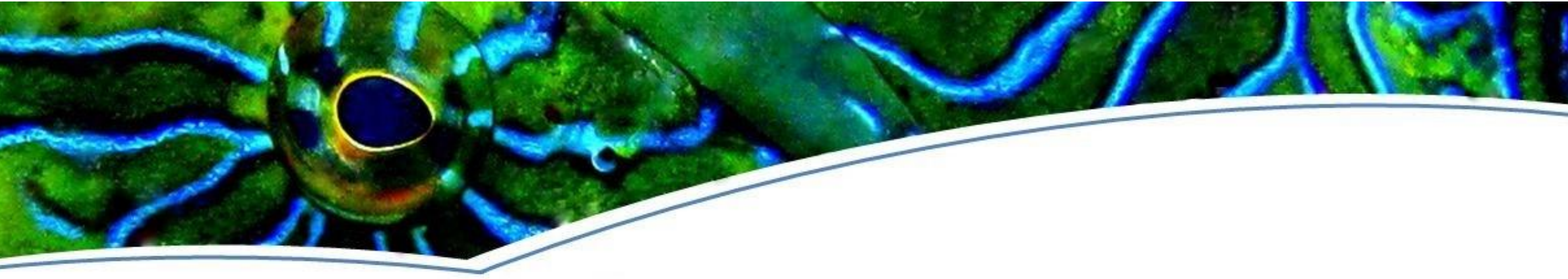
Palacios Otero, Maria del Mar (2017) ***Controlling mesopredators: importance of behavioural interactions in trophic cascades.*** **PhD thesis, James Cook University.**



Controlling Mesopredators: importance of behavioural interactions in trophic cascades

Thesis submitted by

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January 2017

for the degree of **Doctor of Philosophy in Marine Biology**

ARC Centre of Excellence for Coral Reef Studies

College of Science and Engineering

James Cook University

Dedicated to the ones I love …

To my amazing mom, dad and brother

who infused my childhood with science, oceans, travel and.

To my beloved partner for all his emotional and scientific support.

Acknowledgements

I owe a debt of gratitude to many people who have contributed to the success of this PhD thesis and who have made this one of the most enjoyable experiences of my life.

Firstly, I would like to thank my supervisor Mark McCormick for embarking with me on this ‘mesopredator’ journey. I greatly appreciate all his expertise, insight, knowledge and guidance. My gratitude is extended to all my co-authors, Donald Warren, Shaun Killen, Lauren Nadler, and Martino Malerba who committed to my projects and shared with me their knowledge, skills and time. Special thanks to Lauren Nadler, for all her friendship and valuable help organizing fieldtrips, proof reading manuscripts, and rehearsing for conferences.

This thesis involved extensive fieldwork (9 months), so I have infinite gratitude to all the people that volunteered and helped me execute each of the lab and field experiments (Donald Warren, Diego Lozano-Cortes, Pascal Sebastian, Maddison Craig, Christopher Mirbach, Diletta Carmellini, Fernando Zapata). I would like to particularly thank Donald Warren and Maddison Craig for all their commitment, enthusiasm, and hard work in the field. I am additionally grateful to the directors (Lyle Vail & Anne Hoggett) and staff of Lizard Island Research Station which greatly facilitated the logistics of my research. To my lab-mates, Bridie Allen, Lauren Nadler, Donald Warren, Govinda-das Lienart and Ryan Ramasamy, thank you so much for the help light-trapping, fish sorting and tagging, plumbing tanks and capturing dottybacks. I greatly appreciate the work of my peer editing friends (Lauren Nadler, Steve Doo & Paloma Matis), which have greatly improved the quality of drafts from my thesis, manuscripts, and grant proposals. The feedback provided by the two external reviewers of this PhD thesis allowed me to greatly improve the quality of my research. I am grateful to them for all their comments and suggestions.

I would warmly like to thank my parents, Yolanda Otero and Jairo Palacios, who showed me to love science and always encouraged me to pursue my academic dreams. Also, to my marine biologist aunt and uncle, Martha Palacios and Enrique Pena, who first exposed me (> 20 years ago) to marine labs, algae cultures and aquariums. Lastly, I am forever grateful to my partner Martino Malerba for being my greatest scientific and emotional ally and always filling my life with happiness and joy.

Statement of Contribution of Others

This thesis includes collaborative work with my supervisor Prof Mark McCormick (James Cook University, AU). Together, we conceived and designed the projects. I collected and analysed the data, interpreted the results and wrote initial versions of the manuscripts. Mark provided feedback on the manuscripts, along with intellectual, technical, and financial support.

Several collaborators provided additional technical and editorial guidance on specific chapters:

- **Chapter 2:** Mr. Donald Warren (James Cook University, AU)
- **Chapter 4:** Dr. Shaun Killen (University of Glasgow, UK)
  Dr. Lauren Nadler (James Cook University, AU)
  Dr. James White (James Cook University, AU)
- **Chapter 5:** Dr. Martino Malerba (James Cook University, AU)

Throughout the thesis, I received statistical support from Dr. Martino Malerba and assistance in data collection from Donald Warren, Maddison Craig, Christopher Mirbach, Diego Lozano-Cortes, Pascal Sebastian, Fernando Zapata, Diletta Carmellini, Bridie Allan and Lauren Nadler. A peer-editing group composed by Lauren Nadler, Steve Doo and Paloma Matis provided editorial help.

Financial support for these projects was provided by an Ian Potter Doctoral fellowship at Lizard Island (M.M. Palacios), Australian Coral Reef Society research award (M.M. Palacios), James Cook University Graduate Research Scheme (M.M. Palacios), International Postgraduate Research Scholarship - James Cook University (M.M. Palacios), International Doctorate Scholarship - Department of Science, Technology and Innovation of Colombia - COLCIENCIAS (MM. Palacios), Australian Research Council Discovery Scheme (M. McCormick) and the ARC Centre of Excellence for Coral Reef Studies (M. McCormick).

All the experiments that encompass this thesis were conducted at Lizard Island Research Station (a facility of the Australian Museum).

Abstract

Trophic cascades illustrate the role of predation on ecological communities. In the absence of top-down control from apex predators, mid ranking species can drastically increase their abundance and foraging activity, creating stronger impacts on bottom prey populations. The consequences of such mesopredator release are difficult to predict, as intraguild predator-predator interactions can alter the impact of mesopredators on prey. Top-predators, through risk effects, can elicit antipredator responses that limit the behaviour and foraging activity of mesopredators. Moreover, positive (e.g., cooperative hunting) or negative behavioural interactions (e.g., interference) among mesopredators can modify each other's feeding rate, and hence any expected impact on prey. Although trophic cascades are of major concern for wildlife conservation and management purposes, there is limited understanding of the behavioural links in food webs and how they may influence ecosystem dynamics.

This thesis examined how behavioural interactions throughout the food web mediate the impacts (lethal and sub-lethal) of mesopredators on their prey. By using a model food chain of coral reef fishes [coral trout (*Plectropomus leopardus*) > dottybacks and groupers (*Pseudochromis fuscus* and *Cephalopholis boenak*, *Epinephelus maculatus*) > juvenile damselfish (*Pomacentrus* spp.)], this research provided key insights on the cascading consequences of top-predator loss on coral reefs. The thesis consists of several field and laboratory experiments that manipulated the lethal and sub-lethal effects of predation to examine the implications for the predators and their prey.

Fishes use visual and chemical stimuli to assess predation risk and develop antipredator responses. However, it remains unknown whether these cues in isolation are sufficient to induce behavioural changes in mesopredator-prey interactions. Hence, in **Chapter 2**, I examined how different combinations top-predator cues (visual, chemical, or both) alter mesopredator behaviour and modify their impact on juvenile damselfish prey. Laboratory results showed that even the independent sight or smell of a top-predator can strongly restrict the distance swum, area explored and foraging activity of mesopredators. In doing so, this mesopredator restriction indirectly triggered a behavioural release (e.g., increased their space use and activity) of their damselfish prey.

To further examine the impact of behavioural trophic cascades on juvenile fishes I tested the consequences of predation risk under natural field settings (**Chapter 3**). Using experimental patch reefs, I assessed the behaviour and survival of juvenile fishes placed on patches where cues from a top-predator fish (visual and chemical stimuli) or controls (PVC pipe or empty patch) had

been deployed. After exposure to top-predator cues for 72 h, damselfishes from top-predator patches had significantly higher space use (46 %), feeding rate (95 %) and survival (67 %) than those from control patches. This study demonstrated that predation risk from large-sized predators can indirectly favour the persistence and behaviour of bottom level prey in the field.

In **Chapter 4**, I extended the results from Chapters 2 and 3 by examining whether cascading effects from top-predators could influence other phenotypic traits of bottom prey, such as physiology. Using intermittent-flow respirometry in a laboratory setting, I measured the oxygen (O_2) uptake (a proxy for aerobic metabolism) of juvenile fish exposed to a combination of mesopredator and top-predator cues. Interestingly, results suggested that top-predators can benefit bottom prey physiologically by indirectly allowing them to maintain routine O_2 uptake, minimising their metabolic stress. This study illustrated for the first time that top-predators can indirectly negate the effect of mesopredators on prey metabolic rates.

Each mesopredator species can impact prey differently. Moreover, their combined effects cannot typically be predicted by their independent contributions. Thus, in **Chapter 5**, I tested for multiple predator effects, exploring how intra- and inter-specific interactions among three mesopredator species influenced their combined lethal impact on bottom prey survival. By manipulating the density and diversity of mesopredators in a mesocosm experiment, I found that behavioral patterns within mesopredator pairs often matched those predicted from their hunting mode. However, the identity of the mesopredator species determined the strength of any positive or negative interactions (e.g., synergies, interference), and thus the nature and magnitude of MPEs on prey survival (i.e., risk-enhancing effects, risk-reducing effects, linear effects). Given the context-dependency of the predator-predator interactions, none of the mesopredators tested are expected to play identical functional roles on natural coral reefs.

The overarching aim of this thesis was to establish the importance of behavioural interactions in the control of mesopredators. This was achieved by conducting field and laboratory manipulations of a three-level food web of coral reef fishes. Results indicated that lethal and sub-lethal impacts of mesopredators on prey can be largely diminished by predation risk from top-predators (**Chapters 2, 3, 4**). However, intra- and inter-specific interactions with other mesopredators most often enhance prey mortality (**Chapter 5**). From a conservation perspective, these findings illustrate how a predatory-release of mid-ranking species could impair the replenishment, fitness, and development of newly-settled fish, and thus indirectly modify the structure and diversity of reef fish communities. From an ecological point of view, these findings highlight the role and importance of behavioural interactions in the balance of complex food webs.

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APPENDIX A

Chapter 1: General Introduction

Trophic cascades are one of the most influential ecological concepts of the last decades (Ripple et al. 2016). Interest in trophic cascades continues to rise with the widespread exploitation of large-carnivores from marine and terrestrial ecosystems and the documentation of their devastating impacts on community structure, function, and diversity (reviewed by Estes et al. 2011, Ripple et al. 2014). Vast correlative and experimental evidence has demonstrated that the depletion of top-predators often results in an explosion of mid-ranking consumers/predators (known as mesopredators) which increase their abundance, foraging activity and habitat use, having strong impacts on bottom trophic levels (Prugh et al. 2009, Ritchie and Johnson 2009). Given that bottom prey is often represented by autotrophs and primary producers, trophic cascades (see Table 1.1 for terminology definitions) can easily tip ecosystems towards alternate states and phase shifts (Brashares et al. 2010, Terborgh and Estes 2010, Estes et al. 2011).

Table 1.1. Definition of terms related to trophic cascades. Modified from Creel and Christianson (2008), Madin et al. (2016) and Ripple et al. (2016).

Term	Definition
Trophic cascades	Indirect species interactions that originate with predators and propagate downward through the food web. They must involve indirect effects, but are not required to begin with apex predators nor end with autotrophs.
Lethal effects	Consumptive effect of a predator on prey due to predation mortality.
Risk effects	Non-consumptive effect of a predator on prey. It is triggered by changes in prey behaviour, physiology or morphology in response to predation risk.
Indirect effects	Interaction between two species with one or more interceding species. It involves two or more adjacent links in an interaction chain.
Mesopredator release	Increase in the abundance and/or behaviour of a mid-ranking (meso) predators.
Behavioural trophic cascade	Trophic cascade driven by changes in mesopredator behaviour.
Numerical trophic cascade	Trophic cascade driven by changes in mesopredator abundance or biomass.

1.1 Controlling mesopredators through Lethal & Risk effects

Top-predators can exert top-down control on mesopredators through several (often coupled) mechanisms. Top-predators control mesopredators through lethal (consumptive) effects when they kill and reduce the mesopredator population, thereby restricting their impact on lower level prey (Abrams 1995, Creel and Christianson 2008). Such trophic cascades driven by changes in mesopredator abundance or biomass, are referred to as Numerical trophic cascades. A well-known example of this is the sea otter/sea urchin/kelp trophic cascade of the Aleutian Islands, in which the collapse of the sea otter population (top-predator) led to the demise of the kelp forest (bottom level species) due to an explosion of overgrazing sea urchins (mesoconsumers; Estes and Palmisano 1974; Fig 1.1a).

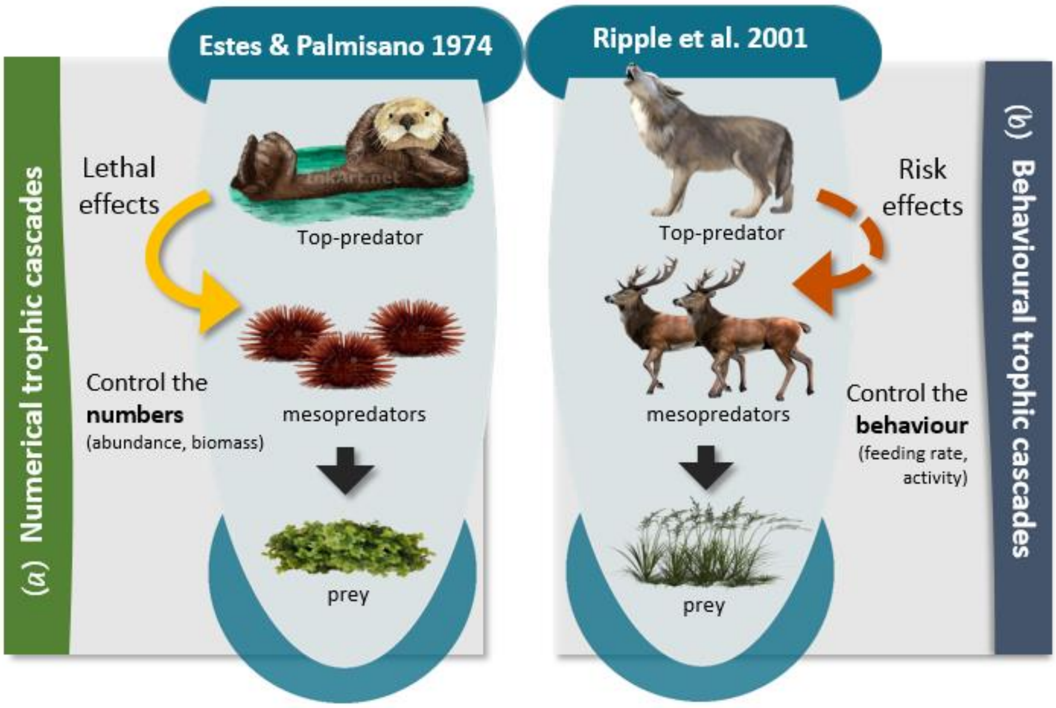

Figure 1.1. (a) Numerical trophic cascade triggered by the lack of lethal (consumptive) effects from sea otters. **(b)** Behavioural trophic cascade elicited by the loss of risk (non-consumptive) effects from wolves.

Interestingly, top-predators can also control mesopredators through risk (non-consumptive) effects (Abrams 1995, Werner and Peacor 2003, Creel and Christianson 2008). Motivated by fear alone, mesopredators can develop costly traits (e.g., changes in behaviour, morphology and physiology) to reduce the predation risk from top-predators (Lima and Dill 1990, Lima 1998b). Such an antipredator response usually restricts their foraging behaviour and habitat use, limiting their impact on lower trophic level species (Schmitz et al. 2004). For example, in North America, wolves are known to limit the habitat use and grazing patterns of ungulate herbivores which indirectly enhances the survival and replenishment of native vegetation (Ripple et al. 2001). Behavioural trophic cascades triggered by changes in mesopredator behaviour can easily propagate in the ecosystem due to their spatial and temporal scope. Predation risk is experienced simultaneously by many prey for prolonged periods of time, it can operate in short temporal scales and can be transmitted from one prey to another (Werner and Peacor 2003, Schmitz et al. 2004, Berger 2010). This can make the non-consumptive effects of top-predators equal or greater than their direct consumptive effects (reviewed by Preisser et al. 2005).

Mesopredators can directly impact the survival and phenotype of low trophic level species through lethal and sub-lethal effects (Abrams 1995, Lima 1998a). However, as illustrated on Figure 1.1, most trophic cascades describe how changes in the suppression of mesopredators alter the lethal impact on bottom prey (e.g., changes in the survival, biomass and/or abundance of plants, birds and small mammals; reviews by Schmitz et al. 2004, Ritchie and Johnson 2009, Estes et al. 2011). In contrast, ecologists have rarely examined the cascading effects of top-predators on the phenotypic traits of low trophic levels (e.g., changes in physiology, behaviour and morphology). With the suppression of mesopredator foraging activity and habitat use, bottom level prey can often reduce their antipredator response and allocate more energy and time to important, fitness-enhancing processes such as feeding, growth, development and reproduction (Lima 1998b, Hawlena and Schmitz 2010). For instance, experimental evidence from Australia showed that the foraging efficiency and habitat breadth of small rodents (bottom level consumers) can be greatly enhanced when dingoes (top-predator) suppress the numbers and activity of mesopredator feral cats (Gordon et al. 2015). To fully understand the indirect effects of top-predators on food webs and ecosystem dynamics, further research needs to explore how trophic cascades can impact phenotypic traits of bottom prey.

1.2 Controlling mesopredators through intraguild interactions

The fact that most prey face multiple mesopredators in natural systems (Schoener 1989, Polis 1991) limits our ability to predict the consequences of a mesopredator release. First, each mesopredator can impact prey differently according to its life-history traits (e.g., identity, hunting mode, foraging behaviour; Schmitz 2007). Second, the combined effects of multiple predators cannot usually be predicted from their independent contributions (Sih et al. 1998). Evidence mainly from invertebrate food webs suggests that behavioural interactions within the mesopredator trophic guild (intra – interspecific interactions) can change the foraging behaviour of mesopredators and their combined impact on prey (e.g., Soluk and Collins 1988, Losey and Denno 1998, Vance-Chalcraft and Soluk 2005, Griffen 2006).

Multiple predators can have linear or non-linear effects on the mortality of their shared prey (Sih et al. 1998; Table 1.2). Linear effects occur when their combined impact on prey mortality is similar to that predicted by the sum of their independent effects (e.g., when each one forages alone; Sokol-Hessner and Schmitz 2002). In contrast, non-linear effects can cause higher or lower mortality levels than those predicted. Positive behavioural interactions among predators (i.e., facilitation) usually allow them to kill more prey than expected (i.e., risk- enhancing effects; Losey and Denno 1998), while negative interactions (i.e., intraguild predation, interference competition) tend to reduce their joint impact on prey mortality (i.e., risk- reduction; Rosenheim et al. 1993, Vance-Chalcraft and Soluk 2005). Behavioural changes of the prey in relation to the predators involved can additionally influence the nature of multiple predator effects (Sih et al. 1998; Table 1.2).

Table 1.2. Multiple predator effects on prey mortality. Modified from Sih et al. (1998).

EFFECT ON PREY MORTALITY	PREDATOR – PREDATOR BEHAVIOUR	PREY ANTIPREDATOR BEHAVIOUR
Linear: Predictable	No interaction	
Risk – enhancing: Higher than predicted	Positive interaction (e.g., cooperative hunting, facilitation)	Conflicting predator-specific defences: prey's response to one predator increases its risk to another predator
Risk – reducing: Lower than predicted	Negative interaction (e.g., interference competition, intraguild predation)	Non-specific defences: prey reduce activity or increase sheltering, becoming less available or conspicuous to predators

Much caution is required when assessing multiple predator effects (MPEs) as their magnitude can be largely influenced by the density of predators and prey, the hunting mode and identity of predators, and the topography of the habitat (reviewed by Sih et al. 1998, Schmitz 2007). Given the complexity of MPEs, only through robust experimental designs and detailed manipulations of the mesopredators (e.g., simultaneous use of additive and substitutive experiments; Griffen 2006, Soomdat et al. 2014) will we be able to comprehend their interactive effects on prey and how they may impact the magnitude of trophic cascades. Studies examining MPEs on high order species (e.g., vertebrates) and complex food webs are additionally required to fully encompass the extent of behavioural interactions on natural ecosystems (Sih et al. 1998).

1.3 Trophic cascades in coral reefs

Coral reefs harbour the most diverse food webs of marine fishes, with up to 200 macro-carnivore fishes playing different functional roles and interacting in complex networks (Bellwood et al. 2004, Helfman et al. 2009). Unfortunately, on most unprotected reef systems, species playing top-predatory roles have been over harvested and exploited by the fishing industry (e.g., reef sharks and large-bodied groupers; Jackson et al. 2001, Myers and Worm 2003, Robbins et al. 2006, DeMartini and Smith 2015). Unequivocally, such a widespread decline of apex piscivores has reduced their functional role in ecosystems by weakening the lethal and risk effects they exert on the fish food web.

Particularly in coral reef fishes, trophic cascades appear to be mainly triggered by the loss of risk effects from large piscivores. Except for Mumby et al. (2012), which used a 7-year data set to illustrate a numerical trophic cascade of reef fish in the Caribbean, most studies fail to detect indirect numerical changes (e.g., biomass, abundance, percent cover) of bottom level species due to the loss of lethal effects from large piscivores (Russ and Alcala 1998, Mumby et al. 2006, Newman et al. 2006, Sandin et al. 2008). It is believed that successive lethal links along the fish food web are often shaded by: (i) the lack of baseline data prior to top-predator declines, (ii) the importance of bottom-up processes (productivity), and (iii) the prevalence of omnivory, opportunism, dietary breadth and ontogenetic diet shifts among marine fishes (reviewed by Baum and Worm 2009). In contrast, recent research has shown that loss of risk effects allows small-bodied herbivores to increase their feeding rates, foraging grounds, and activity periods leading to stronger impacts on macroalgae prey (e.g., Madin et al. 2010a, Rizzari et al. 2014, Catano et al. 2016, Kindinger and Albins 2017). This evidence indicates that the presence of large piscivores can favour the survival, biomass and/or distribution of bottom level prey through the suppression of herbivores. More research, however, needs to address how such behavioural suppression applies to small-sized carnivores (mesopredators) and whether it can dampen both the lethal and the sub-lethal effects on their juvenile fish prey (Stallings 2008).

The indirect consequences of predator loss are difficult to predict in coral reefs. The complexity of the fish mesopredator guild (reviews by Prugh et al. 2009, Ritchie and Johnson 2009) and the paucity of research on behavioural linkages among multiple trophic levels (Dill et al. 2003, Schmitz et al. 2004, Preisser et al. 2005, Baum and Worm 2009), prevents us from understanding the net effect that a mesopredator release will have on the recruiting community of juvenile fish. The speed at which populations of large fish are being reduced (Jackson et al. 2001, Myers and Worm 2003) highlights the need to increase our understanding of behavioural mesopredator interactions in trophic cascades. Studying predator interactions will better inform predictions about the consequences of predator loss and largely improve the efficiency of fisheries and management policies on coral reefs (Dill et al. 2003, Heithaus et al. 2008, Madin et al. 2016).

1.4 Study system

This thesis was conducted at Lizard Island, northern Great Barrier Reef (14°40´S, 145°28´E), during the austral summer months (October-January). At this location, all the fish species employed are consistently abundant at shallow patch reefs where they frequently interact through predator – prey dynamics (Caley and St John 1996, Stewart and Jones 2001). Throughout the chapters of this thesis I used the following three-level food web of coral reef fish to address my research aims.

- **Top-predator:** The leopard coral trout, *Plectropomus leopardus*, was used as the top-predator species (Fig 1.2a). This conspicuous reef piscivore reaches > 60 cm standard length (SL), feeds predominantly on small sized fish (St. John 2001), has few natural predators, and occupies the same trophic level as reef sharks (Frisch et al. 2014, Frisch et al. 2016a). Despite coral trouts being relatively common on the GBR (up to 240 fish ha -1; Kingsford 2009, Miller et al. 2012), they are classified as Near threatened as per IUCN criteria due to the high fishing pressure and poor fisheries management populations experience at some locations of the Indo-Pacific (Sadovy de Mitcheson et al. 2013).

- **Mesopredators**: The dottyback, *Pseudochromis fuscus*, was used as the primary focal mesopredator species as it thrives under laboratory conditions and its ecology, biology, and behaviour have been extensively studied (e.g., Beukers and Jones 1998, Holmes and McCormick 2010, Feeney et al. 2012, Ashworth et al. 2014, Cortesi et al. 2015). Dottybacks are small-bodied (< 12 cm total length; TL; Fig. 1.2b), site-attached carnivores that voraciously consume newly-settled fishes using ambush and pursuit techniques (Feeney et al. 2012). Additional mesopredator species used in these studies included the groupers *Cephalopholis boenak* and *Epinephelus maculatus* (only juvenile stage individuals, ~6-12 cm TL) which also co-occur on shallow patch reefs and are known to frequently hunt juvenile reef fishes (Stewart and Jones 2001, Beukers-Stewart and Jones 2004).

- **Bottom level prey:** Juveniles of the whitetail damselfish, *Pomacentrus chrysurus,* and the ambon damselfish*, P. amboinensis,* were used as bottom prey (Fig. 1.2c). Larvae of these planktivorous fishes (10 - 15 mm SL; Kerrigan 1996) settle to coral and rubble reefs during the austral summer months. At this point, they are subject to extremely high rates of predation by small reef piscivores such as groupers, dottybacks and lizardfishes (Almany and Webster 2006). Studying the cascading lethal and sub-lethal impacts that these juvenile fish face, is a priority given that any community changes at this initial life-stage may have a disproportionate influence on the attributes of the adult fish assemblage (Caley 1998).

The terms 'top-predator > mesopredators > bottom prey' refer to the hierarchy and trophic status among the species used in this thesis and is not meant to imply that they have a fixed trophic category in natural systems. For instance, despite trout species usually hold top-predatory roles in coral reefs (similar to that of reef sharks; Frisch et al. 2014, Frisch et al. 2016b), they might occupy lower mesopredatory levels if they co-occur with larger-sized piscivores (e.g., tiger sharks, *Galeacerdo cuvier*).

Figure 1.2. Reef fish species. **(a)** Coral trout, *Plectropomus leopardus*, **(b)** dottyback, *Pseudochromis fuscus* and **(c)** ambon damselfish, *Pomacentrus amboinensis*. Photographs by M.M Palacios (a,c) and Christopher Mirbach (b).

1.5 Aims & thesis outline

The overarching aim of this thesis was to establish the importance of behavioural interactions in the control of mesopredators. I designed my research aims from two perspectives. First, I examined how interactions between mesopredators and bottom prey may be mediated by risk effects from top-predators (**Chapter 2**; Fig. 1.3b) and how this cascades to the prey of mesopredators through lethal and sub-lethal effects (**Chapters 3** and **4**). Second, I studied how intra- and inter-specific interactions with other mesopredators also mediate a mesopredator's impact on their prey (**Chapter 5**; Fig. 1.3c). The specific aims of the four data chapters of this PhD thesis are as follows:

- **Chapter 2:** Examines the relative importance of visual and chemical cues in the top-down control of mesopredators.
- **Chapter 3:** Explores the cascading consequences of mesopredator control on the survival and behaviour of bottom prey.
- **Chapter 4:** Investigates potential cascading impacts of mesopredator control on prey physiology.
- **Chapter 5:** Tests how behavioural interactions among mesopredator species influence the nature and magnitude of multiple predator effects (MPEs).

Overall, this research filled knowledge gaps on how species along a food web interact behaviourally and how populations change in response to perturbations in the trophic links.

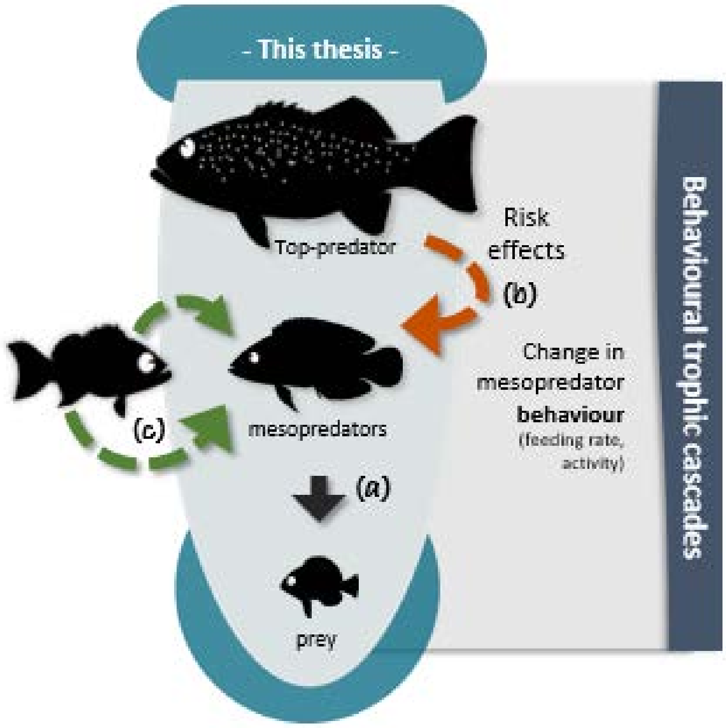

Figure 1.3. Interaction between a mesopredator and **(a)** its prey, which can be modified by risk effects from a **(b)** top-predator and/or **(c)** behavioural interactions with other mesopredators.

1.5 Publication Details

Each data chapter is presented as a stand-alone scientific article. Thus, the contextualization of the work in each chapter was broadened to fit the scope of the target journal. At present, **Chapter 2** has been published in *Oikos* (Palacios et al. 2016b) and **Chapter 4** in the *Journal of Animal Ecology* (Palacios et al. 2016a). **Chapters 3** and **5** have been submitted for publication in *Proceedings of the Royal Society B* and *Oecologia*, respectively.

Chapter 2: Sensory cues of a top-predator indirectly control a reef fish mesopredator

Published
Palacios, M.M., D.T. Warren & M.I. McCormick. 2016
Sensory cues of a top-predator indirectly control a reef fish mesopredator
Oikos 125: 201-209
http://onlinelibrary.wiley.com/doi/10.1111/oik.02116/full

2.1 Summary

Behavioural trophic cascades highlight the importance of risk effects in the maintenance of healthy trophic level links in complex ecosystems. However, there is limited understanding on how the loss of indirect top-down control can cascade through the food web to modify lower level predator-prey interactions. Using a reef fish food web, our study examines behavioural interactions among predators to assess how fear elicited by top-predator cues (visual and chemical stimuli) can alter mesopredator behaviour and modify their interaction with bottom prey. Under experimental conditions, the presence of any cue (visual, chemical, or both) from the top-predator (coral trout, *Plectropomus leopardus*) strongly restricted the distance swum, area explored and foraging activity of the mesopredator (dottyback, *Pseudochromis fuscus*), while indirectly triggering a behavioural release of the bottom prey (juveniles of the damselfish *Pomacentrus chrysurus*). Interestingly, the presence of a large non-predator species (thicklip wrasse, *Hemigymnus melapterus*) also mediated the impact of the mesopredator on prey, as it provoked mesopredators to engage in an inspection behaviour, while significantly reducing their feeding activity. Our study describes for the first time a three-level behavioural cascade of coral reef fish and stresses the importance of indirect interactions in marine food webs.

2.2 Introduction

The indirect effects of predators influence the structure and dynamics of ecological communities by affecting fear and risk landscapes for prey (Schmitz et al. 2004, Creel and Christianson 2008). Evidence from both terrestrial and aquatic ecosystems, suggests that risk imposed by top-predators is strong enough to cascade through food webs, controlling the behaviour of mid trophic level species (i.e., mesopredators, mesoconsumers) and mediating their impact on bottom prey (reviewed by Vance-Chalcraft et al. 2007). Under risk of predation, mesopredators undergo costly behavioural changes (antipredator response) in which they attempt to avoid a top-predator at the expense of foraging or other fitness-enhancing activities (Sih 1980, Lima and Dill 1990). For example, research on the wolf/ungulate/aspen system in North America shows that the removal of predation risk by depletion of large carnivores triggers an ecological and behavioural release of mesopredators, which often leads to a depletion of bottom prey species (reviews by Prugh et al. 2009, Ritchie and Johnson 2009, Estes et al. 2011, Ripple et al. 2014). While the theory of trophic cascades is well developed, there is limited field evidence on the connections among trophic levels and how populations change in response to perturbations in the trophic links.

Coral reefs are among most the diverse ecosystems, with up to 200 species of fish macro-carnivores interacting in complex food webs (Bellwood et al. 2004). The balance of the coral reef food web could be, however, under threat as the overexploitation of large predatory fishes (Jackson et al. 2001, Myers and Worm 2003, Robbins et al. 2006) may eliminate the predation risk that behaviourally controls many marine mesopredators (reviewed by Heithaus et al. 2008). Recent field-evidence already shows that in the absence of top-predators, mid-size fishes increase their feeding rates, foraging area and activity periods, likely having negative effects on the population of bottom prey (Stallings 2008, Madin et al. 2010b, McCauley et al. 2010, Ruppert et al. 2013b, Rizzari et al. 2014). Nevertheless, because much of this research has focused on mid-level herbivores (which feed on behaviourally simple organisms such as plants or algae; e.g., Madin et al. 2010a, Rizzari et al. 2014) we have limited understanding on how indirect effects can be transmitted though the fish food web or how mesopredator release can lead to strong behavioural suppression of prey fish populations. Despite many authors highlighting the strength of behavioural cascades and the importance of trait mediated indirect interactions (reviewed by Werner and Peacor 2003, Schmitz et al. 2004), most evidence comes from terrestrial and freshwater ecosystems. Marine ecologists have seldom studied the behavioural linkages between more than two trophic levels simultaneously (reviewed by Preisser et al. 2005), and never (to our knowledge) using a three level food chain of fish. Detailed studies of mesopredator fish behaviour are needed to elucidate cascading indirect fear effects in coral reefs and understand the indirect consequences of large-carnivore declines in marine systems (Heithaus et al. 2008, Baum and Worm 2009).

To understand the nature of the linkages among trophic groups it is necessary to first comprehend how predators and prey interact. Aquatic vertebrates typically assess predation risk through chemical and visual stimuli (Helfman 1989, Murphy and Pitcher 1997, Kats and Dill 1998, Brown et al. 2011) which contain different information on the type, temporal resolution and intensity of the threat (Smith and Belk 2001). Chemical cues, such as predator released odours (i.e., kairomones) or conspecific damage-release alarm cues inform prey of the characteristics of the threat over large spatio-temporal scales (Kats and Dill 1998, Wisenden 2000, Ferrari et al.

2010, Brown et al. 2011). These cues are usually complemented by visual stimuli (e.g., sight of predator) which are indicative of immediate threat and give more accurate information on the location, behaviour and motivation of the predator (Helfman 1989, Murphy and Pitcher 1997, Hartman and Abrahams 2000, Smith and Belk 2001). The dominance of one or the other cue depends on both the environment and context of the cue transition (McCormick and Lönnstedt 2013). Determining how mesopredators respond to sensory cues from top-predators is key to understanding the behavioural mechanisms that underlie indirect risk effects.

To extend our understanding of the indirect top-down control of fish community dynamics, we studied how risk elicited by top-predator cues (chemical and visual stimuli) affected mesopredator behaviour and modified their interaction with bottom prey. Using experimental mesocosms, we used a three level food-web of coral reef fishes (coral trout/dottyback/damselfish juveniles) to address the following questions: (a) how is the activity, space use and feeding behaviour of a mesopredator modified by cues from a top-predator? (b) does the magnitude of the antipredator response by mesopredators vary with the type of cue present (chemical or visual stimuli) or the interaction amongst cues? (c) are top-predator cues enough to behaviourally control the mesopredator foraging activity? and (d) how is the behaviour of bottom prey modified by changes in mesopredators behaviour that results from the presence of top-predator cues? Answering these questions allowed us to describe for the first time a three level behavioural cascade of coral reef fish and stress the importance of indirect interactions in marine food webs.

2.3 Materials & Methods

Study species & Fish handling

Fish were collected from the lagoon of Lizard Island (14°40´S, 145°28´E), northern Great Barrier Reef (GBR), during the austral summer months (October-January). *Pseudochromis fuscus* (brown dottyback) was used as the focal mesopredator species, as it is a small site-attached carnivore (up to 10 cm total length; TL) known to voraciously consume newly settled fishes in both the laboratory and the field (Beukers and Jones 1998, Feeney et al. 2012). Adult dottybacks (6.9 ± 0.09 cm, mean TL ± SE, N=50) were captured from shallow patch reefs by SCUBA divers using hand nets and an anaesthetic clove oil solution. Bottom prey consisted of planktivorous juveniles of the whitetail damselfish, *Pomacentrus chrysurus* (1.3 ± 0.003 cm, mean standard length SL ± SE, N=300), collected with light traps just before their recruitment on the reef. Traps were moored overnight at least 100 m away from the reef edge and collected at dawn. The top-predator used was the leopard coral trout, *Plectropomus leopardus*. This large carnivore is relatively common on the GBR (3.5 to 9.0 fish 1,000 m^2; Ayling et al. 2000), reaches more than

50 cm SL, and feeds predominantly on small sized (< 9 cm) reef associated fish (St. John 2001). The thicklip wrasse, *Hemigymnus melapterus*, was used as the non-predator species. Despite its large size (> 30 cm TL) it feeds mainly on small invertebrates like crustaceans, polychaete worms and molluscs (Randall 2013). Individuals of *P. leopardus* (39.3 ± 2.6 cm, mean TL ± SE, N=4) and *H. melapterus* (33.4 cm TL) were caught using hand lines (with barbless hooks) and barrier nets, respectively.

Fishes were maintained in separate tanks at the Lizard Island Research Station, in a flow-through seawater system at ambient temperatures and light photoperiods. Damselfish juveniles were fed *Artemia* spp. twice daily, while the rest of the collected fishes (dottybacks, coral trouts and the thicklip wrasse) were daily fed thawed prawn or squid. All fish were released at the end of the experiment back to their collection site.

Experimental overview

Changes in the behaviour of the focal mesopredator (dottybacks) to five experimental treatments were quantified: (i) visual cue of the top-predator; (i.i) chemical cue (odour) of the top-predator; (iii) simultaneous chemical and visual cues of the top-predator; (iv) simultaneous chemical and visual cues of the non-predator; and (v) chemical and visual cues of an empty tank. The first three treatments (i, ii, and iii) corresponded to top-predator treatments, while the last two (iv and v) served as controls. Behavioural observations were recorded on six bottom prey (damselfish juveniles) while the mesopredators were exposed to the experimental treatments. Ten replicate trials were undertaken for each cue treatment, with all fish being tested only once to maintain independence among trials.

Observation mesocosms

The experiments were undertaken in five replicate 368-l pools (referred hereafter as mesopredator arena; 112.5 cm diameter x 40 cm height; water height ~ 28 cm), containing a piece of rubble head (20 x 20 x 15 cm), an air stone, a 2 cm layer of sand, a 30 cm ruler (used for reference in the video recording) and two glass tanks located on opposite ends (a rectangular for the top-predator and a cylindrical for the bottom prey; Fig. 2.1). The top-predator tank (also used for the non-predator; 36 x 47 x 40 cm) was screened on all sides with removable black Perspex panes, while the cylindrical prey tank (24 cm diameter x 38 cm height) was clear plastic with 32 small holes (< 0.5 cm) to allow exchange of chemical cues between the mesopredators and the bottom prey. Two video cameras were positioned on tripods over the observational mesocosms; one was high above the mesopredator arena to record the movements of the dottybacks, while the other was placed over the prey tank to document the behaviour of the damselfish juveniles and the feeding strikes of the dottybacks on the tank. The five experimental treatments were randomly assigned daily to each of replicate observational pools. After running each trial the seawater from the pool was completely exchanged and all the objects (e.g., rubble head, ruler) were thoroughly rinsed with saltwater to remove residual chemical cues.

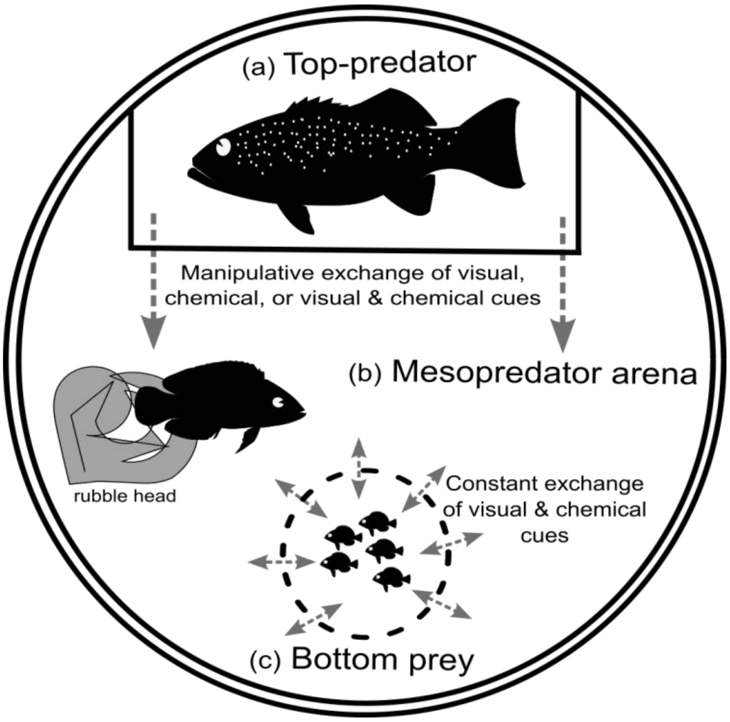

Figure 2.1. Experimental mesocosms constructed to assess the indirect interactions between a three-level food web of coral reef fish. **(a)** Rectangular tank to hold the top–predator (coral trout, *Plectropomus leopardus*) or the non-predator (thicklip wrasse, Hemigymnus melapterus); **(b)** Mesopredator arena where *Pseudochromis fuscus* (dottybacks) could swim freely and indirectly interact with top-predator and/or prey; and **(c)** Cylindrical tank to hold the bottom prey (damselfish juveniles, *Pomacentrus chrysurus*). The top-predator tank (also used for the non-predator; 36 x 47 x 40 cm) was screened on all sides with removable black Perspex panes, while the cylindrical prey tank (24 cm diameter x 38 cm height) was clear plastic with 32 small holes (< 0.5 cm) to allow exchange of chemical cues between the mesopredators and the bottom prey.

Experimental protocol

Before running experimental trials, damselfish juveniles were trained to recognize cues from the dottybacks as their collection prior to reef settlement may have prevented them from learning the visual and chemical identity of reef associated predators. Naïve juvenile fishes can learn the identity of a novel predator by pairing simultaneously conspecific damage-released chemical cues (indicative of threat) with visual and/or chemical cues of a predator (Brown and Chivers 2005, Ferrari et al. 2010, Lönnstedt et al. 2013a). To prepare the damage-released chemical cues, three damselfish per training session (1.2 – 1.4 cm SL) were euthanized with a quick blow to the head (following James Cook University animal ethics permit: A1720) and placed in a petri dish where 10 superficial cuts were made to the skin of each donor fish (5 cuts per flank). Fish were then rinsed with 10 ml of sea water (previously obtained from their tank) creating a solution of damage-release alarm cues. To obtain the mesopredator odour (kairomones), four dottyback*s* were randomly selected and kept for at least 12 h in a tank containing 4-l of aerated seawater. Training of the damselfish juveniles occurred by introducing simultaneously in their 22-l holding tank (with water flow suspended): 10 ml of the conspecifics damage-released chemical cues; 30 ml of the dottyback odour; and a live dottyback placed in a sealed ziploc bag (serving as a visual cue). After 10 min all cues were removed from the tank, and water flow was restored. Three training sessions were carried out in total, each one comprising the training of ~ 100 damselfish juveniles.

Experimental trials were initiated with a 5 min acclimation period of the coral trout and the six damselfish juveniles (instead of just one, to further stimulate the activity of the dottyback) in their respective observation tanks. A dottyback (previously starved for 48 h) was then introduced in to the arena and its pre-stimulus behaviour was video recorded for 10 min. The assigned stimuli were revealed afterwards by: (a) removing the black panes from the top-predator tank thereby allowing the mesopredator sight of the top-predator; (b) overflowing the top-predator tank to spill coral trout odour (chemical cue) into the mesopredator arena; or (c) simultaneously performing (a) and (b) to expose the dottybacks to both visual and chemical cues from the top-predator (or the non-predator species or the empty tank). Immediately after exposure to the cues, the post-stimuli behaviour of the dottybacks was video recorded for 10 min. During this last sampling period, a second video camera positioned over the damselfish juveniles recorded their activity. A total of 50 dottybacks and 300 damselfish juveniles were used in the experiments. Due to the availability of only four coral trouts, these were randomly assigned to the three top-predator treatments.

Behavioural assessment

Five behavioural attributes of the dottybacks foraging activity were quantified from the pre- and post-stimulus 10 min video recordings: (a) percentage of time spent in shelter; (b) total distance moved (cm); (c) distance swum per trip outside the shelter (cm); (d) total area explored (cm^2); and (e) number of feeding strikes (#/10 min). Measures of space use (b, c and d) by the dottyback were obtained from the recordings using ImageJ software to calculate the length of each trip (distance swum per trip outside the shelter), the total distance swum (sum of all the individual trips) and the area explored (area of the convex polygon formed when joining the outermost locations ventured).

The antipredator behaviour of the six damselfish juveniles was assessed during the 10 min post-stimulus period (while dottybacks were under the effect of the experimental treatments) by estimating their use of space under maximum predation risk. For videos in which the dottybacks struck the damselfish tank (e.g., during post-stimulus periods without top-predator cues), the exact moments of the strikes were designated as instants of maximum predation risk, and among these, 10 frames were chosen haphazardly for analysis. Conversely, for videos that lacked dottyback strikes (e.g., during many post-stimulus periods where top-predator cues were present), we assumed that the maximum predation risk was constant throughout the time, and we selected haphazardly 10 frames to analyse. For each video frame assessed, the position of each damselfish was recorded in relation to the source of risk (location of the dottyback or its strike) and classified into seven distance bands (-12 cm, -9 cm, -6 cm, 0 cm, +6 cm, +9 cm, +12 cm) radiating from the centre of the cylindrical prey tank (24 cm diameter). Fish located from the centre of the tank (0 cm band) towards the source of risk were assigned to a positive (+) band, with the +12 cm band being indicative of a specimen located at the minimum distance from the source of risk. In contrast, a damselfish within the -12 cm band was positioned the furthest away from the dottyback and considered to have the strongest antipredator behaviour.

This approach was specifically chosen as it allowed a comparison of the damselfish antipredator behaviour (measured as space use) across all treatments while indirectly including the inherent variations in the predation pressure between trials. For trials in which the dottyback was exerting high predation pressure (due to the lack of top-predator cues) the maximum predation risk was when the dottyback struck, however, for trials in which the dottyback was exerting low predation pressure and never struck (due to top-predator cues), the predation risk (exerted by the activity and presence of the dottyback alone) was constant throughout all frames and any could be selected as a moment of maximum risk.

Statistical analysis

Changes in the dottyback behaviours between the pre- and post-stimulus observation periods were calculated and used as raw data in the analyses. The five behaviours were not independent from each other so they were compared among treatments using a one-way multivariate analysis of variance (MANOVA). Subsequently, ANOVAs and orthogonal planned comparisons were used to contrast the behaviours between specific pairs of treatments: Non-predator treatment (iv) vs. Empty tank treatment (v); Control treatments (iv, v) vs. Top-predator treatments (i, ii, iii); Visual cue treatment (i) vs. Chemical cue treatment (i.i); Isolated top-predator cue treatments (i, ii) vs. Combined chemical and visual cue treatment (iii). The antipredator response of the damselfish juveniles was compared among treatments with a one-way analysis of covariance (ANCOVA) using the number of feeding strikes during the pre-stimulus period as a covariate. This approach was used to reduce within-treatment error variance and to take into account any effect that pre-stimulus strikes could have on the post-stimulus behaviour (through conditioning or learnt behaviour of the damselfish juveniles). The ANCOVA was followed by the same planned comparisons used for the dottybacks. Residual analyses were used to examine if the data satisfied the assumptions of normality and homoscedasticity. All the behavioural data from the dottybacks were normal and homoscedastic, however, data from the damselfish juveniles was square root transformed to meet the assumptions of parametric tests.

2.4 Results

The behaviour of the dottybacks was significantly affected by the experimental cue treatments (MANOVA, $F_{6,40} = 3.23$, $P < 0.001$) and all five variables measured, were in part, responsible for this difference (ANOVAs, $P < 0.001$; Table 2.1). Dottybacks exposed to cues from the top-predator had contrasting behavioural changes to fish exposed to both control treatments. During the pre-stimulus period, all dottybacks were very active exploring the arena and striking at the damselfish juveniles. However, dottybacks exposed to top-predator cues during the post-stimulus period exhibited a significant increase in the percentage of time spent sheltering as well as a reduction in the total distance swum, the total area explored, the distance swum per trip and the number of feeding strikes in relation to those exposed to control treatments (Fig. 2.2a, c, e, g, i; planned comparisons, $P < 0.001$; Table 1). It was noteworthy, however, that both control treatments showed different patterns for certain behaviours. Dottybacks exposed to cues from the non-predator significantly reduced the distance swum per trip and the number of feeding strikes in comparison to those exposed to the empty tank (Fig. 2.2g, i; planned comparisons, $P < 0.05$; Table 2.1).

Dottybacks had very similar behavioural changes when exposed independently to chemical or visual cues from the top-predator (Fig. 2.2 b, d, f, j; planned comparisons, $P > 0.05$; except for distance/trip, Fig. 2.2h; Table 2.1). The presence of any of these predatory cues restricted the activity and foraging of the dottybacks, and increased the time spent in shelter by almost 40 %. For three out of the five behaviours, the response of the dottybacks differed between the isolated cue treatments and the combined visual and chemical cue treatment. Pairing of both sources of risk elicited a stronger behavioural response measured as a significant increase in the percentage time spent sheltering, and significant reductions in the total area explored and the distance swum per trip (Fig 2.2 b, f, h; planned comparisons, $P < 0.05$, Table 2.1).

Table 2.1. Summary of F-values for the ANOVAs, ANCOVA and planned comparisons examining five behaviours of the dottybacks (mesopredators) and the space use (distance from source of risk) of the damselfish juveniles (bottom prey). ANOVAs were run to test the behaviours of the dottybacks among the five treatments, while an ANCOVA was used to test the space use of the damselfish juveniles. The number of feeding strikes in the pre-stimulus period was used as the covariate in the ANCOVA, however, this variable did not significantly explain the space use of the damselfish juveniles during the post-stimulus period ($F_{1,44}$ = 0.08, P = 0.77). Treatments: C= Chemical cues (top-predator released odours), V= visual cues (sight of the top-predator). Asterisks indicate significant differences where * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.

		Mesopredator					**Bottom prey**
Analysis		**Time in shelter (%)**	**Total distance (cm)**	**Total area (cm^2)**	**Distance / trip (cm)**	**Feeding strikes (#/10 min)**	**Distance from the source of risk (cm)**
ANOVA/ ANCOVA		12.72***	7.04***	9.59***	11.06***	7.29***	**16.08*** **
Planned Comparisons	Empty tank **vs.** Non-predator treatment	0.1	1.06	3.86	4.76*	12.13**	**4.11* **
	Control treatments **vs.** Top-predator treatments	44.11***	24.70***	23.2***	24.42***	14.77***	**57.02*** **
	C **vs**. V	0.71	1.81	3.95	10.74**	0.29	**0.06**
	C, V **vs.** C + V	5.97*	0.59	7.35**	4.33*	1.72	**0.45**

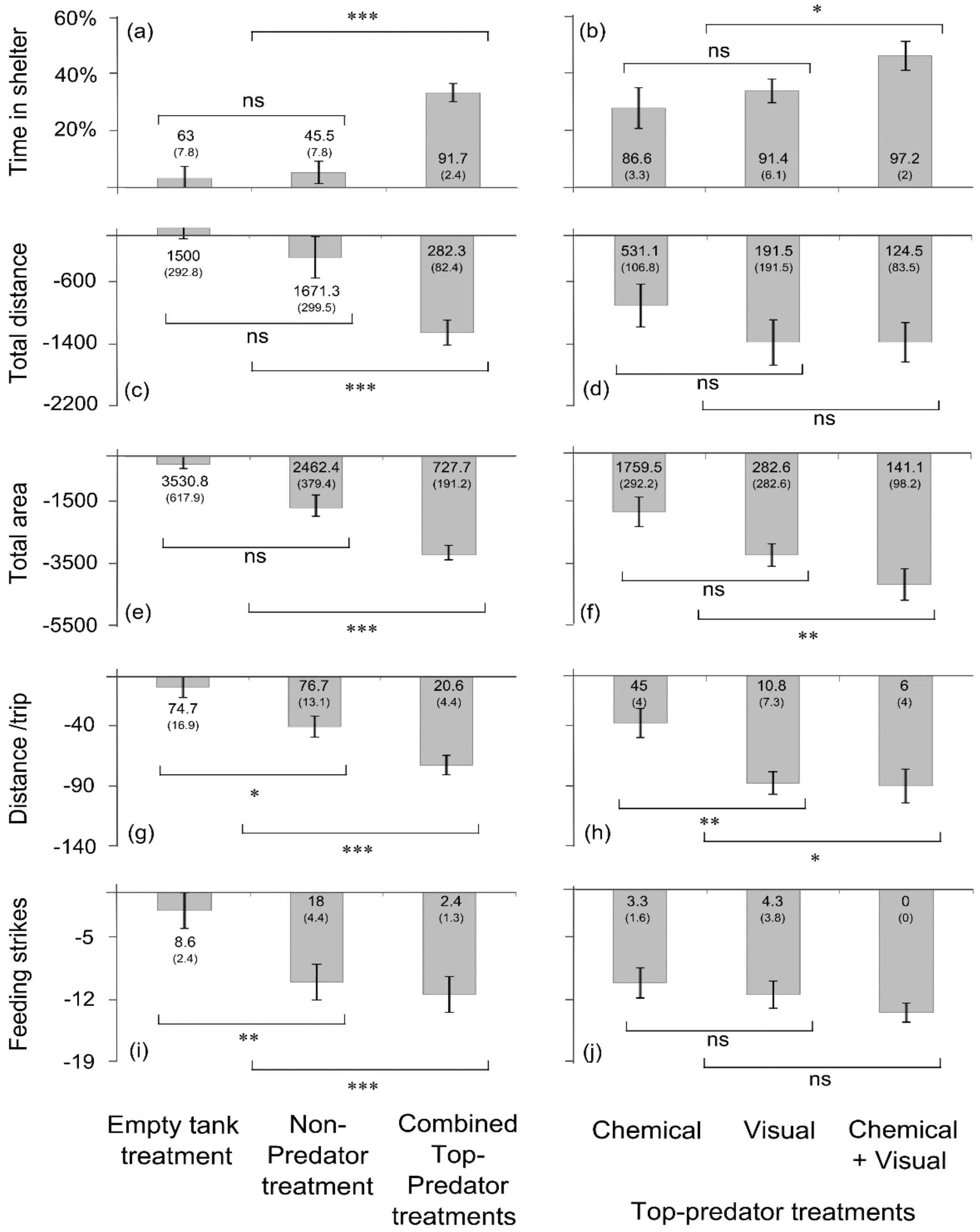

Figure 2.2. Change (mean ± SE) in **(a, b)** time spent in shelter (%), **(c, d)** total distance moved (cm), **(e, f)** total area explored (cm^2), **(g, h)** distance swum per trip (cm), and **(i, j)** feeding strikes (#/10 min) by dottybacks (mesopredators) between the 10-min pre and post-stimulus periods. Fish were exposed to: the visual cue of a top-predator (coral trout); the chemical cue (odour) of a top-predator; the co-occurrence of chemical and visual cues of a top-predator; the co-occurrence of chemical and visual cues of a non-predator (thicklip wrasse); and the co-occurrence of chemical and visual cues of an empty tank. A positive value indicates an increase in activity and a negative value indicates a decrease in activity. Asterisks above or below the bars represent significant differences between the planned comparisons (* = P < 0.05; ** = P < 0.01; *** = P < 0.001). Numbers inside the bars indicate the values recorded during the post-stimulus period [mean (± SE)].

The space use of the damselfish juveniles during the post-stimulus period was not explained by the number of pre-stimulus strikes ($F_{1,44} = 0.08$, $P = 0.77$), but it significantly differed among the five experimental treatments (ANCOVA, $F_{4,44} = 16.08$, $P < 0.001$). During the control treatments damselfish juveniles stayed far away from the strikes of the dottybacks (using mostly the negative bands; -4.7 ± 0.3 cm), while in the top-predator treatments they remained significantly closer to the source of risk (+1.5 ± 0.7 cm; planned comparison, $P < 0.001$, Fig 2a; Table 2.1). Although the space use of the damselfish was relatively similar among the three top-predator treatments (planned comparisons, $P > 0.05$, Fig 2.3b; Table 2.1), we did detect differences between the two control treatments (planned comparison, $P < 0.05$, Fig 2.3a; Table 2.1). Damselfish positioned themselves significantly closer to the source of risk when the dottybacks were being exposed to the non-predator (thicklip wrasse) than when exposed to the empty tank.

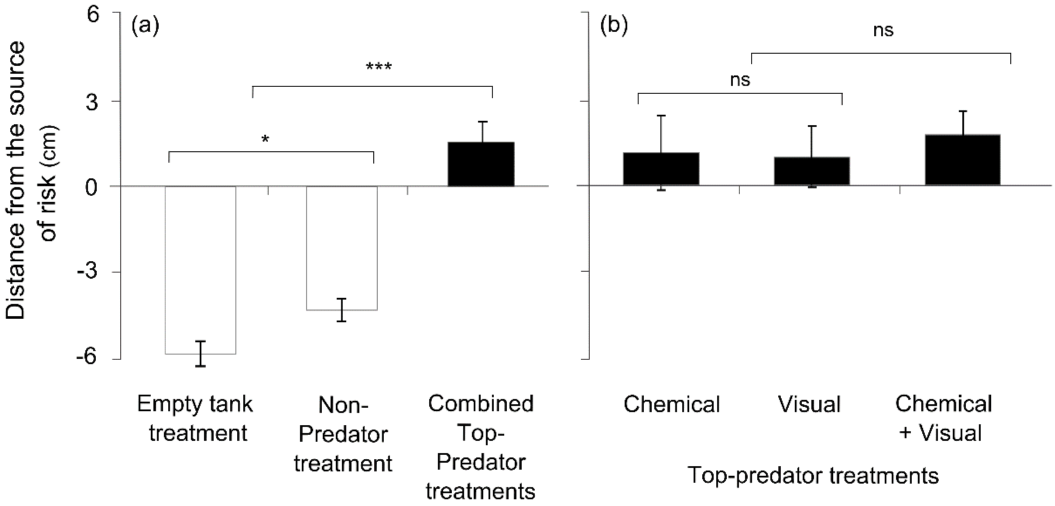

Figure 2.3. Distance (mean ± SE) from the source of risk (location of the dottyback or its strike) at which damselfish juveniles (bottom prey) were positioned during moments of maximum predation risk within the post-stimulus period. Dottybacks (mesopredators) were under the effect of the five experimental treatments: the visual cue of a top-predator (coral trout); the chemical cue (odour) of a top-predator; the co-occurrence of chemical and visual cues of a top-predator; the co-occurrence of chemical and visual cues of a non-predator (thicklip wrasse); and the co-occurrence of chemical and visual cues of an empty tank. Positive values are indicative of fish with low predator avoidance behaviour (black bars) located close to the risk source, while negative ones are indicative of fish with higher predator avoidance behaviour (white bars) located far away from the source of risk. Asterisks above the bars represent significant differences between the planned comparisons (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

2.5 Discussion

Our study demonstrated a cascade of indirect risk effects in which the behaviour of mid- and low- trophic level species was determined by the risk of predation from top-predators. When experimentally exposed to predatory cues from a macro-carnivore (coral trout), dottybacks altered their foraging behaviour, decreasing by more than 70 % the distance swum, area explored and feeding attacks on damselfish juveniles (bottom prey). Suppression of the mesopredator activity correspondingly led to a decline in the level of risk they posed to damselfish, as the juveniles altered their behaviour by coming closer to the source of risk (location of the dottyback or its strike) by 37 %. While a previous study illustrated links between risk-mediated mesopredator behavioural changes and the recruitment of reef fishes (possibly through direct, lethal effects; Stallings 2008), the present study is the first to provide evidence that such behavioural alterations can simultaneously modify the behaviour of lower trophic level species.

Visual or chemical cues from the predatory coral trout triggered a strong antipredator response in the dottybacks, suggesting the presence of indirect top-down control over this mesopredator. Exposure to either of the predatory cues independently was enough to reduce the overall activity and feeding rate of the dottybacks, however, the strongest response occurred when visual and chemical cues were presented simultaneously. Many studies on marine and fresh water fishes have shown that multiple sensory cues provide organisms more complete information than any one cue in isolation (Smith and Belk 2001, Amo et al. 2004, Dalesman and Inchley 2008, McCormick and Manassa 2008). It was noteworthy, however, that the presence of independent predatory cues was enough to also trigger behavioural changes in the dottybacks that resulted in significant reductions in the overall activity and feeding rate. These mesopredator behavioural modifications triggered behavioural changes in the damselfish juveniles, which significantly decreased their predator avoidance behaviour. Taking into account that organisms make trade-offs between predator avoidance and other fitness-enhancing activities (Sih 1980, Lima and Dill 1990), the reduction of the antipredator response could be particularly beneficial to the fitness of individuals in vulnerable early ontogenic stages (Almany and Webster 2006). They could potentially increase their space use, activity and foraging which would likely correspond to a higher energy allocation to growth and later reproduction. Although visual cues, such as the sight of the predator, are indicative of high risk of predation (Helfman 1989, Murphy and Pitcher 1997, Hartman and Abrahams 2000), two laboratory experiments also show no clear difference in the magnitude of behavioural changes elicited by isolated visual and chemical cues (McCormick and Manassa 2008, Milano et al. 2010). The magnitude of the antipredator response is usually dependent on the concentration of risk cues (Dupuch et al. 2004, Zhao et al. 2006, Holmes and McCormick 2011), so it is likely that the relatively strong behavioural changes triggered by chemical cues in these latter studies are due to a high concentration of predator odour or alarm cues. Chemical cues appear to be resembling a threat as eminent as the sight of the top-predator.

Unexpectedly, the large non-predator also modified the behaviours of the mesopredator. The thicklip wrasse triggered changes in the feeding rate and the distance swum per trip of dottybacks. This was because the dottybacks without reducing their overall activity, shifted their interest from attacking the damselfish juveniles to engaging in an inspection behaviour with the large non-predator. Such behaviour is frequently observed in freshwater fish as they tentatively approach a novel predator or potential threat to acquire extra information (e.g., Murphy and Pitcher 1997, Smith and Belk 2001, Brown and Magnavacca 2003). Although non-predator species not always trigger behavioural changes on their prey (McCormick and Manassa 2008), a recent study also found that non-predators can reduce the prey foraging activity. Marsh-Hunkin et al. (2013) report that although their non-predator species (French grunt, *Haemulon flavolineatum*) did not trigger a reduction in the movement of prey gobies (in comparison to predators like the red lionfish and the Nassau grouper), it did cause a decrease of their feeding activity similar to the one caused by the predators. More research is needed to understand the behavioural effects that non-predators have on lower trophic levels, however, recent evidence suggests that large non-predators might be playing an important role distracting mesopredator fish from their foraging activities. In this way, large organisms (despite their trophic status) could also be mediating the impact of mesopredators on bottom prey. Although in the present study their effect was not as strong as the presence of the top-predator, the wrasse led indirectly to positive changes in the behaviour of the damselfish juveniles. These results have important implications for management as they show that not only can the loss of macro-carnivores trigger behaviourally induced cascades, but the loss of macro-invertivores or herbivores may also lead to the release of mesopredators. To judge the importance of indirect effects of predators on lower trophic groups we also require an understanding of the relative magnitude of the behavioural effects of non-predators.

Changes in the mesopredator behaviour due to top-predator cues, allowed damselfish juveniles to modify their space use and exhibit significantly lower antipredator behaviour. Although the presence of the non-predator did not have an effect as strong as the top-predator, it also led indirectly to positive changes in the space use of the damselfish juveniles. Taking into account that organisms make trade-offs between predator avoidance and other fitness-enhancing activities (Sih 1980, Lima and Dill 1990), the reduction of the antipredator response could be particularly beneficial to the fitness of individuals in vulnerable early ontogenetic stages (Almany and Webster 2006). They could potentially increase their space use, activity and foraging which should lead to a higher energy allocation to growth and later reproduction.

Our results must be considered in the context of previous studies, as the strength of the mesopredator's antipredator response to predatory cues may be influenced by the species, the behavioural history of the individuals, the habitat conditions, and even the personality of the fish (Chivers et al. 2001, Lima and Steury 2005, Ferrari et al. 2010, Martin et al. 2010, Marsh-Hunkin et al. 2013). Laboratory based experiments have limitations, such as the ecological relevance of the concentrations of cues, the context of the trials (e.g., procedure, size of the tanks), and the stress of captivity, which can all influence the overall outcome and result in exaggerated manipulations of the natural systems (Irving and Magurran 1997, Blanchet et al. 2007, Kim et al. 2009, Rizzari et al. 2014). For the present experiment, the concentration of the top-predator cues and the stress that fish normally undergo in captivity may have affected the results. Although our experimental setup could have allowed a potential indirect effect of the coral trouts on the damselfish juveniles (Fig. 2.1), the coral trouts here employed (~ 40 cm TL) usually prey on bigger damselfish (> 5 cm SL; Kingsford 1992, St. John 2001) and do not represent a major threat to the juveniles used in our study (< 1.4 cm SL). Furthermore, because the damselfish juveniles were predator naïve and only conditioned to recognize the smell and sight of dottybacks, it is unlikely they identified the coral trout as their predator. Despite their shortcomings, laboratory experiments are an important tool to study trophic cascades of indirect effects as they allow detailed behavioural observations and strict control over the risk of predation. We consider that only through the combination of field- and laboratory- based approaches will we be able to understand the mechanisms underlying the indirect effects of mesopredator control (Schmitz et al. 2004) and the community dynamics in ecosystems as complex as coral reefs.

Mesopredators constitute an important functional group in most food webs due to their high abundances, population growth rates, voracity and activity levels (Prugh et al. 2009, Ritchie and Johnson 2009, Ripple et al. 2014). In coral reef systems, their behavioural release could represent a major threat to the assemblage of post-settlement fishes (bottom prey) as mesopredators have strong negative effects on the persistence (direct effects; Carr and Hixon 1995, Almany and Webster 2006, Feeney et al. 2012), phenotypic selection (Gagliano et al. 2007b) and behaviour of low trophic level species (indirect effects; McCormick and Manassa 2008, Holmes and McCormick 2011, Marsh-Hunkin et al. 2013). Although intra- and interspecific interactions (i.e., hunting synergies or interference competition; Sih et al. 1998) will affect how mesopredator release is manifest, our results suggest that the reduction of top-predators may have important effects on coral reef fish interactions, as even odours from top-predators can suppress the activity of mesopredators and lower their impact on juvenile fishes. Although top-down processes and trophic cascades are difficult to detect and study in natural marine ecosystems (Heithaus et al. 2008, Baum and Worm 2009), comparable behavioural links to those found here have already been reported in the field. For example, tiger sharks are known to indirectly influence the habitat selection of mesopredators (dolphins) and herbivores (dugongs), whose spatial distributions are sensitive to densities of top-predators (Heithaus and Dill 2006, Wirsing et al. 2007). Evidence also shows that the presence of fur seals reduces the foraging effort of morwong fish on turf algae (Connell 2002) and that the presence of Pacific sleeper sharks indirectly protects the walleye pollock from predation by the harbor seals (Frid et al. 2007). Further studies are required to fully understand indirect risk effects in marine ecosystems, however, evidence is starting to reveal the importance of top-predators in the protection of low trophic level guilds and the key role that risk effects might play in marine trophic cascades.

Given the speed at which populations of large predators are being depleted (Jackson et al. 2001, Ceballos and Ehrlich 2002, Myers and Worm 2003, Robbins et al. 2006) and at which phase shifts are occurring worldwide (Hughes et al. 2007, Estes et al. 2011) it is imperative to understand the effects of predator removal on community dynamics. The indirect release of mid-ranked herbivores from both terrestrial and marine ecosystems has been relatively well studied in comparison to the release of mid-ranked carnivores (Ritchie and Johnson 2009). Our study expands the work of Stallings (2008) and suggests that in marine systems even isolated predatory cues, such as the sight or the odour of a macro-carnivore, can suppress the foraging behaviour of mid-ranked fish and relax their indirect effect on low trophic level species. Strong indirect effects, such as the ones found here, have the potential of strengthening trophic cascades triggered by the loss of top-predators. Understanding behavioural dynamics among predators will not only serve to better predict the consequences of predator loss, but also aid the management of coral reef fisheries (Dill et al. 2003, Heithaus et al. 2008).

Chapter 3: Positive indirect effects of top-predators on the survival & behaviour of juvenile fishes

Submitted
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Positive indirect effects of top-predators on the survival & behaviour of juvenile fishes
Proceedings of the Royal Society B

3.1 Summary

Top-predators can suppress mesopredator behaviour through risk effects. However, there is limited understanding on whether such behavioural suppression can dampen the lethal and sub-lethal effects of mesopredators on bottom level prey. Here, we document a field experiment that examines how the presence of top-predator cues (visual and chemical stimuli from a coral trout) indirectly influences the behaviour and survival of juvenile fish prey from different species (*Pomacentrus amboinensis* and *P. chrysurus*) and sizes (small and large; ~ 0.15 cm SL difference). Results showed that after exposure to reef mesopredators for 72 h, juvenile fish had higher space use (~46 %), feeding rate (~95 %), and survival (~67 %) on habitat patches where top-predator cues had been deployed. Survival was always higher for individuals of *P. amboinensis* and of large-size independent of the presence or absence of predation risk cues from the top-predator. We hypothesize predation risk from the large-sized predator indirectly favoured the persistence and behaviour of juvenile fishes, by promoting risk-averse behavioral responses in mesopredators. Our results evidence a potential mechanism through which risk effects can cascade through the food web and highlight the consequences that harvesting top-predators may have on the replenishment of bottom prey populations.

3.2 Introduction

Top-predators can indirectly influence food web dynamics though the risk effects they exert on ecosystems (Abrams 1995, Schmitz et al. 2004). However, with the accelerating depletion of large top-carnivores, predation risk patterns are expected to change allowing species of mid-trophic status (i.e., mesopredators, mesoconsumers) to strengthen their sub-lethal and lethal impacts on bottom level prey (Ritchie and Johnson 2009, Estes et al. 2011, Ripple et al. 2014). It is now a research priority to understand how changes in predation risk will indirectly impact the community dynamics of bottom level prey, especially in complex marine systems where human activities are already altering risk landscapes (Madin et al. 2016).

Predation risk plays an important role in coral reefs (Dill et al. 2003, Madin et al. 2016). Several field studies have shown that large-bodied predators can limit the foraging activity of mid-ranking herbivores and indirectly favour the persistence and distribution of macroalgae (e.g., Madin et al. 2010a, McCauley et al. 2010, Rizzari et al. 2014, Catano et al. 2016). Surprisingly, only Stallings (2008) has examined how these behavioural trophic cascades could influence complex, mobile, bottom level prey such as juvenile fishes. Newly-settled fishes already suffer major predation pressure from mid-ranking piscivores (e.g., small cods, wrasses and lizardfishes), which can reduce their population by more than 50 % in 2 days (Caley 1998, Almany and Webster 2006). Although the adaptive foraging of mid-ranking piscivores subject to risk from top-predators can favour juvenile fish recruitment (Stallings 2008), it is yet to be determined whether this risk can dampen both lethal (i.e., intensity of mortality, selective-loss of individuals) and sub-lethal effects (i.e., behaviour, physiology) on juvenile fish prey. Community changes at this initial life-stage may have disproportionate influence on the attributes of the adult fish assemblage (Caley 1998).

Mortality caused by mesopredators is both extremely high and often selective for individual attributes such as fish body size (e.g., small fish; Booth 1995a, McCormick and Hoey 2004) or species (e.g., cardinalfishes; Webster and Almany 2002). Increased mortality of a specific prey type can occur due to: (i) increased vulnerability via differential performance or antagonistic interactions (e.g., reduced burst speed, displacement to riskier habitats; Walker et al. 2005, McCormick 2009); and (ii) active selection by mesopredators (McCormick and Meekan 2007, Holmes and McCormick 2010). We expect that under predation risk the selective loss of juvenile prey might change as mesopredators (a) lose strict preferences and target the easiest available prey, or in contrast, (b) increase their selectivity attempting to maximize the energy intake per prey (reviewed by Stephens and Krebs 1986, Lima 1998b). However, little is known of how predation risk from top-predators can cascade through the food web to actually modify the nature and magnitude of selectivity by mesopredators for their juvenile fish prey.

Mesopredators are also known to impose sub-lethal effects on the behaviour and physiology of juvenile fishes. Laboratory manipulations show that the presence of mesopredators or any of their cues (e.g., visual, chemical) triggers a strong antipredator response in newly-settled fishes, which causes them to drastically reduce their feeding rate, distance swum, and height above substratum (e.g., Holmes and McCormick 2011, Lönnstedt et al. 2012). Additional to these behavioural constraints, mesopredators can also impact juvenile fish physiology by boosting their stress levels and metabolism by more than 35 % (Palacios et al. 2016a). It is still unknown, however, whether any of these sub-lethal effects on the phenotype of juvenile prey can be mitigated by the presence of top-predators on natural coral reefs.

To address these knowledge gaps, we explored the extent to which predation risk from top-predators can alter sub-lethal and lethal processes that affect bottom level prey. Using experimental habitat patches and a three-level food web of reef fishes (coral trout> mesopredators> juvenile damselfish prey) we addressed the following questions: (a) does predation risk from a top-predator indirectly favour the survival of juvenile prey (via mesopredator suppression)? (b) is the size- and species-selective loss of juveniles modified by the predation risk from top-predators? and (c) can the behavioural trophic cascade dampen sub-lethal effects on the space use, foraging and competition of juvenile fish? Lastly, we explored if any of these indirect effects of predation risk are consistent among two reef sites with different species composition of piscivores. Answering these questions allowed us to extend our understanding of the indirect top-down control on fish community dynamics.

3.3 Materials & Methods

Study sites and species

Juveniles of the damselfishes *Pomacentrus amboinensis* and *P. chrysurus* were selected as bottom level prey. Both species coexist among coral reefs in the Indo-Pacific, settling in high numbers on the shallow patch reefs of Lizard Island (14°40´S, 145°28´E; northern Great Barrier Reef, Australia) during the austral summer months (Stewart and Jones 2001, Webster 2002). After settling, these fishes exhibit high site fidelity (< 1 m diameter home range), differ in coloration (*P. amboinensis* = yellow; *P. chrysurus* = brown-grey), feed mainly on plankton, frequently compete for shelter and are subject to high mortality from reef predators (Webster 2002, Almany et al. 2007).

The leopard coral trout (*Plectropomus leopardus*, Serranidae) was used as the top-predator species. This conspicuous reef piscivore reaches > 60 cm standard length, has few natural predators, and isotopic assessments suggest they forage at the same trophic level as reef sharks (Frisch et al. 2016b). Coral trouts are known to mainly feed on mid-sized reef fishes (~9 cm TL) including Pomacentrids, Labrids, Synodontids and Scarids (St. John 2001). During the day, coral trouts can be commonly found stationary next to patch reefs or the edge of the main reef (MIM, *personal observation*).

The present study was conducted on November 2016 at two reef locations at Lizard Island, Vicki's Reef and Lagoon Reef (Appendix A: Fig. A.1a). At each location, we constructed 18 small habitat patches (20 cm height x 20 cm diameter) on the sandy bottom along the continuous reef edge using live and dead colonies of the bushy hard coral, *Pocillopora damicornis*. Replicate patches were standardized for composition and complexity, positioned 2 m from the reef edge and separated by 10 m (Appendix A: Fig. A.1b,c). Previous studies suggest that migration of juvenile damselfish between patches or to the main reef is negligible (Hoey and McCormick 2004). Habitat patches were cleared of all fishes and invertebrates prior to release of the experimental fishes.

Mesopredator assemblage

To quantify the diversity, size range and abundance of piscivorous predators on each reef site, scuba divers conducted 16 visual surveys along 30 x 5 m belt transects. Each transect was surveyed twice. The first survey recorded only mobile and conspicuous piscivore species. The second survey focused on benthic and cryptic piscivores, through a thorough examination of the coral colonies and sea bottom. Fish species were considered piscivores following Stewart and Jones (2001). Divers visually estimated piscivore body size and classified fish as small ($S \leq 10$ cm TL), medium ($10 < M > 20$ cm TL) or large ($L \geq 20$ cm TL). Transects were located randomly along the reef edge, in order to assess the area that would have the greatest influence on the experimental habitat patches. Half the surveys were conducted in the morning (7:00 – 12:00 h) and half in the afternoon (12:00 – 17:00 h) to obtain a uniform distribution of observations during the day. Given that belt transects can often underestimate small-cryptic piscivores (Stewart and Beukers 2000), a series of baited point surveys were conducted to corroborate the richness and relative densities of this group of piscivores (N= 8 per reef site). Both methods yielded relatively similar estimates of richness and density of benthic piscivores, so only results from belt transects were considered in this study.

Juvenile fish collection and tagging process

Juvenile damselfishes were captured with light traps just before their recruitment on the reef. Traps were moored overnight at least 50 m away from the reef edge and collected at dawn (6:00 – 7:00 h). Fishes were transported to Lizard Island Research Station (LIRS), where they were fed *Artemia* spp. and kept in flow-through tanks with aerated seawater at ambient temperatures and light photoperiod. Twenty-four hours after capture, juvenile fishes were separated by species and measured for standard length (SL ± 0.001 cm). Following Holmes and McCormick (2006), fish were classified into small or large categories, always maintaining a ~ 0.1 cm size difference between individuals within a species pair. Juvenile fish were arranged in groups of four including: 1) a large *P. amboinensis* (1.31 ± 0.004 cm, mean SL ± SE), 2) a small *P. amboinensis* (1.17 ± 0.004 cm SL), 3) a large *P. chrysurus* (1.34 ± 0.005 cm SL) and 4) a small *P. chrysurus* (1.20 ± 0.006 cm SL). To achieve individual identification, fish were placed into a clip-seal plastic bag containing aerated seawater and injected with a subcutaneous fluorescent tag (visual implant elastomer; VIE) in the dorsal musculature using a 27G hypodermic needle (as per Holmes and McCormick 2006). Within each group of four individuals, damselfish were distinguishable by having a 1.5 – 2 mm long tag of different colour (e.g., red, blue, green, orange or yellow). Tag colours were alternated between replicate patches and treatments to avoid the possibility of mesopredators selecting juvenile prey based on tag colour. Previous studies on newly collected juvenile damselfish have found that tagging in this way does not affect their growth or survival (Hoey and McCormick 2006).

Field experiment

The effect of a top-predator on the survival and behaviour of juvenile prey was assessed *in situ* by assigning three experimental treatments to the habitat patches and monitoring the fate of four juvenile fishes for up to 72 h. The three treatments comprised: a) top-predator cues, b) a control PVC pipe, or c) an empty control (Fig. 3.1). The top-predator treatment consisted of professionally made, 30 cm total length, 3D fiberglass model of a common coral trout, *Plectropomus leopardus* (Serranidae). The trout model was anchored with wire (2 mm thick) to a brick buried at approximately 10 -15 cm from the habitat patch. The thin wire allowed the model to float at 10 -15 cm from the substratum and easily move with the currents and water flow. To complement the visual cue (model) of a coral trout, we attached a 150 ml agar cube designed to release coral trout odour. A preliminary laboratory experiment showed that the agar cube (composed of 150 ml of trout odour and 8 g of agar) took from 4 to 12 h to dissolve depending on the currents and water flow. When tested on a focal mesopredator species, odour released from the agar cube led to behavioural changes of similar magnitude than those from the direct exposition to coral trout odour (Appendix A: Fig. A.2). A (sham) control for the top-predator treatment consisted of a 30 cm long PVC pipe (10 cm diameter) anchored to float, allowing it to move comparably to the coral trout model. The PVC pipe also had an agar cube attached to it, but in this case, it was made with seawater (rather than water containing coral trout odour). The true control treatment consisted of habitat patches that had no floating stimulus or agar cube nearby.

Predator models are frequently employed to study and manipulate predation risk in marine systems (e.g., Rizzari et al. 2014, Catano et al. 2016). However, due to their stationary nature, fishes may habituate to their presence. To reduce the chances of mesopredator habituation we (i) used coral trout odour to complement the visual cues from the model, (ii) kept trials relatively short (72 h) and (ii) facilitated a side to side motion of the decoy by deploying it against the current.

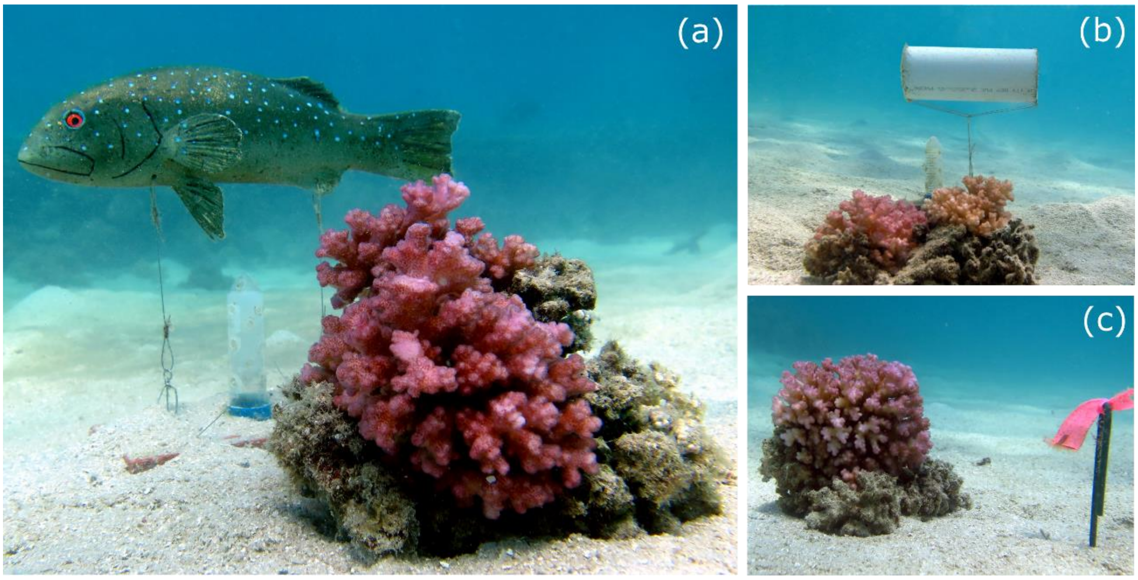

Figure 3.1. Habitat patches constructed from live and dead coral showing the three experimental treatments: **(a)** top-predator cues, **(b)** PVC pipe, and **(c)** control. The top-predator treatment consisted of a fiberglass model of a coral trout, *Plectropomus leopardus* (30 cm SL) paired with agar cube that released coral trout odour (See Methods). In contrast, the PVC pipe treatment included a 30 cm long tube (10 cm diameter) along with a seawater agar cube. For the control, patches were left intact.

To begin the experiment, an experimental group of four tagged juvenile fish (as described above) was placed onto an experimental patch. Juvenile fishes were transported from the laboratory to the habitat patches in 1-L labelled clip-seal bags filled with aerated seawater and released at their assigned patches between 15:00 – 17:00 h. During the release, scuba divers shielded the juvenile fish from mesopredators for 10 min while they acclimated to the new environment. Survival of each juvenile fish was monitored at 12, 24, 36, 48, 60 and 72 h by visual surveys. At 36 h, the activity and behaviour of each juvenile was video recorded for three minutes. Divers positioned a GoPro camera 30 cm away from the coral patch and directed it towards the location of the juvenile fishes (i.e., usually up-current of the patch). Two clear acrylic sticks of 15 cm length were also installed to serve as scale during the video analysis. Once the recording began, divers left the area and remained > 10 m away from the patch to avoid any disturbance on the fishes. Preliminary observations showed juvenile fish acclimated fast (< 1 min) to the novel objects and the presence of the divers. Three aspects of activity and behaviour were estimated: a) space use (average height above substratum, cm), foraging (feeding strikes/ 2 min) and c) interactions (displays and chases/ 2 min). Video processing was done manually in VLC media player.

Due to logistical constrains associated to the number of patches to monitor at a given time (N= 36) and the number of available top-predator models (N = 12), the experiment was replicated five times (five rounds) to achieve a total of 30 replicates per treatment per reef site.

After each round (72 h), all juvenile fish remaining were moved to the continuous reef and patches were re-configured and re-used for further replicates. The treatment assigned to each habitat patch was systematically rotated to avoid the possibility of site-attached mesopredators learning about the presence of prey. All trials were run between the 10th and the 29th November 2016, with each round lasting four days and starting the day immediately after the conclusion of the previous round.

Statistical analysis

The predator assemblage at Vicki's and Lagoon was compared in terms of size structure and species relative abundance. We used two-sample t-tests to examine differences in the richness (spp. /150 m^2), density (total predatory fish/150 m^2), and relative abundance of small, medium and large sized predators between reef sites. Variation in predator species abundance and composition was assessed using an Analysis of Similarity (ANOSIM), based on the Bray-Curtis similarity index (considers the relative abundance of each taxa). Finally, a Similarity Percentage Analysis (SIMPER) was used to examine which species contributed most to the dissimilarities between both reef sites.

The survival of juvenile fish was analysed by fitting a generalized linear model (with a binomial distribution) including all combinations of main effects (i.e., reef site; treatment; fish prey size; fish prey spp.) and 2-, 3-, 4- way interactions. Akaike's information Criterion (Hunsicker et al. 2011) was used to determine the best fitting model from all possible candidates, obtained by removing single or combination parameters from an initial fully parameterized model. We checked for any systematic trends between the residuals of the best fitting model and the experimental 'rounds'. Consistently AIC selected against adding 'trial' as random effect to explain juvenile fish survival. The overall effects of the explanatory variables were evaluated from the best fitting model using a likelihood ratio test (LRT). Kaplan-Meier survival plots were used to illustrate juvenile prey survival trajectories among reef sites and treatments.

Finally, the behaviour of the surviving damselfish was compared using a two-way ANOVA with reef site (2 levels: Vicki's, Lagoon) and treatment (3 levels: top-predator cues, PVC pipe, control) as factors. The average height above substratum (cm), feeding strikes (strikes/2 min) and interactions (number/ 2 min) were used as dependent variables. All significant differences detected in the ANOVAs were further explored using Tukey's HSD post-hoc test. Residual analyses were used to examine whether data satisfied the assumptions of normality and homoscedasticity. All data from mesopredator density and damselfish behaviour was square-root transformed to meet the assumptions of parametric tests. Except for the ANOSIM and SIMPER, which were ran in the PAST software package (Hammer et al. 2001), all analyses and graphs were performed in the statistical computer software R (R Core Team 2015).

3.4 Results

Mesopredator assemblage

A total of 596 individuals of 33 piscivore species from 10 families were surveyed at Vicki's and the Lagoon sites (Appendix A: Table A.1). Both sites had a similar mean species richness (Vicki's = 8.6 ± 0.5; Lagoon = 6.8 ± 0.8 spp. /150 m^2 ± SE; t = 2.03, P = 0.051) and density (Vicki's = 20.3 ± 2; Lagoon =16.9 ± 2.3 ind/150 m^2 ± SE; t = 1.22, P = 0.23). However, the size structure of piscivores differed markedly (Fig. 3.2a). While Vicki's had a higher abundance of mid-sized fishes (t = 4.27, P < 0.001), most piscivores at the Lagoon were large-sized individuals (t = -3.11, P < 0.01). Small piscivores tended to be more abundant at Vicki's, but this pattern was not significant (t = 1.16, P = 0.25).

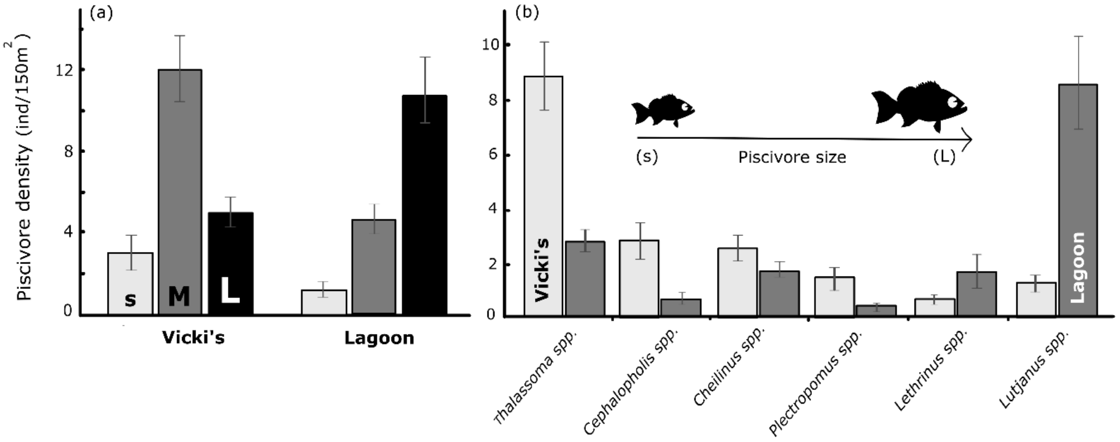

Figure 3.2. Density of piscivores (mean ± SE) at Vicki's and the Lagoon reef sites, as function of **(a)** their size structure (Small ≤10 cm TL; 10 < Medium > 20 cm TL; Large ≥ 20 cm TL) and (b) taxonomic genera. Only the six most representative fish taxa are displayed (Appendix A: Table A.1). These are ordered from left to right with respect to their average size.

The piscivore assemblage at Vicki's and the Lagoon also differed in the relative abundance of the piscivore species (ANOSIM; R = 0.57, P < 0.0001; Fig. 3.2b). The SIMPER analysis revealed that 60 % of the dissimilarity was due to differences in the density of *Thalassoma lunare* (20.3 % contribution), *Lutjanus gibbus* (16.1 %), *L. carponotatus* (6.5 %), *Cephalopholis cyanostigma* (6.2 %), *Cheilinus chrorurus* (5.5 %) and *Plectropomus leopardus* (5.3 %). The medium-sized wrasses (*T. lunare* and *C. chlorurus*) and rock-cods (*C. cyanostigma* and *C. microprion*) were particularly abundant at Vicki's, where they represented 60.6 % of the piscivores observed. We recorded relatively low densities of large mobile predators, except for the coral trout (*P. leopardus*) which, at this place, is known to approach divers in search for food.

In contrast, the piscivore community at the Lagoon was dominated by snappers (*L. gibbus* and *L. carponotatus*) and lethrinids (*Lethrinus atkinsoni* and *L. nebulosus*) which together represented 41.3 % of the total piscivore abundance. Small wrasses and rock- cods were also present at this site, but at lower densities than at Vicki's.

Prey Survival & Selectivity

Variation in juvenile fish survival was most parsimoniously explained by the main effects alone (i.e., reef site, treatment, fish prey size, fish prey spp.). The AIC model selection criteria selected against any 2-, 3-, and 4- way interaction effects, implying that the impact of each main effect remained consistent across all levels of the other variables.

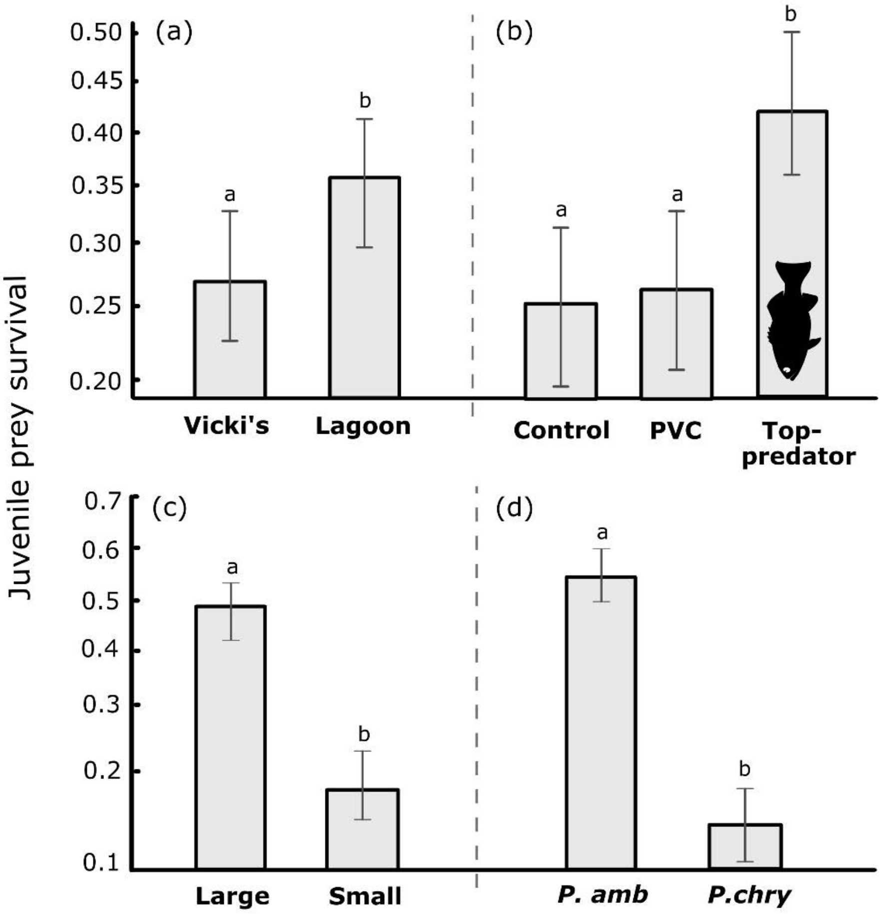

Figure 3.3. Survival (mean ± SE) of juvenile damselfish prey at the 72 h survey, according to the **(a)** reef site (Vicki's, Lagoon), **(b)** treatment (control patches, PVC pipe patches, top-predator cue patches), **(c)** prey size class (small, large: ~ 0.15 cm SL difference) and **(d)** prey species (*Pomacentrus amboinensis, P. chrysurus*). Bars with the same lowercase letter did not differ significantly according to the Tukey's HSD post-hoc means comparisons.

In this model, juvenile fish survival was significantly influenced by the reef site, as prey from the Lagoon had marginally higher survival than prey at Vicki's (LRT $X^2_{df\,1} = 3.9$, $P = 0.046$; Fig. 3.3a). Survival was also found to vary with the experimental treatments (LRT $X^2_{df\,2} = 16.6$, $P < 0.001$; Fig. 3.3b). In the control and PVC pipe treatments, damselfish experienced similar survival rates of 24.9 % and 26.4 %, respectively (Tukey's test, $P > 0.05$). However, prey survival was significantly higher in the top-predator treatment, where 42.7 % of the juvenile fish remained alive at the 72 h survey (Tukey's test, $P < 0.001$). Interestingly, survival trajectories showed that patterns of survival among treatments were established within the first night (Fig. 3.4). At the 12 h survey, most patches (control and PVC tube) had already lost > 50 % of the fishes, while those deploying the top-predator cues exhibited < 40 % mortality. After this time, differences in survivorship among treatments remained relatively constant, with ~ 0.5 % drops in survival occurring gradually over the following 60 h.

Survival was also dependent on the size and species of the damselfish prey (Fig. 3.3c,d). While small-sized prey only had a 18.05 % survival, the larger-sized counterparts reached 47.6 % (LRT $X^2_{df\,1} = 61.9$, $P < 0.0001$). In a similar way, 55.1 % of *P. amboinensis* individuals were still alive by the 72 h survey while more than 75 % of *P. chrysurus* had already been lost (LRT $X^2_{df\,1} = 129.7$, $P < 0.0001$).

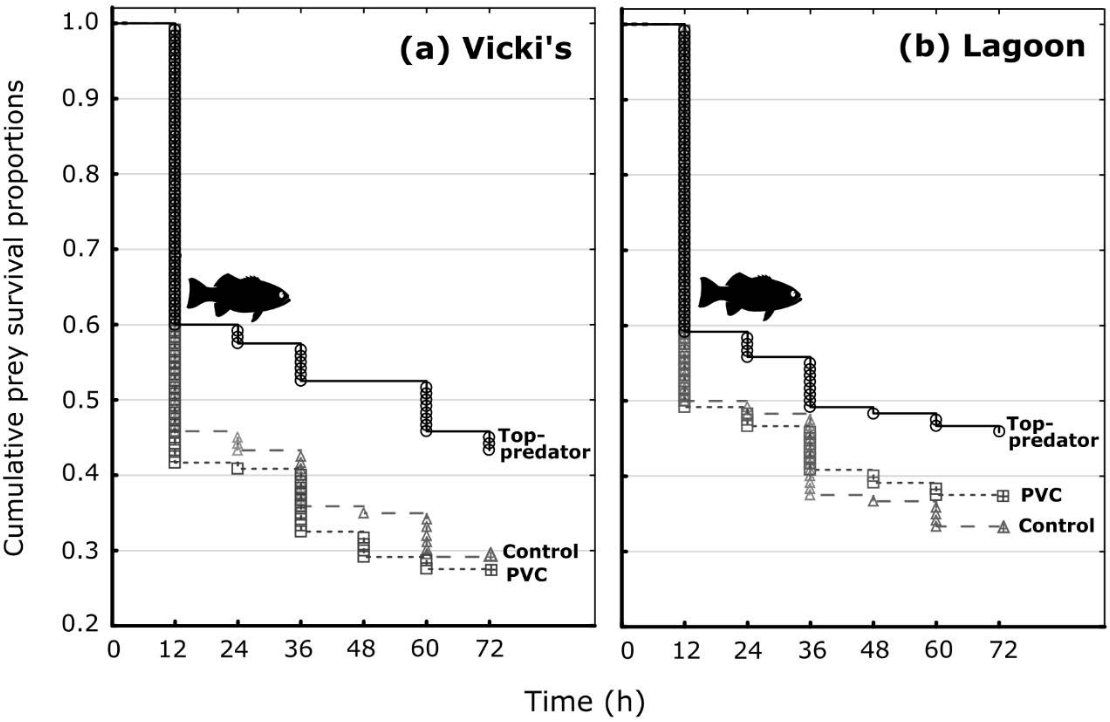

Figure 3.4. Survival trajectories of juvenile damselfish prey at **(a)** Vicki's and the **(b)** Lagoon reef sites for the three experimental treatments: control patches (light grey; broken line), PVC pipe patches (dark grey; dotted line) and top-predator habitat patches (black; continuous line).

Prey behaviour

Behaviour was only recorded for large individuals of *P. amboinensis*, which were the most frequent survivors at the 36 h survey. Changes in damselfish space use, feeding rate and aggressive interactions were explained by the reef site and/or the treatment (Table 3.1; main effects, $P < 0.05$), but not by their interaction (Table 3.1; Reef * Treatment interaction, $P > 0.05$). Behavioural differences among reef sites occurred as juvenile fish at the Lagoon tended to swim higher above the substrate and exhibit more aggressive displays, than those from Vicki's (Fig. 3.5a,c; Reef, $P < 0.05$). In addition, there were significant differences in the space use and feeding rate of fish according to the treatment (Fig. 3.5a,b; Treatment, $P < 0.05$). Juvenile fish from patches with top-predator cues used significantly higher sections of the coral and fed at greater speeds than those from control or PVC patches (Tukey's tests, $P < 0.05$). As expected, juvenile fish from control and PVC patches exhibited similar behaviours (Tukey's test, $P > 0.05$). The aggressiveness of fish was highly variable and was not significantly affected by the treatments (Fig. 3.5c).

Table 3.1. Results of the ANOVA used to compare the behaviour of *Pomacentrus amboinensis* between reef site (2 levels: Vicki's, Lagoon) and treatment (3 levels: control, PVC pipe, top-predator cues). Behaviours analysed included space use (height above substratum; cm), foraging (feeding strikes / 2 min), interactions (displays + chases/ 2 min).

Sources of variation	**Space use**		**Foraging**		**Aggressive interactions**	
	F	**P**	**F**	**P**	**F**	**P**
Reef	$F_{(1, 132)} = 59.6$	**<0.001**	$F_{(1, 132)} = 1.5$	0.23	$F_{(1, 95)} = 5.0$	**0.03**
Treatment	$F_{(2, 132)} = 17.9$	**<0.001**	$F_{(2, 132)} = 12.1$	**<0.001**	$F_{(2, 95)} = 0.4$	0.70
Reef x Treatment	$F_{(2, 132)} = 1.6$	0.20	$F_{(2, 132)} = 0.9$	0.41	$F_{(2, 95)} = 0.8$	0.46

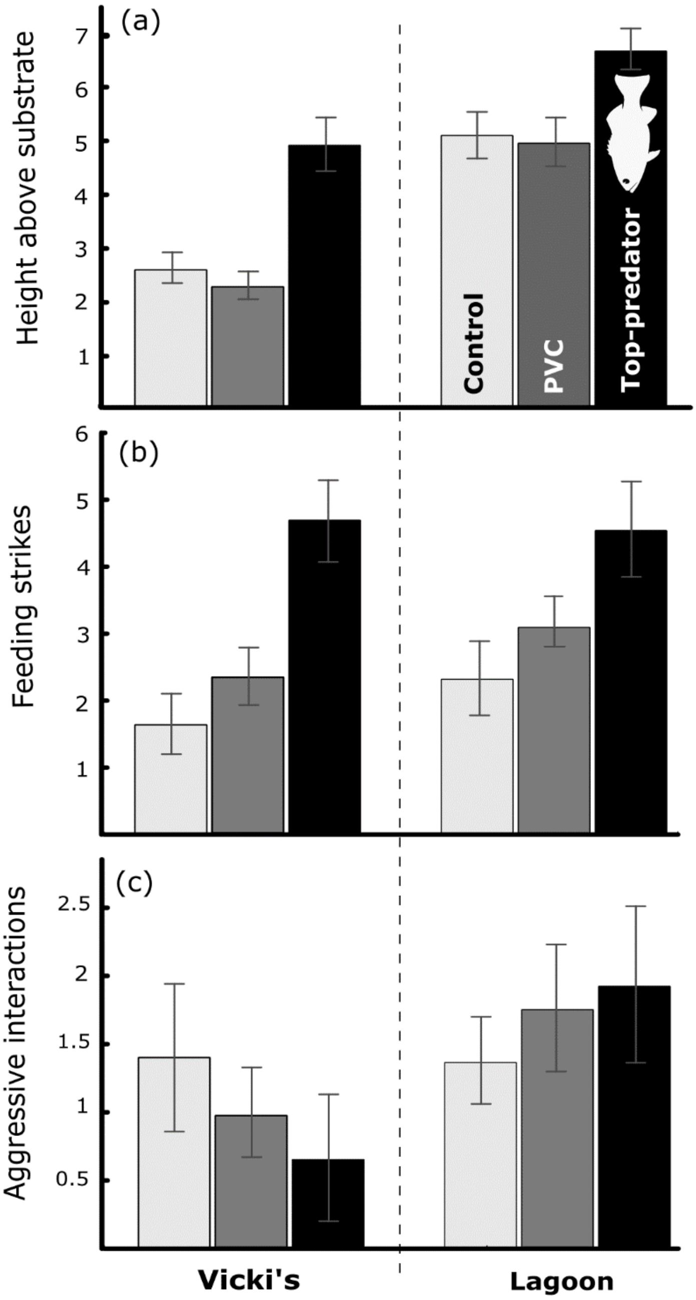

Figure 3.5. Mean (± SE) **(a)** space use (height above substratum, cm; N = 138), **(b)** foraging (feeding strikes/ 2 min; N = 138) and **(c)** aggressive interactions (displays and chases/ 2 min; N = 101) recorded for juveniles of *Pomacentrus amboinensis* at Vicki's and the Lagoon reef sites. Juvenile damselfish were under the effect of three experimental treatments: control patches (white bars), PVC pipe patches (grey bars) and top-predator patches (black bars). All the behaviours were recorded 36 h after the start of the experiment. Aggressive interactions were only recorded when the pair of conspecific *P. amboinensis* remained alive at the 36 h survey.

3.5 Discussion

Our study explored the extent to which predation risk from top-predators can indirectly mediate lethal and sub-lethal effects on bottom level prey. We found that juvenile fish prey exhibited ~ 66.5 % higher survivorship, ~ 46 % greater space use, and ~ 94.5 % higher feeding rate on experimental habitat patches where top-predator cues were present (i.e., visual and chemical stimuli from a coral trout). While a previous study illustrated the indirect link between the large piscivores and the recruitment of juvenile fish (i.e., through risk-mediated behavioural changes in mesopredators; Stallings 2008), this study evidences that such behavioural cascade can potentially impact both the behaviour and survival of the newly-settled communities of reef fish.

Risk effects on prey survival & selectivity

Juvenile prey experienced high rates of mortality on control and PVC pipe habitat patches (~ 72 % mortality after 72 h). However, such mortality was significantly lower on patches where top-predator cues were deployed. We hypothesize that such reduction in juvenile mortality was a result of the foraging restrictions and risk-adverse behaviours that predation risk commonly induces on mid-ranking fish. Although we did not record the behaviour of the mesopredatory fishes to directly attribute causation, we speculate this is the most likely mechanism given that small reef predators are the main cause of mortality of juvenile fish (Caley 1998, Almany and Webster 2006) and these mesopredators are known to suppress their foraging activity in the presence of risk cues from large piscivores (**Chapter 2**). Furthermore, a patch reef experiment from the Caribbean has already shown that the behavioural control of small piscivores can directly favour the persistence of newly-settled fishes. Stallings (2008) found that the abundance of fish recruits was highest (> 40 fish/patch) on patches with high densities of large-sized Nassau groupers that could effectively suppress the foraging of small-sized graysby and coney groupers. In a similar way, we propose that the survival of juvenile damselfish on our patches was favoured by the presence of risk cues from a top-predator which limited the foraging visits of smaller-sized piscivores.

The survival of the juvenile prey differed between the two reef sites, with prey from Vicki's reef experiencing the highest mortality. Piscivore surveys showed that Vicki's had the largest proportion of small-bodied wrasses and grouper species (mesopredators), particularly known to target and consume juvenile fishes (e.g., Beukers-Stewart and Jones 2004, Holmes et al. 2012). Thus, not surprisingly, the predation pressure and mortality of juvenile prey was the highest at this reef site. Similar to previous studies, we found that the Lagoon was dominated by large-bodied piscivores and had relatively low levels of juvenile fish mortality in comparison to other reef sites of Lizard Island (Stewart and Beukers 2000, Holmes and McCormick 2006). Although large fish species (e.g., emperors, snappers) can potentially feed on juvenile-sized prey (< 2 cm SL; Kulbicki et al. 2005), this is unusual given the vast asymmetry between the energy spent hunting and the energy gained (Optimal Foraging theory; Stephens and Krebs 1986), especially in coral reefs where juvenile fish effectively shelter in crevices and branches (Roberts and Ormond 1987).

The size and species-selective loss of juvenile fishes was consistent across all experimental treatments and reef sites, with small-sized juveniles and *P. chrysurus* individuals always experiencing the highest mortalities. Unfortunately, this consistent pattern of selective mortality does not allow us to discriminate which mechanism was the main driver; i.e., whether (i) increased vulnerability via antagonistic interactions or (ii) active selection by predators. The finding that small-sized juveniles and *P. chrysurus* individuals had the highest mortalities aligns with the vulnerability hypothesis (mechanism i) given that they are indeed the weakest and least competitive of the group of fish deployed on each patch. Size is known to confer significant advantage to strength, aggressiveness and competitive abilities so large-sized juveniles most often win the battle for quality shelter (Booth 1995b, Poulos and McCormick 2014). Additionally, *P. amboinensis* is a strong aggressive and competitor known to displace congeneric species to riskier habitats (McCormick and Weaver 2012, Kok et al. 2016). However, the fact that the most abundant mesopredator (i.e., the moon wrasse *Thalassoma lucasanum*) is known to prefer small-sized juveniles (< 1.2 cm SL; McCormick and Meekan 2007, Holmes and McCormick 2010) suggests that selectivity by predators (mechanism ii) may also be a contributing factor to the patterns observed. Without direct observations of the predation events we can only speculate on the relative importance of predator-driven phenotypic selection. Moreover, because mesopredators that capitalize on juvenile fishes are diverse and common, knowledge is required on the relative contribution of each mesopredator species to the juvenile mortality and the presence predator-predator interactions that might change the magnitude or direction of their lethal effects.

Risk effects on prey behaviour

In patches where top-predator cues had been deployed, juvenile prey exhibited ~ 46 % greater space use and ~ 94.5 % higher feeding rates. Assuming the same mechanistic links of Stallings (2008), these results suggest that risk effects from top-predators (i.e., via mesopredator behavioural suppression) can cascade to indirectly dampen both the lethal and sub-lethal effects on juvenile fishes. While two laboratory based studies have shown that behavioural trophic cascades triggered by risk cues from top-predators can favour the space use and metabolism of newly-settled fish (Palacios et al. 2016a, Palacios et al. 2016b), this is the first time field-based research reports equivalent patterns. Here, we hypothesize that the behavioural control of mesopredators reduced the level of risk exerted on juvenile prey, allowing them to exploit food resources furthest from the shelter and allocate more time and energy on foraging activity than in predator avoidance (Lima 1998b, Brown et al. 1999). Movements higher above the substrate and increased feeding rates are common behaviours of juvenile planktivore fishes under low stress levels or reduced predation risk (e.g., Holmes and McCormick 2011, Lönnstedt et al. 2012). For many animals, including fishes, enhanced levels of activity and foraging can allow greater access to higher quality food sources, which can significantly boost an individuals' body condition, growth rate, competitive advantage, and survival (Connell 1998, Steele and Forrester 2002, Hoey and McCormick 2004).

Aggression between resident prey was not significantly affected by the presence of top-predatory cues, however, it did differ between reef sites (Vicki's = low number of aggressive interactions vs. Lagoon = higher number of interactions). This could suggest that antagonistic interactions among juvenile fish are being influenced by differences in predation pressure from mesopredators. One possibility is that the enhanced activity and space use of juveniles at the Lagoon (due to the low numbers of mid-sized piscivores) may have increased the encounter rates and spatial overlap among conspecifics, thus favouring a high number of aggressive interactions at this location. At the same time, it is possible that the large assemblage of mid-sized piscivores at Vicki's often distracted and interrupted interactions among juvenile fish, hence reducing the number of aggressions recorded (Sih et al. 1985, Gurevitch et al. 2000). To clearly elucidate the impact that predation risk from mesopredators has on juvenile fish interactions, a better understanding of the intrinsic (e.g., size and condition of the opponents) and extrinsic factors (e.g., density, habitat complexity) that influence reef fish competition is required (Bonin et al. 2015).

Conclusion

Humans have exploited larger-sized carnivores from most natural ecosystems worldwide (Estes et al. 2011, Ripple et al. 2014). The consequent loss of top-down control from apex predators has allowed an explosion of mid-ranking species that dramatically increase their abundance, distribution, and foraging activity (Ritchie and Johnson 2009). As shown here for coral reefs, a release of reef mesopredators could greatly threaten the survivorship of juvenile fishes and jeopardize the replenishment of reef fish communities. Negative consequences on newly-settled fishes are compounded if we consider the sub-lethal effects on prey behaviour. Reduced space use and feeding rate along with increased stress and self-maintenance costs (Palacios et al. 2016a) could impair the body condition, fitness, and growth of newly-settled fishes (Connell 1998, Steele and Forrester 2002, Abdulla 2004) and have carryover effects to subsequent life stages (Gagliano et al. 2007a). Overall, these findings underscore the importance of top-predators and the extent of risk effects in complex marine communities. While more empirical research will allow us to fully understand how the loss of top-predators will cascade to impact marine populations and ecosystems, it is critical that conservation strategies target the preservation of predatory species, and the myriad of direct, indirect, lethal, and risk-associated links they maintain in healthy food webs.

Chapter 4: Top- predators negate the effect of mesopredators on prey physiology

Published
Palacios, M.M., S.S. Killen, L.E. Nadler., J.R. White & M.I. McCormick. 2016
Top- predators negate the effect of mesopredators on prey physiology
Journal of Animal Ecology 85:1078-1086
http://onlinelibrary.wiley.com/doi/10.1111/1365-2656.12523/full

4.1 Summary

Predation theory and empirical evidence suggest that top-predators benefit the survival of bottom prey through the suppression of mesopredators. However, whether such behavioural suppression can also affect the physiology of bottom prey has yet to be examined. Using a three-tier reef fish food web and intermittent-flow respirometry, our study examined changes in the metabolic rate of bottom prey exposed to combinations of mesopredator and top-predator cues. Under experimental conditions, the mesopredator (dottyback, *Pseudochromis fuscus*) continuously foraged and attacked bottom prey (juveniles of the damselfish *Pomacentrus amboinensis*) triggering an increase in prey O_2 uptake by 38 ± 12.9 % (mean ± SE). The visual stimulus of a top-predator (coral trout, *Plectropomus leopardus*) restricted the foraging activity of the mesopredator, indirectly allowing bottom prey to minimise stress and maintain routine O_2 uptake. Although not as strong as the effect of the top-predator, the sight of a large non-predator species (thicklip wrasse, *Hemigymnus melapterus*) also reduced the impact of the mesopredator on prey metabolic rate. We conclude that low trophic level species can benefit physiologically from the presence of top-predators through the behavioural suppression that top-predators impose on mesopredators. By minimising the energy spent on mesopredator avoidance and the associated stress response to mesopredator attacks, prey may be able to invest more energy in foraging and growth, highlighting the importance of the indirect, non-consumptive effects of top-predators in marine food webs.

4.2 Introduction

Top-predators can drive food web dynamics through a variety of direct and indirect effects (Abrams 1995). The indirect effects of predation can be observed in three-tier trophic cascades, where a direct negative link between the top-predators and intermediate level species (e.g., mesopredators, mesoconsumers) often indirectly favours the next consecutive trophic level of bottom prey (Werner and Peacor 2003, Schmitz et al. 2004). For instance, correlative evidence from temperate forests suggests wolves limit habitat use and grazing patterns of ungulate herbivores, which in turn indirectly enhances the survival and recruitment of native vegetation (Ripple et al. 2001). Few studies have assessed a fully trait-mediated pathway in which successive predator – prey interactions are driven by predation risk, with impacts on the behavioural, physiological or morphological traits of the species. Recently, Gordon et al. (2015) and (Palacios et al. 2016b) showed that in particular desert and coral reef food webs, top-predators can alter the non-consumptive effects (predation risk) of mesopredators and indirectly affect the behaviour of bottom prey (e.g., increased habitat breadth, reduced anti-predator behaviour). Given that organisms make costly energetic trade-offs between predator avoidance and self-maintenance activities (reviewed by Lima 1998b, Brown and Kotler 2004), top-predators could have positive indirect effects on the lifetime fitness of bottom prey (e.g., mating success, fecundity, reproductive rate). However, this can only be determined by detailed examinations of the effects of risk-induced trophic cascades on different behavioural, physiological and morphological traits of prey.

Prey physiology is strongly affected by the presence of predators (reviewed by Hawlena and Schmitz 2010, Zanette et al. 2014). In vertebrates, physiological responses to predation risk include altered cardiovascular activity, ventilation and metabolism (e.g., Ward et al. 1996, Cooke et al. 2003, Hawkins et al. 2004, Steiner and Van Buskirk 2009). These physiological mechanisms can improve the prey's probability of escaping an attack, but can be energetically costly and may decrease the surplus of energy available for other tasks such as activity, growth, maintenance or reproduction (Houston et al. 1993, DuRant et al. 2007). Consequently, chronic and/or frequent exposure to predation stress can reduce the energy allocation for essential physiological functions (Hawlena and Schmitz 2010). For example, under chronic predation risk, snowshoe hares experience a reduction in their body condition index, leucocyte counts and reproductive output (Boonstra et al. 1998, Sheriff et al. 2009). In addition, larval and juvenile marine fishes that experience frequent exposure to predator cues display reduced growth and lipid stores (Killen and Brown 2006, Killen et al. 2007). While non-consumptive predator-prey interactions are physiologically costly for prey, it is yet unknown if such costs can be ameliorated when the predator itself is under behavioural suppression by a higher level predator. Given that the anti-predator response of animals is proportional to the level of predation risk (Helfman 1989), we hypothesise that any restrictions in the activity and foraging of the mesopredator should reduce predator-induced stress and energy expenditure of the prey.

To address this knowledge gap, we explored a potential mechanism through which risk-driven effects may cascade to influence the metabolic rate of bottom prey. Using a three-level food web of coral reef fishes as a model system, we experimentally examined how risk elicited by a top-predator altered mesopredator behaviour and consequently modified their influence on bottom prey activity and oxygen uptake. We specifically aimed to: (a) determine whether acute predation risk by a top-predator (coral trout) affects the behaviour of a mesopredator (dottybacks); and (b) quantify how the altered behaviour of the mesopredator affects the metabolic rate (estimated oxygen uptake) of bottom prey (damselfish juveniles). The terms 'top-predator > mesopredator > bottom prey' refer to the hierarchy and trophic status among the three species used in the study, and is not meant to imply that they have a fixed trophic category in their natural ecosystem (e.g., the coral trout could be the top-predator in one system but the mesopredator in another). At any given point these terms could be replaced by 'high trophic level species > intermediate level species > bottom level species' respectively.

4.3 Materials & Methods

Experimental overview

Changes in the metabolic rate (oxygen uptake) of damselfish juveniles were measured and compared among six experimental treatments crossing the presence of a mesopredator (2 levels: dottyback, goby) with a top-predator (3 levels: coral trout, thicklip wrasse, empty tank). The goby and thicklip wrasse served as non-predator species to control for the meso- and top-predator, respectively. Behavioural observations were recorded both on the damselfish juveniles and the mesopredators (dottybacks/gobies). Eight to nine replicate trials were undertaken for each treatment, with all fish being tested only once to maintain independence among trials. Routine metabolic rate was calculated given its common use as an indicator of stress and energy expenditure in response to predation risk (Chabot et al. 1996, Ward et al. 1996, Holopainen et al. 1997, Steiner and Van Buskirk 2009) and their correlation to a number of ecologically relevant behaviours and life-history traits (Biro and Stamps 2010, Burton et al. 2011, Killen et al. 2013).

Study species & Fish Handling

Juveniles of the common Indo-Pacific damselfish, *Pomacentrus amboinensis,* were used as the bottom prey. This benthic species is a site-attached omnivorous demersal spawner with a bipartite life history. When the larvae (10 - 15 mm SL; Kerrigan 1996) settle to shallow reefs during the austral summer months (October-January), they are subject to extremely high rates of predation by small reef piscivores such as cods, dottybacks and lizardfishes (Almany and Webster 2006). These damselfish juveniles can learn to recognise reef predators, have strong anti-predator behaviour and exhibit threat-sensitive responses to predation risk (Holmes and McCormick

2011). The dottyback (*Pseudochromis fuscus*) was used as the focal mesopredator species, as it is a small (< 10 cm TL) site-attached carnivore that voraciously consumes newly settled fishes using ambush and pursuit techniques (Feeney et al. 2012). It acclimates well to aquarium conditions and is known to respond to visual and chemical cues from top-predators (**Chapter 2**). The leopard coral trout (*Plectropomus leopardus*) was used as the top-predator species. This large (> 30 cm SL) reef piscivore is relatively common on the Great Barrier Reef (GBR; Ayling et al. 2000) and consumes predominantly small-sized reef fish (3 - 7 cm SL; St. John 2001). The non-piscivorous reef-fish species selected to experimentally control for the presence of the meso and top-predator, were the white-barred goby (*Amblygobius phalaena*) and the thicklip wrasse (*Hemigymnus melapterus*), respectively. The goby (< 15 cm TL) feeds mainly on algae and copepods (Sano 1984), while the wrasse (> 30 cm TL) usually consumes small crustaceans, polychaete worms and molluscs (Randall 2013). Although both non-predators are frequently found around patch reefs and in close proximity to newly settled fish, they are not known to prey on them.

All fishes were collected from the lagoon of Lizard Island (14°40′S, 145°28′E), northern GBR, during the second week of November 2014. Damselfish juveniles (1.37 ± 0.008 cm, mean SL ± SE, N = 54) were captured from the reef edge with light traps moored overnight, while both dottybacks (7.03 ± 0.05 cm, mean TL ± SE, N = 26) and gobies (7.5 ± 0.1 cm, mean TL ± SE, N = 28) were collected from patch reefs by SCUBA divers using hand nets and a mild anaesthetic clove oil solution. Specimens of *P. leopardus* (39.4 ± 1.07 cm, mean TL ± SE, N = 5) and *H. melapterus* (28.3 ± 2.3 cm, mean TL ± SE, N = 4) were caught using hand lines (with barbless hooks) and barrier nets, respectively. Fishes were maintained at the Lizard Island Research Station in separate holding tanks. Coral trouts and thicklip wrasses were individually kept in 300 L round tanks, dottybacks were isolated individually in porous 1 L containers in groups of 10 in 68 L tanks and all of the damselfish juveniles were kept together in a 22 L aquarium (~3 fish per L). All tanks had a flow-through seawater system at ambient temperatures (27.5 - 29°C) and light photoperiods (12 h light: 12 h dark). Damselfish juveniles were fed *Artemia* spp. twice daily, while the rest of the fishes were fed prawn or squid.

Before the onset of experimental procedures, damselfish juveniles were trained to recognise cues from the dottybacks as their collection prior to reef settlement may have prevented them from learning the identity of reef-associated predators. Naïve juvenile fishes can learn the identity of a novel predator by simultaneously presenting conspecific damage-release chemical cues (indicative of threat) with visual and/or chemical cues of a predator (Brown and Chivers 2005). Similar to the protocols followed by McCormick and Holmes (2006) and Lönnstedt et al. (2012) damselfish juveniles were trained by exposing them concurrently to a variety of cues, including: 10 mL of the conspecifics damaged-released chemical cues, 30 ml of the dottyback odour and a live dottyback placed in a sealed ziploc bag (serving as a visual cue). After 10 min, all cues were removed from the tank and water flow was restored. To prepare the damage-released chemical cues, three damselfish per training session (12 – 14 mm SL) were euthanized with a quick blow to the head and placed in a petri dish where 10 superficial cuts were made to the skin of each donor fish (5 cuts per flank). Fish were then rinsed with 10 ml of seawater (previously obtained from their tank) creating a solution of damage-release alarm cues. To obtain the mesopredator odour, four dottyback*s* were randomly selected and kept for at least 12 h in a tank containing 4 L of aerated seawater.

Experimental setup

Experiments were undertaken in four pairs of replicate glass tanks (25 x 60 cm; 30 cm water). Each pair of tanks consisted of a mesopredator tank and a top-predator tank positioned next to each other along their longest side (Fig. 4.1). Except on the face they shared, both tanks were completely shielded from external disturbances by opaque curtains. Each mesopredator tank contained a layer of sand, a shelter for the mesopredator (PVC tube: 8 cm length x 3 cm diameter), a resin branching coral (14 × 11.5 × 5 cm; item no. 21505; Wardleys/TFH) and a sealed glass respirometry chamber for the damselfish juvenile (described below). Two removable opaque panels were used to modulate the interactions and cue exchange between the fish. The first panel was positioned between the mesopredator and the top-predator tanks allowing an exchange of visual cues only when it was removed. A previous study with the same study species showed that visual cues from the top-predator are sufficient to achieve behavioural suppression of the dottyback (**Chapter 2**). The second panel divided the mesopredator tank transversally into two sections, separating the mesopredator and its shelter from the damselfish juvenile. Only when this panel was removed could the mesopredator approach and interact with the damselfish juvenile. Video cameras installed over each pair of experimental tanks recorded the behaviour of each fish. As in the holding tanks, all experimental tanks had constant flow-through seawater at ambient temperature.

Intermittent-flow respirometry was used to measure oxygen uptake of damselfish juveniles as proxy for aerobic metabolism. This technique allows continuous monitoring of dissolved oxygen levels inside a respirometry chamber that is intermittently flushed with oxygenated water to measure oxygen decline in the absence of hypoxia (Svendsen et al. 2016). Oxygen uptake is a good approximation for aerobic metabolic rate as oxygen is consumed in the breakdown of stored energy in order to fuel many of the most important processes that affect fitness, including locomotor activity, growth and maintenance (Chabot et al. 2016, Nelson 2016). In this study, respirometers consisted of individual cylindrical glass chambers (11 cm length x 2 cm diameter; total volume of chamber plus associated tubing = ~70 mL) protected externally with half-cylinders of clear acrylic (11 cm length x 5.5 cm radius). Water flow through the chambers was driven by an external pump set to alternately turn on (2 min) and off (8 min) throughout the measurement periods. This allowed water oxygen content to be measured every 2 s for 8 min while the respirometer was in the closed state, after which the respirometer was flushed with aerated water for 2 min to prevent it from reaching hypoxic levels. Water mixing within each respirometer was achieved with a pump that moved water through the chamber and around an external circuit of gas-impermeable tubing. Also located within the circuit for each respirometer was a flow-through cell that housed an oxygen-sensing optode attached to an oxygen sensor (Firesting 4-Channel oxygen metres; Pyro- science, Germany) and a computer. To correct for background bacterial respiration, oxygen uptake was recorded for 30 min at the beginning and end of each trial in each chamber without fish. Every day the respirometers, flow-through cells and tubing were thoroughly cleansed with soap, bleach and hot water.

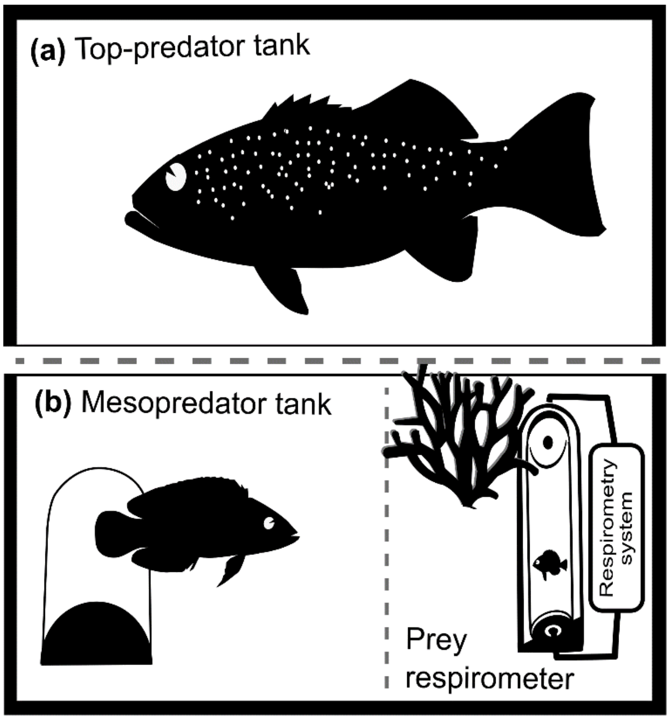

Figure 4.1. Experimental setup used to assess the indirect interactions between a three level food web of coral reef fish. The set up includes **(a)** a top-predator tank to hold the top–predator (coral trout, *Plectropomus leopardus*) or the non-predator (thicklip wrasse, *Hemigymnus melapterus*) and **(b)** a mesopredator tank where the mesopredator (dottyback, *Pseudochromis fuscus*) or the small non-predator (goby, *Amblygobius phalaena*) could swim freely and interact with top-predator and/or prey. Each mesopredator tank contained a layer of sand, a shelter for the mesopredator, a resin branching coral and a respirometry chamber to hold the bottom prey (damselfish juveniles, *Pomacentrus amboinensis*). A removable opaque panel was positioned between the top-predator and mesopredator tanks (grey discontinuous line) allowing an exchange of visual cues only when it was removed. A second panel divided the mesopredator tank, and only with its removal could the mesopredator approach and interact with the damselfish.

Experimental protocol

All juveniles were starved for 24 h prior to experimentation in order to ensure that they were in a post-absorptive state (Niimi and Beamish 1974). Experimental trials began with a pre-stimulus period, in which a damselfish juvenile was introduced into the respirometer and left undisturbed for 2 h while recording its oxygen uptake and activity. After 2 h, the assigned mesopredator (dottyback/goby) and top-predator (coral trout/ thicklip wrasse) were introduced into the tanks and left to acclimate for 20 min. The post-stimulus period was initiated by removing the two opaque panels thereby allowing the mesopredator to simultaneously (a) interact with the damselfish juvenile and (b) receive visual cues from the top-predator. For the following 2 h, the behaviour of the mesopredator and the post–stimulus oxygen uptake and activity of the damselfish were recorded. Each trial lasted approximately 4 h 20 min. All damselfish juveniles were then weighed to determine wet body mass. A total of 53 trials were executed over nine days, running simultaneously four trials in the morning and four in the afternoon. Every day the six experimental treatments were randomly assigned to the two periods of the day (am/pm) and the four pairs of replicate tanks.

Metabolic and behavioural assessment

Measures of metabolic rate (oxygen uptake; mg O_2 h^{-1}) and activity (line crosses) were estimated for each damselfish juvenile. Routine metabolic rates were estimated as the mean level of oxygen uptake in the 1 h before and after exposure to the predator cues. Rates during each closed phase were calculated using linear least-squares regression, excluding the first and last minute of each closed phase. In total, 6 measures of oxygen uptake were collected per hour and used to calculate the oxygen uptake of each prey damselfish. Activity was measured by quantifying the number of times the damselfish juvenile crossed five equidistant lines that transversally divided the respirometer chamber into six 1.8 cm-width sections. Line crosses were only assessed during the first 10 min of the pre- and post-stimulus 1 h periods selected for metabolic analysis. Pilot observations showed this sample period (10 min) was representative of the activity of the damselfish during the correspondent hour.

Three behavioural attributes of the mesopredators (dottybacks/gobies) were quantified from the 10 min post-stimulus period: (a) time spent inside shelter (min); (b) time spent near the respirometer containing the prey (min); and (c) number of strikes to the respirometer. The time near the respirometer included all of the time the mesopredator was closer than one body length from the chamber, while the number of strikes considered all of the attacks in which the mesopredator hit the chamber with its mouth.

Statistical analysis

Changes in the damselfish metabolic rate between the pre- and post-stimulus observation periods were calculated (Δ O_2 uptake; mg h^{-1}) and compared using a two-factor analysis of covariance (ANCOVA) with mesopredators (2 levels: dottyback, goby) and top-predators (3 levels: coral trout, thicklip wrasse, empty tank) as factors. Damselfish wet body mass (g) and change in activity (Δ line crosses) were used as covariates in the analysis to correct for the effects of body size and movement. Additionally, a general linear model (GLM) was used to examine the relationship between the change in activity (Δ line crosses) and the change in oxygen uptake (Δ O_2 uptake; mg h^{-1}) of damselfish juveniles exposed to the six treatments. Mesopredator behaviour was analysed among treatments using a two-factor analysis of variance (ANOVA) with either time spent inside shelter or time spent near the respirometer as dependent variables. Gobies never attacked the damselfish juveniles, so the number of strikes was analysed only for the dottybacks through a one-way ANOVA. All significant differences detected were further explored using post-hoc Tukey's HSD for unequal N. Residual analyses were used to examine if the data satisfied the assumptions of normality and homoscedasticity. Data from the damselfish juveniles were normal and homoscedastic, however, data from the mesopredators were square root transformed to meet the assumptions of parametric tests.

4.4 Results

Changes in the oxygen uptake of the damselfish juveniles (ΔMO_2) between the pre- and post- stimulus periods were significantly influenced by the mesopredator (dottyback/goby), the top-predator (coral trout/ thicklip wrasse/ empty tank) and their interaction (Fig. 4.2, Table 4.1a; ANCOVA main effects and interaction, $P < 0.05$). When damselfish juveniles were exposed to the goby (small non-predator) their oxygen uptake remained relatively constant independent of the presence of an empty tank, a wrasse or a trout (Fig. 4.2; grey bars are not significantly different, Tukey's HSD test, $P > 0.05$). Nevertheless, in the presence of a dottyback (mesopredator) the oxygen uptakes of damselfish were influenced by the top-predator treatments (Fig. 4.2; black bars significantly different, Tukey's HSD test, $P < 0.05$). Oxygen uptakes of damselfish increased by 38 ± 12.9 % (mean ± SE) if they were exposed to the dottyback alone (cues of an empty tank), yet remained constant if the dottyback was under the effect of a coral trout (cues of a top-predator). Damselfish increased oxygen uptake to intermediate levels if the dottyback was under the effect of the wrasse.

Changes in activity of the damselfish (ΔAc; line crosses) did have a significant effect on the ΔMO_2 (Table 1a; $F_{1,42} = 4.85$, $P < 0.05$). Overall, the ΔAc were positively correlated with the

ΔMO_2 (Fig. 4.3). Although the relation between the ΔAc and the ΔMO_2 did not statistically differ among the six treatments (Table 4.1b; GLM interaction, $F_{5,38} = 0.48$, $P > 0.05$), there were interesting qualitative differences in the responses observed during exposure to the goby and the dottyback (Fig. 4.3). In the presence of the non-predatory goby, decreases in the activity (negative change) of the damselfish were most often associated with decreases in oxygen uptake. However, in the presence of the mesopredator (dottyback) many damselfish increased oxygen uptake despite reducing their activity. Further, damselfish that increased their activity usually had higher levels of oxygen uptake if exposed to a dottyback alone (control) or a dottyback under the effect of the wrasse (Fig. 3).

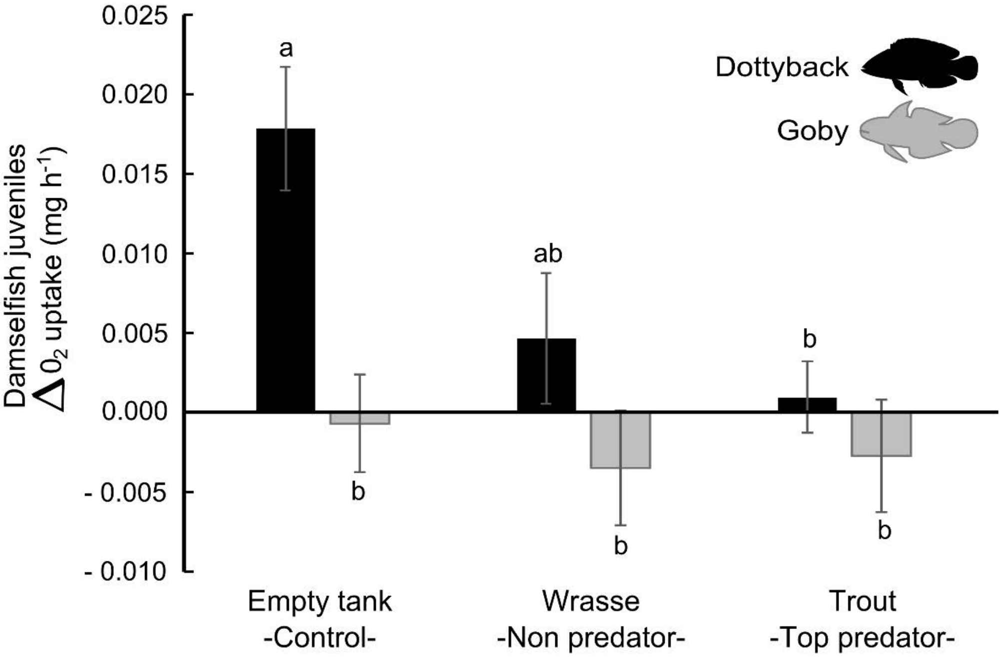

Figure 4.2. Change (mean ± SE) in the oxygen uptake ($\Delta MO2$; mg O_2 h^{-1}) of damselfish juveniles between 1 h pre- and post-stimulus periods. During the post-stimulus periods damselfish were exposed to the combination of a mesopredator (dottyback/goby) and a top-predator (coral trout/ thicklip wrasse/ empty tank). The goby and thicklip wrasse served as non-predator species to control for the meso- and top-predator, respectively. Black bars correspond to the dottybacks (mesopredator) while grey bars to the gobies (small non-predator). A positive value indicates an increase in oxygen uptake while a negative value indicates a decrease. Bars with the same lowercase letter did not differ significantly according to the Tukey's HSD post-hoc test.

The behaviour of the dottyback and goby were affected differently by the top-predator (Table 2; ANOVA interaction, $P < 0.05$, Fig. 4a, b). Under control conditions (cues of an empty tank), dottybacks were significantly more active than the gobies, spending more than 70 % of the time exploring the arena and constantly approaching the damselfish chamber (~ 20 % of the time, Fig. 4a, b; black and grey bars in the control treatment are significantly different, Tukey's HSD test, $P < 0.05$). However, under the effect a large fish (either wrasse or trout) the behavioural differences disappeared, as dottybacks and gobies spent a similar percentage of time active in the tank and near the damselfish (Fig. 4.4 a, b). Gobies never attacked the damselfish (as expected by their non-piscivorous food preferences), so the number of strikes was only recorded for the dottybacks. Under control conditions dottybacks frequently struck at the damselfish chamber. However, when dottybacks were simultaneously exposed to the trout, the total number of strikes was significantly reduced by 83.6 % (Fig. 4.4c; Table 4.2; ANOVA, $F_{2,22} = 6.9$, $P < 0.01$). Dottybacks struck at the damselfish an intermediate number of times when exposed to the wrasse (top-predator control).

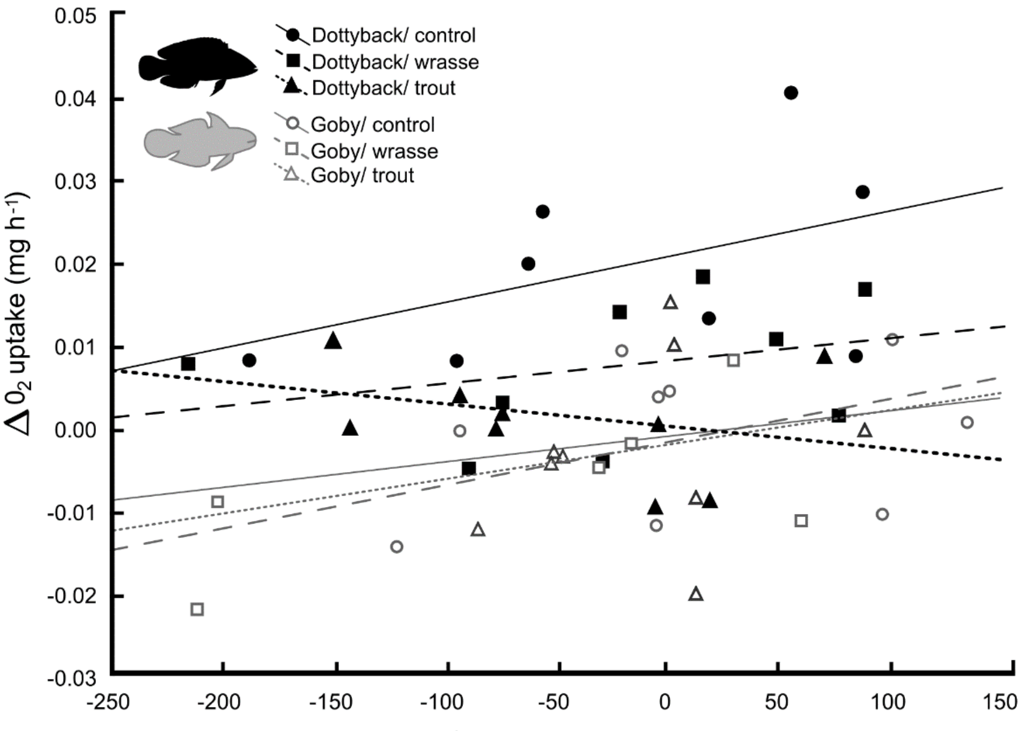

Figure 4.3. Relationship between the change in activity (ΔAc; line crosses) and the change in oxygen uptake (ΔMO2; mg h-1) of damselfish juveniles exposed to six treatments combining the presence of a mesopredator (dottyback/goby) and a top-predator (coral trout/ thicklip wrasse/ empty tank) during the post-stimulus period. The goby and thicklip wrasse served as non-predator species to control for the meso- and top-predator, respectively. Black symbols correspond to the dottybacks (mesopredator) while grey symbols to the gobies (small non-predator). For both axes positive values indicate an increase of the variable (activity or O_2 uptake) during the post-stimulus period while negative values indicate a decrease.

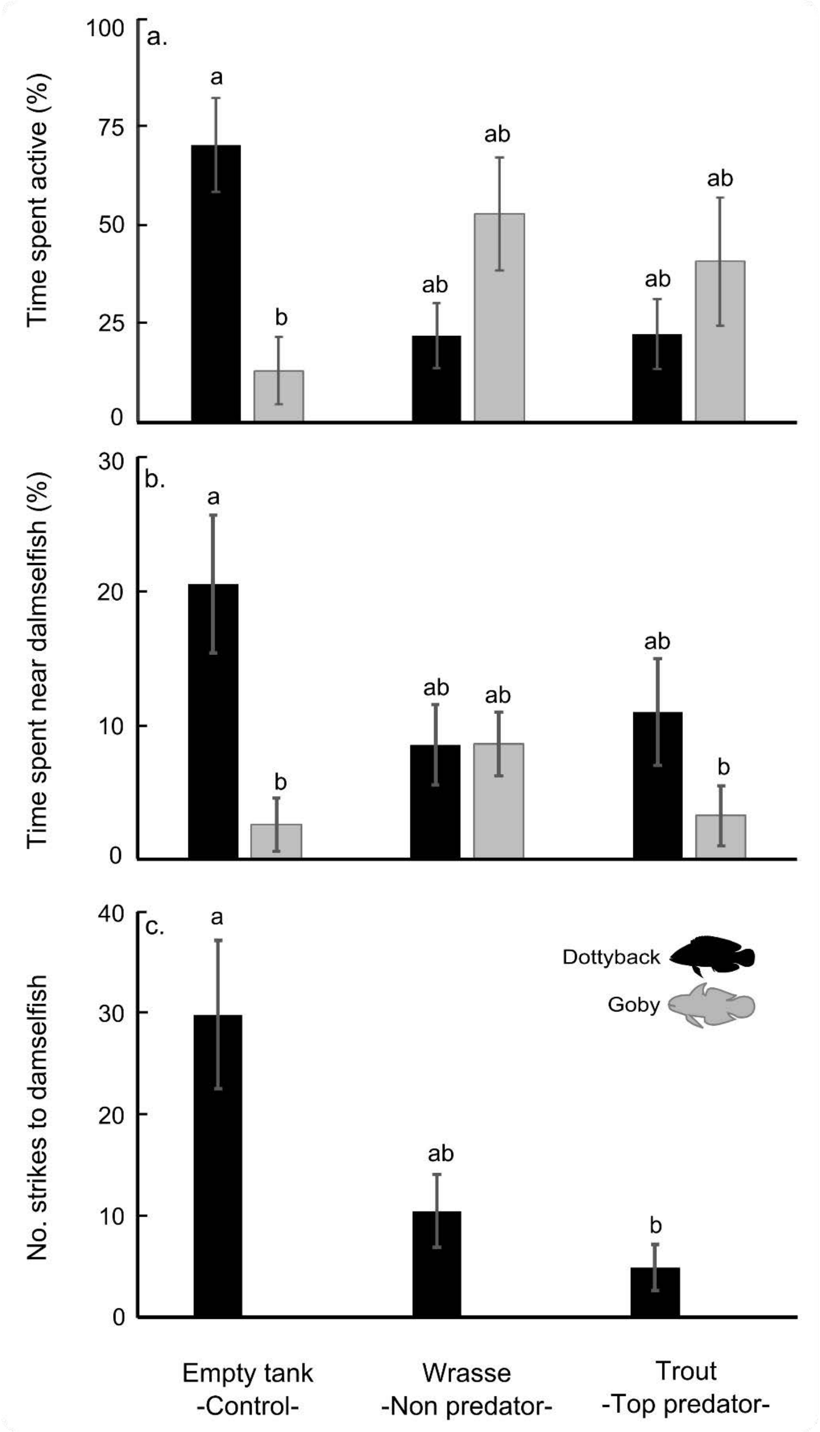

Figure 4.4. Mean (± SE) **(a)** time spent active (%), **(b)** time spent near the damselfish chamber (%), and **(c)** number of strikes (#/10 min) recorded for dottybacks (mesopredator) and gobies (small non-predator) exposed to an empty tank (control conditions), a thicklip wrasse (large non-predator) or a coral trout (top-predator). Black bars correspond to dottybacks, while grey bars to gobies. Bars with the same lowercase letter did not differ significantly according to the Tukey's HSD post-hoc test.

Table 4.1. **(a)** Parameter estimates of the ANCOVA used to test the change in oxygen uptake (ΔMO_2; mg h^{-1}) of the damselfish juveniles (bottom prey) exposed to six treatments combining a mesopredator (dottyback/goby) with a top-predator (coral trout/ thicklip wrasse/ empty tank). The variables body mass and change in activity were used as covariates in the assessment of metabolic change for the damselfish. **(b)** Parameter estimates are also shown for the GLM examining the relation between the change in activity (ΔAc; line crosses) and the change in oxygen uptake (ΔMO_2; mg h^{-1}) of damselfish juveniles exposed to the six treatments. Asterisk indicate significant differences where * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

a. ANCOVA

Sources of variation	SS	DF	MS	F	P	
(a) Mesopredator	1.68E-03	1	1.68E-03	19.43	0.000	***
(b) Top-predator	7.45E-04	2	3.72E-04	4.30	0.020	*
(a) x (b)	5.90E-04	2	2.95E-04	3.41	0.042	*
Covariates:						
Body Mass (g)	1.19E-04	1	1.19E-04	1.37	0.248	ns
Δ activity (line crosses)	4.20E-04	1	4.20E-04	4.85	0.033	*
Error	3.64E-03	42	8.66E-05			

b. GLM

Sources of variation	SS	DF	MS	F	P	
(a) Treatment	3.03E-03	5	6.05E-04	6.52	0.000	***
(b) Δ activity (line crosses)	2.52E-04	1	2.52E-04	2.71	0.108	ns
(a) x (b)	2.25E-04	5	4.49E-05	0.48	0.786	ns
Error	3.53E-03	38	9.29E-05			

Table 4.2. Parameter estimates of the ANOVAs examining three behavioural attributes (Time spent active, Time spent near damselfish, No. strikes) of the mesopredators (dottybacks/gobies) exposed to a top-predator (coral trout/ thicklip wrasse/ empty tank). Asterisk indicate significant differences where * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Time spent active (s)	SS	DF	MS	F	P	
(a) Mesopredator	367.61	1	367.61	0.07	0.793	ns
(b) Top-predator	3541.58	2	1770.79	0.34	0.717	ns
(a) x (b)	80946.19	2	40473.10	7.67	0.001	**
Error	248131.54	47	5279.39			

Time spent near damselfish (s)	SS	DF	MS	F	P	
(a) Mesopredator	71.34	1	71.34	10.12	0.003	**
(b) Top-predator	11.78	2	5.89	0.84	0.440	ns
(a) x (b)	53.78	2	26.89	3.81	0.029	*
Error	345.41	49	7.05			

No. strikes to damselfish	SS	DF	MS	F	P	
Top-predator	3.40	2	1.70	6.90	0.005	**
Error	5.42	22	0.25			

4.5 Discussion

Our study demonstrated a cascade of trait-mediated effects through which top-predators indirectly negated the effect of mesopredators on prey metabolic rate. Under experimental conditions, the mesopredator (dottyback) frequently attacked bottom prey (damselfish juveniles) triggering a marked increase in their metabolic rate. As hypothesised, however, acute risk from the top-predator (visual cues of a coral trout) restricted the mesopredator behaviour (reduction in activity and feeding strikes), indirectly allowing bottom prey to reduce physiological stress and minimise routine metabolic rate.

The cascade of non-consumptive effects documented here begins with the behavioural suppression of the mesopredators by the top-predator. Dottybacks exposed to acute predation risk from the top-predator allocated less time to foraging (i.e., less time near the prey and a lower number of attacks) as the threat of predation from the coral trout induced them to shelter and remain inactive most of the time. Similar trade-offs and behavioural changes have been observed in a wide range of taxa, as prey often reduce their activity, space use and foraging under predation risk (e.g., Orrock et al. 2004, Valeix et al. 2009). Interestingly, when foraging restrictions occur on intermediate-level species, they often result in a reduction of the consumptive effects they impose on the next lower trophic level (e.g., Schmitz et al. 1997, Turner 1997, Trussell et al. 2002). For example, in coral reef ecosystems top-predator fishes are known to restrict foraging of mid-size carnivores (e.g., coneys, *Cephalopholis fulva*; graysbys, *C. cruentata*; Stallings 2008) and mid-size grazers (e.g., blackbar damselfish, *Plectroglyphidodon dickii*; Madin et al. 2010b), thereby having positive indirect effects on the survival of juvenile fishes and density of algae, respectively.

In the present study, the behavioural suppression of the mesopredator by the top-predator modified its non-consumptive impact on the physiology of the bottom prey. Active and foraging mesopredators induced an increase in the routine metabolic rate of the bottom prey that was likely due to at least two main sources: a) increased locomotor activity while avoiding the mesopredator strikes; and b) an increased autonomic stress response. Similar predator-induced respiratory responses have been recorded for many vertebrates (Chabot et al. 1996, Ward et al. 1996, Holopainen et al. 1997, Hawkins et al. 2004). Animals have a finite-energy budget to distribute between self-maintenance and investment processes (Stearns 1992, Ricklefs and Wikelski 2002); therefore, increases in routine oxygen uptake may limit the allocation of energy to somatic growth, reproduction and storage (Houston et al. 1993, DuRant et al. 2007). Negative effects of predation risk could be further exacerbated by other primary and secondary physiological stress responses (i.e., increased cortisol, heart rate, ventilation), which are known to alter food assimilation, body condition and immunocompetence (Höjesjö et al. 1999, Pijanowska and Kloc

2004, Killen and Brown 2006, Killen et al. 2007, Sheriff et al. 2009). These effects may be especially problematic for early juvenile fishes which face a large number of predators (Caley 1993, Almany and Webster 2006, Hixon 2015) and are under pressure to a) rapidly grow and escape gape-limited predation (Bailey 1989, Holmes and McCormick 2010), b) increase the array of food items they can potentially utilize (Fuiman 1994) and c) improve their body condition to better cope with additional stressors (e.g., competition; Booth and Beretta 2004, Hoey and McCormick 2004). Frequent interruptions to routine foraging (behavioural restrictions), with accompanying increases in metabolic rate due to stress and activity, could have consequences for juvenile fitness and survival. Thus, we hypothesise that by minimising increases in prey metabolic rate caused by mesopredator attacks, top-predators could have positive indirect effects on the physiology and perhaps fitness of bottom prey.

Interestingly, the top-predator species was not the only treatment to alter the behaviour of the mesopredators and affect the metabolic rate of the prey. Results showed that mesopredators exposed to the invertivorous wrasse reduced their foraging activity, which lead to an intermediate level of oxygen uptake by bottom prey. We hypothesize, however, that the behavioural changes of the mesopredators were not triggered by an anti-predator response (as occurs in the presence of coral trout), but by their engagement in inspection behaviours towards the wrasse. Many vertebrates commonly inspect large, novel species (through tentative approaches and follow ups) to acquire extra information on the potential threat that they pose (e.g., FitzGibbon 1994, Fishman 1999, Walling et al. 2004). In this case, although the invertivorous wrasse did not represent a threat to the mesopredator, it seemed to play a key role distracting the mesopredator, limiting its attacks on the bottom prey and indirectly allowing prey to mount a lower physiological stress response. These findings are in line with previous studies, which suggest that large-sized individuals (regardless of their trophic status or diet) could hinder the foraging impact of intermediate-sized species on bottom prey (**Chapter 2**, Marsh-Hunkin et al. 2013). However, this hypothesis needs to be tested in future research to determine the extent in which inspection behaviours can divert the attention of mesopredator from activities such as foraging.

Our results show a potential mechanism through which top-predators, or even large-sized non-predatory individuals, can indirectly influence the physiology of bottom trophic level species. However, the nature and magnitude of the indirect effects reported should be considered in the context of the trials (e.g., procedure, experimental setup) and characteristics of the species employed. It must be taken into account that anti-predator behaviours and physiological responses can depend on the intrinsic phenotypic traits of the animal (e.g., size, body condition; Lönnstedt and McCormick 2011, Preisser and Orrock 2012, Wormington and Juliano 2014), the level of predation risk present in the sampled population (high vs low risk environments; Brown et al. 2005, Bell et al. 2010, Clinchy et al. 2011) and the duration of the predation risk (acute vs chronic;

Holopainen et al. 1997, Steiner and Van Buskirk 2009). Experimental manipulations are an useful initial step in understanding the non-consumptive links within trophic levels (Schmitz et al. 2004); however, future studies should address how these indirect effects may apply to more natural and complex scenarios. Field-based approaches will be indispensable to accurately extrapolate our results to coral reef systems where: a) food webs have multiple complex trophic levels, b) consumptive and non-consumptive interactions can occur simultaneously and c) the duration and strength of predation risk is highly variable. Though much evidence exists on the effects of predators on prey behaviour and physiology (single predator – prey interaction; reviewed by Hawlena and Schmitz 2010, Zanette et al. 2014), future research should build on results presented here to determine how these effects may be modified by multiple trophic levels of predator-prey interactions.

In summary, our study examined whether the non-consumptive effects of top-predators could cascade through the food web to impact the physiology of bottom prey. We found that acute predation risk from a top-predator reduced the predatory behaviour of the mesopredators and thereby minimised the impact of the mesopredator on the oxygen uptake, activity and physiological stress of the bottom prey. These results suggest that a release of mid-ranking piscivores, due to the overexploitation of large piscivores and the alteration of predation risk in marine food webs (Madin et al. 2016), could largely increase their non-consumptive impact on bottom prey. Increasing levels of predation risk from mesopredators are expected to reduce feeding opportunities, which along with high self-maintenance costs could impair the fitness, growth and survival of the recruiting fishes. Although logistically challenging, the incorporation of predator-induced plasticity into theoretical models (e.g., Abrams 1990, Bolker et al. 2003) and empirical research of predator–prey interactions (e.g., Schmitz et al. 1997, Heithaus et al. 2007) is critical for the realistic evaluation of the effects of predators on prey populations and community dynamics.

Chapter 5: Multiple predator effects on juvenile prey mortality

Submitted
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Multiple predator effects on juvenile prey mortality
Oecologia

5.1 Summary

Loss of top-predators often leads to mesopredator release in marine systems. Understanding the net effect that mesopredator release may have on lower level coral reef fishes is a key issue, but can be challenging to predict. Empirical evidence indicates that interactions among predators (e.g., interference, facilitation) can alter predation rates and modify any expected linear effects on prey survival. However, few studies explicitly examine the behavioural mechanisms that lead to these non-linear changes in predation. Using an additive-substitutive design, we experimentally manipulated reef piscivores (*Pseudochromis fuscus* [F], *Cephalopholis boenak* [B], *Epinephelus maculatus* [M]) to determine how behavioural interactions among predator pairs modify their combined impacts on juvenile fish prey. Predators seldom engaged in aggressive interactions (except for FF) and were, in contrast, often found to: (i) increase their foraging effort when placed in pairs (e.g., strikes in B almost doubled when in BB) and (ii) facilitate the strike success of their partner (e.g., F increased capture success of M when in FM). However, the identity of the predator species determined the strength of such interactions, and thus, the nature of MPEs (i.e., risk-enhancing effects: treatments BB, MM and FM; risk-reducing: BM; linear: FF, FB). Despite predators F and M appearing to be functionally redundant, they do not play identical functional roles as they produce vastly diverse effects when paired with additional predator species. In this way, knowledge of the identity of the predator species and the behavioural interactions among them is crucial to successfully predicting multiple predator effects (MPEs) in coral reefs. Research on the non-linear effects on prey mortality is key to understanding the potential effects of biodiversity loss on ecosystem function.

5.2 Introduction

Populations of apex predators have been decimated worldwide (Estes et al. 2011, Ripple et al. 2014). The resulting loss of lethal and/or risk effects from top-down predation has often led to a release of mid-ranking species (e.g., mesopredators), which drastically increase their impact on bottom prey populations (Prugh et al. 2009, Ritchie and Johnson 2009, Estes et al. 2011). Food web theory often assumes that ecologically similar species (e.g., mesopredators) can be treated collectively as a single functional unit (Fretwell 1987). However, due to differences in life history traits (e.g., hunting modes, habitat domain), predator species within a functional unit can exert different impacts on prey (e.g., Schmitz and Suttle 2001, Chalcraft and Resetarits 2003). Moreover, their combined effect cannot necessarily be predicted from the sum of their independent contributions (known as linear effects). Behavioural predator-predator interactions (e.g., facilitation, interference) can often increase (risk-enhancing effects) or decrease (risk-reducing effects) their collective impact on prey mortality (generally referred to as non-linear effects; Sih et al. 1998, Schmitz 2007). Given that most prey species are targeted by multiple mesopredator species, understanding the nature and magnitude of multiple predator effects (MPE) is a critical issue for community ecology (Sih et al. 1998).

Reef fish communities are ideal for examining MPEs. Mid-sized piscivores represent an abundant and diverse trophic guild which strongly limit the population size of fish prey (Hixon 1991, Stewart and Jones 2001, Almany and Webster 2006). Several studies have provided insights into the combined effects of multiple reef predators (Hixon and Carr 1997, Ford and Swearer 2012, Stier et al. 2013, Stier and White 2014). However, none have: (i) taken detailed behavioural observations of the predators involved to accurately interpret the MPEs; (ii) run in tandem additive and substitutive designs to distinguish the effects of predator density from that of predator richness; or (iii) tested for functional substitutability among predators. Given the ongoing exploitation of marine macro-carnivores (Jackson et al. 2001, Myers and Worm 2003) it is now imperative to examine the net effect that a release of meso-piscivores might have on ecosystem structure and function. Only with detailed observations and controlled manipulations of predator density and richness will we understand the complex behavioural pathways by which MPEs are generated (Griffen 2006, Byrnes and Stachowicz 2009, Soomdat et al. 2014).

In this study, we examined the combined effect that multiple benthic piscivores have on the survival of shared fish prey. Using three mesopredator fishes (active predator: *Pseudochromis fuscus*, ambush predators: *Cephalopholis boenak* and *Epinephelus maculatus*) and all the possible paired combinations, we explored how intra and interspecific behavioural interactions influence the nature and magnitude of MPEs. Specifically, we ran in tandem an additive (maintaining relative density but increasing total density) and substitutive (maintaining total density but increasing relative density) experimental design to fully understand the combined effects of the three mesopredators. While the additive design tests for non-linear effects due to interspecific interactions, the substitutive design (also known as a replacement series) tests for functional substitutability among predator species by comparing the effects of interspecific interactions against that of intraspecific interactions (Sih et al. 1998, Griffen 2006, Byrnes and Stachowicz 2009). Additionally, we explore how mesopredators with different functional traits (hunting mode: active vs. ambush predators) can influence the nature of MPEs (Sih et al. 1998, Sokol-Hessner and Schmitz 2002, Schmitz 2007).

5.3 Materials & Methods

We conducted an experiment to examine the interactions and foraging behaviour among three mesopredator species (*Pseudochromis fuscus*, F; *Cephalopholis boenak*, B; *Epinephelus maculatus*, M) and their combined effect on the mortality of a common fish prey (juvenile *Pomacentrus amboinensis*). Prey were exposed to nine predatory treatments that included each single mesopredator on its own (F, B, M) and all possible intraspecific (FF, BB, MM) and interspecific (FB, FM, BM) mesopredator pairs (Fig. 1). This experimental design included both additive and substitutive components to test for multiple predator effects (MPEs) on prey survival. Preliminary trials showed a non-predator control treatment was unnecessary as prey survival was 100 % in the absence of mesopredator(s). Each treatment included 10 – 12 replicates, with each individual fish being used in only a single trial.

Study species & fish handling

The mesopredators *P. fuscus, C. boenak* and *E. maculatus* were selected as focal study species because they co-occur at shallow patch reefs on the northern Great Barrier Reef and voraciously forage on small juvenile fish (Stewart and Jones 2001). At Lizard Island, the dottyback *P. fuscus* can be found at an average density of 2.3 ind/100 m^2 while the groupers *C. boenak* and *E. maculatus* occur at 1.9 and 0.03 ind/100 m^2 respectively (Stewart and Jones 2001). Higher densities are usually reported on shallow patch reefs of the lagoon (~2 ind/ m patch), where they tend to aggregate in response to the high availability of prey (Beukers and Jones 1998, Webster 2002). The dottyback, *P. fuscus* is a highly active and mobile species that stalk and chase prey using mainly pursuit techniques (Feeney et al. 2012). In contrast, grouper species, like *C. boenak* and *E. maculatus,* are considered ambush predators as they are cryptic, usually hiding in holes within the reef matrix and only striking at prey that is in their vicinity (Hobson 1979, Shpigel and Fishelson 1989). Both groupers can reach more than 20 cm SL, sit at high trophic levels, and represent a potential threat to dottybacks (Beukers-Stewart and Jones 2004, Beukers-Stewart et al. 2011), however during their juvenile life-stage (< 12 cm SL) they occupy the same mesopredator guild as dottybacks. Combinations of these mesopredator species (adult dottybacks + juvenile groupers) are commonly employed to study predation on juvenile coral reef fishes (Beukers and Jones 1998, Webster 2002, Webster and Almany 2002, Almany 2003).

Focal prey consisted of juveniles of the damselfish *P. amboinensis* (1.29 ± 0.003 cm, mean SL ± SE) which commonly settle to patch reefs and are frequently preyed upon by resident mesopredators (Webster 2002). All fishes were collected from Lizard Island (14°40′S, 145°28′E), northern Great Barrier Reef, Australia. Mesopredator fishes of similar size (adults of *P. fuscus*, 6.82 ± 0.07 cm; juveniles of *C. boenak*, 7.11 ± 0.12 cm; juveniles of *E. maculatus*, 6.69 ± 0.08 cm; mean TL ± SE) were captured using hand nets and an anaesthetic clove oil solution, while juvenile damselfishes were collected as they came into the reef at the end of their larval phase using light traps. Fishes were transported to Lizard Island Research Station within 1 h of capture, and maintained in individual aquaria with flow-through aerated seawater and ambient temperature and photoperiod. Mesopredators were fed squid daily, while damselfish were fed *Artemia* spp. twice daily. All fish were fed to satiation.

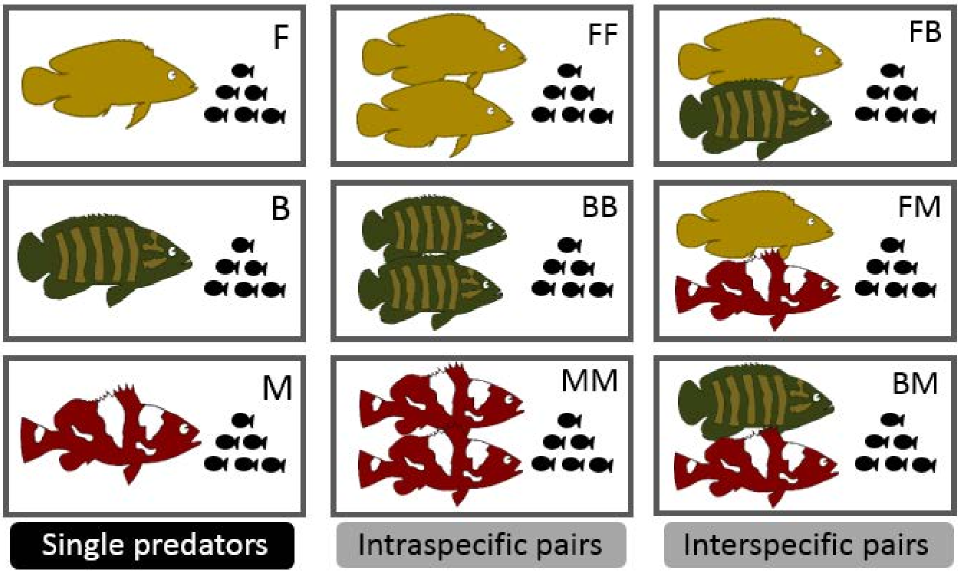

Figure 5.1. Experimental design including nine predatory treatments. Treatments comprise single mesopredators (*Pseudochromis fuscus*, F; *Cephalopholis boenak*, B; *Epinephelus maculatus,* M) foraging on their own and all possible intraspecific (FF, BB, MM) and interspecific pairs (FB, FM, BM). Predator(s) foraged on six juvenile damselfish prey (*Pomacentrus amboinensis*).

Experimental trials

Experimental trials were run in five 368-L grey pools. Each pool contained a 3 cm sand-layer as substrate, two large rubble heads to shelter predators (15 cm height x 20 cm diameter), a small piece of the live bushy coral *Pocillopora damicornis* to shelter prey (5 cm height x 10 cm diameter), rubble and 5 to 7 pieces of PVC tube (standardized by total volume) randomly scattered in the arena to increase habitat complexity. Mesopredator(s) were not fed for 24 h prior to being introduced into the pool at 17:00 – 18:00 h, and allowed to acclimate for 13 h. The following morning (7:00 – 8:00 h), six damselfish prey were introduced into each pool and protected for 10 min within a clear bucket (38 cm height x 24 cm diameter) while they acclimated to the experimental setup. After 10 min, the bucket was lifted, allowing the mesopredator(s) to access the prey for a total of 6 h (trial duration).

Preliminary assessments suggested that most of the predator-prey and predator-predator interactions occurred within the first hour of trial, as they tended to decrease over time due to predator satiation and/or prey depletion. Given that all trials were watched live by the same observer (to maintain consistency in the behavioural measures), mesopredator behaviuor was only recorded during the first hour of trial. Throughout this time, the observer (M.M.P.) recorded: (i) the number of successful and non-successful feeding strikes by each mesopredator, and (ii) the number of chases, bites and displays between mesopredators (as a measure of aggressive interactions). While mesopredator individuals were not tagged, the observer was able to keep separate the behaviour of each individual predator during monospecific trials due to natural markings on the individuals. Each hour the number of juvenile fish surviving was recorded.

Statistical analysis

Prey survival

Mean prey survival probabilities and 95 % confidence intervals (CI) were computed for each predatory treatment, by analysing the time-series of prey survival with a Cox regression model (Cox and Oakes 1984). This ecological modelling approach allows the analysis of prey survival rates from time-dependent data and is able to handle functional responses of any shape (Allison 1995, Moya-Laraño and Wise 2000). The assumption that hazards were proportional across all treatments was verified with a test on weighted residuals (Grambsch and Therneau 1994). Prey survivals calculated with the Cox regression model were subsequently compared to predicted values from a null multiplicative risk model (MRM) which assumes independent-linear effects among predators (Soluk and Collins 1988). Following Soomdat et al. (2014), we inferred a pair of predators had linear effects on prey survival if the expected survival (calculated with the MRM) fell inside the 95 % CI of the observed effects (calculated with the Cox regression). The MRM is the most common technique to analyse MPEs (e.g., Soluk and Collins 1988, Soluk 1993, Sih et al. 1998, Griffen 2006) however, given that it assumes constant per capita prey mortality through time (linear functional response) and/or no prey depletion, it can overestimate the occurrence of risk-enhancement effects in additive designs and risk-reduction effects in substitutive designs (see discussion by McCoy et al. 2012). To avoid any misinterpretation we analysed our results in the light of both designs the additive and substitutive design.

- ***Mesopredator density (additive model).*** We used the additive design and the probability of prey survival caused by each mesopredator species hunting independently (e.g., F, B, M), to examine whether increasing total mesopredator density (single predator vs. predator pairs) elicited non-linear effects. Analysis was made for both intraspecific and interspecific predator pairs. However, it must be noted that for interspecific pairs this model confounds the effects of increasing predator density to that of increasing predator richness. The expected survival for each of the intraspecific pairs ($E_{i,i}$; FF, BB, MM) was calculated as $E_{i,i} = P_i \times P_i$, where P_i is the probability of prey surviving when foraged on by single individuals of F, B or M. The expected survival for each interspecific pair ($E_{i,i}$; FB, FM, BM) was estimated as $E_{i,j} = P_i \times P_j$, where P_i and P_j is the probability of prey surviving when foraged on by single individuals of F, B or M.

- ***Mesopredator diversity (substitutive model).*** To examine the effects of mesopredator diversity (increase in richness) we employed the substitutive design. This approach experimentally controlled for total predator density, while comparing the effects of interspecific pairs to those observed on intraspecific pairs. The expected survival for interspecific pairs was calculated as $E_{i,j} = (P_{i,i} \times P_{j,j})^{0.5}$, where $P_{i,i}$ and $P_{j,j}$ is the probability of prey surviving when foraged on by pairs of intraspecific mesopredators (FF, BB, MM). Following Sih et al. (1998), species were considered 'substitutable' if the effect of an interspecific pair on prey survival (e.g., BM) was inherently similar to the effects caused by each predator species in an intraspecific pair (e.g., BB and MM).

Mesopredator behaviour

To determine whether intra- and inter-specific interactions affect the predatory behaviour of each mesopredator species, we used one-way analyses of variance (ANOVA) to compare the number of total strikes and successful strikes among four treatments. For each mesopredator species, the treatments included: (1) the mesopredator alone (i.e., i); (2) the mesopredator in an intraspecific pair (i.e., ii); and (3, 4) the mesopredator in the two different interspecific pairs (i.e., ij and ik). All significant differences detected in the ANOVAs were subsequently explored using post-hoc Tukey's HSD for unequal N. We further compared the total strikes and successful strikes between the dominant and subordinate individuals of each intraspecific pair (FF, BB, MM), using independent *t*-tests with Bonferroni corrections. The dominant fish was considered the one that initiated most of the aggressive interactions and sheltered closest to the location of the prey. This approach was only used for intraspecific pairs as the contrasting behaviour of interspecific pairs hindered a correct distinction between dominant and subordinate fish (e.g., F is usually more active and aggressive than B or M, despite being the subordinate fish). Residual analyses were used to examine if the data satisfied the assumptions of normality and homoscedasticity. Behavioural data (strikes and successful strikes) were square root transformed to meet the assumptions of parametric tests. The number of aggressive interactions was analysed among treatments using a non-parametric Kruskal-Wallis one-way ANOVA given the heteroscedasticity of the data. All statistical analyses were performed in R version 3.2.2 (R Core Team 2015) using the packages survival (Therneau and Grambsch 2000, Therneau 2015) and rms (Harrell 2015).

5.4 Results

Acclimation to experimental arenas

All mesopredators acclimated well. The morning of the trial, mesopredators were seen regularly swimming through the PVC pipes and making use of all the experimental arena and shelter provided. In the 10 min before the beginning of the trial (once prey was introduced into the clear bucket), mesopredators had already approached prey or even start striking at them (through the clear bucket). Once the trial started all mesopredators foraged as expected according to their hunting mode. The pursuit predator (*P. fuscus*, F) was constantly swimming actively and chasing the prey around the arena, while the ambush predators (*C. boenak,* B and *E. maculatus,* M) remained inside the shelter occasionally coming out to attack the prey. When in pairs, mesopredators displayed distinctive behaviours: either directly interacting (e.g., through displays and chases) or deliberately avoiding the location of the other mesopredator.

Prey survival

- ***Mesopredator density (additive model).*** The effect of mesopredator density on prey survival differed according to the identity of mesopredators paired (Fig. 5.2; blue lines). For intraspecific mesopredator pairs, prey survival either matched (for FF; *P. fuscus* + *P. fuscus*) or was lower (for ambush predators; *C. boanak* + *C. boenak*, BB; and *E. maculatus* + *E. maculatus,* MM) than expected from the individual contribution of the predators involved. In contrast, each of the three interspecific predator pairs exerted a different effect on prey mortality; the pair FB (*P. fuscus* + *C. boenak*) achieved the expected capture success, FM (*P. fuscus* + *E. maculatus*) caused lower prey survival, and pair BM (*C. boenak* + *E. maculatus*) caused greater prey survival than expected.

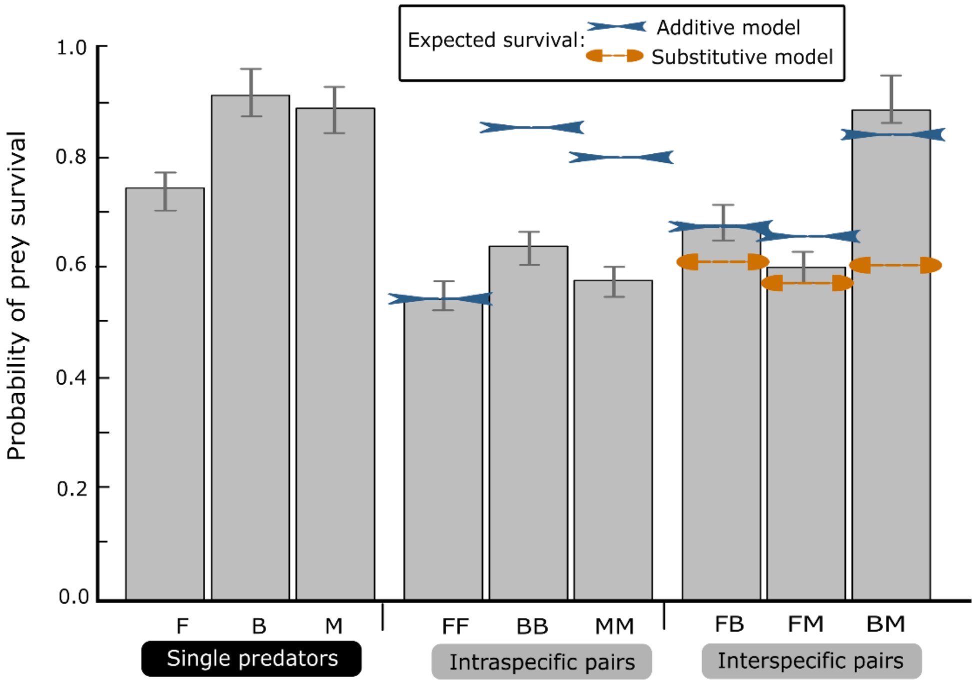

Figure 5.2. Probability of prey survival (mean ± 95 % confidence intervals) for each predatory treatment. Treatments included single mesopredators (*Pseudochromis fuscus,* F; *Cephalopholis boenak,* B; *Epinephelus maculatus,* M) foraging each on their own and all possible intraspecific (FF, BB, MM) and interspecific pairs (FB, FM, BM). Horizontal lines indicate the expected level of prey survival according to the additive model (blue) or the substitutive model (orange).

- ***Mesopredator diversity (substitutive model).*** Interspecific pairs of mesopredators killed less prey than intraspecific pairs (Fig. 5.2; for each interspecific pair, the mean survival is higher than the orange line predicted from the intraspecific pairs). Prey foraged on by FB (*P. fuscus* + *C. boenak*) or BM (*C. boenak* + *E. maculatus*) had higher survival probabilities than if foraged on by the same mesopredator species but on intraspecific pairs. The interspecific pair FM (*P. fuscus* + *E. maculatus*), was the only case of mixed species where the prey survival observed matched that predicted from the intraspecific pairs FF (*P. fuscus* + *P. fuscus*) and MM (*E. maculatus* + *E. maculatus*).

Mesopredator behaviour

Mesopredator *P. fuscus* (F) undertook approximately 6 times more strikes than *C. boenak* (B) or *E. maculatus* (M), however, the capture success rate of the three predators was of a comparable magnitude (Fig. 5.3). The predatory behaviour of F, B and M was differentially affected by the presence of intraspecific and interspecific species (Fig. 5.3). Predation by the pursuit mesopredator (*P. fuscus,* F) remained relatively constant independently of the presence of additional predators of the same or different species (Fig. 5.3 *i, iv*; Table 5.1; P > 0.05). In contrast, the strikes and success of ambush mesopredators (*C. boenak, B* and *E. maculatus,* M) increased significantly when paired with the pursuit species (*C. boenak* + *P. fuscus*, BF in Fig. 5.3 *ii* and *E. maculatus* + *P. fuscus,* MF in Fig. 5.3 *iii*, *vi*; Table 5.1) and improved marginally when foraging in intraspecific pairs (*C. boenak* + *C. boenak*, BB in Fig. 5.3 *ii* and *E. maculatus* + *E. maculatus,* MM in Fig. 5.3 *iii*, *vi*; Table 5.1). The only exception to this was the capture success of *C. boenak* (B), which remained relatively constant across all treatments (Fig. 5.3 *v*; Table 5.1). Interestingly, however, if both ambush species were placed together in an interspecific pair their predatory behaviour (strikes and success) was similar to when each one foraged alone (B and BM in Fig. 5.3 *ii, v*; M and MB in Fig. 5.3 *iii*, *vi*; Table 5.1).

Table 5.1. Parameter estimates of the ANOVAs examining the strikes and success of three mesopredator species *Pseudochromis fuscus* (F), *Cephalopholis boenak* (B), or *Epinephelus maculatus* (M) foraging in intraspecific and interspecific pairs. Asterisks indicate significant differences where * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.

Meso-predator	**Effect**	**Strikes**					**Successful strikes**				
		SS	**DF**	**MS**	**F**	**P**	**SS**	**DF**	**MS**	**F**	**P**
F	Treat	6.6	3.0	2.2	0.5	0.7	0.6	3.0	0.2	0.6	0.6
	Error	277.3	58.0	4.8			19.2	58.0	0.3		
B	Treat	23.8	3.0	7.9	11.0	***	1.3	3.0	0.4	1.5	0.2
	Error	31.9	44.0	0.7			12.4	44.0	0.3		
M	Treat	35.8	3.0	11.9	7.4	***	4.0	3.0	1.3	5.1	**
	Error	74.0	46.0	1.6			12.1	46.0	0.3		

Table 5.2. Parameter estimates of the t-tests examining the strikes and success between the dominant (dom) and subordinate (sub) individuals from intraspecific mesopredator pairs. Mesopredator species included *Pseudochromis fuscus* (F), *Cephalopholis boenak* (B), or *Epinephelus maculatus* (M).

Pairwise t - test			**Strikes**		**Successful strikes**	
			t	**P**	**t**	**P**
F (dom)	**vs.**	**F (sub)**	-3.20	0.003	-2.89	0.007
B (dom)	**vs.**	**B (sub)**	-0.15	0.87	0.23	0.82
M (dom)	**vs.**	**M (sub)**	-2.47	0.023	-2.08	0.052

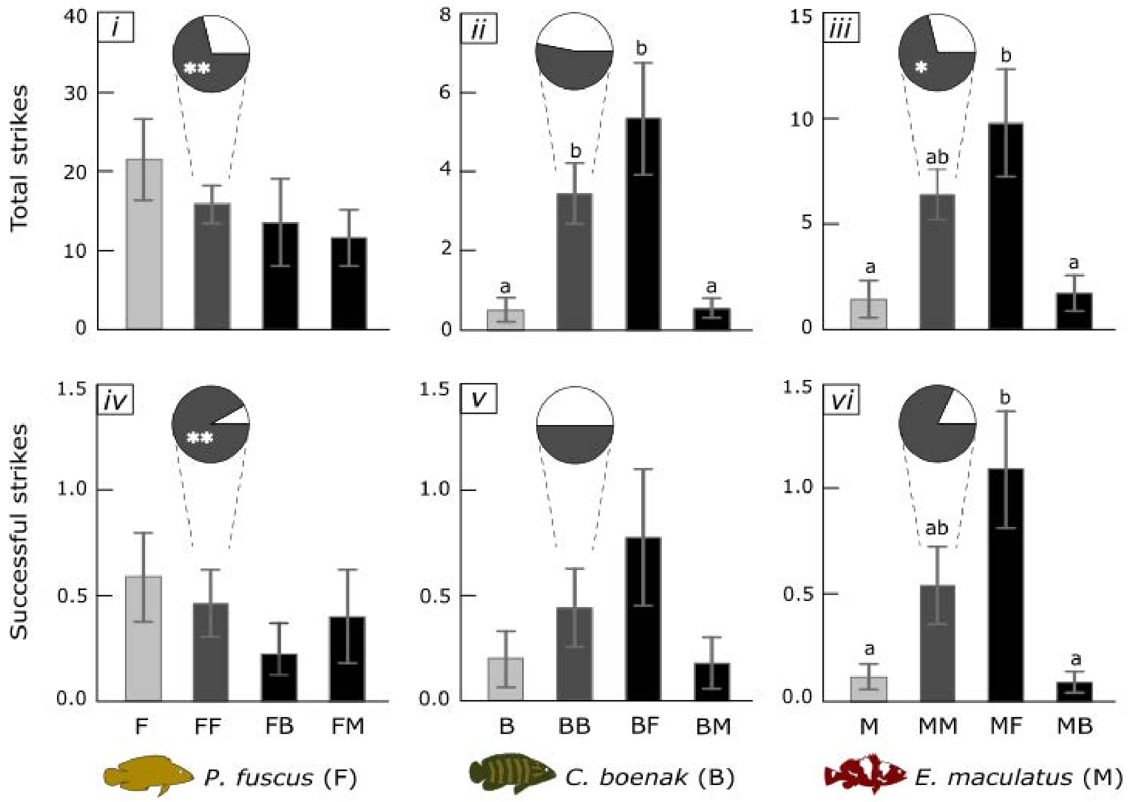

Figure 5.3. Number of strikes (mean ± SE, i, ii, iii) and successful strikes (iv, v, vi) for each mesopredator species *Pseudochromis fuscus* (i, iv), *Cephalopholis boenak* (ii, v), or *Epinephelus maculatus* (iii, vi) when foraging alone (light grey bars), on intraspecific pairs (dark grey bars) or on interspecific pairs (black bars). Significant differences among the treatments (ANOVAs) were detected on panels ii, iii, and vi. Bars with the same lowercase letter correspond to the same homogenous group (Tukey's HSD; $P < 0.05$). Pie charts indicate the proportion of strikes (i, ii, iii) or successful strikes (iv, v, vi) that the dominant (grey) and subordinate (white) individuals of each intraspecific pair contributed to the total. Asterisks inside the pie represent significant differences according to the t-tests (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Depending on the mesopredator species, dominant and subordinate individuals of intraspecific pairs (i.e., FF, BB, MM) had different contributions to the total strikes and success achieved by the pair. For example, in intraspecific pairs of FF (*P. fuscus* + *P. fuscus*) and MM (*E. maculatus* + *E. maculatus*), the dominant individual of the pair exerted most of the strikes and had the most success (pie charts in Fig. 5.3 *i, iii, iv, vi*; Table 5.2; t-tests $P < 0.05$). In contrast, both individuals (dominant and subordinate) of BB (*C. boenak* + *C. boenak*) had very similar contributions to prey mortality (pie charts in Fig. 5.3 *ii, v*; t-tests $P > 0.05$). Among the six pairs of mesopredators tested, aggressive interactions (display, chases, bites) were relatively low except for the intraspecific pair of pursuit mesopredators (FF) which had insignificantly higher aggressive interactions than the rest (Fig. 5.4; Kruskal- Wallis; $H = 27.00$, $P < 0.001$).

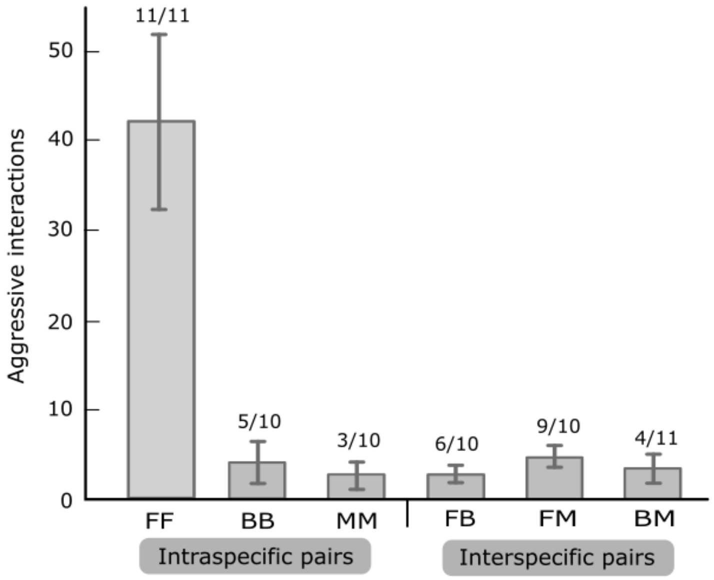

Figure 5.4. Number of aggressive interactions (mean ± SE) including chases, bites and displays recorded for intraspecific and interspecific mesopredator pairs. Mesopredators included *Pseudochromis fuscus* (F), *Cephalopholis boenak* (B), and *Epinephelus maculatus* (M). Numbers over the bars indicate the number of trials in which aggressive interactions occurred.

5.5 Discussion

This study examined the behavioural interactions among three mesopredator species (active predator: *Pseudochromis fuscus,* F; ambush predators: *Cephalopholis boenak*, B; *Epinephelus maculatus*, M) and their combined lethal effects on six fish prey. We found that although behavioural patterns within mesopredator pairs matched those predicted from their hunting mode, the identity of the mesopredator species determined the strength of any positive or negative interactions, and thus, the nature and magnitude of MPEs on prey survival (i.e., risk-enhancing effects: treatments BB, MM and FM; risk-reducing: BM; and linear effects: FF, FB). Given the context-dependency of the predator-predator interactions, none of the mesopredators tested are expected to play identical functional roles on natural coral reefs. Data suggests that it will be difficult to predict the effects of any disturbances to the abundance and richness of the mesopredator assemblage (e.g., mesopredator invasions and extirpations, top-predator loss and mesopredator release) on assemblages of newly-settled fishes.

Our study demonstrates that the behavioural patterns among mesopredators can be predicted from knowledge of their hunting modes (Sih et al. 1998, Schmitz 2007). However, the correct interpretation of MPEs requires a detailed understanding of the behavioural interactions among predators. For example, dottybacks (*P. fuscus,* F) are very active, mobile and territorial predators (Feeney et al. 2012), so it was not surprising to observe frequent and intense aggressive interactions (i.e., chases, bites, displays) within a FF pair. Although such aggressiveness should lead to reduced foraging and lower prey mortality (risk-reducing effects), we instead found linear effects (i.e., typical of non-interacting individuals). Exhaustive behavioural observations on each individual indicated that while the subordinate dottyback within the pair reduced its foraging activity, the dominant significantly increased its foraging effort and strike success. Therefore, although the subordinate dottyback barely hunted, the expected overall prey mortality was met because the dominant dottyback drastically enhanced its foraging effort.

An equivalent case of misleading behavioural patterns was shown with the pairs of groupers (e.g., BB, MM, BM). As predicted from their sedentary behaviour and ambush techniques (Hobson 1979, Shpigel and Fishelson 1989), groupers seldom interacted and spent most of the time sheltering. This lack of positive or negative interactions should have led to linear effects on prey survival, however, we found both risk-enhancing (intraspecific pairs: BB, MM) and risk-reducing effects (interspecific pair: BM). We hypothesize that groupers passively encouraged or deterred each other's foraging behaviour depending on whether they were paired with a conspecific or a heterospecific. Given that most *Cephalopholis* and *Epinephelus* species live in small haremic groups (Shapiro et al. 1994, Liu and Sadovy 2005), it is not surprising that the presence of a conspecific had indirect positive effects on their behaviour. In contrast, the risk-reducing effects detected for the *C. boenak-E. maculatus* pairs are unexpected as we are not aware of antagonistic interactions occurring among interspecific groupers. In fact, Ormond (1980) describes that reef based species such as *Cephalopholis argus*, *Aethaloperca rogaa* and *Plectropomus maculatus* may briefly hunt in what appear to be cooperative interspecific pairs.

Interestingly, even when behavioural patterns are consistent with predator hunting mode and MPE theory, the specific identity of the predators determined the strength of the behavioural interactions, and thus, the nature of MPEs. Reef predators with different hunting modes (active dottyback + ambush grouper: treatments FB and FM) lead to enhanced capture success of groupers through two mechanisms: (i) prey escaping the chases of the dottyback were more likely to approach the fixed position of the ambush grouper, thus increasing the grouper's encounter and strike rates; and (ii) due to the distraction of the prey by the most imminent threat (active dottyback), strikes by the grouper were more likely to succeed. Although these behavioural patterns were evident for both FB and FM, only the pair FM caused higher prey mortalities than expected (risk-enhancing effects). Field experiments on pelagic and benthic piscivore guilds have also found that interspecific synergies not always lead to risk-enhancing effects (Hixon and Carr 1997, Ford and Swearer 2012). Here, it seems that the identity of the grouper species determined the efficiency of the synergy and whether the MPE would occur or not.

Ecologically similar species (i.e., mesopredators) are often expected to have analogous and substitutable effects on ecosystem function (e.g., Sokol-Hessner and Schmitz 2002, Hooper et al. 2005). Our substitutive model suggested that the dottyback *P. fuscus* (F) and the grouper *E. maculatus* (M) are functionally substitutable species, given that the mortality caused by their interspecific pair (FM) was inherently similar to the average mortality caused by each species within an intraspecific pair (FM = FF/2 x MM/2). Despite being functionally similar species, we do not consider mesopredators F and M to be functionally identical because the pairing of each of these species with the grouper *C. boenak* (B) produces contrasting effects. While F paired with B caused linear effects on prey mortality, M paired with B led to risk-reducing effects. Our work suggests that even when two mesopredator species have substitutable effects on prey mortality, caution must be taken when assigning them into functional groups as each one can have vastly diverse effects when interacting with additional mesopredator species. Although simplification is fundamental when conceptualizing community structure and dynamics (Levin 1992), functional redundancy seems to be rare in many complex ecosystems (Chalcraft and Resetarits 2003).

Results from our system suggest that the composition of the mesopredator guild (i.e., which species are present and who they interact with) determines the magnitude of predation on newly-settled fishes. This is relevant to many coral reef studies that have examined predation on artificial patch reefs using a subsample of mesopredator species and non-consistent relative densities (Webster 2002, Webster and Almany 2002, Almany 2003). It is likely that vastly different predation rates could be obtained depending on the identity of the mesopredators employed and the ratio among them (e.g., 1:1 vs. 3:1). From a conservational perspective, our results suggest that any changes in the richness and density of mesopredators, through extirpations (e.g., the grouper *Hypoplectrus castroaguirrei*; Anderson 2015), invasions (e.g., the lionfish *Pterois volitans*; Schofield 2009), or lack of top-down control (e.g., mesopredator release of the coney *Cephalopholis fulvus*; Mumby et al. 2012), are likely to alter the dynamics of predator-predator interactions, and thus the MPEs on newly-settled fishes. For instance, the widespread depletion of marine top-predators (Jackson et al. 2001, Myers and Worm 2003) is expected to trigger trophic cascades, whose outcome and magnitude might depend on the behavioural interactions that arise among mesopredator species. If positive mesopredator interactions are favoured (e.g., synergies, facilitations) the recruiting population of reef fish is likely to experience a dramatic increase of lethal and sub-lethal effects (e.g., enhanced mortality, reduced foraging, high metabolism). In contrast, if negative interactions are favoured (e.g., interference, intraguild predation) trophic cascades might be attenuated. Given the population bottleneck that small reef predators already exert on newly-settled fishes (Almany and Webster 2006), further research should explore how changes to the community composition of marine piscivores could favour one type of interactions or the other. Processes that interfere with survival of fish at crucial life-history transitions may have serious implications for the replenishment and persistence of reef fish communities.

Experimental manipulations are a useful tool to investigate MPEs as they allow detailed behavioural observations and strict control over mesopredator density and diversity (Sih et al. 1998, Schmitz 2007). It is critical, nevertheless, to test how the predator-predator interactions described here scale up in natural coral reefs where the assemblage of reef mesopredators is highly diverse (Hixon 2015), and the piscivore behaviour varies across space, time and ontogeny (Kingsford 1992, St John 1999, Kulbicki et al. 2005, Ashworth et al. 2014). While predator-predator interactions may be favoured during the summer months when many generalist predators target newly-settled fish (Beukers-Stewart and Jones 2004, Feeney et al. 2012), they may be dampened on a daily basis by the opposing diel patterns of many predator species (e.g., dottybacks and wrasses mainly forage at midday, while groupers mostly hunt at dusk and dawn; Feeney et al. 2012, Holmes et al. 2012, Bosiger and McCormick 2014). Furthermore, our results should be tested under natural prey assemblages. The community of newly-settled fish prey is composed of a number of interacting species with different density and behaviour (Webster 2002, Almany and Webster 2006, Heinlein et al. 2010), however most coral reef studies employ a single prey species (e.g., *Pomacentrus amboinensis*, this study; *Chromis cyanea*, Hixon and Carr 1997; *Thalassoma bifasciatum*, Stier and White 2014). Without the use of an ecologically relevant diversity of prey many important aspects of predator-prey interactions may be overlooked (e.g., resource complementary among predator species; Snyder et al. 2006, Griffin et al. 2008).

Mesopredators constitute an important functional group in most food webs. Their high abundances, voracity and activity levels allows them to greatly impact low trophic levels (reviewed by Prugh et al. 2009, Ritchie and Johnson 2009). Knowledge of the identity of the mesopredator species involved, and the behavioural interactions among them, is crucial to realistically predicting MPEs in biodiverse communities such as coral reefs (Soluk 1993, Sih et al. 1998, Chalcraft and Resetarits 2003, Schmitz 2009). While obtaining detailed information on the biology, behaviour, distribution and population dynamics of predatory species may be difficult in many systems, this information will greatly contribute to predictive models that refine the mechanistic understanding of how mesopredator communities might operate under the anthropogenic depletion of top-predators.

Chapter 6: General Discussion

6.1 Key findings & Implications

Behavioural interactions play a critical role in the functioning of complex food webs (Abrams 1995). Hence, their investigation can greatly advance our understanding of natural ecosystems and our ability to protect them (Dill et al. 2003, Schmitz et al. 2004, Preisser et al. 2005). While numerous studies have explored the lethal effects of predator-prey interactions (reviewed by Pinnegar et al. 2000, Baum and Worm 2009), the risk effects that predators exert are often overlooked (Werner and Peacor 2003, Creel and Christianson 2008, Peckarsky et al. 2008). An understanding of these risk effects and behavioural interactions is integral to predicting how populations may respond to perturbations (e.g., loss of top-predators, mesopredator invasions), and thus to improve wildlife conservation efforts (Heithaus et al. 2008, Madin et al. 2016).

The overarching aim of this thesis was to establish the behavioural links that mediate mesopredator behaviour. This was achieved through four related studies that revealed new insights concerning how mesopredators exert lethal and sub-lethal on bottom level prey. Building on the growing body of literature on risk effects (reviewed by Werner and Peacor 2003, Preisser et al. 2005), the first chapters revealed that independent sensory cues from top-predators were enough to limit mesopredator activity and foraging behaviour (**Chapter 2**) while indirectly favouring bottom level prey survival, behaviour, and physiology (**Chapters 3** and **4**). Using additive and substitutive designs (Sih et al. 1998, Griffen 2006), **Chapter 5** explored multiple predator effects (MPEs) by manipulating mesopredator density and diversity. This study illustrated how behavioural interactions within the mesopredator guild also influence mesopredator foraging behaviour and usually lead to enhanced mortality of their prey.

6.1.1 Controlling mesopredators through Risk effects

To study the behavioural responses of mesopredators to top-predators we investigated behavioural trophic cascades. These occur when risk effects originated by top-predators are transmitted through the food web due to changes in species' phenotypic responses (Abrams 1995, Werner and Peacor 2003). Most of the existing research has focused exclusively on the phenotypic plasticity of mid-ranking species (i.e., mesopredator); thus, limiting our understanding of how risk effects propagate to the rest of the food web and impact bottom level prey.

By examining this process via experimental manipulations, this thesis is one of the first studies to describe a behavioural trophic cascade with two successive trait-mediated links. In the first trophic link predation risk from top-predators restrained mesopredator foraging behaviour. In line with optimal foraging theory (Stephens and Krebs 1986, Brown et al. 1999), the risk allocation hypothesis (Lima and Bednekoff 1999, Sih et al. 2000), and previous experimental evidence (reviewed by Lima and Dill 1990, Brown and Kotler 2004, Verdolin 2006) **Chapter 2** described an antipredator response in which mesopredators allocated most of their time to predator vigilance/avoidance while sacrificing their overall activity and foraging behaviour.

In the following downward trophic link I explored how the behavioural plasticity of mesopredators could propagate to impact several phenotypic traits of bottom level prey **(Chapter 3, 4).** Despite the vast amount of literature on the sub-lethal impacts of predator-prey interactions and the myriad of responses of prey to predators (behavioural, morphological and physiological responses; reviewed by Lima 1998b, Hawlena and Schmitz 2010), little research has examined how phenotypic changes in the mesopredators themselves impact trait plasticity of complex bottom level prey (Werner and Peacor 2003). Novel results from this study illustrated how top-predators, through the behavioural suppression of mesopredators, can both enhance the behaviour (i.e., increase foraging activity and habitat use) and reduce the physiological stress (i.e., diminished routine metabolic rate) of bottom trophic level species. These findings provide clear evidence of the importance of trait plasticity and the extent of risk effects in complex ecological communities. To further advance our understanding of behavioural trophic cascades studies should explore how population and community dynamics of bottom prey could be influenced by these indirect effects on their phenotypic traits.

6.1.2 Controlling mesopredators through intraguild interactions

A rich history of experiments has described predator-prey interactions using a single species of predator. However, most studies have largely overlooked the fact that prey are targeted simultaneously by an array of different predator species with different hunting modes, size, or habitat domains (reviewed by Sih et al. 1998, Schmitz 2007, Griffin et al. 2013). Through a study of MPEs (**Chapter 5**) I examined whether behavioural interactions among mesopredators altered their foraging activity, and hence their combined lethal impact on prey. In five out of the six mesopredator pairs examined, behavioural interactions between predators modified their predicted impacts on prey, causing either risk-reducing or risk-enhancing effects. Such results highlighted the important role of intraguild interactions and their potential to modify the magnitude of trophic cascades.

Predicting the nature and magnitude of multiple predator effects is one of the key issues in community ecology. Knowledge of the predator hunting mode and habitat domain can often allow us to predict the combined effects of multiple predators (Schmitz 2007). However, as demonstrated in **Chapter 5** the nature of MPEs (i.e., risk enhancing vs. risk-reducing) depends on the identity of the predator species involved (Soluk 1993, Chalcraft and Resetarits 2003, Schmitz 2009). Relative predator density also plays an important role as it determines the ratio of conspecific and heterospecific interactions. In alignment with several studies on intertidal and terrestrial invertebrates, we found that increasing predator diversity weakened the suppression of prey (Snyder and Ives 2001, Finke and Denno 2004, Siddon and Witman 2004, Vance-Chalcraft et al. 2004, Finke and Denno 2005). However, in contrast to these studies, our results were not caused by interspecific interference among predator species, but by strong facilitation occurring only within the conspecific predator pairs. Given that conspecific competition is known to drastically increase with predator density (Griffen and Williamson 2008, Stier and White 2014), further research needs to address whether such positive interactions remain under natural predator densities.

The variety of behavioural interactions that can modify the impact of mesopredators on prey has important implications for food web ecology and trophic cascades. Loss of top-predators can often have more significant impacts than expected, as interactions that arise within the mesopredator assemblage under a relaxed threat of predation mainly seem to favour risk-enhancing effects (e.g., enhanced foraging activity, social facilitation; **Chapter 5**). When predicting the consequences for trophic cascades, it is critical to account not only for the vertical chain of trophic interactions (top-predators> mesopredator> prey), but also the horizontal links within each trophic guild (e.g., mesopredator – mesopredator interactions; Duffy et al. 2007). As demonstrated by this thesis, these latter links, most often forgotten, have the potential to change the impact on organisms within the bottom trophic levels, and hence the magnitude of trophic cascades.

6.1.3 Trophic cascades in coral reefs

Mid-ranking predatory fishes such as groupers, wrasses and dottybacks (Serranidae, Labridae, Pseudochromidae) exert strong lethal and sub-lethal effects on juvenile fish populations (e.g., Beukers-Stewart and Jones 2004, Holmes and McCormick 2011, Feeney et al. 2012, Holmes et al. 2012, Lönnstedt et al. 2013b). As changes in the juvenile fish community can strongly influence the composition of adult populations, it is important to be able to predict the consequences of mesopredator release for juvenile coral reef fishes. Increased knowledge of the behavioural links of mid-ranking piscivores (**Chapters 2, 3, 4, 5**) greatly advances our understanding of how the widespread loss of large marine piscivores will impact reef fish food webs.

First, the lack of lethal effects from large piscivores can trigger an increase in the abundance of smaller-bodied, intermediate piscivores (Chiappone et al. 2000, Graham et al. 2003, Mumby et al. 2012). As a result, the encounter rates and behavioural interactions among mesopredators are likely to increase due to the finite number of prey (juvenile fish) and habitat resources available. As evidenced in **Chapter 5**, the combined impact of mesopredators on prey (e.g., risk-enhancing, risk-reducing, or linear) will largely depend on the specific identity and relative abundance of the species within the mesopredator assemblage. Although high predator densities might favour interference interactions and risk-reducing effects (Stier and White 2014), most field observations and MPE research indicate that linear or risk-enhancing effects could be a common outcome (Hixon and Carr 1997, Lukoschek and McCormick 2002, Ford and Swearer 2012, Stallings and Dingeldein 2012, Stier et al. 2013). Interestingly, data from **Chapter 5** also suggested that changes in the mesopredator assemblage such as the loss of mesopredator diversity (e.g., reductions in richness due extinction or invasion of certain species) can modify the ratio of interspecific to intraspecific interactions and consequently the overall impact on juvenile prey.

The loss of large piscivores can additionally allow a behavioural release of small-sized piscivores (Stallings 2008). According to **Chapters 2** and **3**, lack of predation risk triggers 70 % increases in mesopredator foraging activity and approximately 67 % higher mortality in juvenile fish. Given that small piscivores already reduce > 50 % of the population of juvenile fishes within the first 48 h on coral reefs (Caley 1998, Almany and Webster 2006), any increases in prey mortality would further strengthen the mortality bottleneck on recruiting populations. Another noteworthy point is that those juvenile fish that survive the lethal impacts of mesopredators are still subject to strong impacts on their behaviour and physiology (**Chapters 3, 4**). Risk- induced reductions in the feeding rates and activity of juvenile fish can limit their acquisition of rich-energetic resources, and therefore negatively impact the fish's energy income, body condition and growth (Connell 1998, Steele and Forrester 2002, Abdulla 2004). The concurrent presence of physiological stress responses can further heighten these impacts. Neuroendocrine processes underlying physiological stress involve the allocation of energy towards emergency functions (e.g., increased cardiovascular activity and respiration), which despite enhancing the immediate chances of surviving, limit the resources available for maintenance processes such as growth, development, food assimilation, reproduction and immune function (reviewed by Hawlena and Schmitz 2010, Zanette et al. 2014). It is likely that the enhanced predation risk from behaviourally released mesopredators could impair juvenile prey fitness, increase their susceptibility to biotic and abiotic stressors, and lead to even higher levels of mortality (e.g., Booth and Beretta 2004, Hoey and McCormick 2004).

Behavioural trophic cascades require more attention from coral reefs scientists. In coral reefs, the loss of large piscivores rarely causes a numerical increase of fish abundance that negatively impacts bottom trophic levels (e.g., Russ and Alcala 1998, Mumby et al. 2006, Sandin et al. 2008). Successive lethal links on fish food webs are often overshadowed by: (i) the lack of baseline data prior to top-predator declines, (ii) the importance of bottom-up processes (productivity), and (iii) the prevalence of omnivory, opportunism, dietary breadth and ontogenetic diet shifts among marine fishes (reviewed by Baum and Worm 2009). In contrast, as shown throughout this thesis, the behavioural release of mid-ranking predatory fish can greatly impact the survival and phenotypic traits of newly-settled fishes (Stallings 2008, **Chapters 3** and **4**). These results, paired with predation risk research on other trophic groups (e.g., herbivores; Madin et al. 2010a, Madin et al. 2010b, McCauley et al. 2010, Rizzari et al. 2014, Catano et al. 2016, Kindinger and Albins 2017), provide compelling evidence of the importance of risk effects in marine ecosystems and the need to further advance our understanding of behavioural trophic cascades on coral reefs (reviewed by Dill et al. 2003, Heithaus et al. 2012, Madin et al. 2016).

6.2 Future directions

To further advance the understanding of the relative importance of behavioural links in food webs and address some of the caveats of the current research, future research should address the following aims:

- **Relevance to larger temporal and spatial scales.** Data for this thesis was obtained through short term experimental manipulations in mesocosm (**Chapters 2** and **5**), laboratory (**Chapter 4**) and patch reef set-ups (**Chapter 3**). Although this research provided details on the mechanisms that control mesopredator behaviour that are impossible to obtain otherwise, it is important to also test how the nature and magnitude of our effects hold up at longer term and larger spatial scales. Some evidence suggests that animal phenotypic responses can be potentially biased during experimental manipulations due to stress from captivity (Morgan and Tromborg 2007) and the context of the trial (Schmitz 2007).

  Effective no-take marine reserves are a useful approach to scale up the spatial context. Several studies have already employed these natural experiments to study patterns of mid-ranking fishes along gradients of human pressure or top-predator removal (e.g., Graham et al. 2003, Mumby et al. 2006, Sandin et al. 2008, Madin et al. 2010a, Boaden and Kingsford 2015). To scale up time frames, studies of numerical trophic cascades can employ long term data sets of fish abundance and biomass (Russ and Alcala 1998, Mumby et al. 2012, Ruppert et al. 2013a). Unfortunately, most monitoring programs do not record any behavioural measures of the fishes surveyed. Without this information it is complicated to examine whether changes in predator abundances or environmental conditions are already altering the space use and activity of species. Would it be possible to include some key, but simple, behavioural assays during monitoring surveys? At population level, behavioural measures such as feeding rate, number of aggressive interactions, home range size, and activity budget could be recorded for certain fishes (e.g., species of ecological importance or high abundance) during established focal sampling periods. Additionally, long term repetitive measures on marked individuals could provide useful information on the plasticity of behaviour between life-history stages, and within social structure (reviewed by Clutton-Brock and Sheldon 2010). Regardless of the logistical constrains, the information generated would greatly improve our understanding of the long term behavioural links in marine systems.

- **Strengthening trait-based research.** In contrast to the behavioural and trait-mediated approach of this thesis, most studies explore only the lethal links and density dependent interactions in food webs (reviewed by Peckarsky et al. 2008). This could be related to fact that: (i) trophic cascades were often defined as density-mediated indirect interactions (Oksanen et al. 1981, Pace et al. 1999, Polis et al. 2000), (ii) basic ecological models such as Lotka-Volterra equations are governed by changes on species densities (Volterra 1928), and (iii) changes in abundance or biomass are usually easy to experimentally detect and measure (e.g., reduction in specimen counts or percent cover). However, ecological communities are thought to be replete with trait-mediated links (Werner and Peacor 2003, Schmitz et al. 2004). To get a full understanding of food web dynamics and ecological communities, more research needs to examine the impact of phenotypic plasticity (morphological, physiological, life history) on species interactions.

  Moreover, in studies where lethal effects are the focus (i.e., MPEs) detailed behavioural data is critical to understanding and interpreting the mechanisms driving density dependent patterns (Sih et al. 1998). For example, behavioural observations were critical to determine that the linear predation effects observed in conspecific pairs of *Pseudochromis fuscus* were caused by strong foraging dominance in only one of the predators, rather than evenly distributed between the individuals in the pair (see discussion of **Chapter 5**). Studies that overlook the importance of behavioural plasticity in predator-predator and predator-prey interactions can lead to incorrect interpretations and understandings of natural systems (Sih et al. 1998, Werner and Peacor 2003, Schmitz et al. 2004, Schmitz 2007).

- **Examining complex & dynamic systems.** Most ecosystems harbour complex food webs that dynamically link bottom level autotrophs to high level vertebrates (Schmitz et al. 2000, Miner et al. 2005, Duffy et al. 2007). Nevertheless, most of the framework for empirical and applied ecology comes from studies carried out on static links (Lima 2002) and/or invertebrate species (Schmitz et al. 2004, Schmitz 2007). Similar to the research conducted in this thesis, it is critical to show how the density and phenotypic plasticity of predators and prey change in relation to one another (Lima and Dill 1990, Abrams 1995, Lima 1998b, 2002, Schmitz et al. 2004). Further, research should employ food webs that include organisms other than terrestrial arthropods and intertidal crustaceans (Sih 1980). For example, our understanding of community ecology would greatly be extended by studies that include high order vertebrates (e.g., wolves and sharks) as they tend to directly and indirectly interact with a large number of species (given their location higher up the trophic chain; Ritchie and Johnson 2009, Estes et al. 2011, Ripple et al. 2014).

- **Testing other anthropogenic impacts.** Predator loss and mesopredator release do not occur in isolation. Humans are concurrently destroying, polluting, and fragmenting ecosystems; hence, indirectly modifying the links among species (reviewed by Brooks et al. 2002, Fahrig 2003, Ripple et al. 2014). For coral reef fishes changes in the water quality (Pratchett et al. 2015, Wenger et al. 2015) and the habitat structure are of major concern (Wilson et al. 2006, Rogers et al. 2014). Numerous studies have proven that even subtle increases in temperature, CO_2 (pH reduction) and turbidity modify predator-prey interactions due to the impairment of risk assessment, social learning, and escape performance of prey (e.g., Ferrari et al. 2011, Wenger et al. 2013, Allan et al. 2015). Furthermore, recent studies have highlighted how degradation of coral reef habitats (i.e., coral death and transition to a rubble system) can drastically diminish the ability of many species to detect, learn and respond to alarm cues, thus having immediate consequences for their survival (Lönnstedt et al. 2013a, Chivers et al. 2016).

Although predator-prey interactions have been extensively studied, further research needs to elucidate how lethal and risk effects on food webs will change under projected environmental conditions. Laboratory experiments are an important tool to test the performance of species under manipulated scenarios of climate change and habitat degradation. However, caution must be taken when interpreting these results given short acclimation times, the artificial set-ups, the lack of concurrent climate and non-climate stressors, and the little scope for adaptation (Wernberg et al. 2012, Munday et al. 2013, Browman 2016). As mentioned before (see Expanding temporal and space scales above), a potential solution would be to monitor species interactions through ecologically relevant time frames. In the future, this could allow us to correlate environmental changes to variations in the physiological traits and the characteristics of predator-prey interactions (Schmitz and Barton 2014).

Trophic cascades are mainly expected to occur when humans fish *down* the food web (i.e., top-predators are the main target; Pauly et al. 1998). However, many fisheries nowadays also exploit species *throughout* the food web (i.e., catches include top-predators, but also expand to lower trophic levels; Essington et al. 2006). Under these circumstances trophic cascades should be rare as the fishing pressure and removal of individuals is spread across several trophic levels. Future research should explore how different fishing regimes affect marine food webs to fully understand how assemblages of coral reef fishes will be affected worldwide.

6.3 Concluding remarks

Collectively, the results of this thesis described how behavioural links in the food web affect mesopredator behaviour. Mesopredator foraging activity, and thus their lethal and/or sub-lethal impacts on prey are determined by behavioural interactions with higher trophic guilds (top-predators; **Chapters 2, 3, 4**) or with the same guild (other mesopredators; **Chapter 5**). Alongside climate change, the depletion of top-predators is one of the most significant anthropogenic impacts on nature (reviewed by Estes et al. 2011, Ripple et al. 2014). Empirical evidence contained within this thesis suggests that the loss of top-predators will likely trigger a release of mesopredator behaviour, with potentially grave consequences for the persistence and fitness of organisms within bottom trophic levels. The conservation of large-carnivores (e.g., wolves, sharks, lions, groupers) needs to become the focus of widespread public recognition and a priority for conservation efforts. As underscored by this thesis, it is critical that management strategies are targeted towards the preservation not only of species, but of the myriad of direct, indirect, lethal, and risk-associated links they maintain in healthy food webs.

‘What escapes the eye... is a much more insidious kind of extinction:
the extinction of ecological interactions’ Daniel H. Janzem

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Appendices

Appendix A

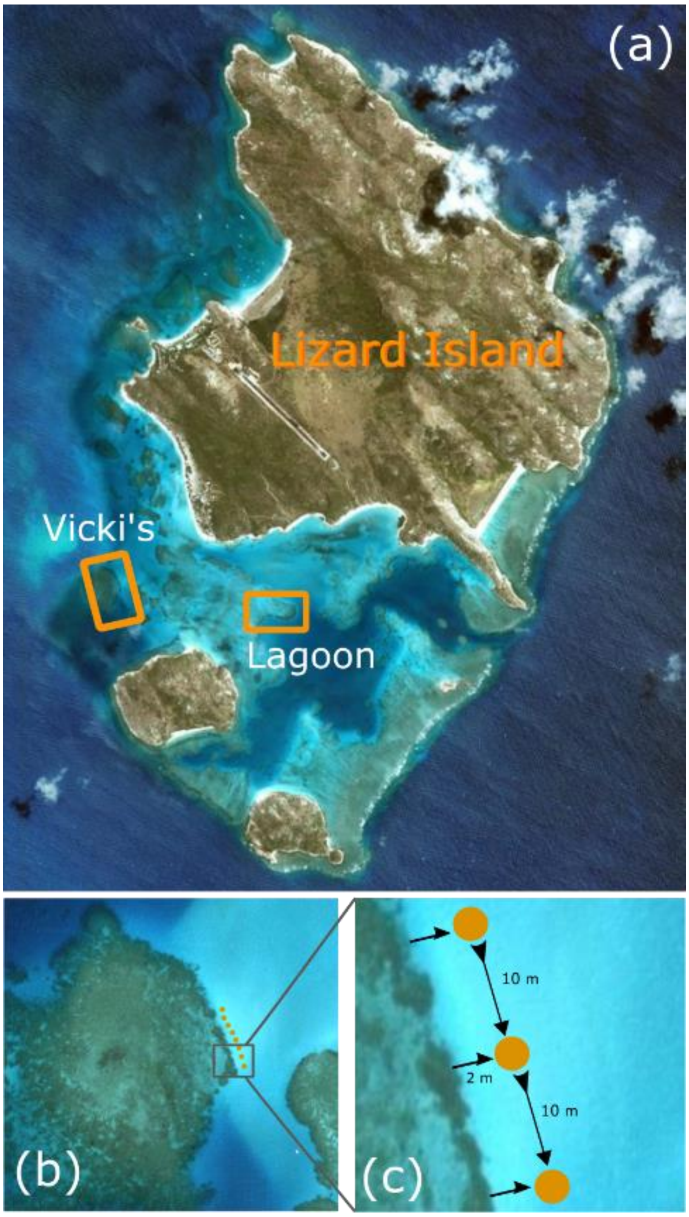

Figure A.1. Map of Lizard Island showing the location of the two reef sites (a) Vicki's Reef and Lagoon Reef. The spatial arrangement of the patch reefs (orange circles) along the reef edge (b) and the distance among individual patches (c) are also illustrated. Figures at different scales. Photo credit GeoEye satellite image.

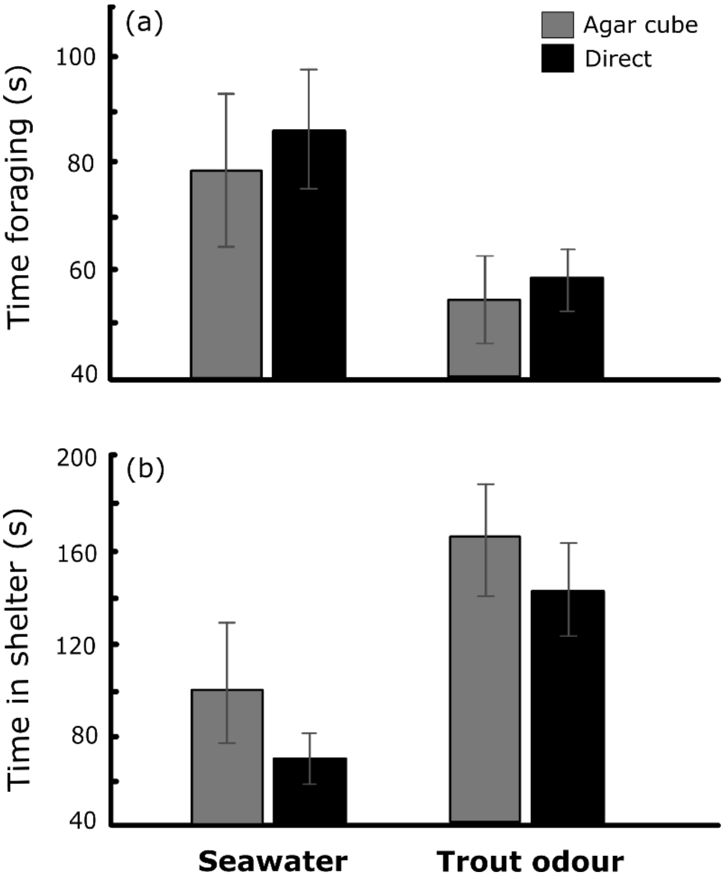

Figure A.2. Time (mean ± SE) that a focal mesopredator *Pseudochromis fuscus*, spent (a) foraging and (b) inside shelter when exposed to control seawater or seawater with coral trout odour. Cues injected into the mesopredator tank were obtained directly from the source (tap seawater vs. tank holding a trout; black bars) or from a dissolved agar cube (grey bars). Agar cubes where prepared 2-3 h before use by mixing 8 g of agar with 150 ml of control seawater or water collected from a 368-l pool containing a coral trout, *Plectropomus leopardus*. Factorial ANOVAs (origin of cue: 2 levels= seawater vs. coral trout odour; type of cue: 2 levels= direct vs. agar cube) conducted on log-transformed data indicated the coral trout odour had a significant effect on the time foraging (control seawater vs. coral trout odour: $F_{1,35}=4.9$, $P<0.05$) and the time inside shelter (control seawater vs. coral trout cues: $F_{1,35}=12.5$, $P<0.01$). For both behaviours, the effect remained consistent independent of the use of direct cues or cues from dissolved agar cubes (interaction Origin X Type of cue; $P> 0.05$). The two types of cues (direct vs. agar cube) generated responses of similar magnitude for both behavioural responses (time foraging: $P> 0.05$; time inside shelter: $P> 0.05$).

Table A.1. Density (mean no. / $150m^2$) of piscivorous species surveyed at Vicki's and the Lagoon sites. Families with the greater number of species are displayed first. Each fish surveyed was classified as small (S ≤10 cm TL), medium (10 < M > 20 cm TL) or large (L ≥ 20 cm TL). The most common size for each species is displayed. Species with asterisk (*) had one of the six highest total abundances.

Piscivore density	Vicki's reef		Lagoon reef		Size
	Ẋ	± SE	Ẋ	± SE	
Labridae (wrasses)					
* *Chelinus chrorurus*	1.63	0.38	1.44	0.27	M
Chelinus diagrammus	0.25	0.11	0.19	0.10	M
Chelinus trilobatus	0.69	0.25	0.00	0.00	M
Chelinus undulatus	0.00	0.00	0.07	0.06	L
Epibulus insidiator	0.50	0.16	0.00	0.00	M
Thalassoma amplycephalum	0.06	0.06	0.07	0.06	M
Thalassoma hardiwcke	0.88	0.22	0.06	0.06	M
* *Thalassoma lunare*	7.88	1.23	2.56	0.42	M
Serranidae (groupers)					
Cephalopholis boenak	0.00	0.00	0.07	0.06	M
* *Cephalopholis cyanostigma*	1.94	0.50	0.44	0.20	M
Cephalopholis microprion	0.88	0.27	0.19	0.10	M
Epinephelus cyanopodus	0.13	0.09	0.00	0.00	L
Epinephelus merra	0.19	0.10	0.07	0.06	M
Plectropomus leopardus	1.44	0.43	0.31	0.15	L
Lethrinidae (emperors)					
* *Lethrinus atkinsoni*	0.31	0.12	0.56	0.27	L
Lethrinus harak	0.25	0.11	0.25	0.14	L
Lethrinus nebulosus	0.06	0.06	0.88	0.42	L
Lethrinus obsoletus	0.06	0.06	0.28	0.17	L
Lutjanidae (snappers)					
Lutjanus bohar	0.81	0.28	0.25	0.14	L
* *Lutjanus carponotatus*	0.44	0.18	1.81	0.51	L
Lutjanus fulviflamma	0.00	0.00	1.93	1.60	L
* *Lutjanus gibbus*	0.00	0.00	4.13	1.15	L
Lutjanus quinquelineatus	0.00	0.00	0.31	0.15	L
Synodontidae (lizardfishes)					
Synodus variegatus	0.31	0.20	0.13	0.09	S
Synodus dermatogenys	0.19	0.14	0.00	0.00	M
Holocentridae (squirrelfishes)					
Myripristis murdjan	0.50	0.18	0.07	0.06	M
Neoniphon sammara	0.19	0.10	0.13	0.09	M
Sargocentron espiniferum	0.13	0.09	0.00	0.00	M
Pinguipedidae (sandperches)					
Parapercis cylindrica	0.25	0.19	0.44	0.27	S
Parapersis queenslandica	0.19	0.10	0.00	0.00	M
Pseudochromidae (dottybacks)					
Pseudochromis fuscus	0.13	0.09	0.19	0.14	S
Carangidae (jacks)					
Trachinotus baillonii	0.06	0.06	0.00	0.00	L
Callionymidae (dragonets)					
Diplogrammus goramensis	0.00	0.00	0.07	0.06	S