids are either small species (eg. *L. fulviflamma, L. lutjanus* & *L. quinquelineatus*), or occur only as juveniles or sub-adults (majority < 25cm TL) on these fringing reefs and move to deeper water when they reach a larger size (> 35cm TL) (eg. *L. russelli, L. lemniscatus, L. sebae, L. vitta*). Most of these species are not actively sought after by fishers, and only larger individuals (>

25cm TL) are retained (Williams & Russ 1994; *Pers. Obs*. 1996-2009). Subsequently, the level of fishing mortality imposed on small lutjanids on these fringing reefs is low, and it could be expected that reserve effects, if any, would be weak.

Two primary target species were included in the Lutjanidae group, the mangrove jack (*Lutjanus argentimaculatus*) and the red emperor (*Lutjanus sebae*). *L. argentimaculatus* were only occasionally sighted in the surveys and were most often solitary adults greater than 40cm in length. *L. argentimaculatus* generally displayed strong diver negative behaviour and counts were too low to draw any inferences about zoning effects. Only sub-adult *L. sebae* to a maximum length of approximately 40cm TL were sighted during the 1999/2000 sampling periods. There were no obvious differences in the relative abundance of sub-adult *L. sebae* between NTR and fished zones of either island group.

Like several other Lutjanidae species, the life history of *L. sebae* includes ontogenetic shifts in habitat as the fish grow and mature. Once sub-adult fish reach sexual maturity (approx. 45cm TL) they migrate from the shallow lagoons and front reef slopes to deep inter-reefal areas where they often form large aggregations (McPherson *et al.* 1988; Watson & Goeden 1989; Williams & Russ 1994; Brown *et al.* 1995). Only low levels of fishing mortality are imposed on *L. seabae* on these shallow inshore reefs as the vast majority of fish captured by hook and line fishers are below the previous minimum legal size of 45cm (increased to 55cm in July 2004) and are usually returned to the water alive (*Pers. Obs.* 1998-2008). This may largely account for the lack of detectable reserve effects on this species.

Population density of the Labridae group was found to be significantly higher in fished zones than in NTRs of both island groups. Within the Labridae group, only the Humphead Maori Wrasse

(*Chelinus undulatus*) was a primary fishery target species at the time of these surveys. Low numbers of *C. undulatus* were recorded in the surveys due to low population density and strong diver negative behaviour (Kulbicki 1998, Pers. Obs. 1998-2008). Although not presented in the results due to the low number of sightings and high variability of the counts, *C. undulatus* were sighted more frequently in NTRs than in fished areas of both island groups. *C. undulatus* was declared as a no-take protected species in Queensland waters in 2004.

Members of the genus *Choerodon* made up the majority of the counts for the Labridae group. Most *Choerodon* spp. are considered secondary target species. *Choerodon* spp. are benthic carnivores and several species (*Choerodon anchorago, C. graphicus* and *C. cyanodus*) are commonly sighted foraging in rubble areas, sand areas or seagrass beds (Randall *et al.* 1990; Pers. Obs. 1996-2004). The lower live coral cover recorded for fished zones in both island groups may be a contributing factor in the patterns of abundance observed for the Labridae group. A further potential contributing factor is higher predation pressure on smaller labrids from large piscivores such as coral trout inside the protected reserves (Kingsford 1992; Caley 1993; Jennings & Polunin 1997; St. John *et al.* 2001; Stewart & Jones 2001; Webster & Almany 2002; Graham *et al*. 2003; Dulvy *et al.* 2004). Detailed studies into fish community interactions and dynamics within protected and fished zones of the GBRMP over extended time scales should shed more light on the ecological processes driving these observed effects.

The density and biomass of the Haemulidae group was slightly higher, but not significantly, in NTRs than in fished zones of both island groups. Although *Diagramma pictum* and *Plectorhinchus* spp. are commonly captured by line fishers and make easy targets for spear fishers, they are not targeted or retained by the majority of fishers on the GBR. These haemulid species are not highly

regarded for their eating qualities in this region and are thus subject to relatively low levels of fishing pressure (*Pers. Obs*. 1996-2008).

ANCOVA revealed a significant positive interaction between haemulid population density and live coral cover (hard and soft coral combined). During surveys for the present study, *Diagramma pictum* and *Plectorhinchus* spp. were most often sighted in the vicinity of large massive *Porites* spp. colonies and in areas of structurally complex habitat with generally high live coral cover. Live coral cover was slightly higher in NTR than fished zones in both island groups and it is probable that this habitat effect has influenced haemulid population density on these fringing reefs to a greater degree than fishing effects.

The predator group included most recorded piscivores and benthic carnivores but excluded coral trout (*Plectropomus* spp.) (Appendix 1). Population density of predators was lower, but not significantly, in NTRs than in fished zones of both the Palm and Whitsunday Island groups. Conversely, predator group biomass estimates were higher, but not significantly, in NTRs than in fished zones of both island groups. This pattern suggests that larger predators were more abundant in the protected reserves, while smaller predators (eg. *Choerodon* spp., small *Lethrinus* spp. & small *Lutjanus* spp.) were more abundant in the fished zones. Again, a possible contributing factor to this pattern may be predation pressure or competition from coral trout within the reserves (Kingsford 1992; Caley 1993; Jennings & Polunin 1997; St. John *et al*. 2001; Steward & Jones 2001; Webster & Almanny 2002; Graham *et al*. 2003; Dulvy *et al.* 2004).

Estimates of abundance of Chaetodontidae were significantly higher in protected reserves than in fished zones of the Palm Island group and higher, but not significantly, in NTRs of the Whitsunday group. Many chaetodontid species are coral polyp feeders (eg. *Chaetodon rainfordi* – hard coral;

Chaetodon aureofasciatus – hard coral / generalist; *C. melannotus* – soft coral) (Randall *et al*. 1990; Pratchett 2005). Furthermore, population densities of chaetodontid species have been shown to correlate closely with live coral cover (Chabanet *et al*. 1997; Lewis 1997; Pratchett *et al*. 2006). In the present study, chaetodontid density was found to be influenced significantly and positively by both live hard coral cover and total live coral cover (hard & soft coral combined). Live coral cover was higher in protected reserves than in fished zones of both island groups. It could therefore be expected that the abundance of coral feeding chaetodontids would also be higher in the protected reserves.

Population density and biomass estimates for Siganidae and the non-target fish group did not differ significantly between protected and fished zones of either the Palm or Whitsunday Island groups. However, both siganid and non-target fish density and biomass were significantly higher in the Palm Islands than in the Whitsunday Islands in 1999-2000. The present study has not revealed any clear factors contributing to the large regional differences observed in the relative abundance and biomass estimates for these two groups.

The results presented in this chapter suggest that over time, adequately patrolled and protected NTRs will support higher population densities and biomass of targeted reef fish species. The findings are consistent with those of numerous other studies conducted on both tropical and temperate reefs (*see reviews by*; Roberts & Hawkins 2000; Russ 2002; Gell & Roberts 2003). This study, along with others presented in this thesis, is the first in the GBR region to use reliable estimates of coral trout abundance collected before management zoning was implemented on these fringing reefs, and after a reasonable period of time to detect strong reserve effects. The data presented in this chapter have provided a solid baseline for temporal monitoring of fish and coral communities on inshore fringing reefs of the GBR Marine Park. Chapters 4 and 5 of this thesis

utilise temporal monitoring data to explore fish community dynamics and the persistence of the reserve effects reported here.

Chapter 4: Short-term temporal dynamics of fish and benthic communities within the Orpheus Island no-take marine reserve and the Pelorus Island fished zone.

4.1. INTRODUCTION

In recent years, no-take marine reserves (NTRs) have attracted much attention from politicians, resource managers, scientists, fishers, environmentalists and the wider community. This interest has been fueled by an extensive and still growing body of literature examining the effects of NTR protection on biodiversity, fishery resources and socio-economics (*see reviews by*; Roberts & Hawkins 2000; Russ 2002; Gell & Roberts 2003; Lubchenco *et al.* 2003; Roberts et al. 2005; Mora *et al*. 2006). However, the majority of studies examining reserve effects on exploited fish populations have involved comparisons at one time of sites with and without NTR protection. Few studies are sufficiently spatially or temporally replicated and fewer still conform to complete Before-After-Control-Impact Pair (BACIP) experimental designs (Jones *et al.* 1993; Russ 2002; Willis *et al.* 2003b).

Despite these limitations, there is a wealth of convincing data supporting the positive benefits of marine reserve protection on exploited fish populations (Roberts & Hawkins 2000; Gell & Roberts 2003; Day *et al.* 2003; Lubchenco *et al.* 2003). Surprisingly however, there is a relative paucity of studies examining marine reserve effects in the worlds' largest marine park, the Great Barrier Reef Marine Park (GBRMP). The few studies which have examined the effects of GBRMP reserves have often yielded equivocal results or detected only weak effects of protection (Williams & Russ 1994; Zeller & Russ 1998; Adams *et al.* 2000; Ayling *et al.* 2000; Mapstone *et al.* 2003). The

majority of these studies have been conducted on mid-shelf platform reefs which are generally more than 40km from the coast and are primarily harvested by commercial hook and line fishers (Ferreira & Russ 1995; Gribble & Robertson 1998; Mapstone *et al.* 2003).

The Orpheus Island NTR was established in 1987 and by the time this monitoring program began, the reserve had been formally protected for nearly 11 years. Although low levels of poaching by recreational fishers have previously been recorded inside the Orpheus Island reserve, fish stocks within the reserve have received a consistently high level of protection in comparison to more remote reefs of the GBRMP (Davis *et al.* 2004; Evans & Russ 2004; Williamson *et al.* 2004).

The objectives of this study were;

1. To examine short-term temporal dynamics of fish populations within protected NTR and fished zones of the Palm Island group.
2. To examine persistence of the effects of NTR protection on fish stocks of the Palm Island group between 11 and 13 years post reserve establishment.
3. To examine short-term temporal variability in benthic habitat characteristics (live coral cover & habitat structural complexity).
4. To gain insight into the levels of compliance by recreational fishers to NTR zoning in the Palm Island group, quantify fishing effort and incidences of poaching.

4.2. METHODS

4.2.1. Study Locations

Study locations and site descriptions are described in Chapter 2 (General Methods).

4.2.2. Data collection

The method of data collection is described in Chapter 2 (General Methods). In addition, during six sampling periods between 1998 and 2000 (32 days in total), records were kept of the number of vessels sighted actively fishing the fringing reefs of Pelorus Island and Orpheus Island. These observations were collated and estimates of mean daily and yearly recreational fishing effort were calculated.

4.2.3. Sampling design

Surveys of reef fish populations and benthic communities were conducted on the back reef (leeward) sections of fringing reefs surrounding Orpheus Island and Pelorus Island within the Palm Island archipelago (see Chapter 2 – General Methods). Temporal monitoring of reef fish populations and benthic communities, involving two survey trips per year, was conducted in 1998, 1999 and 2000. It must be noted that the experimental design utilised in the present study was pseudo-replicated as only 1 no-take reserve (Orpheus Island) and 1 fished area (Pelorus Island) were monitored. This was unavoidable as at the time of sampling, Orpheus Island was the only NTR within the Palm Island group. A breakdown of the distribution of sampling effort during the monitoring period is provided in Table 4.1.

Survey Time	Protected NTR (Orpheus Island - leeward)	Fished (Pelorus Island - leeward)
1. June 1998	6	6
2. August 1998	6	6
3. May 1999	6	6
4. September 1999	6	6
5. June 2000	6	6
6. September 2000	6	6

Table 4.1: Locations and numbers of sites used to survey reef fish populations and benthic communities in protected and fished zones of the Palm Island group in 1998, 1999 and 2000.

4.2.4. Data handling and analysis

In this chapter, the analysis is restricted to two target fish groups, coral trout (*Plectropomus* spp. – comprised of *Plectropomus maculatus*, *P. leopardus* and *P. laevis*) and stripey snapper (*Lutjanus carponotatus*), and two non-targeted fish groups, rabbitfish (Siganidae – comprised of *Siganus doliatus* and *S. lineatus*) and butterflyfish (Chaetodontidae – comprised of *Chaetodon aureofasciatus*, *C. melannotus*, *C. rainfordi* and *Chelmon rostratus*) and the sessile benthic community.

Due to large between-transect variation within sites, assumptions of homogeneity of variance for ANOVA could not be met by using standard data transformations when attempting to analyse the data at the transect level. Thus, transect data were pooled at the site level. This made the 6 randomly selected sites within each zone the replicates. All variates (density and biomass of fish groups, live coral cover, structural complexity and underwater visibility) were analysed by orthogonal, two-factor univariate repeated measures ANOVAs (fixed factors: zones, trips). Cochran's tests were used to assess homogeneity of variances and quantile-quantile plots were used to determine whether or not the data were normally distributed.

Repeated measures analysis of co-variance (ANCOVA) were performed on density and biomass of fish surveyed between June 1998 and September 2000, with total live coral cover (hard and soft coral combined) used as the co-variate. Interactions between variates and co-variates in the ANCOVA were tested by examining the B-weights and beta weights. Following ANOVAs, means were compared using Tukey's HSD tests.

Density estimates of all fish species and groups were analysed using untransformed data as they conformed to the assumptions of normality and homogeneity of variances. Biomass estimates of the *Plectropomus* spp., group were square root (x + 1) transformed to satisfy ANOVA assumptions. Biomass estimates for *Lutjanus carponotatus* and the remaining fish groups were analysed using untransformed data. Length-frequency distributions of *Plectropomus* spp. in the Orpheus Island protected reserve and the Pelorus Island fished zone for each survey time, were compared using 2-sample Kolmogorov-Smirnov tests.

4.3. RESULTS

4.3.1. Effects of NTR protection on the density and biomass of fish species and groups

Plectropomus spp. (coral trout)

Between June 1998 and September 2000, population density and biomass estimates of *Plectropomus* spp. consistently remained significantly higher ($p < 0.001$) in the Orpheus Island NTR than in the Pelorus Island fished zone (Figure 4.1 & Table 4.2). During the three years of monitoring, population density ranged between 1.47 (May 1999) and 3.43 (June 1998) times higher

in the NTR than in the fished zone. Biomass estimates were between 2.73 (May 1999) and 7.62 (June 1998) times higher in the NTR than in the fished zone (Figure 4.1).

Within the Orpheus Island reserve, estimates of *Plectropomus* spp. population density increased significantly (*Tukey's test:* $p < 0.01$) between June 1998 and June 2000, then decreased again, but not significantly, by September 2000 (Figure 4.1). Biomass estimates of *Plectropomus* spp. remained relatively stable inside the protected reserve during the monitoring period. Neither density nor biomass of *Plectropomus* spp. varied significantly within the Pelorus Island fished zone throughout the monitoring period. There were no significant interactions between trip and zone for *Plectropomus* spp. density or biomass (Figure 4.1 & Table 4.2).

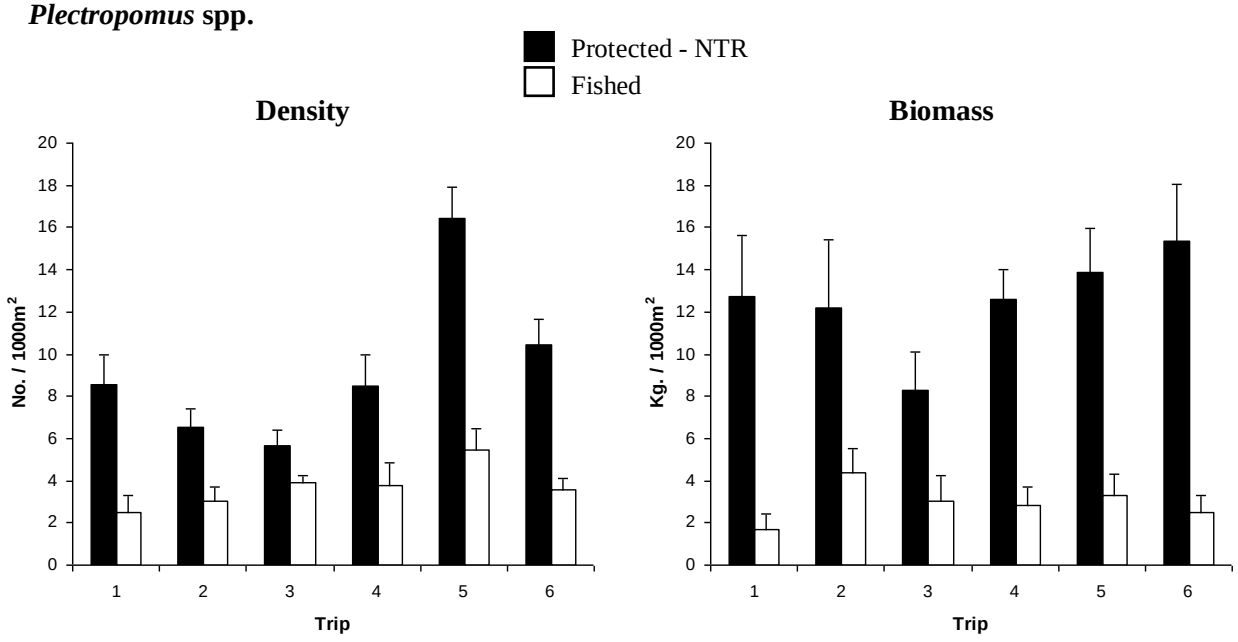


Figure 4.1: Temporal estimates of mean (± 1SE) density (number / 1000m^2) and biomass (kg / 1000m^2) of *Plectropomus* spp. in the Orpheus Island no-take reserve and the Pelorus Island fished zone.

Repeated measures ANCOVA revealed no significant effects of live coral cover (hard and soft coral combined) on population density or biomass of the *Plectropomus* spp. group (Table 4.3). Disparities between the extent of zone and trip effects detected for *Plectropomus* spp. by repeated measures ANOVA and ANCOVA were due to the reduction in the degrees of freedom available for the comparisons when the co-variate (live coral cover - LCC) was included in the data matrix. This statistical effect also applied to all other fish species and groups.

During May and September 1999, and September 2000, length-frequency distributions of *Plectropomus* spp. differed significantly between the Orpheus Island NTR and the Pelorus Island fished zone (2-sample Kolmogorov-Smirnov test critical values = 0.33 in May 1999; 0.31 in September 1999; 0.28 in September 2000; $p < 0.05$ in all cases). During June & August 1998, and June 2000, no significant differences in *Plectropomus* spp. length-frequency distributions were detected between the NTR and the fished zone (Figure 4.2).

In the Orpheus Island NTR, the mean modal length (across six trips: 1998-2000) of *Plectropomus* spp. was 36.7cm, whereas in the Pelorus island fished zone the mean modal length was 28.3cm. On average, 70% of coral trout sighted in the reserve were 35cm TL or above (ie. the majority of those fish were reproductively mature), whereas in the fished zone, only 46% of fish were over 35cm TL (Table 4.4).

Between 1998 and 2000, 55.3% of all *Plectropomus* spp. sighted on the leeward fringing reefs were *P. maculatus* (bar-cheek coral trout), 44.2% were *P. leopardus* (common coral trout) and less than 1% were *P. laevis* (footballer / blue-spot coral trout).

Source of Variation	Trip * Zone (5, 50 *df*)	Trip (5, 50 *df*)	Zone (1, 10 *df*)
Plectropomus spp. density	1.78 (ns)	5.52 (***)	22.45 (***)
Plectropomus spp. biomass	1.00 (ns)	1.15 (ns)	28.01 (***)
Lutjanus carponotatus density	0.58 (ns)	0.52 (ns)	5.47 (*)
Lutjanus carponotatus biomass	0.55 (ns)	0.93 (ns)	2.77 (ns)
Chaetodontid density	1.77 (ns)	13.88 (***)	5.26 (*)
Chaetodontid biomass	1.77 (ns)	13.88 (***)	5.26 (*)
Siganid density	1.15 (ns)	7.04 (***)	2.06 (ns)
Siganid biomass	1.15 (ns)	7.04 (***)	2.06 (ns)
Live hard coral cover	0.32 (ns)	2.91 (*)	0.66 (ns)
Live coral cover (hard & soft)	0.46 (ns)	4.41 (**)	0.03 (ns)
Structural complexity index	0.10 (ns)	1.38 (ns)	4.29 (ns)
Under water visibility	1.73 (ns)	2.73 (*)	1.75 (ns)

Table 4.2: Results of two-factor univariate repeated measures ANOVA on the density and biomass of fish species and groups, benthic habitat variates and underwater visibility within the protected and fished zones of the Palm Island group over 6 trips between 1998 & 2000. Numerical figures are F values. Symbols in brackets are significance levels of tests; * = < 0.05; ** = < 0.01; *** = < 0.001; ns = non significant.

Source of Variation	**Trip * Zone (5, 20 *df*)**	**Trip (5, 20 *df*)**	**Zone (1, 4 *df*)**	**LCC (T1) (1, 4 *df*)**	**LCC (T2) (1, 4 *df*)**	**LCC (T3) (1, 4 *df*)**	**LCC (T4) (1, 4 *df*)**	**LCC (T5) (1, 4 *df*)**	**LCC (T6) (1, 4 *df*)**
Plectropomus spp. density	1.14 (ns)	1.20 (ns)	10.96 (*)	0.18 (ns)	0.75 (ns)	0.01 (ns)	0.88 (ns)	0.42 (ns)	0.21 (ns)
Plectropomus spp. biomass	1.19 (ns)	0.38 (ns)	23.74 (**)	0.54 (ns)	0.69 (ns)	0.12 (ns)	1.92 (ns)	2.12 (ns)	0.80 (ns)
Lutjanus carponotatus density	0.30 (ns)	0.44 (ns)	1.11 (ns)	0.10 (ns)	0.10 (ns)	0.02 (ns)	0.02 (ns)	0.03 (ns)	0.13 (ns)
Lutjanus carponotatus biomass	0.21 (ns)	0.33 (ns)	0.80 (ns)	0.10 (ns)	0.37 (ns)	0.36 (ns)	0.01 (ns)	0.07 (ns)	0.34 (ns)
Chaetodontid density	0.43 (ns)	0.85 (ns)	29.88 (**)	0.24 (ns)	0.12 (ns)	0.43 (ns)	14.33 (*)	0.79 (ns)	10.19 (*)
Chaetodontid biomass	0.43 (ns)	0.85 (ns)	29.88 (**)	0.24 (ns)	0.12 (ns)	0.43 (ns)	14.33 (*)	0.79 (ns)	10.19 (*)
Siganid density	0.75 (ns)	1.12 (ns)	2.31 (ns)	11.52 (*)	0.61 (ns)	13.11 (*)	4.65 (ns)	1.45 (ns)	2.52 (ns)
Siganid biomass	0.75 (ns)	1.12 (ns)	2.31 (ns)	11.52 (*)	0.61 (ns)	13.11 (*)	4.65 (ns)	1.45 (ns)	2.52 (ns)

Table 4.3: Results of two-factor univariate repeated measures ANCOVA on the density and biomass of fish species and groups with live coral cover (LCC) used as the covariate within the protected and fished zones of the Palm Island group over 6 trips between 1998 & 2000. Numerical figures are F values. Symbols in brackets are significance levels of tests; * = < 0.05; ** = < 0.01; *** = < 0.001; ns = non significant.

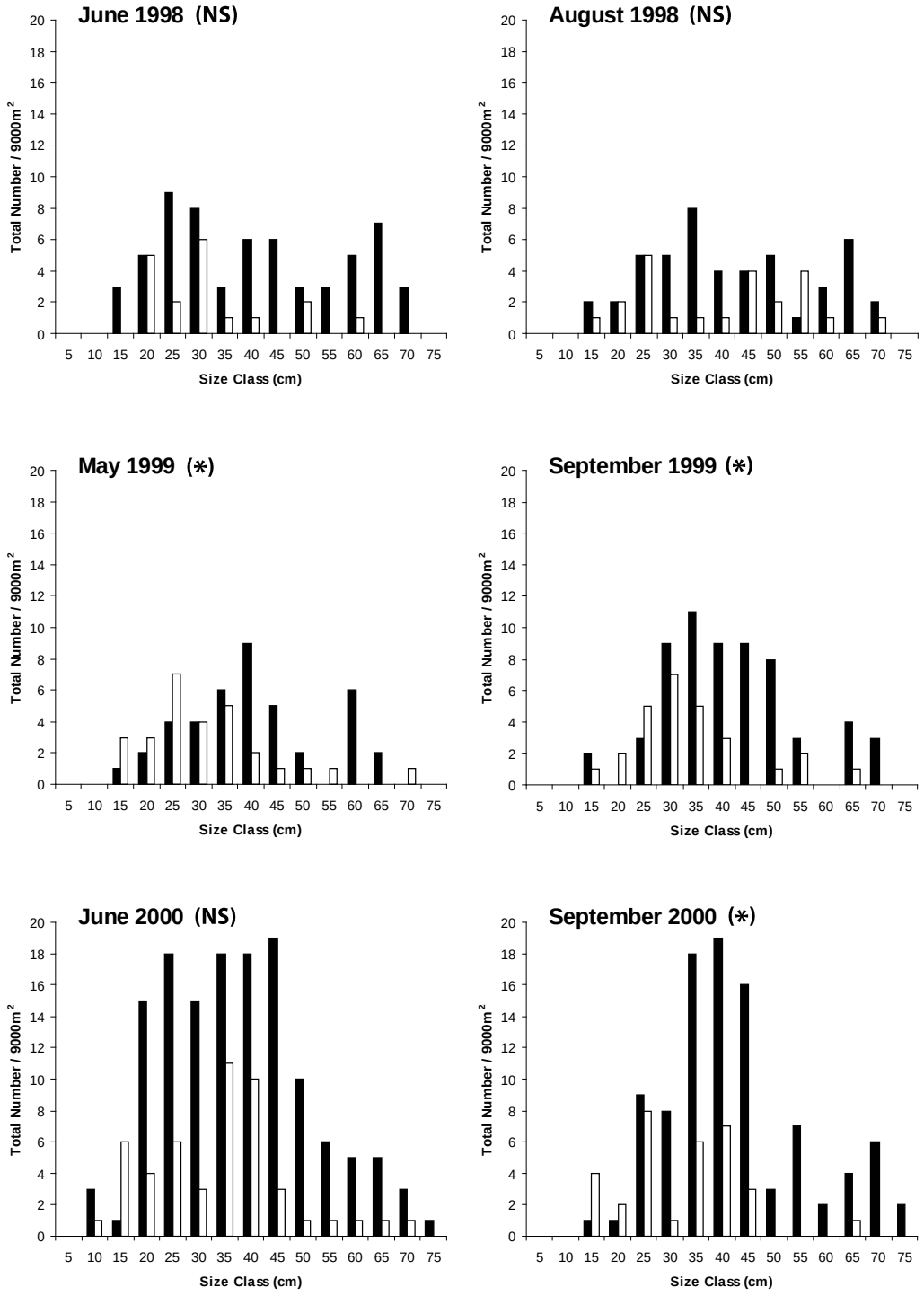


Figure 4.2: Length-frequency distributions of *Plectropomus* spp. within the Orpheus Island no-take reserve (black bars) and the Pelorus Island fished zone (white bars) throughout the monitoring period (1998-2000). Results of Kolmogorov-Smirnov tests comparing distributions between protected and fished zones are also shown: * = significant ($p < 0.05$); NS = non significant.

ZONE	TL.	June 1998	Aug. 1998	May 1999	Sept. 1999	June 2000	Sept. 2000	Mean
Orpheus Island (Protected)	< 35cm	40%	30%	27%	23%	38%	20%	**29.7%**
	> 35cm	60%	70%	73%	77%	62%	80%	**70.3%**
	Mode	25cm	35cm	40cm	35cm	45cm	40cm	**36.7cm**
Pelorus Island (Fished)	< 35cm	72%	46%	61%	56%	41%	47%	**53.8%**
	> 35cm	28%	54%	39%	44%	59%	53%	**46.2%**
	Mode	30cm	25cm	25cm	30cm	35cm	25cm	**28.3cm**

Table 4.4: Relative abundances of sub-minimum legal size (< 35cm TL) and legal size and above (> 35cm TL) *Plectropomus* spp. within the Orpheus Island no-take reserve and the Pelorus Island fished zone between June 1998 and September 2000. Modal lengths of *Plectropomus* spp. sighted in each zone on each survey occasion are also shown.

* Note that the minimum legal size for *P. leopardus* and *P. maculatus* in Queensland state waters is 38cm TL. These two species comprise > 99% of the *Plectropomus* spp. grouping utilised in this study.

Lutjanus carponotatus (Stripey Sea Perch)

Estimates of *Lutjanus carponotatus* population density were significantly higher in the Orpheus Island NTR than in the Pelorus Island fished zone during four of the six survey trips. Biomass estimates of *L. carponotatus* were consistently higher in the NTR than in the fished zone, but these differences were only significant during the final two survey trips, June and September 2000 (Figure 4.3 & Table 4.2). Repeated measures ANOVA revealed no significant differences in *L. carponotatus* population density or biomass between survey trips (Figure 4.3 & Table 4.2). There were no significant interactions between trip and zone for either density or biomass (Table 4.2). ANCOVA detected no significant effects of live coral cover on the density or biomass of *L. carponotatus* (Table 4.3).

Lutjanus carponotatus

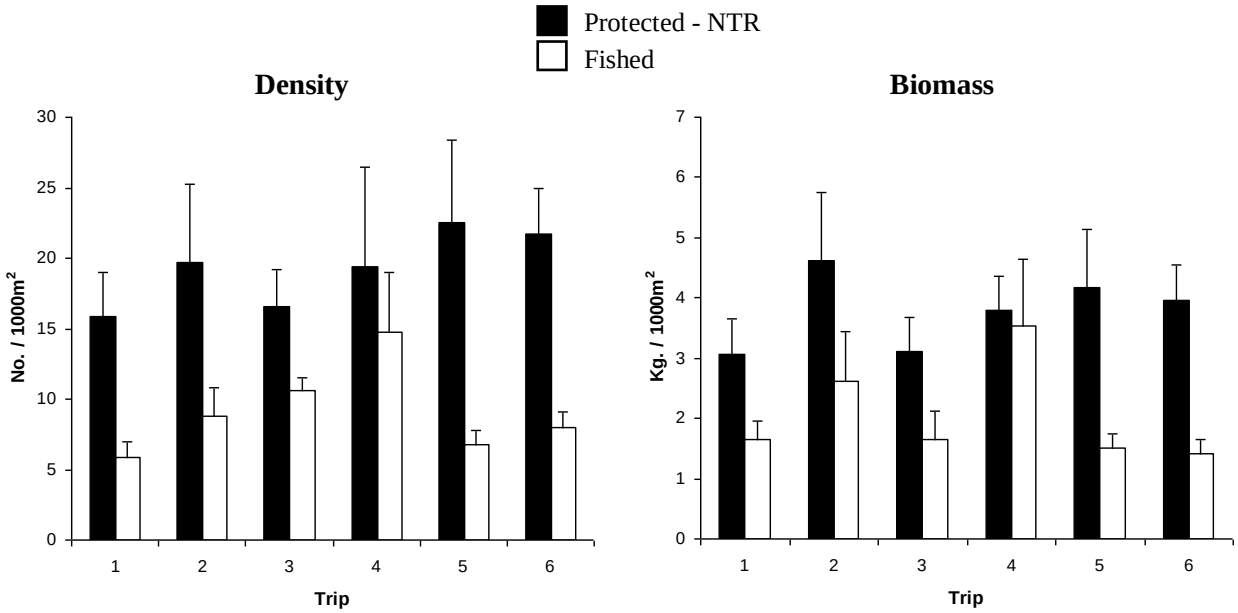


Figure 4.3: Temporal estimates of mean (± 1SE) density (number / 1000m^2) and biomass (kg / 1000m^2) of *Lutjanus carponotatus* in the Orpheus Island no-take reserve and the Pelorus Island fished zone.

Chaetodontidae (Butterfly fishes)

Population density and biomass estimates of the chaetodontid group were consistently higher within the Orpheus Island NTR than in the Pelorus Island fished zone (Figure 4.4 & Table 4.2). Within the reserve, chaetodontid population density and biomass increased significantly (*Tukey's test:* $p < 0.001$) between June 1998 and September 2000. Chaetodontid density and biomass were more stable within the Pelorus Island fished zone, with only one significantly lower estimate recorded in May 1999 (*Tukey's test:* $p < 0.05$) (Figure 4.4 & Table 4.2). Significant positive effects of live coral cover (hard & soft coral combined) on chaetodontid population density and biomass were detected in September 1999 and in September 2000 ($p < 0.05$ in both cases) (Table 4.3).

Chaetodontidae

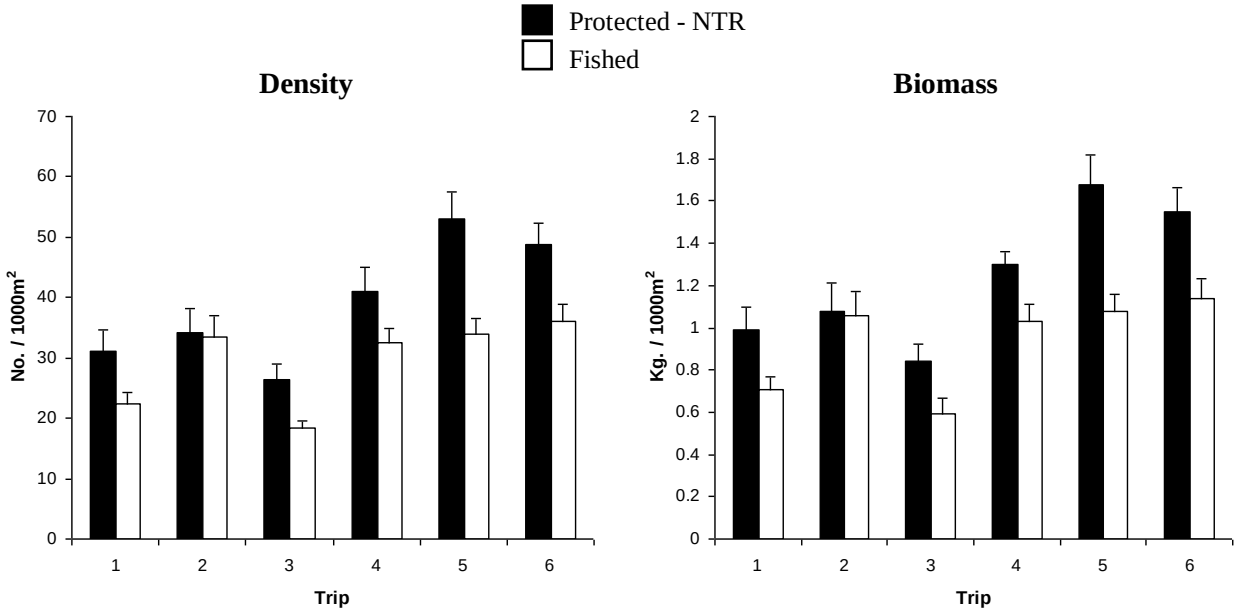


Figure 4.4: Temporal estimates of mean (± 1SE) density (number / 1000m^2) and biomass (kg / 1000m^2) of the Chaetodontidae group in the Orpheus Island no-take reserve and the Pelorus Island fished zone.

Siganidae (Rabbit fishes)

Population density and biomass estimates of the Siganidae group were not significantly different between the Orpheus Island NTR and the Pelorus Island fished zone throughout the monitoring period (Figure 4.5 & Table 4.2). Siganid population density and biomass increased significantly within the Pelorus Island fished zone between June 1998 and September 2000 (*Tukey's test:* $p <$ 0.001). Within the Orpheus Island reserve, siganid density and biomass estimates did not vary significantly throughout the monitoring period (Figure 4.5 & Table 4.2). Significant negative effects of live coral cover (hard & soft coral combined) on siganid population density and biomass were detected in June 1998 and in May 1999 ($p < 0.05$ in both cases) (Table 4.3).

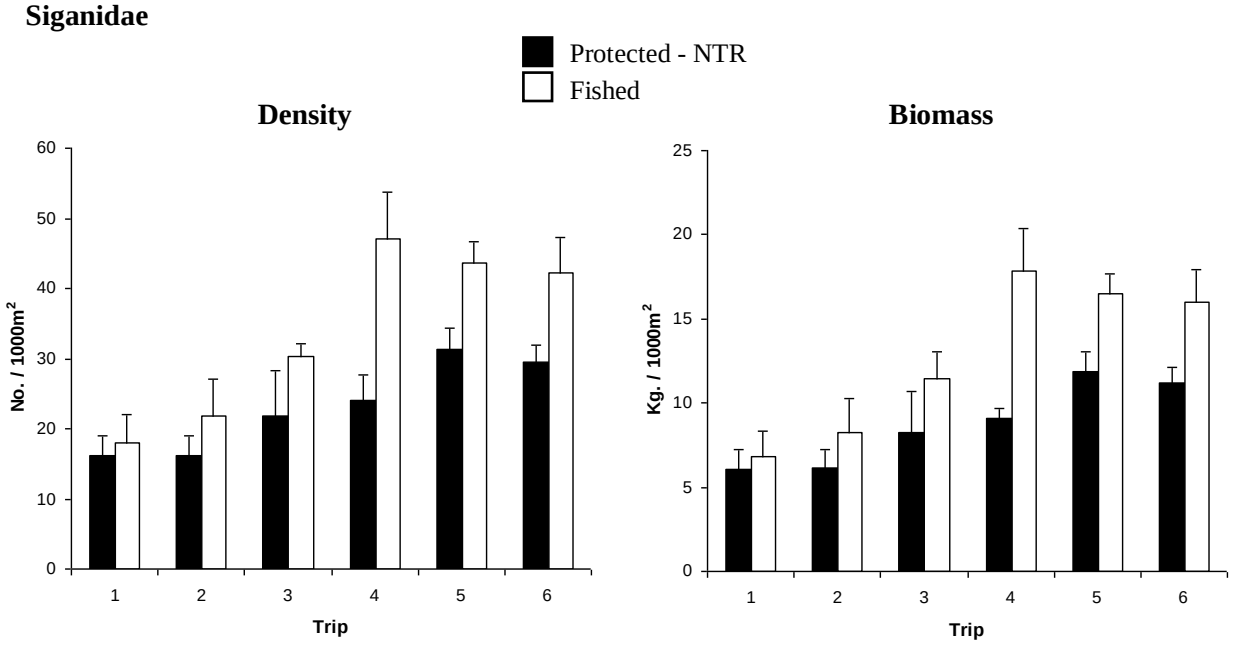


Figure 4.5: Temporal estimates of mean (± 1SE) density (number / 1000m^2) and biomass (kg / 1000m^2) of the Siganidae group in the Orpheus Island no-take reserve and the Pelorus Island fished zone.

4.3.2. Temporal variations in benthic cover and structural complexity

No significant differences in mean live hard coral cover (LHC) or mean total live coral cover (hard & soft coral combined - LCC) were detected between the Orpheus Island reserve and the Pelorus island fished zone (Figure 4.6 & Table 4.2). Estimates of LHC increased significantly (*Tukey's test:* $p < 0.05$) within the Pelorus Island fished zone between May 1999 and June 2000, but fell slightly again by September 2000 (Figure 4.6 & Table 4.2). LHC estimates did not vary significantly within the Orpheus Island NTR during the monitoring period. Within the Orpheus Island NTR and the Pelorus Island fished zone, estimates of mean total live coral cover (LCC)

increased significantly (*Tukey's test:* $p < 0.01$) between August 1998 and June 2000, before falling slightly by September 2000 (Figure 4.6 & Table 4.2).

Throughout the monitoring period, mean estimates of habitat structural complexity were consistently higher, but not significantly higher, in the Pelorus Island fished zone than in the Orpheus Island NTR. Mean structural complexity did not change significantly within each zone during the monitoring period (Figure 4.6 & Table 4.2).

4.3.3. Variations in underwater visibility between survey trips

Within each survey period, no significant differences in mean underwater visibility were detected between the NTR and the fished zone (Figure 4.6 & Table 4.2). Mean underwater visibility was lowest in June 2000 in both the NTR and the fished zone (Figure 4.6). In both the reserve and fished zone, underwater visibility was significantly lower in June 2000 than in September 1999 and in September 2000 (*Tukey's test;* $p < 0.05$ in both cases).

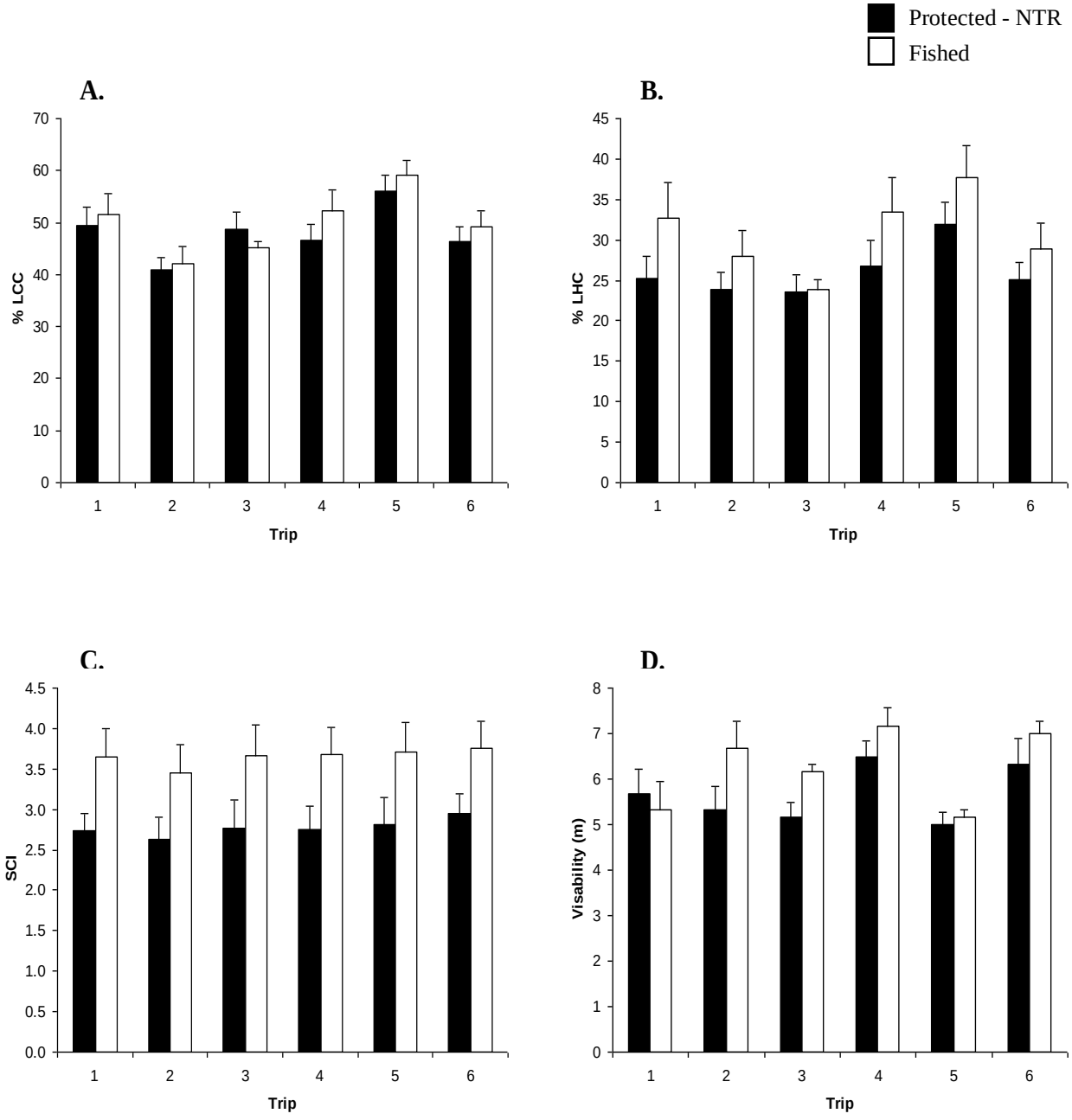


Figure 4.6: Temporal estimates of benthic habitat characteristics and underwater visibility in the Orpheus Island no-take reserve and the Pelorus Island fished zone.. **A**. Mean (± 1SE) percent total live coral cover (% LCC - hard and soft coral combined). **B**. Mean (± 1SE) percent live hard coral cover (% LHC). **C**. Mean (± 1SE) structural complexity indices (SCI). **D**. Mean (± 1SE) underwater visibility.

4.3.4. Recreational fishing effort and poaching in the Palm Island group

During the six survey trips between 1998 and 2000 a total of 32 days were spent collecting data at Orpheus and Pelorus Island. During this period, 102 recreational fishing vessels were sighted fishing the Pelorus Island fringing reef and 8 vessels were sighted illegally fishing the reefs within the Orpheus Island reserve. A summary of the number of vessels recorded fishing and extrapolations of mean daily and yearly fishing effort on the fringing reefs of Pelorus and Orpheus Islands is provided in Table 4.5.

Island	No. Vessels Recorded	Mean No. Vessels / Day	Mean No. Vessels / Year	% Weekday	% Weekend
Orpheus Is	8	0.25 (± 0.09)	91.3 (± 32.8)	77.3%	22.7%
Pelorus Is	102	3.19 (± 0.41)	1164.2 (± 150.7)	64.7%	35.3%

Table 4.5: Total number of vessels observed fishing on fringing reefs of Orpheus and Pelorus Islands during 32 days of observations between June 1998 and September 2000. Estimates of mean daily and yearly fishing effort (vessels / day and vessels / year) and the relative proportion of total fishing effort attributable to weekdays and weekends is also provided.

4.4. DISCUSSION

Spatially and temporally replicated studies of NTRs and surrounding fished areas can yield much information on the fishery effects and benefits of reserve protection, the dynamics of fish populations and fish community and habitat interactions (Cole *et al.* 1990; Ferreira & Russ 1995;

Edgar & Barrett 1999; Nardi *et al.* 2004). This study set out to quantify persistence of the effects of no-take marine reserve protection on fish populations and to examine fish community dynamics and interactions over a temporal scale of 3 years.

Temporal monitoring of the Orpheus Island NTR and the Pelorus Island fished zone has revealed consistent positive effects of protection on targeted reef fish species. Between June 1998 and September 2000, coral trout (*Plectropomus* spp.) were consistently more abundant within the Orpheus Island NTR. On average, coral trout population density was 2.54 times higher in the NTR than in the fished zone. Furthermore, coral trout were larger and heavier within the NTR with over 70% of fish sighted likely being reproductively mature (at least 35cm in length), and mean biomass estimates 4.66 times higher than in the fished zone. Evidently, after 11-12 years of adequate protection, coral trout populations within the NTR had responded strongly and the patterns of relative abundance first observed in June 1998 had persisted through the three year monitoring period.

The mean abundance of coral trout within the NTR generally increased throughout the monitoring period but reached a peak in June 2000, before falling slightly by September 2000. Within the reserve, coral trout density was 22% higher, and biomass was 21% higher during September 2000 than it was in June 1998. The June 2000 population density peak was driven by significant increases in the relative abundances of mid-sized coral trout between 20cm and 45cm TL, the majority of which were presumably female fish (Ferreira & Russ 1992; Adams *et al*. 2000) (Figure 4.2). The most parsimonious explanations for the high numbers of mid-sized coral trout sighted in June 2000, are both high recruitment success during the 1999/2000 Summer and movement from windward or deeper areas into sheltered fringing reef areas shallower than 12m. By September 2000 it could be expected that a proportion of the coral trout population inside the reserve had

moved again to spawning aggregation sites in areas other than the survey sites utilised in this study (Samoilys 1997; Zeller & Russ 1998; Russell 2001). Spawning associated movements may partly explain the reduction in the mean density of coral trout between June and September 2000.

Of potential significance, mean underwater visibility during June 2000 surveys was the lowest of any survey trip and was significantly lower (by 1-2m) than during both September 1999 and September 2000. An alternative hypothesis for the very high density estimate of *Plectropomus* spp. in June 2000, may be that during lower visibility conditions, coral trout tend to approach divers more closely and thus enter the transect survey area with greater frequency than they do when the visibility is better. Although not presented in this thesis, repeated measures ANCOVA's were also carried out on all fish groups using underwater visibility as the co-variate. There were no statistically significant effects detected in any of these analyses. Continued monitoring of these reefs should provide insight into any potential correlations between underwater visibility and visual counts of *Plectropomus* spp. It may be that on these inshore reefs, variations in underwater visibility of as little as 2m can strongly influence the 'sightability' of coral trout, potentially in a counter-intuitive way.

Although NTR effects at Orpheus Island were clearly well established by 1998, it is possible that the population of *Plectropomus spp*. within the Orpheus Island reserve had not yet reached 'natural' maximum carrying capacity by September 2000. Russ & Alcala (2004) predicted that populations of large predatory reef fish (ie. Serranidae, Lutjanidae, Lethrinidae) within Philippine marine reserves may take between 13 and 40 years to recover to natural states of carrying capacity. Clearly, continued monitoring of these fringing reefs in the longer term (5-10 years or more) is required in order to achieve greater insight and resolution into the relatively short-term patterns of temporal change presented here.

In addition to the strong reserve effects detected for coral trout, it is evident that the Orpheus Island NTR has also created benefits for the stripey sea perch (*Lutjanus carponotatus*). Mean density and biomass estimates of *L. carponotatus* remained consistently higher in the NTR than in the fished zone between June 1998 and September 2000. Although generally not specifically targeted, most captured *L. carponotatus* which are above the minimum legal length of 25cm TL are retained by fishers (Williams & Russ 1994; *Pers. Obs* 1996-2008). Within the Pelorus Island fished zone 52.1% (total mean across 6 trips) of the *L. carponotatus* population was at or above the minimum legal length of 25cm between 1998 and 2000. Interestingly, only 44.8% of the *L. carponotatus* population within the Orpheus Island NTR was at least 25cm TL during the monitoring period. *L. carponotatus* density or biomass estimates did not show any consistent temporal patterns during the monitoring period and there were no significant effects of survey trip detected. This was primarily due to large between-site variability in *L. carponotatus* counts within each survey trip.

Throughout the monitoring period, butterfly fish (Chaetodontidae) were consistently more abundant within the NTR than within the fished zone. It is most probable that this pattern is linked with the dynamics of the coral community. Of the four species of chaetodontid surveyed in this study, two species are predominantly generalist benthic invertebrate feeders (*Chaetodon rainfordi* and *Chelmon rostratus*), while *C. aureofasciatus* feed predominantly on the live polyps of hard corals (eg. *Acropora* spp. and *Goniopora* spp.) and *C. melannotus* feed primarily on the polyps of soft corals (eg. *Sarcophyton* spp. and *Sinularia* spp.) (Allen & Steene 1996; Pratchett 2005). The lack of significant variation in live coral cover (hard and soft coral combined) between the reserve and the fished zone complicates the interpretation of this pattern of chaetodontid abundance. Interestingly however, both the population density of chaetodontids and live coral cover increased significantly within the Orpheus Island reserve between 1998 and 2000.

Data presented here suggests that mean live coral cover (hard and soft coral combined) had fallen to approximately 40% by August 1998 following a mass coral bleaching event on the GBR during March 1998 (Baird & Marshall 1998; *Pers. Obs.* 1998). By June 2000, mean live coral cover in the Orpheus Island NTR had recovered to 56.1%. ANCOVA revealed significant positive effects of live coral cover on chaetodontid population density. Therefore, it is probable that the increase in coral cover between 1998 and 2000 has facilitated the increase in chaetodontid abundance, particularly for the two most commonly sighted species *C. aureofasciatus* and *C. melannotus.*

The two species of herbivorous rabbit fish (Siganidae) surveyed during the monitoring period were consistently more abundant within the Pelorus Island fished zone than in the Orpheus Island reserve. Although popular for their eating qualities throughout Asia and the islands of the Pacific, siganids are not sought after or captured by fishers in the GBR region and as such, it was not expected that NTR effects would be detected for these species. A potential explanation for the higher abundance of siganids within the fished zone is reduced rates of predation by large piscivores on juvenile rabbit fish inside the fished zone (Kingsford 1992). Although no supporting data is presented here, a second likely contributing scenario is strong associations of siganid populations with the relative cover of turfing and macro algae. Longer term and more detailed monitoring programs should provide greater insight into fish community effects within marine reserves and associations between functional groups of fishes and the habitat.

Monitoring of fishing effort around Pelorus Island and Orpheus Island has highlighted the fact that there is a high level of fishing pressure being applied to these inshore reefs. The vast majority of this effort is applied by the recreational fishing sector. Furthermore, although incidences of poaching within the reserve have been recorded, the NTR at Orpheus Island has received a

consistently high level of protection and is potentially one of the most adequately protected NTRs within the GBR Marine Park (Davis *et al*. 2004; Williamson *et al*. 2004).

The major limitation to the generality of the results presented here, is that the design of the monitoring program did not conform to a complete Before-After-Control-Impact Pair (BACIP) experimental design (Jones *et al.* 1993; Russ 2002; Willis *et al.* 2003b). Only one protected reserve and one fished 'control' area were monitored between 1998 and 2000. This limitation was largely unavoidable, as Orpheus Island was the only protected marine reserve within the Palm Island group. Despite this limitation, this study has added to the evidence that given time and adequate protection, NTRs will provide a spatial refuge in which exploited fish populations can build up to a point where they may function to sustain if not enhance surrounding fisheries.

This study has added strength to the debate of the effectiveness and reliability of underwater visual census (UVC) methodologies in coral reef fishery stock assessments. The UVC methodology used here has produced clear and consistent estimates of population abundance and size distributions of target and non-target reef fishes. The fact that all surveys were conducted by a single observer eliminates the potentially large source of variability introduced when using multiple observers, especially over extended temporal scales. Observer training and guidance in the early stages is essential if continuity of results is to be maintained in any ongoing monitoring project. Furthermore, it is critical that future surveys on these fringing reefs are not conducted when underwater visibility is below 5m. This study has provided a solid baseline for continued monitoring of these high-use inshore fringing reefs.

Chapter 5: Long-term effects of no-take marine reserve protection on reef fish and benthic communities in the GBR Marine Park.

5.1. INTRODUCTION

In an adaptive management context it is essential to establish performance monitoring programs which are designed to assess the ecological and social implications of management strategies (Hilborn & Walters 1992). Furthermore, effective management of marine resources is reliant on accurate estimates of ecosystem resilience, standing stock biomass of species targeted by fisheries and an understanding of the temporal dynamics of fish and invertebrate communities (Hughes *et al.* 2003, 2005; Bellwood *et al*. 2004). Within the Great Barrier Reef Marine Park (GBRMP) there have been surprisingly few studies undertaken with sufficient spatial and temporal replication to allow estimates of ecosystem resilience, benthic community dynamics and reef fish population structure and dynamics in the face of exploitation. Furthermore, there has been a relative paucity of studies specifically examining the effects of no-take marine reserve (NTR) protection on fish populations and community dynamics on GBRMP reefs. Of the few studies that have been conducted, most have generated highly equivocal results (e.g. Williams & Russ 1994; Zeller & Russ 1998; Adams *et al.* 2000; Ayling *et al.* 2000; Davies 2000; Mapstone *et al.* 2003, 2008).

Chapter 3 of this thesis and Williamson *et al*. (2004) drew on data collected by Ayling and Ayling in 1983/84 (3 – 4 years prior to the establishment of the no-take marine reserves) and provided a spatially and temporally replicated assessment of coral trout populations on fished and protected inshore GBR fringing reefs after 12 – 13 years of NTR protection. Significant increases in the

mean population density and biomass of coral trout (*Plectropomus* spp.) and stripey snapper (*Lutjanus carponotatus*) were detected in NTR zones of the Palm and Whitsunday Island groups. Evans and Russ (2004) reinforced the findings of Williamson *et al*. (2004) by demonstrating that the mean population biomass of both species was significantly higher in protected zones than in fished zones of both the Palm and Whitsunday Island groups and in a third island group, the Keppel Islands, located approximately 400km south of the Whitsunday Islands. Furthermore, these studies found that the mean density and biomass of non-target fish species (Siganidae and Chaetodontidae) did not differ between protected and fished zones at any of the three island groups. These studies have provided convincing evidence that after 12 to 14 years of adequate protection, the NTRs on these inshore reefs had provided substantial benefits for populations of target species within reserve boundaries.

In this chapter, long-term temporal monitoring data are presented for two fishery target species (coral trout; *Plectropomus* spp. group and stripey snapper; *Lutjanus carponotatus*), several non-targeted fish species (Siganidae and Chaetodontidae groups) and the sessile benthic community in NTR and fished zones of the Palm, Whitsunday and Keppel Island Groups between 1999 and 2007. This study employs a Before-After-Control-Impact-Pairs (BACIP) experimental design to provide an assessment of the long-term effects of no-take marine reserve protection on coral trout (*Plectropomus* spp.) populations. For all other fish groups and the coral community however, baseline data from prior to the establishment of the protected areas was not available.

The specific objectives of this study were to assess the long-term effects of marine reserve protection on populations of target and non-target reef fishes, and to examine the temporal dynamics of several key indicator fish species and the sessile benthic community on inshore fringing reefs of the GBRMP.

5.2. METHODS

5.2.1. Study Locations

This study was carried out in the Palm, Whitsunday and Keppel Island groups. Further details of the study locations and site descriptions are provided in Chapter 2 (General Methods).

5.2.2. Data Collection

The method of data collection is described in Chapter 2 (General Methods). In addition however, the findings presented in this chapter draw on the baseline provided by the pre-zoning underwater visual census (UVC) data for coral trout (*Plectropomus* spp.) collected by Dr. Tony Ayling in the Palm and Whitsunday Island groups during 1983 and 1984. The UVC methods utilised for the collection of the baseline data set are described in Chapter 3. The remainder of the UVC data presented in this chapter (1999 – 2007) were collected by David Williamson (DW) and Richard Evans (RE).

5.2.3. Sampling design

A description of the sampling design utilised for the 1983/84 baseline data set is provided in Chapter 3. Between 1999 and 2007, UVC data collection was conducted at the same 24 sites per island group in both the Palm and Whitsunday Islands. A description of these sites is provided in Chapter 3. In addition, data collected from 12 sites in the Keppel Island group are also presented in this chapter. Within the Keppel Islands, 4 sites were located within the protected NTR at Middle Island, 2 sites were located within the Halfway Island NTR, and 6 sites were located within fished zones surrounding Great Keppel Island. Maps of the island groups, annotated with the 1987 – 2004

management zoning plan, and the approximate position of sites within each island group are provided in Figure 2.1 (Chapter 2).

Since 1999, data collection trips have been conducted periodically at each island group. Research funding allocations, weather conditions and availability of personnel have determined the frequency at which these sites have been sampled. A summary of the survey trips undertaken since 1999 is provided in Table 5.1.

Island Group	1999	2000	2001	2002	2003	2004	2005	2006	2007
Palm	×	✓	✓	✓	✓	×	×	×	✓
Whitsunday	✓	×	✓	✓	✓	×	×	×	✓
Keppel	×	×	×	✓	×	✓	×	✓	✓

Table 5.1: Underwater visual census (UVC) survey trips conducted on near-shore reefs of the Great Barrier Reef Marine Park between 1999 and 2007. ✓ = data collected at long-term monitoring sites; × = no data collected.

5.2.4. Data handling and analysis

In this chapter, UVC data from the long-term monitoring sites in the Palm, Whitsunday and Keppel Island groups are presented. The analysis is restricted to two target fish groups, coral trout (*Plectropomus* spp. – comprised of *Plectropomus maculatus*, *P. leopardus* and *P. laevis*) and stripey snapper (*Lutjanus carponotatus*), and two non-targeted fish groups, rabbitfish (Siganidae – comprised of *Siganus doliatus* and *S. lineatus*) and butterflyfish (Chaetodontidae – comprised of *Chaetodon aureofasciatus*, *C. melannotus*, *C. rainfordi* and *Chelmon rostratus*) and the sessile benthic community. Temporally replicated estimates of mean density, biomass and percent cover

within NTR and fished zones of all island groups combined and for each separate island group are presented graphically.

Mean density and biomass of fish, and percent cover estimates of benthos, for the three island groups combined were calculated using data from 2002 and 2007 as these were the two years in which the long-term monitoring sites were surveyed in all three island groups in the same year (Table 5.1). In order to balance the statistical analysis between island groups, 6 protected and 6 fished sites (12 sites) were randomly selected from the total 24 sites surveyed at each of the Palm and Whitsunday Island groups. Therefore, 6 protected and 6 fished sites from each of the three island groups were included in the statistical analysis. Within each individual island group, the data utilised in the analysis and presented in the figures includes all long-term monitoring sites at all survey occasions.

Pre-zoning baseline UVC data collected by Dr. Tony Ayling in 1983/84 for the *Plectropomus* spp. group were only available for the Palm and Whitsunday Island groups (see Chapter 3). These data have been included in the temporal dynamics figures for the Palm and Whitsunday Island groups, but have not been included in the statistical analysis. Analysis of the pre-zoning *Plectropomus* spp. data is presented in Chapter 3 and in Williamson *et al*. (2004). Pre-zoning baseline data were not available for fish species other than *Plectropomus* spp.

Statistical comparisons of density, biomass and percent cover were made using univariate repeated measures ANOVA. Before proceeding with ANOVA, all data were examined for homogeneity of variance using Cochrans test, normality using normal probability plots and sphericity using the Mauchley test. In cases where raw data did not meet ANOVA assumptions, data were transformed ($Log(x + 1)$). Tukey's post-hoc tests were used to detect significant differences between means.

The statistical software package *STATISTICA* was used for all analyses and a significant difference was considered to exist if $p < 0.05$. All data presented in the text and figures are the mean ± 1 standard error (SE) of untransformed data.

5.3. RESULTS

5.3.1. Effects of no-take reserve protection on fish species and groups

Plectropomus spp. (Coral Trout)

The density of coral trout (*Plectropomus* spp.) varied significantly between island groups (regions), between protected and fished zones and through time (Table 5.2; Figure 5.1a & Figure 5.2). *Plectropomus* spp. biomass varied significantly between zones and years, but did not vary significantly between regions (Table 5.2; Figure 5.1b & Figure 5.2). The mean density of *Plectropomus* spp. was consistently higher in the Keppel and Palm Island groups than in the Whitsunday Island group (Table 5.2; Figure 5.2). However, *Plectropomus* spp. were larger on average in the Whitsunday Island group than in the Keppel and Palm Island groups, thus producing no significant difference in biomass between regions (Table 5.2; Figure 5.2).

In two of the three island groups, the density of *Plectropomus* spp. was significantly higher in protected zones than in fished zones (Table 5.3; Figure 5.2). The one exception was during 2002 in the Keppel Islands, where the density of *Plectropomus* spp. was higher, but not significantly so, in fished zones than in protected zones. The biomass of *Plectropomus* spp. was significantly higher in

protected zones than in fished zones of all three island groups, a pattern that was consistent through time (Table 5.3; Figure 5.2).

For the three regions combined, a significant increase in density and biomass of *Plectropomus* spp. was detected in protected zones, but not in fished zones, between 2002 and 2007 (Table 5.2; Figure 5.1). It should be noted however, that in both the Palm and Whitsunday Island groups, the increases recorded in protected zones between 2002 and 2007 were preceded by declines in density and/or biomass between 1999/2000 and 2002 (Figure 5.2). These declines were not detected within fished zones of the Palm or Whitsunday Island groups (Figure 5.2a & 5.2b).

In the Palm Island group, a significant decline in the density of *Plectropomus* spp. was recorded in protected zones between 2000 and 2001. Although recruitment success and adequate survivorship led to a steady increase in density in both protected and fished zones from 2001 to 2007, biomass continued to decline until at least 2003 (Figure 5.2a). A similar pattern was recorded in the Whitsunday Islands, where the biomass of *Plectropomus* spp. declined steadily in protected zones between 1999 and 2002 (Figure 5.2b). In both the Palm and Whitsunday Islands, peak biomass estimates were recorded during 1999/2000 (Figure 5.2a & 5.2b). Although not presented here, length frequency data revealed a significant decrease in the abundance of large fish (55 – 75 cm TL) between 1999/2000 and 2002 in protected zones of both the Palm and Whitsunday Island groups. In the Keppel Island group, large temporal variability in both density and biomass has been detected in protected zones and to a lesser degree in fished zones (Figure 5.2c). Large between-site variability however, has produced marginally significant or non-significant ANOVA results (Table 5.3).

Pre-zoning, baseline *Plectropomus* spp. data from the Palm and Whitsunday Island groups highlights the strong effects of zoning protection on these primary target reef fish species (Figure 5.2a & 5.2b). In NTR zones of the Palm Island group, the highest recorded density of *Plectropomus* spp. (2000) was 4.82 times higher than the density in 1984. In 2001, when density in protected zones was at its lowest recorded level to date, the density remained 2.68 times higher than in 1984. In 2000, the biomass of *Plectropomus* spp. in protected zones of the Palm Islands was 7.44 times higher than in 1984. At its lowest level in 2003, biomass remained 2.88 times higher than in 1984. Similar patterns were recorded in the Whitsunday Island group, where density and biomass of *Plectropomus* spp. in protected zones in 1999 were 3.96 and 7.72 times higher respectively, than in 1984. In 2002, when mean values for protected zones were at their lowest, density and biomass remained 2.89 and 3.17 times higher respectively than in 1984 (Figure 5.2a & 5.2b). In both the Palm and Whitsunday Island groups, the variation in density and biomass between the 1984 baseline and post 1999 fished zones remained small and non-significant (Figure 5.2a & 5.2b).

Species / Group	**Region** 2,30 *d.f.*	**Zone** 1,30 *d.f.*	**Region x Zone** 2,30 *d.f*	**Year** 1,30 *d.f.*	**Year x Region** 2,30 *d.f.*	**Year x Zone** 1,30 *d.f.*	**Year x Region x Zone** 2,30 *d.f.*
Plectropomus spp. Density	**6.86** (**)	**12.93** (**)	1.21 (ns)	**5.57** (*)	2.02 (ns)	**4.68** (*)	2.12 (ns)
Plectropomus spp. Biomass	3.25 (ns)	**38.04** (***)	0.33 (ns)	**5.83** (*)	1.09 (ns)	0.49 (ns)	0.89 (ns)
L. carponotatus Density	**9.65** (***)	**17.01** (***)	1.71 (ns)	1.38 (ns)	0.07 (ns)	1.40 (ns)	0.02 (ns)
L. carponotatus Biomass	1.08 (ns)	**28.18** (***)	**4.70** (*)	**6.94** (*)	0.15 (ns)	0.08 (ns)	0.55 (ns)
Siganid Density	**3.93** (*)	0.25 (ns)	0.80 (ns)	0.35 (ns)	0.46 (ns)	0.01 (ns)	0.21 (ns)
Chaetodontid Density	**24.21** (***)	1.62 (ns)	0.99 (ns)	**5.71** (*)	0.74 (ns)	3.93 (ns)	0.47 (ns)
% LCC	3.29 (ns)	0.27 (ns)	0.09 (ns)	0.26 (ns)	0.11 (ns)	0.03 (ns)	0.46 (ns)
% HCC	**20.80** (***)	0.81 (ns)	1.67 (ns)	0.11 (ns)	0.08 (ns)	0.08 (ns)	0.18 (ns)
% SCC	**18.01** (***)	3.24 (ns)	**3.46** (*)	**7.07** (*)	0.93 (ns)	2.42 (ns)	0.87 (ns)
% MAC	**12.01** (***)	0.71 (ns)	**6.18** (**)	**4.41** (*)	**17.14** (***)	4.05 (ns)	0.49 (ns)

Table 5.2: Results of univariate repeated measures analysis of variance on density and biomass of fish species or groups, and on percent cover of benthic variables across all island groups (regions) in two years (2002 and 2007). Values given are *F* ratios (probability results are shown in brackets); *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns: not significant. Statistically significant results are presented in bold text. ANOVA degrees of freedom (*d.f.)* are shown in column headings. LCC = live coral cover; HCC = hard coral cover; SCC = soft coral cover; MAC = macro-algal cover.

Region	Species / Group	Year P & W: 4,88 *d.f.* K: 3,30 *d.f.*	Zone P & W: 1,22 *d.f.* K: 1,10 *d.f.*	Year x Zone P & W: 4,88 *d.f.* K: 3,30 *d.f.*
Palm Is. Group	*Plectropomus* spp. Density	**5.92** (***)	**50.64** (***)	1.34 (ns)
	Plectropomus spp. Biomass	**5.32** (***)	**39.75** (***)	**4.21** (**)
	L. carponotatus Density	**6.54** (***)	**10.02** (**)	**3.09** (*)
	L. carponotatus Biomass	**5.45** (***)	**12.68** (**)	**3.55** (**)
	Siganid Density	**15.38** (***)	1.89 (ns)	0.29 (ns)
	Chaetodontid Density	**29.92** (***)	**7.81** (*)	1.09 (ns)
	% LCC	**11.16** (***)	2.86 (ns)	0.46 (ns)
	% HCC	**7.64** (***)	0.29 (ns)	0.83 (ns)
	% SCC	**4.65** (**)	**5.11** (*)	1.66 (ns)
	% MAC	**4.34** (**)	3.63 (ns)	0.94 (ns)
Whitsunday Is. Group	*Plectropomus* spp. Density	**4.23** (**)	**20.19** (***)	1.55 (ns)
	Plectropomus spp. Biomass	**6.36** (***)	**42.90** (***)	1.54 (ns)
	L. carponotatus Density	1.17 (ns)	0.70 (ns)	0.86 (ns)
	L. carponotatus Biomass	**3.29** (*)	3.58 (ns)	0.38 (ns)
	Siganid Density	**13.26** (***)	0.15 (ns)	**3.24** (*)
	Chaetodontid Density	**18.66** (***)	**5.77** (*)	1.48 (ns)
	% LCC	1.99 (ns)	2.92 (ns)	1.55 (ns)
	% HCC	1.27 (ns)	2.88 (ns)	0.50 (ns)
	% SCC	**5.69** (***)	0.38 (ns)	2.31 (ns)
	% MAC	**6.73** (***)	**5.34** (*)	0.48 (ns)
Keppel Is. Group	*Plectropomus* spp. Density	2.75 (ns)	3.22 (ns)	2.68 (ns)
	Plectropomus spp. Biomass	**4.33** (*)	**18.59** (**)	0.16 (ns)
	L. carponotatus Density	0.56 (ns)	2.97 (ns)	0.69 (ns)
	L. carponotatus Biomass	1.32 (ns)	**16.98** (**)	1.18 (ns)
	Siganid Density	0.78 (ns)	0.01 (ns)	0.94 (ns)
	Chaetodontid Density	**10.26** (***)	0.02 (ns)	0.74 (ns)
	% LCC	2.61 (ns)	0.17 (ns)	0.20 (ns)
	% HCC	2.40 (ns)	0.58 (ns)	0.18 (ns)
	% SCC	1.87 (ns)	1.54 (ns)	0.62 (ns)
	% MAC	**7.14** (***)	1.48 (ns)	1.87 (ns)

Table 5.3: Results of univariate analysis of variance on density and biomass of fish species or groups, and on percent cover of benthic variables within each island group. Values given are *F* ratios (probability results are shown in brackets). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns: not significant. Statistically significant results are presented in bold text. ANOVA degrees of freedom (*d.f.)* are shown in column headings; P & W = Palm and Whitsunday Island groups; K = Keppel Island group. LCC = live coral cover; HCC = hard coral cover; SCC = soft coral cover; MAC = macro-algal cover.

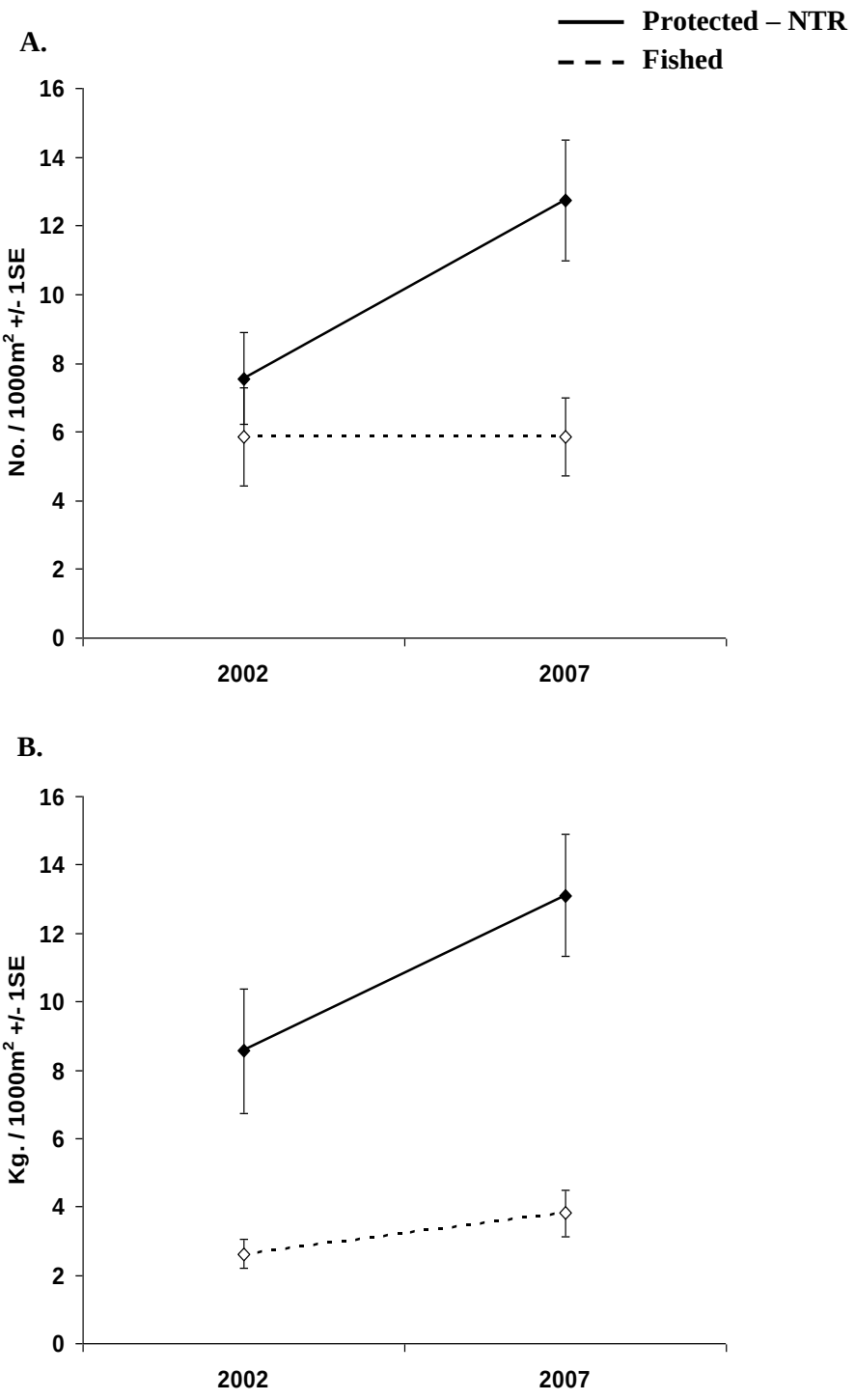


Figure 5.1: Mean (±1SE) density (A) and biomass (B) of *Plectropomus* spp. in combined protected (NTR) zones and fished zones of the Palm, Whitsunday and Keppel Island groups in 2002 and 2007.

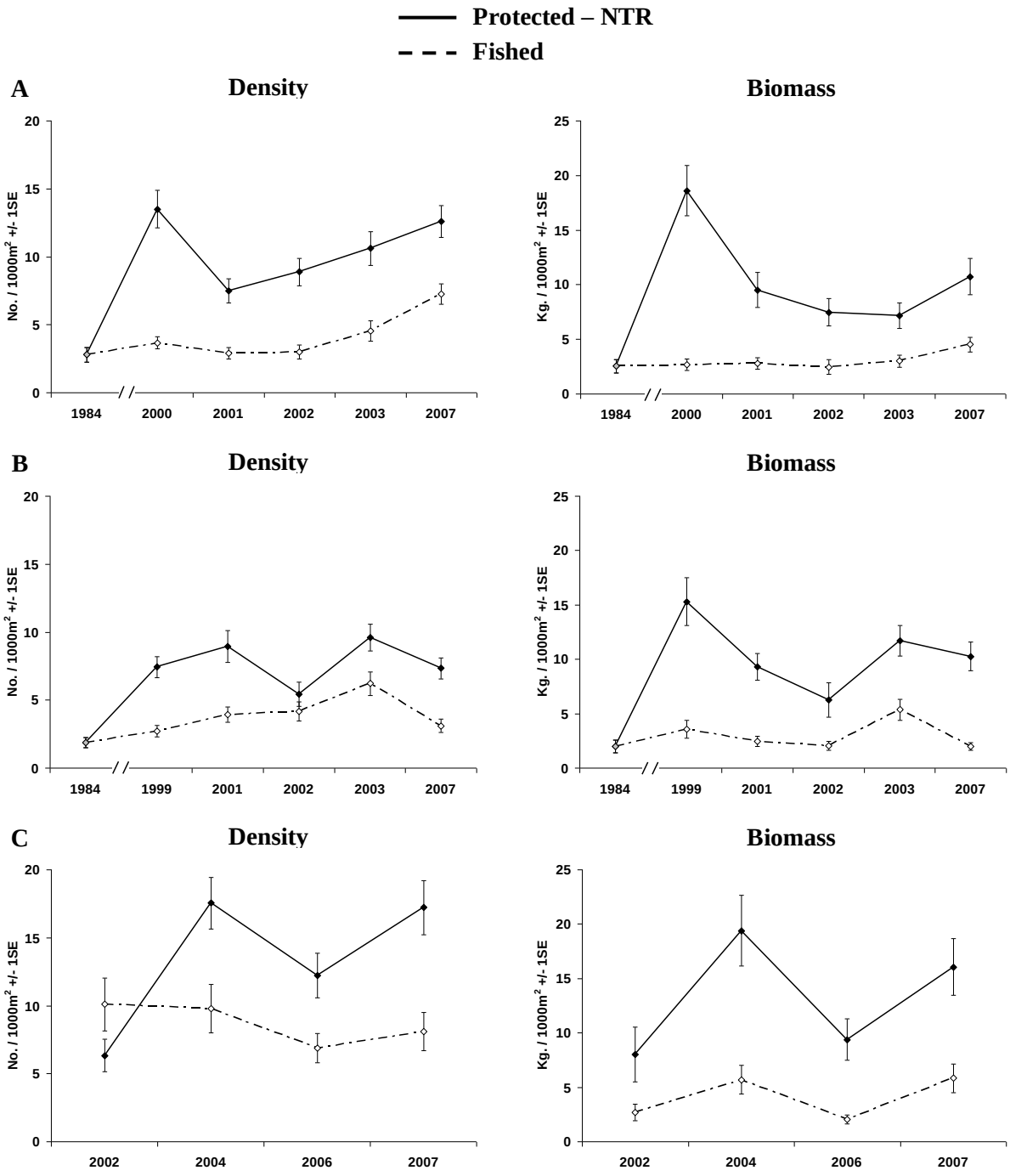


Figure 5.2: Temporal dynamics in mean (± 1SE) density and biomass of *Plectropomus* spp. in protected (NTR) zones and fished zones of the Palm (A), Whitsunday (B) and Keppel (C) Island groups. Mean density and biomass estimates are also provided for the pre-protection period (1984) in the Palm and Whitsunday Island groups.

***Lutjanus carponotatus* (Stripey Sea Perch)**

Across all regions combined, density and biomass of *L. carponotatus* were significantly higher in protected zones than in fished zones (Table 5.2; Figure 5.3). However, this pattern was largely driven by the Palm Island group, where mean density was significantly higher in protected zones than in fished zones in two out of five survey years (Table 5.3; Figure 5.4a). *L. carponotatus* density remained consistently higher but not significantly so, in protected zones than in fished zones of the Whitsunday and Keppel Island groups (Table 5.3; Figure 5.4). Overall mean size of *L. carponotatus* was larger in protected zones than in fished zones and biomass was significantly higher in protected zones than in fished zones of the Palm and Keppel Island groups, but not the Whitsunday Island group (Table 5.3; Figure 5.4).

Temporal variability in *L. carponotatus* density and biomass was greatest in the Palm Island group, where a significant decline was recorded in protected zones but not in fished zones between 2000 and 2001 (Table 5.3; Figure 5.4). The population of *L. carponotatus* within the Orpheus Island reserve recovered between 2001 and 2007, with density and biomass returning to the peak levels detected in 2000 (Figure 5.4a). Within protected zones of the Whitsunday and Keppel Island groups and within fished zones of all three island groups, *L. carponotatus* density and biomass remained relatively stable throughout the monitoring period (Figure 5.4).

Significantly higher densities of *L. carponotatus*, were recorded in the Palm and Keppel Island groups than in the Whitsunday Island group, however, like *Plectropomus* spp., fish were larger on average in the Whitsunday Islands and no significant regional differences in biomass were detected (Table 5.2; Figure 5.4).

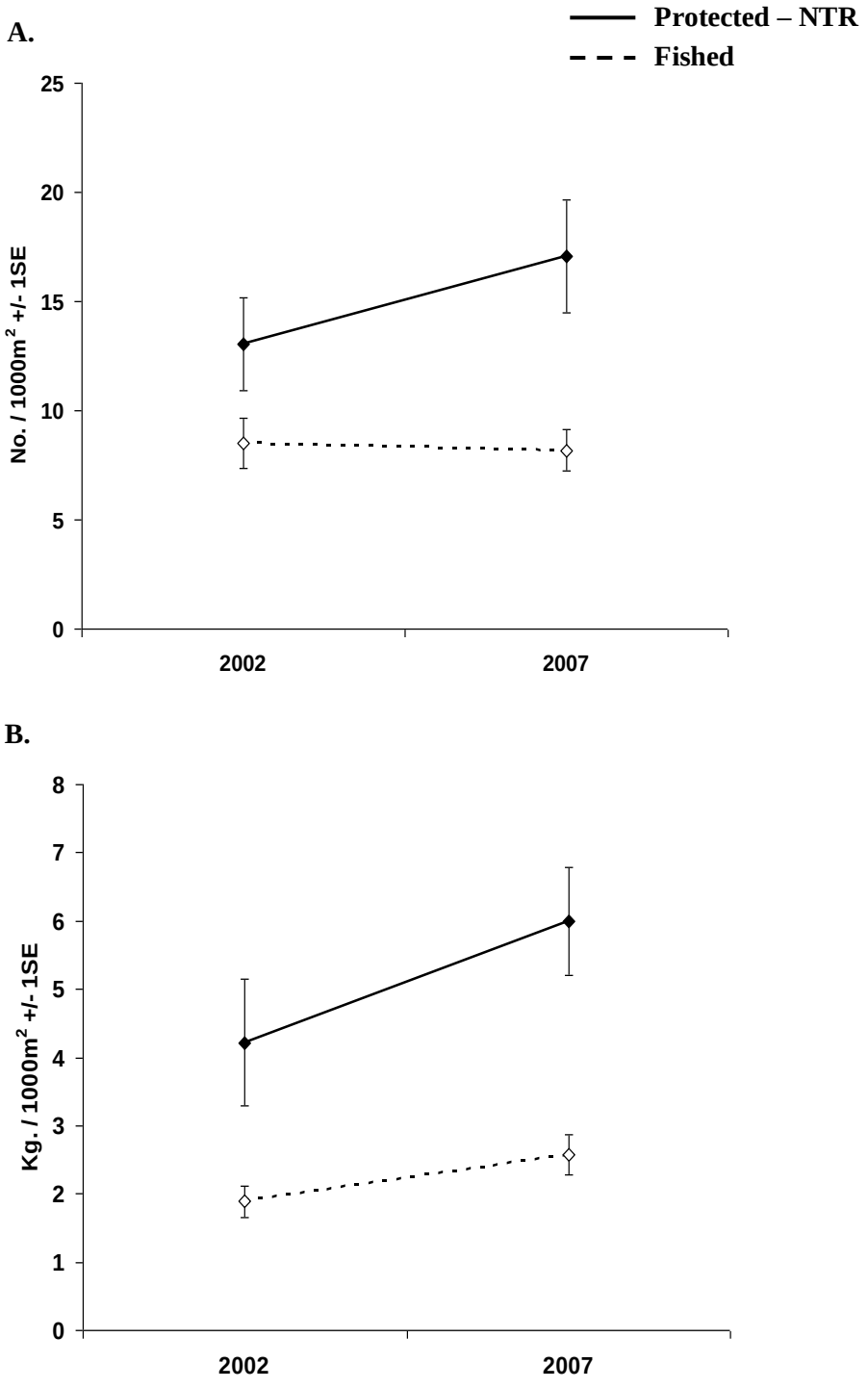


Figure 5.3: Mean (±1SE) density (A) and biomass (B) of *Lutjanus carponotatus* in combined protected (NTR) zones and fished zones of the Palm, Whitsunday and Keppel Island groups in 2002 and 2007.

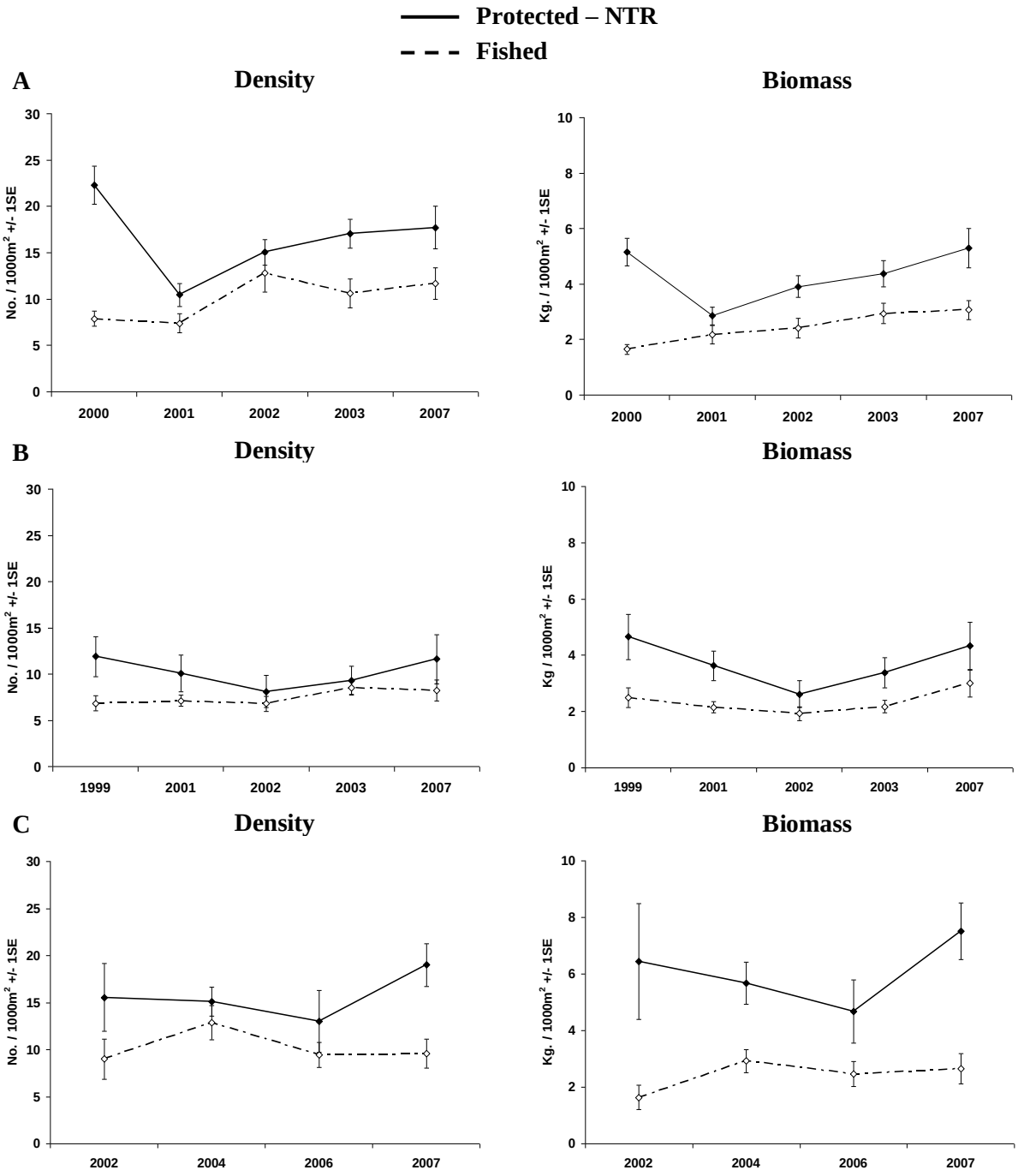


Figure 5.4: Temporal dynamics in mean (± 1SE) density and biomass of *Lutjanus carponotatus* in protected (NTR) zones and fished zones of the Palm (A), Whitsunday (B) and Keppel (C) Island groups.

Siganidae (Rabbit fishes)

No significant differences were detected in the density of the Siganidae group between protected and fished zones of any island group at any time (Tables 5.2 & 5.3; Figures 5.5a & 5.6). Siganid density was significantly higher in the Palm Islands than in the Keppel Islands, and higher, but not significantly, than in the Whitsunday Islands (Table 5.2, Figure 5.6). Significant temporal variation in the density of the Siganidae was detected in the Palm and Whitsunday Island groups, but not in the Keppel Islands (Table 5.3, Figure 5.6).

Chaetodontidae (Butterfly fishes)

Significant variation in the density of Chaetodontidae was detected between regions, with reefs of the Keppel Island group supporting higher overall abundances of butterflyfish than those of the Palm and Whitsunday Island groups (Tables 5.2 & 5.3; Figure 5.7). In both the Palm and Whitsunday Island groups, chaetodontid density remained higher in protected zones than in fished zones throughout the monitoring period, however, this difference was only significant on two occasions at both island groups (Palm Islands – 2000 & 2007; Whitsunday Islands – 2003 & 2007) (Table 5.3; Figure 5.7a & 5.7b). In the Keppel Island group, no significant differences in chaetodontid density were detected between protected and fished zones (Table 5.3; Figure 5.7c). Significant temporal variability in chaetodontid density was detected in all three island groups (Table 5.3; Figure 5.7).

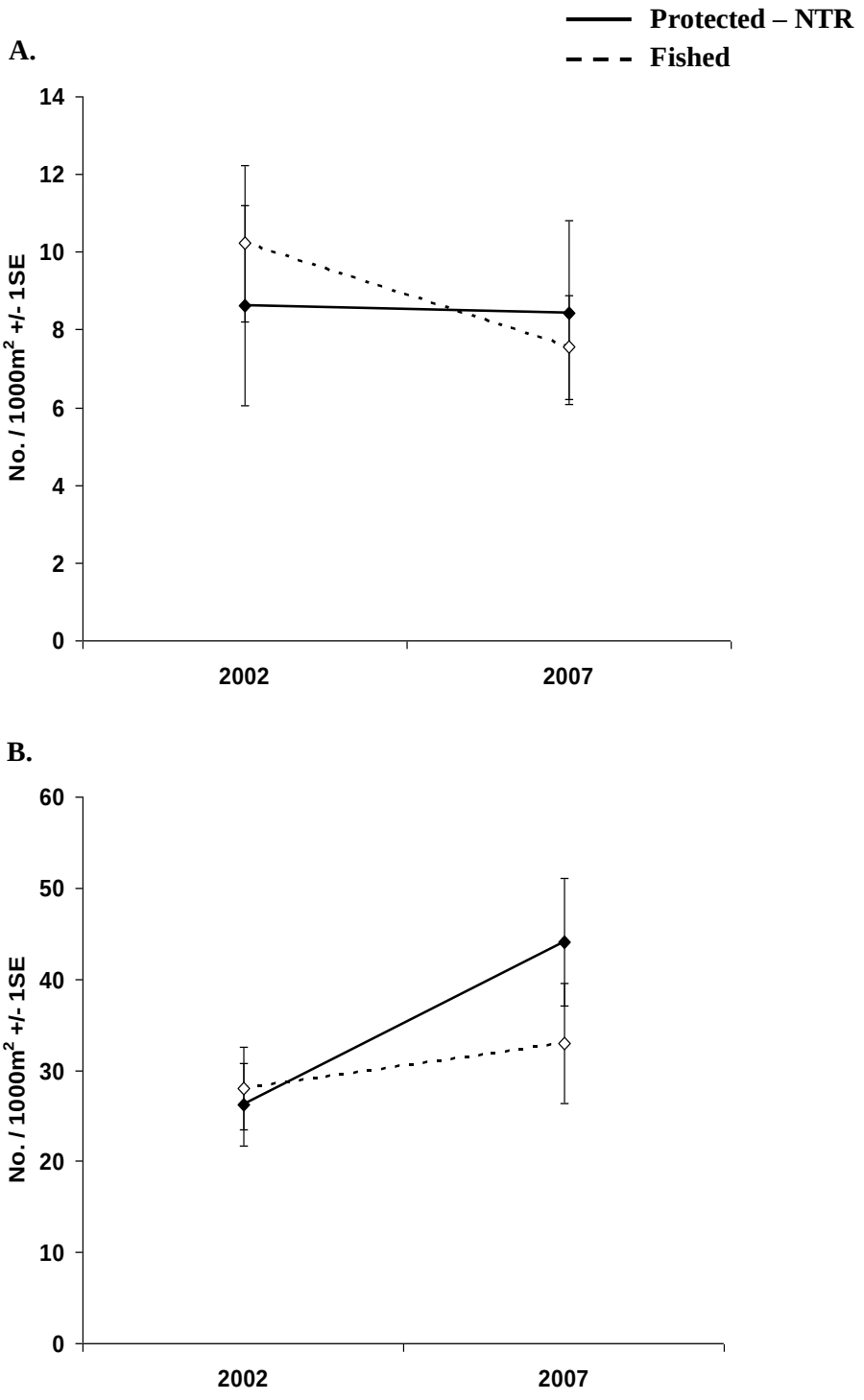


Figure 5.5: Mean (+/- 1SE) density of non-target fish species; Siganidae (A) and Chaetodontidae (B) in combined protected (NTR) zones and fished zones of the Palm, Whitsunday and Keppel Island groups in 2002 and 2007.

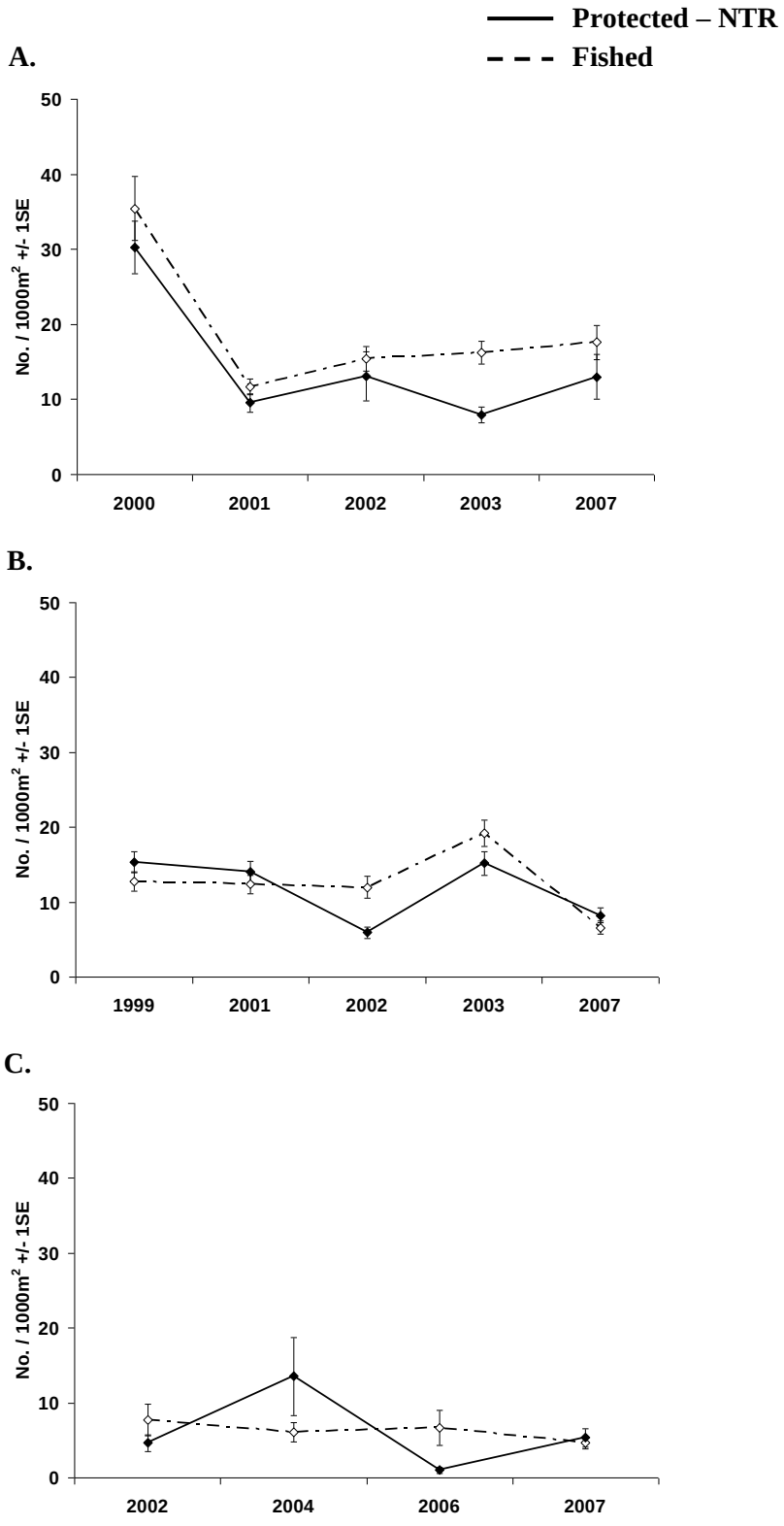


Figure 5.6: Temporal dynamics in mean (+/-1SE) density of the Siganidae group in protected (NTR) zones and fished zones of the Palm (A), Whitsunday (B) and Keppel (C) Island groups.

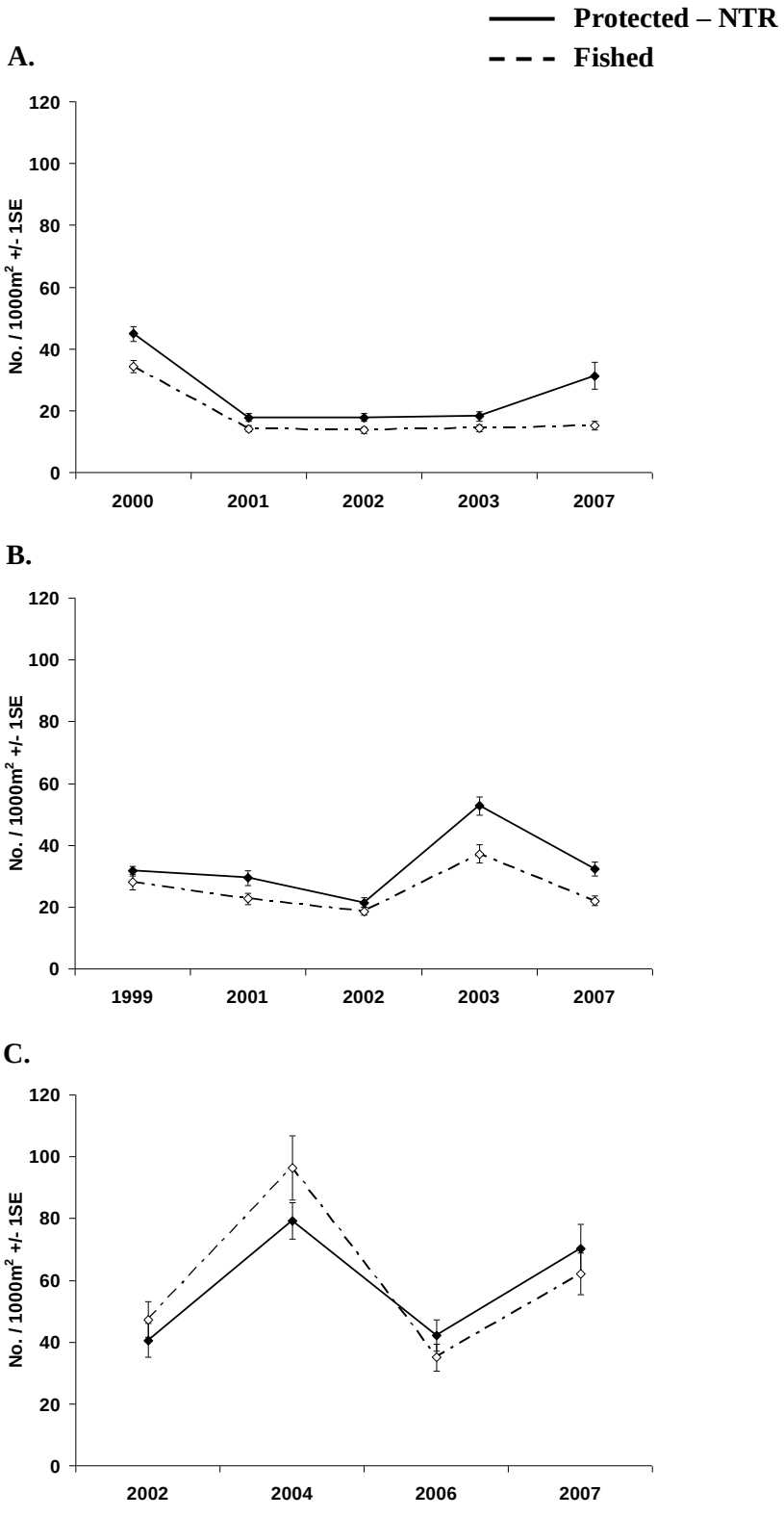


Figure 5.7: Temporal dynamics in mean (+/-1SE) density of the Chaetodontidae group in protected (NTR) zones and fished zones of the Palm (A), Whitsunday (B) and Keppel (C) Island groups.

5.3.2. Variations in benthic cover between years, regions and zones

Live coral cover (LCC) was relatively high at all three island groups throughout the monitoring period (Figures 5.8 & 5.9). The lowest values were recorded in the Palm Island group, where LCC ranged between 46% and 61%; while the highest values were recorded in the Keppel Island group where LCC ranged between 54% and 73%. A mass coral bleaching event occurred on the Great Barrier Reef in early 2002 and some reefs in the Palm, Whitsunday and Keppel Island groups were affected. In March 2006, a localised but relatively severe coral bleaching event occurred in the Keppel Island group. The temporal dynamics outlined below were largely driven by these coral bleaching events. No significant differences were detected in LCC between protected and fished zones of any island group (Tables 5.2 & 5.3; Figures 5.8 & 5.9).

A significant steady decline in LCC was recorded in the Palm Islands between 2000 and 2007 and this decline was accompanied by a significant increase in the cover of macro algae (Table 5.3; Figure 5.9a). Across all protected and fished sites within the Palm Island group, a significant decline in LCC of approximately 15% occurred between September 2000 and April 2002. After some limited recovery in 2003, LCC fell again and remained at approximately 46% through until 2007 (Figure 5.9a).

In the Whitsunday Islands, LCC remained relatively stable throughout the monitoring period but there was a significant increase in macro algal cover, predominantly in fished zone sites which were located along the eastern coast of Whitsunday Island (Table 5.3; Figure 5.9b).

In the Keppel Island group, monitoring began in October 2002, approximately 8 months after the 2002 mass coral bleaching event. It is evident that the reefs were still in the recovery phase at that

time. A decline in mean LCC of approximately 20% occurred during 2006 following the localized coral bleaching event. This decline was not statistically significant however due to large between site variability in the scale of the decline (Table 5.3; Figure 5.9c). Several of the sites which had experienced declines in LCC during 2006 had largely recovered to pre-bleaching levels by December 2007. Recovery of the reefs was not uniform however, and overall mean LCC remained below pre-bleaching levels in December 2007. The majority of the sites which recovered successfully from the 2006 bleaching event were in fished zones, while recovery of sites within protected zones was not as pronounced (Figure 5.9c).

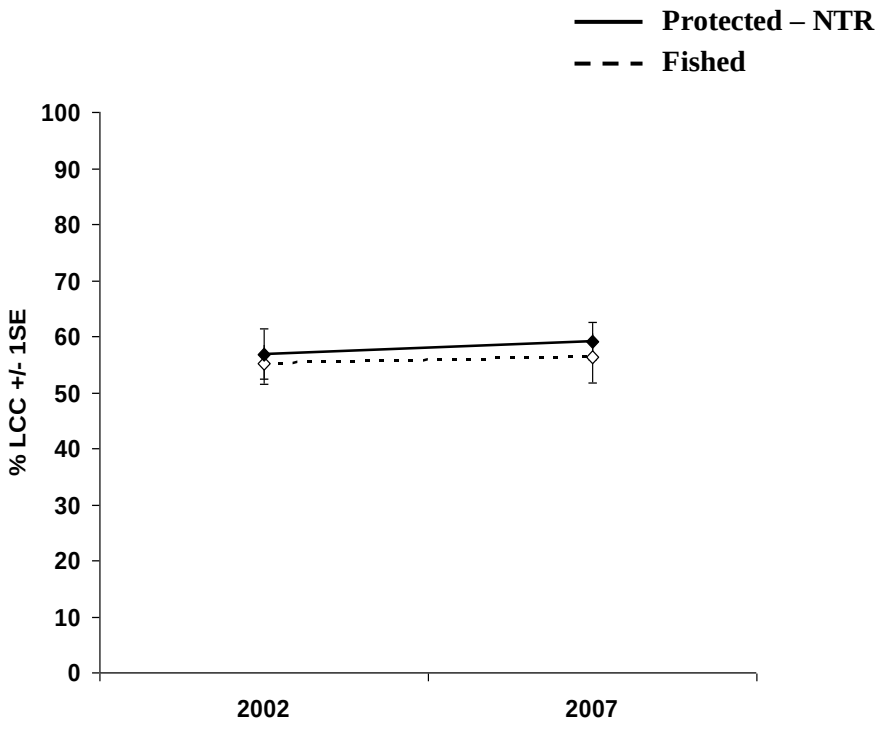


Figure 5.8: Mean (+/- 1SE) percent live coral cover (% LCC) in combined protected (NTR) zones and fished zones of the Palm, Whitsunday and Keppel Island groups in 2002 and 2007.

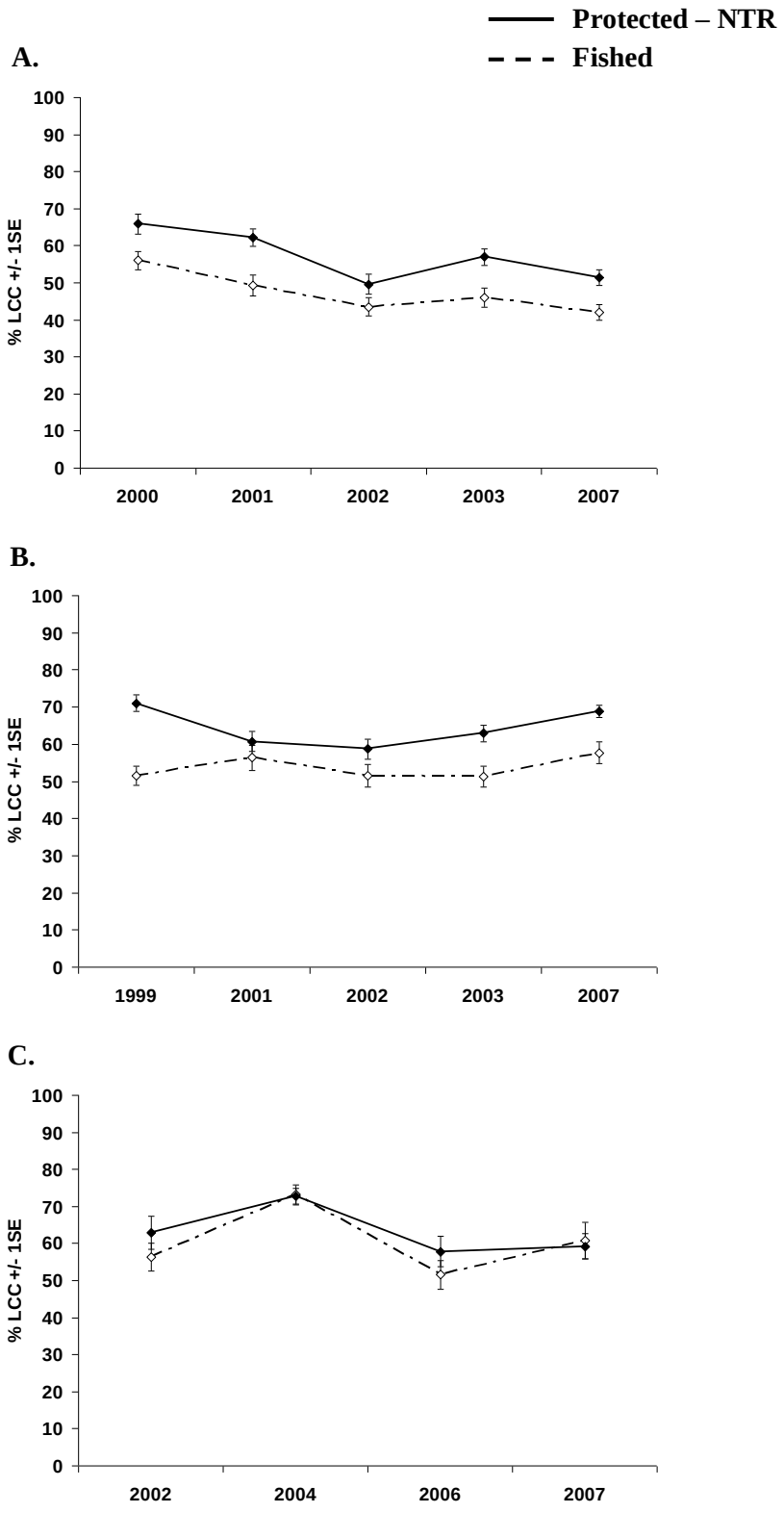


Figure 5.9: Temporal dynamics in mean (+/- 1SE) percent live coral cover (% LCC) in protected (NTR) zones and fished zones of the Palm (A), Whitsunday (B) and Keppel (C) Island groups.

5.4. DISCUSSION

No-take marine reserve protection has produced consistently strong effects on populations of the key fishery target species, coral trout (*Plectropomus* spp.) on the near-shore reefs of the Great Barrier Reef Marine Park (GBRMP). This general effect of management zoning is consistent with previous work presented in this thesis and with several publications produced by our research group (Evans & Russ 2004; Williamson *et al*. 2004; Russ *et al*. 2008).

The inclusion of reliable baseline data showing relatively low coral trout density in 1983/84 (pre-zoning) in the Palm and Whitsunday Island groups, strongly suggests that no-take zoning was the most likely cause of increased coral trout density, mean size and biomass recorded consistently within the protected NTR zones from 1999 onwards. The data presented here has demonstrated that the effects of NTR protection on coral trout populations have persisted for almost a decade (1999 – 2007) on these inshore fringing reefs. Furthermore, populations of *Plectropomus* spp. have remained relatively stable in fished zones, where the recent estimates of density and biomass (1999 – 2007) were not significantly different from baseline estimates collected prior to the implementation of the first GBRMP zoning plan in 1987.

Although fishing pressure on these inshore reefs has increased since 1984 (Higgs & McInnes 2003), it is evident that populations of *Plectropomus* spp. were already depressed within fished zones at least as early as 1984. It is unclear at this stage whether or not the no-take protected zones have contributed to sustaining coral trout populations on the fished reefs. However, given recent insights into patterns of adult fish movement, larval dispersal and rates of self recruitment, it is highly likely that enhanced populations of exploited fish species within NTRs are contributing

both post-settlement fish and larvae to surrounding fished areas (Almany *et al*. 2007; Jones *et al*. 1999, 2005; Zeller & Russ 1998). This is an area requiring further investigation.

Also consistent with previous findings is the significant effect of marine reserve protection on the secondary target fish species, the stripey snapper (*Lutjanus carponotatus*). The results obtained for *L. carponotatus* largely reflect the results for *Plectropomus* spp. Although the effects of NTR protection were not as pronounced for this species, *L. carponotatus* were consistently more abundant and were larger on average in NTRs than in fished zones of all three island groups throughout the monitoring period. As stated in Chapters 3 and 4, it is evident that the benefits of effective NTR protection can extend to a range of species beyond those which are most favoured and sought after by fishers (Williamson *et al*. 2004).

As expected, no clear effects of reserve protection were detected for the Siganidae or Chaetodontidae groups. Siganids are not targeted by fishers on the Great Barrier Reef, and these findings are consistent with the assumption that NTRs have little direct effect on fish species that are not exposed to fishing mortality (Evans & Russ 2004; Williamson *et al*. 2004). Furthermore, St. John *et al*. (2001) found that Siganids constitute less than 0.5% of the prey items of *Plectropomus* spp. on the Great Barrier Reef. Although some preliminary data has been presented for predator-prey effects on certain fish species within no-take reserves, it is likely that predation pressure on siganids is low, even in protected areas where predator abundances are high (Graham *et al*. 2003).

As many species of butterfly fish feed predominantly on scleractinian corals and utilise the reef matrix as a refuge, temporal dynamics of populations are expected to be closely linked with variations in live hard coral cover (Pratchett *et al*. 2006, 2008). In the Palm, Whitsunday and Keppel Island groups, the most numerically abundant chaetodontid species is *Chaetodon*

aureofasciatus, and the patterns presented here are largely driven by this species. Although *C. aureofasciatus* has often been classified as an obligate hard-coral feeder, recent work has determined that this species also feeds on a range of non-coral prey, particularly zooanthids (Pratchett 2005). The capacity for prey switching in *C. aureofasciatus* may thus make this species more resilient to declines in hard coral cover than other obligate corallivores (Pratchett *et al*. 2006). Although the data has not been presented here, a significant decline in the species richness of chaetodontids and a decline in the abundance of obligate corallivores accompanied the 2006 coral bleaching event in the Keppel Island group. Following the bleaching event, the most heavily impacted chaetodontid species were the obligate hard coral feeders, *Chaetodon baronessa*, *C. lunulatus*, *C. plebius* and *C. trifascialis* (Williamson, Evans & Russ, *unpublished data*). These results mirror the findings of Pratchett *et al*. (2006).

A mass coral bleaching event occurred on the Great Barrier Reef during January and February 2002. Less than 30% of reefs in the Palm Island and Whitsunday Island groups were bleached, but greater than 60% of reefs in the Keppel Island group were affected (Berkelmans *et al*. 2004). A second coral bleaching event occurred in the Keppel Island group in March 2006 and although the spatial extent of this bleaching event was far more restricted than the 2002 event, mortality of hard corals was relatively high in the affected areas (Diaz-Pulido *et al. in press*).

The temporal dynamics of live coral cover (LCC) detected here were largely attributable to mortality of hard corals during and soon after the 2002 mass coral bleaching event in the Palm and Keppel Island groups and the 2006 bleaching event in the Keppel Island group. The 2002 bleaching event led to a significant decline in LCC in the Palm Island group and no appreciable long-term recovery has been recorded since 2002. In 2007, mean LCC was approximately 14% lower in both protected and fished zones of the Palm Islands than it was in 2000. Although a number of reefs in

the Whitsunday Islands were classified as bleached during the 2002 event (Berkelmans *et al.* 2004), subsequent recovery was generally high and there was no apparent long-term impact on LCC in the sites monitored during this study. In the Keppel Island group, monitoring was initiated in October 2002, approximately 8 months after the 2002 mass bleaching event. In October 2002, LCC on these reefs had likely recovered to close to pre-bleaching levels and LCC continued to rise until the next bleaching event occurred in March 2006. Although the recovery was not uniform across all sites, this remarkable rate of recovery, particularly of branching *Acropora* spp. corals was again recorded on the reef slopes of the Keppel Islands following the 2006 bleaching event. This pattern of coral mortality and subsequent rapid recovery in the Keppel Islands has also been documented in a recent publication by Diaz-Pulido *et al.* (2009).

The reefs of the Keppel Island group have been exposed to repeated impacts from both thermally-induced coral bleaching and Fitzroy River flood events. It is now apparent that the *Acropora* spp. corals which dominate these reefs have developed an incredible capacity to recover from these impacts through active re-growth from small sections of remnant living coral tissue on dead branches which are covered in turf algae (Diaz-Pulido *et al.* 2009). The data presented here further demonstrates that NTRs provide little, if any, increased resilience to the coral communities of these reefs. It is evident, that climate change and the projected increased frequency of mass coral bleaching events will likely undermine many of the benefits of NTR protection on exploited fish populations and biodiversity (Jones *et al.* 2004; Munday & Holbrook 2006; Munday *et al*. 2007, 2008; Emslie *et al*. 2008; Russ *et al*. 2008).

As expected, some year to year variation has been recorded in the fish populations of these inshore reefs. It is evident that movements of fish due to variations in local conditions, feeding and spawning activities, and natural variability in recruitment and mortality rates are all factors that

have contributed to the temporal dynamics documented here. In addition, observer biases and variability in sampling efficiency due to weather conditions, currents and underwater visibility, have undoubtedly also contributed to some of the temporal variability. Continued monitoring of long-term protected sites, recently protected sites and fished sites will facilitate greater resolution of the dynamics of fish populations on these reefs and yield further insight into the longer-term effects of management zoning and the resilience of these reefs to the larger scale impacts of climate change.

Chapter 6: Transgenerational marking of marine fish larvae: stable isotope retention, physiological effects and health issues.

6.1. INTRODUCTION

Quantifying larval dispersal distances, rates of self recruitment to natal locations and connectivity of populations of marine organisms represent some of the greatest challenges facing marine ecologists and resource managers (Cowen *et al*. 2000; Halpern & Warner 2003; Palumbi 2004; Sale *et al*. 2005). Empirical measurements of these parameters are essential for validating predictive biophysical models of larval dispersal and for the optimal design of networks of marine reserves (Jones *et al*. 1999, 2005; Palumbi 2004; Sale *et al*. 2005; Cowen *et al*. 2006). Because of the difficulties associated with conducting mark-recapture studies on pelagic larvae, few have successfully achieved in situ tracking of larvae from their natal location to where they settle (Swearer *et al*. 2002; Thorrold *et al*. 2002; Almany *et al*. 2007). Mass marking of larval marine fishes has previously been achieved by immersing embryos in a solution of a fluorescent compound (tetracycline) (Jones *et al*. 1999, 2005). However, this technique is limited to demersally spawning fishes whose embryos are readily available for experimentation.

Thorrold *et al*. (2006) successfully used an enriched stable isotope barium chloride ($BaCl_2$) solution to provide a maternally inherited chemical mark to batches of larvae of both demersal and pelagic spawning reef fish species. The transgenerational marker operates by altering barium isotope ratios in otolith cores of the offspring spawned by females exposed to an enriched barium isotope spike. Once deposited in the core of the embryonic otoliths, the isotope signature remains intact

throughout the life of the fish. Otoliths are then collected from larvae or juveniles and barium isotope ratios in the cores are determined using laser ablation inductively coupled plasma mass spectrometry (ICP-MS). This technology has provided a powerful new tool for use in empirical investigations of larval dispersal, connectivity of fish populations and export effects of marine reserves. Recently, Almany *et al*. (2007) successfully applied the technique to wild populations of *Amphiprion percula* (Pomacentridae) and *Chaetodon vagabundus* (Chaetodontidae) on reefs in Papua New Guinea.

In order to safely and successfully apply this larval marking technique to wild populations, it is essential to first test the effectiveness of the markers on focal species under captive conditions. Not only is it imperative to establish that larvae are being effectively marked and not adversely affected by the mark (Thorrold *et al.* 2006), but it is also important to investigate potential negative effects of $BaCl_2$ injection on the condition of adult females. Furthermore, high dose exposure to barium has been shown to produce a range of adverse health effects in humans (Koch *et al*. 2003; NTP 1994; Roza & Berman 1971; Wetherill *et al*. 1981), therefore the potential health issues that may result from consumption of injected fish must also be considered. While likely not an issue for small reef fish, total barium doses for larger fishes targeted by artisanal, recreational or commercial fishes may be more problematic.

Background exposure of the general human population to barium has been estimated to be 9 – 26 µg Ba / kg of body weight / day (ATSDR 2005; IPCS 2001). Estimates of exposure levels that pose a minimal risk to humans (MRLs) have been defined for barium by the U.S. Department of Health and Human Services (Agency for Toxic Substances and Disease Registry). An MRL of 0.7 mg Ba / kg of body weight / day has been set for intermediate-duration (15 – 364 d) oral ingestion of barium in foods and water (ATSDR 2005). The International Program for Chemical Safety (IPCS)

defines 0.21 mg Ba / kg body weight / day as the no observable adverse effect level (NOAEL) for long-term (lifetime) exposure to barium. In line with these values, a 70 kg human can orally ingest 15 – 49 mg of barium per day, depending on the duration of exposure, without appreciable risk of adverse health effects (ATDSR 2005; IPCS 2001).

With growing interest in the use of enriched stable isotopes as transgenerational markers for fish larvae, this study examined the extent of physiological responses in $BaCl_2$ injected fish, retention of barium in various fish body tissues and potential exposure levels for humans who may consume treated fish. The specific objective of this study was to investigate potential physiological effects in adult coral trout (*Plectropomus leopardus*, Lacepède) and to measure residual barium concentrations in fish body tissues over an 8 week period following a single low dose injection of enriched stable isotope $BaCl_2$ solution.

6.2. METHODS

6.2.1. Study species

The common coral trout (*Plectropomus leopardus*; family Serranidae) is a protogynous hermaphroditic fish that occurs on coral reefs throughout the Indo-Pacific region. *P. leopardus* is an important fisheries species throughout its range and on Australia's Great Barrier Reef, it is the primary target species of a commercial hook and line fishery in which they comprise approximately 33% of the total catch (Williams 2002).

6.2.2. Sourcing and treatment of experimental fish

Thirty adult *P. leopardus* were captured by a commercial fisher on reefs offshore from Townsville (Queensland, Australia) using hook and line. Fish were maintained in holding tanks on board the vessel for a maximum of three days before being transported to the James Cook University's (JCU) marine aquarium facility. Total lengths of fish ranged from 380 – 420 mm and weights ranged from 705 – 1130 g. Restrictions on total biomass loading of the aquarium facility meant that it was not possible to hold more than 30 fish of this size.

Ten fish were haphazardly allocated into each of three separate treatment groups. Two groups were administered with $BaCl_2$ solution (*i.e.* 2 mg ^{138}Ba / kg body weight [T2] or 4 mg ^{138}Ba / kg body weight [T4]), while a third 'control' group were administered with an equivalent volume of 0.9% sodium chloride ($NaCl_2$) solution. All solutions were administered via intra-peritoneal injection, and injection volumes ranged from 1 – 3 ml depending on fish weight and treatment group. The enriched isotope barium dosage rates used here have been shown to produce clearly distinguishable markers in larvae of both demersal and pelagic spawning reef fish (Thorrold *et al.* 2006). Following injection, all fish were tagged with two external t-bar tags (Hallprint, Victor Harbour, Australia) before being placed for 30 min into a quarantine bath to remove ectoparasites. The quarantine solution consisted of 25 ml of 40% formaldehyde dissolved in 100 L of 10 ppt seawater. Fish were then released into one of two sections of a 100,000 L outdoor aquarium. The two sections were of equal size and were separated by plastic mesh (5 x 5 cm). Each section housed ten treatment fish and five control fish.

Fish were fed once daily with pieces of pilchard (*Sardinops* spp.) and squid (*Loligo* spp.). Water temperature within the aquarium system ranged from 25.5 - 28.5 ^{o}C, while salinity (35 ppt) and pH

(8.2) were consistent during the experimental period. Dissolved ammonia (NH_3), nitrate (NO_3) and nitrite (NO_2) concentrations remained below 0.03 mg / L throughout the study.

6.2.3. Sampling

Two fish from each of the two barium dosage treatments and two fish from the control treatment were harvested at post-injection intervals of 48 hours, 1 week, 3 weeks, 5 weeks and 8 weeks. Fish were removed from the aquarium using hook and line and anaesthetised via immersion for approximately 1 min in a tank containing 20 ml of a 1:1 emulsion of clove oil and 100% ethanol, dispersed in 30 L of seawater. Once anaesthetised, each harvested fish was weighed and measured.

Anaesthetised fish were secured in a foam cradle and two separate blood samples (2 ml each) were collected from the caudal artery using a hypodermic needle (22 gauge) attached to either an ordinary 3 ml syringe, or a 3 ml syringe infused with fluoride heparin (Sigma, St. Louis, U.S.A.). In the former case, blood was immediately transferred to an EDTA Microtainer (Becton Dickinson, Franklin Lakes, U.S.A.). Whole blood was then temporarily stored for up to 6 hr at approximately 15 ^{o}C. In the latter case, blood was immediately transferred to a 2 ml vial and centrifuged at 3000*g* for 5 minutes to separate plasma which was subsequently stored at –20 ^{o}C until analysis.

Following extraction of blood samples, mortality was induced by re-immersing fish in the anesthetic solution tank for approximately 2 minutes. Once deceased, fish were placed into ice slurry for approximately 30 minutes before dissection. On dissection, both lobes of the gonad were removed, one lobe was placed in a vial and fixed in 10% phosphate buffered formalin for histological assessment (sexing). The other lobe of the gonad was placed into a vial and frozen (-20 ^{o}C). The whole liver was removed from each fish and frozen, as was a sample of the muscle (fillet)

tissue. Two vertebrae were removed from the mid region of the vertebral column of each fish; muscle and connective tissue was stripped from the bone samples using small forceps before freezing.

6.2.4. Analysis of residual barium in fish tissues

Wet weights of frozen samples of muscle, gonad, liver and bone were recorded before the samples were freeze dried for approximately 72 hours. Once dry, samples were weighed again and crushed to a fine powder using a rock mill. Tissue samples were then dissolved by open vessel, microwave-assisted digestion. Analysis of residual barium concentrations and Ba^{2+} isotope ratios within fish tissues was conducted using a Varian UltraMass 700 inductively coupled plasma – mass spectrometer (ICP-MS).

6.2.5. Whole blood analysis

Haemoglobin (Hb) and haematocrit (Hct) concentrations, and counts of red blood cells (RBC) and white blood cells (WBC) were determined using a Coulter HmX Heamatology Analyser. Because the haematology analyser was calibrated for human blood cells, RBC and WBC counts for *P. leopardus* blood samples were cross validated by manual microscopic reading of stained blood films. Blood films were stained using the May-Grunwald and Giemsa stains and buffered using Sorenson's buffer before drying and reading.

6.2.6. Steroid hormone analysis

To assess the potential impact of $BaCl_2$ on endocrine control processes, the plasma concentrations of a range of steroid hormones were measured: estradiol-17β (E_2), testosterone (T), 11-

ketotestosterone (*11KT*) and cortisol (*F*). These steroids were chosen because of their dominant roles in the regulation of reproduction (E_2, *T and 11KT*) and stress (*F*) in teleost fishes (Frisch 2004; Frisch & Anderson 2005). Plasma steroid concentrations were measured by radioimmunoassay (RIA) following extraction from plasma with ethyl acetate using the protocol described by Pankhurst & Carragher (1992). Extraction efficiency was determined by recovery of [^{3}H]-labeled steroid from triplicates of a plasma pool, and assay values for each steroid were adjusted accordingly. Assay specificity was verified by confirming parallelism in the binding curves of serially diluted plasma extracts and steroid standards (Frisch *et al*. 2007). The minimum detectable concentration for each assay was 0.075 ng / ml.

6.2.7. Gonad histology

Histological assessment was carried out in order to determine the sex and reproductive condition at the time of harvest of each of the 30 experimental fish. Histological analysis of *P. leopardus* gonads was carried out in accordance with the methods described by Samoilys & Roelofs (2000). Three to four transverse sections of 2 – 4 mm thickness were taken from the medial section of a single gonad lobe for each individual experimental fish. Tissue sections were placed into tissue processing cassettes and placed in 70% alcohol for 3 hr. Gonad tissue sections were then dehydrated and processed to paraffin wax using an automatic tissue processor and embedded in paraffin wax using stainless steel moulds. Tissue samples were then sectioned at 5 µm using a rotary microtome and mounted on glass microslides. Tissue sections were stained using Mayers Haematoxylin and Young's Eosin-Erythrosin stains (Winsor 1994; Samoilys & Roelofs 2000). Slides were examined microscopically and the sex and reproductive stage of each fish was determined according to the criteria defined by Samoilys & Roelofs (2000).

6.2.8. Statistical analysis of data

All data were analysed using two-factor univariate ANOVA to assess the effects of treatment and time. Assumptions of ANOVA were tested using Levene's test for homogeneity of error variances and normal probability plots. Data which did not satisfy the assumptions of ANOVA were transformed using a log (x + 1) function. Post-hoc comparisons of group means were conducted using Tukeys' HSD tests. The statistical software package *STATISTICA* was used for all analysis and a significant difference was considered to exist if $p < 0.05$. All data presented in the text and figures are the mean ± standard error (SE) of untransformed data.

6.3. RESULTS

6.3.1. Tissue Barium concentrations following $BaCl_2$ injection

The ^{138}Ba spike administered as a $BaCl_2$ solution to *Plectropomus leopardus* through intra-peritoneal injection was quickly absorbed into fish body tissues. Elevated tissue barium concentrations were detected within 48 hours of injection in muscle, gonad, liver and bone samples of both 2 mg Ba / kg (T2) and 4 mg Ba / kg (T4) treatment fish (Figure 6.1; Table 6.1). After initial peaks in barium concentrations at 48 hours or 1 week, concentrations declined in all sampled tissues other than bone (Figure 6.1).

Mean barium concentrations in muscle tissue samples of both T2 and T4 treatment fish remained elevated above control group concentrations throughout the 8 week study period. Peak mean residual barium concentrations in muscle tissue were detected 1 week post $BaCl_2$ injection in both T2 and T4 treatment fish. The highest mean barium concentrations recorded in muscle tissue

samples were 0.12 mg Ba / kg in T2 treatment fish and 0.29 mg Ba / kg in T4 treatment fish. Tukey's post-hoc tests revealed that muscle tissue barium concentrations in T2 treatment fish were only significantly higher than control group fish at the 48 hour and 1 week post-injection time intervals. Beyond this time barium concentrations in muscle tissue of T2 treatment fish were not significantly elevated above levels in control fish. In T4 treatment fish, barium concentrations remained significantly higher than in control group fish up until 5 weeks post injection (Figure 6.1a; Table 6.1).

Mean residual barium concentrations in gonad tissues of both T2 and T4 treatment fish peaked at 48 hours post injection and remained higher than in control fish gonads throughout the duration of the trial. However, due to a high level of variance in the data, these differences were not statistically significant (Figure 6.1b; Table 6.1). Mean barium concentrations in gonad tissue had returned to close to control levels by 8 weeks post injection. However, ^{138}Ba:^{137}Ba ratios remained elevated at 8 weeks post injection when they were still approximately 3.2 times higher in T2 treatment fish and 6.1 times higher in T4 treatment fish than in control group fish.

Peak barium concentrations in liver tissue were detected at 48 hours post injection in both T2 and T4 treatment fish. However, the only statistically significant difference detected within each sampling period was between the T4 treatment and control fish at 48 hours post injection (Tukey's post-hoc test, $p = 0.002$). Residual barium concentrations declined quickly in liver tissue. By 1 week post injection and for the remainder of the study period, barium concentrations in liver tissues of $BaCl_2$ injected fish were not significantly higher than in control fish (Figure 6.1c; Table 6.1).

Barium was readily incorporated into bone tissue within 48 hours of $BaCl_2$ injection. Residual barium concentrations and ^{138}Ba:^{137}Ba ratios in bone samples of both T2 and T4 treatment fish

remained significantly higher than in control fish for the duration of the study. There were no indications that barium concentrations in bone tissues were returning toward baseline (control) levels (Figure 6.1d; Table 6.1).

	Treatment × Time (8, 15 df)	***Treatment (2, 15 df)***	***Time (4, 15 df)***
Muscle	3.67 (∗)	53.47 (∗∗∗)	9.42 (∗∗∗)
Gonad	0.47 (ns)	3.57 (ns)	2.21 (ns)
Liver	4.37 (∗∗)	13.96 (∗∗∗)	14.00 (∗∗∗)
Bone	2.34 (ns)	1001.27 (∗∗∗)	1.91 (ns)

Table 6.1: Results of two-factor univariate ANOVA on residual barium (Ba^{2+}) concentrations in *Plectropomus leopardus* tissue samples. Numerical figures are *F* values. Symbols in brackets are significance (*p*) levels of the tests; ∗ = < 0.05; ∗∗ = < 0.01; ∗∗∗ = < 0.001; ns = not significant.

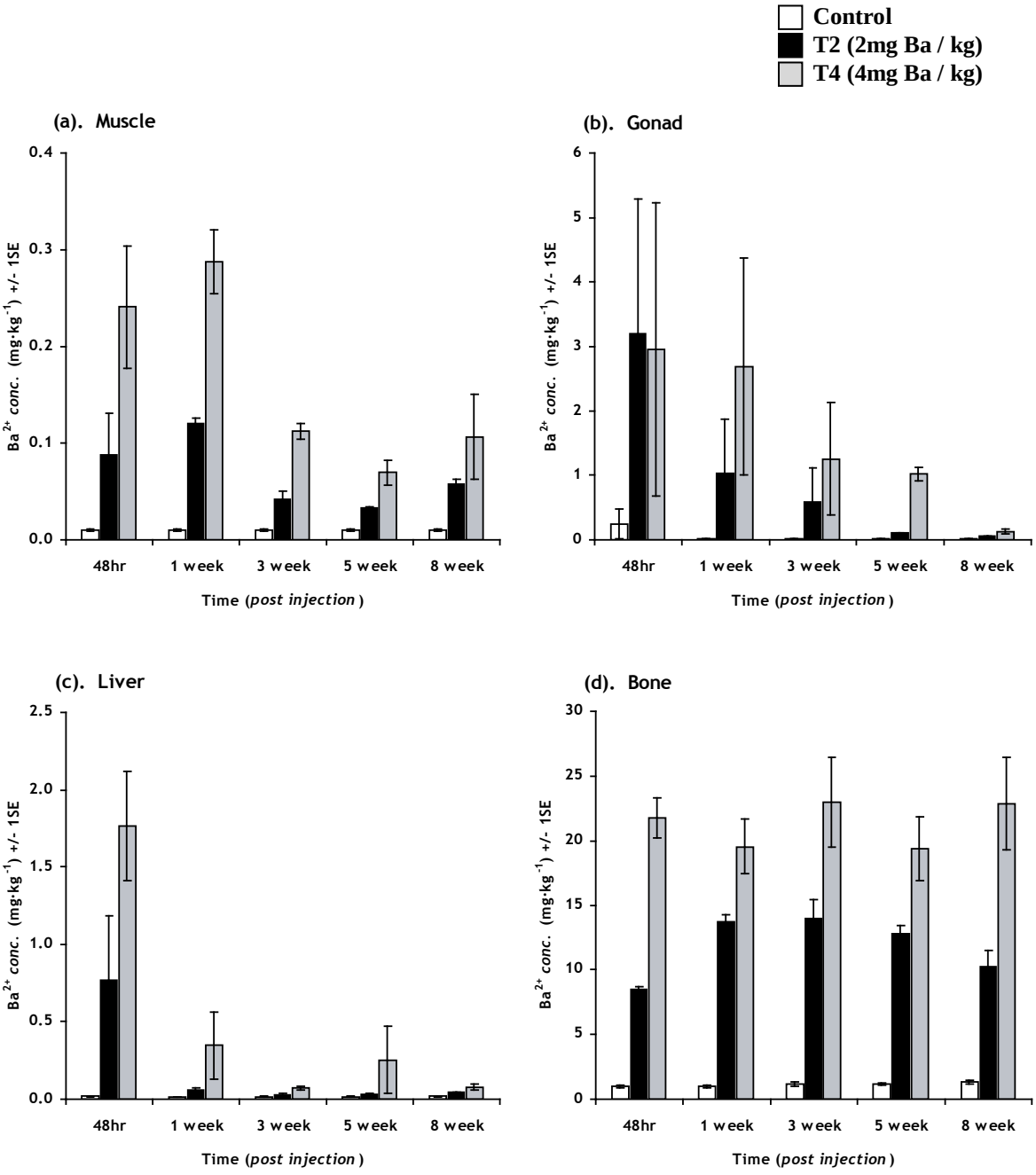


Figure 6.1: Mean (+/- 1 SE) residual barium (Ba^{2+}) concentrations (mg / kg wet weight) in *Plectropomus leopardus* tissues in five successive post injection intervals. **(a)**. Muscle (fillet); **(b)**. Gonad; **(c)**. Liver; **(d)**. Bone.

6.3.2. Effects of $BaCl_2$ injection on whole blood parameters

No statistically significant effects of $BaCl_2$ injection were detected on the four measured whole blood parameters; red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) and haematocrit (Hct) concentrations. Significant variations in all four blood parameters were detected through time within each treatment. However, within each sampling period, no significant differences in whole blood parameters were detected between $BaCl_2$ injected fish and control fish (Figure 6.2; Table 6.2).

	Treatment × Time (8, 15 df)	***Treatment (2, 15 df)***	***Time (4, 15 df)***
Red blood cell (RBC)	0.89 (ns)	0.12 (ns)	7.54 (**)
White blood cell (WBC)	1.02 (ns)	1.53 (ns)	25.98 (***)
Haemoglobin (Hb)	0.99 (ns)	0.31 (ns)	10.39 (***)
Haematocrit (Hct)	0.86 (ns)	0.44 (ns)	7.76 (**)

Table 6.2: Results of two-factor univariate ANOVA on *Plectropomus leopardus* whole blood indices. Numerical figures are *F* values. Symbols in brackets are significance (*p*) levels of the tests; ** = < 0.01; *** = < 0.001; ns = not significant.

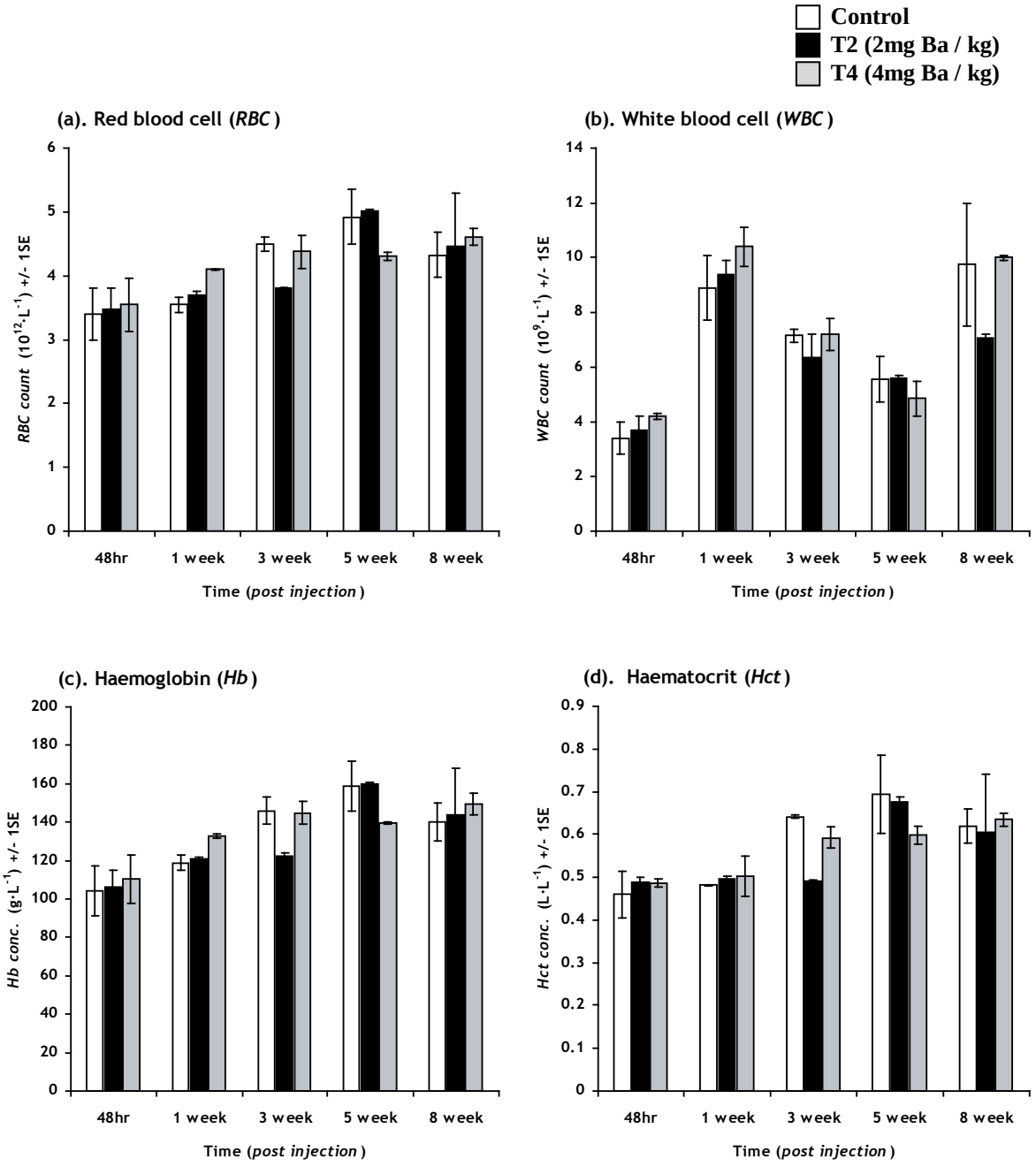


Figure 6.2: Mean (+/- 1 SE) values for *Plectropomus leopardus* whole blood indices in five successive post injection intervals. **(a).** Red blood cell (RBC) count; **(b).** White blood cell (WBC) count; **(c).** Haemoglobin (Hb) concentration; **(d).** Haematocrit (Hct) concentration.

6.3.3. Effects of $BaCl_2$ injection on blood plasma steroid concentrations

No statistically significant variations in blood plasma steroid concentrations were detected between $BaCl_2$ injected and control fish within each sampling time or within each treatment group across time (Figure 6.3; Table 6.3).

Peak estradiol-17β (E_2) concentrations were recorded in control group fish at the 3, 5 and 8 weeks post injection sampling periods. However, due to high error variance, these observed differences were not statistically significant (Figure 6.3a; Table 6.3). Peak cortisol (F) concentrations were recorded in T2 group fish at the 1 and 8 week sampling periods. Similarly, due to large error variances these differences were not significant (Figure 6.3d; Table 6.3).

	Treatment × Time (8, 15 df)	***Treatment (2, 15 df)***	***Time (4, 15 df)***
Estradiol - 17β (E_2)	0.74 (ns)	2.44 (ns)	0.73 (ns)
Testosterone (T)	1.01 (ns)	0.01 (ns)	1.79 (ns)
11 Ketotestosterone (11KT)	0.90 (ns)	0.36 (ns)	1.35 (ns)
Cortisol (F)	1.30 (ns)	2.71 (ns)	0.56 (ns)

Table 6.3: Results of two-factor univariate ANOVA on *Plectropomus leopardus* blood plasma steroids. Numerical figures are *F* values. Symbols in brackets are significance (*p*) levels of the tests; ns = not significant.

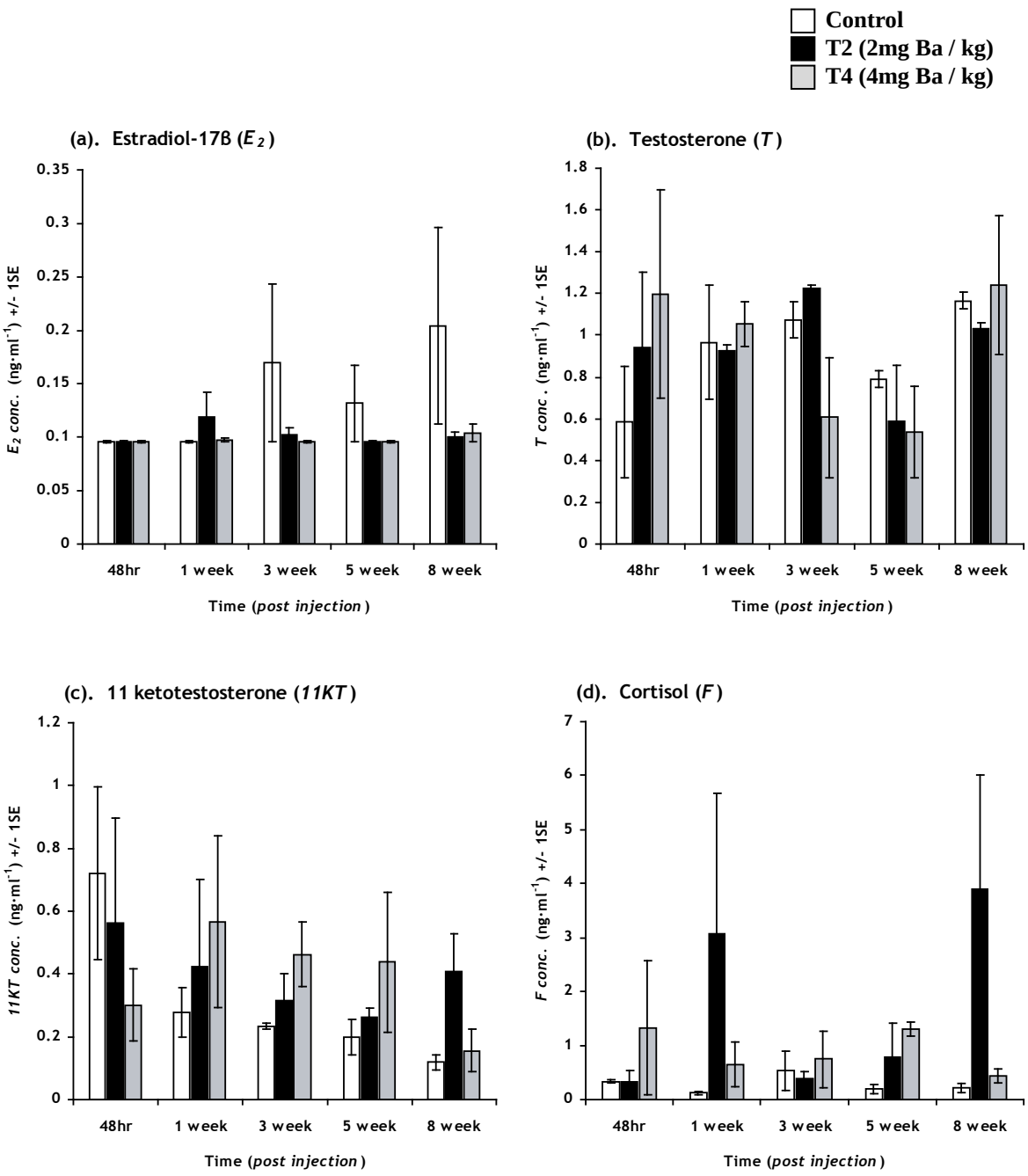


Figure 6.3: Mean (+/- 1 SE) concentrations of *Plectropomus leopardus* blood plasma steroids in five successive post injection intervals. **(a).** Estradiol-*17β* (E_2); **(b).** Testosterone (*T*); **c).** 11-ketotestosterone (*11KT*); **(d).** Cortisol (*F*).

6.3.4. Effects of $BaCl_2$ injection on body and liver weight

Body weight changes of experimental fish were highly variable throughout the study period. However, ANOVA revealed no significant differences between treatment groups and control fish within each sampling time or within groups across time (Figure 6.4a; Table 6.4). Overall mean values for each treatment group across all sampling times were; control group +1.8% bodyweight, T2 –0.7% bodyweight, T4 –2.9% bodyweight.

Liver weight as a percentage of total body weight varied significantly within each group across the 8 week study period. However, there were no significant differences detected between treatment groups and control group fish within each sampling time (Figure 6.4b; Table 6.4).

	Treatment × Time (8, 15 df)	***Treatment (2, 15 df)***	***Time (4, 15 df)***
% change in body weight	1.50 (ns)	1.43 (ns)	2.42 (ns)
% liver of body weight	0.93 (ns)	0.08 (ns)	13.73 (***)

Table 6.4: Results of two-factor univariate ANOVA on *Plectropomus leopardus* percentage change in body weight and percent liver weight of body weight. Numerical figures are *F* values. Symbols in brackets are significance (*p*) levels of the tests; *** = < 0.001; ns = not significant.

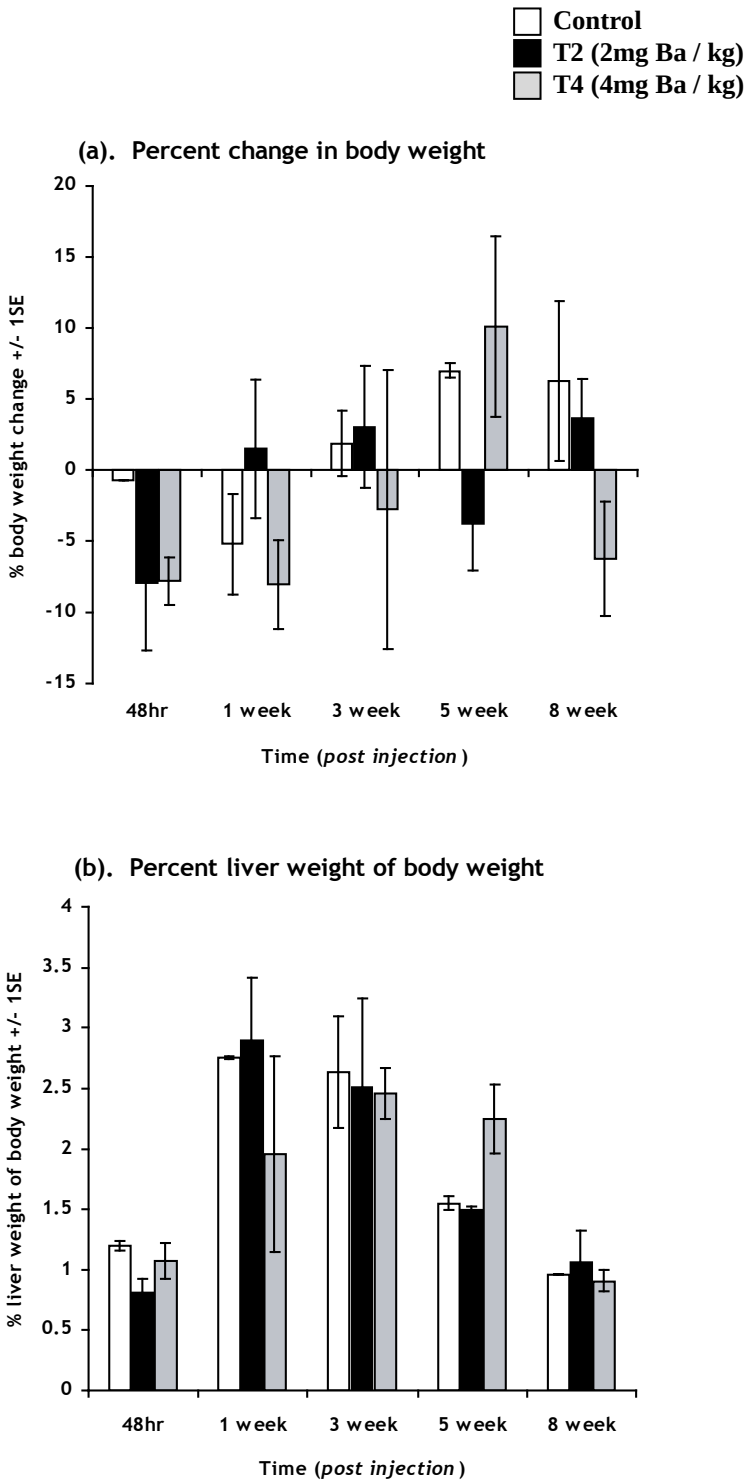


Figure 6.4: (a). Mean (+/- 1 SE) percent change in fish body weight and **(b).** Mean (+/- 1 SE) percent liver weight of fish body weight in five successive post injection intervals.

6.3.5. Histological assessment of fish gonads

Histological analysis of *P. leopardus* gonads revealed that 93% (28/30) of the experimental fish were mature females. Of the 28 females, 23 were determined to be in resting reproductive condition, while five were in ripe reproductive condition at the time of harvest. Both of the male fish identified were in ripe condition at harvest. Both resting and ripe condition female fish were distributed more or less evenly among control and barium injection treatments. All experimental fish were of a similar size (380 – 420 mm TL) and within this size range, no sexual dimorphism was externally apparent in *P. leopardus*.

6.4. DISCUSSION

There is increasing interest in the use of new technologies for estimating dispersal distances and demographic population connectivity for marine organisms, particularly in relation to the design of marine protected area (MPA) networks (Jones *et al*. 1999, 2005; Gell & Roberts 2003; Palumbi 2004; Almany *et al*. 2007). Of particular interest is establishing the fate of larvae produced by adults in protected areas and estimating their contribution to sustaining fisheries in areas outside of the reserves (Sale *et al.* 2005). The development of a method of using enriched stable isotope solutions for transgenerational mass marking of fish otoliths provides the first direct means of answering this question. However, such techniques should only be embraced provided that they can be applied without significant harm to either the fishes or to people who might consume them. This study confirmed that dosages of a $BaCl_2$ solution up to 4 mg Ba / kg female weight have no detectable short-term physiological effects on adult fish.

Adverse effects on humans or other animals that consume fish injected with a $BaCl_2$ solution are possible, as existing studies indicate that barium is predominantly absorbed from the gastrointestinal tract and the respiratory tract (Cuddihy & Griffith 1972; Leggett 1992). High-dose exposure to barium has been shown to result in a number of effects within the body including ventricular tachycardia, hypertension and/or hypotension, muscle weakness and paralysis (Koch *et al*. 2003; Roza & Berman 1971; Wetherill *et al*. 1981). However, Wones *et al*. (1990) found no adverse effects on the cardiovascular system of humans following daily exposure to low doses (5-10 ppm) of $BaCl_2$ in drinking water (1.5 L d^{-1}) for 10 consecutive weeks. Several animal studies have examined the oral systemic toxicity (McCauley *et al*. 1985; NTP 1994; Perry *et al*. 1989; Tardiff *et al*. 1980), neurotoxicity (NTP 1994), and reproductive and developmental toxicity (Dietz *et al*. 1992) of barium. The results of these studies suggest that the kidney is the most sensitive target of toxicity following intermediate-duration oral exposure to barium. One 2-year study found no evidence of carcinogenic effects of $BaCl_2$ administered daily in drinking water to rats and mice at dosage rates of up to 75 mg and 200 mg / kg body weight / day respectively (NTP 1994).

Data presented here demonstrates that the dosage levels of enriched isotope barium required to mark fish larvae are far too low to represent a health risk to humans who may consume treated fish. Peak muscle tissue concentrations of barium were detected one week after injection in both experimental dosage treatments, after which it steadily declined. A peak residual barium concentration of 0.29 mg Ba / kg was detected in muscle tissue of *P. leopardus* administered with a dosage of 4 mg Ba / kg. At this tissue concentration, a 70 kg human would need to consume approximately 170 kg of muscle tissue in a single day to reach the 0.7 mg Ba / kg / day intermediate duration MRL (ATDSR 2005). More conservatively, to remain below the 0.21 mg Ba / kg / day long-term exposure NOAEL, a 70 kg human could consume up to 50 kg of muscle tissue per day, every day, over consecutive years. Obviously both these scenarios are extremely unlikely

and we deduce that at the concentrations tested here, it would not be possible for a human to be adversely effected by consuming fish that had been injected with an enriched stable isotope $BaCl_2$ solution used to tag embryonic otoliths.

Gonad tissue absorbed barium within 48 hours of $BaCl_2$ injection in treatment fish. Mean residual barium concentrations in gonad tissue then steadily fell in both $BaCl_2$ treatment groups and by the 8 week post injection sampling time, concentrations in treatment fish gonads were almost equivalent to control fish gonads. However, ^{138}Ba:^{137}Ba ratios remained elevated at 8 weeks post $BaCl_2$ injection in both 2 mg and 4mg ^{138}Ba / kg treatment fish. This result may suggest that for *P. leopardus,* the effective embryo marking time of enriched $BaCl_2$ solution is at least 8 weeks post injection at the dosage rates used here. The effective marking time of a single enriched isotope $BaCl_2$ injection is an aspect of this technology which requires further research.

Although barium was absorbed into the livers of treatment fish within 48 hours of injection, it was processed and largely eliminated within 1 week. By the 8 week post injection sampling time, mean barium concentrations within the livers of treatment fish had almost completely returned to the concentrations detected in control fish. Previous studies have demonstrated that the primary route of excretion of barium or $BaCl_2$ from mammals is in faeces (ATSDR 2005; IPCS 2001; NTP 1994; Stoewsand *et al*. 1988). It is likely that this same mechanism is operating in fishes, but this has yet to be tested.

Barium was quickly absorbed into bone tissue of treatment fish and concentrations remained relatively stable throughout the 8 week study duration. Unlike other tested body tissues, there was no clear indication that barium concentrations in bone tissue were falling throughout the experimental period. These findings are consistent with several previous studies that have detected

significant and sustained increases in barium concentrations in bone tissues of rats, mice and dogs orally administered with $BaCl_2$ in drinking water or as a component of feeds (Cuddihy & Griffith 1972; NTP 1994; Stoewsand *et al*. 1988). In humans, it has been shown that approximately 90% of the barium in the body is in bone tissue, while only 1–2% of the total body burden is generally found in muscle, adipose, skin, and connective tissue (Miller *et al*. 1985; Schroeder *et al*. 1972).

We detected no physiological responses in *P. leopardus* attributable to a single injection of $BaCl_2$ solution at a dosage rate of up to 4 mg Ba / kg. No effects of $BaCl_2$ injection were detected on RBC or WBC counts, on Hb or Hct concentrations (whole blood parameters), or on concentrations of plasma steroid hormones. While significant variation was observed in the four measured whole blood parameters throughout the 8 week trial period, no significant effect of either $BaCl_2$ treatment was detected. This suggests that variation in whole blood parameters through time was a function of environmental variability, or other unmeasured physiological or immunological stressors that affected both control and treatment group fish.

Although elevated cortisol concentrations are indicative of physiological stress (Barton 2002; Frisch & Anderson 2005), mean cortisol levels in blood plasma were not different among experimental treatments. Furthermore, cortisol concentrations in both control and treatment groups were typical of those found in aquarium acclimated *P. leopardus* (Frisch & Anderson 2005). With respect to the reproductive steroids (E_2, T and 11KT), there were no differences among experimental groups, and mean levels for all groups were typical for this species at this time of year (Frisch *et al*. 2007). Together, these results suggest that small doses of $BaCl_2$ do not adversely impact the endocrine control processes of *P. leopardus*.

This study has provided the first empirical investigation into the effects of low dose $BaCl_2$ injection on the toxicological and physiological responses of a marine fish. We conclude that a single intra-peritoneal injection of $BaCl_2$ solution (up to 4 mg Ba / kg) has no significant effects on the physiology of *P. leopardus*. Further, the risk to humans who may consume treated fish is minimal based on these results. Thus, enriched $BaCl_2$ solutions, at dosage rates appropriate for maternal transmission of a chemical larval marker, appear to be a safe and reliable way to mass-mark larvae of marine fishes subject to potential human consumption.

Chapter 7: An experimental evaluation of Transgenerational Isotope Labeling (TRAIL) in a coral reef grouper.

7.1. INTRODUCTION

The establishment of marine parks, including networks of no-take reserves (NTRs), has been widely advocated to stem global declines of fishery resources and marine biodiversity (Agardy 1994; Dayton et al. 2000; Russ 2002; Roberts *et al.* 2005; Mora *et al.* 2006). The benefits of NTR protection have been well documented for exploited species within reserve boundaries (Halpern & Warner 2002; Russ 2002; Williamson *et al.* 2004; Roberts *et al.* 2005; Russ *et al.* 2008), however, estimates of the degree to which reserves contribute to sustaining fish stocks in surrounding fished areas have remained elusive (McClanahan & Mangi 2000; Gell & Roberts 2003; Sale *et al.* 2005; Abesamis *et al.* 2006). For fishes whose numbers increase within NTR areas, the potential for augmenting fish populations in surrounding areas comes largely from larval export (Russ 2002). Despite general agreement that larval export and recruitment subsidies from NTRs can occur, the lack of empirical larval dispersal and demographic population connectivity data has clearly impeded the uptake of NTR networks in fisheries management (Sale *et al.* 2005). Furthermore, the optimal location, size and spacing of NTRs within the network must be based in part on estimates of larval retention within NTRs and connectivity between NTRs and fished areas (Jones *et al.* 2007; Cowen *et al.* 2000, 2006; Mora & Sale 2002; Gell & Roberts 2003; Halpern 2003; Palumbi 2004).

Definitive empirical estimates of levels of self-recruitment, dispersal and demographic connectivity among populations are rare, primarily due to the difficulties associated with tracking microscopic larvae from their natal origins, through the pelagic environment to their eventual settlement locations (Thorrold *et al.* 2002, 2006; Jones *et al*. 1999, 2007). For fishes, the extent of connectivity has been estimated using a range of techniques including genetic analyses that use mitochondrial and microsatellite markers (Jones *et al.* 2005; Purcell *et al.* 2006; Gerlach *et al.* 2007), biophysical and hydrodynamic modelling (Cowen *et al.* 2000, 2006; James *et al.* 2002), and natural geochemical tags in calcified structures of fish and invertebrates (Swearer *et al*. 1999; Zacherl 2005). However, with all of these approaches there is usually some level of uncertainty in measuring retention and connectivity on the spatial scales that NTRs are typically implemented.

Recent approaches to mass-marking of embryonic fish with artificial tags provide a direct means of estimating larval retention and connectivity. Mass marking of larval marine fishes has been achieved by temporarily immersing embryos in a solution of a fluorescent compound (tetracycline) before they are released to the pelagic environment (Jones *et al.* 1999, 2005). However, this technique is limited to those species whose embryos are easily collected and manipulated, such as benthic-spawning damselfish (Pomacentridae). Thorrold *et al.* (2006) introduced a new marking technique, transgenerational isotope labeling (TRAIL), and demonstrated that maternal injections of an enriched barium stable isotope in a chloride solution provided an inherited chemical tag to larvae of a benthic-spawning clown fish (*Amphiprion melanopus*) and a pelagic-spawning serranid (*Centropristis striata*). The transgenerational marker passes from mother to offspring and permanently alters the barium isotope ratios in otolith cores of the offspring. Otoliths can then be extracted from larvae or juveniles and barium isotope ratios in the otolith cores can be determined using laser ablation inductively coupled plasma mass spectrometry (ICP-MS). Recently, Almany *et al.* (2007) successfully applied this technique to wild populations of a clownfish (*Amphiprion*

percula) and a butterflyfish (*Chaetodon vagabundus*) and demonstrated high levels of larval retention within a small island MPA.

The integration of empirical measurements into coupled biophysical models of larval dispersal is a fundamental step in achieving optimal design and functioning of marine reserve networks (Botsford *et al.* 2009). The wider application of the transgenerational marking technique to ecologically and commercially important fish species must first be reliant on conducting rigorous testing under controlled experimental conditions. The overall objective of this study was to test the efficacy of the TRAIL technique for a large pelagic-spawning reef fish species *Epinephelus fuscoguttatus* (Serranidae - Epinephelinae). Many species of *Epinephelus* are heavily exploited in commercial, recreational and artisanal fisheries, and depleted numbers have lead to several recently being classified as threatened or endangered species on the IUCN Red List (Roberts & Hawkins 1999; Sadovy & Vincent 2002; Baillie *et al.* 2004; Cheung *et al.* 2005; IUCN 2009). *E. fuscoguttatus* is also one of the larger reef fish species that has been successfully reared in captivity and large numbers are produced in mariculture facilities throughout South Asia (Sadovy 2000; Marte 2003; Yap *et al*. 2006). The present study provides the first test case of mass marking larvae via maternal transmission of stable isotopes for this important coral reef fish genus.

The specific questions addressed in this study were:

1. Does injection of barium isotopes (135Ba and 137Ba) produce unequivocal marks on the otoliths of offspring?
2. Are there any potential negative effects on reproductive performance, egg size, condition or larval growth due to injection of adult female fish with barium chloride solution at dose rates appropriate for transgenerational marking of offspring?

7.2. METHODS

7.2.1. Study location and focal species

This study was carried out at the Gondol Research Institute for Mariculture (GRIM), which is located on the northern coast of Bali, Indonesia (8º 9.254'S; 114º 42.912'E). The study species, the brown-marbled grouper (*Epinephelus fuscoguttatus* - Serranidae) is associated with coral reefs and distributed throughout the Indo-Pacific where it is a target of commercial, recreational and artisanal fisheries across its range, as well as being widely used in commercial mariculture facilities (Rimmer 2000; Sadovy & Vincent 2002). *E. fuscoguttatus* is a protogynous hermaphroditic species which reaches a maximum length of approximately 120 cm TL and an age of over 40 years. The mean size and age of first reproduction in female *E. fuscoguttatus* has been estimated to be approximately 500 mm TL and 4 years (Pears *et al.* 2006).

7.2.2. Broodstock description and handling

Broodstock *E. fuscoguttatus* were originally wild captured fish, but they had been held in captivity at the GRIM for approximately 7 years at the time of this study. The weight of female *E. fuscoguttatus* ranged between 5 and 9 kg, while males were between 6 and 9 kg. Broodstock were held in four outdoor 100,000 L concrete tanks which were equipped with anti-jump nets and shade cloth covers. A flow-through seawater system was utilised for the broodstock tanks and ambient water temperature was approximately 28.5 °C during the study period. Broodstock fish were fed twice daily with fresh fish and squid.

In order to establish the experimental treatments, fish were captured using large scoop nets after the water depth in the tanks had been lowered to approximately 0.5 metres. Fish were transferred in small groups to a 1000 L holding tank where they were lightly anaesthetised using 2-Phenoxyethanol in seawater at a concentration of approximately 200 µl / L.

To determine the sex and reproductive condition of each individual, fish were cannulated by inserting a fine, flexible plastic tube through the urino-genital opening and into the ovary or testis, where small egg or sperm samples were extracted. Oocyte samples were taken for microscopic measurements of egg diameters. All fish were then placed into a fine mesh cradle and weighed (to the nearest 0.5 kg) using a clock face spring balance. After weighing, male fish were transferred directly to their allocated treatment tank; female fish were transferred to a 1000 L recovery tank in preparation for isotope injection. Seven female and four male fish were assigned into each treatment.

7.2.3. Injection of isotope markers

While immersed in the recovery tanks, female *E. fuscoguttatus* were held inverted (ventrally upward) in a mesh cradle by two assistants. We injected an enriched stable Ba isotope solution in chloride form (Oak Ridge National Laboratory, Tennessee, U.S.A.), or 0.9% sodium chloride (NaCl) solution, by lifting a pelvic fin upward and inserting a 23g hypodermic needle through the body wall and into the coelomic cavity. For treatment fish, the appropriate volume of $BaCl_2$ solution was injected to deliver a dosage of Ba isotope corresponding to 0.5 or 2 mg Ba / kg of body weight (treatment T0.5 or T2 respectively). A total of seven female fish were given the T0.5 treatment and four females were given the T2 treatment administered with $^{137}BaCl_2$ solution. In addition, three T2 treatment females were injected with $^{135}BaCl_2$ instead of $^{137}BaCl_2$. Seven female

control fish were injected with 0.9% NaCl solution at a volume equal to the volume utilised for the T2 treatment. After injection, female fish were released into the allocated broodstock tank for each group. Injections of broodstock fish were conducted approximately 10 days prior the new moon in May 2006.

7.2.4. Spawning of E. fuscoguttatus broodstock and rearing of larvae

Spawning occurred 'naturally', without the need to induce fish using hormone injections or to manually strip gametes. Spawning of *E. fuscoguttatus* broodstock occurred at night and began approximately three days after the new moon in May 2006. Spawning continued over three successive nights.

Eggs were collected using 400 μm mesh egg collector nets, positioned within 1000 L egg collecting tanks adjacent to each broodstock tank. Eggs were supplied from the surface of the broodstock tank via a spillway to the egg collecting tank. Due to the setup of the broodstock tanks and egg collectors, it was not possible to separate the eggs produced by each individual female within each treatment group. Thus all eggs produced from each treatment group were pooled. Once collected, eggs were transferred to an incubation tank containing separate 400μm mesh nets on frames. Fertilised eggs were maintained in the egg incubation tank for approximately 12 hours before being transferred to the hatchery. Hatching of eggs occurred in the larval rearing tanks approximately 18 hours after spawning.

One 6000 L and one 1000 L larval rearing tank were used for each treatment. Larval rearing tanks were semi-static systems, with regular water exchanges (~ 10 – 20%) being conducted during cleaning from approximately day 10 to day 40. Daily 100% water exchanges were conducted from

day 40 through to final harvest at day 50. Larval rearing tanks were maintained at 27 – 30˚C and were supplied with constant aeration and filtered natural light.

Larvae were reared under 'green water' culture conditions (Rimmer 2000; Liao et al. 2001). From day 2 through until approximately day 30, micro-algae and rotifers were maintained within the larval rearing tanks. Rotifers were maintained at a concentration of 5 – 10 individuals / ml from first feeding stage (day 3 – 4) to day 10; and at 15 – 20 individuals / ml from day 10 to day 30. Copepod nauplii were also supplied to the larvae from day 3 to day 15. Feeding with brine shrimp (*Artemia salina*) nauplii was introduced at day 15 and was continued through until the completion of the larval rearing phase at day 50. Mysid shrimp were introduced from approximately day 40 and continued through until day 50. Nursery rearing stage (day 50), juvenile *E. fuscoguttatus* were fed on pelletised feeds and pieces of fin fish and squid. A small number of individuals were maintained in the nursery system and reared through until day 100.

7.2.5. Analysis of maternally inherited barium isotope markers in larval otoliths

The analysis methods utilised for *E. fuscoguttatus* otoliths in the present study, were carried out in accordance with the methods described by Thorrold *et al.* (2006). Thirty, 40 day old *E. fuscoguttatus* larvae (mean 21.1 mm TL) were selected from the pool of samples for each of the treatment and control groups. Sagittal otoliths were extracted, cleaned and rinsed with Milli-Q water and dried. Nine individual otoliths were selected from each group, mounted on petrographic slides using cyanoacrylic glue and then polished down to the mid-plane with 3 µm lapping film. After triple rinsing with Milli-Q water, otoliths were dried for 24 hours in a class 100 laminar flow cabinet. Otoliths were then remounted on petrographic slides using double-sided tape.

A 50 µm x 50 µm raster was ablated at the core of each prepared otolith using a New Wave Research UP213 laser ablation system coupled to a Thermo Finnigan Element 2 inductively coupled plasma mass spectrometer (ICP-MS). We measured ^{135}Ba, ^{137}Ba and ^{138}Ba in otolith samples, a dissolved otolith reference material (Sturgeon *et al.* 2005), and a 2% HNO_3 instrument blank using a wet aerosol technique for both laser and solution analyses as outlined in FitzGerald *et al.* (2004). Barium isotope ratios were calculated from blank-corrected intensities following correction for instrument mass bias. The mass bias correction was calculated from the otolith reference material, dissolved in 2% $HN0_3$ and diluted to a final Ca concentration of 40 µg / g. We assumed that the otolith reference material contained naturally invariant isotope ratios of 6.385 for $^{138}Ba/^{137}Ba$, 10.877 for $^{138}Ba/^{135}Ba$, and 1.704 for $^{137}Ba/^{135}Ba$. External precision (relative standard deviation) of the isotope ratio measurements, determined by periodically assaying a second otolith reference throughout ICP-MS runs, was 1% for ^{138}Ba / ^{137}Ba, 2% for ^{138}Ba / ^{135}Ba and ^{137}Ba / ^{135}Ba.

Barium isotope ratios for each otolith were plotted and compared against theoretical mixing curves between the enriched Ba spikes and natural Ba isotope ratios (Almany *et al.* 2007). Single factor univariate ANOVA was used to test for statistical differences in ^{138}Ba / ^{137}Ba and ^{137}Ba / ^{135}Ba isotope ratios between treatment and control groups.

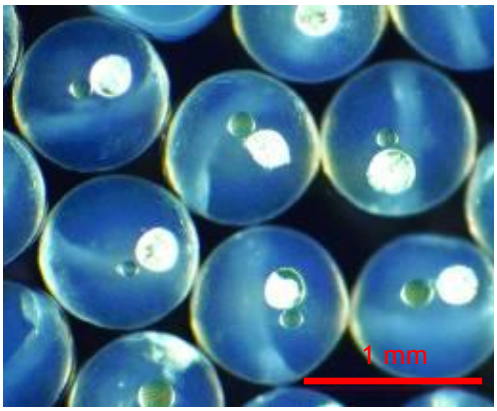
7.2.6. Morphological assessment of eggs and larvae

In order to examine potential maternally transmitted effects of $BaCl_2$ treatment on the quality of eggs and larvae, eggs and pre-feeding (yolk sac) larvae (days 1 – 3) were randomly sampled from treatment and control larval rearing tanks and fixed in 80% ethanol. Fifty eggs and day 1 – 3 larvae were selected from the pool of samples for each treatment and control group. Eggs and larvae were then placed into cavity slides, examined under a stereo dissecting microscope and photographed.

Morphological measurements were conducted using the software program ImageTool (UTHSCSA). The morphological parameters measured were; egg diameter and area, larval standard length (SL), head depth, eye diameter, yolk sac area and oil globule area. Images of fertilised *E. fuscoguttatus* eggs and yolk sac (Day 1 – Day 3) larvae are shown in figure 7.1.

Differences in morphological parameters between treatments were assessed graphically and compared statistically using two factor univariate ANOVA. Following ANOVA, comparison of within and between group means was conducted using Tukey's post-hoc tests. Linear regression plots of the relationship between yolk sac area and standard length (SL) were produced for the control and treatment groups. ANCOVA was used to test for significant homogeneity of slopes between groups. All statistical analyses were conducted using the software program STATISTICA (StatSoft Inc.). In all cases, a statistically significant difference was considered to exist if $p < 0.05$.

A.



B.

C.

D.

Figure 7.1: Microscopic images of *Epinephelus fuscoguttatus* eggs and yolk sac larvae. **A.** Fertilized eggs; **B.** Day 1 larvae; **C.** Day 2 larvae; **D.** Day 3 larvae.

7.3. RESULTS

7.3.1. Validation of barium isotope markers

Otoliths of day 40 *E. fuscoguttatus* larvae from both the T0.5 and T2 treatment groups carried unequivocal altered barium isotope ratio signatures. Mean ^{138}Ba / ^{137}Ba isotope ratios in otolith cores of T0.5 and T2 treatment group larvae produced by females injected with the $^{137}BaCl_2$ spike were significantly lower than in control group larvae by factors of 78.3% and 87.2% respectively ($F_{2, 24} = 310.8$; $p < 0.001$). Furthermore, the mean ^{137}Ba / ^{135}Ba isotope ratio in otoliths of control group larvae was 87.6% lower than in T0.5 treatment group larvae and 91.7% lower than in T2 treatment group larvae. ANOVA revealed that these differences were highly significant ($F_{2, 24} = 15.38$; $p < 0.001$). In both cases, mean ^{138}Ba / ^{137}Ba and ^{137}Ba / ^{135}Ba isotope ratios did not vary significantly between otoliths of T0.5 and T2 treatment groups (Figure 7.2a; Table 7.1).

The mean ^{137}Ba / ^{135}Ba isotope ratio in otoliths from T2 treatment group fish produced by females injected with the $^{135}BaCl_2$ spike was 93.0% lower than the ratio detected in otoliths of control group larvae. This difference was also highly significant ($F_{1, 16} = 9723.1$; $p < 0.001$) (Figure 7.2b; Table 7.1).

	^{138}Ba / ^{137}Ba	**^{137}Ba / ^{135}Ba**
Control	6.44 (± 0.04)	1.69 (± 0.01)
T0.5	1.39 (± 0.28)	13.66 (± 3.12)
T2 ($^{137}BaCl_2$ spike)	0.82 (± 0.11)	20.38 (± 2.78)
T2 ($^{135}BaCl_2$ spike)	6.18 (± 0.06)	0.12 (± 0.01)

Table 7.1: Mean (± 1SE) barium isotope ratios in the control group and in the T0.5 and T2 treatment groups.

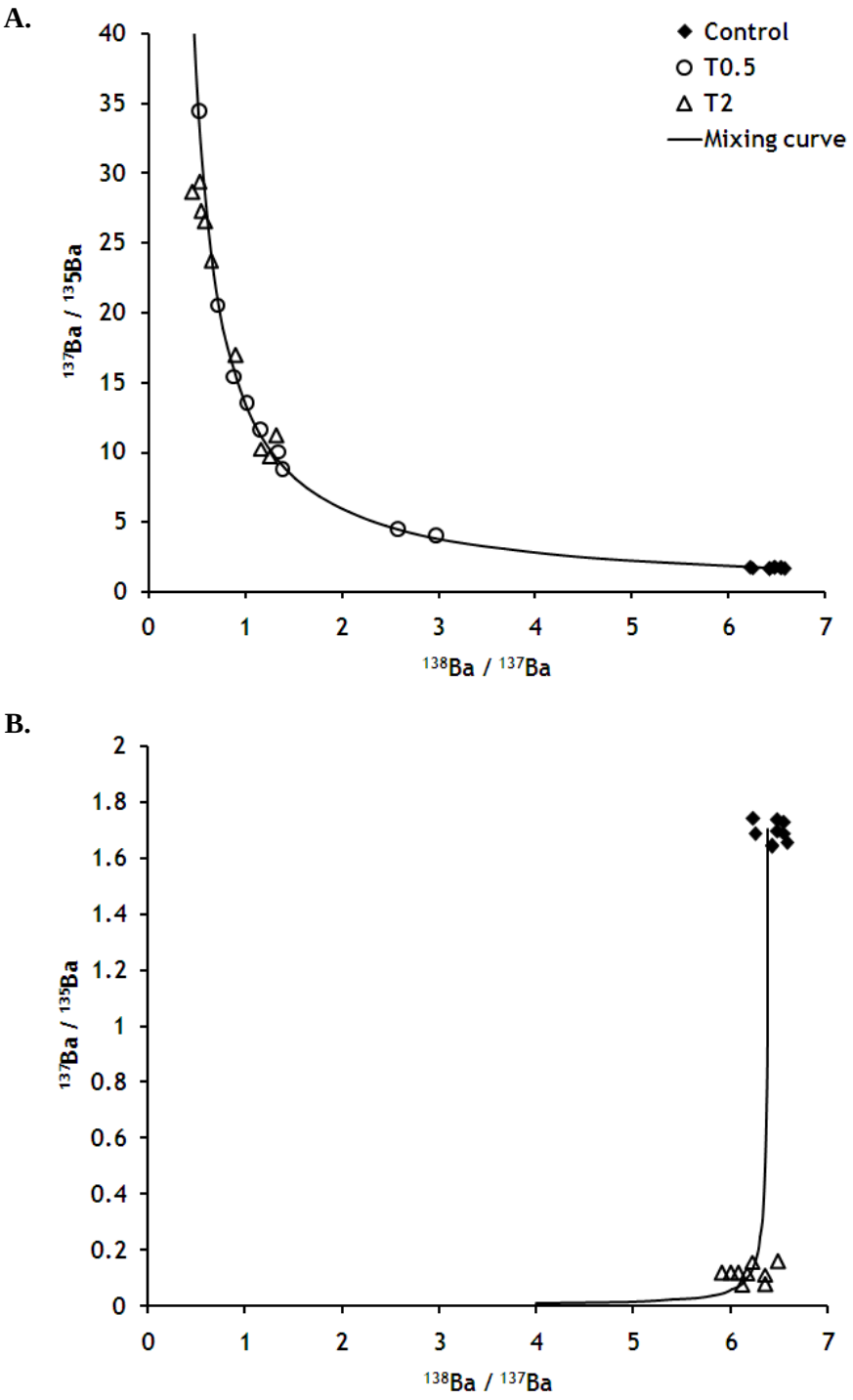


Figure 7.2: Barium isotope ratios in otolith cores of 40 day old *Epinephelus fuscoguttatus* larvae from **A.** Control group, 0.5 mg ^{137}Ba / kg (T0.5) and 2 mg ^{137}Ba / kg (T2) treatment groups; **B.** Control group and the 2 mg ^{135}Ba / kg (T2) treatment group.

7.3.2. Reproductive performance of adults and condition of eggs and pre-feeding larvae

The mean body weight of female *E. fuscoguttatus* broodstock was 6.74 kg (+/- 1.23 kg SD). Mean oocyte diameter across all groups at cannulation was 408.57 µm (+/- 86.85 µm SD). Single-factor ANOVA revealed no significant difference in female body weight ($F_{2, 18} = 0.093$; $p = 0.911$) or oocyte diameter ($F_{2, 18} = 0.083$, $p = 0.921$) between groups prior to $BaCl_2$ injection. The first post-injection spawning occurred synchronously for T0.5, T2 and control group fish. There was no apparent effect of $BaCl_2$ injection on the timing of spawning.

The mean size of fertilized eggs produced by both T0.5 and T2 treatment *E. fuscoguttatus* were not significantly different than those produced by control group fish. Variability in egg size was similar between groups and ANOVA revealed no significant differences in egg area between treatment and control groups ($F_{2, 147} = 0.200$; $p = 0.788$) (Figure 7.3).

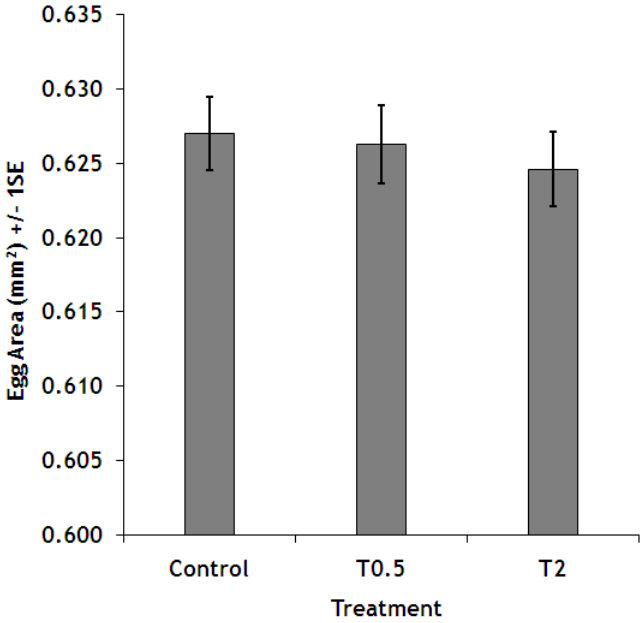


Figure 7.3: Mean area (mm^2 +/- 1SE) of fertilised *Epinephelus fuscoguttatus* eggs from control, 0.5 mg ^{137}Ba / kg (T0.5) and 2 mg ^{137}Ba / kg (T2) treatment groups.

Significant variations in the standard length, head depth, yolk sac area and oil globule area of pre-feeding *E. fuscoguttatus* larvae were detected between control and treatment groups. No significant differences in mean eye diameter were detected between groups.

The mean standard length of pre-feeding larvae from the T2 treatment group was significantly shorter at day 2 than both T0.5 and control group larvae (Tukey's: $p < 0.01$). Although T2 group larvae remained shorter than both T0.5 and control group larvae at day 3, this difference was not significant (Figure 7.4a; Table 7.2).

Day 2 larvae from the T0.5 and T2 treatment groups had significantly narrower mean head depths than control fish (Tukey's: $p < 0.05$ and $p < 0.01$ respectively). These differences did not persist however, as by day 3, no significant differences in head depth were present (Figure 7.4b; Table 7.2).

One day old larvae from the T2 treatment group had significantly smaller mean yolk sac areas than both control group and T0.5 treatment group larvae (Tukey's: $p < 0.01$). However, no differences in yolk sac area were present between treatment and control groups at day 2 or day 3 (Figure 7.4d; Table 7.2). ANCOVA did detect significant heterogeneity between linear regression slopes of yolk sac area versus standard length for the control and treatment groups ($F_{2, 444} = 15.790$; $p < 0.001$). This difference was again due to day 1 larvae (</= 2.2 mm SL) from the T2 treatment group having significantly smaller yolk sac areas for a given standard length than both control group and T0.5 treatment group larvae (Figure 7.5). The relationship between yolk sac area and standard length was not significantly different between the control group and the T0.5 treatment group. Yolk reserves were almost completely expended by day 3 and by this stage the larval jaw structure had

formed, mouths were open and eyes were pigmented. All viable larvae began feeding late on day 3 or on day 4.

Day 2 larvae from the T2 treatment group also had significantly smaller oil globule areas than did larvae from the T0.5 treatment and control groups (Tukey's: $p < 0.01$ for both groups). Oil globules were completely absorbed by day 3 in both treatment and control groups (Figure 7.4e; Table 7.2).

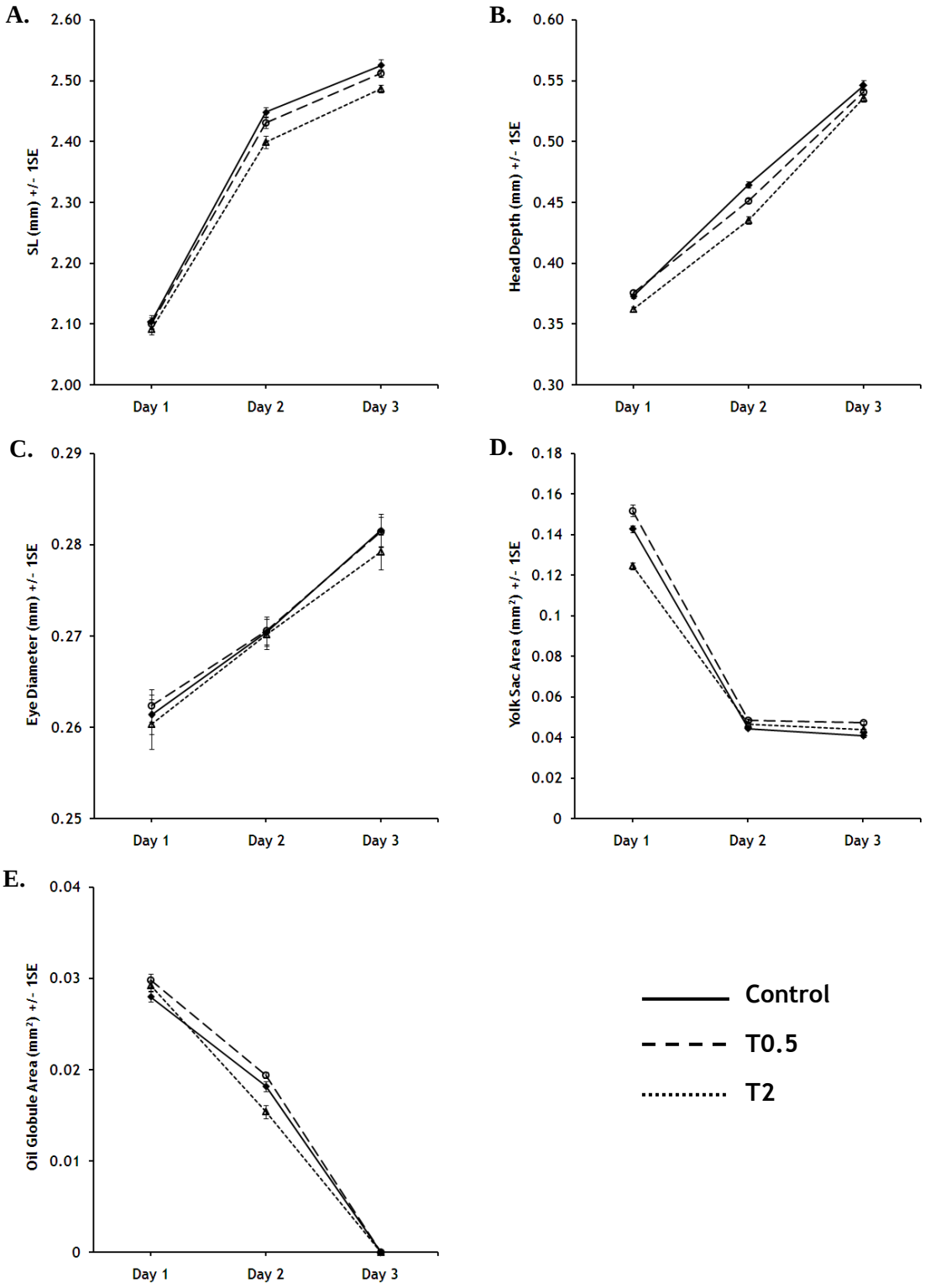


Figure 7.4: Mean (+/- 1SE) **A**. standard length (SL, mm); **B**. head depth (mm); **C**. eye diameter (mm); **D**. yolk sac area (mm^2) and **E**. oil globule area (mm^2) of yolk sac (day 1 – 3) *Epinephelus fuscoguttatus* larvae from control, 0.5 mg ^{137}Ba / kg (T0.5) and 2 mg ^{137}Ba / kg (T2) treatment groups.

	Treatment 2, 441 *d.f.*	Age (days) 2, 441 *d.f.*	Treatment x Age 4, 441 *d.f.*
Standard length	9.9 (***)	1657.2 (***)	1.1 (ns)
Head Depth	28.6 (***)	2857.4 (***)	4.8 (***)
Eye Diam.	0.6 (ns)	79.3 (***)	0.1 (ns)
Yolk-sac Area	44.9 (***)	4374.4 (***)	28.1 (***)
Oil Globule Area	7.8 (***)	2755.3 (***)	6.9 (***)

Table 7.2: Summary results of two-factor univariate analysis of variance on morphological parameters in yolk sac (day 1 – 3) *Epinephelus fuscoguttatus* larvae. Numerical values are F ratios (probability results are shown in brackets). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns: not significant. ANOVA degrees of freedom (*d.f.)* are shown in column headings.

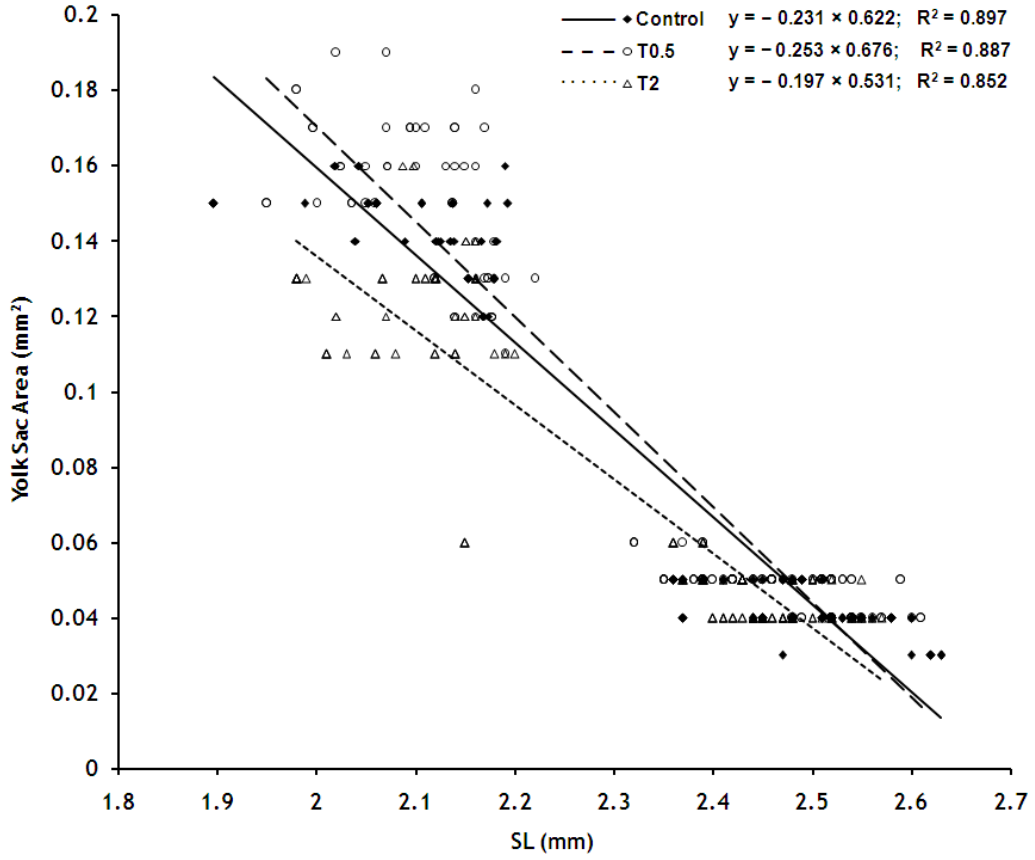


Figure 7.5: Linear regression plot of yolk sac area (mm^2) versus standard length (SL, mm) of yolk sac (day 1 – 3) *Epinephelus fuscoguttatus* larvae from control, 0.5 mg ^{137}Ba / kg (T0.5) and 2 mg ^{137}Ba / kg (T2) treatment groups.

7.4. DISCUSSION

This study extends the findings of Thorrold *et al.* (2006), demonstrating that the injection of enriched stable barium isotopes is 100% successful in the geochemical tagging of the larvae of a large pelagic spawning coral reef fish. Low dose injections of both $^{137}BaCl_2$ and $^{135}BaCl_2$ administered into the body cavities of gravid female *E. fuscoguttatus* produced distinct maternally inherited tags in all of the offspring examined. Once embedded in the calcium matrix of the otolith core, the Ba isotope marker is permanent and it can potentially be detected at any life stage of the offspring fish (Thorrold *et al.* 2006).

The provision of dose rates as low as 0.5 mg Ba / kg of female body weight generated unequivocal tags in larvae which were spawned within 20 days of maternal injection. A key question requiring further investigation is whether or not larvae from successive spawning events are tagged subsequent to the first post-injection spawning event. Thorrold *et al.* (2006) demonstrated that a single injection of $^{137}BaCl_2$ at dose rates as low as 0.45 mg Ba / kg can reliably produce 100% marked larvae in successive clutches of *Amphiprion melanopus* for at least 60 days post maternal injection. Furthermore, a recent study by Williamson *et al.* (2009) (Chapter 6) demonstrated that ^{138}Ba / ^{137}Ba isotope ratios in ovary tissues of adult female coral trout (*Plectropomus leopardus*) injected with $^{138}BaCl_2$ at a dose rate of 2 or 4 mg Ba / kg remained significantly different (3 – 6 fold) from ratios in control group fish, up to 8 weeks post injection. Although no *P. leopardus* larvae were produced in that study, the elevated isotope ratios in ovaries of treated female fish suggests that the maternally transmitted marker may be effective for at least 8 weeks post injection in this species. Depending on the specific application, an effective marking time of several weeks to months would likely be most useful in the majority of field studies, particularly in tropical

regions where many fish species, including *E. fuscoguttatus* and other serranids, have extended spawning seasons (Pet *et al.* 2005; Pears *et al.* 2006).

The present study has demonstrated that the 0.5 mg Ba / kg dose rate was equally as effective as the 2 mg Ba / kg dose rate in producing tagged larvae. However, the results also suggest that increasing the $BaCl_2$ concentration above 2 mg Ba / kg may generate negative effects on egg and larval quality. Injection of $BaCl_2$ at a dose rate of 2 mg Ba / kg potentially contributed to small reductions in yolk sac area, oil globule area, standard length and head depth of pre-feeding *E. fuscoguttatus* larvae. These effects appeared to be transitory however, as they had disappeared by the time larvae had reached the first feeding stage at day 3. Although no significant differences were detected between the morphological measures of control, T0.5 and T2 treatment group larvae at the first feeding stage, the significantly smaller yolk reserves of day 1 and day 2 larvae in the T2 treatment could potentially translate into reduced survivorship of wild larvae in a field setting (Green & McCormick 2005).

No significant between group differences in mean female body weight or oocyte size and development stage were detected prior to $BaCl_2$ injection, however it cannot be excluded that the observed variability in larval condition indices between groups were at least partially due to variability in maternal condition. Maternal condition can be influenced by intra and inter-specific interactions, food availability and/or other physical environmental factors, and egg and larval quality has been shown to be strongly linked to maternal condition (Green & McCormick 2005; Gagliano & McCormick 2007). In the present study it was not possible to assign eggs produced from each treatment group to individual females and thus link egg and larval quality directly to maternal condition. However, given the clarity and consistency of the results presented here, it is evident that larval quality was likely affected by treating female *E. fuscoguttatus* with the 2 mg Ba /

kg dose rate. The 0.5 mg Ba / kg dose rate effectively tagged embryonic otoliths, thus it is clearly possible to keep the Ba dose rate below the level that could affect larval growth or survival. Further studies are required to explicitly test potential effects of low-dose $BaCl_2$ injection on maternal condition and egg and larval quality for a range of fish species.

Another fundamental consideration for field applications of the TRAIL technique is the potential adverse effects of $BaCl_2$ injection on both adult fish, and on humans who may consume those fish. Williamson *et al.* (2009) (Chapter 6) demonstrated that an injection of enriched $BaCl_2$ spike solution at dose rates up to 4 mg Ba / kg produced no detectable physiological effects in adults of another serranid species, *Plectropomus leopardus*. Furthermore, the concentration of residual barium in muscle tissue of injected *P. leopardus* was extremely low and it was concluded that treated fish presented no consumption risk for humans.

Given the increasing reliance on spatial management systems for both biodiversity conservation and enhanced sustainability of exploited fish stocks, empirical data of larval dispersal patterns and demographic population connectivity is urgently required (Sale *et al.* 2005). Incorporation of empirical larval dispersal data into biophysical models is of critical importance as it will greatly improve our understanding of the structure and functioning of marine populations, communities and ecosystems; and ultimately lead to optimal design and effectiveness of NTR networks. This study has demonstrated the efficacy of a single low-dose injection of enriched stable isotope $BaCl_2$ in reliably producing marked larvae in a large pelagic spawning reef fish. The results of the present study and those of several other recent studies (Thorrold *et al.* 2006; Almany *et al.* 2007), should instill confidence in the use of this technique for mass marking larvae of a range of reef fish species. The next step is to apply this larval marking technique to large reef fishes in the field. This is the topic of the next chapter of this thesis.

Chapter 8: Larval retention and export in a network of no-take marine reserves: a preliminary analysis.

8.1. INTRODUCTION

During the past few decades the body of evidence documenting the positive effects of no-take marine reserves (NTRs) has grown considerably and there is now overwhelming evidence that populations of exploited species will increase within adequately protected reserves (Roberts & Hawkins 2000; Halpern & Warner 2002; Gell & Roberts 2003; Russ *et al*. 2002, 2008; Lubchenco *et al*. 2003; Russ & Alcala 2003, 2004; Williamson *et al.* 2004). The expectation is that enhanced populations within marine reserves should lead to increased reproductive output per unit area, potentially providing recruitment subsidies to surrounding fished areas and increasing the overall sustainability of exploited stocks (Mora & Sale 2002; Russ *et al.* 2002; Gell & Roberts 2003; Roberts *et al.* 2001, 2005; Botsford 2005; Evans *et al.* 2008).

The importance of generating empirical data of larval dispersal and demographic connectivity of marine populations is widely recognised (Cowen *et al.* 2000, 2006; Russ 2002; Gell & Roberts 2003; Shanks *et al.* 2003; Palumbi 2004; Sale *et al.* 2005). However, due to the difficulties associated with tracking larval dispersal within marine environments, very few studies have achieved in situ tracking of larvae from natal reefs to settlement locations and none have achieved it for large commercially important fish species (Jones *et al.* 1999, 2005; Almany *et al.* 2007; Planes *et al*. 2009). This lack of empirical data remains an impediment to the implementation of NTR networks and wider acknowledgement of the potential contribution of reserves to achieving sustainable fisheries management (Gell & Roberts 2003; Willis *et al*. 2003b; Sale *et al.* 2005).

Throughout this thesis it has been demonstrated that NTRs on inshore reefs of the Great Barrier Reef Marine Park (GBRMP) have provided significant and persistent benefits for populations of exploited species within reserve boundaries (Chapters 3, 4 & 5). It has also been established from laboratory work that recently developed enriched stable isotope larval marking techniques are safe and effective for use on large commercially important fishes (Chapters 6 & 7). The logical extension of this series of studies involves applying the new larval marking technologies to begin examining larval dispersal, demographic connectivity and export effects of NTRs in the field. This study is the first to apply the larval marking technique to populations of large commercially and recreationally targeted fish species, within a network of marine reserves. Furthermore, this study also examines movement patterns and home ranges of adult fish within NTRs and spill-over from NTRs to surrounding fished areas.

The aim of this chapter is to describe the first field experiment carried out to examine how individual NTRs may contribute larvae and adults of commercially important fishes to surrounding exploited reefs. Although this project is still a work in progress, details of the experiment will be described and preliminary findings presented.

The specific objectives of this study were to:

1. Utilise recently developed larval marking technologies to track larvae of target fish species from their natal reef of origin within NTRs to their settlement locations.
2. Measure demographic population connectivity, larval dispersal patterns and rates of self-recruitment within a network of marine reserves.
3. Assess movement patterns of adult fish within NTRs and estimate rates of flux across reserve boundaries to surrounding fished areas.

8.2. METHODS

8.2.1. Study location

This research project was carried out in the Keppel Island group (23°10´ S, 150°57´ E) within the Great Barrier Reef Marine Park (GBRMP). The Keppel Islands consist of 16 continental islands and several isolated rocky outcrops that are located between 10 km and 30 km from the mainland. The majority of the islands within the group are uninhabited National Parks, however a resort, airstrip, several guest houses and some private dwellings are located on Great Keppel Island. The Keppel Island group is a high-use recreational area and the fringing coral reefs which surround the islands are popular destinations for fishers, divers, boaters and tourism operators. Commercial aquarium fish and invertebrate collectors also operate within the Keppel Islands.

Four NTRs were utilised in this study, Middle Island, Halfway Island, Clam Bay and Egg Rock (Figure 8.1). Middle Island, Halfway Island and Egg Rock were designated as NTRs in 1987, while the Clam Bay NTR was established in July 2004. Data presented in Chapter 5 of this thesis and in Russ *et al.* (2008) have demonstrated that the mean abundance and average sizes of target fish species are significantly higher within NTRs of the Keppel Island Group than in surrounding fished areas.

Figure 8.1: Map of the Keppel Island group, showing post July 2004 zoning information. Green shaded areas are no-take marine reserves (Marine National Park Zone), blue (Habitat Protection Zone) and yellow (Conservation Park Zone) shaded areas are open to fishing. Dashed circles indicate the locations of adult fish tagging, red markers indicate the general locations in which recruit fish were collected. The regional setting of the Keppel Island group is shown in figure 2.1 of Chapter 2 (General Methods).

8.2.2. Adult fish capture and tagging

Two 14 day adult fish tagging trips were conducted, the first in November 2007 and the second in January 2008. Recreational fishers were sourced from local sportfishing clubs to assist with the tagging trips. Fishers used their own private vessels and fishing equipment, but were provided free accommodation, meals, fishing bait and fuel. Capture, tag and release fishing was conducted on fringing reefs within NTRs at Middle Island, Halfway Island, Clam Bay and Egg Rock (Figure 8.1). The total fishing effort during the two fishing trips was 196 vessel-days or 503 fisher-days.

Fish were captured using hook and line, to a depth of approximately 20 metres. Captured fish were placed into aerated seawater holding tanks for up to 5 minutes while their condition was monitored. Fish that had experienced swim bladder over-inflation during capture were vented using a 16 gauge hypodermic needle. Fish were then placed onto large foam mats and the total length and fork length were measured and recorded. Each fish was then tagged with a unique number-coded external t-bar tag. A phone number and website address were printed on all external tags to facilitate reporting of adult fish recaptures by the general public.

All captured individuals of three species, *Plectropomus maculatus* (Bar-cheek coral trout), *Lutjanus carponotatus* (Stripey Snapper) and *Epinephelus quoyanus* (Long-finned rock cod) were administered with an injection of enriched stable isotope barium chloride ($BaCl_2$) solution. Small volumes of $BaCl_2$ solution were injected into the body cavity at a dose rate of 1mg Ba / kg of body weight. Three distinct barium isotope markers were utilised, ^{137}Ba at Middle Island, ^{138}Ba at Halfway Island and Clam Bay, and a 1:1 mixture of ^{135}Ba / ^{138}Ba at Egg Rock. In addition, a small sample of pectoral or dorsal fin was removed from each individual of the three target species for population-based genetic analyses. Fin clip samples were fixed in 80% high-grade ethanol.

The first spawning of potentially barium marked eggs occurred during December 2007. Data presented in chapters 6 and 7 of this thesis and in Williamson *et al*. (2009a, 2009b), suggests that the effective marking time of a single enriched isotope $BaCl_2$ injection is approximately 2 months in large groupers. Given this information, it is expected that the final spawning of barium marked eggs occurred in March 2008.

8.2.3. Estimating adult fish population size and total proportion tagged

Underwater visual census (UVC) surveys were conducted to determine the size of *P. maculatus, L. carponotatus* and *E. quoyanus* populations within each marine reserve and the relative proportion of the populations which had been tagged. Visual surveys were conducted within two weeks of the completion of the fishing periods. Several long meandering swims of approximately 60 minutes duration were conducted within each reserve location, covering both reef slope and reef flat habitats. All adult individuals of the three target species were recorded and their total lengths estimated. All fish were recorded as tagged or un-tagged.

A simple Petersen type estimate of the adult fish population size within each reserve location was calculated for each of the target species by dividing the total number of fish tagged and released within each location by the relative proportion of tagged fish re-sighted during UVC surveys within each location. The following formula was used:

$$N = \frac{M}{R / 100}$$

Where:

N = Total population size
M = Total number of individuals marked in each population
R = Percentage of tagged individuals sighted in UVC recapture sample

8.2.4. Recruit fish sample collection and processing

Collection of juvenile (recruit) *P. maculatus, L. carponotatus* and *E. quoyanus* was conducted during May 2008 and February 2009. Recruit fish of all three species were collected using spears, clove oil, dip nets and fence nets. The fork length of collected recruits ranged between 21 mm and 300 mm. The maximum age of recruit fish which were potentially barium marked was

approximately 150 days during the May 2008 collection period and 420 days during the February 2009 collections.

Age determination studies of juveniles of the three target fish species have been conducted by our research group between 2006 and 2009 using samples collected from the Keppel Island group. The results of these studies are currently unpublished, but they did function to define the target length range of potentially barium marked recruits of the three target species. The target length range for *P. maculatus* was 45 – 160 mm in May 2008 and 190 – 300 mm in February 2009, *L. carponotatus* was 25 – 100 mm in May 2008 and 120 – 200 mm in February 2009, *E. quoyanus* was 50 – 75 mm in May 2008 and 80 – 140 mm in February 2009. Similar numbers of *P. maculatus* and *L. carponotatus* target length range recruits were collected in May 2008 and February 2009. However, due to the significantly slower growth rate and more cryptic behaviour of *E. quoyanus* than both *P. maculatus* and *L. carponotatus*, the vast majority of *E. quoyanus* recruit samples were collected in February 2009.

The fork length and total length of each collected recruit fish was measured and recorded. The sagittal otoliths of each fish were extracted, cleaned, and stored dry in numbered and grid-referenced 96-well microtitre plates. Fin-clip samples were taken from each individual and fixed in 80% high-grade ethanol.

8.2.4. Analysis of otolith samples

One otolith from each pair of sagittae was mounted on a glass microscope slide and polished until the daily otolith rings were visible. Daily otolith rings were counted in order to determine the exact

age of each sampled recruit fish. Age determination work was carried out in accordance with the methodologies described by Lou & Moltschaniwskyj (1992).

The methods of processing and analysis utilised for assessing barium markers in recruit fish were conducted according to the methods described in chapter 7 of this thesis and by Thorrold *et al.* (2006). Otoliths were cleaned and mounted on petrographic slides and polished down to the midplane with 3 µm lapping film. After triple rinsing with Milli-Q water, otoliths were dried for 24 hours in a class 100 laminar flow cabinet. Otoliths were then remounted on petrographic slides using double-sided tape. The core of each prepared otolith was scanned using a New Wave Research UP213 laser ablation system coupled to a Thermo Finnigan Element 2 inductively coupled plasma mass spectrometer (ICP-MS). The concentration of ^{135}Ba, ^{137}Ba and ^{138}Ba in each otolith core was measured and barium isotope ratios were calculated. Barium isotope ratios for each otolith were plotted and compared against a theoretical mixing curve between the enriched Ba spikes and natural Ba isotope ratios, thus determining if each individual was tagged or not. All chemical analysis was carried out at the Woods Hole Oceanographic Institution (Woods Hole, Massachusetts).

8.2.5. Estimating movement patterns of adult reef fish

Tagged fish which were recaptured within the marine reserves during the experimental fishing periods were re-measured and the tag number and the GPS location of each recapture was recorded. Recaptures of tagged fish which have moved to areas outside of the marine reserves are being reported by the general public via a free-call hotline or the internet. Analysis of adult fish movement data is currently still in progress. Tag and recapture data has been uploaded to a global information system (GIS) data base. The movement patterns of a number of species including *P.*

maculatus, L. carponotatus and *E. quoyanus* are being analysed using the GIS software program ArcView (ESRI). Preliminary fish movement data is presented in this chapter, however a complete analysis will be carried out in future months and reported elsewhere.

8.3. RESULTS

8.3.1. Adult fish tagging and estimates of population size

A total of 6,166 reef fish were captured, tagged and released during the two 14 day tagging periods. Twenty nine fish species from 5 families (Lutjanidae, Lethrinidae, Serranidae, Labridae, Haemulidae) were represented in the catch. The total sampling effort contributed to the project was 503 fisher-days which equated to an overall catch rate of 12.3 fish / fisher / day.

The three target species, *Plectropomus maculatus, Lutjanus carponotatus* and *Epinephelus quoyanus* represented 78.8% of the catch. *Epinephelus quoyanus* was the most readily captured species and comprised 50.1% of the catch. Approximately 22% of the catch was *Lutjanus carponotatus*, while 6.6% of the catch was *Plectropomus maculatus*. A summary of the total hook and line catch and the total number of recaptures for the three target species is provided in figures 8.2 and 8.3.

UVC surveys revealed that across all four marine reserve locations, 25.8% (± 9.3% SE) of the adult *P. maculatus* population was tagged, 41.8% (± 8.6% SE) of *L. carponotatus* were tagged and 70.4% (± 8.2% SE) of *E. quoyanus* were tagged. A breakdown of the total proportion of each

marine reserve population and the estimates of total adult population size for each of the three target species are provided in table 8.1.

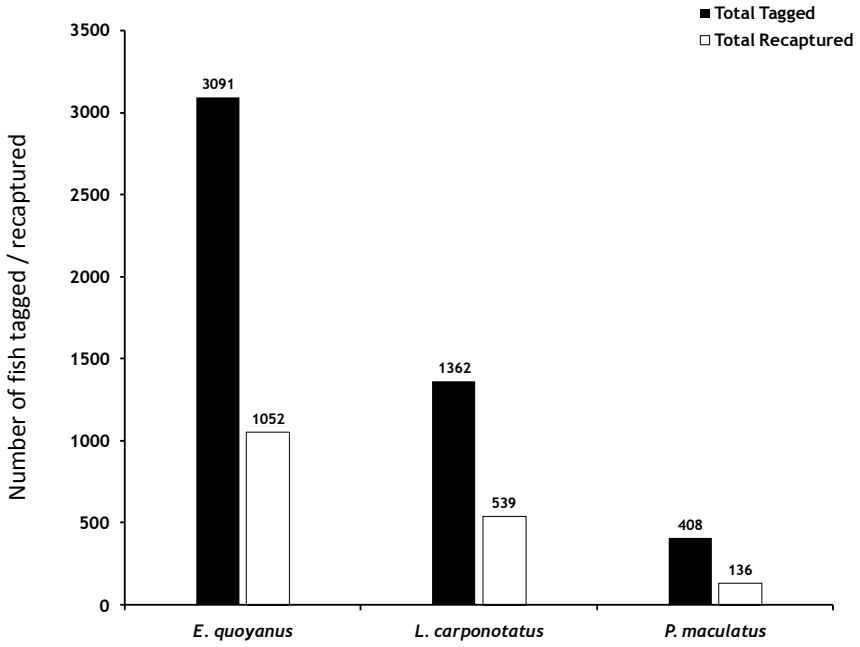


Figure 8.2: Total number of *Plectropomus maculatus*, *Lutjanus carponotatus* and *Epinephelus* captured, tagged and released and the total number of recaptures in no-take marine reserves of the Keppel Island group during November 2007 and January 2008.

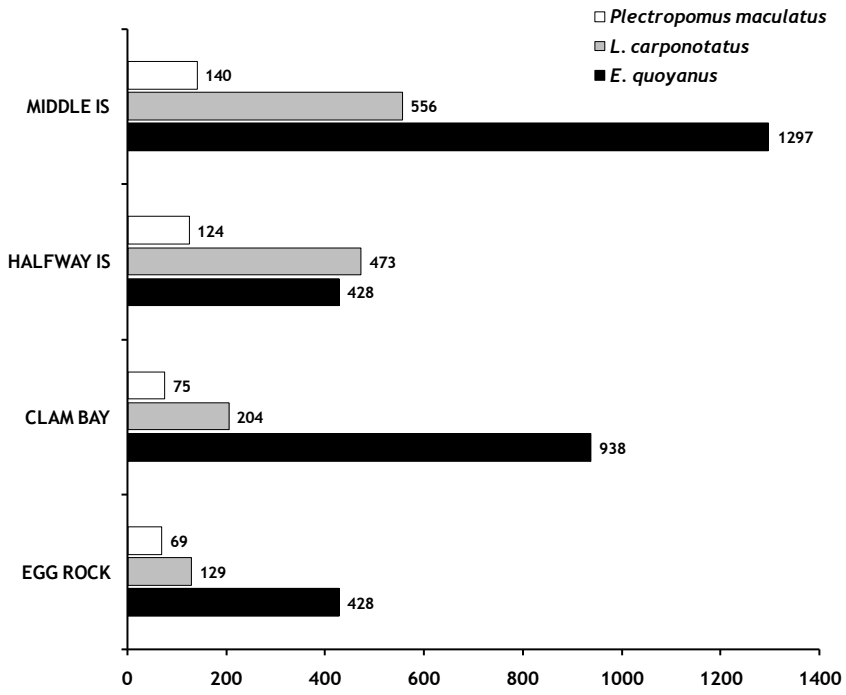


Figure 8.3: Total number of *Plectropomus maculatus, Lutjanus carponotatus* and *Epinephelus quoyanus* captured, tagged and released within each marine reserve location of the Keppel Island group during November 2007 and January 2008.

Species	Location	Total No.	Estimate of	Estimate of
	Middle Island	140	13.0%	1075 (± 122)
	Halfway Island	124	37.4%	332 (± 36)
P. maculatus	Clam Bay	75	7.1%	1063 (± 111)
	Egg Rock	69	45.7%	151 (± 17)
	Total	**408**	**25.8%**	**2621 (± 286)**
	Middle Island	556	50.8%	1095 (± 121)
	Halfway Island	473	60.5%	782 (± 87)
L. carponotatus	Clam Bay	204	21.6%	946 (± 103)
	Egg Rock	129	34.6%	373 (± 41)
	Total	**1362**	**41.8%**	**3196 (± 352)**
	Middle Island	1297	71.2%	1821 (± 203)
	Halfway Island	428	87.5%	489 (± 54)
E. quoyanus	Clam Bay	938	82.8%	1133 (± 126)
	Egg Rock	428	50.8%	843 (± 93)
	Total	**3091**	**70.4%**	**4286 (± 476)**

Table 8.1: Total number of adult *P. maculatus*, *L. carponotatus* and *E. quoyanus* tagged within each location, UVC estimates of the total proportion tagged within each location, and the inferred Petersen estimates of total adult population size within each location.

8.3.2. Recruit fish collections and analysis of dispersal patterns using barium markers

Collections of juvenile (recruit) *P. maculatus*, *L. carponotatus* and *E. quoyanus* were conducted during May 2008 and February 2009. Collections were carried out in both fished (non-reserve) areas and within NTRs. Analysis of otolith samples is still in progress and only a proportion have currently been analysed for barium isotope markers. Two barium tagged recruits have been detected thus far, however this number is likely to increase as further otolith samples are analysed. A summary of the results to date is provided in table 8.2. It is expected that all analyses will be completed by September 2009.

Both of the barium tagged recruits obtained to date were tagged with ^{138}Ba which was specific to the Clam Bay and Halfway Island NTR. The barium tagged *P. maculatus* recruit was collected on the back reef (non-reserve) at Halfway Island, so had travelled a maximum linear distance of approximately 2.5 km from where it was spawned. The tagged *L. carponotatus* recruit was collected on the reef flat within the Clam Bay NTR, hence it was retained within the source NTR. Again, the maximum distance from the spawning site to settlement location was approximately 1.5 km.

Species	No. Recruits Collected	Size Range (FL)	No. Analysed	No. Tagged
P. maculatus	350	45 – 300 mm	63	1
L. carponotatus	284	25 – 200 mm	35	1
E. quoyanus	177	50 – 140 mm	23	0

Table 8.2: Total number and size range of juvenile (recruit) *P. maculatus*, *L. carponotatus* and *E. quoyanus* collected from the Keppel Island group in May 2008 and February 2009. The number of samples of each species which have been analysed to date and the total number of barium tagged individuals detected is also shown.

8.3.3. Movements of adult fish and spill-over from NTRs

Analysis of adult fish movements within marine reserves and from reserves to surrounding fished areas is still in progress. The results of this component of the study should be completed by June 2009, and will not be reported in this thesis.

The results currently available demonstrate significant movements of *P. maculatus*, *L. carponotatus* and *E. quoyanus* within the NTRs of the Keppel Island group. In addition, a number of tagged fish have been recaptured outside of the NTRs and reported. To date, 12 *E. quoyanus* and 3 *L. carponotatus* have been captured in close proximity to the marine reserve boundary which runs between the western end of Clam Bay and the northern end of Halfway Island. In these 15 cases, the total distance moved from tagging to recapture location is less than 1 km.

Three *P. maculatus* have also been captured outside of marine reserve boundaries. Two of these fish moved a distance of approximately 2 km, from within the Clam Bay reserve, to areas outside the reserve. One individual *P. maculatus* which was tagged at the Middle Island reserve (23°10.066´ S, 150°55. 042´ E) in January 2008 was recaptured at Middle Rock (23°59.799´ S, 151°46.531´ E) in November 2008. This fish was 430 mm in total length, and the movement undertaken represents a straight line distance of approximately 125 km.

8.4. DISCUSSION

Although the results of this study are currently incomplete, a significant amount of valuable information has already been obtained. Of fundamental importance, is the demonstration that the

trans-generational larval marking technique which was trialed and reported in chapters 6 and 7 of this thesis, can be successfully applied to large, pelagic spawning reef fish species in the field. This larval marking technique has previously been applied successfully in populations of a butterflyfish (*Chaetodon vagabundaus*) and an anemone fish (*Amphiprion percula*) in Papua New Guinea (Almany *et al.* 2007). However this study is the first to apply the technique to populations of commercially and recreationally targeted fish species, within a network of marine reserves. Further weight is added to this study as it has been conducted within the iconic Great Barrier Reef Marine Park.

Even with the limited results which are currently available, this study has confirmed that there is some level of retention of *P. maculatus* and *L. carponotatus* larvae within the Keppel Island group. Furthermore, it has been successfully demonstrated that eggs and larvae produced from fish populations within marine reserves can be dispersed to surrounding areas which are open to fishing and to other marine reserves within the network. The findings presented here concur with those of several previous larval fish tagging studies which have demonstrated higher than expected rates of self-recruitment, restricted larval dispersal distances and high local connectivity of populations (Jones *et al*. 1999, 2005; Almany *et al*. 2007; Planes *et al.* 2009).

Once completed, the larval dispersal data generated from this study will be incorporated into a coupled biophysical model which is currently being developed by colleagues at the Australian Museum. The development of such models which incorporate empirical physical and biological data is of fundamental importance to understanding and accurately predicting demographic population connectivity. Ultimately, this kind of information will be integral to effective marine resource management, particularly in relation to the optimal design of marine reserve networks (Cowen *et al.* 2000, 2006; Sale *et al*. 2005; Jones *et al.* 2007).

Although preliminary, data generated from the adult movement component of this study have already yielded several interesting results. Significant movements of fish have been recorded within and between NTRs and preliminary analyses indicate considerable flux of fish across reserve boundaries. In addition, the one adult *P. maculatus* that travelled approximately 125 km, is the longest recorded movement for this and other *Plectropomus* species (Samoilys 1997a; Zeller & Russ 1998).

It is evident from the data presented in this thesis, that the NTRs of the Keppel Island group are providing significant benefits for populations of target fish species within reserve boundaries. Furthermore, there is some suggestion in the preliminary data presented here that fish populations within NTRs are providing both recruitment subsidies and adult fish biomass to surrounding fished areas. This evidence is consistent with the expectation that adequately protected NTRs can provide benefits beyond their boundaries (Russ 2002; Gell & Roberts 2003).

The involvement of recreational fishers in the fish tagging phase of this study has provided a demonstration of the potential benefits of the research sector actively engaging with the wider community. In this case, the collaboration led to increased sampling effort and research capacity, increased awareness, mutual trust and improved dissemination of information from researchers to the community and vice versa. In short, without the involvement of experienced local fishers, we would not have achieved as much as we have.

Although the findings presented in this chapter are preliminary, I have attempted to provide an overview of the work that has been completed so far. Analysis of samples and data will continue

through 2009 and 2010, but once complete, I expect to have a broad range of robust results to report.

Chapter 9: General Discussion

This thesis has demonstrated persistent and widespread effects of no-take marine reserve (NTR) protection on populations of exploited reef fish species in the Great Barrier Reef Marine Park (GBRMP). Estimates of mean abundance, size and biomass of coral trout (*Plectropomus* spp.) on fringing reefs within NTR boundaries were consistently higher than on the fished reefs of three inshore island groups. Temporal monitoring documented considerable dynamics within coral trout populations on these inshore reefs, however the positive effects of NTR protection persisted during the 5 – 10 year monitoring period.

The effects of NTR protection were less pronounced or consistent for secondary target species such as the stripey snapper (*Lutjanus carponotatus*). Generally however, population density, mean length and biomass were all consistently higher in NTRs than in fished zones. It is evident that the benefits of NTR protection can extend to a range of fish species beyond those most favoured and sought after by fishers. Overall, the findings add to a growing body of evidence that given time and adequate protection, populations of exploited fish species can increase considerably within NTRs, eventually leading to higher reproductive output per unit area and potential recruitment subsidy and spill-over benefits for surrounding areas which remain open to fishing (Roberts & Hawkins 2000; Russ 2002; Gell & Roberts 2003; Halpern 2003; Sobel & Dahlgren 2004; Roberts *et al.* 2005).

Species of fish not targeted by fisheries, such as the Siganidae and Chaetodontidae, are largely unaffected by NTR protection. As expected, the abundance of these non-target groups, particularly the Chaetodontidae, was influenced to a greater degree by benthic (coral) community dynamics

than by NTR protection. Furthermore, the data suggested that top-down predation and/or competitive pressure, driven largely by *Plectropomus* spp., may be contributing to some of the observed patterns of abundance in certain fish groups such as siganids, small serranids (*Cephalopholis* spp.) and labrids (*Choerodon* spp.). These potential secondary effects of NTRs on warrant further investigation. There is also preliminary evidence that habitat degradation reduces, but does not eliminate the positive effects of NTR status on exploited predators.

The inshore fringing reef NTRs examined in this study are some of the most effectively protected within the GBRMP. The relatively close proximity to human population centres and high accessibility of these areas means that formal surveillance operations are relatively effective in comparison to more remote locations of the GBRMP (Gribble & Robertson 1998; Davis *et al.* 2004; Williamson *et al.* 2004; Russ *et al.* 2008). Furthermore, the fishing pressure applied to these reefs is almost exclusively recreational, compliance with zoning regulations is generally high and poaching (zoning infringement) rates are relatively low (Higgs & McInnes 2003; Davis *et al.* 2004; Chapter 4). Much of the early evidence of the effects of NTR protection on target fish species within the GBRMP was highly equivocal (Williams & Russ 1994). The majority of those studies were conducted on mid-shelf platform reefs which are located between 40 and 200km from the coast and where fishing effort is dominated by commercial hook and line fishers.

Data presented in this thesis have provided some of the most compelling evidence to date that NTRs in the GBRMP have generated significant benefits for populations of exploited fish species within reserve boundaries. It should not be assumed however, that the extent of the positive effects of NTR protection presented here are more broadly representative of the GBRMP as a whole. Although Russ *et al*. (2008) demonstrated rapid increases in *Plectropomus* spp. populations following NTR establishment on inner and mid-shelf GBRMP reefs, temporal replication of that

study was limited to 2 years post-zoning. Continued monitoring of long-term protected NTRs, recently protected NTRs and fished areas will yield further insight into the effects of management zoning and the ecological processes structuring the fish and benthic communities of these reefs.

This thesis and several publications arising from it have contributed to the current state of knowledge on the effects of NTR protection on fish populations. Such effects are now well documented in the international literature and there is little doubt that adequately sized, located and protected NTRs can produce considerable benefits for exploited species within reserve boundaries (Jennings 2000; Roberts & Hawkins 2000; Russ 2002; Halpern 2003; Sobel & Dahlgren 2004; Roberts *et al.* 2005;). However, there remains a critical lack of empirical data sets which demonstrate subsidies from NTRs to surrounding fished areas (Russ 2002; Gell & Roberts 2003; Willis *et al.* 2003b; Roberts *et al.* 2005; Sale *et al*. 2005; Steneck *et al.* 2009). The establishment of NTR networks displaces fishing effort and reduces total fishable area (Hilborn *et al*. 2006). The degree to which NTRs can compensate for these losses by providing recruitment subsidy and spill-over to fished areas requires further investigation. Empirical demonstrations of the scales of larval dispersal and demographic population connectivity for a range of marine species are also urgently required. The integration of such measurements into coupled biophysical models of larval dispersal is a fundamental step in achieving optimal design and functioning of marine reserve networks (Cowen *et al.* 2000; Sale *et al.* 2005; Jones *et al.* 2007; Botsford *et al.* 2009).

The development and testing of the transgenerational larval marking technique presented in chapters 6 and 7 of this thesis has confirmed that the technique is effective and safe for use on large commercially important reef fish species. It was demonstrated that low dosage injections of enriched stable isotope barium chloride solution into gravid female fish produces a reliable and permanent geochemical tag in the otoliths of offspring. Furthermore, administration of barium

chloride solution at dose rates up to 4 mg Ba / kg body weight was shown to produce no detectable physiological effects on treated fish and presents no appreciable risk for humans who may consume treated fish. The results presented in this thesis and those of several other recent studies (Thorrold *et al.* 2006; Almany *et al.* 2007), should instill confidence in the use of this technique for mass marking larvae of a range of reef fish species.

The adult fish tagging and larval tracking experiment outlined in chapter 8 is currently incomplete. However, early results from this project are encouraging. One barium tagged *Plectropomus maculatus* recruit and one tagged *Lutjanus carponotatus* recruit spawned within NTRs of the Keppel Island group have been recaptured in reef areas both within and beyond reserve boundaries. This is one of the first times larval export has ever been measured empirically from a NTR to a fished area anywhere in the world. To have achieved this for such an iconic and commercially significant species as coral trout has substantial significance for the management of the GBRMP and the hook and line fishery. Furthermore, adult fish tagged within NTRs have also been recaptured in areas outside of reserves.

Although preliminary, these results represent a significant advance in our capacity to measure patterns of larval dispersal, demographic population connectivity and export effects of NTRs. Fundamentally, it has now been demonstrated that it is possible to tag and recapture larvae of large pelagic spawning fish species in the field. Additionally, it is clear that there is some level of larval retention within the Keppel Island group and that the spatial scales of larval dispersal and recruitment subsidies from NTRs may be more localised than previously expected. These preliminary findings concur with those of several previous studies which have employed larval marking techniques to track larval reef fish from spawning locations to settlement locations (Jones *et al.* 1999, 2005, 2007; Almany *et al.* 2007; Planes *et al.* 2009). Further work is required on larval

connectivity and it is important not to overstate the significance of the results presented in this thesis. Analysis of samples is currently still underway and it is expected that all data from this larval connectivity project will be collated by mid 2010.

A considerable amount of valuable information has been produced from the research presented in this thesis. Data generated by the inshore reefs monitoring program was utilised by the Great Barrier Reef Marine Park Authority (GBRMPA) during the planning and design phase of the Representative Areas Program (RAP) re-zoning of the marine park in 2004. Furthermore, the results of this work have appeared frequently in the media and have been cited both in Australia and internationally. This thesis has contributed to the understanding of the ecological effects of NTR protection and it is hoped that this work will assist managers in conveying details of the potential effects of NTRs to stakeholders and local communities. Effective management is most often reliant on community support and understanding. This thesis expands the knowledge base required to increase support for wider utilisation of networks of NTRs in marine resource management strategies.

Although this research has made a valuable contribution to our understanding of the effects of NTR protection, it is evident that further work is required on fish and benthic community monitoring. Continued UVC monitoring of the inshore reefs of the GBRMP will provide greater resolution of the longer term effects of NTR protection and a more thorough understanding of the temporal dynamics of reef fish and benthic communities. Given the current projections of increased frequency and severity of disturbances on coral reefs due to climate change (eg. coral bleaching events and cyclones), it is crucial that long-term monitoring of these reefs be maintained. Such data will provide a solid framework in which to investigate reef community responses to major

disturbance events and it will provide managers with some of the information necessary for making policy decisions which may contribute to increasing the long-term resilience of the ecosystem.

It is also critically important that further work be conducted to investigate larval dispersal patterns and demographic connectivity of populations. Due to the significant logistical and financial requirements for large-scale connectivity studies, it will not be possible to conduct such studies everywhere. However, if suitable study locations are identified and adequate funding and resources are allocated, it is evident that such studies can yield exceptional results. In order to extrapolate empirical larval dispersal and demographic connectivity data to broader spatial and temporal scales, it will be necessary to integrate the data into coupled biophysical models. Once validated, the resulting models may potentially be used to optimize the size, location and spacing of NTRs for generating maximum benefit for exploited species, biodiversity protection and ecosystem integrity.

NTRs are clearly not a panacea for addressing all of the pressures impacting coral reef ecosystems. However, given the current degraded condition of many coral reef ecosystems and dire projections of the future impacts of ocean warming, acidification and resource exploitation, a whole suite of management actions are necessary. Among these, appropriately designed and effectively implemented NTR networks can significantly contribute to the long-term sustainability of populations of exploited species, reduce anthropogenic degradation of habitats and protect or restore natural states of biodiversity. On their own, networks of NTRs represent one of the few management options for which positive outcomes can be readily and immediately demonstrated. However, the benefits of NTR networks will be greatest when integrated with additional management actions that aim to reduce the human footprint on coral reefs and provide long-term sustainability of coral reef resources.

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***Appendix 1*: List of surveyed fish species, the status of each species in the GBR recreational fishery and the analysis group/s to which species were assigned.**

T = Target species; ST = Secondary Target species; NT = Non-target species; P = Protected species. ◆ = Species excluded from density and biomass calculations; ✶ = Species excluded from biomass calculations.

Family / Species	Fishery Status	Analysis Groups
Chaetodontidae		
Chaetodon aureofasciatus	NT	Chaetodontid; Non-Target
C. melannotus	NT	Chaetodontid; Non-Target
C. rainfordi	NT	Chaetodontid; Non-Target
Chelmon rostratus	NT	Chaetodontid; Non-Target
Centropomidae		
Psammoperca waigiensis	ST	Predator
Haemulidae		
Diagramma pictum	ST	Predator
Plectorhinchus chrysotaenia	ST	Predator
P. chaetodonoides	ST	Predator
P. flavomaculatus	ST	Predator
Labridae		
Cheilinus fasciatus	NT	Labridae; Non-target
C. undulatus ✶	P	Labridae; Predator
Choerodon anchorago	ST	Labridae; Predator
C. cyanodus	ST	Labridae; Predator
C. fasciatus	NT	Labridae; Non-target
C. graphicus	ST	Labridae; Predator
C. monostigma	ST	Labridae; Predator
C. schoenleinii	ST	Labridae; Predator
Lethrinidae		
Lethrinus atkinsoni	ST	Predator
L. harak	ST	Predator
L. laticaudus	T	Predator
L. lentjan	ST	Predator
L. miniatus	T	Predator
L. nebulosus	T	Predator
L. obsoletus	ST	Predator
L. olivaceus	ST	Predator
L. ornatus	ST	Predator

Appendix 1: Continued

Family / Species	Fishery Status	Analysis Groups
Lutjanidae		
Lutjanus argentimaculatus	T	Lutjanid; Predator
L. carponotatus	ST	Lutjanid; Individual
L. fulviflamma ◆	ST	
L. fulvus	ST	Lutjanid; Predator
L. kasmira	ST	Lutjanid; Predator
L. lemniscatus	ST	Lutjanid; Predator
L. lutjanus ◆	ST	
L. monostigma	ST	Lutjanid; Predator
L. quinquelineatus	ST	Lutjanid; Predator
L. russelli	ST	Lutjanid; Predator
L. sebae	T	Lutjanid; Predator
L. vitta ◆	ST	
Serranidae		
Anyperodon leucogrammicus	ST	Serranid; Predator
Cephalopholis boenak	ST	Serranid; Predator
C. cyanostigma	ST	Serranid; Predator
C. microprion	ST	Serranid; Predator
C. miniata	ST	Serranid; Predator
Cromileptes altivelis	P	Serranid; Predator
Epinephelus caeruleopunctatus	ST	Serranid; Predator
E. coioides	ST	Serranid; Predator
E. fuscoguttatus ✱	ST	Serranid; Predator
E. merra	ST	Serranid; Predator
E. ongus	ST	Serranid; Predator
E. polyphekadion	ST	Serranid; Predator
E. quoyanus	ST	Serranid; Predator
Plectropomus laevis	T	Serranid; *Plectropomus* spp.
P. leopardus	T	Serranid; *Plectropomus* spp.
P. maculatus	T	Serranid; *Plectropomus* spp.
Siganidae		
Siganus doliatus	NT	Siganid; Non-target
S. lineatus	NT	Siganid; Non-target