Hall, April Elizabeth (2015) ***Top-down control, trophic interactions, and the importance of predatory fishes on coral reefs.*** **PhD thesis, James Cook University.**

Top-down Control, Trophic Interactions, and the Importance of Predatory Fishes on Coral Reefs

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September 2015

For the degree of Doctor of Philosophy

College of Marine and Environmental Science

James Cook University

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge the dedication given to this project by my primary supervisor and mentor, Professor Michael Kingsford. Mike has played an integral role in my development as a scientist, and his input and enthusiasm made this project not only possible, but enjoyable. I thank Mike for the insight he provided into various developmental and analytical aspects of the project, and also for the substantial periods of time spent assisting me with fieldwork, including many enjoyable hours spent underwater.

I would like to thank Dr Timothy Clark for his input into the physiological aspects of this project, particularly for his technical assistance in setting up and running respirometry experiments, but also for his analytical and editorial assistance. Thank you to Rhondda Jones for assistance with statistics, to Sue Reilly for assistance with histology, and to Eleanor Gregory for assistance with media releases. I thank the staff at the beautiful research stations I was fortunate enough to visit during this research (Lizard, Orpheus, and One Tree Island) for your logistical support.

This project involved extensive field work, and I am grateful for the assistance I received on field trips from Mark O'Callaghan, Dave Hall, Lisa Boström-Einarsson, Amelia Wenger, Tiffany Sih, Hannah Epstein, Eric Brown, Emily Gerard and David Williamson, as well as many others who provided assistance on field trips. I would particularly like to thank Amelia Wenger and Lisa Boström-Einarsson, whose support, friendship and scientific insight are invaluable to me. Finally, I would like to thank my husband Dave Hall; not only for the enormous amount of personal support and encouragement he has provided, but also for his direct input and assistance into many aspects of this project. Without my incredible and clever group of friends and family, this achievement would never have been possible, and I am indebted to them for helping me to reach this milestone.

CONTRIBUTION OF OTHERS

This work is the result of collaborations with my supervisors Professor Michael Kingsford and Professor Garry Russ, as well as Dr Timothy Clark (external collaborator). In particular, Michael Kingsford played a critical role in the development and implementation of project ideas for the thesis, and assisted with field and laboratory work, data analysis, and editing of Chapters One, Two, Three, Four and Six. Dr Timothy Clark contributed to the design, implementation, and analysis of the experiments presented in Chapter Five, and provided editorial assistance for this chapter.

I received a stipend through the Australian Postgraduate Award administered by James Cook University (JCU), as well as a Smart Futures Scholarship from the Queensland Government. My research was funded primarily by an Australian Research Council Centre of Excellence for Coral Reef Studies Grant to Michael Kingsford. Additional funding was provided by the Royal Zoological Society of NSW, the Wildlife Preservation Society of Qld, the Ecological Society of Australia, and the Graduate Research Scheme (JCU). This research was carried out using internal infrastructure at JCU (e.g. laboratory and computing resources), as well as external infrastructure at the Lizard Island, Orpheus Island and One Tree Island research stations.

All images used in this thesis were obtained under appropriate copyright licences. The image of *Pseudochromis fuscus* and *Halichoeres argus* (used in Chapters Four and Five) were obtained with permission under creative common licences from the Freshwater and Marine Image Bank at the University of Washington. All other images were obtained from the public domain, with the exception of the cover image of *Plectropomus areolatus,* which was photographed by myself.

DECLARATION OF ETHICS

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the JCU Animal Ethics Committee- Approval Number # A1808.

GENERAL ABSTRACT

Predators play critical roles in terrestrial and marine ecosystems, and can be responsible for maintaining balance and stability in ecological communities. Predators may exert top-down control on prey communities through predation on species at lower trophic levels, however the importance of top-down effects in structuring ecological communities has been widely debated by ecologists. Coral reefs contain a high diversity and abundance of piscivorous fishes, which primarily consume smaller fishes. The objective of this thesis was to examine the role of piscivorous fishes in structuring reef fish populations through predator-prey interactions. Focus was given on examining predator-prey interactions at a variety of spatial and biological scales. At large spatial scales, the importance of piscivores in structuring prey communities was examined by comparison of fish assemblages amongst management zones with varying predator densities (Chapter Two). At regional scales, the sub-lethal effects of predators on prey were examined by comparison of the population demographics of a prey species in areas of high and low predator biomass (Chapter Three). These studies were then followed up by a closer examination of predator-prey interactions using manipulative aquarium experiments (Chapters Four and Five).

Removal of apex predators has resulted in a variety of cascading effects in a range of systems, and studying the effects of such extirpations can give insight into the ecological role of predators. On the Great Barrier Reef (GBR), piscivorous fishes are the primary target of both recreational and commercial fisheries, and piscivore densities can be depleted on heavily fished reefs. Marine reserves however, have been effective in protecting and restoring piscivore populations, so there may be great variation in the density and biomass of piscivores between fished and unfished zones. These variations provide a robust experimental template to study to role of predatory fishes on coral reefs. In Chapter Two, the role of piscivores in influencing prey communities was examined by a comparison of fish assemblages in three management zones which represent a gradient of fishing intensity: marine reserves (no fishing), limited fishing zones (moderate fishing intensity) and open zones (highest fishing intensity). Fish counts and habitat surveys were conducted in each management zone at four locations on the

GBR. There was great variation in predator biomass amongst zones, and strong evidence of associated prey release in heavily fished areas. The trophic composition of reef fish assemblages varied amongst zones; reefs open to fishing had much lower densities of piscivores, and higher densities of prey and herbivorous fishes compared to marine reserves.

In Chapter Three, the lethal and sub-lethal effects of predators were examined at a regional scale, by comparing the population demographics of the prey species *Scolopsis bilineatus* amongst management zones at the Palm Islands. The biomass of predators varied greatly between marine reserves and fishes zones at this location, as described in Chapter Two. For *Scolopsis bilineatus,* despite no variation in numerical abundance and mortality rates, there were strong differences in a variety of demographic traits for *S. bilineatus* between multiple areas of high and low predator biomass. These sub-lethal effects were sex dependant, and impacted females more strongly than males. Chapters One and Two, therefore, highlight the importance of predators in influencing prey communities on coral reefs, through lethal and sub-lethal top-down effects.

Coral reef fishes may be vulnerable to both predation and competition during the early life stages, and these processes may interact to influence mortality, growth and behaviour. Chapter Four compared the relative and interacting effects of competition and predation on two competing species of small damselfish; *Pomacentrus amboinensis* and *P. moluccensis*, which both exhibited patterns of prey release in heavily fished areas from Chapter Two. Using a multifactorial experiment, fish were subjected to the sight and smell of a known predator (*Pseudochromis fuscus*), the presence of the heterospecific competitor, or a combination of the two. Both predation and competition impacted the growth and behaviour of prey, and the presence of the predator tended to exacerbate competitive effects. There were strong differences between species according to dominance hierarchies, and subordinate fish suffered greater reductions in growth compared to dominant fish. These data highlight the importance of predator/competitor synergisms in influencing key behaviours and demographic parameters for juvenile coral reef fishes.

Chapter Five examined the physiological responses of predators to prey, and explored the mechanisms underpinning the behavioural response of prey to a predator. I used intermittent flow respirometry to demonstrate a strong metabolic response of *P. amboinensis* to visual predator cues. *P. amboinensis* had elevated metabolism for a 24 hour period when faced with a predator, but metabolism was not elevated when presented with an olfactory cue only, or when presented with non-predatory fish. These data highlight the energetic costs associated with predator-prey interactions, and demonstrate the capacity for prey to discriminate between predatory and non-predatory fish, and respond accordingly. Outcomes from Chapters Four and Five demonstrated that behavioural and physiological mechanisms may underpin the response of prey to a predator, and this can ultimately scale up to the variations in abundance and demography observed in previous chapters.

In conclusion, this thesis provides a detailed examination of the importance of predator-prey interactions on coral reefs, and highlights the important role that predators play in regulating prey at multiple spatial and biological scales. These data are relevant to conservation and management of coral reefs, and reinforce the importance of preserving and restoring top-down trophic interactions in ecological systems

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CHAPTER 1: GENERAL INTRODUCTION

A central goal of ecology is determining how ecosystems function, and how interactions amongst species can influence natural systems. As human populations increase, and anthropogenic impacts on ecosystems become more prevalent, the work of ecologists has shifted to focus more strongly on how such impacts can influence ecosystem function. Globally, ecosystems are subject to a myriad of human induced impacts, including climate change, pollution, habitat destruction, and overharvesting of natural resources (Pauly et al. 1998; Jackson et al. 2001; Duffy 2003; Hughes et al. 2003; Thomas et al. 2004; Hughes 2008; Estes et al. 2011). One of the most pervasive impacts that has been observed is the extirpation of apex predators, which has occurred in almost all biomes on earth. Reductions or removals of apex predators have caused documented ecosystem change in many terrestrial, marine and freshwater systems (Pauly et al. 1998; Jackson et al. 2001; Duffy 2003; Terborgh et al. 2010; Estes et al. 2011). The ubiquity of flow-on effects from such disturbances demonstrates the integral role of predators in stabilising ecosystems, and promoting biodiversity. Understanding and documenting the important role of predators in community dynamics has, therefore, become an important component of conservation based ecology.

1.1 OVERHARVESTING OF PREDATORS AND CASCADING ECOLOGICAL EFFECTS

Predators perform crucial roles in natural systems, primarily through their ability to regulate populations of species at lower trophic levels. Predators may exert top-down control on their prey by the direct act of predation, or through a variety of indirect effects. Indirect effects often involve cascading trophic effects, whereby the consequences of predator-prey interactions are felt at lower trophic levels (Terborgh et al. 2010). Such cascading effects can be critical to the maintenance of stability and equilibrium in many food webs, however, the importance of these interactions is often not detectable until predators are heavily depleted from systems (Pinnegar et al. 2000; Carr et al. 2002; Beschta and Ripple 2009; Estes et al. 2011). In recognition of the importance of top-down effects, many predators are considered “keystone species”, critical to the ongoing stability and health of their ecosystems (Paine 1969). Implicit in this concept is the recognition that removal of top predators can result in changes to the community structure of an ecosystem. Such top-down effects have been observed most commonly in freshwater and intertidal systems, and may be mediated or interact with bottom-up effects such as variation in primary production or nutrient supply (McQueen et al. 1989; Hunter and Price 1992; Strong 1992; Hunter et al. 1997). Variations in habitat quality may also ameliorate or exacerbate the importance of top-down effects, depending on the strength of habitat associations (Wilson et al. 2008a; Wilson et al. 2008b). Understanding the role of top-down and bottom-up effects in natural systems is increasingly important, especially given the propensity for human activities to substantially disturb these processes.

Recognition of the importance of predators has primarily come about due to observations of the changes which occur when predators are depleted or even lost from systems. Such "trophic downgrading" (*sensu* Estes et al. 2011) has led to significant changes in terrestrial, aquatic, and marine systems from the poles to the tropics. In marine systems, fishing is a significant threat to marine predators, and intensive fishing of apex predators has led to dramatic reductions in predatory fish populations (Pauly 1995; Pauly et al. 1998; Browman and Stergiou 2004). Pelagic predators such as tuna and billfish, and coral reef predators such as grouper and snappers are the primary targets of many fisheries, and an estimated 75% of global fish stocks are now considered depleted or fully exploited (Hilborn et al. 2003; GBRMPA 2009; Branch et al. 2010; Essington 2010; FAO 2012). Such practices have often led to the economic extinction of predator-focussed fisheries, which are increasingly forced to target lower trophic level fishes. In addition to these economic consequences, severe ecological consequences can accompany such predator reductions, and the "ecological extinction" (*Sensu* Jackson et al. 2001) of overharvested predators is a serious conservation concern.

1.2 THE ROLE OF PREDATORY FISHES ON CORAL REEFS

Coral reefs contain a particularly high diversity of predatory fishes, which may constitute a substantial proportion of total fish biomass on pristine reefs (Sandin et al. 2008; Friedlander et al. 2010). Despite their prevalence, there is significant dichotomy amongst coral reef ecologists concerning the importance of such predators and top-down effects on coral reefs. Tropical systems such as rainforests and coral reefs have historically been considered unlikely to experience strong top-down effects, due to the enormous diversity and high functional redundancy present (Shurin et al. 2010). In such diverse systems, the loss of a particular predator species may be compensated for by another, which may diminish the importance of top-down effects on prey (Salomon et al. 2010). Despite this, strong top-down effects have been demonstrated in coral reefs at a number of locations including Kenya (McClanahan and Shafir 1990), the Caribbean (Hughes 1994), and the Red Sea (Roberts and Polunin 1992). Cascading trophic effects on coral reefs often occur in areas where sea urchins are the primary grazers, and may come about due to variation in the abundance of fishes which consume sea urchins (McClanahan and Shafir 1990; Hughes 1994; McClanahan 1994). McClanahan and Shafir (1990) documented strong top-down interactions between triggerfishes, urchins and turf algae on reefs in Kenya, demonstrating that fishing of triggerfishes resulted in lower coral cover and an increase in algal cover. Complex top-down relationships between predatory and herbivorous fishes, urchins and macroalgae have also been demonstrated on reefs in the Caribbean, where both predatory and herbivorous fish are targeted by fisheries (Hughes 1994; Mumby et al. 2006). In contrast, comparatively fewer studies have clearly demonstrated strong top-down effects from piscivores on small prey fishes on coral reefs. Depletion of piscivores has resulted in greater densities of prey fishes on reefs in the Red Sea (Roberts and Polunin 1992), and in Kenya (McClanahan et al. 1999), however, no such effects were detectable in similar studies in Fiji (Jennings and Polunin 1997) the Seychelles (Jennings et al. 1995) and the Philippines (Russ and Alcala 1998). Such contrasting outcomes highlight the need to better understand the regulatory role of predatory fishes on coral reefs, especially in light of ongoing fishing-related disturbances to predatory fish populations.

On the Great Barrier Reef (Queensland, Australia; GBR), both commercial and recreational fisheries focus predominantly on targeting predatory (piscivorous) fishes (Mapstone et al. 2004). Recreational fishing is one of the most popular activities on the GBR, and a variety of predators including coral trout (*Plectropomus* spp. and *Variola* spp.), snappers (*Lutjanus* spp.) and emperors (*Lethrinus* spp.) are targeted (GBRMPA

2011, 2014). A similar suite of predatory fishes are targeted by the Coral Reef Fin Fishery, which is the principle commercial fishery targeting coral reef fishes on the GBR (Mapstone et al. 2004). The Great Barrier Reef Marine Park (GBRMP) was created in 1975 to conserve and protect coral reef ecosystems on the GBR (GBRMPA 2014). The GBRMP encompasses the entire GBR (>2000km in length) and consists of a multi-use zoning system, which designates allowable activities within each zone. Approximately one third of the GBRMP is zoned as no-take marine reserves (green zones), where no fishing is allowed at any time. Other areas are managed primarily through gear restrictions, such as the conservation park (yellow) zones, which restrict the number of hooks/lines per person. Conservation park areas are predominantly used by recreational fishers. Open (blue) zones, allow for both commercial and recreational fishing activities to occur, however, catch and bag limits do apply across all zones (GBRMPA 2014). Management zones within the GBRMP represent a gradient of fishing intensity, from open zones (most heavily fished) to marine reserves (unfished). These zones represent an excellent experimental template to study the ecological effects of fishing on coral reefs.

The combination of predator-focused fisheries, and effective marine reserves on the GBR has resulted in great variation in the biomass of targeted predators amongst management zones. Although previous studies (e.g. Williamson et al. 2004; Russ et al. 2008) have shown that marine reserves are effective in restoring the biomass of targeted predators, there is currently little understanding of how such variations may affect the structure of coral reef fish assemblages on the GBR through cascading trophic effects. A single study by Graham et al. (2003) demonstrated increases in the abundance of select small pomacentrid and labrid species in heavily fished areas on inshore reefs around the Palm Islands and Whitsundays. These results provide an indication that fishing of piscivores can affect small prey species, however, further studies are needed to better understand the generality of top-down effects on reefs on the GBR, and how such variations in predator-prey interactions may affect the structure of reef fish communities. The magnitude of trophic effects may also depend on the intensity of fishing, and/ or the effectiveness of protection within a given location. The Great Barrier Reef Outlook Report (GBRMPA 2014), identified extraction of predators as an ongoing threat to the natural and heritage values of the GBR. In order to fully evaluate the impacts of exploitation of predatory fishes, an understanding of how predators respond to fishing pressure at broad spatial scales, and consequently how reef fish communities vary over a gradient of fishing intensity (and therefore predator biomass) is needed.

1.3 SUB-LETHAL EFFECTS OF PREDATORS ON PREY

Predators affect prey species primarily through the process of direct predation, however, there are a number of important sub-lethal top-down effects which can also occur. Predators may influence important demographic traits such as growth rates, size and age structures, condition and fitness, and reproductive output of prey (Connell 1996; Webster 2002; Ruttenberg et al. 2011; Walsh et al. 2012; Davenport and Chalcraft 2013). Given the importance of such demographic traits to population dynamics, sub-lethal effects may eventually lead to variations in prey density, but may be detectable long before a numerical response in evident. Sub-lethal effects may come about due to predator mediated behaviours exhibited by prey, as behaviours may change significantly due to the fear of predation (Preisser et al. 2005; Madin et al. 2010; Madin et al. 2012). Optimal foraging behaviour requires a trade-off between accessing the food required for energy stores, and sheltering from predators to avoid predation. When predators are abundant, small prey species may shift their behaviour to spend more time sheltering, which can result in reduced access to feeding opportunities, and reductions in overall foraging rates. Indeed, numerous studies have demonstrated the capacity of predatory fishes to supress the foraging rates of their prey (e.g. McCormick and Holmes 2006; Bosiger et al. 2012; Lönnstedt et al. 2012; Mitchell et al. 2013; Rizzari et al. 2014). Such behavioural cascades are an important component of top-down control, and can have significant consequences on the population demographics of small prey species. Behavioural cascades can be an important mechanism underpinning top-down effects, which highlights the importance of understanding predator effects at multiple spatial and biological scales (Preisser et al. 2005; Madin et al. 2010; Madin et al. 2012).

1.4 INTERACTING ECOLOGICAL PROCESSES: THE ROLE OF PREDATOR-COMPETITOR SYNERGISMS

Predators can also influence prey species indirectly when predation interacts with other ecological processes. Competition can be a fundamental process shaping ecological communities, as individuals compete for finite resources such as food, mates, or shelter space (Connell 1978). Competitive processes can be important in regulating populations, by limiting the capacity of individuals or species to grow and reproduce (Cappuccino 1995). Competition and predation often directly interact to determine mortality rates, and the combination of predation and competition can often have synergistic effects (Hixon and Carr 1997; Hixon and Jones 2005). The role of predator/competitor synergisms may vary according to the limiting resource that is driving competition. If competition occurs primarily for predator-free shelter space, the presence of a predator may increase the intensity of competitive interactions, since the consequences of losing a competitive interaction are likely fatal. For example, Holbrook and Schmitt (2002) demonstrated that predation was the primary cause of density dependent mortality for two species of tropical damselfish *(Dascyllus flavicaudus and D. trimaculatus).* Competitive interactions may also increase the vulnerability of prey to predators, particularly if competitive interactions reduce the growth rate and condition of subordinate competitors (Jones 1987; Persson 1988; Figueira et al. 2008). Such interactions often occur for teleost fishes, where size- selective predation is commonly observed, and reductions in growth and condition can significantly decrease the chance of survivorship (Sogard 1997; Booth and Hixon 1999; Hoey and McCormick 2004).

On coral reefs, juvenile fishes may be particularly vulnerable to interactions between predation and competition due to their bi-partite life cycle. As an individual transitions from its pelagic larval stage to its demersal reef-associated stage, it is vulnerable to a host of unfamiliar predatory fishes, and must compete with other juveniles for access to a predator-free shelter site in order to survive (Holbrook & Schmitt 1989, Holbrook & Schmitt 2002). This early life stage represents a significant population bottleneck for these fishes, and mortality rates in the first 24 hours can be extremely high (Sale and Ferrell 1988; Holbrook and Schmitt 2003). Populations of fishes in this post-settlement stage may be regulated by interactions between predation and competition, as young fishes compete for critical resources such as food and shelter space (Jones 1987). Competition amongst conspecifics (intraspecific competition) and heterospecifics (interspecific competition) is often intense, and dominance hierarchies are formed very quickly (Jones 1988; McCormick and Weaver 2012). Variations in demographic parameters such as growth rates in this early post-settlement stage can be extremely important in structuring fish assemblages at local scales, since life time survivorship and reproductive output can be influenced by the growth and condition of juveniles (McCormick 1998; Booth and Hixon 1999; Caselle 1999). Variations in survivorship and reproductive output of local populations can be an important determinant of community structure on coral reefs, so understanding the effects of predator-competitor synergisms for juvenile fishes is a critical component of coral reef ecology.

1.5 USE OF NOVEL TECHNIQUES TO UNDERSTAND PREDATOR-PREY INTERACTIONS

Traditionally, ecological studies concerning predator-prey interactions have focussed on documenting changes to the abundance and/or behaviour of prey according to varying predator densities. Documenting the ecological effects of predators on prey in this way is critical in determining how the outcomes of predator-prey interactions can translate into changes in ecological communities. In order to fully understand the mechanisms underpinning the response of prey to predators, however, more novel techniques are needed. The development and use of scientific techniques such as stable isotope and fatty acid biomarkers have facilitated an increased understanding of the connections within food webs, and can provide a clearer picture of links between various trophic levels (Pinnegar and Polunin 2000; Estrada et al. 2005; Hall et al. 2006). These techniques can provide important information about energy and nutrient flows within food webs, but to understand the mechanisms underpinning predator-prey interactions, a more focussed approach is necessary.

For fishes, development of otolith analysis techniques, and use of direct physiological measurements have the potential to provide detailed quantification of the effects of predators on prey. Such detailed data can provide a mechanistic understanding of links between physiology, biology, behaviour and ecology in the context of top-down effects and trophic interactions. Otoliths are calcium carbonate structures present in the ear apparatus of fishes that are responsible for balance and orientation (Green et al. 2009). As fish grow, additional layers of calcium carbonate are added to otoliths, and this process results in the formation of both daily and annual growth rings that are visible in the cross-section of an otolith. Otolith growth is related to somatic growth, so measurement of the distance between daily rings can give an accurate representation of the growth of an individual over time (Pitcher 1988; Thresher 1988; Fowler 1990; Kingsford et al. 2011). Development of this innovative technique has led to significant increases in our understanding of fish demographics, and can be useful for obtaining time integrated information on growth effects due to experimental treatments, or natural disturbances. In addition, otolith annuli can be used to provide accurate age estimates of fish, enabling quantification of mortality rates, age structures, and recruitment dynamics (Green et al. 2009).

The ability to calculate reliable metrics such as age, growth and mortality has provided enormous advantage to ecologists, and allows the population dynamics of study species to be compared amongst different natural or experimental scenarios. Despite this opportunity, comparatively few studies on coral reefs have considered the effects of predators on demographic traits of prey at the population level using otolith analysis techniques. Although numerous experimental and observational studies have demonstrated the effects of piscivores on prey demographics, many of these studies were conducted at small spatial scales, and/or have measured the condition or fitness of prey without associated demographic data (Connell 1997; Connell 1998; Hoey and McCormick 2004; Walsh et al. 2012). At broader scales, predator-induced demographic shifts have been recorded in the Line Islands, whereby demographic patterns for non-targeted fishes varied between fished and unfished atolls (Ruttenberg et al. 2011); however, it is not known if similar patterns may occur as a consequence of fishing on the GBR. To address this knowledge gap, further research is needed which specifically quantifies the effects of predators on prey demographics at the population level, and otolith analysis techniques are an integral tool for this purpose.

To accurately understand and interpret the behavioural reactions of prey to a predator, an understanding of the physiological response of prey to predator stimuli is needed. In natural systems, organisms use a variety of stimuli to perceive and interpret information from their environment (Ydenberg and Dill 1986; Munoz and Blumstein 2012). For prey to survive, predator stimuli must be correctly detected and interpreted, and must be accompanied by an appropriate behavioural response. While under-responding may result in death, over-responding may compromise energy acquisition through reductions in foraging rates (Ydenberg and Dill 1986; Lima and Dill 1990; Cooke et al. 2003). For coral reef fishes, the high diversity of predators present on reefs means prey must quickly learn to assess the relative danger of situations and respond appropriately to optimise energy usage. The ability for an individual to successfully navigate this trade-off may have important implications for lifetime growth and fitness, so understanding the physiological response of prey to a predator can aid in interpreting the potential consequences of predator-prey interactions. This is best achieved through experimental trials whereby the physiological response of prey to a stressor is directly measured.

Previous studies have demonstrated the metabolic response of prey to predator stimuli by measurement of ventilatory frequency, heartbeat and opercular beat rate in response to predator stimuli (Barreto et al. 2003; Gibson and Mathis 2006; Oulton et al. 2013). Such measurements can provide an indication of how the metabolism of prey is altered by predator threat, but may have limited accuracy, and often do not provide a time-integrated measure of metabolic rate (Barreto and Volpato 2004; Clark et al. 2013). Intermittent flow respirometry is an effective technique for measuring metabolic rate in a time integrated manner (Clark et al. 2013). This technique allows measurement of the oxygen consumption rate of an individual through time. Since oxygen consumption is a reliable proxy for metabolic rate, respirometry techniques can be used to detect the metabolic response of individuals to experimental treatments (Clark et al. 2013). Such techniques can give significant insight into mechanisms underpinning the behavioural response of prey to a predator, and can provide important information for trophic ecology studies in the future.

1.6 RESEARCH AIMS AND THESIS STRUCTURE

The overarching objective of this thesis was to examine the importance of predatory fishes on coral reefs, specifically with regards to how predator-prey interactions shape coral reef fish communities. This research focussed on examination of predator-prey relationships at multiple spatial and biological scales, which are presented in this PhD thesis in four data chapters. In combination, these data chapters provide a detailed analysis of the role of predatory fish on coral reefs, including consideration of lethal vs. sub-lethal effects, direct and cascading trophic effects, and the role of behaviour and physiology in predator-prey interactions. The specific aims of this thesis were to:

1. Examine the role of predatory fishes in structuring prey assemblages, by comparison of reef fish assemblages amongst management zones on the GBR (Chapter Two);
2. Investigate the role of sub-lethal top-down effects on prey fishes, by comparison of the population demographics of a prey species in areas of high and low predator biomass (Chapter Three);
3. Compare the relative and interacting effects of predation and competition on the early life history stages of prey (Chapter Four);
4. Explore the metabolic costs of predator-prey interactions by examining the physiological response of prey to the presence of a predator (Chapter Five).

Chapter Two examined the importance of predatory fishes in structuring prey assemblages at large spatial scales (up to 1200 km). In this chapter, I used variation in predator biomass amongst management zones on the GBR as an experimental template to examine the importance of top-down effects on coral reefs. I hypothesised that in areas where fishing intensity was high, depletion of piscivorous fishes would lead to prey release for lower level prey fishes, and changes to the trophic composition of reef fish assemblages. To address this, I used visual surveys of reef fish assemblages and benthic habitats at four locations on the GBR, separated by hundreds to thousands of kilometres. This chapter provides detailed information about trophic relationships on reefs, and constitutes a large proportion of this thesis.

Chapter Three further explored top-down effects by examining the importance of sub-lethal effects at a more regional scale (at the Palm Islands). In this chapter, I examined the impact of predators on the population demographics of the prey species *Scolopsis bilineatus* (Nemipteridae: bridled monocle bream), by comparing areas of high and low predator biomass (as identified in Chapter Two). I hypothesised that in areas with high predator biomass, the mortality, growth, condition and reproductive potential of *S. bilineatus* would be reduced. I used a variety of methods such as otolith ageing techniques, histology and calculation of condition indices to address this hypothesis. Chapter three provides insight into how predators can influence prey at a sub-lethal level.

Chapter Four also examines sub-lethal effects, but focuses on interactions between predation and competition at local scales. I hypothesised that the presence of a predator would exacerbate competitive effects, and have a synergistic effect on the growth of prey. To test this, I used a multifactorial aquarium experiment, and tested the effects of a small coral reef predator (Serranidae: *Pseudochromis fuscus)* on two competing damselfish species; *Pomacentrus amboinensis* and *P. moluccensis*. I used otolith microstructure analysis accompanied with behavioural data to assess the effects of predation and competition on the growth and behaviour of prey. Chapter Four provides insight into the important interacting effects of predation and competition for juvenile reef fishes.

Chapter Five examined predator-prey relationships at the smallest scale (within an organism), and focussed on the metabolic cost of predator-prey interactions. I hypothesised that prey exposed to visual and/or olfactory predator stimuli would exhibit a physiological response, resulting in elevated metabolism. To test this, I used intermittent flow respirometry to detect changes in oxygen consumption for *P. amboinensis* when subjected to the sight and/or smell of *P. fuscus*. This is an innovative approach, which can provide an understanding of the physiological mechanisms underpinning predator-prey interactions. Data from this chapter demonstrate the energetic costs associated with predator-prey interactions, and provide unique insight into the metabolic response of prey to predators.

CHAPTER TWO: PREDATORS DRIVE COMMUNITY STRUCTURE IN CORAL REEF FISH ASSEMBLAGES

† **Boaden AE,** Kingsford MJ (2015) Predators drive community structure in coral reef fish assemblages. Ecosphere 6:art46

2.1 ABSTRACT

The importance of top-down effects in structuring ecological communities has been widely debated by ecologists. One way in which to examine these processes is to study the secondary effects of predator removal on communities. This study examined the role of predatory fishes in structuring communities of coral reef fishes, by using a network of marine reserves (the Great Barrier Reef Marine Park) as a natural experiment. I hypothesised that reefs with high densities of piscivores (marine reserves) would have distinct fish communities from those where piscivores have been depleted through fishing, due to variation in predation pressure. I predicted that predator depletion would result in "prey release", and a corresponding increase in prey densities along a gradient of fishing intensity, causing a change in the community composition of reef fishes. To address this, fish counts and habitat surveys were conducted at four locations on the Great Barrier Reef. At each location, comparisons were made amongst three marine park zones that varied in their exposure to fishing practices; no- take marine reserves, limited fishing areas, and open fishing areas.

The density and biomass of predators varied consistently amongst zones at each location. Furthermore, there was strong evidence for prey release at all four locations, resulting in distinct fish assemblages amongst zones. Reefs open to fishing had much lower densities of piscivores, and higher densities of prey and herbivorous fishes compared to marine reserves. This broad pattern was consistent amongst locations, and persisted at the level of species, trophic groups, families and communities. Habitat characteristics did not vary significantly amongst zones in a consistent manner amongst locations. Although habitat relationships were strong for specialist species such as butterflyfishes, densities of predators were stronger predictors of prey density for most species, and the trophic composition of reef fish communities differed significantly amongst zones at all locations. Results from this study support the concept that top-down effects can be strong drivers of prey populations and influence community structure in highly diverse systems. These data emphasise the vital role of predators, and reinforce the importance of preserving and restoring top-down trophic interactions in ecological systems.

2.2 INTRODUCTION

Predators perform crucial roles in both marine and terrestrial ecosystems, and the pervasive loss of apex predators is of global conservation concern. Major reductions in predator populations have occurred in almost every ecosystem on the planet, as a result of hunting, fishing, habitat destruction, and a myriad of other anthropogenic effects (Pauly et al. 1998; Jackson et al. 2001; Duffy 2003; Terborgh et al. 2010; Estes et al. 2011). The role of predators in structuring ecological communities has been a recurring concept in ecological theory, and the loss of predators may provide an opportunity to examine how predators shape natural systems (Terborgh et al. 2010). Reductions or removals of apex predators have caused documented changes to communities in terrestrial, freshwater, and marine systems (Shears and Babcock 2002; Shears and Babcock 2003; Beschta and Ripple 2009; Babcock et al. 2010; Estes et al. 2011). Although the nature and complexity of these changes may vary from one system to another, the ubiquity of flow-on effects demonstrates the importance of key predators in stabilising natural systems.

2.2.1 TOP-DOWN EFFECTS AND TROPHIC CASCADES

Predators may exert top-down control on ecosystems by interacting with species at lower trophic levels, primarily through predation. However, the importance of this process in structuring communities has long been debated by ecologists. Nelson Hairston and others proposed the “Green World Hypothesis” in 1960, suggesting that global plant biomass is controlled indirectly by predators, which limit herbivore populations (Hairston et al. 1960). Robert Paine coined the term “trophic cascade” to describe this process, through his experimental work on “keystone” predators of marine intertidal systems (Paine 1980, 1988). Trophic cascades typically involve interactions between predators, primary consumers and primary producers, and by definition must include three or more trophic levels which are connected by predation (Paine 1980, 1988; Pinnegar et al. 2000). Implicit in this concept is the notion that removal of top predators could result in changes to the community structure of an ecosystem. Such “top-down effects” from predation may be mediated by or interact with “bottom-up effects” such as variation in primary production and nutrient supply. For many systems it is likely that both top-down and bottom-up effects interact to structure communities (Hunter et al. 1997; Shurin et al. 2010). Further, variations in habitat quality may interact to either ameliorate or exacerbate top-down effects, depending on the nature of habitat associations (Wilson et al. 2008b). Since overexploitation of species and habitat degradation are the leading causes of species extinctions, it is critical to determine the relative importance of top-down effects across a wide range of habitats (Dulvy et al. 2003; Wilson et al. 2008b).

Although debate on the importance of top-down effects continues, there is little doubt that the “trophic downgrading” (*sensu:* Estes et al. 2011) of ecosystems has led to significant changes in terrestrial, aquatic and marine ecosystems worldwide. Instances of trophic cascades can be found in almost all biomes, from the poles to the tropics, and can lead to landscape scale changes, demonstrating the importance of top-down effects (Shurin et al. 2010; Estes et al. 2011). For example, in the North Pacific, the collapse of sea otter populations resulted in dramatic increases in the abundance of sea urchins, and subsequent overgrazing and degradation of kelp forests (Estes et al. 1978; Estes and Duggins 1995; Estes et al. 2011). In the USA, extirpation of large predators from national parks has resulted in major impacts to plant communities through overgrazing by ungulates (Beschta and Ripple 2009). For example, loss of wolves from Yellowstone national park in the early 1900s resulted in recruitment failure for Aspen due to overgrazing by Elk (NRC 2002; Beschta and Ripple 2009). These examples demonstrate the importance of top-down effects across a wide range of ecosystems.

In marine systems, intensive fishing of apex predators has resulted in systematic reductions of predatory fish populations (Pauly et al. 1998). Globally, over 75% of fish stocks are depleted or fully exploited, and it is common practice to principally target apex predators such as tuna, billfish, and on coral reefs, groupers, jacks and snappers

(GBRMPA 2009; Essington 2010; FAO 2012). This can lead to the "ecological extinction" of overharvested predators (*Sensu:* Jackson et al. 2001*)*, with severe ecological and economic consequences (Pauly 1995; Pauly et al. 1998; Jackson et al. 2001; Estes et al. 2011). Marine reserves have been developed as a critical tool to address this issue, and some well managed reserves have been successful in restoring high numbers of piscivorous fishes through prohibition and regulation of fishing (Russ and Alcala 2004; Mumby et al. 2006; Russ et al. 2008; Babcock et al. 2010). Apart from the clear conservation benefit that this creates, this restoration of higher trophic levels means that reserves can be used as an effective comparison to adjacent fished areas, making marine reserve networks a powerful scientific tool to investigate trophic interactions (Graham et al. 2003; Micheli et al. 2005). The Great Barrier Reef Marine Park (GBRMP) has the potential be a particularly useful tool for use in such investigations.

The GBRMP is the largest network of marine reserves in the world, and is considered a well-managed and effective marine reserve network (Russ et al. 2008). On the Great Barrier Reef (GBR), densities of targeted piscivores such as coral trout and snapper are two to three fold higher in marine reserves compared to adjacent fished areas (Mapstone et al. 2004; Williamson et al. 2004). The GBRMP consists of multiple zones which vary in the fishing activities allowed within them, resulting in a gradient of fishing intensity (GBRMPA 2009). Approximately one third of the total area of the GBRMP is designated as marine reserve, and this area is representative of all major habitat types that occur on the GBR (GBRMPA 2009). The result is a well replicated suite of sites with different levels of fishing mortality, which can facilitate investigation of the ecological role of predators on coral reefs. As such, the design and effectiveness of the GBRMP makes it an ideal template on which to study the importance of top-down effects in coral reef systems.

2.2.2 TOP-DOWN EFFECTS IN COMPLEX SYSTEMS: THE CASE FOR REEFS

The strength of top-down effects may vary amongst reef systems depending on species diversity and the complexity of trophic interactions. On temperate reefs in Australia and New Zealand, long term studies of no-take marine reserves have demonstrated strong top-down effects of predators (Babcock et al. 1999; Babcock et al. 2010). Inside marine reserves, re-establishment of predatory lobster and fish populations resulted in decreases in herbivores such as urchins and abalone, and a corresponding increase in macroalgal cover (Edgar and Barrett 1999; Shears and Babcock 2003; Barrett et al. 2009). In these cases, there was a strong and direct link between predators, herbivores and the benthos. Conversely, tropical systems such as coral reefs and rainforests have historically been considered unlikely to be strongly influenced by top-down effects due to their complexity and very high diversity (Strong 1992; Polis and Strong 1996). Such species-rich systems often have a high rate of omnivory, as well as ontogenetic and environmentally induced diet shifts, which can prevent the establishment of discrete trophic levels and obscure top-down effects (Polis and Strong 1996; Shurin et al. 2010). Coral reefs have a naturally high abundance and diversity of piscivorous fishes, which may constitute a significant proportion of fish biomass on protected reefs (Sandin et al. 2010). As such, there may be a high level of functional redundancy in the predator guild, and it has been postulated that removal of a predatory species may simply result in replacement by another (Shurin et al. 2010). Piscivorous fishes on coral reefs are also known to be highly opportunistic, and often include a wide variety of prey species in their diet (Kingsford 1992; Kulbicki et al. 2005). Furthermore, the complex and shelter-rich substrate of coral reef habitats may mediate predator-prey interactions, and many coral reef fishes are strongly influenced by variation in benthic habitat (Bouchon-Navaro and Bouchon 1989; Garpe and Öhman 2003; Jones et al. 2004). All of these characteristics may act to dampen top-down effects, however, despite this, they have been documented in coral reef ecosystems in Kenya (McClanahan and Shafir 1990), the Caribbean (Hughes 1994), the Great Barrier Reef (Graham et al. 2003) and the Red Sea (Roberts and Polunin 1992).

Studies that have demonstrated top-down or cascading trophic effects on coral reefs thus far generally hold the common property of having urchins as the primary grazer in the system, and involve fisheries where predators of urchins such as triggerfishes, are heavily targeted (McClanahan and Shafir 1990; Hughes 1994; McClanahan 1994). In Kenya, fishing of triggerfishes has led to an increase in urchin density in fished areas, resulting in bio-erosion of coral, and an increase in filamentous algae, which is more resistant to urchin grazing (McClanahan and Shafir 1990). As such, fished areas have a lower coral cover and topographic complexity compared to marine reserves (McClanahan 1994). Conversely in the Caribbean, urchins are important in controlling macroalgae and mediating competition between coral and algae (Sammarco 1980; Hughes 1989). Fishing of both predatory and grazing fishes here has resulted in urchins being the primary regulators of algae (Hughes 1989; Hughes 1994). When urchin populations were decimated by disease, overgrowth of macroalgae followed, resulting in large declines in live coral cover (Lessios et al. 1984; Hughes et al. 2007b). The consistency of top-down and cascading trophic effects in systems involving fish-urchin interactions is high in both tropical and temperate systems, however, studies concerning top-down effects of piscivores on prey fishes have had conflicting results (Pinnegar et al. 2000). Depletion of piscivores through fishing was found to have no detectable effect on prey fishes at spatial scales of tens of kilometres in the Seychelles (Jennings et al. 1995), Fiji, (Jennings and Polunin 1997) and the Philippines, (Russ and Alcala 1998). Conversely, greater densities of prey fishes in predator depleted areas at similar spatial scales has been found for grazing surgeonfishes in the Red Sea (Roberts and Polunin 1992), small damselfish and labrid species on the Great Barrier Reef (Graham et al. 2003), and diodontids, small labrids and pomacentrids in Kenya (McClanahan 1994). None of these studies, however, have demonstrated prey release along a gradient of fishing intensity, or assemblage level changes due to predator depletion. Furthermore, with the exclusion of Graham et al. (2003), all of these studies involve fisheries where fishes from multiple trophic levels are targeted, which can increase the difficulty of detecting top-down effects.

Although top-down effects have been difficult to demonstrate on coral reefs at large spatial scales (i.e. kilometres to hundreds of kilometres), numerous small scale studies have demonstrated the importance of predator-prey interactions in structuring fish communities. Top-down effects of piscivores on prey have been demonstrated at scales of metres to tens of metres on natural patch reefs in Moorea (Holbrook and Schmitt 2003), and on constructed reefs in the Virgin Islands (Hixon and Beets 1993), whereby prey abundance and/or species richness was highly correlated with local piscivore abundance. Experimental manipulation of small patch reefs (a few metres in diameter) through predator removal or exclusion, has similarly resulted in changes in the abundance, species richness, and mortality of prey species on reefs with natural recruitment (Doherty and Sale 1985; Caley 1993; Connell 1998), and with stocked prey (Carr and Hixon 1995). These studies have been effective in determining the importance of predators in structuring prey communities at small spatial scales, particularly in the early post-settlement stage. However, it is difficult to “scale up” the implications of such studies to a spatial scale that is ecologically relevant to both understanding population drivers of coral reef communities, and management of marine systems. At broad scales, ecological processes on coral reefs occur against a backdrop of abiotic influences such as storms, cyclones, pollution, sedimentation, and climate change impacts, which can cause significant changes to coral reefs (Rogers et al. 1983; Hughes 1994; Hughes et al. 2003; Wenger et al. 2012; Woolsey et al. 2012). Coral reefs continue to be at risk from an ever increasing number of such threats, which could affect coral reef organisms at multiple trophic levels. Understanding the relative importance of top-down effects on coral reefs is vital when considered in light of such threats, and will increase the ability of ecologists to predict the outcomes of future perturbations to coral reef communities.

The GBRMP provides a unique opportunity to study predator-prey interactions and the importance of top-down effects on coral reefs. This marine reserve network can act as a natural experiment, allowing investigation of these ecological interactions at a broad spatial scale that is both ecologically relevant, and applicable to management. The GBRMP is the ideal template on which to test the importance of top-down effects on coral reefs for a number of reasons. Firstly, both recreational and commercial fisheries focus primarily on piscivorous reef fishes; herbivorous and small bodied fishes are not major targets (Taylor et al. 2010; GBRMPA 2011). This focus on removal of higher trophic levels allows investigation of top-down effects without the dampening effect of removal of lower trophic levels. Secondly, most coral reef fishes are removed by line fishing, a method that is unlikely to have large effects on habitat structure compared to other fisheries methods such as trawling, netting, or explosive fishing, so habitat structure should not vary as a direct result of fishing (Jennings and Kaiser 1998; GBRMPA 2011). As such, changes in prey assemblages across a gradient of fishing intensity can be attributed to predator removal, rather than habitat destruction. Thirdly, the zoning plan of the GBRMP was designed such that each zone contains a representative area of habitat types occurring on the GBR (GBRMPA 2009). Therefore, use of a selection of reefs/sites from within the GBR can be considered an accurate representation of processes at a broader scale. This combination of factors allows the GBRMP to be used as an excellent template for investigating the importance of top-down effects on coral reefs.

The objective of this study was to investigate the importance of top-down effects on coral reefs, using the GBRMP as a natural experiment in predator removal. The specific aims of this study were as follows:

1. Estimate the density and biomass of piscivorous fishes and their prey, across a gradient of fishing intensity.
2. Estimate the importance of top-down (predation) effects on prey fishes from a variety of trophic groups.
3. Investigate the relative importance of habitat on prey fishes, and how this may interact with top-down effects.
4. Investigate the impacts of predator removal on the trophic structure and species assemblage structure of fishes on the Great Barrier Reef.

Given the focus that recreational fisheries have on piscivorous fishes on the GBR, coupled with previously demonstrated differences in the biomass of key piscivores (Evans and Russ 2004; Russ et al. 2008), I hypothesised that patterns of predation would be significantly different amongst management zones on the GBR. Furthermore, I hypothesised that these differences in predation pressure would result in changes to prey assemblages, and that the nature of such changes would differ according to both the vulnerability of prey trophic groups to predation, and the strength of habitat associations. Small bodied prey such as pomacentrids and juvenile herbivores (e.g. scarids) feature prominently in the diet of key piscivores such as coral trout (Kingsford 1992), and as such I hypothesised that these groups would be strongly influenced by top-down effects, while habitat specialists such as chaetodontids would be more influenced by variation in benthic habitats, such as the cover of live coral (Bouchon-Navaro and Bouchon 1989; Fowler 1990). Finally, I hyopthesised that these variations in the response of prey groups to predator removal would result in the formation of distinct fish assemblages amongst management zones at the four locations surveyed. From this, I made the following four predictions:

Prediction 1) *Depletion from fishing would result in a reduction in predator densities in heavily fished zones, and related variation in overall predator density and biomass amongst management zones.*

Prediction 2) *Predator depletion would result in a corresponding increase in the density of small prey fishes such as pomacentrids and herbivorous fishes, resulting in an increase in prey densities in depleted zones.*

Prediction 3) *Species that associate strongly with habitat characteristics such as live coral cover (e.g. chaetodontids) would be strongly influenced by variation in benthic habitat, and these habitat effects may diminish the importance of top-down effects.*

Prediction 4) *The composition of fish communities would vary amongst management zones due to predator depletion and associated changes in prey assemblages.*

2.3 METHODS

2.3.1 STUDY AREA

The Great Barrier Reef Marine Park (GBRMP), Queensland Australia, consists of a network of marine protected areas, and is managed through a zoning plan which designates allowable activities within each zone. Zoning of the GBRMP allowed me to study reefs with varying predator numbers and therefore with related variation in predation pressure. This study focussed on three zones that are subject to a gradient of fishing pressure: no-take marine reserves, limited fishing zones and open fishing zones. No- take marine reserves are closed to all forms of fishing at all times, and will be referred to hereafter as “marine reserves”. Limited fishing zones allow limited recreational fishing (with gear restrictions) and open areas allow for both recreational and commercial fishing including line fishing, trawling and trolling (GBRMPA 2009).

Coral reefs on the GBR are subject to both commercial and recreational fisheries. The main commercial fishery operating on coral reefs with the GBRMP is the coral reef fin fishery; predominantly a hook and line fishery which operates mostly at offshore locations. There is also a substantial amount of recreational fishing activity, particularly near populated areas (Taylor et al. 2010). Both commercial and recreational fisheries largely target piscivorous reef fishes, namely coral trout (a group including seven serranid species from the genera *Plectropomus* and *Variola*), red-throat emperor (*Lethrinus miniatus*), stripey snapper (*Lutjanus carponotatus*), and a variety of other snappers and emperors (GBRMPA 2009, Taylor et al. 2010). The GBRMP was formed in 1975 to provide a network of marine reserves, and was rezoned in 2004 to increase protection to its current level, with 30% of its area now designated as no-take marine reserves (GBRMPA 2009). The majority of the marine reserves surveyed in this study had been protected for at least 25 years. With the exception of One Tree Island, and two of the marine reserve sites in the Whitsundays, marine reserves surveyed in this study were established in 1986-1988. One Tree Island was protected as a scientific research zone (no-take) in 1978, and the two Whitsundays sites were established as part of the rezoning in 2004.

Studies have shown that no-take marine reserves have been effective in re-establishing populations of targeted fishes such as piscivores (Evans and Russ 2004; Russ et al. 2008). In order to assess the importance of piscivores in structuring prey assemblages, surveys were conducted in zones within the GBRMP that were expected to vary in the density and biomass of piscivorous fishes. As fishing practices on the GBR are heavily targeted towards piscivorous fishes, I predicted that piscivore densities would vary with fishing intensity. Fish and habitat surveys were conducted within three management zones which varied in fishing intensity: marine reserves (predicted to have high densities of piscivores), limited fishing zones (predicted to have moderate piscivore densities) and open fishing zones (predicted to have low piscivore densities). To allow assessment of the spatial generality of patterns, surveys were conducted at four locations, separated by hundreds of kilometres; the Capricorn Bunkers Group in the southern GBR, the Whitsundays and Palm Islands in the central GBR and the Ribbon Reefs in the northern GBR (Appendix A). Hierarchical sampling designs were used at each location to examine processes at multiple spatial scales and to provide tests for each of these levels; residual variance measured variation amongst replicate belt transects. Tests are provided for sites within reefs (separated by hundreds of metres to kilometres), reefs within management zones (separated by kilometres), and management zones within each location (separated by tens of kilometres; Table 2.1); the entire design was repeated at four locations. Sampling designs varied amongst locations according to the local zoning plan, and all three management zones were surveyed at each location with the exception of the Ribbon Reefs, which does not have limited fishing zones (Table 2.1). At each site, fish were counted and habitat surveyed along belt transects, as described below. Five transects (25 x 5m) were placed haphazardly along the reef crest at each site, in a depth range of 3-10 metres. All fish counts and habitat surveys were conducted by a single observer for the duration of the study. Methods were tailored to test the predictions of the study as follows.

Table 2.1 Sampling design of surveys showing the zones surveyed, and nested design of reefs within zones, and zones within reefs, as well as total sites for each location.

	Zones surveyed			**Samples sizes**		
Location	**MR**	**LF**	**OP**	**Reefs per zone**	**Sites per reef**	**Total sites**
Capricorn bunkers	x	x	x	2	3	18
Whitsundays	x	x	x	1	5	15
Palm Islands	x	x	x	1	5	15
Ribbon Reefs	x		x	2	2	8
					Totals for all locations	56

MR= marine reserve, LF= limited fishing, OP= ope

2.3.2 PREDICTION ONE AND TWO

Depletion from fishing would result in reduced predator densities, and a corresponding increase in prey densities in heavily fished areas.

2.3.2.1 FISH COUNTS

I hyopthesised that fishing would cause a reduction in piscivorous fish densities, and result in a corresponding increase in prey densities along a gradient of fishing intensity. To test this, fish assemblages were quantified by surveying fishes using underwater visual census along belt transects. Surveys were focussed on estimating the density and size of piscivorous fishes as well as that of common prey groups, and herbivorous fishes. A total of 150 species were surveyed throughout the study, including large piscivores targeted by fisheries (Serranidae, Lutjanidae, and Lethrinidae), smaller meso-predators such as *Cephalopholis, Epinephelus*, *Pseudochromis* spp. (Serranidae), and small piscivorous labrids, small prey species such as pomacentrids and chaetodontids, and larger herbivorous fishes such as scarids, acanthurids, and siganids; taxonomy was according to Randall et al. (1997). Counts were restricted to reef associated species, and did not include transient pelagic species (e.g. carangids). Transect sizes varied according to the relative densities and mobility of each species. All piscivorous fishes and mobile herbivores were counted along 25 x 5m transects, chaetodontids and pomacentrids were counted along 10 x 5m transects, and highly abundant and site attached pomacentrid species were counted along 2 x 10m transects; the latter two sized transects were within the area of the 25 x 5m transect. The size of each fish (total length) was estimated, and small fishes were placed into the following size categories: recruits (<15mm), small (15-29mm), medium (30-59mm), large (60-100mm) and extra-large (>100mm). These methods allowed me to estimate both the density and biomass of each species.

2.3.2.2 TROPHIC GROUPINGS

After all data were collected, fish were categorized into trophic groupings to allow comparisons of the trophic structure of fish assemblages amongst zones. These groups were as follows (number of species in parentheses): targeted piscivores (16), non-target piscivores (25), omnivorous (21), planktivorous (11), and herbivorous pomacentrids (13), corallivorous (15), and benthic feeding chaetodontids (10), and scraping (18) cropping (6), grazing (5) and farming (10) herbivores. Categorization of species into trophic groups was decided based on published accounts and personal observations of diet and feeding behaviour (Ceccarelli et al. 2005; Pratchett and Berumen 2008; Green and Bellwood 2009; Froese and Pauly 2013). Targeted piscivores were defined as those species commonly targeted by either recreational or commercial fisheries, and fishes were categorized as piscivorous if fishes constituted the majority of their diet (GBRMPA 2009, 2011). Biomass estimates of each species were calculated from length estimates of fishes by using length-weight relationships provided in the online resource Fishbase (Froese and Pauly 2013).

2.3.2.3 DATA ANALYSIS

I predicted that the densities and biomass of fishes would vary amongst management zones and that this would be consistent by location and replicate zones within locations. Fully nested analysis of variance (ANOVA) was used to compare the density and biomass of fishes, and habitat characteristics amongst zones, reefs and sites at each location. This analysis also allowed me to use variance components to estimate the percentage of the total variation that could be attributed to different levels of the design; raw data were used to calculate variance components (Underwood and Petraitis 1993). Because the sampling design varied slightly due to the number of management zones that occur within each location, locations were analysed separately (see Tables 2.1 and 2.2). Assumptions of homogeneity of variance for ANOVA were tested using Cochran's test (Underwood 1997). Normality of the data was assessed by a visual examination of the distribution of the residuals; data were transformed when necessary. Post-hoc analyses were performed to determine how zones differed from each other using the Student-Newman-Kuels (SNK) test. Results from post-hoc analyses were used to group zones.

2.3.3 PREDICTION THREE

Species that associate strongly with habitats such as live coral would be more strongly influenced by variation in benthic habitat than by top-down effects.

2.3.3.1 BENTHIC HABITAT SURVEYS

To characterise the benthos and reef structure at each site, the percentage cover of benthic habitat types, and the rugosity and vertical relief of the substratum were estimated. These have been demonstrated to be important habitat characteristics and determinants of the distributions and diversity of many reef fishes (Luckhurst and Luckhurst 1978; Holbrook et al. 2002). All habitat data were collected along five 25 x 5m transects at each site. Substratum cover was recorded using the linear point intercept method, which has been demonstrated to be a precise and time-efficient method for obtaining estimates of coral cover (Nadon and Stirling 2006). Cover was estimated by recording the dominant substratum at 20 to 35 points along each transect. Coral cover was recorded according to morphology, as described by Veron (2000). This included live and dead forms of the following: branching, massive, foliose, laminar, encrusting, and free living hard corals, and branching, foliose and encrusting soft corals. In order to most accurately record the available habitat types for fishes, where dead corals were covered with encrusting organisms (e.g. algae, sponges), the encrusting organisms were recorded, and dead coral was only recorded as such if it was bare. The cover of red, green and brown algae was also recorded, and separated into filamentous, branching, coralline, and macroalgae, and the cover of sand and rubble was also recorded.

As well as benthic cover, the structural complexity of reefs can be an important determinant of fish densities, as complex structures may increase the availability of shelter sites (Hixon and Beets 1993; Beukers and Jones 1998). Two measures were used to estimate the structural complexity along each transect; rugosity and vertical relief. Rugosity measures give an estimate of the overall complexity of the substratum; low rugosity values indicate that the substratum is flat and homogenous with a low number of shelter sites, while high rugosity values indicate a complex substratum with a variety of holes and crevices, which can be used by fish for shelter sites (Nash et al. 2013). The observer was trained to accurately estimate rugosity along transects by first measuring rugosity using the traditional chain-and-tape methods (Risk 1972), and then using these values as a guide to visually estimate rugosity on subsequent reefs. These pilot studies encompassed reefs which varied in their rugosity and vertical relief, and rugosity was found to be estimated with a high level of accuracy using this method. Mean vertical relief of each transect was calculated by estimating the reef height at five metre intervals along each 25 x 5m transect. Rugosity and vertical relief estimates were converted into categories, 1-4 for rugosity and 1-5 for vertical relief. For each site, an overall structural complexity index was calculated by multiplying the rugosity and vertical relief categories, and the index ranged from 1 (lowest) to 20 (highest). Both the structural complexity index, and the values for rugosity and vertical relief were used for analyses in this study. The structural complexity was used in ANOVAs to compare broad patterns of complexity amongst zones, reefs and sites. The raw rugosity and vertical relief values were used as descriptors for the multiple regressions and BIO-ENVIRON analysis (Clarke and Ainsworth 1993) in order to get a more detailed understanding of how habitat variables influenced fish densities.

2.3.3.2 DATA ANALYSIS

I predicted that the relative importance of predators vs. habitat would vary amongst prey species, depending on the strength of their habitat associations. To test this, linear regression models were used to test the explanatory value of predator densities and habitat characteristics for a number of prey species and groups. Stepwise linear regressions were used to analyse relationships between predators, prey and habitat, and determine the best combination of variables to predict prey densities. Stepwise regression was used rather than multiple regressions to allow the best combination of habitat and species density variables to be determined for the regression model, allowing me to identify the most important characteristics for species/groups. The following predictor variables were used in the analysis: percentage cover of live hard coral, live soft coral, dead coral and algae, as well as vertical relief and rugosity, and densities of coral trout (*Plectropomus* spp.), *L. carponotatus*, and total piscivores. Square root and log transformations were applied to variables to meet the assumptions of normality for General Linear Models.

2.3.4 PREDICTION FOUR

The composition of fish communities would vary amongst management zones due to predator depletion and associated changes in prey assemblages.

I predicted that variation in the densities of predatory fishes would be a good predictor of reef fish assemblages. The trophic structure of fish assemblages amongst zones and reefs were compared using non-metric multi-dimensional scaling (nMDS) and Permutational Analysis of variance (PERMANOVA) (Anderson 2001). All analyses were performed separately for species-level data and trophic structure. To compare the trophic structure, species were pooled into trophic groups (as described above) and the analysis was run on the pooled data. Data were square root transformed to decrease the influence of highly abundant species and matrices of similarity were calculated using the Bray-Curtis similarity coefficient. Nonmetric multidimensional scaling (nMDS) was used to produce ordinations of the rank orders of similarities amongst zones; stress levels in the nMDS were used to evaluate the robustness of the test, and were considered robust if stress was <0.2. Differences amongst zones within each location were formally tested using Permutational ANOVA (PERMANOVA), which is considered a robust method of multivariate analysis of variance (Anderson 2001). Percentage of similarity analysis (SIMPER) was used to compare the dissimilarity of communities amongst zones, and the contribution of species and groups to this dissimilarity. BIO-ENV analysis (Clarke and Ainsworth 1993) was used to determine the extent to which multivariate patterns of fish densities were driven by habitat characteristics, and to determine the best environmental descriptors for the biological data.

2.4 RESULTS

2.4.1 PREDICTION ONE

Depletion from fishing would result in variation in overall predator density and biomass amongst management zones.

2.4.1.1 PISCIVORES

Piscivore densities differed significantly amongst management zones at three of the four locations across the GBR (Fig. 2.1 and Table 2.2). The density of all piscivores combined was a minimum of two times greater in no-take marine reserves compared to other zones in both the Capricorn Bunkers, and Whitsundays, while at the Palm Islands, piscivore density was greatest in the open fishing zones, and at the Ribbon Reefs no significant differences in piscivore density occurred amongst zones. At the Palm Islands, the density of non-targeted piscivores was inversely related to targeted piscivores. Greater overall densities of piscivores in the open fishing zones at the Palm Islands occurred due to increases in species not targeted by fisheries; targeted species had significantly greater densities in marine reserves (Fig. 2.2 and Table 2.2). Such patterns were not found at the other three locations. The biomass of piscivores was significantly greater in marine reserves than fished zones at all four locations (Fig. 2.3 and Table 2.2). In the Capricorn Bunkers, piscivore biomass decreased significantly along the gradient of fishing pressure (open < limited fishing < marine reserves). Overall, densities of piscivores varied consistently by zone, with little variation attributed to reefs within zones (0-2%). Zones explained 14-31% of the variation; variation was moderate at the site level (12-16%), and high at the residual level (56-70%), as would be expected for aggregating fish, but this did not prevent detection of significant zone effects in the ANOVA.

2.4.1.2 KEY FISHERIES TARGETS

The densities of coral trout (*Plectropomus* spp.) were up to five times greater in marine reserves compared to open zones. At all four locations, the density and biomass of coral trout was significantly greater in marine reserves than limited fishing and open zones (Fig. 2.1 and 2.3 and Table 2.2). At the Capricorn Bunkers the density and biomass of coral trout decreased along a gradient of fishing intensity (open < limited fishing < marine reserves). Densities of coral trout overall were much greater at this location (Fig. 2.1 and Table 2.2). Zoning explained a greater proportion of variance (24-44%) than either reefs (0-3%) or sites within reefs (13-28%); variation amongst replicates was again great (residual; 48-70%). The density and biomass of stripey snapper (*Lutjanus carponotatus*) was also greater in marine reserves in the three locations where this species occurs. Although zoning effects were significant for this species, residual variation was very high (up to 85%) due to their aggregative behaviour, however, little variation occurred amongst reefs and sites within zones (0-8%).

Table 2.2 Results from ANOVAs on fish groups and habitat characteristics by location. Analyses for fish species/groups are on density data unless otherwise specified. Analyses on habitat refer to percentage cover, apart from complexity, which is the structural complexity index. Coral trout = summed density of all *Plectropomus* spp.

Location	**Capricorn Bunkers**			**Whitsundays**		**Palm Islands**		**Ribbon Reefs**		
Dependent variable	**Zone** (2,72)	**Reef** (3,72)	**Site** (12,72)	**Zone** (2,12)	**Site** (12,60)	**Zone** (2,12)	**Site** (12,60)	**Zone** (1,32)	**Reef** (2,32)	**Site** (4,32)
Piscivores	***	NS	*	**	*	*	NS	NS	NS	NS
Targeted	***	NS	NS	***	*	**	*	*	NS	NS
Non-targeted	NS	**	**	NS	*	**	*	NS	NS	NS
Coral trout	***	NS	*	***	***	***	NS	***	NS	NS
L. carponotatus	**	NS	NS	**	NS	*	NS	Not present		
Total prey	***	*	***	*	NS	**	NS	**	NS	NS
Pomacentridae	***	NS	***	*	NS	**	NS	**	NS	NS
Planktivorous	**	***	***	*	NS	*	*	*	NS	NS
Omnivorous	***	NS	***	*	NS	***	**	*	NS	NS
Herbivorous	**	***	***	***	NS	***	*	NS	***	*
P. moluccensis	***	NS	**	***	***	*	NS	***	***	**
P. wardi	**	**	***	***	***	**	***	**	***	NS
A. polyacanthus	NS	*	NS	**	*	***	NS	NS	NS	NS
A. curacao	**	NS	***	*	**	**	***	**	**	NS
Chaetodontidae	*	**	NS	NS	NS	**	**	*	*	NS
Total herbivores	**	NS	*	**	***	***	**	NS	NS	*
Scraping	***	*	**	***	***	NS	NS	NS	NS	NS
Grazing	***	***	***	**	NS	NS	NS	NS	NS	NS
Cropping	*	NS	*	NS	*	NS	NS	NS	NS	NS
Farming	**	***	***	***	NS	***	**	NS	***	*
BIOMASS										
Piscivores	***	NS	*	***	NS	***	***	**	**	**
coral trout	***	NS	**	***	**	***	NS	***	**	**
L. carponotatus	*	**	**	**	NS	**	*	Not present		
Total herbivores	**	NS	*	NS	*	NS	NS	NS	*	*
Live hard coral	NS	***	**	NS	*	NS	**	NS	NS	NS
Live soft coral	***	**	***	***	***	***	***	NS	**	***
Algae	*	NS	**	NS	**	NS	**	NS	*	**
Complexity	*	NS	**	*	NS	**	**	***	***	**

*p<0.05, **p<0.01, p<0.001.

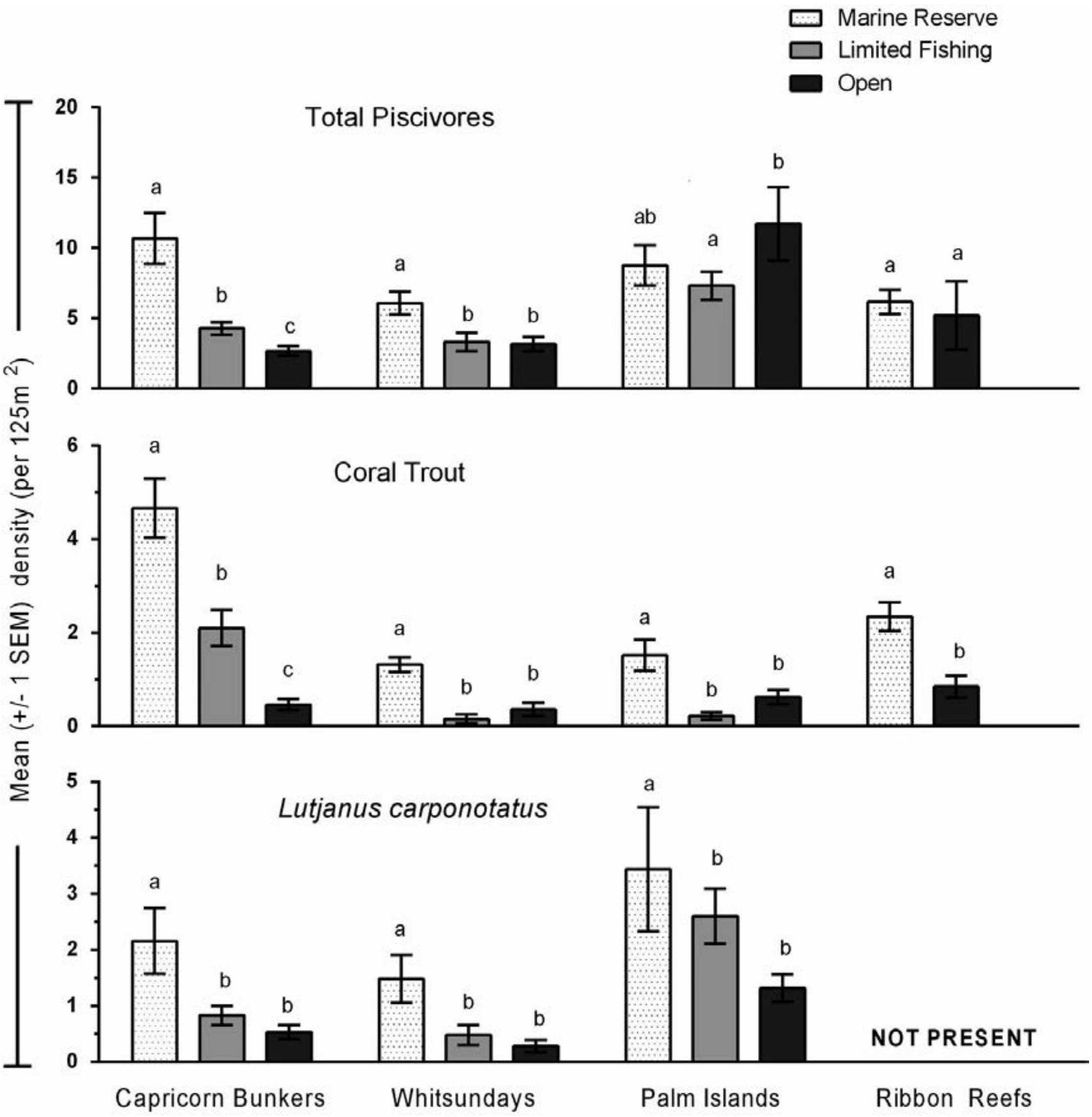

Figure 2.1 Mean density (±1SE) of total piscivorous fishes, and key species targeted by fisheries; coral trout (*Plectropomus* spp.), and stripey snapper (*Lutjanus carponotatus*), amongst marine reserve, limited fishing and open zones at all four locations. *L. carponotatus* does not occur at the Ribbon Reefs. Letters above bars indicate groupings based on post-hoc analysis: different letters indicate significant differences amongst zones.

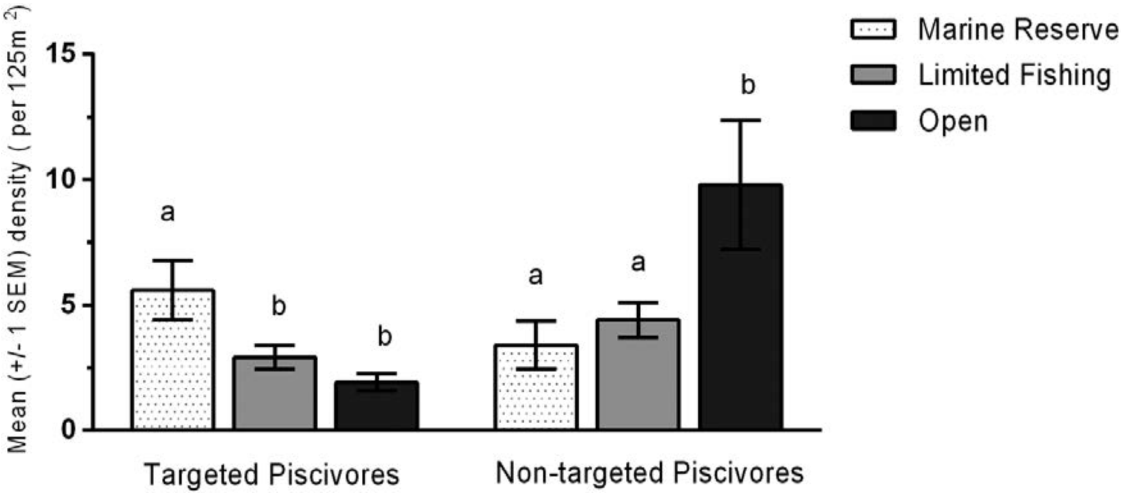

Figure 2.2 Mean density (±1SE) of targeted and non-targeted piscivorous fishes amongst management zones at the Palm Islands. Letters above bars indicate groupings based on post-hoc analysis: different letters indicate significant differences amongst zones

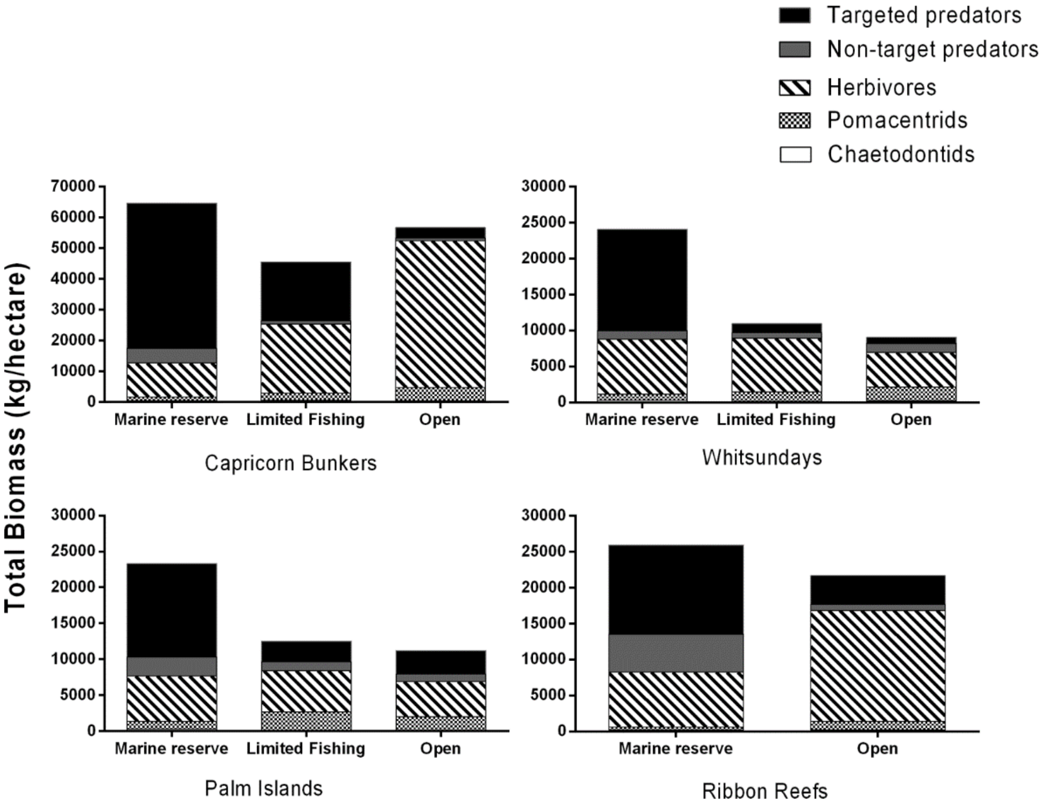

Figure 2.3 Total biomass (kg/hectare) of fishes amongst zones at all four locations, separated by families/trophic groups. Note the differences in axis scales amongst locations.

2.4.2 PREDICTION TWO

Predator depletion would result in an increase in the density of small prey fishes and an overall increase in prey densities in depleted zones.

2.4.2.1 POMACENTRIDS

Densities of total prey and pomacentrids were strongly influenced by zoning and had an inverse relationship to marine reserve protection; densities were significantly greater in fished zones at all four locations (Fig. 2.4 and Table 2.2). At the Whitsundays, pomacentrid densities in limited fishing zones did not differ significantly from either marine reserves or fished zones, however, open fishing zones had significantly greater densities compared to marine reserves. At all other locations densities were significantly greater in both limited fishing and open zones. Zoning explained 17-30% of the variation in pomacentrid density. There was little variation in pomacentrid densities amongst reefs or sites within zones (0-9%), with the exception of the Capricorn Bunkers, where densities differed significantly amongst sites, which accounted for 41% of the total variation (Table 2.2). There were great differences in densities amongst replicates, which explained 40-70% of the variation, but this did not mask zoning effects.

Pomacentrids demonstrated differences in their response to zoning when separated into trophic groups. Densities of omnivorous pomacentrids were greater in fished zones at all four locations, and increased along a gradient of fishing intensity (marine reserves < limited fishing < open), at the Capricorn Bunkers and Palm Islands (Fig. 2.5). Planktivorous and herbivorous pomacentrids showed greater variability in their zoning relationships. Planktivorous pomacentrids had significantly greater densities in fished zones at the Palm Islands and Ribbon Reefs, and in limited fishing zones at the Capricorn Bunkers, but showed the opposite relationship at the Whitsundays, where densities were significantly lower in limited fishing zones compared to marine reserves and open zones. Densities of herbivorous pomacentrids were greatest in limited fishing zones at the Whitsundays, and in open zones at the Palm Islands, but showed no zoning-related patterns in the Ribbon Reefs or Capricorn Bunkers (Fig. 2.5).

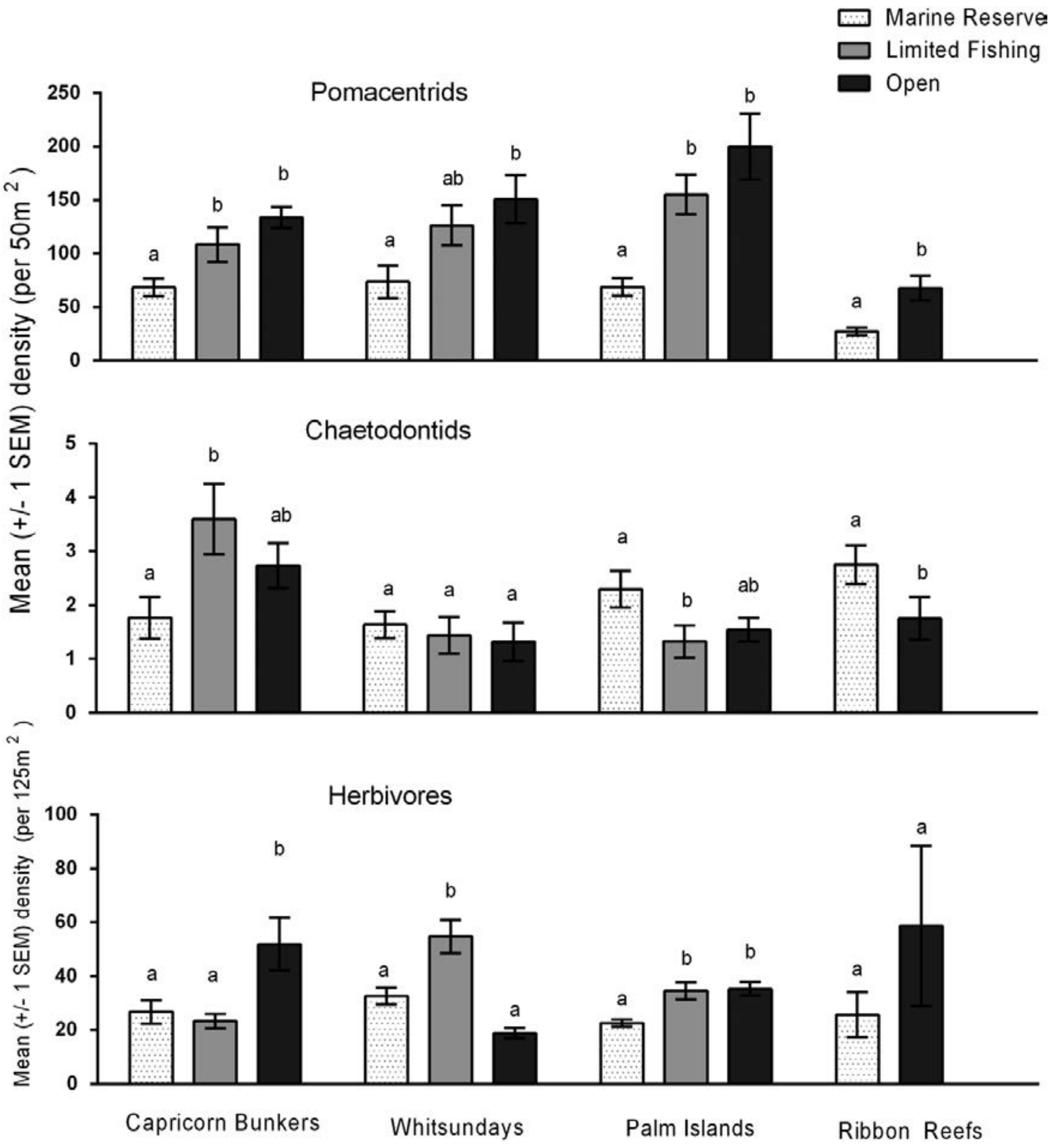

Figure 2.4 Mean density (±1SE) of pomacentrids, chaetodontids, and herbivorous fishes amongst marine reserve, limited fishing and open zones at all four locations. Letters above bars indicate groupings based on post-hoc analysis: different letters indicate significant differences amongst zones.

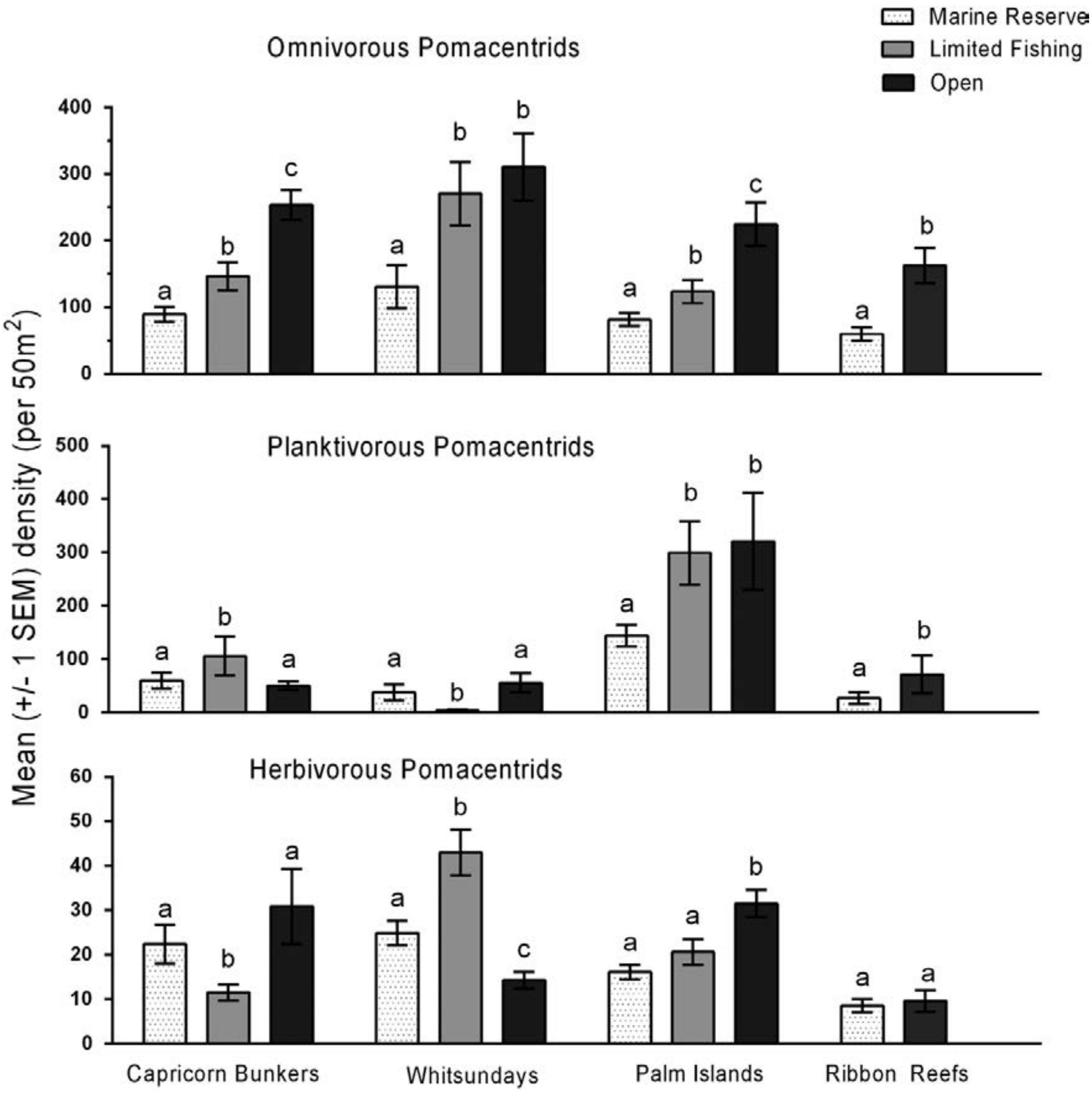

Figure 2.5 Mean density (±1SE) of omnivorous, planktivorous and herbivorous pomacentrids amongst marine reserve, limited fishing and open zones at all four locations. Letters above bars indicate groupings based on post-hoc analysis: different letters indicate significant differences amongst zones.

Zoning had a strong impact on densities of individual pomacentrid species (e.g. *Pomacentrus moluccensis, P. wardi, Amblyglyphidodon curacao, Acanthochromis polyacanthus;* Fig. 2.6 and Table 2.2). Overall, 50% of pomacentrid species exhibited zoning-related patterns whereby they had significantly greater densities in fished zones at most locations. Species that exhibited differences made up 75% of the total pomacentrid densities, and had a strong effect on patterns for pomacentrids overall.

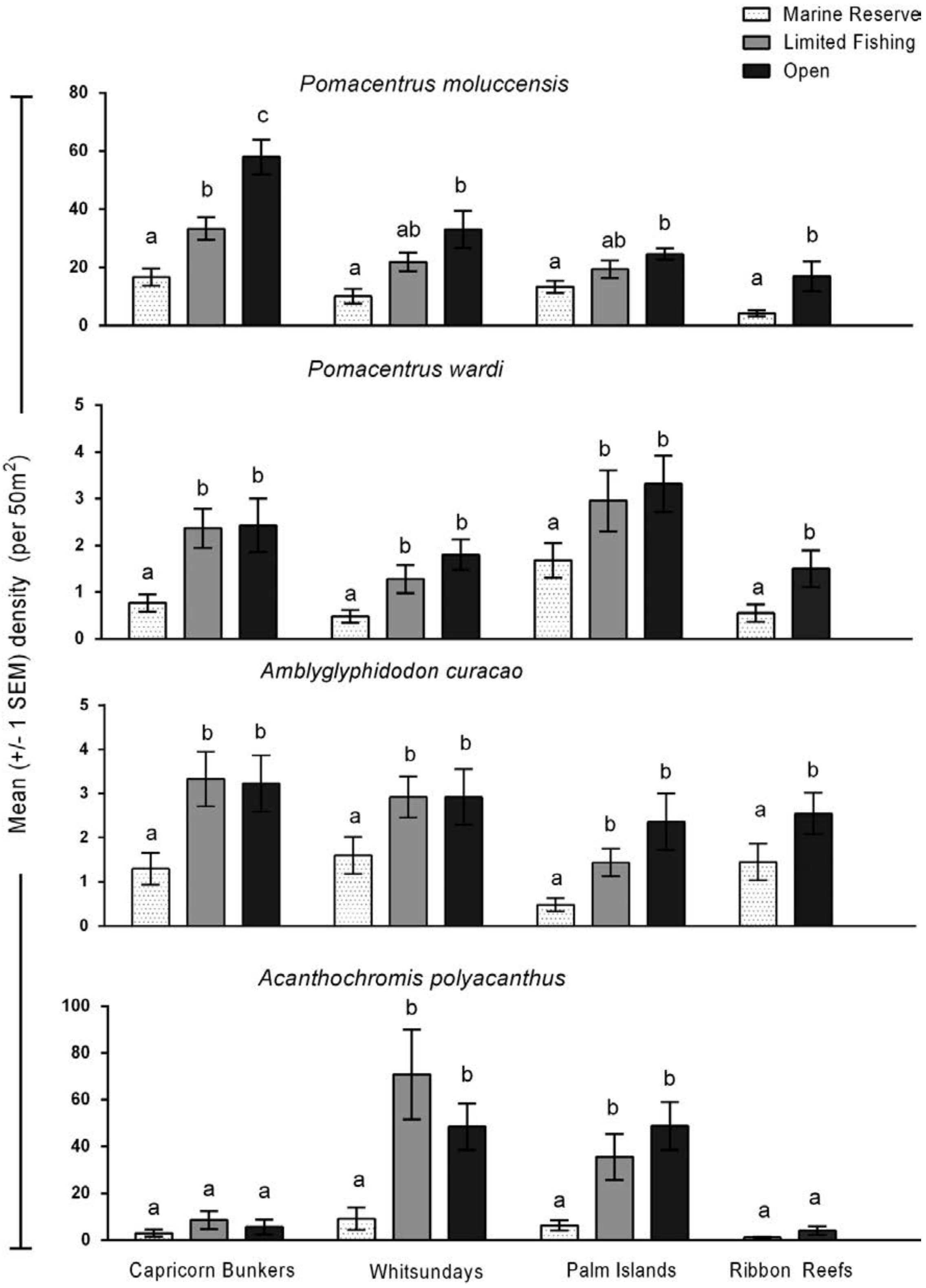

Figure 2.6 Mean density (±1SE) of the key prey species; *Pomacentrus moluccensis, P. wardi*, *Amblyghlyphidodon curacao*, and *Acanthochromis polyacanthus* amongst marine reserve, limited fishing and open zones at all four locations. Letters above bars indicate groupings based on post-hoc analysis: different letters indicate significant differences amongst zones.

2.4.2.2 CHAETODONTIDS

Although the density of chaetodontids did differ amongst the fishing zones, these patterns were not consistent amongst the four locations. The density of chaetodontids was significantly greater in marine reserves at the Palm Islands and Ribbon Reefs compared to fished zones (Fig. 2.4 and Table 2.2). At the Capricorn Bunkers densities were greatest in limited fishing zones, while at the Whitsundays, densities were very similar amongst zones. Densities of chaetodontids differed significantly amongst reefs within zones at the Capricorn Bunkers and Ribbons, where reefs were responsible for 15-24% of the total variation. Variation amongst sites was high at the Palm Islands, accounting for 21% of total variation, and was great amongst replicates at all locations (residual level = 65-90%).

2.4.2.3 HERBIVOROUS FISHES

Herbivorous fishes were strongly influenced by zoning, and had greater densities in fished zones compared to marine reserves at the Capricorn Bunkers, Whitsundays and Palm Islands (Fig. 2.4 and Table 2.2). At the Ribbon Reefs there was a strong trend of greater densities of herbivores in open zones, however, this was not significant due to very patchy distributions, as indicated by high residual variation (85%). Patterns of variation in densities by zones differed amongst locations. At the Capricorn Bunkers, herbivore densities were significantly greater in open zones compared to marine reserves and limited fishing zones, and the total herbivore biomass was more than four times greater than in marine reserves (Fig. 2.3 and 2.4, Table 2.2). This pattern was consistent for all herbivore trophic groups (scraping, grazing, cropping and farming herbivores). At the Whitsundays, total herbivore density was greatest in the limited fishing zones, and there were no significant differences between open zones and marine reserves (Fig. 2.4 and Table 2.2). This pattern was consistent for all herbivore groups except for cropping herbivores, but did not result in a significant difference in total biomass (Fig. 2.3 and Table 2.2). At the Palm Islands, total densities of herbivores were greater in limited fishing zones and open zones, however, this pattern was driven largely by farming herbivores, mostly territorial grazing pomacentrids (Fig. 2.4 and Table 2.2). Densities of scraping, grazing and cropping herbivores did not differ significantly by zone, and the total biomass of herbivores was similar amongst zones (Fig. 2.3 and Table

2.2). Overall, zoning effects were strong, accounting for up to 20% of total variation, while variation amongst reefs within zones was low (0-2%). Variation in herbivore density was great amongst sites (18-64%), and at the residual level (29-80%), due to their strong schooling behaviour.

2.4.3 PREDICTION THREE

Species that associate strongly with habitats such a live coral would be more strongly influenced by variation in benthic habitat than by top-down effects.

2.4.3.1 VARIATION IN BENTHIC COVER AMONGST MANAGEMENT ZONES

Fishing did not have a strong effect on habitat, and no consistent patterns were found amongst management zones at the four locations. Live hard coral cover was consistent amongst all three zones at all locations. It did however, vary significantly amongst reefs at the Capricorn Bunkers (16% of total variation) and sites at the Capricorn Bunkers (11%) and Whitsundays (40%; Fig. 2.7 and Table 2.2). There were some differences in soft coral and algal cover amongst management zones, however, there was no consistent pattern amongst the four locations. Live soft coral cover differed by zone at all locations except the Ribbon Reefs, however, patterns were not consistent amongst locations, and varied amongst reefs (20-40% of total variation) and sites (10-30%). Soft coral cover was lower in limited fishing zones at the Capricorn Bunkers compared to both marine reserves and open zones and had the lowest cover in open zones at the Whitsundays. At the Palm Islands soft coral cover was lower in both limited fishing and open zones, however, no zoning patterns occurred at the Ribbon Reefs (Fig. 2.7 and Table 2.2). Overall, soft coral cover was much greater in the Whitsundays compared to other locations. Algae were less abundant in both limited fishing and open zones at the Capricorn Bunkers, however, percentage cover did not differ significantly amongst zones at any other location. Algal cover differed significantly sites at all locations, and variation amongst sites accounted for up to 85% of the total variation.

Structural complexity differed significantly amongst zones, however, the nature of this varied amongst locations. At the Whitsundays and Palm Islands, complexity was lowest in open fishing zones, however, the opposite pattern was observed for the Capricorn

Bunkers and Ribbon Reefs (Fig. 2.7). Structural complexity varied greatly amongst sites within reefs (up to 40% of total variation), and was lower in open zones at both the Whitsundays and Palm Islands (Fig. 2.7 and Table 2.2). Conversely, At the Capricorn Bunkers and Ribbon Reefs, complexity was greatest in open zones, however, was not significantly different compared to marine reserves at the Capricorn Bunkers. There was great variation in the distributions of benthic cover and structural complexity at the residual level, which accounted for 9-79% of total variation.

2.4.3.2 HABITAT ASSOCIATIONS VERSUS PREDATOR-PREY RELATIONSHIPS

There was a strong negative relationship between the density of predators and prey at all four locations, and predator density had more predictive value than habitat for small prey species and groups such as pomacentrids (damselfishes) (Table 2.3 and 2.4). Densities of small prey species such as pomacentrids were negatively related to densities of key predators such as coral trout (*Plectropomus spp.*), and stripey snapper (*L. carponotatus*) at all four locations (Fig. 2.8 and Table 2.3 and 2.4). This relationship was particularly strong at the Ribbon Reefs, where densities of total prey, and total pomacentrids were strongly negatively related to densities of coral trout (*P. leopardus, P. laevis* and *P. areolatus*) which explained 82-83% of the variation. This relationship was stronger for omnivorous pomacentrids, compared to planktivores and herbivores. Total predator density was the strongest predictor for the lemon damsel (*P. moluccensis*, 49.4%), and the density of *P. leopardus* was the strongest predictor for the spiny chromis (*A. polyacanthus,* 32.5%).

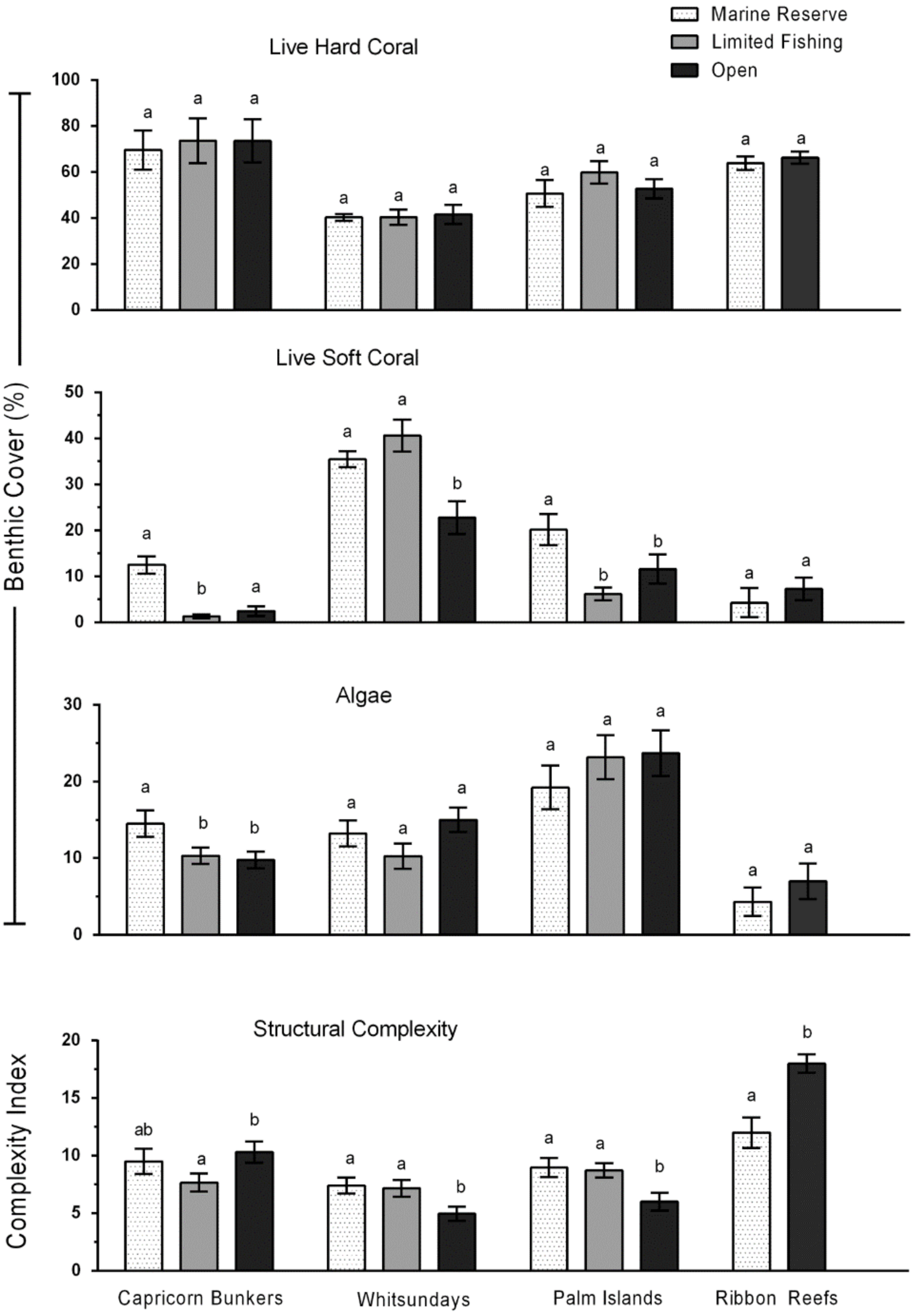

Figure 2.7 Mean percent cover (±1SE) of live hard and soft coral and algae, and mean structural complexity of reefs amongst zones at all four locations. Letters above bars indicate groupings based on post-hoc analysis: different letters indicate significant differences amongst zones.

At the Palm Islands, the regression model did not explain a large proportion of variation, however, there was a significant negative relationship between coral trout (*P. maculatus* and *P. leopardus*) and total prey, pomacentrids (including omnivores and herbivores) *P. moluccensis* and *A. polyacanthus* (Fig. 2.8 and Table 2.4). The density of *P. maculatus* explained 15-34% of the total variation for these species/groups. In the Whitsundays, the density of the stripey snapper (*L. carponotatus*) was a strong negative predictor for densities of small prey species/groups. *L. carponotatus* density was the primary predictor for densities of total prey, omnivorous pomacentrids and *P. moluccensis*, and explained 40-50% of variation (Fig. 2.8 and Table 2.3). In the Capricorn bunkers, the density of coral trout (*P. leopardus* and *P. laevis*) was the primary predictor for the density of pomacentrids (including planktivores, omnivores and herbivores). The density of coral trout explained 30-46% of the variation in density for these species/groups. Habitat variables had less predictive value for pomacentrids than piscivore densities did, and no consistent relationships with habitat were detected amongst the four locations (Table 2.3 and 2.4). Hard coral cover was a primary predictor for total prey (31.6%), and a secondary predictor for *P. moluccensis* (31.4%) at the Capricorn Bunkers, and for *A. curacao* (17.2%) at the Whitsundays.

Chaetodontid densities were strongly related to habitat characteristics, including hard coral, algae, rugosity and vertical relief. Hard coral cover was the primary predictor for the density of chaetodontids at the Capricorn Bunkers, Whitsundays and Palm Islands and explained 33-76% of the variation in the density of chaetodontids overall (Fig. 2.8 and Table 2.3 and 2.4). No strong predator-prey relationships occurred for chaetodontids, and piscivore densities held little predictive value for this group. Patterns of density for chaetodontids differed at the Ribbon Reefs, and were positively related to densities of coral trout, but not significantly related with habitat.

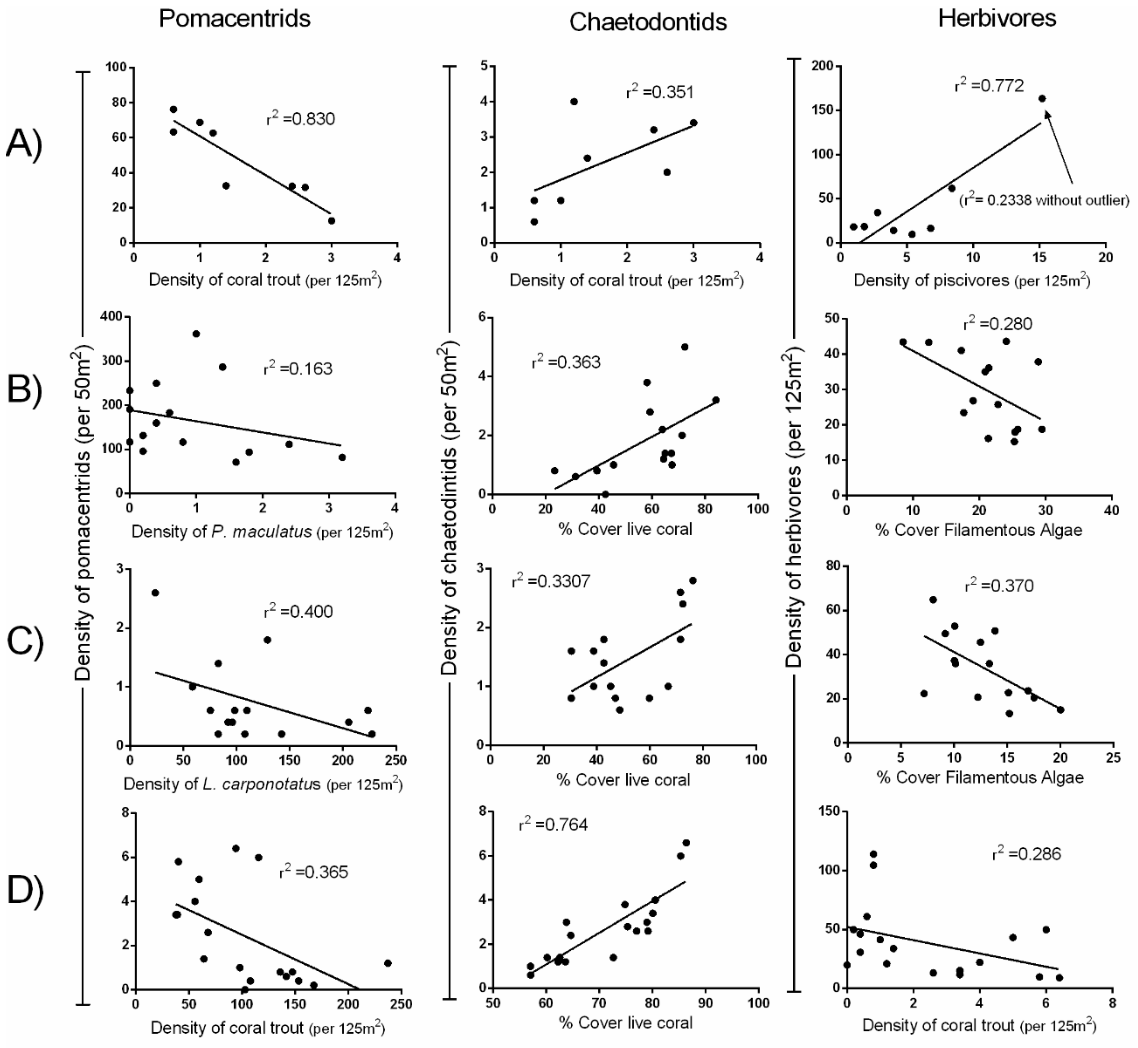

Figure 2.8 Linear regressions of pomacentrids, chaetodontids, and herbivorous fishes at each location (zones pooled). A= Ribbon reefs, B= Palm Islands, C= Whitsundays, and D = Capricorn Bunkers. Fish groups are plotted against their primary predictor from the multiple regressions models, using site means. All regressions had slopes significantly different from zero (ANOVA).

Herbivores displayed great variation in patterns amongst locations, and predator densities and a variety of habitat characteristics were primary predictors for herbivore groups. At the Whitsundays and Palm Islands, the total density of herbivores had a significant negative relationship with the cover of filamentous algae, and the cover of algae was lowest where densities of herbivores were great (Fig. 2.8 and Table 2.3 and 2.4). At the Palm Islands there was a secondary positive relationship between herbivore abundance and live coral cover, the opposite of the pattern observed for filamentous algae (Table 2.4). These patterns were not consistent, however, when herbivores were analysed as trophic groups, for example only farming herbivores had a negative relationship with algae at the Whitsundays (Table 2.3). At the Capricorn Bunkers densities of total herbivores were negatively associated with coral trout abundance, which explained 28.6% of the variation. At the Ribbon Reefs, herbivores had a strong positive relationship with piscivores, which explained 77.2% of variation, however, this pattern was mostly driven by a single site which had high densities of both predatory and herbivorous fishes; without this data point the relationship was poor (r^2= 0.2338). There were no clear trends in relationships between habitat variables, predator densities and herbivore groups (grazers, scrapers, browsers and farmers). Scrapers and croppers had a negative relationship with coral trout at the Capricorn Bunkers but this was not consistent amongst locations (Table 2.3 and 2.4). Herbivore groups were associated with a variety of habitat variables such as live coral, dead coral, vertical relief, rugosity, and filamentous algae; however, no clear trends occurred amongst groups within or amongst locations, or within groups amongst locations and within locations.

Table 2.3 Results of stepwise multiple linear regressions on the density of prey species and groups, and herbivore functional groups for the Capricorn Bunkers and Whitsundays. Only significant independent variables and models are given. Signs for the coefficient of each independent variable are in parentheses. The r^2 values have been multiplied by 100 to represent the percentage of variation each independent variable explains. L. carp = *Lutjanus carponotatus*. "Coral trout" refers to the summed density of all *Plectropomus* spp.

Location	**Capricorn Bunkers**			**Whitsundays**		
Dependant variable	**Independent variable**	**Partial r^2 (x100)**	**Model r^2 (x100)**	**Independent variable**	**Partial r^2 (x100)**	**Model r^2 (x100)**
Total prey	Hard coral (+)	31.6	31.6	*L. carp* (-)	40.0	40.0
Pomacentrids	Coral trout. (-)	36.5	36.5	*L. carp* (-)	40.7	40.7
Planktivores	Coral trout (-)	42.11	42.11	Not significant		
Omnivores	Coral trout. (-)	42.7	42.7	*L. carp* (-)	24.5	24.5
				Hard coral (+)	19.5	44.0
				Coral trout (-)	13.8	57.8
Herbivores	Coral trout. (-)	28.55	28.55	Algae (-)	45.2	45.2
P. moluccensis	Coral trout. (-)	44.8	44.8	*L. carp* (-)	50.5	50.5
	Hard Coral (+)	31.4	58.2	Coral trout (-)	16.8	67.3
P. wardi	Algae (-)	16.8	16.8	*L. carp* (-)	14.1	14.1
A. polyacanthus	Coral trout. (-)	46.1	46.1	*L. carp* (-)	42.7	42.7
A. curacao	Relief (+)	15.5	15.5	Coral trout (-)	32.0	32.0
				Hard coral (+)	17.2	49.2
Chaetodontids	Hard coral (+)	76.4	76.4	Hard coral (+)	33.1	33.1
	Algae (-)	7.8	84.2	Rugosity (+)	5.7	38.8
	Relief (+)	6.5	90.7			
Total Herbivores	Coral trout (-)	28.6	28.6	Algae (-)	37.0	37.0
"Grazers"	Algae (-)	24.4	24.4	*L. carp* (+)	31.7	31.7
	Dead Coral	23.6	48.0			
"Scrapers"	Coral trout (-)	23.2	23.2	Dead coral (+)	21.5	21.5
	Relief (-)	23.2	46.4			
"Croppers"	Coral trout (-)	14.3	14.3	Not significant		
"Farmers"	Relief (-)	31.8	31.8	Algae (-)	42.6	42.6

Table 2.4 Results of stepwise multiple linear regressions on the density of prey species and groups, and herbivore functional groups for the Palm Islands and Ribbon Reefs. Only significant independent variables and models are given. Signs for the coefficient of each independent variable are in parentheses. The r^2 values have been multiplied by 100 to represent the percentage of variation each independent variable explains. L. carp = *Lutjanus carponotatus*. “Coral trout” refers to the summed density of all *Plectropomus* spp.

Location	**Palm Islands**			**Ribbon Reefs**		
Dependant variable	**Independent variable**	**Partial r^2 (x100)**	**Model r^2 (x100)**	**Independent variable**	**Partial r^2 (x100)**	**Model r^2 (x100)**
Total prey	Coral trout (-)	15.5	15.5	Coral trout (-)	82.2	82.2
Pomacentrids	Coral trout (-)	16.3	16.3	Coral trout (-)	83.0	83.0
Planktivores	Not significant			Coral trout (-)	32.9	32.9
Omnivores	Coral trout (-)	33.4	33.4	Coral trout (-)	75.0	75.0
Herbivores	Coral trout (-)	20.7	20.7	Piscivores (-)	32.3	32.3
	Algae (-)	15.7	36.4			
P. moluccensis	Coral trout (-)	18.8	18.8	Piscivores (-)	49.4	49.4
P. wardi	*L. carp* (-)	16.6	16.6	Not significant		
A. polyacanthus	Coral trout (-)	23.4	23.4	Coral trout (-)	32.5	32.5
A. curacao	Coral trout (-)	17.2	17.2	Not significant		
Chaetodontids	Hard Coral (+)	36.3	36.3	Coral trout (+)	35.1	35.1
Total Herbivores	Algae (-)	28.0	28.0	Piscivores (+)	77.2	77.2
	Hard coral (+)	4.0	32.0			
“Grazers”	Rugosity (+)	25.6	25.6	Relief (-)	42.1	42.1
	Hard Coral (-)	12.3	37.9			
“Scrapers”	Rugosity (+)	10.8	10.8	Hard coral (-)	34.1	34.1
	Relief (-)	9.2	20.0			
	Hard coral (-)	3.8	23.8			
“Croppers”	Not significant			Not significant		
Total prey	Rugosity (-)	29.8	29.8	Algae (+)	71.1	71.1

2.4.4 PREDICTION FOUR

The composition of fish communities would vary amongst management zones due to predator depletion and associated changes in prey assemblages.

The structure of fish assemblages varied significantly amongst zones in the composition of both species and trophic groups (Fig. 2.9 and Table 2.5). At the Capricorn Bunkers and Palm Islands, open zones had a significantly different composition of trophic groups compared to both marine reserves and limited fishing zones. This relationship differed slightly when comparing species compositions, which were distinct in marine reserves compared to limited fishing and open zones at both locations (Table 2.5). In the

Whitsundays all three zones had distinct species and trophic assemblages and at the Ribbon Reefs the trophic composition differed amongst zones (marine reserve and open), however, the species composition did not. Variation in the trophic composition of species assemblages by zone was driven strongly by prominent pomacentrid groups such as omnivorous, planktivorous and herbivorous pomacentrids (Table 2.6). These three groups combined were responsible for up to 70% of the dissimilarity amongst management zones. Non-target predators (Palm Islands), and grazing herbivores such as surgeonfishes (Ribbon Reefs) were also responsible for differences amongst zones. At the species level, four common pomacentrid species (*P. moluccensis, C. atripectoralis, C. nitida,* and *A. polyacanthus*) were responsible for the majority of dissimilarity amongst zones. The relationship between habitat and the composition of fish assemblages was weak and habitat was not a significant driver of the composition of species assemblages or trophic groups at any of the locations. BIOENVIRON analysis indicated that habitat variables were not driving the zoning-related trends in fish assemblages. There were no significant relationships between habitat characteristics and assemblage structure, and habitat did not vary amongst zones at the multivariate level. There were no consistent patterns in the best environmental descriptors amongst locations; a combination of benthic cover and structural complexity characteristics best described the patterns of fish density, however, none of these were significant.

Table 2.5 Results of permutational ANOVA (PERMANOVA) on fish densities by trophic groupings, and by species. Letters indicate groupings based on post-hoc analysis.

	PERMANOVA		**Post-Hoc analysis (groups)**		
Location	**Dependant variables**	**p**	**MR**	**LF**	**OP**
Capricorn Bunkers	Trophic groups	0.0092*	a	a	b
	Species	0.0006*	a	b	b
Whitsundays	Trophic groups	0.0006*	a	b	c
	Species	0.0005*	a	b	c
Palm Islands	Trophic groups	0.0110*	a	a	b
	Species	0.0004*	a	b	b
Ribbon Reefs	Trophic groups	0.0041*	a	NA	b
	Species	0.6993	a	NA	a

MR= Marine Reserve, LF= Limited Fishing, OP= open.

*Analysis significant (p<0.05).

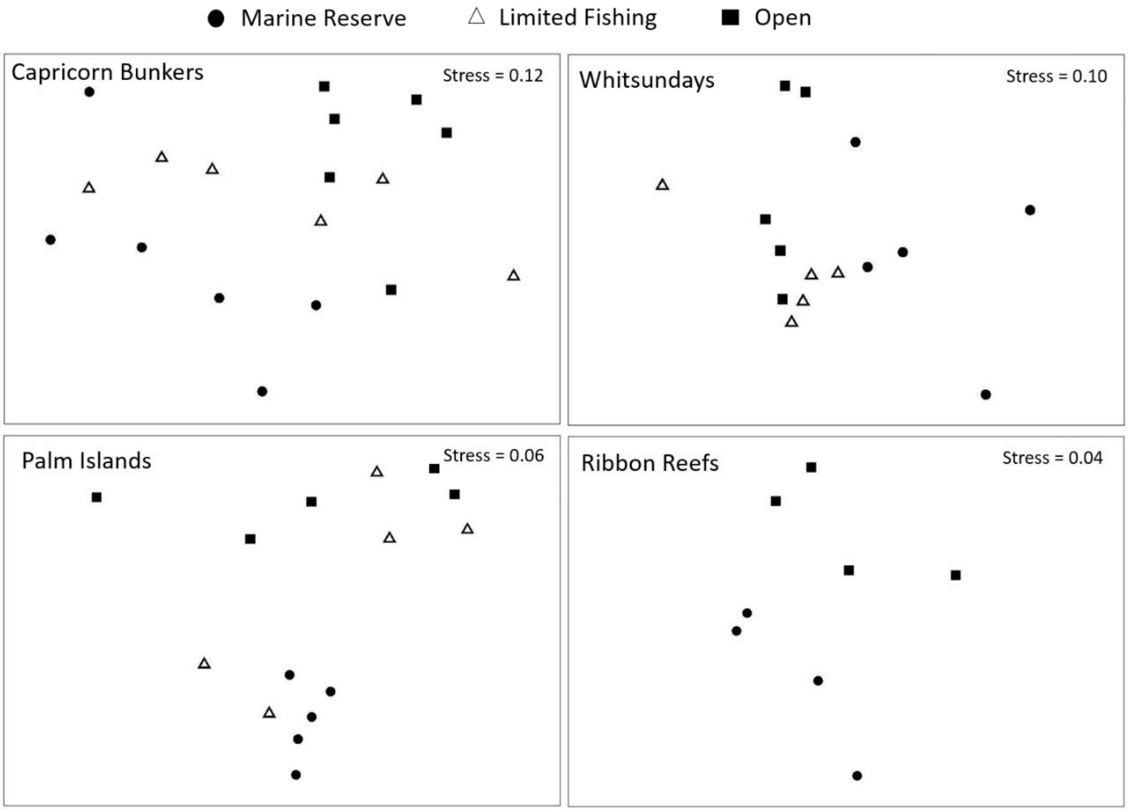

Figure 2.9 Ordination plots (nMDS, nonmetric multidimensional scaling) of fish assemblages amongst marine reserves, limited fishing, and open zones at the Capricorn Bunkers, Whitsundays, Palm Islands and Ribbon Reefs. Symbols represent sites within each management zone, and data have been pooled into trophic groups. Stress values indicate the level of fit between the Bray-Curtis similarity rankings and distance rankings in the ordination plot. Stress values <0.2 indicate a useful ordination.

Table 2.6 Results of multivariate SIMPER analysis on the density of species and trophic groups amongst zones at each location. Dissimilarity values are the percentage dissimilarity amongst zones that were significantly different from PERMANOVA analysis. The three species/groups that were responsible for the largest amount of this dissimilarity are listed for all significant analyses.

Location	Dependant variable	Dissimilarity	Top 3 groups/species accounting for differences
Capricorn Bunkers	Trophic groups	22-28%	Omnivorous (31-33%), planktivorous (14-22%) and herbivorous (12%) pomacentrids
	Species	56-59%	*P. moluccensis* (10-12%), C. *atripectoralis* (7-12%) and *C. nitida* (9-10%)
Whitsundays	Trophic groups	21-27%	Omnivorous (27-39%), planktivorous (19-22%) and herbivorous (7-21%) pomacentrids
	Species	36-44%	*A. polyacanthus* (10-17%), *P. moluccensis* (9-14%) and *C. nitida* (10-13%)
Palm Islands	Trophic groups	21-26%	Planktivorous (40-47%) and omnivorous (18-27%) pomacentrids, non-target predators (8%)
	Species	33-38%	*Neopomacentrus* spp. (14%), *A. polyacanthus* (8-12%) and *C. nitida* (4-6%)
Ribbon Reefs	Trophic groups	56%	Omnivorous (33%), planktivorous (20%) pomacentrids, grazers (9%)
	Species	NS	

NS= not significant

2.5 DISCUSSION

This study has provided strong evidence supporting the ecological importance of top-down control in ecosystems, and demonstrated the role of predatory fishes in structuring coral reef fish assemblages. These findings concur with studies from terrestrial systems (Beschta and Ripple 2009), lakes (Carpenter et al. 2010), and temperate reef systems (Edgar and Barrett 1999; Shears and Babcock 2003), which have demonstrated the role of predators in controlling populations of species at lower trophic levels. Coral reefs are complex systems, with a high degree of functional diversity and variability in trophic interactions**.** The strength and consistency of predator-prey relationships found in this study is remarkable in light of this complexity, providing strong evidence that piscivores are important in structuring prey communities.

2.5.1 PREDICTION 1 AND 2: PREDATOR-PREY INTERACTIONS AND PREY RELEASE

Global reductions in predator populations have resulted in significant changes to ecosystems, primarily through alteration of predator-prey interactions, and subsequent flow-on effects (Estes et al. 2011). In this study, I predicted that predator depletion from fishing would cause an overall reduction in piscivore densities, and a corresponding increase in prey densities along a gradient of fishing intensity. There was strong evidence to support these predictions at a range of spatial scales. Prey generally had greater densities in heavily fished zones where predators were depleted, and predator density was a strong predictor of prey density for many species. As expected, coral reef fish communities were spatially heterogeneous, and varied amongst reefs and sites within management zones. These variations, however, did not prevent detection of strong zoning-related effects. Given the prevalence of significant zoning-related patterns (e.g. 29 out of 32 tests for pomacentrid species), it is highly unlikely that these results could have been due to chance and the conclusions, therefore, are robust.

While changes in prey density in the opposite direction to predators suggests that predator-prey interactions are the cause, these data are correlative, and may co-vary with habitat. However, there was no evidence of a consistent zoning effect on habitat at the univariate or multivariate level. Furthermore, prey release was documented along a gradient of fishing intensity for species such as damselfishes, providing strong evidence that release from predation was the likely mechanism underpinning the observed patterns. These data demonstrate the important role of top predators, and challenge the notion that top-down effects are likely to be weak in complex systems (Polis and Strong 1996; Shurin et al. 2002; Shurin et al. 2010). Findings from this study are consistent with ecological models from terrestrial, freshwater and temperate marine systems, in which top predators are considered strong regulators of community structure (Sinclair et al. 2003; Carpenter et al. 2010). While top-down control has been demonstrated quite consistently in these systems, the case for coral reefs has been less clear. Data from this study supports findings from coral reef ecosystems in the Caribbean (Hughes 1994), Kenya (McClanahan and Shafir 1990) and the Red Sea (Roberts and Polunin 1992), which all demonstrated some level of top-down control by predators on coral reefs. These data also concur with and expand on the only previous study to consider such trophic interactions on the Great Barrier Reef (Graham et al. 2003) and demonstrate that predators can have important regulatory roles in complex and diverse ecosystems.

In addition to direct effects on prey, apex predators can have a significant influence on community organization through interactions with meso-predators. The "Meso-predator Release Hypothesis" predicts that populations of small predators will increase as they are released from competition with apex predators, which can in turn affect prey species (Soule et al. 1988). Such interactions are most commonly observed for mammalian carnivores in terrestrial systems. For example, in California declines in coyote populations led to increases in native (skunks, racoons, foxes) and exotic (feral cats, opossums) meso-predators causing local extinctions of scrub-feeding birds (Crooks and Soule 1999). Similar interactions have occurred for Tasmanian devils and quolls in Australia, following the near extinction of Tasmanian devils due to a disease outbreak (Hollings et al. 2014). In this study, I found evidence to suggest that predator/meso-predator interactions occurred at the Palm Islands between large fisheries-targeted predators such as coral trout and snappers, and smaller non-target meso-predators such as small groupers and cods. Such patterns may have come about due to competitive release, or release from juvenile predation, as larger predators were removed through fishing. Fear mediated responses might also occur, as meso-predators alter their behaviour in the presence of apex predators (Ritchie and Johnson 2009). Despite the fact that targeted predators and meso-predators share similar prey sources (Kingsford 1992 (*Plectropomus leopardus*); Beukers-Stewart and Jones 2004 *(Cephalopholis cyanostigma, C. boenak*); Feeney et al. 2012 (*Pseudochromis fuscus*)), prey release still occurred in fished areas. This suggests that overall predation pressure remained much lower in these areas, and that large targeted predators play a stronger role in regulating prey compared to meso-predators, probably due to their greater overall biomass and associated high prey consumption.

Fish populations can be regulated by a complex variety of pre-settlement and post-settlement processes including larval supply, and mortality from predation. Larval supply may be an important factor determining densities of prey, as described in the “Recruitment-limitation Hypothesis” (Doherty 1981; Doherty and Fowler 1994). Predators can influence the effect that larval supply has on populations of post-settlement fishes through mortality, and indirect effects on intraspecific and interspecific competition (Connell 1998; Holbrook and Schmitt 2002; Hixon and Jones 2005). As such, in areas of very low predator density, prey density may be strongly related to larval supply. It is possible that recruitment pulses in heavily fished areas, in combination with low piscivore densities, resulted in greater densities of prey in this study. However, it is unlikely that larval supply was a contributing factor to differences in the zoning-related patterns observed. Considering the great variation in oceanographic processes that occur amongst the locations surveyed, it is highly unlikely that the same management zones would be subject to similar oceanographic conditions, especially considering that consistent patterns were found at locations separated by hundreds of kilometres (Wolanski 2001). Furthermore, available data indicate that densities of key predators at these locations have remained relatively stable over recent years, whereas larval supply and related recruitment is by nature highly variable (Doherty et al. 2004; Kingsford 2009). The evidence strongly suggests, therefore, that post-settlement mortality arising from predation was the primary process regulating densities of prey.

The body size and home range of prey species/groups may influence their relative susceptibility to predation. Coral reefs have a high diversity of fishes at all trophic levels, which encompass a range of body sizes which may vary in their susceptibility to predation (Munday and Jones 1998). Such diversity has been suggested to result in strong interactions between top-down effects and habitat variations, as species that grow to a larger size become less susceptible to predation, and more influenced by habitat and/or food availability (Sinclair et al. 2003). Studies in the Serengeti have demonstrated such interactions between ungulates of various sizes, and their canine and feline predators (Sinclair et al. 2003). In the present study, large herbivorous fishes such as grazers (Acanthuridae), scrapers (Scaridae) and croppers (Siganidae, Acanthuridae) had highly variable responses to habitat and predators. These herbivores are vulnerable to predation in the juvenile phase, as evidenced by high numbers of juvenile scarids in the gut contents of coral trout (Kingsford 1992), however may suffer lower overall mortality from predation compared to species with smaller maximum body sizes such as damselfishes (Hambright et al. 1991).

Home range may also influence the effect to which species are affected by either top-down or habitat effects at local scales. Many herbivorous fishes have large home ranges (Welsh and Bellwood 2012), which may make the effects of both local predator densities, and local habitat characteristics difficult to detect. This would explain the variable and inconsistent relationships with predators and habitat found for herbivores amongst locations in this study. Despite these variations, however, densities of herbivores did vary at broad spatial scales in relation to zoning and predator density, particularly at the Capricorn Bunkers where biomass increased threefold from marine reserves to open zones. This suggests that if variation in predator densities occurs at large spatial scales (e.g. depletion of predators at the reefal scale), this can influence even far ranging species with highly variable distributions.

2.5.2 PREDICTION THREE: TOP-DOWN VERSUS HABITAT EFFECTS

For many species, both top-down processes and habitat variation may interact to regulate populations, and the relative importance of each process may vary amongst species and trophic levels (McQueen et al. 1989; Brown et al. 2013). I predicted that the relative importance of top-down (predation) vs. habitat effects on prey would vary amongst species according to the strength of habitat relationships. That is, habitat specialists would be more likely to be driven by variation in benthic habitats, while generalists would be more strongly affected by predation. I found some evidence of this, however, this varied amongst fish families. For example, within the Pomacentridae (damselfishes), the lemon damsel (*P. moluccensis*) is considered a habitat specialist that predominantly occurs on live hard coral (Booth 2002; Pratchett et al. 2012). Despite these habitat associations, densities of *P. moluccensis* were strongly influenced from the top-down, and predators such as coral trout were strong predictors for the density of this species. Conversely, chaetodontids were most influenced by habitat characteristics, and associated strongly with live hard coral, with no clear detectable predator effects. The relative importance of top-down vs. habitat effects on prey may be reliant on whether habitat is important to a species for shelter, food, or both. Both *P. moluccensis* and chaetodontids associate with live hard coral, however, *P. moluccensis* relies on this resource for shelter, while many chaetodontids also rely on hard corals as a primary food source, as well as shelter (Pratchett and Berumen 2008). Further, their pairing behaviour may also provide protection from predation (Brandl and Bellwood 2013b). The combined characteristics of habitat specialisation, corallivory, and pairing behaviour may make these fishes less vulnerable to predation, and therefore strongly influenced by variations in benthic habitat.

2.5.3 PREDICTION FOUR: PREDATOR-PREY INTERACTIONS AND COMMUNITY LEVEL CHANGE

Changes in the abundance or distributions of apex consumers can cause major shifts in patterns of predation and alter the structure of communities (Hairston and Hairston 1997; Estes et al. 2011). Recognition of these top-down effects led to the development of broad ecological theories to describe global patterns of productivity, such as Hairston's "Green World Hypothesis" (Hairston et al. 1960). At a more regional scale, I predicted that depletion of predators on coral reefs would cause a change in the community composition of prey fishes, and there was strong evidence of this at all four locations. Marine reserves were characterised by a large biomass of piscivores, and a moderate number of herbivores and prey. With increasing fishing pressure, piscivore densities decreased and fish assemblages changed considerably. Numbers of herbivorous fishes increased up to two-fold, constituting a large amount (up to 80%) of total biomass at fished reefs in some locations (e.g. Capricorn Bunkers). Small prey fishes such as pomacentrids also increased greatly in number. From the analyses, habitat had little to do with this change in fish assemblages; instead community level changes were strongly driven by small pomacentrid species and groups. Although the importance of individual interactions between species and habitat cannot be denied, overall changes in species assemblages amongst MPA zones could not be explained by these interactions, and the representation of habitats was similar amongst sampling units. Instead, it is likely that prey release due to predator depletion is responsible for this, as evidenced by the large change in predator biomass amongst zones, and the corresponding but inverse response of prey.

Results from this study support the concept that top-down effects can be strong drivers of prey populations and community structure, even in highly diverse systems. There was strong evidence of top-down control at multiple spatial scales, and for a variety of prey species/groups. This study supports the findings of numerous experimental studies which have highlighted the importance of piscivores in structuring fish communities at small spatial scales (i.e. tens of metres) (Hixon 1991; Hixon and Beets 1993; Connell 1998; Holbrook and Schmitt 2003), and demonstrates that these processes can be scaled up and observed in broad scale field studies spanning kilometres to hundreds of kilometres. Species in diverse systems such as coral reefs and rainforests have evolved a remarkable variety of ecological traits and adaptations that allow them to co-exist in these environments. Prey species on coral reefs are no exception to this, and have evolved a spectacular array of physical, behavioural and physiological adaptations that help them to escape from the diversity of predatory species which threaten them.

Fish may use camouflage (Feitosa et al. 2012), morphological adaptations (Lönnstedt et al. 2013; Robertson 2013), and behavioural responses such as pairing and shoaling to escape predation (Connell 2000; Brandl and Bellwood 2013a). They exhibit an impressive variety of escape responses, and can learn quickly about the dangers of predators and adapt their behaviours accordingly (Lönnstedt et al. 2012). Additionally, the extremely high structural complexity that coral reef mosaics exhibit supplies a seemingly infinite number of shelter holes which help prey to escape from predation (Hixon and Beets 1993; Holbrook et al. 2002). All of these factors may act to dampen top-down effects, and could prevent landscape/seascape level changes from trophic cascades occurring, as is witnessed in lower diversity systems. However, results from this study suggest that top-down effects can be strong drivers of community structure on coral reefs, and that diverse systems may not always be as resilient to trophic perturbations as was previously imagined (Polis and Strong 1996; Loreau et al. 2002).

2.5.4 TOP-DOWN VERSUS CASCADING EFFECTS

Although top-down effects were very strong in this study, there was not unequivocal evidence of these effects cascading down a further trophic level and influencing habitats. Cascade effects must, by definition, involve three or more trophic levels, and generally involve changes in habitat which occur due to interactions between herbivores and predators (Paine 1980; Pinnegar et al. 2000). On the GBR, herbivorous fishes such as scarids (parrotfishes), acanthurids (surgeonfishes) and siganids (rabbitfishes) are important regulators of algal growth, and can mediate competition between algae and coral, facilitating coral survival (Mumby et al. 2006; Hughes et al. 2007a). Conversely, smaller herbivores such as territorial damselfishes may promote algal growth through their "farming" behaviour, whereby they defend and tend to small patches of turf algae (Ceccarelli et al. 2005). Hence the role of herbivorous coral reef fishes in influencing primary production and habitat may be more complex than in other systems. In this study, relationships between piscivores and herbivores occurred whereby herbivorous fishes had greater densities in fished zones where predators were depleted, most likely due to lower juvenile mortality. At the Capricorn Bunkers, evidence for predator/herbivore interactions were further strengthened by the occurrence of direct negative relationships between piscivores such as coral trout, and densities of herbivore functional groups such as scrapers and croppers. Additionally, algal cover was significantly lower in fished areas where herbivore density was high. Hence open zones had fewer predators, more herbivores and lower algal cover, suggesting that top-down effects may be cascading down multiple trophic levels and influencing the benthos at this location. However, it should be noted that other habitat characteristics such as the cover of live soft coral also varied by zone at this location, which is unlikely to be related to herbivores, and that there was no evidence of greater live coral cover in fished areas as a result of lower algal cover. Furthermore, while predators had a strong negative influence on herbivores in the multiple regression models, herbivore/benthos relationships could not be consistently demonstrated in a similar manner. These data allow clear inferences to be made about the importance of top-down effects, and suggest the potential for trophic cascades involving piscivores, herbivores, and algal cover, however, further studies would be required to provide a clear and direct link between herbivores and benthic cover in order to confirm these cascading effects.

This study has provided valuable insight into the importance of top-down effects on coral reef fishes. Other studies around the world have demonstrated top-down and cascading trophic effects on coral reefs, however, these examples typically involve predator effects on grazing urchins, and associated changes in algal cover (McClanahan 1994; Hughes et al. 2007a). Prior studies that have investigated the top-down effects of coral reef fishes using *in-situ* survey data have had varying outcomes (Jennings et al. 1995; Russ and Alcala 1998; Pinnegar et al. 2000). This study found strong, consistent evidence of top-down effects for coral reef fish at multiple spatial scales and for many species and groups, resulting in distinct fish assemblages as a function of fishing and predator removal. The difference between the outcomes of this study and previous studies may be due to the specific nature of fisheries on the GBR, coupled with the existence of a well-managed marine reserve network. Fisheries on the GBR target piscivorous fishes heavily (GBRMPA 2009, 2011), and consistent differences in piscivore numbers were found according to fishing intensity. This situation is in contrast to other studies, which have typically involved fisheries that target multiple trophic levels, and have already begun to “fish down the food web” (Pauly et al. 1998) or involve small, potentially ineffective marine reserves (McClanahan and Shafir 1990; Russ and Alcala 1998; Mumby 2006; Newman et al. 2006).

Results of this study support that of Graham et al. 2003 which demonstrated changes in the density of select prey species between open zones and marine reserves on the GBR. Graham et al. (2003) found consistent trophic effects for six prey species (four pomacentrids, one labrid and one scarid), as well as a direct negative relationship between the pomacentrid *Acanthochromis polyacanthus* and coral trout, however, could not extrapolate this to changes in assemblages, or prey groups overall. The present study has expanded on these initial findings, and indicates that these patterns are much more consistent amongst species, groups and locations than expected. Furthermore, prey release was demonstrated over a gradient of fishing intensity, and over an exceptionally large spatial scale, providing strong evidence that fishing effects occur at the ecosystem level.

2.5.5 MARINE RESERVE NETWORKS AS SCIENTIFIC TOOLS

Results from this study support the use of marine reserve networks as scientific tools for investigating ecological processes. In this study, fishing had a strong impact on piscivore populations, and these impacts were consistent amongst zones at all of the study locations, providing a strong background template to investigate predator-prey interactions. Trophic effects often take decades to eventuate (Babcock et al. 2010), however, the majority of marine reserves surveyed in this study have been in effect for more than 25 years, and despite high natural variation amongst reefs, the effects of fishing and MPA zoning were clear for fishes from multiple trophic groups. The marine reserve network was useful as a natural experiment, and also was an effective impact study to determine the impacts of predator removal. Contrary to the typical BACI model, where sampling is done before the impact (Green 1979), this study was limited to spatial inference as the zoning had been in place for multiple years. However, in this case spatial inference was very strong because the study was done at multiple latitudes over very broad spatial scales and multiple reefs with different zones within latitudes. This broad scale and nested design allowed the investigation of ecological processes at multiple spatial scales, and allowed hypotheses from experiments regarding the importance of predators to be tested in a field scenario. The enigmatic nature of predator effects is such that they are often difficult to detect without manipulation (Estes et al. 2011). In this case, fishing provided consistent manipulation of assemblages by removing piscivores at large spatial scales, however, this would not have been effective if not complemented with well managed marine reserves. Given the limitations of correlation, it is doubtful that the importance of predator-prey interactions could have been detected without this manipulation. Marine reserves are beneficial for conservation, management, recreation and stewardship of our marine resources and biodiversity (Jennings 2000; Lubchenco et al. 2003; Russ et al. 2004; Russ et al. 2008), and these results suggest yet another benefit of the creation of networks of marine reserves; their ability to enhance scientific knowledge and understanding (Carr et al. 2011).

2.5.6 MANAGEMENT IMPLICATIONS

Results from this study hold clear management implications, and reinforce the importance of ecosystem-based management of fisheries and marine reserves. Fisheries on the GBR are considered to be fairly sustainable (GBRMPA 2009, 2011), however, the prevalence of trophic effects found in this study imply that this should be assessed at the ecosystem level rather than just for the targeted species. While the total catch of piscivorous fishes on the GBR may be low in relation to other fisheries worldwide, fishing has caused a change in a key ecological process; predation. Ecological processes must be preserved just as importantly as species are, and overfishing can lead to "ecological extinction" of predators, a state from which it can be difficult to return (Jackson et al. 2001). While at present there is probably little risk that fisheries on the GBR are approaching this point, these data indicate that caution would be judicious and that adaptation of an ecosystem level approach would be most effective for fisheries management. On the other hand, these data also highlight the effectiveness of marine reserves on the GBR in protecting both targeted species, and ecological processes. Importantly, predator-prey interactions changed along a gradient of fishing pressure at some locations in this study, highlighting the validity and utility of having limited fishing zones, which offer an intermediate level of protection, but still allow recreational fishing activities. This study is, to my knowledge, the first to demonstrate such gradient type effects.

While strong trophic effects from fishing were found in this study, it is important to note that these data constitute a subset of locations, without temporal replication. The consistent patterns found in this study from four locations encompassing a large area and latitudinal range suggest that these patterns are likely to occur broadly across the GBR, however, the likelihood of trophic effects occurring may differ amongst individual reefs and in part this may relate to temporal organismal trajectories relating to other impacts. The Great Barrier Reef and other reef systems are under threat from a myriad of stressors such as cyclones, nutrient runoff and sedimentation, and climate change impacts, all of which can have a strong effect on habitats and fish assemblages (Rogers et al. 1983; Hughes 1994; Hughes et al. 2003; Jones et al. 2004; De'ath et al. 2012; Wenger et al. 2012). The current state of reefs is actually the net result of a time series of events as reefs are subjected to perturbations, and decline or recover. Delays in recovery from such stochastic events could add variation to ecological processes amongst locations, especially since major disturbances such as cyclones are often latitude specific (Basher and Zheng 1995). The consistency of trophic effects and ecological interactions amongst latitudes found in this study is remarkable in light of this, however, it is unknown how fisheries-related impacts may interact with other disturbances**.** Cascading trophic effects have been shown to develop over decadal scales, and the frequency of disturbances at some reefs on the GBR may preclude development of such interactions (Babcock et al. 2010). Future research should focus on determining the spatial and temporal generality of trophic effects, as well as their relative importance in disturbed/undisturbed reefs, in order to fully understand the implications of these patterns.

Top-down effects have been demonstrated in multiple terrestrial ecosystems, however, robust predator-related effects have rarely been demonstrated in reef ecosystems. In this multi-spatial scale study I have demonstrated that top-down effects can be important drivers of community structure in complex ecosystems such as coral reefs, and that marine reserve networks can be effective tools for investigating such ecological interactions. Predator removal through fishing resulted in major trophic effects, and marine reserves were effective in restoring and maintaining top-down control. The consistency of trophic interactions found in this study provide compelling evidence of the importance of predators in ecosystems, and hold important implications for the development of ecological paradigms, as well as conservation and management.

CHAPTER THREE: VARIATION IN THE POPULATION DEMOGRAPHICS OF SCOLOPSIS BILINEATUS IN RESPONSE TO PREDATORS

3.1 ABSTRACT

Predatory fishes play critical roles in the trophodynamics of coral reefs, and the biomass of predators can be a strong determinant of the structure of reef fish assemblages. In this study, variations in predator biomass between management zones on the Great Barrier Reef were used to examine how predators influence the biomass, mortality, condition, and reproductive potential of a common prey species *Scolopsis bilineatus* (bridled monocle bream; Nemipteridae). This species is hermaphroditic, and exhibits pre-maturational sex change, so is a suitable study species for investigating the impacts of predators on reproductive biology. Predator biomass was more than three times greater in marine reserves compared to fished areas, however there were no numerical differences in the biomass or mortality of *S. bilineatus.* Despite this, growth rates, size-at-age, condition and reproductive potential were reduced in marine reserves where predator biomass was high. The response of fish to predators was highly sex-dependent, and females suffered the greatest reductions in condition and reproductive potential, particularly from ages 3-11. This study provides strong evidence for the important role that predators play in regulating prey dynamics, and emphasises the importance of understanding top-down control by predators when considering fisheries management techniques and conservation strategies.

3.2 INTRODUCTION

Predators play an integral role in the trophodynamics of ecological systems, and patterns of predation can be a strong determinant of community structure. In marine systems, intensive fishing of apex predators has resulted in systematic reductions of predatory fish populations (Pauly et al. 1998; Jackson et al. 2001; Estes et al. 2011). Significant ecological consequences associated with such predator reductions have served to highlight the important regulatory role of predators in marine food webs

(Babcock et al. 2010; Estes et al. 2011). On coral reefs, predatory fishes (piscivores) can constitute a high proportion of total fish biomass and predator-prey interactions are important in shaping fish communities through time (Hixon and Beets 1993; Sandin et al. 2010). Numerous studies have documented changes in the biomass of targeted predators from fishing practices (e.g. Russ and Alcala 1996; Newman et al. 2006; DeMartini et al. 2008; Sandin et al. 2008; Russ and Alcala 2010), however, fewer studies have considered the potential secondary effects on non-target prey fish species (but see Jennings et al. 1995; Jennings and Polunin 1997; Graham et al. 2003). Top predators such as large groupers, snappers and jacks are generally the most sought after fisheries species, and examples of predator depletion from fisheries impacts are numerous (e.g. Jackson et al. 2001; Dulvy et al. 2004; Salomon et al. 2010; Sandin et al. 2010; Estes et al. 2011). Ecological theory predicts that such reductions in predator abundance should result in increases in prey abundance due to the release of lower trophic levels from predation. Data from Chapter Two in this thesis supported this prediction, and demonstrated the capacity for predatory fishes to exert lethal top-down control on lower level prey species on coral reefs at large spatial scales (Boaden and Kingsford 2015). To further explore this relationship, this chapter focussed on examination of both lethal and sub-lethal top-down effects at a regional scale.

Predators affect prey populations primarily through predation (mortality), but can also influence demographic traits such as growth patterns, size and age structures, condition, and reproductive output (Connell 1996, 1998; Webster 2002; Ruttenberg et al. 2011; Walsh et al. 2012; Davenport and Chalcraft 2013). Variation in these traits may be detectable long before a numeric response in abundance or biomass is evident, and can occur due to variations in prey behaviour due to the presence of a predator (Helfman 1989; Heithaus et al. 2008). When predators are abundant, many small prey fishes exhibit strong anti-predatory behaviours, such as reducing foraging rates, and increasing sheltering behaviour (Heithaus et al. 2008; Madin et al. 2010; Madin et al. 2012). Such “risk effects” can result in reduced net energy intake, if they reduce the energy available for important processes such as growth, energy storage and reproduction (Cooke et al. 2003; Killen and Brown 2006; Heithaus et al. 2008). Although numerous studies have demonstrated the behavioural response of prey fishes to a predator (McCormick and Holmes 2006; Bosiger et al. 2012; Lönnstedt et al. 2012; Mitchell et al. 2013; Rizzari et al. 2014), less is known about the long term (i.e. months to years) sub-lethal effects of predator threat on prey at the population level on coral reefs. Experimental studies conducted on coral patches have demonstrated differential mortality and/or growth for small prey fishes such as pomacentrids due to variation in predator threat (Hixon and Beets 1993; Carr and Hixon 1995; Connell 1996). On larger spatial scales, correlative studies in the Line Islands and at Hawaiian atolls have shown that fishing of top predators can indirectly affect the size and condition of lower level prey species, however, the generality of this trend amongst locations is not known (DeMartini et al. 2005; Ruttenberg et al. 2011; Walsh et al. 2012).

Life history characteristics such as reproductive biology and demography can play a strong role in determining how an individual or population may respond to predator threat. The energy budgets of male and female fish differ, for example, due to the increased amount of energy females require for egg production. As such, the sensitivity of prey species to alterations in energy budgets may vary by sex, with females likely to be more sensitive (Wootton 1979). Coral reefs contain a high proportion of hermaphroditic species, which can have complicated sex changing life-cycles, including protogyny (female to male sex change) and protandry (male to female sex change; Warner 1984; Sadovy de Mitcheson and Liu 2008). Sex change may be functional, whereby an individual functions reproductively first as one sex, and then the other, or pre-maturational, whereby fish contain both male and female sexual organs, but only mature reproductively as either a male or a female (Sadovy and Shapiro 1987; Hamilton et al. 2008; Sadovy de Mitcheson and Liu 2008). Such complex reproductive interactions have the potential to play a strong role in determining how individuals or populations respond to interactions with predators, and a clear understanding of the reproductive biology of prey species is required in order to predict and understand predator effects.

On the GBR, piscivorous fishes such as coral trout, snappers and emperors are important fisheries targets for both commercial and recreational fishers (Taylor et al. 2010; GBRMPA 2011). As a result, piscivore numbers vary significantly according to local fishing pressure, and previous studies have found great differences in piscivore biomass in no-take marine reserves vs. fished zones (Russ and Alcala 2004; Russ et al. 2008). In Chapter Two, I demonstrated the importance of piscivores in regulating prey populations on the GBR, and found strong evidence of prey release in areas where predators were heavily depleted from fishing (Boaden and Kingsford 2015). This variation in predator-prey dynamics has clear conservation and management implications, but also allows the zoning system on the GBR to be used as an experimental template to study the importance of piscivorous fishes in coral reef systems.

The objective of this study was to use variations in predator biomass between management zones as a natural experiment to investigate interactions between predators and prey at the lethal and sub-lethal level. The nemipterid species *Scolopsis bilineatus* was used as a model prey species to investigate these interactions. *S. bilineatus* is an abundant coral reef fish, and has a complex life cycle, which includes pre-maturational sex change (detailed below). One outcome of this reproductive trait is that females tend to exhibit reduced growth trajectories compared to males. The goal of the study was to determine how the reproductive biology of *S. bilineatus* interacted with predator threat to influence demographic processes and overall body condition. Given the focus on piscivorous fishes as fisheries targets, I hypothesised that patterns of predation would differ between management zones. Since prey behaviours tend to be strongly influenced by local predator biomass, I further hypothesised that management zones would present differing levels of perceived predator threat for *S. bilineatus*, and that this variation in threat would affect this prey species at the lethal and sub-lethal level. From this, I aimed to test the following predictions:

1. The biomass of predatory fishes would differ between management zones due to depletion of predators from fishing activities (Aim One);
2. The biomass of *S. bilineatus* would be greater in fished zones due to reduced mortality and/or increased growth rates associated with lower predator biomass (Aim Two);

3. The condition and reproductive potential of *S. bilineatus* would differ between zones due to variation in predation pressure, and the strength of this response would vary by sex (Aim Three).

3.3 METHODS

3.3.1 STUDY DESIGN

Fish surveys and collections took place during November of 2012 at multiple reefs within the Palm Island group, in the central Great Barrier Reef region (18° 30’20.35 S; 152° 05’21.27 E). The Palm Island group includes several inshore islands, and the survey and collection sites used in this study included sites around Orpheus, Pelorus, and Fantome Island (Appendix B). No-take marine reserves and fished zones surrounding these islands were used to compare the biomass of predatory fishes between zones, and investigate the effects of predators on the population demographics of the prey species *S. bilineatus*.

3.3.2 STUDY SPECIES

Scolopsis bilineatus is a mid-sized fish (max size ≈ 20cm) of the family Nemipteridae (monocle breams) inhabiting shallow reef habitats on the GBR (Boaden and Kingsford 2012, 2013). *S. bilineatus* is abundant on reefs in the Palm Island region, and would be a potentially important food source for larger predatory fishes such as coral trout, snappers and emperors, which consume prey of a similar size (Kingsford 1992; Connell 1998). The reproductive biology, demography and distributions of this species in the Palm Island region are well understood (see Boaden and Kingsford 2012, 2013). *S. bilineatus* undergoes pre-maturational sex change, exhibits sex-specific growth rates, and reproduces during the summer months (Boaden and Kingsford 2013). Previous studies provide ample baseline information on demographics and reproductive biology to enable interpretation of how these parameters may be influenced by predators. This species is, therefore, an ideal candidate to investigate the effects of predation on population demographics, particularly with regards to how reproductive parameters such as sex change and sex-specific growth rates can influence predator impacts.

3.3.3 SURVEY METHODS

To estimate the abundance and biomass of fish, as well as the benthic cover in marine reserves and fished zones, underwater visual surveys along belt transects were used. Methods used for visual surveys of fish and benthic habitats were consistent with those described in Chapter Two. Fish surveys were focussed on estimating the density and size (to the nearest 1cm) of predatory fishes and the focal prey species *S. bilineatus*. All reef-associated predatory fishes were counted (total = 42 species), including species targeted by fisheries (e.g. Serranidae, Lutjanidae and Lethrinidae), as well as smaller meso-predators such as *Cephalopholis, Epinephelus* and small predatory labrids. Piscivorous fishes were categorized as such if fishes constituted the majority of their diet (Randall 1967; Kingsford 1992; Froese and Pauly 2013). Benthic cover was estimated using the linear point intercept method, as described in Chapter Two. Fish and benthic cover were recorded along five 25 x 5m transects placed haphazardly in shallow reef habitats at each site. A pilot study revealed significant differences in benthic habitats in exposed vs. sheltered sides of the islands, so survey and collection sites were restricted to the sheltered side, to avoid any confounding habitat effects (Appendix B). Within each of the two zones (fished and marine reserves), five sites were surveyed; sites were separated by hundreds of metres to kilometres.

3.3.4 FISH COLLECTIONS

To investigate the effects of predator biomass on the population demographics of *S. bilineatus*, fish were collected from shallow reef habitats at three sites within each of the two zones. Collection sites were separated by hundreds of metres to kilometres to ensure independence (Appendix B). Sites were selected to be representative of *S. bilineatus* populations within each zone, and contained similar habitats and environmental conditions. This approach enabled a robust comparison of demographic and reproductive parameters between zones. A total of 114 and 108 fish were collected from marine reserve and fished zones respectively. All collections took place after surveys were completed to prevent interference with survey data. Fish were collected using hand spears, and to minimise bias in collections, fish were speared by first encounter, regardless of size. This method ensured that a random sample of fish were collected from sites. Fish were injected with 10% formalin into the visceral cavity immediately after collection to preserve gonad and liver tissue. All tissue samples were preserved in 10% formalin once dissected.

3.3.5 SAMPLE PROCESSING

To estimate age and growth patterns, reproductive maturity and condition of *S. bilineatus* between zones, otoliths, whole gonads, and livers were dissected from collected fish. All fish were measured (total length; mm) and weighed to the nearest 0.1g. Sagittal otoliths were dissected from each fish, cleaned and ground to obtain a thin transverse section through the primordium. One otolith from each fish was sectioned and aged, and annual or daily rings were counted along the longest axis of the otolith section. To remove the possibility of bias, sections were coded so that the identity of each sample was unknown, and all otoliths were analysed by the same observer (myself). To ensure accuracy in age estimates, each section was counted on two occasions, separated by at least one week. If the two counts differed, sections were counted a third time and if after this third time no consistent age was reached, the sample was excluded from analyses.

Whole gonads and livers were dissected from each fish, blotted dry, and then weighed to the nearest 0.001g. Gonads were stored in 10% formalin and transported back to the laboratory to be sectioned for histology. For each gonad, several five-micron sections were taken and stained with Mayer's haematoxylin to produce histology slides. Fish were sexed from examination of these slides, and their stage of reproductive maturity was determined by estimating the percent cover of cell types at each developmental stage, as described in Boaden and Kingsford (2013). The cover of stage 1-4 oocytes (for females) and spermatogonia, spermatocytes, spermatids and spermatozoa (for males) were estimated. This information was then used to categorize fish as immature, resting, ripening (maturing), ripe (mature) or spent (recently reproduced) as described by Webb & Kingsford (1992).

3.3.6 DATA ANALYSIS

S. bilineatus exhibits pre-maturational sex change accompanied with sex-specific growth rates (Boaden and Kingsford 2013), so data for analyses were separated by sex where possible. Data on growth curves, size-at-age, condition indices and reproductive cycles were analysed separately by sex, however, sexes were combined for calculation of mortality rates to provide a large sample size for a robust comparison of mortality rates between zones. The sex of *S. bilineatus* cannot be visually discriminated, so all survey data refers to both sexes combined. For all analyses performed, assumptions of homogeneity of variance were verified using Cochran's test (Underwood 1997). Normality of the data was assessed by visual examination of the distribution of the residuals, and data were transformed when necessary to meet the assumptions of each statistical test.

3.3.6.1 BIOMASS ESTIMATES

Biomass estimates were calculated from estimated lengths using length-weight relationships provided on the online resource FishBase (Froese and Pauly 2013). Nested analysis of variance (ANOVA) was used to compare the biomass of predatory fishes and *S. bilineatus*, as well as benthic cover between marine reserve and fished zones. The factor "zone" had two treatments (marine reserve and fished), and the five survey sites were nested within each of these treatments. Results from Chapter Two indicated that the relationship between zoning and the biomass varied between targeted and non-target predators at the Palm Islands, so analyses were performed on total predator biomass, as well as the biomass of targeted and non-target predators separately.

3.3.6.2 GROWTH AND MORTALITY

Patterns of growth for *S. bilineatus* were described using the von Bertalanffy growth function (von Bertalanffy 1957). The von Bertalanffy growth curve was fitted using the following formula:

$$L_t = L_\infty \left\lfloor 1-e^{-K(t-t_0)} \right\rfloor$$

where $L_\infty =$ the asymptote of the growth curve

$L_t =$ length at age t

$K =$ growth coefficient (rate at which the curve approaches the asymptote)

$t =$ the age of fish (years)

$t_0 =$ theoretical origin of the growth curve

The model was fitted using the methods previously described in Boaden and Kingsford (2013). Growth curves were modelled separately for each sex, and data was separated by zone. Two-way ANOVA (factors= zone and age class) was used to test for differences in growth between zones for each sex, by comparing the mean size of fish within a given age class. Since the sample sizes of fish varied by age class, I used a sub-sample of six fish (for males) and eight fish (for females) per age class for analyses. The instantaneous mortality (Z) of *S. bilineatus* was calculated for each zone using log-linear regression analyses of age-frequency data as per the methods outlined in Kingsford and Hughes (2005). The slope of the regression line between year classes estimated the instantaneous mortality rate (Z). Because there is no fishery for *S. bilineatus*, fishing mortality was assumed to be zero, so Z is an estimate of natural mortality. The slopes of age-frequency relationships were compared using analysis of covariance (ANCOVA), according to the procedures of Zar (1999).

3.3.6.3 CONDITION INDICES

Three metrics were used to compare the condition of *S. bilineatus* between zones: the hepatosomatic index (HSI), gonadosomatic index (GSI) and Fulton's condition factor (K factor hereafter). Formulas for these are as follows:

$$\mathrm{HSI} = \frac{\text{liver mass}}{\text{body mass}} \times 100$$

$$\mathrm{GSI} = \frac{\text{gonad mass}}{\text{body mass}} \times 100$$

$$\text{K factor} = \frac{\text{body mass}}{\text{standard length(mm)}^3} \times 100$$

HSI is a commonly used index for energy reserves, and indicates the mass of fats stored in the liver (Lambert and Dutil 1997; Walsh et al. 2012). GSI can be used as an index for reproductive potential, as it indicates the mass of gonad tissue stored for reproductive purposes (Lloret and Planes 2003; Walsh et al. 2012). The K factor is an index of overall body condition and can be indicative of the total lipid content of fish (Herbinger and Friars 1991; Chellappa et al. 1995; Mozsar et al. 2015). As these condition indices can co-vary with fish length, an analysis of covariance (ANCOVA) was performed on each condition index (factor = zone, covariate = total length of fish). Fish length was not a significant covariate in any of the ANCOVA analyses, which validated the use of the condition indices over the size range of fish collected (Bolger and Connolly 1989). Nested ANOVAs were used to compare condition indices between marine reserve and fished zones. The factor "zone" had two treatments (marine reserve and fished), and the three collection sites were nested within each of these treatments. Data were separated by sex for each analyses, and since the sample sizes of fish of each sex varied amongst sites, I used a randomly selected sub-sample of five fish per site for analyses. To ensure that this sub-sample was representative, the mean of the sub-sample from each site was compared to the mean of all samples. The sub-sample used was found to be representative of the total sample mean for each site.

3.4 RESULTS

3.4.1 BIOMASS AND HABITAT BETWEEN ZONES

Zoning had a significant effect on the biomass of predatory fishes, and total biomass was more than three times greater in marine reserves compared to fished zones (Fig. 3.1 and Table 3.1). This trend was mostly due to the depletion of larger-bodied exploited species such as coral trout and snappers in fished zones; targeted predators had more than a five-fold increase in biomass in marine reserves compared to fished zones (Table 3.1). Although there was a trend for the biomass of smaller, non-target predators to be greater in marine reserves, this pattern was not significant due to high residual variation (Table 3.1). There were no effects of zoning on the biomass of *S. bilineatus*, however, biomass varied greatly amongst sites (Fig. 3.1 and Table 3.1). There were no significant differences in the cover of live and dead coral, and algae between zones, however, benthic habitats varied amongst sites (Table 3.1).

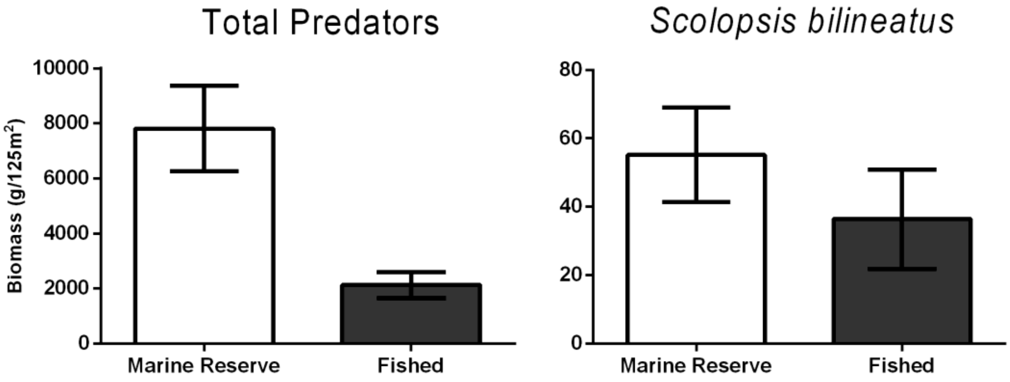

Figure 3.1 Mean biomass (± 1 SE) of total predatory fishes, and *S. bilineatus* in marine reserve and fished zones around the Palm Islands. Data from sites within zones are pooled.

3.4.2 GROWTH AND MORTALITY

Patterns of growth for *S. bilineatus* differed between marine reserve and fished areas for both females and males, which tended to grow faster in fished areas compared to marine reserves (Fig. 3.2 and Table 3.2). Although asymptotic lengths and maximum ages were similar between zones, growth coefficients were higher in fished areas compared to marine reserves for both males and females (Table 3.2). The relationship between zoning and size-at-age differed between sexes. The influence of zoning on the size-at-age of females varied amongst the different age classes, and there was a significant interaction between zone and age class (Fig, 3.3; $F_{(4, 70)} = 2.733$; p= 0.036). Variations in growth were strongest for younger females, which tended to be larger in fished zones compared to marine reserves from ages 3-11; length asymptotes were similar between zones, and the size distribution of older females was more even between zones (Figs 3.2 and 3.3 and Table 3.2).

Table 3.1 Mean values (±1SE) for biomass (g/125m^2), benthic cover (% cover) and condition indices in marine reserve and fished zones (sites pooled), and degrees of freedom (d.f.) and mean squares from analysis of variance (ANOVA) by zone and site. ** p< 0.01; ***p< 0.001

	Mean value (±1 SE)		**Mean squares (ANOVA)**		
Dependant variable	Marine Reserve	Fished	Zone	Site (Zone)	Residual
d.f.			*1*	*8*	*40*
Total predators[1]	7816.33 (±1546.2)	2129.64 (±476.8)	30.421**	3.371	2.267
Targeted predators[1]	6489.27 (±1343.02)	1423.66 (±346.6)	98.457***	9.349	5.374
Non-target predators[1]	1327.06 (±531.9)	626.30 (±122.2)	0.030	5.940	5.818
Scolopsis bilineatus[1]	55.25 (±13.9)	43.40 (±14.3)	7.578	15.210***	3.267
Live coral	50.75 (±2.7)	52.87 (±3.1)	0.006	0.060**	0.014
Dead coral	17.16 (±2.2)	21.77 (±2.7)	0.014	0.041***	0.008
Cover algae	19.22 (±1.8)	23.71 (±2.6)	0.002	0.002	0.001
d.f.			*1*	*4*	*24*
Females- HSI	0.787 (±0.07)	1.164 (±0.06)	0.822**	0.368**	0.059
GSI	2.107 (±0.445)	4.041 (±0.61)	28.045**	15.465**	2.487
K factor	1.53 x10^{-3} (±4.33 x10^{-5})	1.70 x10^{-3} (±3.52 x10^{-5})	0.233**	0.014	0.025
Males - HSI	0.604 (±0.03)	0.750 (±0.05)	0.161***	0.121***	0.008
GSI	0.174 (±0.01)	0.224 (±0.03)	0.339	0.603	0.309
K factor	1.48 x10^{-3} (±4.82 x10^{-5})	1.58 x10^{-3} (±1.0 x10^{-4})	0.073	0.085	0.109

[1] Data log transformed

Male fish also grew more rapidly, and were significantly larger at a given age in fished zones compared to marine reserves (Table 3.2 and Fig. 3.3; $F_{(1,\ 56)} = 15.204$; $p = 0.000$). The relationship between length and zoning was consistent amongst age classes for males, and there was no significant interaction between zone and age class (Fig. 3.3; $F_{(3,\ 56)} = 2.585$; $p = 0.062$). Overall patterns of growth differed between sexes, and males grew more rapidly and reached a larger asymptotic length compared to females (Table 3.2). There were a greater proportion of younger, female fish in marine reserves compared to fished zones, however, the overall age structure of *S. bilineatus* was similar between zones (Fig. 3.4). There were no significant differences in overall mortality rates between zones ($F_{(1,\ 13)} = 4.232$; $p=0.060$); mortality estimates were 0.2192 and 0.2051 in fished zones and marine reserves respectively (Fig. 3.4).

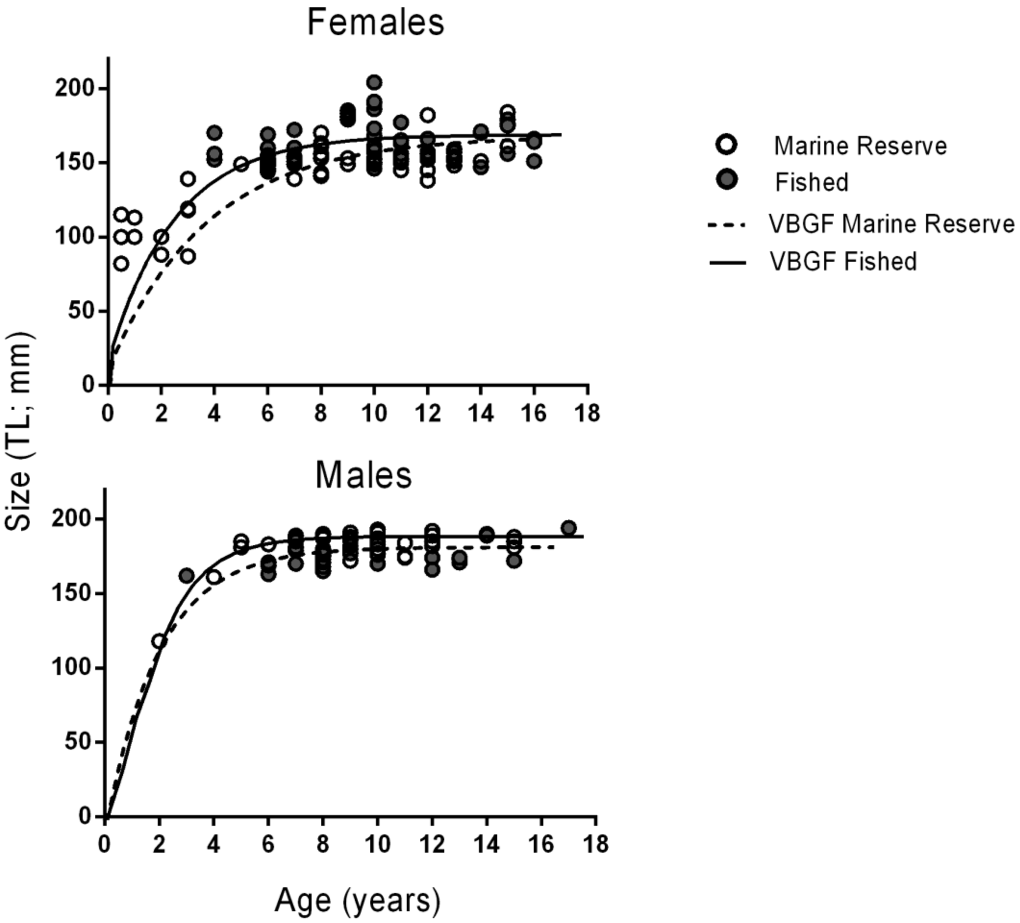

Figure 3.2 von Bertalanffy growth curves of *S. bilineatus* in marine reserve and fished zones, separated by sex.

Table 3.2 von Bertalanffy growth function coefficients and maximum age between zones (sites pooled), by sex.

	Females		Males	
	Marine Reserve	Fished	Marine Reserve	Fished
L∞ (Length asymptote)	163.84	164.82	179.27	183.88
K (growth coefficient)	0.285	0.421	0.521	0.706
Maximum Age	15	16	17	15

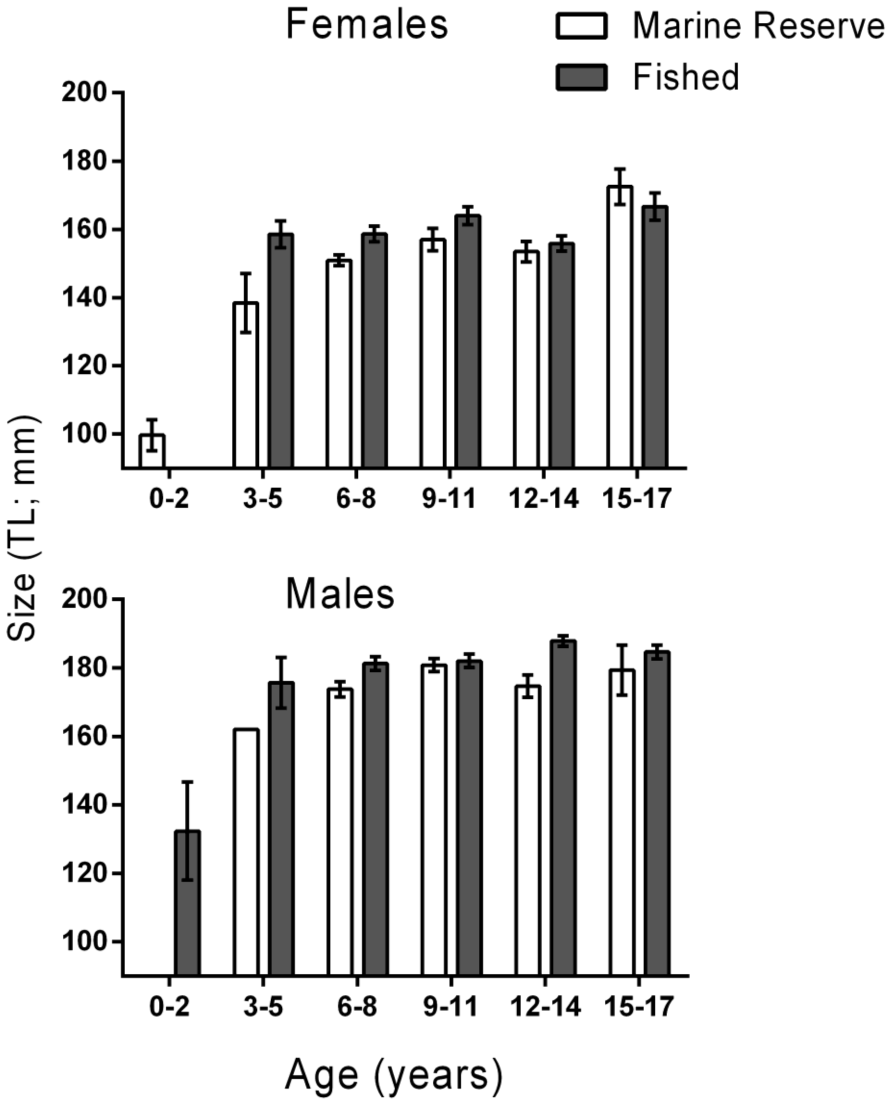

Figure 3.3 Mean (± 1 SE) size of *S. bilineatus* within age categories in marine reserve and fished areas, separated by sex. Data from sites within zones are pooled.

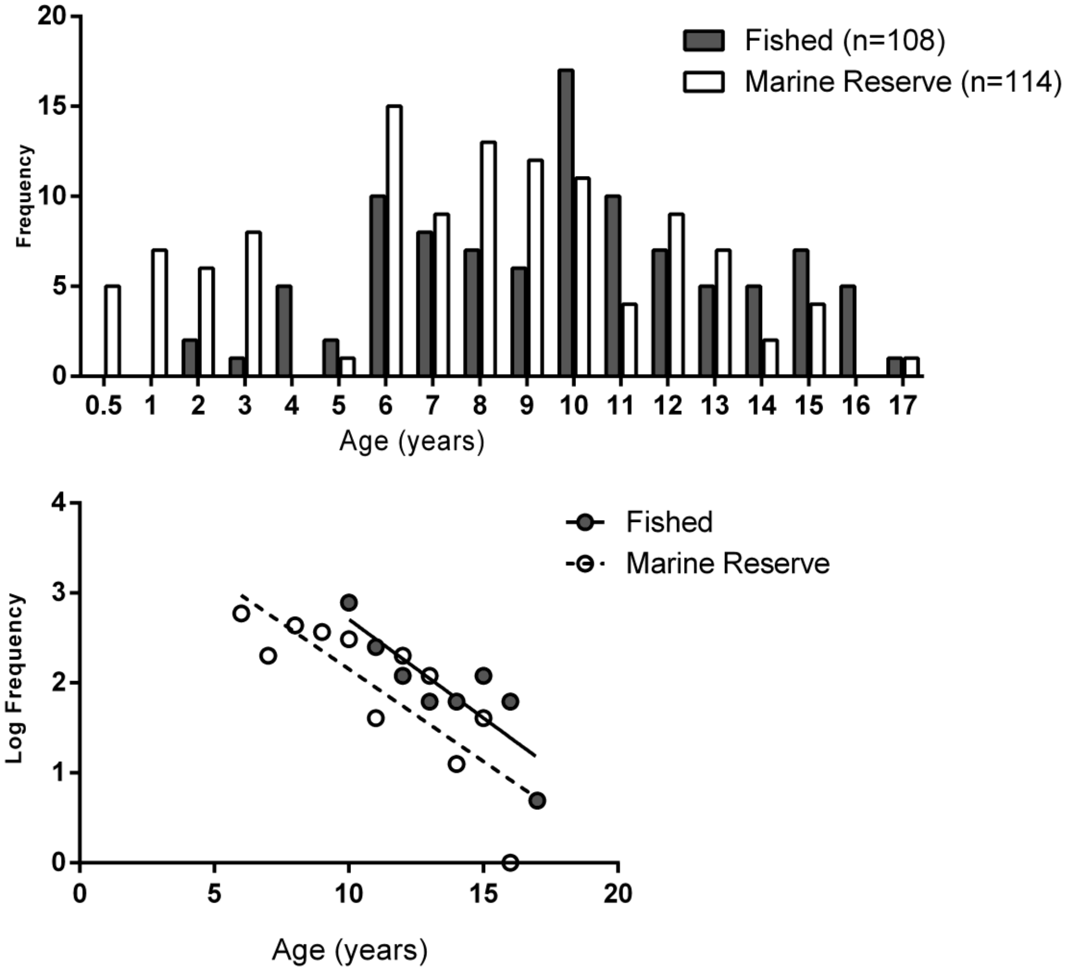

Figure 3.4 Top: Age frequency and bottom: log-linear regressions of age frequency data from *S. bilineatus* collected from marine reserve and fished zones. Data to the left of the age frequency mode have been excluded in the regression analysis to remove sample bias towards larger fish. Data from sites within zones are pooled.

3.4.3 CONDITION AND REPRODUCTION

Zoning had a strong influence on the condition and reproductive potential of *S. bilineatus*, particularly for female fish. The energy reserves (HSI) of both females and males were significantly lower in marine reserves (where predator biomass was greatest) compared to fished areas (Fig. 3.5 and Table 3.1). The reproductive potential of females was significantly reduced in marine reserves, and mean GSI values in fished areas were almost twice that of marine reserves. (Fig. 3.5 and Table 3.1). Males were not similarly affected, and although the

GSI of males was lower in marine reserves compared to fished areas, this pattern was not significant. Similar patterns between zones occurred for body condition; K factor was lower in marine reserves compared to fished areas for both females and males, however, this pattern was only significant for female fish (Fig. 3.5 and Table 3.1). Within zones, there was significant variation amongst sites for the HSI of both sexes, and the GSI and K factor of females.

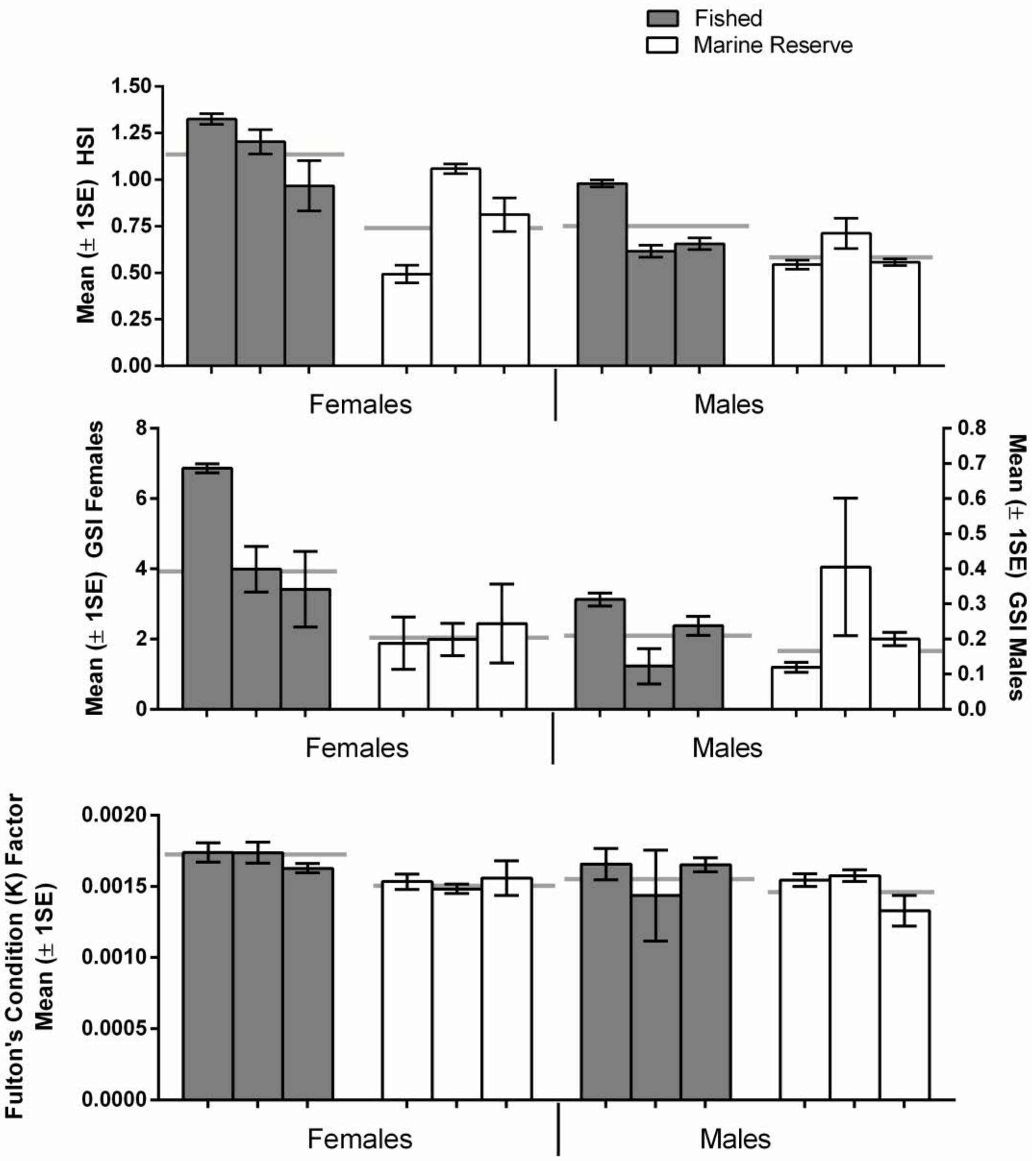

Figure 3.5 Hepatosomatic (HSI) and gonadosomatic (GSI) indices, and Fulton's condition (K) factor of *S. bilineatus* collected from sites within marine reserve and fished zones, separated by sex. Each column represents a collection site; n= 5 fish of each sex per site. Grey bars indicate the pooled mean of each zone.

Zoning also had an effect on the reproductive maturity of females (Fig. 3.6). A high proportion of females were at a sexually mature stage (ripening or ripe) in both zones, and many females showed evidence of recent reproduction (i.e. were spent). A high proportion of females from age four onwards were mature in fished zones, however, in marine reserves only fish aged seven or older were reproductively mature or had reproduced, and none of the 12 fish aged 4-6 were mature (Fig. 3.6). Patterns of reproduction between zones were less clear for males. Unlike females, males were at multiple stages of reproductive maturity at the time of sampling. There was a trend for more males to be sexually mature in marine reserves, however, trends were variable (Fig. 3.6).

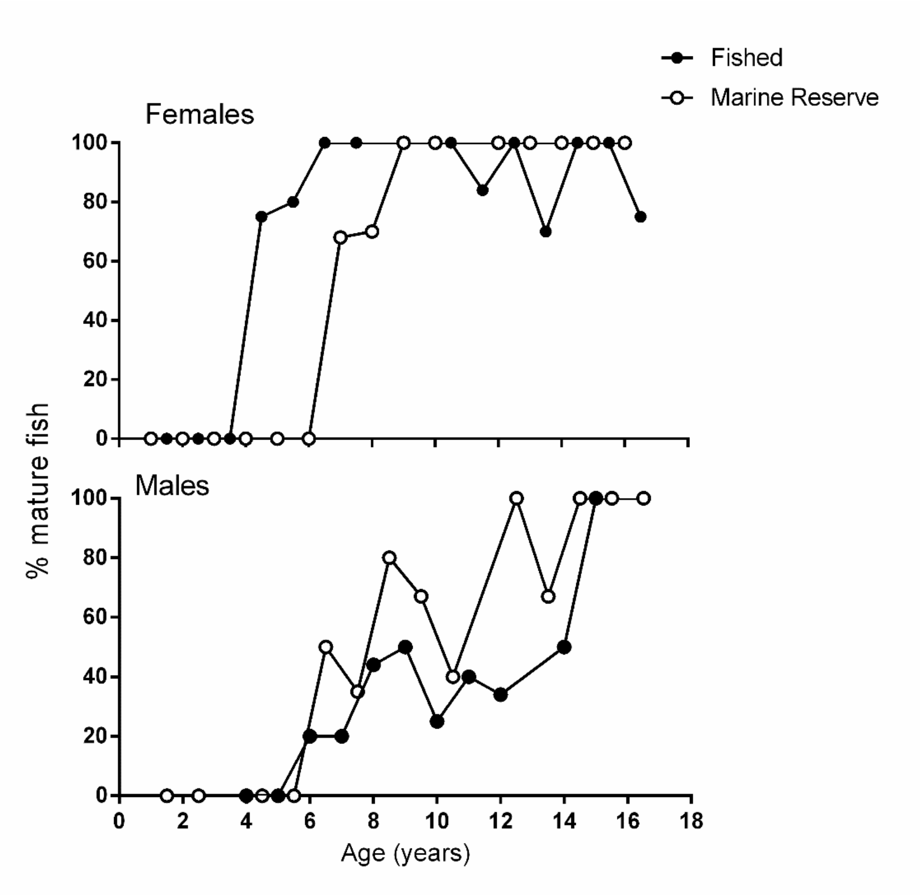

Figure 3.6 Total percentage of mature (ripening, ripe or spent) female and male *S. bilineatus* collected from marine reserve and fished zones, according to age. Data from sites within zones are pooled. Samples sizes within years ranged from 3-6 for females and 2-7 for males.

3.5 DISCUSSION

Predatory fish play important roles in coral reef trophodynamics, and variation in predator densities between management zones on the Great Barrier Reef provides a unique opportunity to examine how patterns of predation can influence key life history traits and demographics of prey. In this study, there were significant differences in the population demographics of *S. bilineatus* between areas of high and low predator biomass, despite a lack of variation in numerical abundance. Although the differences in demographic traits between zones observed in this study cannot be demonstrably linked directly to predator effects, it is highly likely that patterns of predation played a strong role in the demographic differences found, especially given the three-fold increase in predator biomass observed in marine reserves compared to fished areas. It is unlikely that the patterns found happened by chance. Fish surveys and collections took place at multiple sites per zone, and the nested design of the study facilitated a robust comparison of biomass and demographic traits at multiple spatial scales (Kingsford 1998).

Variations in density can cause density dependent mortality and reductions in growth and condition of reef fish, particularly for aggregating species (Booth 1995; McCormick 1998; Holbrook and Schmitt 2002; Boström-Einarsson et al. 2014). In this case, however, it is highly unlikely that the observed demographic differences were attributable to density effects, especially given that the biomass of *S. bilineatus* was similar between zones. *S. bilineatus* adults are strongly site attached, and although adults are commonly encountered, they tend to be well dispersed within shallow reef habitats (Boaden and Kingsford 2012, 2013). As such, mechanisms of density dependence are unlikely to have a strong impact on this species. Habitat characteristics such as the availability of live coral are known to be important determiners for the abundance and diversity of many reef fishes (Luckhurst and Luckhurst 1978; Holbrook et al. 2002), and the quality of habitat can influence demographic processes (Feary et al. 2009). A previous study found some positive associations between *S. bilineatus* densities and the cover of live coral, however, since benthic habitats did not vary by zone, it is unlikely that variations in habitat were the cause of the demographic patterns found (Boaden and Kingsford 2013). Data from this study strongly suggests that variations in predator biomass have resulted in a variety of sub-lethal demographic effects for *S. bilineatus*, and demonstrates the importance of predatory fishes in regulating the population demographics of prey.

Predators can play a strong role in influencing prey densities through top-down control. In Chapter Two, I demonstrated inverse relationships between predatory fishes and their prey on the GBR, including sites around the Palm Islands (Boaden and Kingsford 2015). This data concurred with a previous study by Graham et al. (2003), which found similar effects for predator and prey species at this location. Given this, I predicted that marine reserves would have a higher biomass of predatory fishes, and a lower biomass of *S. bilineatus* due to increased predation pressure and mortality. Although significant differences in predator biomass were found, there were no clear zoning patterns for the biomass of *S. bilineatus* and no differences in mortality rates between zones. These data differ from patterns observed at the Palm Islands for smaller prey species such as pomacentrids, whereby prey abundance was greater in fished areas where predator biomass was lower (Graham et al. 2003; Boaden and Kingsford 2015). The maximum body size of prey species can influence their relative susceptibility to predation, due to the gape limitation of predators (Hambright et al. 1991; Luczkovich et al. 1995; St John 1999). It is possible that *S. bilineatus* may suffer reduced mortality compared to these smaller species, due to their larger maximum body size (approx. 20cm), however, this size range is still well within the size of prey consumed by targeted predators such as coral trout (Kingsford 1992; St John 1999), and newly recruited juveniles (approx. 2cm) would be particularly vulnerable to predation. Despite a lack of numerical response, there was strong evidence for predator-induced demographic shifts between zones, and the growth patterns, condition and reproductive potential of fish differed between zones. Given the importance of growth rates and reproductive output in influencing population dynamics (Hixon 1998; Caselle 1999; Hixon et al. 2012), it is possible that the observed differences in demographic traits will ultimately result in changes in numerical abundance.

The physiological condition of an organism is a major determinant of individual fitness, and can affect patterns of growth, as well as reproductive capability and ultimately survival (Jones and McCormick 2002; Hoey and McCormick 2004; Walsh et al. 2012). The condition and fitness of prey can be strongly influenced by predator biomass and threat if changes in behaviour in response to predators results in lower net energy intake (Helfman 1989; Lima and Dill 1990; Preisser et al. 2005; Madin et al. 2010; Madin et al. 2012). When predator biomass is high, prey may need to allocate more energy to predator avoidance, and may also reduce their energy intake by feeding less, or consuming less nutritious prey (Heithaus et al. 2008). Such predator-induced behavioural modifications can greatly impact energy stores and reduce the amount of energy available for growth and reproduction (Cooke et al. 2003; Killen and Brown 2006; Heithaus et al. 2008). Stress can also directly inhibit reproduction by disrupting hormone pathways responsible for oocyte production or spermatogenesis (Pankhurst and Van der Kraak 1997). I predicted that the condition of *S. bilineatus* would differ between zones due to variation in predation pressure. There was strong support for this prediction across the three key condition proxies, particularly for females. Reductions in condition (K factor) and energy stores (HSI) in areas of high predator biomass mass provides strong evidence to suggest that the presence of predators significantly affected energy acquisition for *S. bilineatus*. Similar patterns have been found in the Line Islands, where the condition of non-target prey species is reduced at isolated atolls with no fishing impact and high predator biomass (Ruttenberg et al. 2011; Walsh et al. 2012). Data from this study adds to the growing body of evidence showing the importance of predators in influencing demographic processes of prey, and the potential indirect effects of fishing predators.

The reproductively biology of an organism can play a strong role in energy allocation, and may influence how the condition of an individual is affected by predator threat. I predicted that *S. bilineatus* would have a sex-specific response to predator threat, and found strong evidence to support this prediction. Females suffered reductions in all three condition indices, and took longer to reach sexual maturity in marine reserves where predator biomass was greatest. The overall maturity levels of females were high, presumably because fish collections were made during a peak reproductive period. The absence of such patterns for male fish is likely due to the lower overall energetic cost of reproduction for male fish, which on average had testes weighing only one fifth the mass of the females' ovaries. For females, predator-induced reductions in condition and energy stores may have led to reductions in the energy available for reproduction, and ultimately reduced reproductive capacity. Reductions in reproductive potential have been documented for coral reef prey fishes in areas of high predator biomass in one previous study in the Line Islands (Walsh et al. 2012), however, the influence of sex on these processes was not investigated. Coral reefs have a high proportion of sex changing fish, and data from this study highlight the importance of understanding how sex change can influence demographic parameters and the response of fish to predator threat.

The influence of 'predator threat' on the reproductive status of fish is not the only factor that could have an impact. Sex change is commonly observed in coral reef fishes, and the reproductive dynamics of sex changing fish can often be influenced by social factors (Warner and Hoffman 1980; Warner and Swearer 1991; Munday et al. 2006). Social interactions can influence sex ratios and reproductive output if sexual maturation and/or sex change is actively supressed or induced by dominant individuals (Jones and Thompson 1980; Hobbs et al. 2004). Intrasex competition can influence local distribution patterns and reproductive dynamics, particularly through active defence of mating territories or spawning sites (Warner and Hoffman 1980; Jones 1981). *S. bilineatus* undergoes sex change, whereby all juvenile fish are immature females, which either undergo pre-maturational sex change to males or continue to mature as females (Boaden and Kingsford 2013). Given that adults are known to form reproductive pairs, and that *S. bilineatus* does not form dense aggregations (Boaden and Kingsford 2013), it is unlikely that social interactions such as intrasex competition were responsible for the variation in reproductive condition observed. Furthermore, the density of *S. bilineatus* did not differ significantly between zones, so it is unlikely that any social influences that may occur would vary systematically by zone. Environmental conditions such as temperature and food availability can also influence the fecundity of teleost fishes (Scott 1979; Wootton 1979), however, since fish collections took place in very similar habitats within a single region it is unlikely that environmental factors were responsible for variation in reproductive condition between zones. These data, therefore, indicate that rather than social interactions or environmental factors variations in predator biomass between zones had a strong influence on reproductive dynamics for *S. bilineatus*, presumably due to energetic trade-offs associated with predator avoidance.

Understanding the ecological impacts of fishing is critical for effective management of fisheries, and the indirect impacts of fishing predators are being increasingly acknowledged as an emerging conservation issue. Ecosystem-based fisheries management requires a detailed understanding of how fishing may affect trophodynamics, and interactions between predators and their prey are a key element of this. This study highlights the variation that can occur between lethal and sub-lethal predator effects for a key prey species. Given such variation, there is a strong case for the inclusion of demographic effects in future studies that consider the trophic impacts of fishing and predator depletion. This study provides valuable insight into sub-lethal predator effects on coral reefs, and suggests the potential for reverse spill over (i.e. from fished to unfished zones) for lower level prey species when growth, condition and reproductive output of prey are enhanced by predator depletion, allowing prey species to be exported out of fished zones. Future studies should consider the potential for such effects on a wide range of species, given the potential implications for connectivity, prey release, and predator-prey dynamics. Understanding the ecological effects of fishing practices, and how they influence the population dynamics of predators and prey will continue to be a critical element in the development and application of ecosystem-based fisheries management.

CHAPTER FOUR: PREDATORS EXACERBATE COMPETITIVE INTERACTIONS AND DOMINANCE HIERARCHIES BETWEEN TWO CORAL REEF FISHES

4.1 ABSTRACT

Predation and competition are critical processes influencing the ecology of organisms, and can play an integral role in shaping coral reef fish communities. This study compared the relative and interacting effects of competition and predation on two competing species of coral reef fish; *Pomacentrus amboinensis* and *P. moluccensis* (Pomacentridae), using a multifactorial experiment. Fish were subjected to the sight and smell of a known predator (*Pseudochromis fuscus*), the presence of a heterospecific competitor (i.e. *P. amboinensis* vs. *P. moluccensis*), or a combination of the two. The sub-lethal effects of predator/competitor treatments were compared with controls; a combination of otolith microstructure analysis and observations were used to determine growth patterns and behaviour. I predicted that the stress of competition and/or predation would result in strong sub-lethal impacts, and act synergistically on growth and behavioural patterns. There was strong evidence to support this prediction, but only for *P. amboinensis,* which suffered reductions in growth in both predator and competitor treatments, with the largest reductions occurring when subjected to both predation and competition concurrently. There was strong evidence of asymmetrical competition between the two damselfish species, with *P. moluccensis* as the dominant competitor, displaying strong aggressive behaviour towards *P. amboinensis*. Growth reductions for *P. amboinensis* in predator/competitor treatments appeared to come about primarily due to increases in shelter seeking behaviour, which significantly reduced the feeding rates of individuals compared with controls. These data highlight the importance of predator/competitor synergisms in influencing key behaviours and demographic parameters for juvenile coral reef fishes.

4.2 INTRODUCTION

Predators play a crucial role in both marine and terrestrial environments, and patterns of predation can be a strong determinant of community structure. Predators may exert top-down control on lower trophic level species through predator-prey interactions, the outcomes of which may be important in shaping communities through time (Hixon and Beets 1993; Beschta and Ripple 2009). Predators influence prey dynamics primarily through predation and direct mortality, however, the presence of a predator may alter the demographic and behavioural traits of prey, resulting a variety of sub-lethal effects. On coral reefs, predatory fishes can play a strong role in regulating prey communities, and key demographic traits such as growth patterns, size and age structures, condition and reproductive output of prey species may be influenced by local predator densities (Connell 1996, 1998; Webster 2002; Ruttenberg et al. 2011; Walsh et al. 2012). Chapters Two and Three of this thesis have demonstrated the capacity for coral reef predators to influence prey species via lethal effects (through variations in density), as well as sub-lethal effects (through variations in population demographics). Such variations in demographic traits are often the result of predator-induced behavioural modifications, which can reduce the availability of energy for growth and reproduction (Cooke et al. 2003; Killen and Brown 2006; Heithaus et al. 2008). When predator biomass is high, prey may need to allocate more energy to predator avoidance, and may also reduce their energy intake by feeding less, or consuming less nutritious prey (Heithaus et al. 2008). Such "risk effects" have been demonstrated in numerous previous studies, which have documented the behavioural and demographic response of juvenile reef fishes to predators (Hixon and Beets 1993; Carr and Hixon 1995; Connell 1996; McCormick and Holmes 2006; Bosiger et al. 2012; Lönnstedt et al. 2012; Mitchell et al. 2013; Rizzari et al. 2014).

Competition can also be a fundamental process shaping communities, as individuals compete for finite resources such as food, mates, or shelter space (Connell 1978). In a competitive interaction, dominant individuals may actively restrict subordinates from accessing resources using aggressive displays, and this process can regulate populations by limiting the capacity of subordinate individuals to grow and reproduce (Cappuccino 1995). This process can result in asymmetrical competition, whereby the subordinate competitor is negatively impacted while the dominant competitor is unaffected (Bonin et al. 2009). In many ecological systems, the processes of competition and predation are tightly linked, and often interact to determine mortality rates and population densities of lower level prey species (Sih et al. 1985; Hixon and Menge 1991; Chase et al. 2002; Hixon and Jones 2005). Interactions between competition and predation may be complex, and the outcomes of such interactions may vary amongst systems, depending on a number of factors such as the limiting resource, the magnitude of predator threat, and the social dynamics of each species (Gurevitch et al. 2000; Chase et al. 2002). Predation may mediate competition amongst prey, by removing individuals and preventing complete dominance of one individual or species, especially when space is the limiting resource (Sih et al. 1985; Gurevitch et al. 2000). Such interactions have commonly been observed in intertidal systems, where competition occurs primarily for optimal position within the intertidal zone (Dayton 1971; Sih et al. 1985; Wootton 1992). Alternatively, predation may increase the intensity of competitive interactions, particularly when species compete for access to predator-free shelter sites (Jeffries and Lawton 1984). Competitive interactions can also increase the vulnerability of small-bodied species to predation, particularly when growth rates are inhibited by competitive processes. Such effects are commonly observed for teleost fishes, where size-selective predation is often observed, and growth and condition are critical factors which influence survivorship of prey species (Sogard 1997; Figueira et al. 2008)

In coral reef fish communities, the combined processes of predation and competition are critical to population regulation (Carr et al. 2002). For coral reef fishes, competitive interactions can be particularly critical to survival, as species compete for shelter space and/or access to critical food resources (Holbrook and Schmitt 1989, 2002). Competitively dominant individuals may increase the mortality of subordinates by restricting access to key shelter sites, thereby increasing predation risk (Hixon and Menge 1991; Holbrook and Schmitt 2002; Hixon and Jones 2005). Competitively dominant group members may gain access to optimal foraging positions, and prevent subordinates from accessing food resources, which can have major consequences for growth rates (Coates 1980; Forrester 1991). Given the importance of a size advantage in avoiding predation, variations in growth rates can have major consequences for survivorship (Sogard 1997; Figueira et al. 2008; Holmes and McCormick 2010a).

The processes of predation and competition may play a particularly strong role in shaping coral reef fish communities, as juveniles are vulnerable due to their bipartite life cycle (Hixon and Beets 1993). As an individual transitions from its pelagic larval stage, to its demersal reef-associated stage, it is vulnerable to a host of predatory fishes, and mortality can be very high in the early life stages (Sale and Ferrell 1988; Holbrook and Schmitt 2003). Surviving fish must then compete for critical resources such as food and shelter space, and competition amongst conspecifics and heterospecifics can be intense, with dominance hierarchies forming quickly after settlement (Jones 1988; McCormick and Weaver 2012). This early post-settlement stage is a critical time for development for small coral reef fishes as reductions in key demographic parameters such as growth rates can influence life time survivorship and reproductive output (McCormick 1998). Since such variations can ultimately regulate prey populations at the community level, understanding the factors influencing demographic processes at these early life stages is a critical component of coral reef ecology.

The outcomes of an interaction between a prey species and a competitor or predator may depend on the behavioural response to each, and the ultimate effect that this may have on demographic processes. When faced with a predator, an individual may exhibit a stress response, increasing their metabolism and exhibiting risk-averse behaviours such as feeding less and sheltering more (Holmes and McCormick 2011; Lönnstedt et al. 2012). In this situation, prey face a trade-off between optimising foraging efficiency, and reducing mortality by sheltering from predators. The presence of a competitor may further complicate this behavioural response, and could either exacerbate or lessen the impact of the predator. The outcomes of this trade off may be influenced by dominance hierarchies, particularly if dominant fish prevent subordinates from gaining access to key resources. In order to understand the effects of predators and/or competitors on prey, the behavioural response of individuals to each stressor, as well as the effects of behaviour on demographic traits such as growth must be determined.

The role of predation and competition in shaping coral reef fish communities has been a key concept debated by ecologists for some time. Numerous studies have documented the effects of either competition or predation on the mortality of juvenile coral reef fishes (Connell 1997) and have explored relationships between predation and density dependence (Holbrook and

Schmitt 2002; Hixon and Jones 2005). Comparatively less is known about the interaction between predation and inter-specific competition for coral reef fishes, particularly with regards to sub-lethal effects such as growth rates. The present study sought to investigate the role of predation and inter-specific competition in influencing the growth and behaviour of two competing damselfish species; *Pomacentrus amboinensis* (ambon damsel) and *P. moluccensis* (lemon damsel). Both of these species showed patterns of prey release from Chapter Two, whereby abundances were greater in fished areas (where predators were depleted) compared to marine reserves (where predators were abundant). These effects were particularly strong for *P. moluccensis*, which showed consistent patterns of prey release at all four locations surveyed.

Since the early life history stages of coral reef fishes can be critical in determining lifetime survivorship and fitness, this study focused on demographic rates and behaviour during the juvenile life stage. The initial predictions were as follows: (1) the presence of a predator or, (2) a heterospecific competitor would result in decreased growth, and changes in feeding behaviour and general activity patterns for juvenile prey and, (3) the presence of both a predator and heterospecific competitor would exacerbate the aforementioned sub-lethal effects. To address these predictions, the specific aims were as follows:

1. Investigate the impacts of predator threat and interspecific competition on the growth of *P. amboinensis* and *P. moluccensis;*
2. Investigate competitive interactions between *P. amboinensis* and *P. moluccensis* and examine competitive behaviours and dominance hierarchies;
3. Compare the relative and interacting effects of predation and interspecific competition on behaviour such as feeding rates, and activity patterns for *P. amboinensis* and *P. moluccensis.*

4.3 METHODS

The objective of the study was to experimentally test the effects of predator threat and interspecific competition on two species of common damselfishes. Since early post-settlement constitutes a critical time for growth of reef fishes, I focussed on predator/competitor effects on juvenile damselfishes. To do this, three species of fish were used in the experiment: juvenile *Pomacentrus amboinensis* (ambon damsel; prey species one), juvenile *P. moluccensis* (lemon damsel; prey species two), and adult *Pseudochromis fuscus* (yellow dottyback; predator). These three species have been used extensively in behaviour-focussed predator-prey experiments, where juveniles of both damselfish species have been reported to exhibit a behavioural response to the sight and smell of *P. fuscus*, the latter being a voracious predator of juvenile damselfishes (Holmes and McCormick 2011; Mitchell et al. 2011; Bosiger et al. 2012; Feeney et al. 2012). *P. amboinensis* and *P. moluccensis* are both small, common, site-attached damselfishes (Pomacentridae) which often co-inhabit coral patch reefs, and may compete for key resources such as food and shelter, particularly in the juvenile stages (McCormick and Weaver 2012). *P. fuscus* (Pseudochromidae) is a small piscivorous predator, and may inhabit patch reefs alongside them, feeding opportunistically on new recruits and juveniles (Feeney et al. 2012). *P. fuscus* has been used extensively in experimental trails, where predation on both *P. amboinensis* and *P. moluccensis* has been commonly observed in aquarium environments (Figueira et al. 2008; Holmes and McCormick 2010a).

This study was conducted from December 2013 to January 2014 at Lizard Island Research Station, at the northern end of the Great Barrier Reef, Australia. All fish were caught from shallow patch reefs at multiple sites around Lizard Island using diluted clove oil and hand nets. Small pieces (≈ 5 x 5 x 5cm) of live *Pocillopora damicornis* (cauliflower coral) were obtained from similar sites using chisels, to use for shelters in the experiment tanks. *P. damicornis* is an abundant coral species around Lizard Island, and commonly inhabited by both damselfish species. Coral pieces were carefully selected to be of similar size and structural complexity. After collection, all fishes/corals were transported immediately back to the research station, and held in flow through aquaria for at least four days before being used in the experiment, to allow them to acclimate to the experimental conditions. All *P. amboinensis* and *P. moluccensis* measured 13-17mm SL, and *P. fuscus* measured 75-90mm at the commencement of the experiment. As both damselfish species were captured off the reef and had likely been there for 20-30 days based on their size, I assumed that they would be familiar with the sight and scent of reef predators such as *P. fuscus. P. amboinensis* and *P. moluccensis* were fed twice daily with 5 ml (per fish) of concentrated *Artemia (*≈ 600 *Artemia* per mL) in all experimental treatments, and *P. fuscus* individuals were fed two damselfish recruits morning and night throughout the experimental and holding period. This is an approximate representation of what *P. fuscus* would consume in the wild (Feeney et al. 2012), which ensured that the predator stimulus was realistic.

4.3.1 EXPERIMENTAL SETUP

The aquarium layout was designed so that prey species could both see and smell the predator, but could not be accessed by it. To achieve this, small experiment tanks (LxWxH = 20 x 10 x 10cm) were placed inside larger, opaque holding tanks (LxWxH =43 x 32 x 31cm) which received flow-through ambient seawater. The smaller, experiment tanks were made of transparent plastic and contained vents, which allowed water to flow freely between the two tanks (Appendix C). The experimental tanks housed the prey species, along with their coral shelter, while the holding tank either contained the predator, or was empty, according to the treatment type. This arrangement allowed both predator and prey to easily see and smell each other, but prevented the predator from accessing and consuming the prey (Appendix C). Competition treatments were created by adding either a conspecific or heterospecific to the experimental tanks (as outlined below). A feeding tube made from soft tubing was attached to the top of each experimental tank, which allowed the *Artemia* to be injected into the tank from a distance, so that the experimenter was not seen, and fish were not disturbed during behavioural trials. The feeding tube was used for the duration of the experiment.

4.3.2 EXPERIMENTAL DESIGN

The aim of the experiment was to test A) the effects of a predator, and B) the effects of a heterospecific competitor on the growth and behaviour of *P. amboinensis* and *P. moluccensis*. To achieve this, a fully orthogonal two-factor design was used for each species. Factors were predator presence (two levels) and competitor presence (three levels; Table 4.1). The orthogonal design comprised of 10 total treatments, with six replicate experiment tanks per treatment (Table 4.1), and the experiment was run for a total of 19 days. This time period is biologically relevant, given the importance of growth during the first few months post-settlement. I hypothesised that if experimental treatments had a strong impact on growth, then variations in growth trajectories would be detectable within this time period. Treatments were randomised amongst tanks, and the allocation of captured fish from different patch reefs was randomized so that each treatment contained a random sample of fish. This method ensured that local variations in predator and/or competitor interactions did not confound experimental treatments. Details of each treatment are outlined below (PA = *P. amboinensis* and PM = *P. moluccensis*).

4.3.2.1 COMPETITION TREATMENTS

In order to test for the effects of interspecific competition, three competition treatments were used for each species as follows: 1) No competitor (PA1 and PM1); 2) paired conspecifics (PA2 and PM2); and; 3) paired heterospecifics (PA1:PM1). The no competitor treatments contained a single fish and were used to test for predator effects only. Paired treatments contained two fish, which inhabited the experimental tank together, and so could directly interact. Paired conspecific treatments were used as a control for density, so that equal densities occurred between paired conspecific and paired heterospecific treatments. This allowed the effects of the heterospecific competitor to be separated from any effects that may be attributable to changes in density, and not to the identity of the competitor *per se* (see Table 4.1). All tanks contained a single *P. damicornis* fragment. Pairs were size matched to 0.1 mm (SL) to remove any effect of a size-advantage on competitive outcomes.

4.3.2.2 PREDATOR TREATMENTS

Two predator treatments (predator present and absent) were used to test for the effects of predator presence on the two prey species (Table 4.1). A single *P. fuscus* was added to the holding tank for each of the predator present treatments, such that it could swim freely around the smaller experimental tank. *P. fuscus* were rotated amongst predator treatment tanks every four days, to remove potential bias associated with individual traits of any predator. To further enhance the predator experience, *P. fuscus* were fed freshly killed juvenile pomacentrids throughout the experiment. Where possible, *P. fuscus* were fed conspecifics (according to each treatment type), with the skin lacerated to ensure that the water was scented with chemical alarm cues (Mathuru et al. 2012).

Table 4.1 Sampling design of experiment, with treatment names as they are referred to throughout the text. PA= *Pomacentrus amboinensis*, PM= *Pomacentrus moluccensis.*

		Fish per replicate tank		Purpose of treatment		
Treatment name	**Predator**	***P. amboinensis***	***P. moluccensis***	**Controls for**	**Tests for**	**Replicates**
No predator PA1	No	1	0	Predator	-	6
No predator PA2	No	2	0	Predator and competitor	-	6
No predator PM1	No	0	1	Predator	-	6
No predator PM2	No	0	2	Predator and competitor	-	6
No predator PA:PM	No	1	1	Predator	Competitor	6
Predator PA1	Yes	1	0	-	Predator	6
Predator PA2	Yes	2	0	Competitor	Predator	6
Predator PM1	Yes	0	1	-	Predator	6
Predator PM2	Yes	0	2	Competitor	Predator	6
Predator PA:PM	Yes	1	1	-	Predator and Competitor	6
					Total	**60**

4.3.3 BEHAVIOURAL OBSERVATIONS AND DOMINANCE HIERARCHIES

4.3.3.1 OBSERVATION PROTOCOL

To evaluate the influence of predator and competitor treatments on prey, behavioural observations were undertaken on day 17 of the experiment. The behaviour of fish in each experimental tank was recorded using GoPro cameras, placed inside the holding tank and facing the experimental tank. Behaviour was recorded for a total of seven minutes, including an initial one minute acclimation period, to allow fish to settle from any disturbance caused by adding the camera to the tank. After this initial minute, recording continued for a further three minutes before food was discretely added to the tank using the feeding tube; recording continued for another three minutes post-feeding. This method allowed behaviour to be recorded for three minutes before and three minutes after feeding. Each recorded video was then watched by the same observer, and the following information recorded: bites taken per minute, total bites (for the three minute observation period), and the activity of the fish (swimming, sheltering, or feeding) every 10 seconds. Feeding behaviour was only recorded in the post-feeding time period; all other behaviours were recorded over the full six minute period. Fish were recorded as sheltering if they were stationary at <1cm from their coral shelters. An average was taken from the 36 time points during the recording to calculate the percentage time spent engaging in each activity for each fish. Agonistic/aggressive interactions (defined as a nip or chase) were recorded per minute in the competition treatments. For each interaction, the individual initiating the nip or chase was recorded, as well as any associated avoidance behaviour.

4.3.3.2 DOMINANCE HIERARCHIES

Establishment of dominance hierarchies is an important component of competitive interactions for coral reef fishes (Forrester 1991). In this study, dominant and subordinate individuals were identified through behavioural observations, and I used associated growth data to test for asymmetrical competition. For paired competitors, each individual was defined as either dominant or subordinate as follows: *Dominant* fish initiated the majority of aggressive interactions (i.e. chases or nips) during competitive interactions. *Subordinate* fish exhibited avoidance behaviour during competitive interactions. Overall dominance hierarchies of the two damselfish species were determined by comparing the outcomes of competitive interactions, as well as associated feeding and movement patterns for each species across all replicates. Dominance hierarchies from behavioural data were compared to growth data to detect evidence of asymmetrical competition. *Asymmetrical competition* was defined as a reduction in a key trait (i.e. growth) for a subordinate species, with no detectable effect on the dominant species. Asymmetrical competition was examined by comparison of growth trajectories for each species when in the presence/absence of a heterospecific competitor. Growth patterns for paired heterospecifics were always compared to paired conspecifics to remove the confounding effect of fish density.

4.3.4 GROWTH EFFECTS: OTOLITH INCREMENT WIDTH ANALYSIS

An important component of this study was the use of otolith microstructure to elucidate sub-lethal impacts of predators and competitors by measuring growth through time. Otoliths are calcium carbonate structures which aid in balance and orientation by fish, and accumulate growth rings on a daily and annual basis (Green et al. 2009). Measurement of the distance between daily rings can give an accurate representation of the somatic growth of the individual on a day-by-day basis (Pitcher 1988; Thresher 1988; Fowler 1990; Kingsford et al. 2011). This innovative technique can be very useful for obtaining time integrated information on growth effects, and can be used to demonstrate impacts on growth from experimental treatments over time. I used daily increment widths from the otoliths of *P. amboinensis* and *P. moluccensis* to measure the growth of individuals during the experiment. Otolith growth is related to somatic growth, and the width of daily increments within the otolith is a commonly used and reliable proxy for growth (Pitcher 1988; Thresher 1988; Fowler 1990; Kingsford et al. 2011). This approach is preferable to using direct measurements of somatic growth since otolith increments give a daily representation of the biological response of the fish to experimental treatments, whereas somatic growth could only be measured at the beginning and end of the experiment.

At the conclusion of the experiment, fish were sacrificed using an ice water bath, measured (SL) to the nearest 0.1mm and their otoliths extracted. Sagittal otoliths were removed, cleaned and ground to obtain a thin transverse section through the primordium. Samples were coded so that the identity of the samples was unknown when measuring increment widths. Otolith sections were then polished until the daily rings were clear, and the daily increment widths (i.e. distance between rings) corresponding to the experimental period were measured using a calibrated computer program.

4.3.5 ANALYSES

The cumulative daily otolith increment widths of each individual were calculated, and used to compare growth of the prey species during the experimental period. Repeated measures analysis of variance (RMANOVA) were used to compare growth trajectories over time in the predator and competitor treatments. Single factor RMANOVA was used to test for the effects of the predator or competitor, and two-factor RMANOVA was used to compare growth trajectories according to predator and competitor treatments. To test for the effects of interspecific competition, growth trajectories for paired conspecific treatments (i.e. PA2 or PM2; density control) were compared against paired heterospecifics (PA1:PM1). Two sample t tests were used to compare the behaviour of *P. amboinensis* and *P. moluccensis* amongst experimental treatments. T tests were used to compare differences in feeding rates, and activity patterns between predator treatments (PA1 and PM1), between species within competition and predator treatments (PA1:PM1), and to compare aggressive interactions between species (PA1:PM1).

I hypothesised that competitive behaviour (either aggression or avoidance) may impact the ability of prey species to feed. To test this, linear regression was used to examine the relationship between bite rates (total bites) and competitive behaviour (aggressive interactions initiated and avoidance behaviour) for both species. Data were pooled between species for the regression analysis to determine the overall feeding consequence of the competitive behaviours. Assumptions of normality and homogeneity of variance were tested using Cochran’s test, as well as visual examination of the distribution of the residuals; data were transformed when necessary. Multivariate tests (Pillai's trace) were used for the within component of the RMANOVA tests because they are more robust to violations of the assumptions of RMANOVA.

4.4 RESULTS

4.4.1 GROWTH

4.4.1.1 EFFECTS OF PREDATOR ONLY

The presence of the predator (*P. fuscus*) had a significant effect on the growth of *P. amboinensis* throughout the experiment (Fig. 4.1A). Growth was reduced in the presence of the predator, and growth trajectories differed significantly between the predator treatments [RMANOVA (Day x predator treatment; $F_{(18,180)} = 2.354$, $p=0.002$; Fig. 4.1A]. Conversely, the presence of the predator had no detectable effect on the growth of *P. moluccensis*, and growth trajectories between predator treatments were similar over time [RMANOVA (Day x predator treatment) $F_{(18,180)} = 0.080$, $p=1.000$; Fig. 4.1B].

4.4.1.2 EFFECTS OF COMPETITOR ONLY

The presence of the heterospecific competitor (*P. moluccensis*) had a significant negative effect on the growth of *P. amboinensis* (Fig. 4.2A). Growth trajectories were significantly lower in the paired heterospecific treatment (PA1:PM1), compared to the paired conspecific (PA2; density control) treatment [(RMANOVA (Day x competitor treatment) $F_{(18,180)} = 11.390$, $p<0.001$; Fig. 4.2A]. Conversely, growth of *P. moluccensis* was not negatively affected by the interspecific competition treatment. Although there was a trend for growth to be slightly greater in the paired heterospecific (PA1: PM1) compared to paired conspecific (PM2; density control) treatments, this was not significant [RMANOVA (Day x competitor treatment) $F_{(18,180)} = 8.390$, $p<0.925$; Fig. 4.2B].

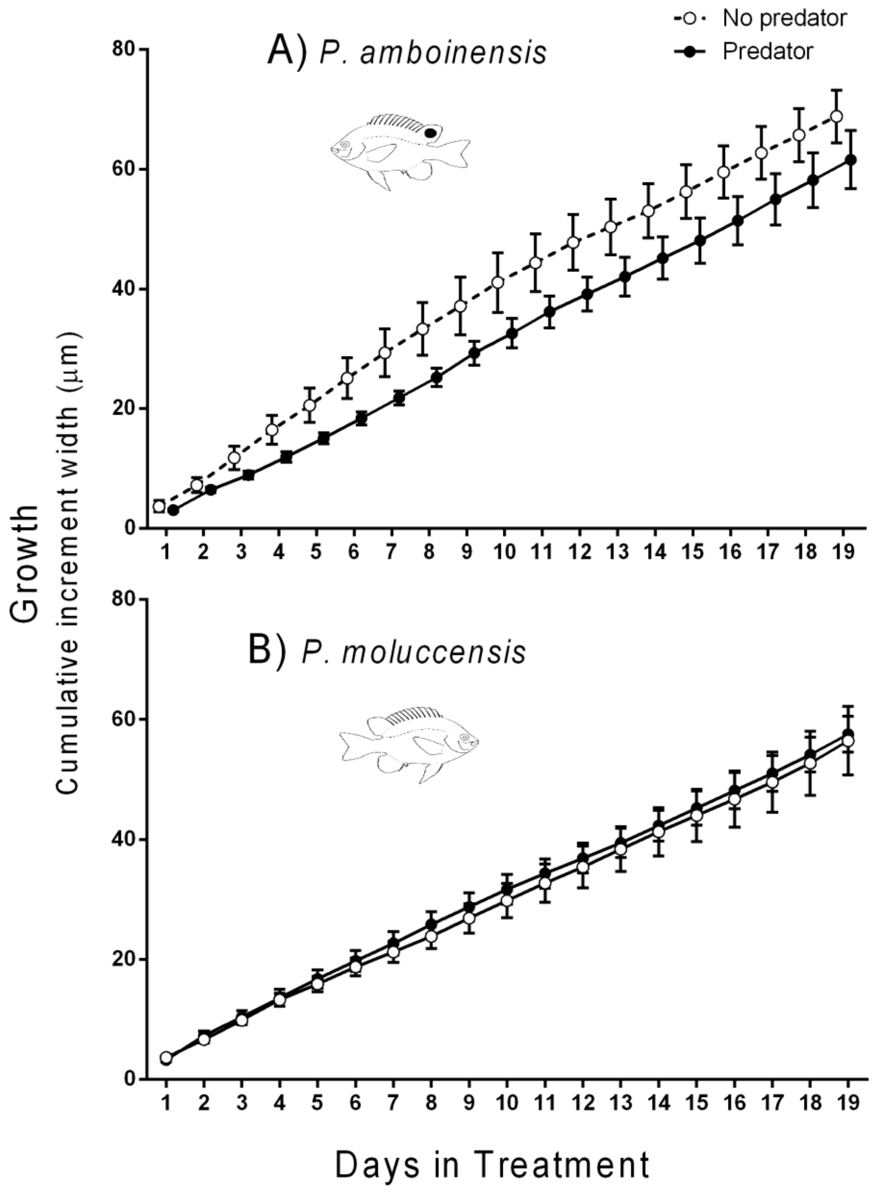

Figure 4.1 Growth (mean cumulative otolith increment width ±1 SE) of A) *P. amboinensis* and B) *P. moluccensis* during the experimental period according to predator treatment. All data are for single fish (PA1 and PM1) only with no competitor present.

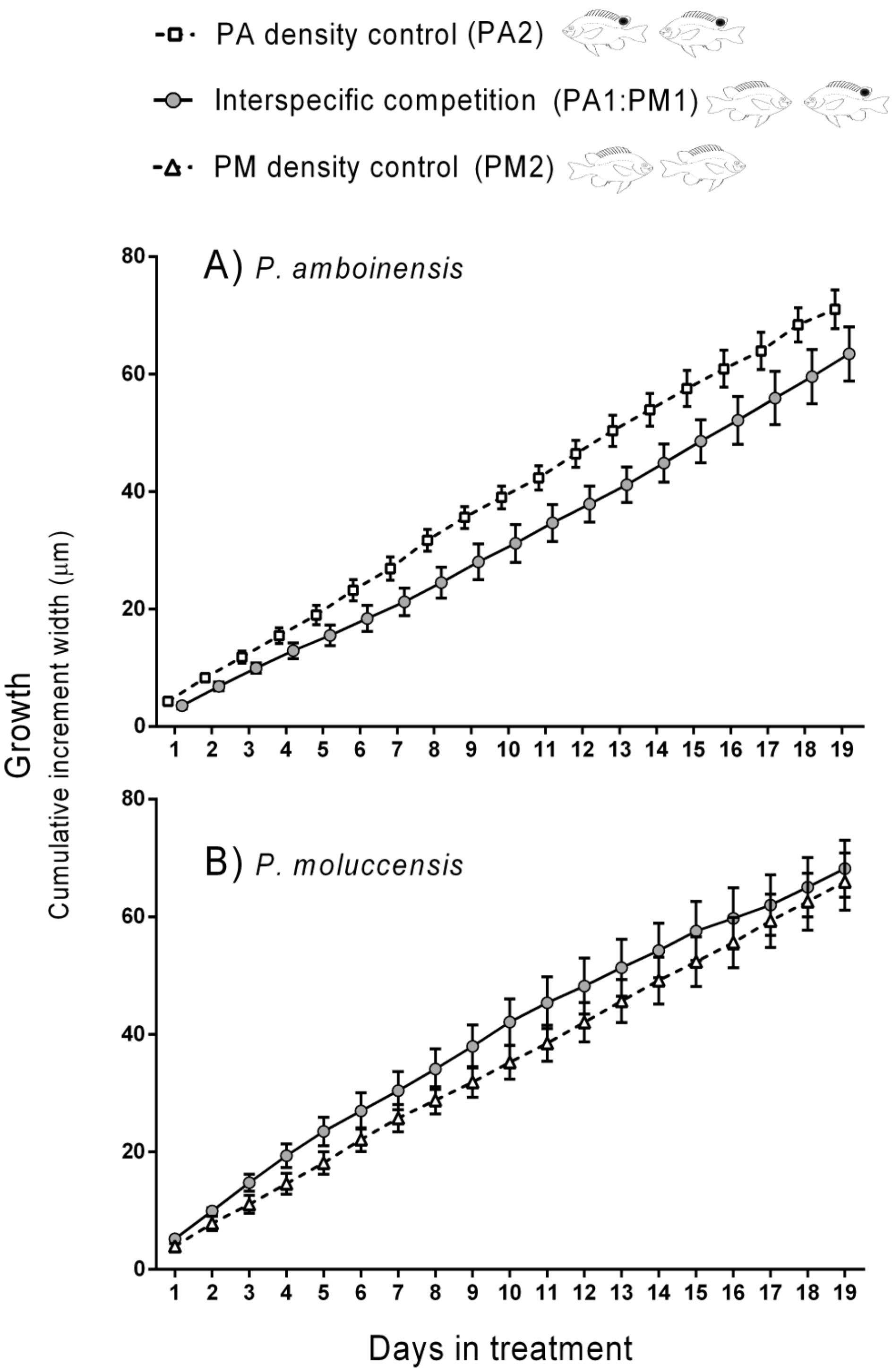

Figure 4.2 Growth (mean cumulative otolith increment width ±1 SE) of A) *P. amboinensis* and B) *P. moluccensis* during the experimental period according to competitor treatment, with no predator present. Paired conspecific treatments (PA2 and PM2) are density controls for the paired heterospecific (interspecific competition) treatment (PA1:PM1). PA= *P. amboinensis,* PM= *P. moluccensis*

4.4.1.3 INTERACTIVE EFFECTS OF PREDATOR AND COMPETITOR

The growth of *P. amboinensis* was affected by interactions between the presence of the predator and interspecific competitor (*P. moluccensis*). In both the interspecific competition treatment (PA1:PM1), and the density control treatment (PA2) growth of *P. amboinensis* was lower in the presence of the predator. The effect of the predator, however, varied amongst competition treatments (Fig. 4.3A). The magnitude of difference between predator treatments was greater when *P. amboinensis* was paired with the heterospecific competitor (PA1:PM1) compared to the density control (PA2; Fig. 4.3A). As such, there was a significant interaction between predation and competition, and growth was lowest when both the predator and interspecific competitor were present [RMANOVA (predator treatment x competitor treatment); $F_{(2, 30)} = 5.895$ $p = 0.007$]. The effect of this interaction varied over time, and growth trajectories between predator treatments in the interspecific competition treatment became more disparate throughout the experimental period [RMANOVA (day x predator treatment x competition treatment); Pillai's trace $_{(36, 28)} = 2.689$, $p = 0.004$]. Thus, the effect of the predator on growth of *P. amboinensis* was exacerbated by the presence of *P. moluccensis*, and the interacting effects of these treatments increased through time (Fig. 4.3A).

In contrast, *P. moluccensis* did not experience significant reductions in growth due to interactions between the heterospecific competitor and predator (Fig. 4.3B). Although there were significant differences in the growth trajectories amongst competition treatments through time [RMANOVA (day x competition treatment); Pillai's trace $_{(36, 28)} = 2.552$, $p=0.006$], growth was actually greater in the heterospecific competition treatments (PA1:PM1) compared to the density control (PM2; Fig. 4.3B). This suggested that *P. moluccensis* was receiving a growth benefit from being in the heterospecific competition treatment. Within competition treatments, growth was lower in the presence of the predator, but the overall effect of the predator on growth trajectories was not significant [RMANOVA (day x predator treatment); Pillai's trace $_{(18, 13)} =2.223$, $p=0.074$].

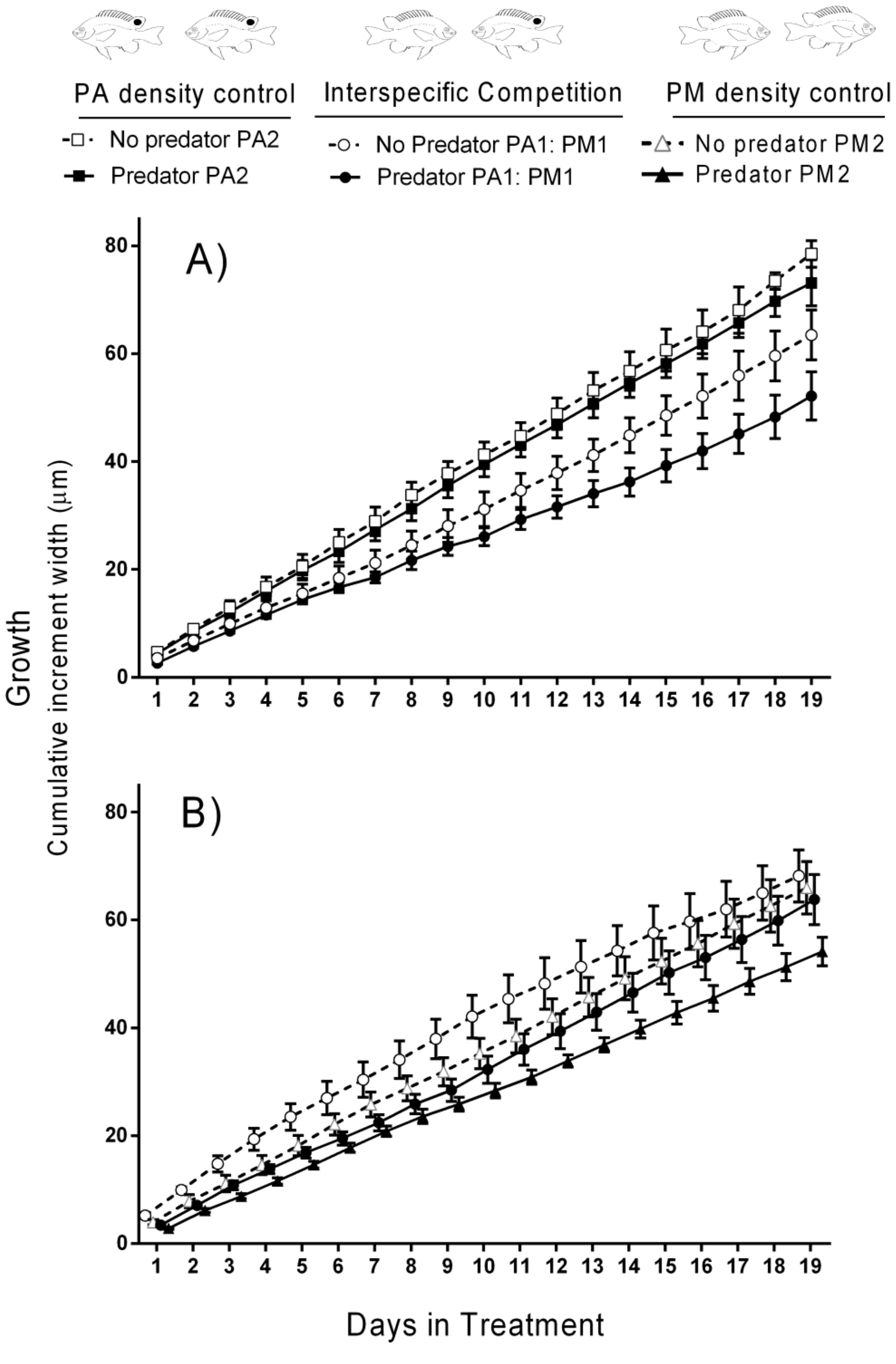

Figure 4.3 Growth (mean cumulative otolith increment width ±1 SE) of A*) P. amboinensis* and B) *P. moluccensis* during the experimental period according to predator and competitor treatments. Paired conspecific treatments (PA2 and PM2) are density controls for the paired heterospecific (interspecific competition) treatment (PA1:PM1). PA= *P. amboinensis,* PM= *P. moluccensis*

4.4.2 BEHAVIOUR

4.4.2.1 BEHAVIOURAL RESPONSE TO PREDATOR ONLY

Feeding rates

Both *P. amboinensis* and *P. moluccensis* changed their behaviour in the presence of the predator; however, behavioural changes were much stronger and more consistent for *P. amboinensis* (Fig. 4.4). *P. amboinensis* significantly reduced their feeding rates in the presence of the predator [(t-test (bites/min) t_{10} = 3.486, p=0.0059, t-test (total bites) t_{10} = 3.164, p=0.0101; Fig. 4.4A]. Mean bite rates (per minute) were reduced by 48%, and overall bites were reduced by 43% (Fig. 4.4A). There was a similar trend for *P. moluccensis*, which exhibited a 29% reduction in bite rate, and an 11% reduction in overall bites in the presence of the predator, however, these differences were not significant [(t-test (bites/min) t_{10} = 1.045, p=0.3266, t-test (total bites) t_{10} = 1.117, p=0.2966; Fig. 4.4B].

Activity patterns

P. amboinensis showed strong changes to their overall activity patterns according to the presence/absence of the predator (Fig.4.4C). *P. amboinensis* spent less time swimming (t-test: t_{10} = 2.628, p=0.0252) and more time sheltering (t-test: t_{10} = 2.540, p=0.0294) when the predator was present. Changes in these activity patterns between predator treatments were substantial. *P. amboinensis* spent around half as much time swimming, and almost triple the amount of time sheltering when the predator was present (mean = 15.6% when predator absent, 45.3% when predator present; Fig. 4.4C). There was also a trend for *P. amboinensis* to spend less time feeding when the predator was present, however, this was not significant (t-test: t_{10} = 1.368, p=0.2013). In contrast, *P. moluccensis* did not show any consistent changes to overall activity patterns according to the predator presence, and the percentage time spent swimming, sheltering and feeding was similar between predator treatments (Fig. 4.4D).

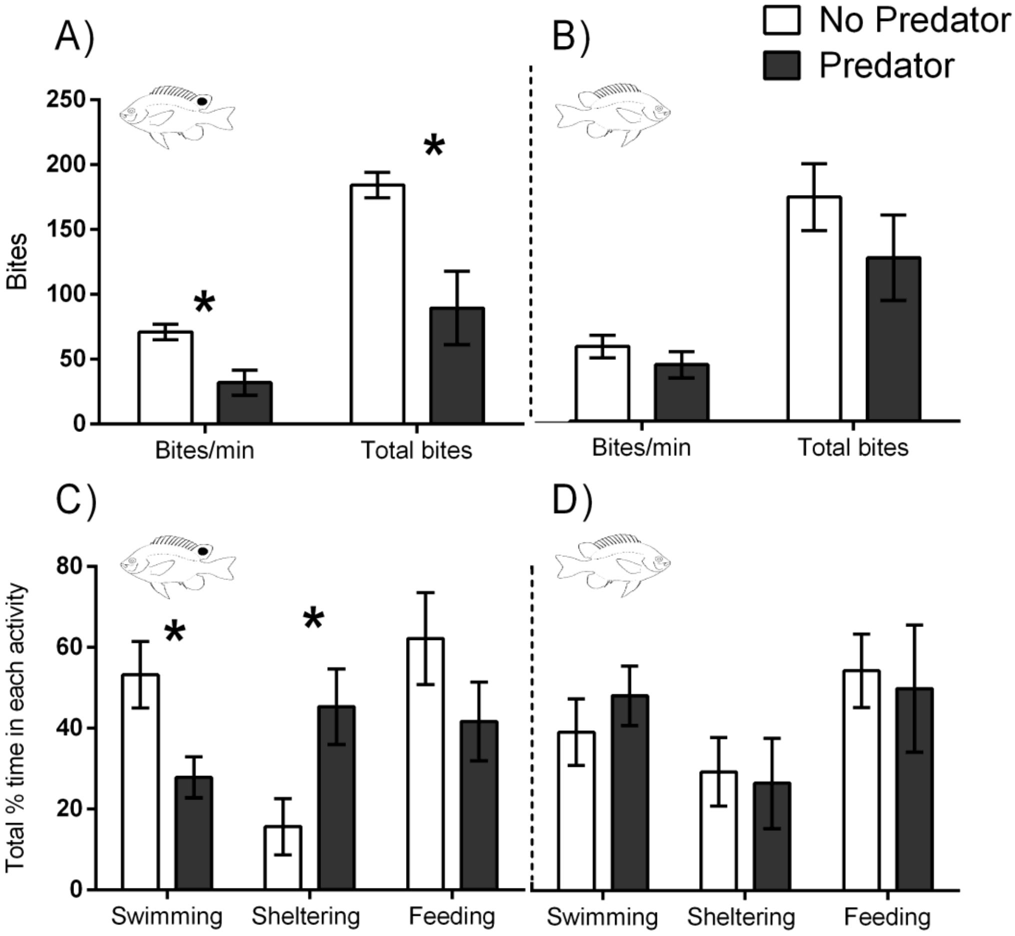

Figure 4.4 Feeding behaviour of *P. amboinensis* (A) and *P. moluccensis* (B), and activity patterns of *P. amboinensis* (C) and *P. moluccensis* (D), between predator treatments. All data are for single fish (PA1 and PM1) only with no competitor present. Asterisks indicate significant differences between predator treatments for each species (t-tests); all bars show means ±1 SE

4.4.2.2 BEHAVIOURAL RESPONSE TO PREDATOR AND COMPETITOR TREATMENTS

Competitive interactions and dominance hierarchies

Agonistic interactions occurred frequently between *P. amboinensis* and *P. moluccensis* in the interspecific competition treatments, and were almost always initiated by *P. moluccensis*, with *P. amboinensis* exhibiting avoidance behaviour (Fig. 4.5). Agonistic interactions generally involved a nip or a chase by the dominant fish, with avoidance behaviour exhibited by the subordinate fish. *P. moluccensis* was the dominant competitor in 10 out of 12 (83%) of competitive pairs, and initiated up to six times as many agonistic interactions as *P. amboinensis* (Fig. 4.5). The presence of the predator strengthened dominance hierarchies; while there was a trend for *P. moluccensis* to initiate more agonistic interactions in both predator treatments, this was only significant when the predator was present (t- test t_{10} = 2.525, p = 0.0355; Fig. 4.5).

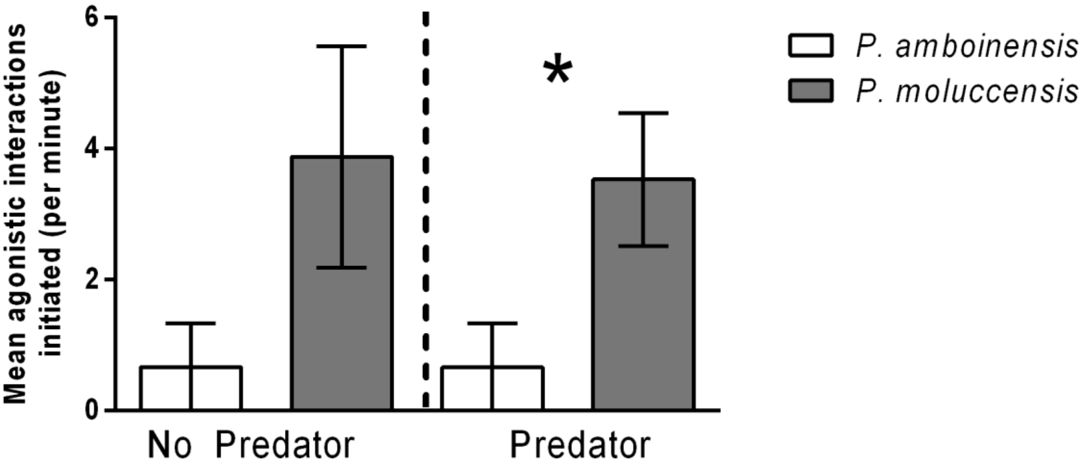

Figure 4.5 Mean (±1 SE) agonistic interactions (i.e. chases) initiated by *P. amboinensis* and *P. moluccensis* in the interspecific competition treatment (PA1:PM1), separated by predator absence or presence. Asterisks indicate significant differences between species within each predator treatment (t-tests)

Feeding rates

P. moluccensis had greater feeding rates compared to *P. amboinensis* in the interspecific competition treatments, and took up to twice as many bites (Fig. 4.6A). In the absence of the predator, *P. moluccensis* took significantly more bites per minute (t-test t_{10} = 3.710, p=0.006), and total bites (t-test t_{10} = 3.289, p=0.011), compared to *P. amboinensis* (Fig. 4.6A). Similar trends occurred when the predator was present, however, this was only significant for bites/min (t- test t_{10} = 2.769, p = 0.0243; Fig. 4.6A). The lower bite rates for *P. amboinensis* were generally associated with competitive interactions, whereby *P. moluccensis* actively prevented *P. amboinensis* from accessing food (Fig. 4.7). There was a significant positive relationship between bite rates and the number of aggressive interactions (chases) initiated (test for slope (ANOVA); p = 0.0238; r^2=

0.2115), and a negative but non-significant relationship between total bites and the number of times a fish exhibited avoidance behaviour (test for slope (ANOVA); $p = 0.292$; $r^2 = 0.0503$). Overall feeding rates were generally lower for both species in the predator present treatment.

Activity patterns

Activity patterns differed between species, and *P. moluccensis* spent more time swimming, and less time sheltering compared to *P. amboinensis* (Fig. 4.6B). This trend occurred in both predator treatments, but was only significant when the predator was absent (t-test (swimming); $t_{10} = 5.486$, $p = 0.0006$, t-test (sheltering); $t_{10} = 6.812$, $p = 0.0003$ Fig. 4.6B). Differences in activity patterns between competitors were substantial, for example *P. moluccensis* spent on average about twice as much time swimming as *P. amboinensis*, and *P. amboinensis* spent on average almost five times as much time sheltering (Fig. 4.6B). There was a trend for *P. moluccensis* to spend more time feeding compared to *P. amboinensis*, however, this was not significant in either predator treatment.

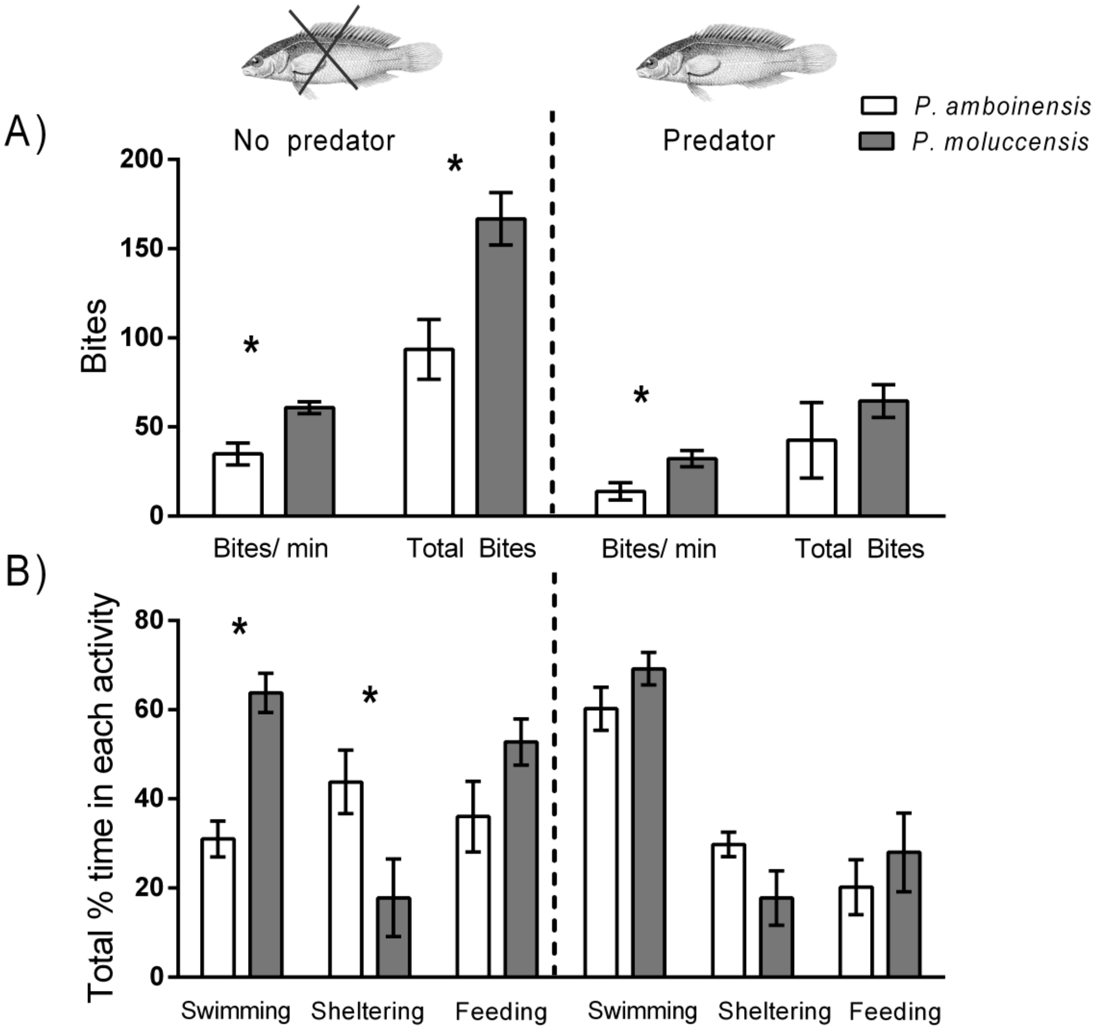

Figure 4.6 A) Feeding behaviour, and B) activity patterns of *P. amboinensis* and *P. moluccensis* in the interspecific competition treatments (PA1: PM1), separated by predator absence (left panels) or presence (right panels). Asterisks indicate significant differences between species within each predator treatment (t-tests); all bars show means ±1 SE

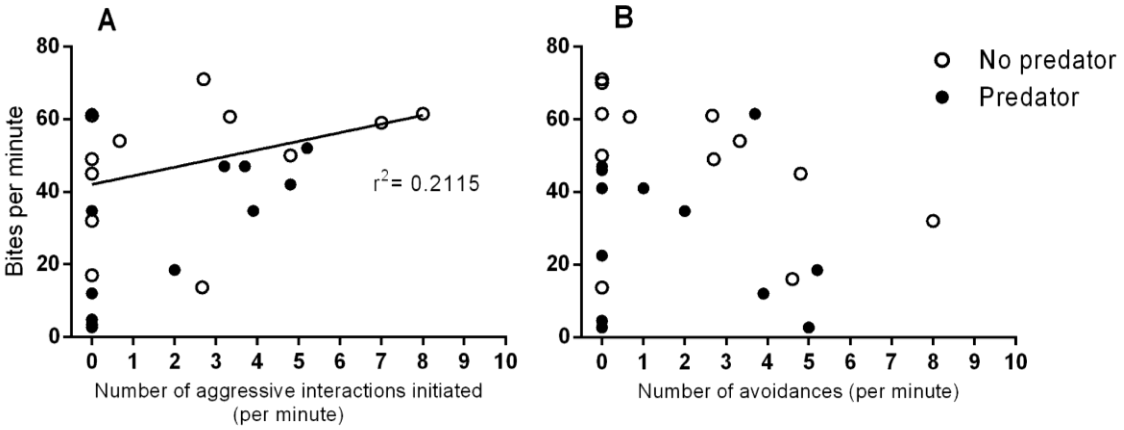

Figure 4.7 Relationship between bites per minute and A) the numbers of agonistic (aggressive) interactions (i.e. chases) initiated, and B) the number of avoidances (i.e. retreats) displayed for *P. amboinensis* and *P. moluccensis* (species pooled) in predator and no predator treatments. Line of fit on panel A represents that the slope is significantly different from zero.

4.5 DISCUSSION

The response of an individual or species to fundamental ecological processes such as predation and competition may vary, and understanding the relative sensitivity of species to such processes is an important step toward predicting how communities or populations may respond to change (Hixon and Jones 2005; Hunsicker et al. 2011) . For reef fishes, the first few months after settlement represents a critical growth period. Variations in growth during this time can impact life-time survivorship, since reductions in growth can increase the period of time for which prey are vulnerable to gape-limited predators (Sogard 1997; Booth and Hixon 1999; Hoey and McCormick 2004). In this study, juvenile damselfishes were used as study prey species, in order to test the effects of competition and predation on growth during this critical period. The experimental duration (19 days) represents a biologically relevant time period over which changes in growth would have severe consequences for damselfish species.

This study used a combination of growth analysis techniques paired with behavioural observations, to gain a detailed understanding of how individuals responded to stress from predator and/or competitor threat. I predicted that the presence of a predator or competitor would cause reductions in growth and changes to the behaviour of both prey species, and that a combination of the two would have a synergistic effect. I found support for these predictions, however, my data suggested that *P. amboinensis* is more sensitive to predator and/or competitor stressors than *P. moluccensis*. As well as between-species differences, I also found differences in how specific behavioural processes were affected by the combination of predator and competitor threat. These data highlight the complex ways in which predation and competition may interact to influence growth and behaviour at the individual and species level, suggesting that detailed species-specific data are needed in order to make predictions about how reef fish communities may respond to changes in these processes on coral reefs.

4.5.1 SPECIES COMPARISONS: RESPONSE TO PREDATOR VERSUS COMPETITOR

Predation is considered to be a critical process influencing the distribution and abundance of reef fishes, and numerous experimental and observational studies have demonstrated the role of piscivorous fishes in influencing prey communities (Hixon and Beets 1993; Carr and Hixon 1995; Jennings and Polunin 1997; Connell 1998; Graham et al. 2003). Chapters Two and Three of this thesis highlighted the important role of coral reef predators in influencing prey assemblages and demographics on the GBR. Given this, I predicted that the presence of a commonly encountered predator (*P. fuscus)* would have considerable sub-lethal effects on both prey species, leading to reductions in growth and behavioural changes. There was support for this prediction, however, significant predator impacts were only observed for *P. amboinensis*. There were marked differences in the response of *P. amboinensis* and *P. moluccensis* to the presence of *P. fuscus*. In the presence of the predator *P. amboinensis* displayed threat-reducing behaviours; feeding less and sheltering more, which resulted in significant growth reductions over the experimental period.

In contrast, *P. moluccensis* showed relatively minor changes to behaviours in response to the predator, with no consequence on growth trajectories. Such variation in responses is surprising, given that *P. fuscus* is a voracious predator of juvenile damselfishes (Feeney et al. 2012) and that both *P. moluccensis* and *P. amboinensis* have been shown to respond to changes in predator abundances on experimental patch reefs

(Caley 1993; Beukers and Jones 1998; Webster 2002) and on natural reefs (Graham et al. 2003). Data from Chapter Two also indicated that both species responded to variations in predator abundance, and were more abundant in areas where predators were depleted. Previous studies have shown that *P. moluccensis* can exhibit a behavioural response when presented with predator and/or conspecific chemical alarm cues (Mitchell et al. 2011; Bosiger et al. 2012), however, data from the present study suggests that this may not necessarily translate into growth reductions for this species on time scales of less than 20 days.

Competitive dominance can be an important factor determining group organisation for many species, however, defining dominance can be problematic. For group living species, the size of an individual often determines their rank within a group, so relative size can be used as a reliable proxy for social rank (Forrester 1991). In some studies, occupation of shelter sites has been used to infer dominance, since access to shelter sites can have a strong impact on survival (Bonin et al. 2009; McCormick and Weaver 2012). A key test of the importance of dominance hierarchies, however, is in the translation of behaviours to demographic outcomes. In this study, I used otolith data to unequivocally demonstrate the effects of predator and competitor treatments on growth trajectories. I controlled for size and focussed on the relationship between behavioural traits and growth outcomes to determine dominance hierarchies between *P. amboinensis* and *P. moluccensis*. Fish were identified as either dominant or subordinate based on behavioural observations. These behavioural data were then compared to growth trajectories to detect asymmetrical competition, and determine whether dominance hierarchies had an effect on growth. These comparisons revealed strong evidence of asymmetrical competition, with *P. moluccensis* as the dominant competitor. Variation in the response of each species to the interspecific competition treatments was largely due to the establishment of clear dominance hierarchies arising from strong interspecific aggression by *P. moluccensis*. As the subordinate competitor, *P. amboinensis* exhibited reductions in growth, coupled with frequent avoidance behaviour and lower bite rates. A direct comparison of competitive behaviours and bite rates demonstrated that *P. moluccensis* was able to gain a competitive advantage by exhibiting strong aggressive behaviour. Aggressive behaviour enabled *P. moluccensis* to increase feeding rates, which ultimately prevented this species from suffering the growth reductions experienced by *P. amboinensis* in the competition treatment.

Historically there has been much debate over the relative importance of competition for reef fishes, as well as the primary limiting resources which species or individuals may compete over (Sale 1977; Doherty and Fowler 1994; Hixon and Webster 2002; Jones and McCormick 2002; Hixon et al. 2012). Optimal foraging behaviour requires a trade-off between sheltering from predators and feeding, so both food and shelter can potentially be important in competitive interactions (Werner et al. 1983). Competition amongst or within coral reef fish species has been shown to occur over reef habitats (Holbrook and Schmitt 2002; Hixon et al. 2012; Boström-Einarsson et al. 2013), and dominant species or individuals may prevent subordinates from accessing key shelter holes (Holbrook and Schmitt 2002).

Studies on gregarious reef fishes have also demonstrated strong competition for optimal feeding positions (Coates 1980; Webster and Hixon 2000; Webster 2004) and the quality of food consumed by an individual may depend on their social rank and physical position within feeding groups (Coates 1980; Forrester 1991). In this study, variations between species in both feeding rates and shelter use occurred for paired fish in competition treatments. *P. moluccensis* had higher bites rates, and occupied shelter habitat less frequently than *P. amboinensis*. There was a clear and direct link between competitive behaviour, bite rates and growth trajectories, suggesting that competition for food was a strong driver of growth. For planktonic species, the position which an individual occupies in the feeding column may determine the quantity and quality of food available for consumption, with individuals sitting higher in the water gaining access to higher quality food resources (Coates 1980; Forrester 1991).

Although increased sheltering behaviour undoubtedly reduces the likelihood of mortality, the reductions in growth which may be associated with sheltering lower down in the water column may be a significant fitness consequence (Booth 1995). Variations in behaviour, feeding and growth between *P. moluccensis* and *P. amboinensis* may be related to differences in how the two species optimise their foraging behaviour in the presence of predators and competitors. These outcomes contrast to previous studies, which have placed *P. amboinensis* as the dominant competitor due to their position lower down on patch reefs and closer to shelter sites (McCormick 2012; McCormick and Weaver 2012). Direct comparisons of shelter use and feeding rates, however, were not considered in these studies. Although growth and mortality were measured, the effects of interspecific competition could not be separated from changes in density, as there was no intraspecific density control (McCormick 2012; McCormick and Weaver 2012). Data from this study highlight the potential complexities surrounding trade-offs between sheltering behaviour and access to feeding which may arise during competitive interactions.

4.5.2 INTERACTING EFFECTS OF COMPETITION AND PREDATION

A key outcome of this study was that the effects of competition were exacerbated by the presence of the predator. I predicted that a combination of predator and competitor threat would have a synergistic effect on growth and behaviour for the prey species. My data supported this prediction; as the subordinate species, *P. amboinensis* suffered greater reductions in growth when exposed to both a predator and competitor, compared to either treatment alone. Although *P. amboinensis* does tend to have faster absolute growth rates compared to *P. moluccensis*, there were still clear reductions in growth trajectories for *P. amboinensis* amongst treatments. *P. moluccensis*, as the dominant competitor, did not experience such reductions in growth trajectories, and in contrast tended to have a growth advantage in the interspecific competition treatments, compared with controls. Given the importance of growth during the early life history stages of reef fishes, such variations in growth trajectories could have severe consequences for fishes later in life (Sogard 1997). These interactions highlight how important competitive dominance can be in gaining a growth advantage, which can ultimately lead to decreased mortality and increased abundance over time.

Data from this study support the emerging notion that competition and predation interact as agents of mortality on coral reefs (Hixon and Carr 1997; Hixon and Jones 2005; Figueira et al. 2008). Although predation is the ultimate cause of mortality, competition over resources such as food or shelter may lead individuals to be more vulnerable to predators, and ultimately increase mortality rates for the subordinate species (Holbrook and Schmitt 2002). The present study provides further evidence that predation and competition can act synergistically at the sub-lethal level, influencing key demographic parameters such as growth. These data highlight and emphasise the complexity of interactions between competition and predation, and suggest that the response of an individual to these processes can depend on their status within competitive hierarchies.

4.5.3 GROWTH VERSUS BEHAVIOURAL RESPONSES

Comparison of growth trajectories, coupled with behavioural observations in this study, facilitated a detailed understanding of how individuals reacted to predator/competitor threat, and how this ultimately translated to changes in growth. There were variations in the degree to which growth and behaviour mirrored one another, depending on the species and the behaviour in question. The behavioural response of *P. amboinensis* to the predator, for example, closely mirrored the growth data; *P. amboinensis* exhibited risk averse behaviour, resulting in lower bite rates when the predator was present, which translated into reduced growth. *P. moluccensis*, in contrast, only displayed minimal changes to behaviour, with no detectable growth effect. The combination of predation and competition resulted in variable outcomes in terms of how behaviours changed. Interestingly, the presence of the predator in competitor treatments exacerbated some behaviours, such as the prevalence of agonistic interactions, but mediated others, such as differences between feeding rates and sheltering behaviour between species. Overall feeding rates were lower in the predator treatments for both competitors, which could explain why the differences in feeding rates between species were lessened. Regardless of these variations, the key result was a decrease in growth for the subordinate species when the predator and competitor were present. These data suggest that behaviour may not always indicate physiological and biological outcomes, and that inferring fitness consequences and other sub-lethal impacts from behavioural data alone may be problematic.

4.5.4 IMPLICATIONS

Understanding the role that predation and competition play in driving ecological communities may be particularly important in environments where human influences have modified these processes. On the Great Barrier Reef, predatory fishes such as groupers, snappers and emperors are heavily targeted by fisheries, resulting in severe predator depletion at heavily fished locations (Williamson et al. 2004; Boaden and Kingsford 2015). This loss of higher trophic levels has resulted in increases in the densities of lower level prey taxa such as damselfishes, and overall changes in the composition of fish communities at both broad and local scales (Boaden and Kingsford 2015). For lower level prey species, this constitutes a change in both predation patterns, as predators are lost, and competitive interactions, as densities of conspecific or heterospecific competitors increase correspondingly.

In addition, the nature of competitive interactions may be influenced by human impacts such as degradation of coral reef habitats, which can influence the strength of competitive interactions and reduce the availability of shelter sites for prey to escape from predators (Boström-Einarsson et al. 2013, 2014). Given the potential impact of human activities on both predation and competitive interactions, an understanding of the relative importance of each process, as well as their potential interactions, will be of great utility when considering the outcomes of future perturbations. This study focussed on the early life history of prey fishes, however, reductions in growth during this phase can influence survivorship, and may ultimately determine the reproductive output of species and influence community composition. This study has demonstrated the important sub-lethal effects of competition and predation on two common coral reef fishes, and these data highlight the need for further species-specific studies to elucidate the relative importance of these critical ecological processes for a range of species, in order to predict how coral reef fish assemblages may respond to future change.

CHAPTER FIVE: SEEING IS BELIEVING: METABOLISM PROVIDES INSIGHT INTO THREAT-PERCEPTION FOR A PREY SPECIES OF CORAL REEF FISH

5.1 ABSTRACT

Responding appropriately to predator threat is a critical survival skill for all organisms. Under-responding can result in death, while continually over-responding can waste precious energy reserves and compromise important life history attributes like growth and reproduction. This trade-off becomes particularly pertinent in predator-rich environments like coral reefs, yet almost nothing is known of the sub-lethal physiological responses that coral reef predators elicit in their prey. To address this knowledge gap, equipment and protocols were designed to measure the metabolic responses of a common coral reef fish (juvenile ambon damsel; *Pomacentrus amboinensis*) to olfactory and visual stimuli of a common predator (adult yellow dottyback; *Pseudochromis fuscus)*. *P. amboinensis* did not exhibit a metabolic response to the olfactory predator stimulus, yet there was a consistent and significant metabolic response to the visual stimulus that endured for at least 24h if the predator remained visible. The oxygen consumption of *P. amboinensis* increased greatly when exposed to the visual threat of the predator, and in some cases doubled compared to the resting rate. A complete lack of metabolic response of *P. amboinensis* to the visual stimulus of a non-predatory wrasse (*Halichoeres argus*) revealed an impressive ability of juvenile *P. amboinensis* to rapidly discriminate between similar-sized predatory and non-predatory fishes. These divergent metabolic responses of *P. amboinensis* were not explained by measureable differences in behaviour in the predator vs. non-predator treatments, as *P. fuscus* and *H. argus* shared similar levels of swimming activity and maintained similar proximities to the respirometry chambers. These findings demonstrate the capacity of coral reef fishes to interpret predator cues and prioritise threats and actions. Moreover, this study is the first to demonstrate that high predator

densities on coral reefs could result in repetitive short-term or even chronic long-term elevations in energy expenditure of prey fishes.

5.2 INTRODUCTION

Predators play essential roles in both aquatic and terrestrial environments, and predation is recognised as a critical process in the preservation of stable ecological systems (Estes et al. 2011). Patterns of predation can be strong determinants of the community structure of an ecosystem, and individual outcomes of predator-prey interactions (both lethal and sub-lethal) can be important in shaping communities through time (Hixon and Beets 1993; Boaden and Kingsford 2015). This process may be particularly important for coral reef fishes due to increased vulnerability as a result of their bipartite lifecycle (Hixon 1991). As coral reef fishes transition from their pelagic larval stage to their demersal reef-associated stage, they are particularly vulnerable to a host of predatory fishes. The resulting high mortality rate can cause a population bottleneck, with losses of up to 50% in the first 48h and continuing high mortality throughout the juvenile phase (Holbrook and Schmitt 2003). In this juvenile phase, how individuals react and respond to predator threats will be critical to shaping lifetime fitness by acting on attributes like foraging, growth and survival (Ydenberg and Dill 1986; Lima and Dill 1990; Cooke et al. 2003). As such, coral reef fishes have evolved a myriad of anti-predator response tactics, including morphological adaptations and behavioural adjustments (Connell 2000; Lönnstedt et al. 2012; Lönnstedt et al. 2013; Robertson 2013).

Animals must gather information from multiple stimuli, including sights, smells and sounds to perceive information about their environment and make appropriate decisions (Ydenberg and Dill 1986; Munoz and Blumstein 2012). Given the high diversity and density of fishes on many coral reefs, prey must quickly learn to assess the relative danger of situations and respond appropriately to maximise survival and fitness. This relies on their ability to process information from predator stimuli, and discriminate between predatory and non-predatory fishes. Numerous studies have used behaviour to document the ability of juvenile coral reef fishes to discriminate between the visual and olfactory stimuli of predatory and non-predatory fishes (Chivers et al. 2001;

McCormick and Holmes 2006; Ferrari et al. 2010; Ward and Mehner 2010; Holmes and McCormick 2011), and to detect and respond to chemical alarm cues that are released from the epidermis of conspecifics when they are injured (Mathuru et al. 2012). When presented with a visual and/or chemical predator stimuli, it has been well-documented that many prey species may change their behaviour by seeking shelter and reducing foraging rates (e.g. Chivers et al. 2001; Ferrari et al. 2010; Mitchell et al. 2011; Lönnstedt et al. 2012). Chapter Four demonstrated the capacity for predators to influence the feeding and sheltering behaviour of a prey species of coral reef fish. The present chapter focussed on exploring the physiological mechanisms that may underpin this behavioural response.

Despite the wealth of behavioural data available, comparatively little is known of any sub-lethal physiological responses that may accompany, or even underpin, the behavioural responses of prey to predators. Critical in this context is the balance between energy acquisition (through food) and energy usage (through activity and stress). The condition and fitness of prey can be strongly influenced by perceived predator threat if changes in behaviour result in lower net energy intake (Helfman 1989; Lima and Dill 1990; Preisser et al. 2005; Madin et al. 2010; Madin et al. 2012). Numerous studies have demonstrated the capacity for predators in aquatic systems to supress the foraging rates of prey (Madin et al. 2010; Madin et al. 2012; Rizzari et al. 2014), and some studies have additionally measured variables like ventilation frequency and heart rate of prey in response to predator threat (Barreto et al. 2003; Gibson and Mathis 2006; Oulton et al. 2013). However, these approaches do not necessarily provide an accurate measure of changes in energy usage in response to predators (Barreto and Volpato 2004). To my knowledge, no prior studies have investigated the direct effects of visual and olfactory predator stimuli on prey energy usage by direct measurement of prey metabolism (i.e. oxygen consumption rates). This represents a significant gap in our understanding of sub-lethal predator-prey interactions, as any repetitive or chronic increase in prey metabolism has the potential to impact lifetime fitness by compromising aerobic processes like growth, locomotion and reproduction (Cooke et al. 2003).

The present study sought to quantify the impacts of predator stimuli on the metabolism of a prey species of coral reef fish. Specifically, I aimed to understand whether an olfactory and/or visual predator stimulus from the yellow dottyback (*Pseudochromis fuscus;* Müller & Troschel, 1849) elicited a metabolic response in juvenile ambon damsels (*Pomacentrus amboinensis;* Bleeker, 1868). Moreover, I was interested in whether *P. amboinensis* could discriminate between *P. fuscus* and a similar-sized non-predatory species (*Halichoeres argus;* Bloch & Schneider, 1801). These study species were chosen based on the outcomes of Chapter Four, where the subordinate competitor (*P. amboinensis*) had the strongest response to the predator (*P. fuscus)* with regards to growth and behaviour. The present chapter focussed on exploring the physiological mechanisms that may underpin this behavioural response, using *P. fuscus* as a model predator species, and *P. amboinensis* as the study (prey) species.

My initial predictions were that (1) olfactory and visual predator stimuli would independently elicit an increase in metabolism in the prey species, (2) a combination of olfactory and visual predator stimuli would elicit a greater metabolic response than either stimulus alone, (3) *P. amboinensis* would visually discriminate and respond appropriately (metabolically and behaviourally) to a predatory vs. a non-predatory fish, and (4) the metabolic response to a visual predator stimulus would subside over time as the prey species recognised it was not accessible to the predator.

5.3 METHODS

5.3.1 ANIMALS AND HOLDING CONDITIONS

This study was conducted at Lizard Island Research Station, at the northern end of the Great Barrier Reef, Australia. Since the objective of this chapter was to explore the physiological mechanisms underpinning the behaviours observed in Chapter Four, I used the same species of prey (juvenile *Pomacentrus amboinensis*) and predator (adult *Pseudochromis fuscus*). Data from Chapter Four, as well as numerous previous experimental studies, have reported behavioural responses of *P. amboinensis* to the sight and smell of *P. fuscus,* the latter being a voracious predator of juvenile damselfishes (Holmes and McCormick 2011; Feeney et al. 2012). *P. amboinensis* were of a similar size (14-18 mm SL) and age (approximately 20-30d), to those used in Chapter Four. Based on their size, it was highly likely that they were familiar with the scent of reef predators as well as conspecific chemical alarm cues. Adult *Halichoeres argus* (argus wrasse) were used as a procedural control. I used the wrasse species *H. argus* as a procedural control, as it is an invertebrate-feeding wrasse that poses no threat to *P. amboinensis* but is of a similar body size and shape to *P. fuscus* and inhabits similar reef habitats (Berkstrom et al. 2012). All fish were caught from the reef using diluted clove oil and hand and/or barrier nets. Fish were held in flow-through tanks at ambient reef water temperature (28±1^{o}C) for at least 48h on a light/dark cycle of 13/11h prior to being used in experiments. *P. amboinensis* were fed concentrated *Artemia* twice daily, but were starved for 24-28h before experiments to minimise any influence of digestion on the results. *P. fuscus* and *H. argus* were fed commercial pellets twice daily, except where outlined below.

5.3.2 RESPIROMETRY SETUP

Measurements of oxygen consumption rate ($\dot{M}o_2$) were used to quantify the aerobic metabolic response of *P. amboinensis* to experimental treatments. All measurements were performed at 28±1^{o}C using intermittent flow-through respirometry, following best practices outlined in Clark et al. (2013). A customised respirometry setup was used to test the independent and synergistic effects of olfactory and visual predator stimuli on *P. amboinensis* metabolism. Six 10ml clear glass vials were used as respirometry chambers, with three chambers submerged in each of two opaque aquaria (L x W x H = 600 x 360 x 380mm, water depth 200mm). The chambers measured 50 mm in length, which provided *P. amboinensis* juveniles with room to move and swim. Each chamber was equipped with an opaque plastic lid (length 13 mm), and had a 12 x 12 mm opaque fibre-optic cable holder mounted on the side (see below), both of which provided cover for the fish to hide behind while in the chambers. One chamber was always kept empty in each trial (randomised between trials) to quantify changes in background (microbial) respiration for subsequent correction of fish $\dot{M}o_2$. Each respirometer was connected via a closed loop to a recirculating pump, which kept the water within each chamber mixed at all times. A flush pump on a 10/10 min on/off cycle was positioned in a flow-through (1 L min^{-1}) reservoir bath (L x W x H = 200 x 200 x 300mm, water depth 25cm) between the two aquaria containing respirometers and was connected to each respirometer with 3 mm vinyl tubing. Excess water pumped into each respirometer during a flush cycle flowed out of a standpipe (3 mm tubing) and dripped back into the reservoir bath.

A second reservoir bath (same dimensions and volume) was also positioned between the two aquaria, which housed an individual *P. fuscus* (predator). The flow of water through this reservoir bath was set at a lower rate (10 ml min^{-1}) to maintain a high concentration of predator scent. Both reservoir baths were aerated at all times to maintain oxygen >95% of air saturation. This arrangement allowed the flush pump and six standpipes to be easily and quietly switched between the two reservoir baths in order to flush the respirometers with either predator-free or predator-scented water. Four *P. fuscus* were housed in individual tanks and cycled through the reservoir bath as necessary throughout the course of the experiments by transferring the fish and its tank water into the reservoir bath. Each *P. fuscus* had been housed in its tank for at least 5 d prior to experiments to ensure a high concentration of predator scent in the water. Moreover, *P. fuscus* were fed three freshly-killed *P. amboinensis* immediately before the flush pump was switched to the predator reservoir; the sides of each *P. amboinensis* were lacerated prior to being fed to *P. fuscus* to ensure that the water was also scented with conspecific chemical alarm cues from the skin (Mathuru et al. 2012). This method ensured that the apparent concentration of both the predator scent and chemical alarm cue (calculated as biomass of predator or dead conspecifics as a function of tank volume) was equal to or above levels that have been documented to cause a behavioural response (Holmes and McCormick 2010b; Ferrari et al. 2012; Lönnstedt et al. 2013).

Temperature-compensated oxygen concentration (mg L^{-1}) of the water within each respirometer was continuously recorded (0.5 Hz) using oxygen-sensitive REDFLASH® dye on contactless spots (2 mm) adhered to the inside of each chamber and linked to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany) via fibre-optic cables. Cables were held in place by a 12 x 12 mm opaque bracket mounted to the side of each chamber. $\dot{M}O_2$ for each fish was calculated from the decline in oxygen concentration in the respirometer between flush cycles. Background respiration was checked in all respirometers before and after each trial to confirm that the single background chamber used in each trial was representative of all chambers. This was found to be the case, so the background respiration from the empty chamber in each trial was used to dynamically correct the $\dot{M}O_2$ of the fish in the other five chambers. The entire system was cleaned with bleach and rinsed thoroughly every 48h or if background respiration reached 10% of fish $\dot{M}O_2$.

5.3.3 EXPERIMENTAL PROTOCOL

The experimental protocol was designed to investigate the metabolic response of juvenile *P. amboinensis* to the sight and smell of the predatory species, *P. fuscus*. For all experiments, five *P. amboinensis* were placed in individual respirometry chambers and allowed to acclimate overnight (at least 16 h) before being subjected to stimuli. All stimuli occurred around the same time of day (12:00) to eliminate any potential confounding diel patterns in metabolism. At the conclusion of the experiments, all *P. amboinensis* were sacrificed using an ice water bath, blotted dry and then weighed to the nearest 0.001 g.

5.3.3.1 Experiment (i): olfactory versus visual predator stimuli

The first series of experiments aimed to investigate whether *P. amboinensis* (16.4 ± 0.2 mm SL, 0.197 ± 0.009 g) elicited a metabolic response to predator odour and conspecific chemical alarm cues. After measuring $\dot{M}O_2$ for at least 16h while the respirometers were intermittently flushed with water from the predator-free reservoir bath, the flush pump and standpipes were carefully moved to the other reservoir bath (containing predator scent and conspecific alarm cues) while the respirometers were on a sealed cycle. After the following flush cycle, I was able to examine the metabolic response of *P. amboinensis* to predator scent and alarm cues in the absence of visual stimuli. $\dot{M}O_2$ measurements continued for 3.5h before one *P. fuscus* was placed into each of the two aquaria containing respirometers, thus exposing *P. amboinensis* to both olfactory and visual predator stimuli. Introduction of each *P. fuscus* was performed carefully to ensure that the experimenters were not seen by the *P. amboinensis* in the respirometers. Each *P. fuscus* was allowed to swim freely throughout the aquaria and interact with the transparent respirometer chambers during 2.5h of $\dot{M}O_2$ measurements before the experiment concluded. The same experimental protocol was repeated to obtain a complete set of $\dot{M}O_2$ data for n=10 *P. amboinensis*. The results of Experiment (i) were used to determine the optimal protocol for subsequent experiments. Since there was no detectable metabolic response by *P. amboinensis* to the olfactory stimulus of the predator (see results), the olfactory stimulus treatment was excluded from Experiments (ii) and (iii).

5.3.3.2 EXPERIMENT (ii): PREDATOR VERSUS NON-PREDATOR VISUAL STIMULI

To examine whether *P. amboinensis* could discriminate between the visual cues of a predatory vs. a non-predatory fish, a separate series of experiments were conducted to compare metabolic responses of *P. amboinensis* (16.5 ± 0.3 mm SL, 0.197 ± 0.008 g) to visual cues of *P. fuscus* (predator) and *H. argus* (procedural control). As above, five *P. amboinensis* were placed individually into respirometers and left to acclimate while the respirometers were intermittently flushed with water from the predator-free reservoir bath. After at least 18 h, either a *P. fuscus* or a *H. argus* was carefully placed into each of the two aquaria containing respirometers and $\dot{M}O_2$ measurements continued for 3.5h (the flush pump remained in the predator-free reservoir). All *P. fuscus* and *H. argus* were of a similar shape and size (75-90 mm), and were rotated throughout the experiment to reduce the potential for results to be strongly influenced by the attributes of an individual. The same experimental protocol was repeated to obtain a complete set of $\dot{M}O_2$ data for n=10 *P. amboinensis* in each of the predator and non-predator groups (n=20 total for the experiment). The swimming activity and proximity to respirometry chambers for *P. fuscus* and *H. argus* were quantified using video footage to validate the use of *H. argus* as a procedural control (see details below).

5.3.3.3 EXPERIMENT (iii): CHRONIC VISUAL PREDATOR STIMULI

A final set of experiments aimed to understand whether metabolic responses of *P. amboinensis* (17.3 ± 0.3 mm SL, 0.207 ± 0.010 g) to visual predator stimuli persisted or dissipated during 24h of exposure. After at least 16h of acclimation to the respirometers, five *P. amboinensis* were exposed to the visual cue of *P. fuscus* by placing one predator per aquarium as outlined above. Water for flushing the respirometers came from the non-predator reservoir bath for the duration of the trial. The experiment ran for 24h following the addition of the predators, during which time $\dot{M}o_2$ was recorded over a full light/dark cycle of 13/11h (lights off between 19:00 and 06:00).

5.3.4 BEHAVIOURAL OBSERVATIONS

To test for relationships between the $\dot{M}o_2$ of *P. amboinensis* and the behaviour of *P. amboinensis*, *P. fuscus* and *H. argus,* the swimming activities of all three species were quantified. I recorded the behaviour of fish in the aquaria and respirometry chambers (n= 3 out of 5 *P. amboinensis* were visible in each trial) using GoPro cameras mounted ~60cm above the water surface. Behaviours of all three species were recorded for the first hour following addition of the predator or wrasse. During this period, I took 5 seconds of behaviour data every minute for one hour (n = 60 time points for each individual fish). Subsequently, the behaviour of *P. amboinensis, P. fuscus and H. argus* were quantified as follows:

5.3.4.1 ACTIVITY CALCULATIONS

The percentage time spent swimming over the one hour period was calculated for all three species. At each 1 min interval, the activity of each species was recorded as either swimming (>0.3 SL s-1) or stationary, and the total percentage of time spent swimming over each one hour period was calculated. To assess relationships between $\dot{M}o_2$ and swimming activity for P. amboinensis, the percentage time spent swimming was also calculated for a 5 min period overlapping the time during which $\dot{M}o_2$ was calculated. Corresponding 5 min values were also calculated for the predator and wrasse to enable comparisons between the swimming activity of the predator/wrasse, and the $\dot{M}o_2$ and swimming activity of *P. amboinensis.*

To determine if the behaviour of the predator or wrasse influenced the swimming characteristics of *P. amboinensis,* and to examine the relationship between swimming characteristics and $\dot{M}o_2$,swimming behaviour were used to derive an activity score for *P. amboinensis*. At each 1 min interval, individuals were assigned an activity score ranging from 0-2, to indicate the gross swimming characteristics of *P. amboinensis* at each time point as follows: 0 = stationary, 1 = swimming slowly, 2 = swimming quickly and/or erratically. Each activity score value was summed over a 5 min period overlapping the time during which $\dot{M}O_2$ was calculated, to calculate a total activity score, ranging from 0-10. The total activity score was used to compare the swimming activities and $\dot{M}O_2$ of *P. amboinensis* at each time point.

5.3.4.2 PROXIMITY CALCULATIONS

During the same 1 min intervals outlined above, the distance of each predator/wrasse from each *P. amboinensis* was calculated using the dimensions of the aquaria as a guide for scale. Each *P. amboinensis* in its individual respirometer was given a proximity score which ranged from 0-3 depending on the distance of the predator/wrasse to its chamber, as follows: <10cm = 3, 10-15cm = 2, 15-20cm = 1, >20cm = 0. The mean and standard error of this proximity score were calculated over the one hour observation period to give overall proximity values. Additionally, to examine relationships between the proximity of the predator/wrasse, and the activity and $\dot{M}O_2$ of *P. amboinensis,* proximity scores over the 5 min period overlapping with $\dot{M}O_2$ measurements were calculated. These values were then plotted against the individual $\dot{M}O_2$ and activity score values for each *P. amboinensis.*

5.3.5 DATA ANALYSIS AND STATISTICS

Respirometry data were analysed using LabChart version 7 (ADInstruments, Sydney, NSW, Australia). The mass-specific $\dot{M}O_2$ (in mg kg^{-1} h^{-1}) was calculated for each fish using the equation in Clark et al. (2013). A Q_{10} of 2.3 was used to adjust $\dot{M}O_2$ to account for any small (±1°C) deviations in water temperature away from 28°C (Norin et al. 2014). Linear mixed effects models were used to compare the $\dot{M}O_2$ of *P. amboinensis* across experimental treatments, and to examine trends within treatments over time (slopes). This is the recommended statistical approach for comparing treatments over time when repeated measurements are made on individuals (Oberg and Mahoney 2007). For each analysis, Akaike Information Criterion (AIC) values were used to determine the best statistical model. The variance amongst treatments was compared using a visual examination of the distribution of the residuals, and the model accounted for instances where variation differed between treatments and time periods. A visual analysis of the raw data was used to detect autocorrelation, and if autocorrelation occurred, it was accounted for in the model using an AR(1) correlation structure This is a standard technique used in time series analysis, which uses lagged values in a sequence as one of its predictors (Pinheiro and Bates 2000).

For each experiment, the model included a combination of random and fixed factors. All models included the random factor “fish conditioned by time period”. This random factor allowed for differences in oxygen consumption between individual fish, as well as the possibility that the magnitude of effects in different time periods (i.e. resting vs. post-stimulus), varied between fish. For each experiment, a period of 5h prior to the addition of any stimulus was defined as the “resting” period, to allow calculation of the resting $\dot{M}O_2$ of *P. amboinensis*. The $\dot{M}O_2$ during experimental treatments was then compared to resting values for each analysis. The fixed factors included in each model varied for each experiment and were as follows:

Experiment (i) compared the $\dot{M}O_2$ of *P. amboinensis* in response to olfactory and visual predator cues. The model had three fixed factors: time, olfactory stimulus and visual stimulus. The resting period was considered a control period. Since the olfactory stimulus remained present when the visual stimulus was added, the effects of olfactory and visual stimuli were modelled as additive. Experiment (ii) compared the $\dot{M}O_2$ of *P. amboinensis* in response to the visual cue from the predator, and compared this with the response to the wrasse (control). For experiment (ii), there were three fixed factors (time, period (pre-stimulus (resting) and post-stimulus)) and treatments (predator or wrasse (control)). For post-hoc analysis, student’s t-tests were used at each time point after the addition of the stimulus to compare $\dot{M}O_2$ between the predator and control (wrasse) treatments. Since multiple comparisons were made, a Bonferroni correction was applied to the critical significance threshold to reduce the probability of type I error. Since 27 comparisons were made, significance was considered at (0.05/27) = $p<0.0018$.To test for the synergistic effects of a visual and olfactory predator cue, and to examine the effects of a visual cue alone, I compared data from experiment (i) (olfactory + visual cues) with those from experiment (ii) (visual cue only). In this model, there were three fixed factors: time, period (pre-stimulus and post-stimulus), and treatment (olfactory + visual or visual stimulus alone).

For experiment (iii), data during the post-stimulus period were divided into three separate time periods as follows: stimulus response (beginning immediately after the visual cue was added), dark cycle (beginning at 19:00), and morning (beginning at 06:00 the following day; see Results). Each of the three post-stimulus time periods were compared individually with the resting period to determine the effect of the visual predator cue over a diel cycle. Separate analyses were used to compare each post-stimulus period with the resting period. For each analysis, there were three fixed factors: time, post-stimulus period, and resting period. Since multiple comparisons were made, a Bonferroni correction was applied to the critical significance threshold to reduce the probability of type I error. Since three comparisons were made, significance was considered at (0.05/3) = $p<0.016$.

Linear regression was used to examine the relationship between behavioural patterns of the predator and wrasse (analysed separately) and the behaviour and $\dot{M}O_2$ of *P. amboinensis.* For all analyses performed, assumptions of homogeneity of variance were verified using Cochran's test (Underwood 1997). Normality of the data was assessed by visual examination of the distribution of the residuals, and data were transformed when necessary to meet the assumptions of each statistical test. Significance was considered at $p<0.05$. Values given in the text are means ± SE unless otherwise indicated.

5.4 RESULTS

5.4.1 EXPERIMENT (I): OLFACTORY VERSUS VISUAL PREDATOR STIMULI

There was no metabolic response of *P. amboinensis* to the olfactory stimulus of the predator ($p=0.9820$), yet the addition of a visual predator stimulus increased $\dot{M}O_2$ by 78 ± 17% compared with resting values (1035 ± 24 mg h^{-1} kg^{-1} vs. 696 ± 15 mg h^{-1} kg^{-1}; $p<0.001$) (Fig. 5.1). The $\dot{M}O_2$ tended to decline over time after the visual predator stimulus (negative slope; $p=0.003$), although values remained elevated above resting levels at the conclusion of the experiment (i.e., 2h post-stimulus). Significant slopes in $\dot{M}O_2$ did not exist within the resting or olfactory-stimulus periods (resting; $p=0.2640$; olfactory; $p=0.9008$). There was no evidence of a synergistic effect of olfactory and visual stimuli on $\dot{M}o_2$; *P. amboinensis* responded similarly to the visual stimulus of the predator regardless of whether it was preceded by an olfactory stimulus (Fig. 5.2; p=0.6254).

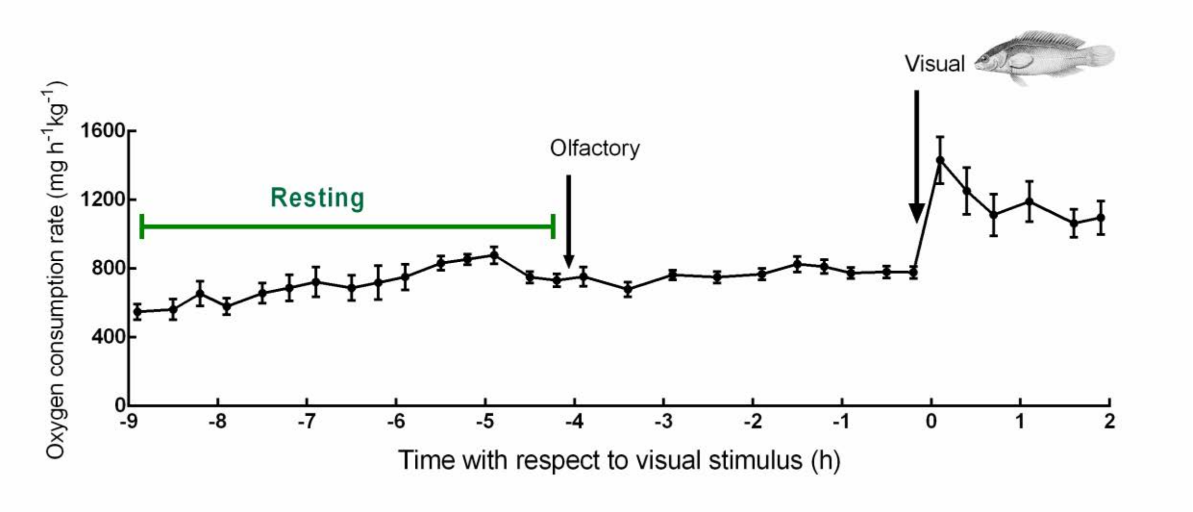

Figure 5.1 Metabolic responses (means ± SE) of *P. amboinensis* to olfactory and visual stimuli of *P. fuscus* (n=10). Oxygen consumption rate ($\dot{M}o_2$; mean ± SE) is shown over time with respect to when the visual stimulus was added. Arrows indicate when visual and olfactory stimuli were added. See text for statistical analyses.

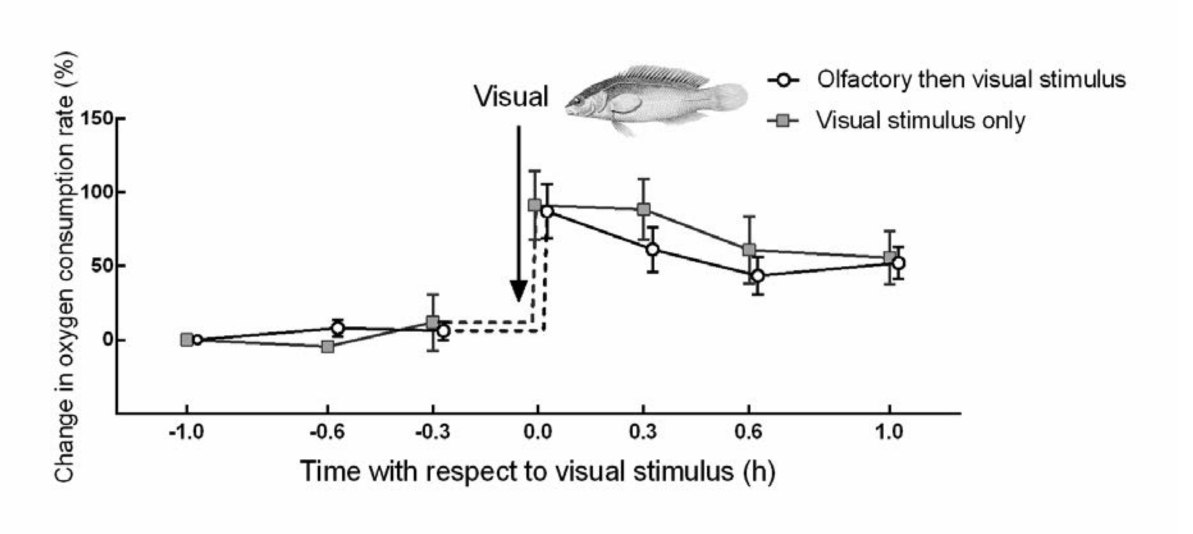

Figure 5.2 Percentage change in the oxygen consumption rate ($\dot{M}o_2$; mean ± SE) of *P. amboinensis* over time with respect to the addition of a visual predator stimulus (n=10). Open circles represent fish that were exposed to an olfactory stimulus followed by a visual stimulus, while closed squares represent fish that were exposed to a visual stimulus only. Percentage change was calculated using one hour before the introduction

of the visual stimulus (i.e., -1.0 h) as a reference point. The visual stimulus was added immediately before time 0; dashed lines indicate the hypothetical trajectories between time -0.3 and time 0 h.

5.4.2 Experiment (ii): Predator versus non-predator stimuli

In this experiment, *P. amboinensis* again responded to the visual stimulus of the predator, increasing $\dot{M}o_2$ by 108 ± 39% compared with pre-stimulus resting rates (Fig. 5.3). In contrast, there was no metabolic response of *P. amboinensis* to the visual stimulus of the non-predatory wrasse (procedural control; Fig. 5.3). Consequently, there was a significant interaction between treatment (predator vs. wrasse) and period (pre vs. post-stimulus; $p<0.0001$). The $\dot{M}o_2$ response to the predator declined over time (negative slope; $p<0.0001$) but remained significantly elevated above the values measured in the control (non-predatory wrasse) treatment for 3h following the introduction of the stimulus (Fig. 5.3).

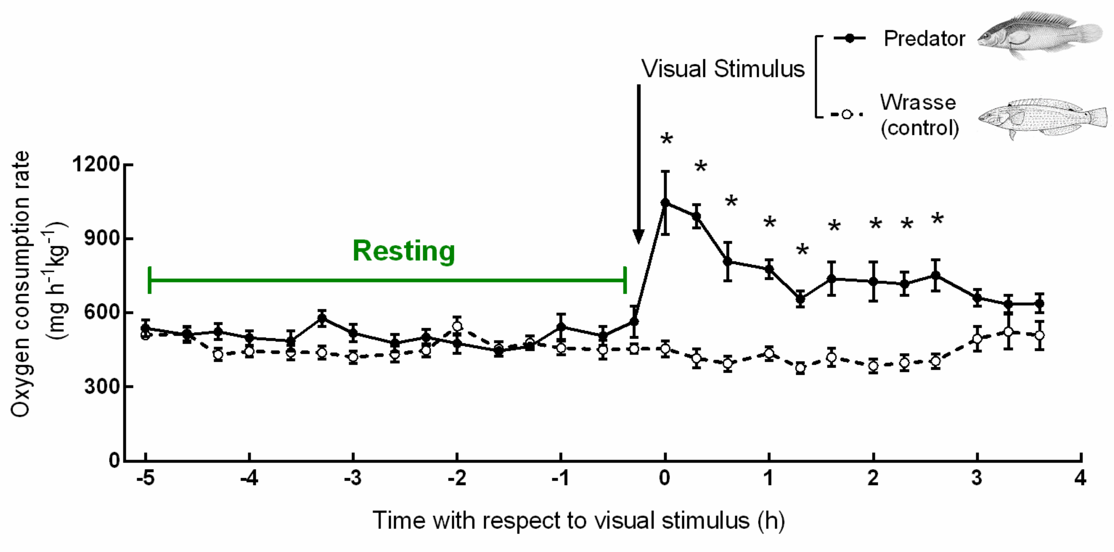

Figure 5.3 Metabolic responses (means ± SE) of *P. amboinensis* to the short-term visual stimulus of a predator (*P. fuscus*; n=9) or wrasse (*H. argus*; procedural control; n=10). Oxygen consumption rate ($\dot{M}o_2$) is shown over time with respect to the time of the visual stimulus treatment. Arrow indicates where the visual stimulus was added, and asterisks indicate significant differences between the predator and wrasse treatments (from linear mixed effects models and post-hoc t tests).

All relationships between $\dot{M}O_2$ and activity levels of *P. amboinensis* showed numerically positive slopes, although only the relationship between $\dot{M}O_2$ and the activity score in the non-predatory wrasse experiments reached statistical significance (p=0.0235; Fig. 5.4B). Notably, $\dot{M}O_2$of *P. amboinensis* was consistently higher when there was a predator rather than a wrasse outside of the respirometry chambers, regardless of the activity patterns of *P. amboinensis* (Fig. 5.4), or the activity patterns or proximity to the chambers of the predator or wrasse (Fig. 5.5C, 5.5F). While the activity levels of the predators were often higher than those of the non-predatory wrasse, there was substantial overlap that allowed direct comparisons (Fig. 5.5A-5.5C). There were no relationships between the behaviour of the predator/wrasse (% time spent swimming or proximity to chambers) and the activity levels of *P. amboinensis* (Fig. 5.5A, B, D, E). Importantly, the predators and wrasses spent a similar amount of time close to respirometry chambers, as highlighted by overlapping proximity scores (Fig. 5.5D-5.5F). These observations highlight that *P. amboinensis* can visually discriminate between a predator and a non-predatory wrasse, and that the metabolic response to the predator was not caused by variations in predator/wrasse behaviour between treatments.

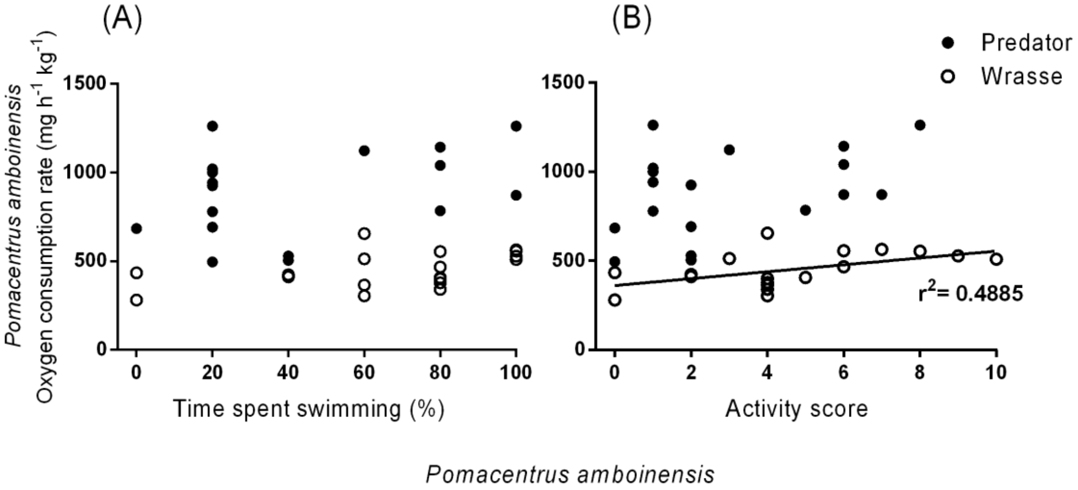

Figure 5.4: Relationship between oxygen consumption rate ($\dot{M}O_2$) and behaviour (time spent swimming (A), and activity score (B)) of *P. amboinensis* in predator and control (wrasse) treatments. Each data point represents a *P. amboinensis* $\dot{M}O_2$ measurement, and the 5 min of behaviour measured for *P. amboinensis* during this time period; n= 6 *P. amboinensis* for each treatment and n= 3 $\dot{M}O_2$ measurements per fish. Fitted line represents a significant linear regression between the $\dot{M}O_2$ and activity score of *P. amboinensis* when visually exposed to non-predatory wrasse.

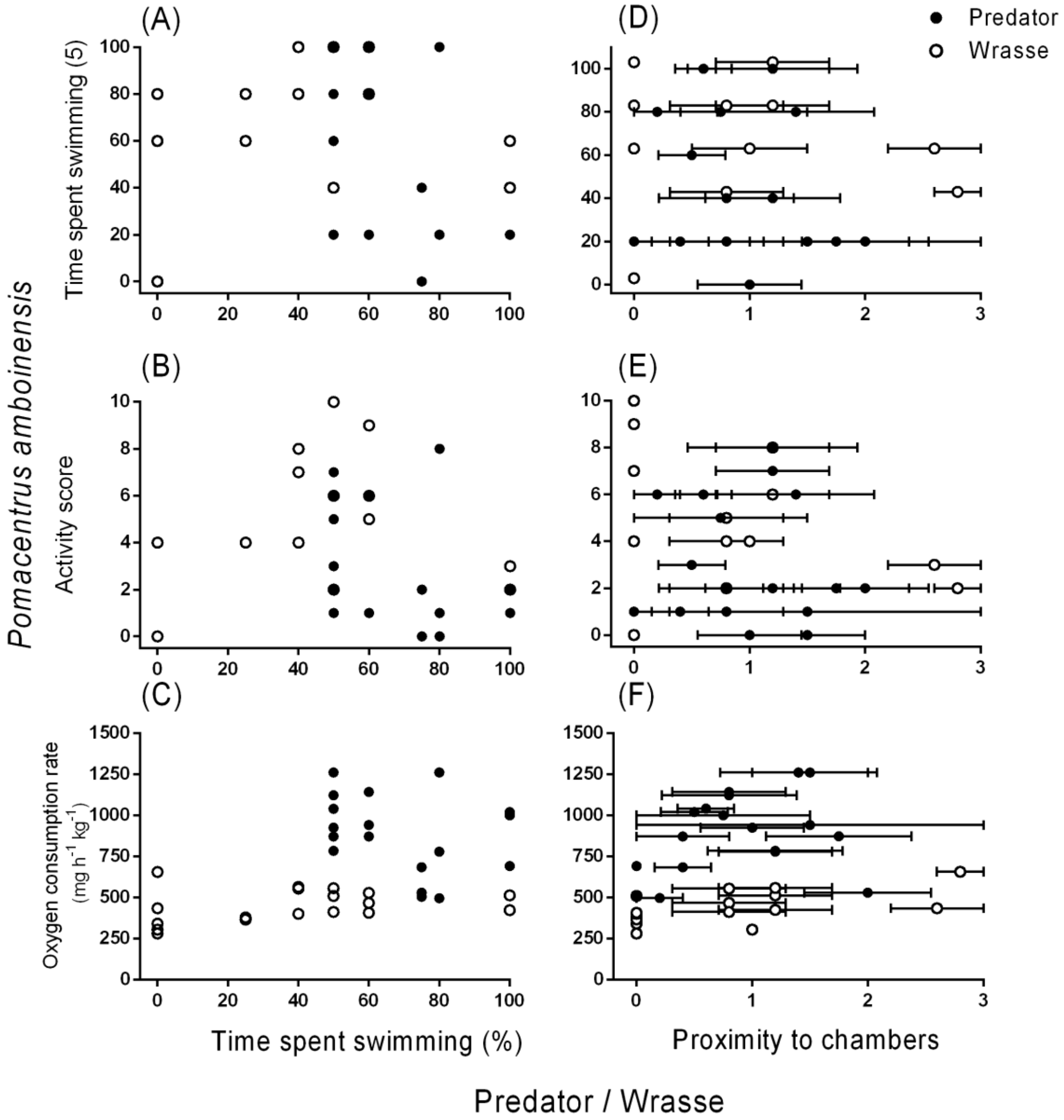

Figure 5.5 Relationships between the behaviour (A, B, D, E) and oxygen consumption rate (C, F) of *P. amboinensis* (vertical axes) and the behaviour of the predator or wrasse (horizontal axes). The percentage time spent swimming of *P. amboinensis* (panels A and D) was calculated for the full hour, while all other panels represent 5 minute time intervals around the *P. amboinensis* $\dot{M}o_2$ measurement period. For panels C and F, n= 6 *P. amboinensis* for each treatment and n= 3 $\dot{M}o_2$ measurements per fish.

5.4.3 EXPERIMENT (III): CHRONIC VISUAL PREDATOR STIMULI

The metabolic response of *P. amboinensis* to the visual stimulus of the predator persisted over a diel cycle (Fig. 5.6). At the beginning of the chronic exposure experiment, the $\dot{M}o_2$ of *P. amboinensis* increased by 82 ± 35% in response to the visual stimulus of *P. fuscus* and was significantly higher during the stimulus response period compared with the resting period (Fig. 5.6; $p<0.0001$). The initial response in $\dot{M}o_2$ was again followed by a gradual decline (negative slope; $p<0.0001$), however, $\dot{M}o_2$ remained elevated above resting values until shortly after the dark cycle commenced (Fig. 5.6). $\dot{M}o_2$ values and individual variability tended to decrease throughout the dark hours, yet overall $\dot{M}o_2$ during the dark cycle did not differ from the pre-stimulus resting period ($p=0.6187$; Fig. 5.6). This suggests that *P. amboinensis* became inactive and predator-induced metabolic stress dissipated when the predator was no longer visible. Once the light cycle recommenced, the $\dot{M}o_2$ of *P. amboinensis* again increased significantly above pre-stimulus resting levels (Fig. 5.6; $p=0.0067$), presumably because the *P. amboinensis* became active again and the predator was visible. Thus, *P. amboinensis* continued to exhibit a metabolic stress response even after 24h in the visual presence of a predator.

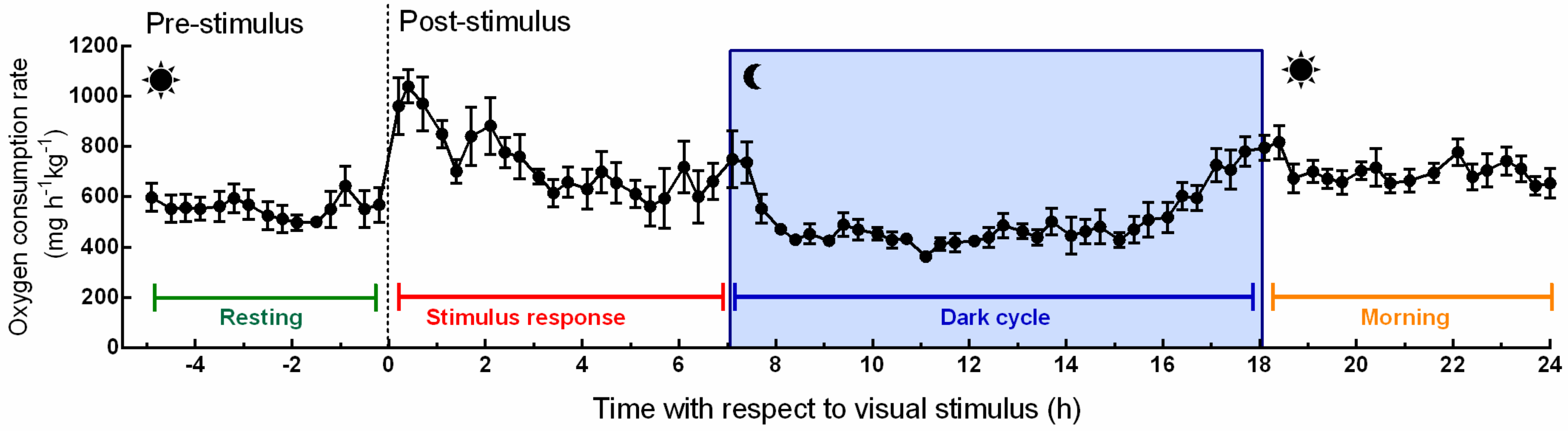

Figure 5.6 Metabolic responses (means ± SE) of *P. amboinensis* to the long-term visual stimulus of a predator (n=5). Oxygen consumption rate ($\dot{M}O_2$) is shown over time with respect to the time of the visual stimulus treatment. Data are divided into one pre-stimulus (resting period), and three post-stimulus periods; stimulus response, dark cycle, and morning. These periods correspond to treatments used in the linear mixed effects models.

5.5 DISCUSSION

Predator encounters are quite literally a matter of life and death. Perhaps more than most environments, coral reefs have high predator densities such that prey species must continually discriminate between high-risk vs. low-risk situations and respond appropriately. Failing to respond to a real threat could obviously result in death, as observed in Chapter Two, which highlighted the capacity for predators to suppress prey abundances on coral reefs. For prey species, continually over-responding to low-risk situations, however, is likely to have considerable sub-lethal impacts such as increased energy usage, compromised growth, and reduced body condition, which can increase the risk of selective predation. Data from Chapters Three and Four highlighted the sub-lethal effects of predators on prey, and documented a variety of demographic, reproductive and behavioural effects resulting from predator-prey interactions. To further explore the mechanisms underpinning such effects, the present study investigated how different predator stimuli impact the aerobic energy usage patterns of a coral reef fish, through direct respirometry measurements over time.

I hypothesised that each of the olfactory and visual predator stimuli would independently elicit an increase in metabolism of *P. amboinensis*, and furthermore that the combination of the two stimuli would have a synergistic effect. Surprisingly, *P. amboinensis* did not exhibit a metabolic response to the olfactory stimulus, yet there was a consistent and strong metabolic response to the visual stimulus. There was no evidence of a synergistic effect from the combination of olfactory and visual stimuli, as *P. amboinensis* responded similarly to the visual stimulus regardless of whether it was preceded by an olfactory stimulus. Similar results have been obtained from behavioural studies on mosquitofish (*Gambusia holbrooki*), which indicated that visual information was of primary importance over short distances (Ward and Mehner 2010). In contrast, behavioural studies on juvenile coral reef fishes have reported strong responses to an olfactory stimulus alone, a visual stimulus alone, or the two stimuli combined (Holmes and McCormick 2011; Lönnstedt et al. 2012; Lönnstedt et al. 2013). Relationships between physiological and behavioural traits can vary greatly (Killen et al. 2013) and this disconnect between physiological and behavioural responses warrants significantly more attention, but it does suggest that physiological perturbations resulting from predator-prey interactions cannot be implied through behavioural observations.

My data suggests that *P. amboinensis* has the ability to rank and prioritise threats, with metabolic responses appropriate to the perceived level of threat. Although previous studies have documented a behavioural response to olfactory predator cues alone (e.g. Holmes and McCormick 2010b), this study shows that this species does not exhibit a significant metabolic response until such time that the predator is visible. Furthermore, these data show that this metabolic response is similar whether preconditioned with an olfactory cue or not. There may be significant variations in the dispersal distance and longevity of visual and chemical stimuli. Chemicals may disperse long distances, and linger long after the predator has left an area, however, visual stimuli represent a more immediate threat (Ferrari et al. 2010; Ward and Mehner 2010). Given this, my results may not be surprising, since *P. amboinensis* commonly inhabits patch reefs along with *P. fuscus* and other meso-predators (McCormick and Holmes 2006) and thus must encounter olfactory predator cues regularly. Presumably, *P. amboinensis* would also come into regular visual contact with *P. fuscus* on the reef, and in these situations a visual assessment of the behaviour of the predator may provide the necessary information to guide the magnitude of the anti-predator response. These data provide evidence that coral reef fishes trade-off threat reduction and energy usage during routine predator-prey interactions.

Increased shelter use is a commonly observed reaction of small prey to the presence of a predator, and access to appropriate shelter habitat is critical for the survival of many prey species (Shulman 1985; Beukers and Jones 1998; Holbrook and Schmitt 2002). In this study, although *P. amboinensis* did utilise the shelter of the opaque respirometer lids and fibre-optic probe holders, there was no coral structure available within the chambers. While future studies should further investigate the metabolic responses of prey to predators in the presence of more natural coral structure (appropriately accounting for the respiration of the coral), the present study was targeted at teasing apart the metabolic responses of prey to olfactory vs. visual predator stimuli as well as predator vs. non-predator visual stimuli. Of course, it is possible that the metabolic response exhibited by *P. amboinensis* in the present study would be reduced on reefs where fish have a better opportunity to exhibit avoidance behaviour and hide in preferred habitat. Having said that, analysis of the behavioural response of *P. amboinensis* to the predator found no evidence that elevated metabolism was an artefact of the fish's inability to appropriately shelter inside the respirometry chambers. That is, the $\dot{M}O_2$ of *P. amboinensis* was elevated when visually exposed to a predator compared with a non-predatory wrasse, regardless of the distance of the predator from the respirometers housing the *P. amboinensis*. The fact that *P. amboinensis* did not exhibit elevated metabolism in response to the procedural controls (wrasse treatments) provides strong evidence that the experimental approaches were robust for addressing the main objectives.

Despite only having spent around 20-30 d on the reef after their pelagic larval phase, this study revealed through metabolic measurements that *P. amboinensis* were able to discriminate between a similar-sized predatory vs. non-predatory fish. This ability to discriminate concurs with behavioural studies on this species (Lönnstedt et al. 2013) as well as other fishes (Kelley and Magurran 2003), yet this approach improved existing knowledge by quantifying prey response patterns through time. Identifying and responding appropriately to predatory vs. non-predatory fishes is essential in the wild, perhaps especially in coral reef environments where the diversity and density of fishes are substantial. It is quite impressive that such young *P. amboinensis* are already so adept at visual discrimination that a metabolic response to the non-predatory wrasse (*H. argus*) was essentially non-existent.

In contrast to my hypothesis that the response of *P. amboinensis* to a visual predator stimulus would subside over time, the fish continued to respond to the sight of the predator throughout the diel cycle, only reaching low and stable levels of metabolism during dark hours when the predator presumably could not be seen, and/or both species were asleep and inactive. This trend reinforces the importance of the visual cue in eliciting a metabolic response, and suggests that metabolic rates of prey species could be chronically elevated in environments rich in actively foraging predators. In such environments, prey species are known to exhibit shelter-seeking behaviour to avoid close encounters with predators, which may give rise to only a transient elevation in metabolism (McCormick and Holmes 2006; Holmes and McCormick 2011). Nevertheless, *P. amboinensis* and *P. fuscus* have a close association on the reef, and thus it is likely that predator encounters would be frequent enough to result in repeatedly or even chronically elevated metabolism. These results suggest that prey species inhabiting predator-rich environments could suffer from elevated daily energy usage and lower growth rates, particularly if this was coupled with lower energy acquisition due to anti-predator behavioural changes that reduce the time spent foraging (Cooke et al. 2003; Killen and Brown 2006). This concept is supported by data from Chapter Four, where *P. fuscus* supressed the foraging rates of *P. amboinensis*. Data from the present chapter provide insight into the physiological processes which may have underpinned the observed changes in behaviour and reductions in growth observed for *P. amboinensis* in Chapter Four, highlighting utility of using biological and physiological data to interpret behaviours.

To conclude, the findings of this study provide new insight into how prey respond to predators at a sub-lethal level. I have shown that visual but not olfactory predator stimuli elicit a significant metabolic response in an abundant coral reef fish, independent of activity levels. Since this response persists even during chronic (24h) predator exposure, this may translate to elevated energy usage and impaired performance (e.g., growth, reproduction) of prey species in predator-rich environments. While this study takes a first step towards understanding the energetic responses associated with predator-prey interactions on coral reefs, future studies should investigate these relationships across life history stages, and with different levels of shelter, to better understand how predators may impact the energy usage and growth patterns of prey species throughout their lifecycle.

CHAPTER SIX: GENERAL DISCUSSION

The loss of predators from natural systems is a significant global conservation concern. Predators perform crucial roles in ecological systems, by exerting top-down control and regulating populations of lower level prey species. This process is important for promoting biodiversity and stability in food webs, and therefore many predators are considered keystone species (Carr et al. 2002; Estes et al. 2011). On coral reefs, predatory fishes may constitute a large proportion of total fish biomass, and are often heavily targeted by both commercial and recreational fisheries (GBRMPA 2011). Given their important functional role, harvesting of such predators has the potential to cause significant impacts due to cascading trophic effects. As such, there is a need to understand the importance of predator-prey interactions on coral reefs, particularly with regards to how predation can influence coral reef fish assemblages.

The overarching objective of the thesis was to examine the importance of predatory fishes on coral reefs of the Great Barrier Reef (GBR), specifically with regards to how predator-prey interactions shape coral reef fish communities. Implicit in this approach was a focus on addressing predatory effects from very broad spatial scales down to individual based responses to the presence of a predator. Data from this thesis highlight the importance of top-down effects on prey at numerous spatial and ecological scales, and demonstrate that densities of predatory fishes can be a key determinant of community structure on coral reefs. In combination, the thesis chapters demonstrate that predators can influence prey communities not only through direct trophic effects (i.e. changes in abundance), but also via fear mediated behavioural, demographic and even physiological effects. This research contributes to ecological theories examining trophodynamics on coral reefs, but also has important implications for management and conservation on the GBR.

6.1 TOP-DOWN CONTROL AND TROPHIC INTERACTIONS ON CORAL REEFS

Top-down control is recognised as a critical process which can determine the trophic structure of ecosystems, however, the importance of top-down control on coral reefs is debated. In Chapter Two, the importance of predatory fishes on coral reefs was examined at broad spatial scales using the zoning design of the Great Barrier Reef Marine Park (GBRMP) as a natural experiment. This chapter used data from extensive fish and benthic surveys to demonstrate that depletion of predatory fishes from fishing resulted in release of prey species from predation, causing significant changes in the trophic structure of fish assemblages. I found strong evidence of prey release at four locations, encompassing large spatial scales (i.e. hundreds to thousands of kilometres). These data provide an important addition to ecological theories with regards to our understanding of the role of predators in biologically diverse systems such as coral reefs. In diverse systems, the importance of top-down effects is predicted to be lower compared to less complex systems, due to the high functional redundancy observed within trophic guilds (Strong 1992; Polis and Strong 1996). Coral reefs have a high diversity of predatory fishes, and it has been postulated that the loss of a one predatory species may be easily compensated for by another (Sandin et al. 2010; Shurin et al. 2010). In contrast to this prediction, data from Chapter Two demonstrated that systematic removal of this key functional group can have important flow-on effects. These data provide a significant demonstration of the importance top-down effects at large spatial scales on the GBR, and emphasise the importance of conserving and protecting predatory fishes.

While predators interact with prey primarily through the direct process of predation, there are also a number of important sub-lethal effects that may occur (Madin et al. 2010; Ruttenberg et al. 2011; Walsh et al. 2012). Chapter Three focussed on these sub-lethal effects, and examined the importance of predators in influencing the population demographics of prey at regional scales (within an island group). In this chapter, I demonstrated that key demographic traits of the prey species *Scolopsis bilineatus* (Nemipteridae) varied according to local predator densities. Despite no differences in biomass or mortality, *S. bilineatus* exhibited significant reductions in growth, size, and condition in marine reserves where predator biomass was high. These data demonstrate the importance of sub-lethal top-down effects in influencing population demographics, and further highlight the important role of predatory fishes on coral reefs. Detection of sub-lethal effects from predators is consistent with our understanding of how "risk effects" operate on coral reefs. Previous studies have shown that predators have the capacity to significantly alter prey communities through predator-mediated behavioural modifications which come about due to the fear of predation (Heithaus et al. 2008; Madin et al. 2010; Madin et al. 2011). In the presence of a predator, prey may alter their behaviour by sheltering more and feeding less, which can have significant consequences for growth and condition, and ultimately impact lifetime survivorship (McCormick and Holmes 2006; Bosiger et al. 2012; Lönnstedt et al. 2012; Mitchell et al. 2013; Rizzari et al. 2014). This process may be particularly critical for coral reef fishes, due to the extreme vulnerability experienced during the early life stages. Data from Chapter Three provide an example of how such risk effects may translate to reductions in key demographic traits for prey species, and this process is explored further using predator-prey experiments in Chapter Four.

Coral reef fishes may be particularly vulnerable to predators due to their bipartite life cycle. As fish transition from their pelagic larval stage to their demersal reef-associated stage, they are vulnerable to a host of unfamiliar predators, and this early life history stage represents a population bottleneck for many species (Sale and Ferrell 1988; Hixon and Beets 1993; Carr and Hixon 1995). For surviving fish, the subsequent few months constitute a critical growth period, as any reductions in growth can result in increased vulnerability to predators. As such, the growth and condition of juvenile fishes can be a key indicator of lifetime survivorship (Sogard 1997; Booth and Hixon 1999). Chapters Four and Five explored the importance of predator-prey interactions for juvenile coral reef fishes at local scales. Using multi-factorial aquarium experiments, I demonstrated in Chapter Four that the presence of a predator (*Pseudochromis fuscus*) had significant consequences on the growth of juvenile prey (*Pomacentrus amboinensis)* due to predator-mediated behavioural modifications. Reductions in growth in predator treatments were associated with reduced feeding rates, lower activity and an increase in the time *P. amboinensis* spent sheltering. Although this experiment focussed on growth during early post-settlement, variations in demographic traits at this time can have significant effects on life-time survivorship (Sogard 1997; Booth and Hixon 1999; Hoey and McCormick 2004). As such, these data highlight the ways in which predators may structure prey populations by influencing behaviour and demographics during the early life stages of prey. Chapter Five then explored the physiological mechanisms underpinning these results, and examined the metabolic response of *P. amboinensis* to the visual and olfactory stimuli of a predator. Using intermittent flow respirometry techniques, I demonstrated that the visual stimulus of a predator resulted in significant increases in the metabolic rate of *P. amboinensis* for periods of up to 24 hours. Furthermore, *P. amboinensis* was able to discriminate between predatory and non-predatory fish, and did not respond metabolically to the presence of a harmless wrasse. These data suggest that fear of predation can have significant energetic consequences for prey species, particularly given the diversity of predators on reefs, and the frequency of predator encounters that prey may experience.

Data from predator-prey experiments in Chapters Four and Five highlight the importance of top-down effects at local scales for juvenile reef fishes. Predation, however, is not the only ecologically relevant process during this time. During early post-settlement, competition within or amongst species can play an important role in regulating juvenile prey densities, particularly when the outcomes of a competitive interaction influence susceptibility to predators (Hixon and Carr 1997; Hixon and Jones 2005). Given these potential interactions, the aims of Chapter Four were twofold. Firstly I aimed to investigate how predators influenced the growth and behaviour of prey, and secondly I aimed to investigate how predation may interact to either exacerbate or mediate competition. A key outcome from this chapter was the prevalence of synergistic effects between competition and predation. For *P. amboinensis*, the presence of a predator exacerbated the effects of a competitor (*P. moluccensis*) on growth. Interactions between competition and predation strengthened dominance hierarchies, and increased the intensity of aggression between competitors. These synergistic effects may be incredibly important for juvenile reef fishes, as reductions in growth or condition during this critical life phase can ultimately translate to reductions in lifetime survivorship or fecundity. Outcomes from this chapter concur with emerging ecological theories which suggest that predation and competition interact as agents of mortality on coral reefs (Hixon and Carr 1997; Holbrook and Schmitt 2002; Hixon and Jones 2005). These data highlight the importance of predator/competitor synergisms during the early life history stages of coral reef fishes.

6.2 PREDATOR-PREY DYNAMICS AT MULTIPLE SPATIAL SCALES

An important component of this thesis was the consideration of the role of predatory fishes at multiple spatial scales. Beginning at the smallest scales (i.e. within an organism), I demonstrated that predators can have strong physiological effects on prey by increasing metabolic rates. Incorporation of a physiology-based chapter was an important component of this research, as physiological effects may underpin the behavioural responses of prey to predators and can influence the energy budgets of prey. At local scales, experiments in Chapter Four showed that this physiological response may be accompanied by a demographic outcome; i.e. reductions in growth in the presence of a predator. Comparisons of prey population demographics at regional scales in Chapter Three allowed extrapolation of this growth effect, and demonstrated that such sub-lethal effects can impact species at the population level. Finally, examination of assemblage-scale responses to variations in predator numbers in Chapter Two showed that the physiological, biological and behavioural changes observed at local scales can result in large scale differences in fish assemblages on coral reefs. These data concur with multiple experimental studies which have explored the role of predators at small spatial scales, and demonstrate that these processes can ultimately scale up and translate to significant changes in fish assemblages (Hixon and Beets 1993; Connell 1998). The multi-scale nature of this thesis provides a robust and holistic examination of predator-prey interactions on coral reefs.

6.3 IMPLICATIONS FOR CONSERVATION AND MANAGEMENT

Predators are increasingly recognised as critical members of ecological communities, and the preservation of predator populations is a global conservation priority. On the GBR, predatory fishes are threatened by fishing practices, due to the predator-focussed nature of recreational and commercial fishing that occurs. In recognition of their important role on reefs, the preservation and management of predatory fishes on the GBR has been identified as an important conservation goal. One of the major aims of establishment of the GBRMP was to maintain key ecological processes on reefs (GBRMPA 2005). In their recent “Outlook Report” the Great Barrier Reef Marine Park Authority (GBRMPA) identified predation as a key ecological process, and expressed concern over the large reductions in predator numbers that have been observed in fished areas (GBRMPA 2014). This focus on conservation of predators, combined with the need for a better understanding of their role on reefs, motivated the development of this PhD research project. Data from Chapters Two and Three of this thesis have a variety of ecological implications which are of direct relevance to management strategies. In these chapters, I have demonstrated that removal of predatory fishes on the GBR through commercial and recreational fishing activities can have important flow-on effects on non-target species. These secondary effects of fishing influence prey at both lethal and sub-lethal levels, and can result in significant changes to the structure of coral reef fish communities. These data indicate that fishing can disrupt trophic interactions, but also highlight the effectiveness of marine reserves in restoring and maintaining predator biomass and associated top-down effects. Results of this research support the continued use of marine reserves for the protection of biodiversity, and indicate that ecosystem-based management of marine reserve networks will play an important role in future management strategies.

Although not directly relevant to management strategies on the GBR, Chapters Four and Five provide significant insight into the biological and physiological underpinnings of predator-prey interactions. Data from Chapter Four highlight the importance of interactions between predation and competition, which may be particularly relevant in future scenarios, given the capacity for human interactions to change competitive outcomes. Recent studies have shown that degradation of coral reef habitats due to anthropogenic impacts can change the nature of competitive interactions on coral reefs (McCormick et al. 2013; Boström-Einarsson et al. 2014). Given this, an understanding of how competition may interact with other ecological processes is therefore relevant for future management of human impacts on coral reef habitats. Changing climates may also impact fish physiology, especially when increased temperatures interact with metabolic processes (Rummer et al. 2014). Although historically physiologists and ecologists have largely worked in separate fields, there is an increasing push for integration of the two fields (Clark et al. 2013). The application of physiological methods to answer ecological questions can facilitate a mechanistic understanding of the processes underpinning ecological interactions. An understanding of the energetic and physiological consequences of predator-prey interactions is therefore important, and can provide key information to aid in understanding trophodynamics on coral reefs.

6.4 CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

This thesis has provided key information relevant to the conservation and management of predatory fishes on coral reefs of the GBR. This research provides a solid foundation for future research projects to further explore the role of predators on the GBR, particularly with regards to future management strategies. While strong trophic effects were found in this study, it is important to note that coral reef systems are subject to a myriad of additional impacts which may interact with trophic effects in complex ways. On the GBR, it would be particularly pertinent to increase our understanding of how disturbances such as cyclones and storms may affect the trophic interactions documented in this thesis. Although I found consistent patterns that related to the level of protection on reefs, the magnitude of differences did vary amongst locations, and this may be partly explained by physical perturbations due to cyclones and recovery processes. A recent paper by Emslie et al. (2015) documented the response of reef fish assemblages to marine reserve protection within the GBRMP, and to cyclone damage. Data from this study indicated there was variation in how non-target fishes responded to marine reserve protection and damage from cyclones (Emslie et al. 2015). Understanding where, how and why trophic effects may vary in their importance amongst reefs, as well as an analysis of how they vary over a range of disturbance regimes, would provide additional insight into these processes and provide important information for ongoing management strategies.

An important finding of this research was the presence of gradient effects of fishing on both predators and prey. The gradient effect indicates that in areas with partial protection (i.e. limited fishing zones), there is some benefit to predators and an associated increase in the strength of top-down control. The clearest gradient was at the Capricorn Bunkers, where predator biomass was much greater than at other locations, and where a large proportion of predatory fish present were commercially important species. It is possible, therefore, that the benefits of partial protection may depend on the structure of predator assemblages at each location, however, this hypothesis has not yet been explored. Implementation of management strategies with relation to marine parks zoning involve a trade-off between allowing utilisation of fisheries resources, and providing protection for reef ecosystems. Partial protection zones may be a valuable tool in achieving this compromise, however, few studies have considered them. Data from this study suggests that partial protection can contribute to conservation goals on the GBR through protection of predators and associated top-down effects. Future research should explore the role of partial protection on the GBR, and should aim to determine the circumstances under which this may be an effective management strategy.

Data from the present study indicate that sub-lethal trophic effects (as observed for *S. bilineatus*) may be detectable before a numerical response is evident in fish assemblages. This suggests that demographic traits may be a more sensitive indicator of changes in trophodynamics on reefs. If so, comparison of demographic traits of non-target prey species may be an important research tool to guide management strategies. An additional future research direction could involve extrapolation of sub-lethal secondary fishing effects to other species/trophic groups, to assess the viability of using demographic metrics as an indicator of trophic changes on reefs. Interestingly, I found contrasting lethal and sub-lethal top-down effects for *S. bilineatus*, which suggests the potential for reverse spill-over for prey species. Reverse spill-over may occur if prey populations are more productive in fished areas due to release from predation, but larvae are exported to marine reserves. Such processes would explain the observed even densities of *S. bilineatus* between zones, despite the existence of significant sub-lethal predator effects. The potential for spill-over involving commercially important species in marine reserve networks has been explored extensively, and has been documented on reefs on the GBR (Harrison et al. 2012). In contrast, the potential for reverse spill-over for prey, due to trophodynamic changes arising from fishing activities, has not been considered. Given the dynamic and highly connected nature of coral reefs, further consideration of processes such as reverse spill-over could aid in understanding how trophodynamic changes within marine reserve networks can occur through time.

To conclude, this research has provided significant insight into the role of predatory fishes on coral reefs. I have demonstrated strong top-down effects at both the lethal and sub-lethal level, which highlight the important role of predators in coral reef fish assemblages. The holistic and multi-scale nature of this thesis provides an integrated understanding of how prey may respond to predators physiologically, biologically and ecologically, which is of direct relevance to conservation and management of the GBR. These data provide a fundamental framework for continued studies focussing on the trophic effects of fishing, and highlight the effectiveness of the Great Barrier Reef Marine Park in preserving this biologically rich natural resource. Outcomes from this research highlight the need for an ecosystem-based approach to management of marine reserve networks, and emphasise the importance of restoring and preserving top-down processes on coral reefs.

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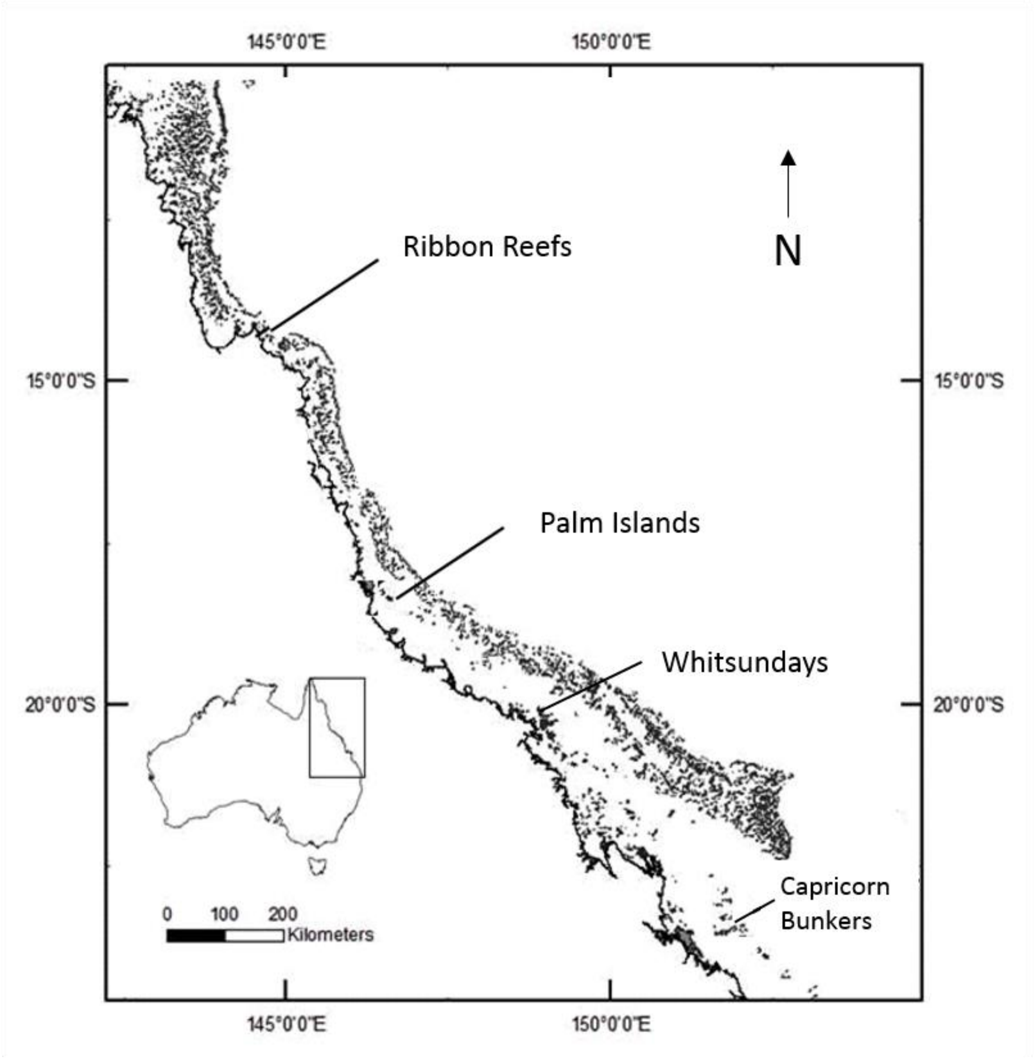
APPENDICES

Appendix A: Map of survey locations for Chapter Two

Appendix B: Map of survey and collection locations for Chapter Three

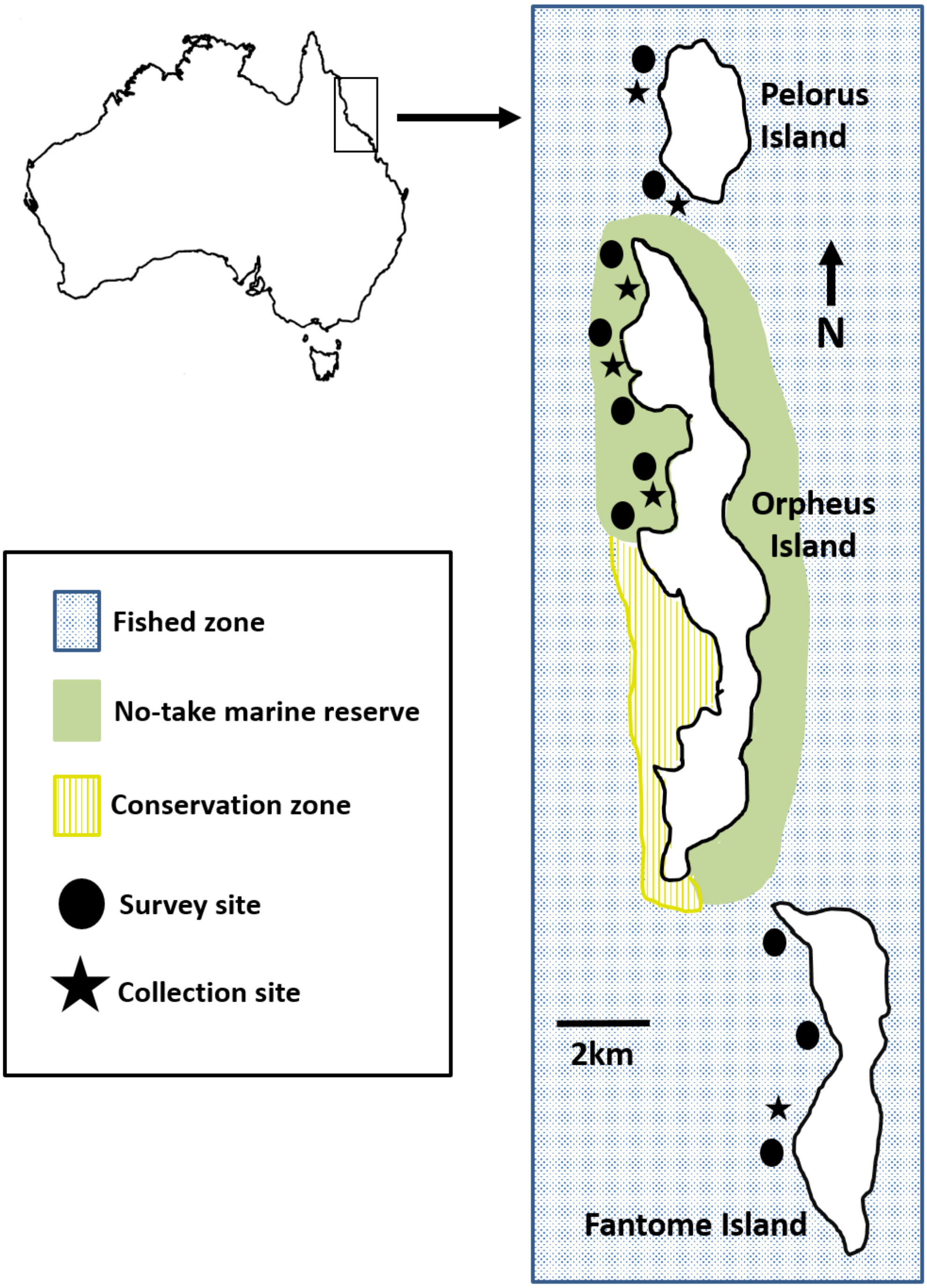
Appendix C: Diagram of experimental setup for Chapter Four

APPENDIX A



Map of locations where fish and benthic surveys took place in Chapter Two, showing their location along the Great Barrier Reef on the Queensland coast

APPENDIX B



Map of survey and collection sites for Chapter Three, showing their location nested within fished and marine reserve zones at the Palm Islands

APPENDIX C

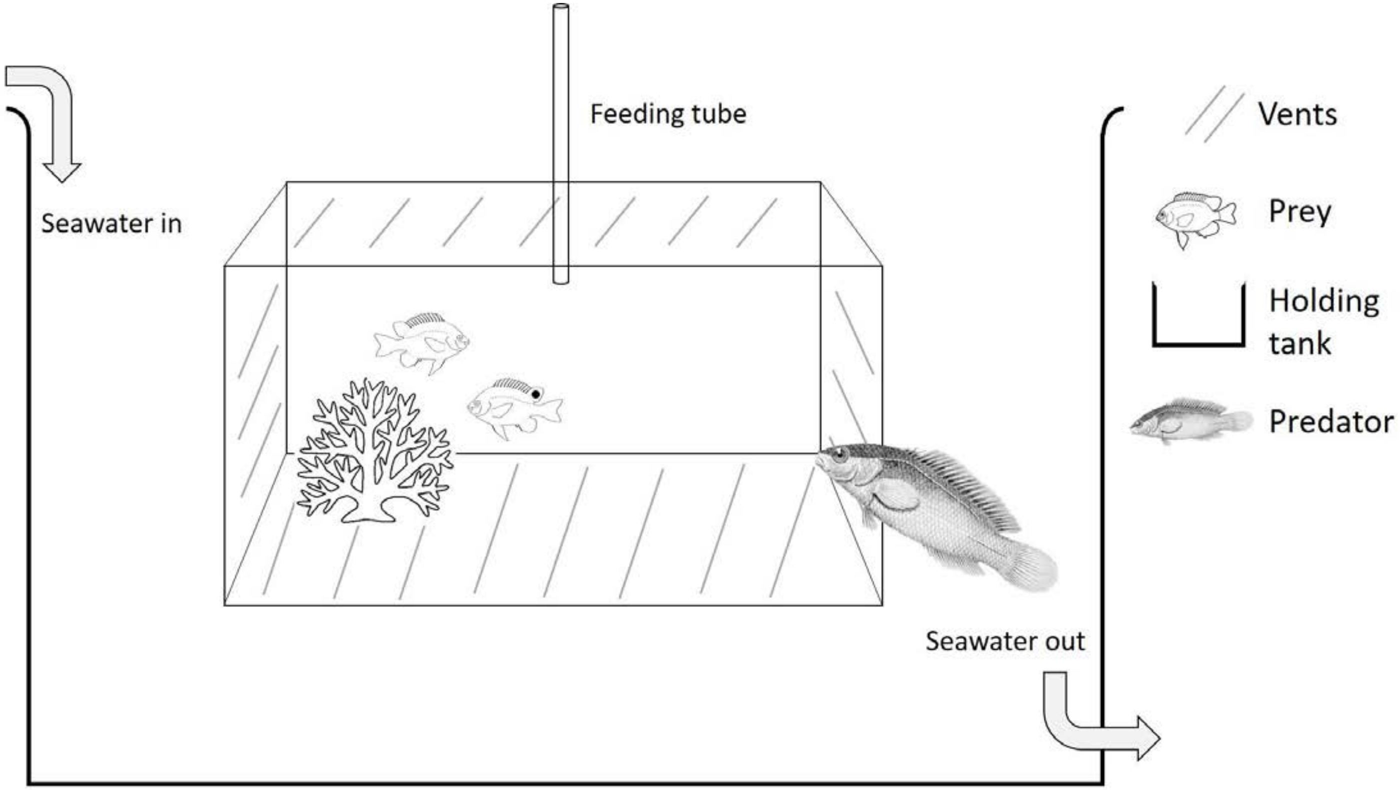
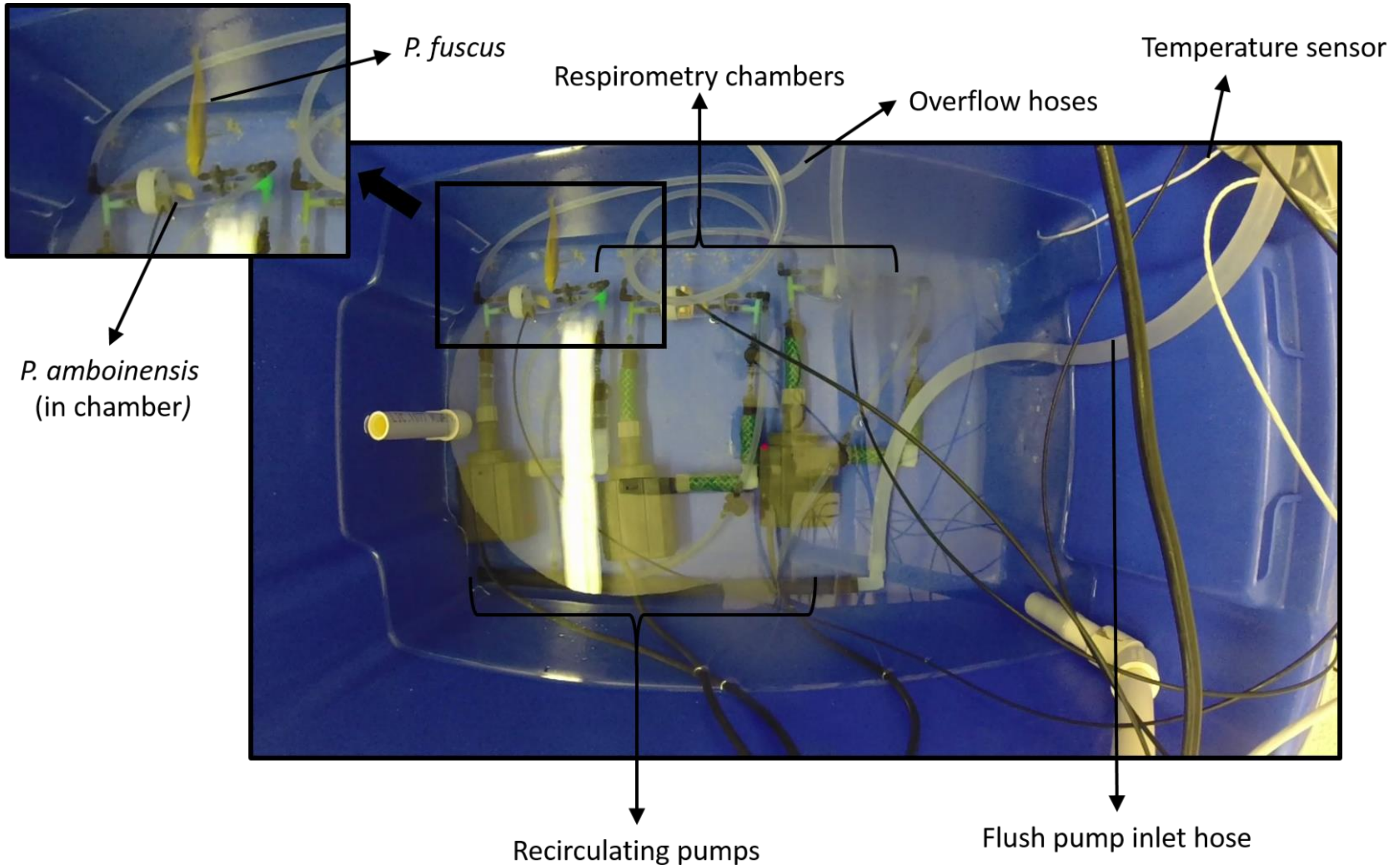


Diagram of experimental setup, showing placement of the smaller experimental tank inside the larger flow-through holding tank.

APPENDIX D



Photograph of experimental setup, showing placement of the respirometry apparatus inside the larger flow-through holding tank