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# **Predator-prey interactions and the importance of sensory cues in a changing world**

Thesis submitted by  
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In December 2013

For the degree of Doctor of Philosophy  
In the School of Marine and Tropical Biology  
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## **Statement on the contribution of others**

This thesis includes some collaborative work with Prof. Mark McCormick, Prof. Douglas Chivers, Prof. Philip Munday, Prof. Mark Meekan and Dr. Maud Ferrari. While conducting these collaborative projects, I was responsible for project design, data collection, analysis, and interpretation of data. My collaborators provided intellectual guidance, financial support, technical instruction and editorial assistance. Aside from standardised formatting for the thesis, all data chapters have been presented as published.

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## General Abstract

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Despite the overwhelming importance of predation in coral reefs, there is a paucity of information on how predators interact with and impact prey populations, and behavioural mechanisms that underlie these interactions. Coral reefs undergo frequent disturbances from biological and environmental agents, and reef inhabitants must continuously adapt and react to their changing environment or die. As the environment changes, the ways in which prey assess the risk of predation are predicted to change as the lucidity of sensory cues will be strongly tied to prevailing habitat features. Making appropriate decisions in the face of predation risk dictates the fate of prey, and for tropical fish predation risk is highest at life history boundaries such as settlement. The overall focus of this study was to examine how the dynamic relationship between predatory fishes and their settlement stage fish prey is influenced by physical and biological disturbances.

The focus of *Chapter 2* was to explore how feeding history influences the relative importance of olfactory and visual sensory modes of learning, and how the experience gained through these sensory modes influences behaviour and predator-related mortality in the field for a settlement stage coral reef damselfish. Both feeding history and experience interacted to have a strong influence on the propensity of newly settling *Pomacentrus wardi* to take risks in their natural environment. Pre-release teaching of two main reef predators to settlement-stage damselfish led to a ~65% survival after 3 days, while predator-naïve fish had <10% survival during the same period of time. This experiment highlights the importance of a flexible and rapid mechanism of learning the identity of predators for survival of young fish during the critical life-history transition between pelagic and benthic habitats.

Non-lethal impacts of predators may also have a major influence on the factors that affect the survival of prey individuals, by subverting growth potential, influencing colour



patterns or long-term behaviour of new settlers. **Chapter 3** examined whether and how the presence of predators indirectly influences prey growth, behaviour and survival in juvenile damselfish, *Pomacentrus amboinensis*. Interestingly, prey exposed to predators for 6 weeks grew deeper bodies and developed larger false eyespots and smaller eyes than fish exposed to herbivores or isolated. Furthermore, when reared with predators, prey hid more, fed less and had an overall lower activity rate than fish from control treatments. Prey from predator treatments also displayed a significantly higher survival once in the field. Clearly, phenotypically plastic development in prey morphology and coloration as well as conservative behaviours can result in dramatic increases in survival for marine prey.

The goal of **Chapter 4** was to examine how experienced and naïve prey individuals respond to different cues that signify the presence of three very different predators (one a highly successful invasive predator, the red lionfish *Pterois volitans*). This study found that *P. volitans* has evolved to circumvent prey risk assessment abilities as it was virtually undetectable by prey. While experienced prey damselfish, *Chromis viridis*, responded with typical antipredator behaviours when exposed to two non-invasive species they failed to visibly react to either the scent or visual presentation of *P. volitans*. No other species has been found to be able to circumvent the sophisticated threat-learning mechanism that fishes possess, and the current findings could be one of the reasons that *P. volitans* is such a successful invader in the Caribbean reef ecosystem.

Habitat degradation is one of the “Big Five” drivers of biodiversity loss. However, the underlying mechanism for this loss and the cascading consequences of habitat degradation on the complex interrelationships between predators and their prey are poorly understood.

**Chapter 5** examines impacts of habitat degradation on risk assessment mechanisms of naïve prey. I found that risk assessment behaviours of new settlers are severely affected by coral degradation. Settlement stage damselfish (*P. amboinensis*) were exposed to visual and

olfactory indicators of predation risk in healthy live, thermally bleached, and dead algal covered coral in a series of laboratory and field experiments. While fish still responded to visual cues in all habitats, they did not respond to olfactory indicators of risk in dead coral habitats, likely as a result of alteration or degradation of chemical cues. These cues are critical for learning and avoiding predators, and as the proportion of dead coral increases, a failure to respond to these crucial threat cues can have dramatic repercussions for survival and recruitment.

While it is known that ocean acidification impairs the ability of prey fish to detect olfactory signposts of risk, it is unknown whether visual information may compensate for the lack of olfactory abilities. **Chapter 6** explored the effects that ocean acidification has on abilities of naïve prey (*P. amboinensis*) to respond to predators. While the visual response to a predator was affected by high CO<sub>2</sub>, it was not entirely lost. Fish exposed to 850 µatm showed reduced antipredator responses, however, exposure to CO<sub>2</sub> did not fully impair responses of the prey to the sight of the predator. These results provide us with a glimmer of hope that fish can adapt and survive through selection in an otherwise very disturbed ecosystem.

The current body of work has provided us with a wider understanding of how biological and physical habitat disturbances can affect species interactions and crucial behavioural processes in a severely stressed ecosystem. I have demonstrated how some of the complex dynamics of coral reef systems will change as these environments continue to be put under stress. By furthering our understanding of the highly complex dynamics of predator-prey interactions we strengthen our ability to interpret the processes that regulate communities and can begin to understand how changes in our natural world will affect these crucial ecological processes.

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## Chapter 1: General Introduction

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All animals must eat to live, and nearly every individual on earth is a potential food source for another animal. This constant threat of predation affects several aspects of an animal's life such as space use, access to food, and allocation of energy into growth, reproduction and maintenance (Sih 1980; Lima & Dill 1990). Ideally, prey should maximize the amount of time spent carrying out vital functions such as feeding and minimize the amount of time they spend undertaking predator avoidance behaviours such as hiding (Ferrari *et al.* 2010a). As the key to surviving predator encounters is early recognition prey must always stay vigilant while undertaking fitness promoting activities to accurately assess risk levels (Helfman 1989; Chivers & Smith 1998). Prey have evolved numerous morphological, chemical and behavioural defences that reduce the chance of an encounter with a consumer and/or increase the chance of surviving an encounter (for comprehensive reviews see: Lima & Dill 1990; Brown & Chivers 2006; Ferrari *et al.* 2010a). It is the behavioural choices that individuals make under the threat of predation that determine the fate of individuals and the genes they hold.

The way that a prey organism behaviourally responds to predators can help determine its resilience to future challenges, such as global environmental change (GEC; CO<sub>2</sub> enrichment, climate, biotic invasions and pollution). How individuals respond to each other often depends on the characteristics of their habitat, and as the habitat characteristics and resource base change so will the interactions that shape communities (Parmesan 2006; Tylianakis *et al.* 2008; Gilman *et al.* 2010). In today's world, we have a poor understanding of how animals will behaviourally respond to changes that are happening in their natural environments. Recent studies have suggested that human-induced habitat change is threatening to perturb the delicate balance between predators and their prey (Parmesan 2006;



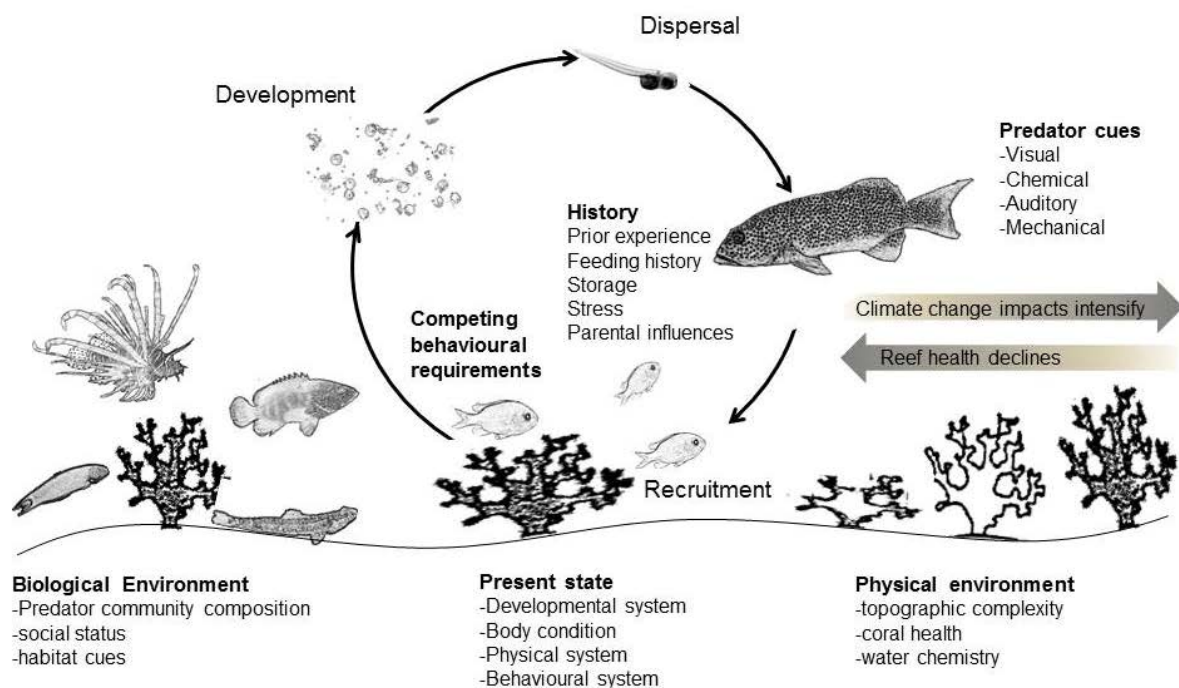
Tylianakis et al. 2008; Munday *et al.* 2012). The unpredicted impacts this may have on community dynamics underscore the need for a detailed understanding of how predators and prey interact with each other and their changing habitats.

### ***Assessing predation risk***

The ability to recognize and avoid predators is of obvious importance to all animals. Prey utilize a variety of sensory inputs to detect and avoid predators including visual, chemical, mechanical, and auditory signals or combinations thereof (Lima & Dill 1990; Ferrari *et al.* 2010a). These sources of information about local predation risk can be received by prey directly from a predator or as a result of a nearby attack on a conspecific (Brown 2003). The importance of each type of signal may vary among different environments and is situation dependent (Fig. 1.1). When sensing danger, prey display species specific ‘anti-predator behaviours’ such as reduced foraging, lowered activity and increased refuge use. In the simplest form, these behaviours are then the sequence of activities that make the difference between being eaten and avoiding the threat. The main senses used for risk assessment in the aquatic environment are visual and chemical cues (Brown & Chivers 2006; McCormick & Manassa 2008; McCormick & Lönnstedt 2013). Chemical cues warn prey of the presence of a predator whilst visual cues provide information that is more specific to the predators’ current motivation and threat (Helfman 1989; Smith & Belk 2001). Separately, these cues induce quite different behavioural responses; however individuals often use multiple cues to accurately assess the level of predation risk (Barbosa & Castellanos 2006).

Studies in low diversity freshwater systems have stressed the context dependent use of chemical and visual cues in prey risk assessment (e.g. Kiesecker *et al.* 1996; Mathis & Vincent 2000; Ferrari *et al.* 2010a). In highly diverse ecosystems such as coral reefs, newly settling prey fish rapidly learn a catalogue of predators by coupling the smell and visual cue

of a potential predator with a chemical alarm cue released from the skin of injured conspecifics (Mitchell *et al.* 2011a). Since these chemical alarm cues are produced in the epidermis of prey and require disruption for their release, they are reliable indicators of the damage of a conspecific (Lönnerstedt & McCormick 2011a). This is an efficient and wide-spread learning mechanism that allows naïve prey to learn the identity of new predators. If this chemical and/or visual learning mechanism is disrupted levels of prey mortality may dramatically increase (Munday *et al.* 2010; Lönnerstedt *et al.* 2014).



**Figure 1.1.** The complexity of factors that influence risk assessment in coral reef fish prey. Almost all reef fishes have complex life cycles, with a 2 to 5 week larval stage dedicated to dispersal, followed by a juvenile and adult reef-associated stage. Because of this open life history, the suite of predators that a newly settled fish is exposed to is likely to be quite different from the ones experienced by their parents. For this reason, coral reef fish have developed flexible mechanisms of predator recognition that enables prey fish to rapidly and correctly identify and respond to local predators. A diversity of factors influence how prey respond to predators including: prey history and present state, predator cues (threats) received, and biological and physical environment. Risk assessment is also balanced against time and energy requirements for other activities such as foraging, mating and maintenance.

***Importance of predation in early life***

Direct (lethal) effects of predators are highest in the younger life stages of animals (Bergenius *et al.* 2002, Gagliano *et al.* 2007a), as young prey are inexperienced, small and less adept at escape (Curio 1976; Vitale 1989). Due to the vulnerable nature of prey many amphibians, invertebrates and some fish will alter their growth (Relyea & Hoverman 2003, Pollock *et al.* 2005), phenotype (Brönmark & Miner 1992, Vaughn 2007, Chivers *et al.* 2008), life history (e.g. timing of hatching, larval duration or maturation; Chivers *et al.* 2001, Relyea 2007; Walsh & Reznick 2008) and behavior (Wisenden *et al.* 1999; Smith & Belk 2001) in response to the presence of predators. These predator induced defenses lower prey vulnerability to consumers. Prey that experience predation threats early in their development have an advantage compared with those prey exposed to predators later in life (Chivers & Smith 1998).

For demersal coral reef fishes, a critical life history stage occurs at the end of the pelagic phase, as fish larvae join the juvenile population. During this time, predator driven mortality produces an exponential mortality schedule, which can result in over 60% loss of a cohort within a single night (Almany & Webster 2006). At this critical life phase, predators are often selective for the attributes of prey, such as size (Holmes & McCormick 2009, 2010) and species (Almany *et al.* 2007). Predator identity will also change as the prey grows or as the prey undergoes shifts in habitat and resource use with ontogeny (Lönnerstedt & McCormick 2011b). For this reason, new settlers must have a flexible mechanism of predator recognition that enables them to rapidly and correctly identify local predators (Fig. 1.1).

Juveniles differ greatly in body condition at settlement (McCormick & Molony 1993, Holmes & McCormick 2006), which may be an indication of recent feeding history. As prey are often more vulnerable to predation while foraging as a result of reduced vigilance and increased conspicuousness (Lönnerstedt & McCormick 2011a), food-restricted fish may take

higher risks when trading off foraging for safety (Booth & Beretta 2004). The degree of the antipredator response elicited by a threat should be commensurate to an individual's relative level of experience as well as its body condition. The relative importance of feeding history and visual and/or olfactory experience with predators for prey survival is currently unknown.

### ***Changing habitats impact predator-prey interactions***

Most natural habitats undergo frequent disturbance from biological and environmental agents (e.g. Mumby *et al.* 2011; Brodie *et al.* 2012) and individuals must continuously adapt and react to their changing environment or die. Today, habitats are changing at far greater rates than during the past 10,000 years which is putting many species at risk as animals are no longer able to cope with environments they have spent thousands of generations specifically adapting to. Indeed, research in terrestrial and marine systems suggests that many organisms face the risk of extinction as the world's habitats continue to change (Thomas *et al.* 2004; Poloczanska *et al.* 2013). However, the mechanisms responsible for this loss of biodiversity are poorly understood. In a world where habitats are rapidly changing, determining the effects that GEC will have on the complex interrelationships between predators and prey is vitally important if we want to understand how communities will respond to our changing world.

Climate impacts on the marine ecosystem include rising sea surface temperatures, changing hydrodynamic regimes, altered ocean chemistry and an increasing prevalence of severe storms (Worm *et al.* 2006; Hughes *et al.* 2010). Furthermore, within the past 2 decades there has been a vast increase in the number of documented marine invasions primarily due to intentional or unintentional transport by humans (Salo *et al.* 2007). An understanding of the ecological effects of predation is made all the more imperative by the often devastating effects of introduced predators and habitat change, destruction and/or loss on native

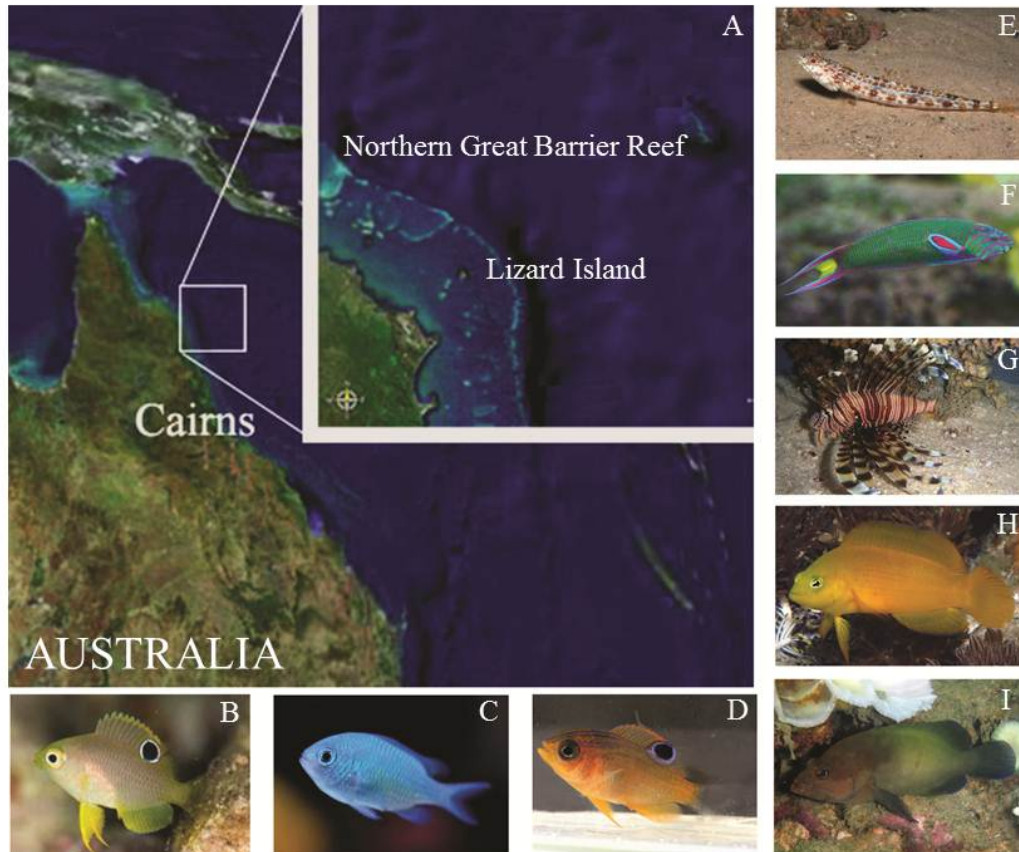
populations (Cox & Lima 2006; Jones *et al.* 2004; Salo *et al.* 2007). As the biological and physical environment changes, the ways in which prey assess the risk of predation is predicted to change as the lucidity of sensory cues will be strongly tied to prevailing habitat features.

The impacts GEC is having on tropical coral reefs has received considerable attention (Hughes *et al.* 2003; Wilson *et al.* 2006; Hughes *et al.* 2010), with several studies revealing significant and widespread declines in the abundance of not just the habitat-forming corals, but also many reef associated organisms such as fish (Jones *et al.* 2004; Pratchett *et al.* 2008). The proximate mechanism for these reductions may be lowered topographic complexity (Alvarez-Filip *et al.* 2009), which alters key population processes such as recruitment and predation (Jones *et al.* 2004; Pratchett *et al.* 2008; McCormick & Lönnstedt 2013). Despite there being a growing body of literature investigating how ocean acidification and increased temperatures impact the biology and physiology of tropical reef fish (see Munday *et al.* 2012 for review), relatively few have considered how ecological processes pivotal to the workings of healthy reef communities are directly affected by habitat degradation and loss. Research is required to identify underlying behavioural processes that may be driving the declines in abundance of coral reef fishes.

### ***Study system***

Coral reefs may be the poster-child for biologically complex ecosystem but they also experience high levels of disturbances from such vectors as coral degradation, ocean acidification, and species invasions (e.g. Hughes *et al.* 2003; 2007; Munday *et al.* 2012). Despite the fundamental role ascribed to predation in the promotion and maintenance of biodiversity in coral reefs, we know little about the ways prey interact with predators and how human induced disturbances will affect these crucial interactions. While habitat

degradation, ocean acidification and species introductions may all interact to yield unpredictable effects on organism biology and physiology, it is crucial that we first understand the impacts of each factor in isolation before addressing the complexity of these synergies.



**Figure 1.2.** Location of the Lizard Island Group on the Great Barrier Reef (a). The three different damselfish used in the experiments: the ambon damselfish (*Pomacentrus amboinensis*; b) the blue-green Chromis (*Chromis viridis*; c), and Wards damselfish (*Pomacentrus wardi*; d). Fish predators of newly-settled reef fish at Lizard Island used in experiments: the sand lizardfish, *Synodus dermatogenys* (e); the moonwrasse, *Thalassoma lunare* (f); the red lionfish, *Pterois volitans* (g), the dottyback, *Pseudochromis fuscus* (h); and the brown rockcod, *Cephalopholis microprion* (i).

All experiments for the present thesis were conducted at Lizard Island (14°40'S, 145°28'E), northern Great Barrier Reef (GBR), Australia during the summer months (Oct-Jan) of 2010-2012. The Lizard Island Group is a mid-shelf reef, situated 30 kilometres from the Australian

mainland and 19 kilometres from the outer barrier reefs (Fig. 1.2a). Most reef types characteristic of the Great Barrier Reef are accessible from the research station. Three different damselfish species (Family Pomacentridae) were used as the model prey. These are all common members of the GBR fish community: the ambon damselfish (*Pomacentrus amboinensis*; Fig. 1. 2b), the blue-green Chromis (*Chromis viridis*; Fig. 2c) and Wards damselfish (*Pomacentrus wardi*; Fig. 1.2d). In the days immediately prior to settlement, these damselfish can be captured away from the reef using light traps (see Meekan *et al.* 2001 for design). Although they have juvenile form and colouration, these individuals are naïve to the suite of predators that await them on the reef. Hence, they provide me with a unique opportunity to examine interactions between predator and prey at a key life stage. A series of different predators were used in the experiments (Fig. 1.2e-i), these predators are known to take a large number of recruits during the settlement season and represent the full range of predatory feeding modes on the reef (Bosiger *et al.* 2012; Feeney *et al.* 2012; Sweatman 1984).

### ***Thesis aims and objectives***

The focus of this thesis is to examine the dynamic relationship between predatory fishes and their fish prey on coral reefs, primarily concentrating on how chemical and visual cues influence the outcome of these interactions. Furthermore, I examine some of the impacts that global environmental change will have on marine fish, and how these impacts may be mediated through intraspecific variation in behaviour. By combining unique and innovative laboratory and field experiments this study aims to answer a series of interrelated questions that build on one another and together form an integrated and comprehensive understanding of how our changing world will influence the dynamic relationship between fish prey and the predators they avoid.

These questions are addressed in a series of five discrete studies which comprise the chapters outlined below. **Chapter 2** explores how feeding history influences the relative importance of olfactory and visual sensory modes of learning, and how the experience gained through these sensory modes influences behaviour and survival in the field for a juvenile coral reef damselfish. **Chapter 3** examines whether and how the presence of predators indirectly influences prey phenotype, behaviour and survival. **Chapter 4** examines how experienced and naïve prey individuals respond to different cues that signify the presence of three very different predators (one a highly successful invasive predator; the red lionfish *Pterois volitans*). The goal was to understand how *P. volitans* have become such efficient predators in non-native regions. In **Chapter 5** I examine the impacts that habitat degradation has on chemical and visual risk assessment mechanisms of naïve prey both in the laboratory and field. Lastly, **Chapter 6** explores the effects that ocean acidification has on abilities of naïve prey to respond to predators.

Enhanced understanding of the interactions between prey and their predators that have shaped the fish communities which inhabit today's coral reefs will aid in predicting how they may respond to future perturbations, such as increased fishing pressure, climate change stressors, or species introductions. By providing information on the process of predation - a process that is fundamental to how communities have developed in the past and will adapt to future changes - this study represents a novel contribution to our understanding of processes regulating marine fish populations. Information on the direct and indirect effects of predators on prey species will allow scientists and managers to better understand the role of particular species in the ecosystem, and how resilience of coral reefs is promoted or compromised.



## Chapter 2

### Predator experience and feeding history determines prey behaviour and survival\*

---

#### 2. 1 Synopsis

Determining how prey learn the identity of predators and match their vigilance with current levels of threat is central to understanding the dynamics of predator–prey systems and the determinants of fitness. Our study explores how feeding history influences the relative importance of olfactory and visual sensory modes of learning, and how the experience gained through these sensory modes influences behaviour and survival in the field for a juvenile coral reef damselfish. We collected young fish immediately prior to their settlement to benthic habitats. In the laboratory, these predator-naïve fish were exposed to a high- or low-food ration and then conditioned to recognize the olfactory cues (odours) and/or visual cues from two common benthic predators. Fish were then allowed to settle on reefs in the field, and their behaviour and survival over 70 h were recorded. Feeding history strongly influenced their willingness to take risks in the natural environment. Conditioning in the laboratory with visual, olfactory or both cues from predators led fish in the field to display risk-averse behaviour compared with fish conditioned with sea water alone. Well-fed fish that were conditioned with visual, chemical or a combination of predator cues survived eight times better over the first 48 h on reefs than those with no experience of benthic predator cues. This experiment highlights the importance of a flexible and rapid mechanism of learning the identity of predators for survival of young fish during the critical life-history transition between pelagic and benthic habitats.

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## 2.2 Introduction

Many organisms live under the constant threat of predation, but for most of these, the magnitude of threats changes through time. Organisms are most vulnerable when they are small or young, or when they undergo ontogenetic shifts in habitat and encounter a new suite of predators. A lack of familiarity with local predators greatly hinders the assessment of risk, particularly when there is a high diversity of potential predators. For this reason, individuals should develop mechanisms that promote the rapid and efficient identification of novel predators to enhance risk assessment and promote survival. Indeed, only prey that recognize the risk associated with specific predators and locations have the ability to fine-tune their behaviour to optimize foraging and reproductive effort in the face of predation.

Understanding how prey learn and modify their behaviour in relation to predators is not only fundamental to the dynamics of predator–prey interactions; it also has repercussions for conservation and management (Mirza & Chivers 2000; Bischof & Zedrosser 2009).

Olfaction and vision are the primary senses used by aquatic prey to assess predation risk. Olfactory cues are thought to be particularly important in aquatic systems because of the limited visibility owing to high topographic complexity, turbidity or low light levels at night. Moreover, many species learn the identity of predators through the coupling of damage-released chemical alarm cues and the odour of predators (Chivers & Smith 1998; Ferrari *et al.* 2010a). This information can then be quickly disseminated to local groups by social learning (Brown & Laland 2003).

For a variety of vertebrate and invertebrate prey, the magnitude of antipredator behaviours elicited by a threat is dependent on the feeding history of the individual (Lima & Dill 1990; Lima 1998). Hungry individuals, or those in poor body condition, take more risks to gain their next meal compared with individuals that have fed recently (Brown & Smith 1996; Chivers *et al.* 2000; Krause *et al.* 2011). Classic examples of this behaviour are shown

by ants and sticklebacks that prefer a profitable but more risky food patch when hungry and a less profitable but safe patch when satiated (Heller & Milinski 1979; Nonacs & Dill 1990). Feeding history also influences memory of a predator encounter (Brown *et al.* 2011) and a prey's ability to evade a predatory attack (McCormick & Molony 1993).

While an individual's history of risk and feeding is predicted to influence fitness and survival, there are few demonstrations of how the two interact to produce a behavioural response. Prey that have experienced predation threats early in their development may have a different response to risky situations compared with those exposed to predators later in life. Similarly, individuals that learn that an animal represents a threat through one sensory mode (e.g. vision) may respond differently to that threat compared with a prey that has learned the threat through a different sensory mode (e.g. olfaction), or through the stimulation of multiple sensory systems. The degree of the antipredator response elicited by a threat should be commensurate to an individual's relative level of experience as well as its body condition. To date, however, no studies have tried to determine the relative importance of feeding history and visual and/or olfactory experience with predators for survival.

Organisms with complex life cycles, such as many insects, amphibians and fishes, are ideal models for the study of learning as they undergo a series of rapid habitat shifts and multiple life-history stages, each exposed to a different set of predators. Decoupling of adult and juvenile life stages through larval dispersal means that adults are often unable to predict the predatory assemblage that newly settling juveniles will experience. For this reason, innate knowledge, or experiences during the larval phase, will be of limited use in risk assessment in the juvenile habitat. Fishes on coral reefs are particularly good models because they live in a food-limited system (Jones & McCormick 2002) and settle with a broad range of body conditions (Hoey & McCormick 2004) into habitat patches that contain a high diversity of potential predators.

Our study explored how feeding history influenced the relative importance of olfactory and visual sensory modes of learning, and how the experience gained through these sensory modes influenced behaviour and survival in the field of the juvenile damselfish, *Pomacentrus wardi*. The experiment manipulated the feeding history of predator-naïve juvenile fish, exposed them to visual and/or olfactory cues of two common predators, and then examined the behaviour and survival of these prey fish in the field. Many studies have shown that fishes, and a wide range of other aquatic organisms, can learn danger through the coupling of chemical or visual cues from a predator with cues from a damaged conspecific (Ferrari *et al.* 2010a). Fewer have shown how feeding history influences the propensity to take risk (Ferrari *et al.* 2010a). Ours is the first to examine the interactive effects of learning and diet to determine the real-world consequences for fitness. We highlight the critical importance of experience with a predator in influencing survival in the field at this key early life-history stage, and the interactive role that motivation to feed has in modifying behaviour and risk.

## 2. 3 Materials and Methods

### *Study species and sampling*

*Pomacentrus wardi* (Pomacentridae) is a site-faithful damselfish that is common on the shallow reefs of the Indo-Pacific. Adults and juveniles occur in shallow lagoons, where they inhabit the reef edge or reef top associated with rubble. Larval duration is 16–21 days, with fish reaching 13–14 mm standard length (SL) at the end of the larval stage (McCormick & Meekan 2010). Fish can potentially disperse hundreds of kilometres from their natal location (Stobutzki & Bellwood 1997), and newly metamorphosed fish settle as solitary individuals into habitats with conspecific adults and sub-adults.

Newly settled *P. wardi* are subject to an array of resident and transient predators. The most common predators at our study site on the shallow (2–3 m depth) coral reefs of Lizard Island, northern Great Barrier Reef, Australia (14°40'S, 145°28'E) are the moon wrasse (*Thalassoma lunare*) a lizardfish (*Synodus dermatogenys* (Holmes & McCormick 2006; Holmes & McCormick 2010a) and an array of flatfishes (family Pleuronectidae). All predators can be seen striking at and occasionally capturing recently settled and juvenile reef fishes during the summer recruitment period. Studies that have monitored newly settled damselfish that were individually identified through tagging have found high levels of mortality in the first few days after settlement, with high levels of variability among sites located hundreds of metres apart (McCormick & Meekan 2010; Holmes & McCormick 2006; McCormick & Hoey 2004).

During October 2010, light traps (see Meekan *et al.* 2001 for design) were used to collect *P. wardi* at the end of their larval phase. Traps were moored at least 100 m away from the reef edge overnight and catches were brought back to the Lizard Island research station just after dawn. Fish were placed into 40-L aquaria with aerated flowing sea water for 24 h (density: approx. 50–100 per 40 l), where they were fed *Artemia* twice per day. Research over the last two decades suggests that fishes collected in light traps are intercepted as they come into the vicinity of the reef to settle, and most are in the process of metamorphosis to their juvenile form (Milicich *et al.* 1992; Wilson & McCormick 1997; McCormick *et al.* 2002). At this stage, they also appear to display active and consistent choices of habitat (Öhman *et al.* 1998; McCormick *et al.* 2010), and have little post-settlement movement over the initial juvenile phase (McCormick 2009), suggesting that they do not attempt to return to the plankton.

Studies of coral reef fishes have found that the pairing of skin extract from prey with a novel predator odour results in an antipredator response in conspecific prey upon exposure to

the novel odour alone (Larson & McCormick 2005; Holmes & McCormick 2010b). Fish can also learn the visual identity of a predator by the pairing of a conspecific skin extract with the sight of the predator (Ferrari *et al.* 2010a; Chivers & Smith 1994).

### ***Experimental protocol***

After initial acclimation, fish were randomly transferred into 60 l aquaria (50 fish per tank, four tanks per treatment) and feeding trials were commenced as per Lönnstedt & McCormick (2011a,b) (table 1 for protocol summary). Fish were pre-conditioned with one of two feeding treatments for 6–8 days (well- and poorly fed fish, 2500 and 320 *Artemia* per litre in the rearing tanks three times daily, respectively). An airstone within each tank kept the *Artemia* in suspension and distributed throughout the tank, so all fish had similar access to food. Prior to being released in the field, fish from both feeding treatments were conditioned to the sight or smell of a predator in a 2 x 2 design, where fish were exposed to (i) a control that received neither visual nor olfactory stimuli from predators (i.e. no visual or chemical learning), (ii) the sight of two key predators (*S. dermatogenys* and *T. lunare* placed in a plastic bag within the conditioning aquarium; the predators often attempted to strike at the prey through the bag; visual learning only), (iii) the odour of the two predators together with skin extracts from *P. wardi* (i.e. an alarm cue; chemical learning only, PO), or (iv) a combination of predator odour (and *P. wardi* skin extracts) and the presentation of predators (both visual and chemical learning). This resulted in eight treatments in total (two feeding levels x four predator cues). Conditioning was undertaken in 15-L aquaria on random samples of three to five fish per treatment 2 h prior to those fish being released on the reef. Group size was random across treatments based on fish availability on the day of conditioning. Olfactory cues were collected from 30-L tanks of aerated sea water containing each of the two predators that had been fed *P. wardi*, with no flow-through water for at least 12 h. Chemical alarm cues were

collected from the skin extracts of a total of 12 *P. wardi* for each treatment tank by making six superficial cuts along the flank of each freshly euthanized donor fish with a clean scalpel and rinsing the fish with 60 ml of sea water. Five millilitres of this chemical alarm cue was injected into the conditioning tanks every 5 min for 30 min for both the olfactory and combined visual and olfactory treatments.

After the 30 min predator conditioning, fish were placed into a labelled plastic bag containing sea water and photographed against a 1 cm grid for the measurement of body size. Fish were then released onto individual patch reefs (25 x 20 x 15 cm) positioned 3 m from the reef edge (2 m apart) within 2 h of conditioning. Patch reefs were composed of a combination of live and dead *Pocillopora damicornis*, a bushy hard coral. A fine mesh cage was placed over the patch reef for 40–60 min to prevent predation during acclimation to their new habitat.

**Table 2.1.** Sequence of methods for the field assessment of the influence of feeding history and predatory experience on the survival of *Pomacentrus wardi*.

Step 1	2	3	4	5	6
collect fish	acclimate	pre-conditioning feeding	predator experience conditioning	field behaviour assessment	monitoring survival (72h)
		well-fed	poorly-fed	olfactory predator cues (yes/no)	visual predator cues (yes/no)

### ***Behavioural assessment***

Following acclimation, the behaviour of fish was quantified for 3 min following previously developed protocols (McCormick & Meekan 2010; McCormick 2009; Fuiman *et al.* 2010; Meekan *et al.* 2010). Briefly, the behaviour of each fish was assessed by a scuba diver positioned approximately 1 m away from the patch. A magnifying glass (4 ×) aided the assessment of bite rates and space use over the 3 min focal animal sampling period for each

fish. Four aspects of activity and behaviour were assessed: (i) bite rate; (ii) total distance moved (cm); (iii) maximum distance ventured from the habitat patch (cm); and (iv) boldness (recorded as a variable on a continuous scale from 0 to 3, where 0 was hiding in hole and seldom emerging; 1 was retreating to a hole when scared and taking more than 5 s to re-emerge, weakly or tentatively striking at food; 2 was retreating to shelter when scared but quickly emerging, with purposeful strikes at food; and 3 was not hiding when scared, exploring around the coral patch and striking aggressively at food). At the end of the 3 min observation period, the fish were approached with a pencil, and the fish's reaction and latency to emerge from shelter was taken into account in the assessment of boldness. This measure of boldness has been found to be repeatable between observers, consistent in the short term for newly settled damselfish and related to survival in the field (McCormick & Meekan 2010; M.I. McCormick 2009, unpublished data). The number of replicate fish for each of the 8 factor combinations (2 food levels x 2 visual levels x 2 olfactory levels) ranged from 23 to 27 depending upon availability of fish in light trap catches.

### ***Survival***

Fish were released onto the reef between 10.00 and 12.00 hrs. Survival was monitored twice a day for 70 to 96 h after release. Previous tagging studies suggest that migration between patches or to the main reef is negligible (McCormick & Meekan 2010; McCormick & Hoey 2004; McCormick 2009). In this study, there were no instances where two fish were found on a single patch reef.

### ***Statistical analyses***

The standard lengths of fish from the two feeding levels (high and low) were compared using an independent-sample t-test. A three-factor MANOVA tested whether the behaviour of fish



differed between the two feeding levels (well-fed versus poorly fed), whether fish had experienced visual (visual predator, or none) or chemical information (predator odour + conspecific skin extract, or sea water), or whether behaviour was affected by the interaction between the three factors. The variables included in the analysis were bite rate, boldness, total distance moved and maximum distance ventured. The last variable was  $\log_{10}(\chi + 1)$  transformed to meet assumptions of normality. Three-factor ANOVAs (type III sums of squares) were employed to examine the nature of the significant difference found by MANOVA. Significant effects in ANOVAs were further explored using unequal-sample Tukey's HSD tests. A Bonferroni-corrected significance level of 0.0125 was used to account for the possible error inflation caused by non-independent variables, and univariate analyses were interpreted in relation to this more conservative alpha level. To further describe how boldness was affected by feeding history and experience with predators, and to examine the potential mechanism underlying the patterns found in mortality (below), two planned comparisons were used: the first examined whether the poorly fed predator-odour-exposed fish differed from the sea water controls (high- and low-fed); the second, whether these three means (pooled) differed from the remaining five treatments.

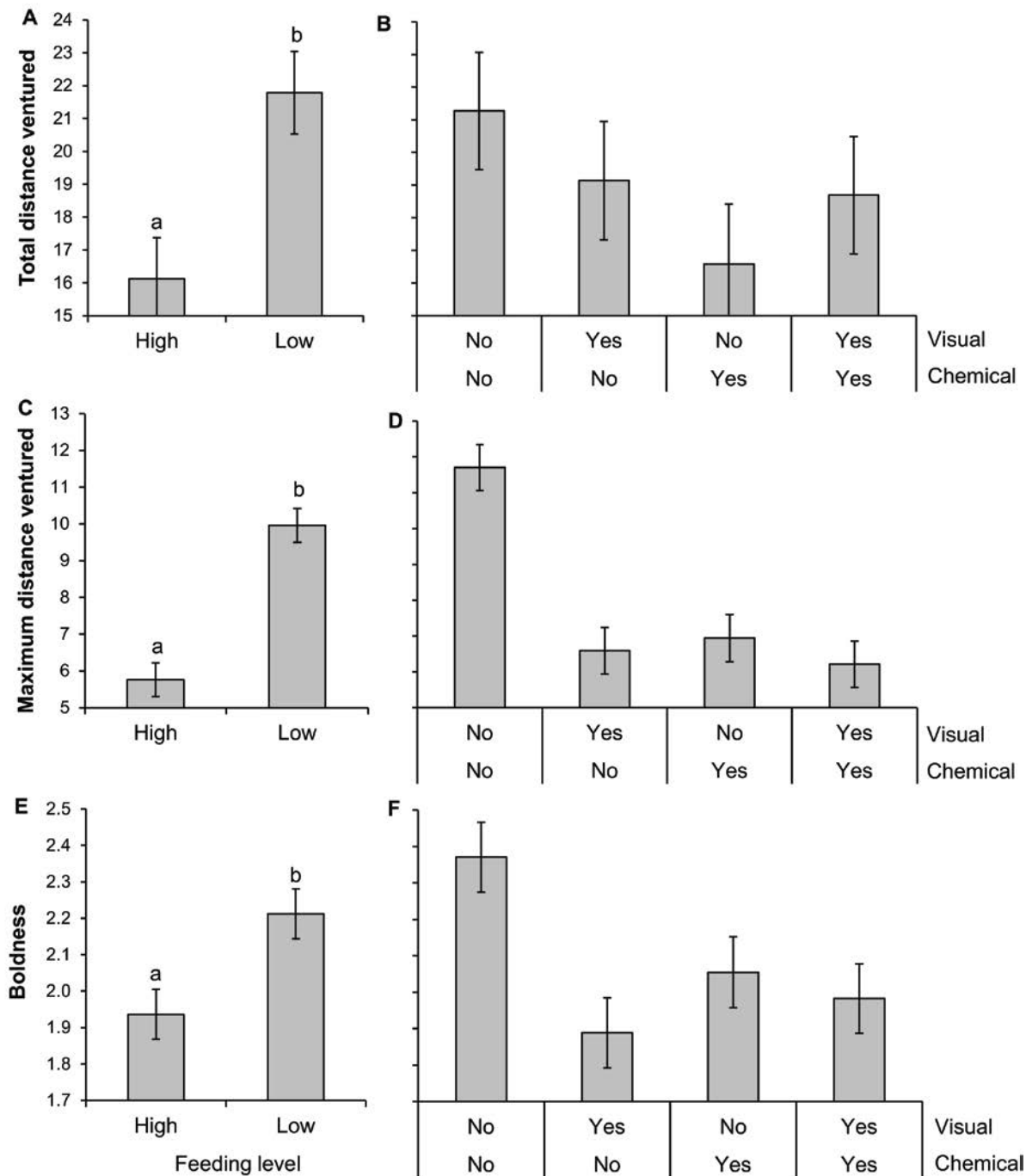
Multi-sample survival analysis using a Cox's proportional hazard model compared the survival of fish in the eight treatments through the 3–4-day census period. In total, there were 213 valid observations, involving 66 censored and 147 complete observations. A Kaplan–Meier survival plot was used to illustrate mortality trajectories. Two further survival analyses were used to determine the nature of the significant difference found among treatments by the first analysis. These determined whether there was any difference in survival among the treatments that fell into two groups that were evident from the Kaplan–Meier plot. The software STATISTICA v. 9.0 was used for all analyses.

## 2.4 Results

Fish used in the field trials from the well fed treatment were slightly larger than fish from the low fed treatment (13.9 mm SL  $\pm$  0.05 s.e. and 13.7 mm SL  $\pm$  0.05 s.e., respectively;  $t_{0.05,211} = 3.917$ ,  $P = 0.0001$ ). There were no significant correlations between standard length of fish and any behavioural variable for either well fed or poorly fed fish ( $n = 102$ ).

There was a strong influence of prior feeding history on the behaviour of *P. wardi* on isolated patch reefs in the field (Pillai's Trace:  $F_{4,194} = 0.173$ ,  $P < 0.0001$ ). ANOVA on the 4 variables showed that there were significant differences in total distance moved in 3 min, maximum distance ventured and boldness between well and poorly fed fish ( $P < 0.004$ ; Fig. 2.1a,c,e). Poorly fed fish were more active, ventured further from shelter and were bolder than well fed fish. While there was a trend towards poorly fed fish having a higher bite rate, this was not significant at the adjusted  $\alpha$  ( $P = 0.044$ ). There were no interactions between food levels and exposure to chemical or visual information, either in the overall analysis of behaviour (MANOVA,  $P > 0.28$ ), or in the univariate analyses on individual behavioural variables ( $P > 0.07$ ).

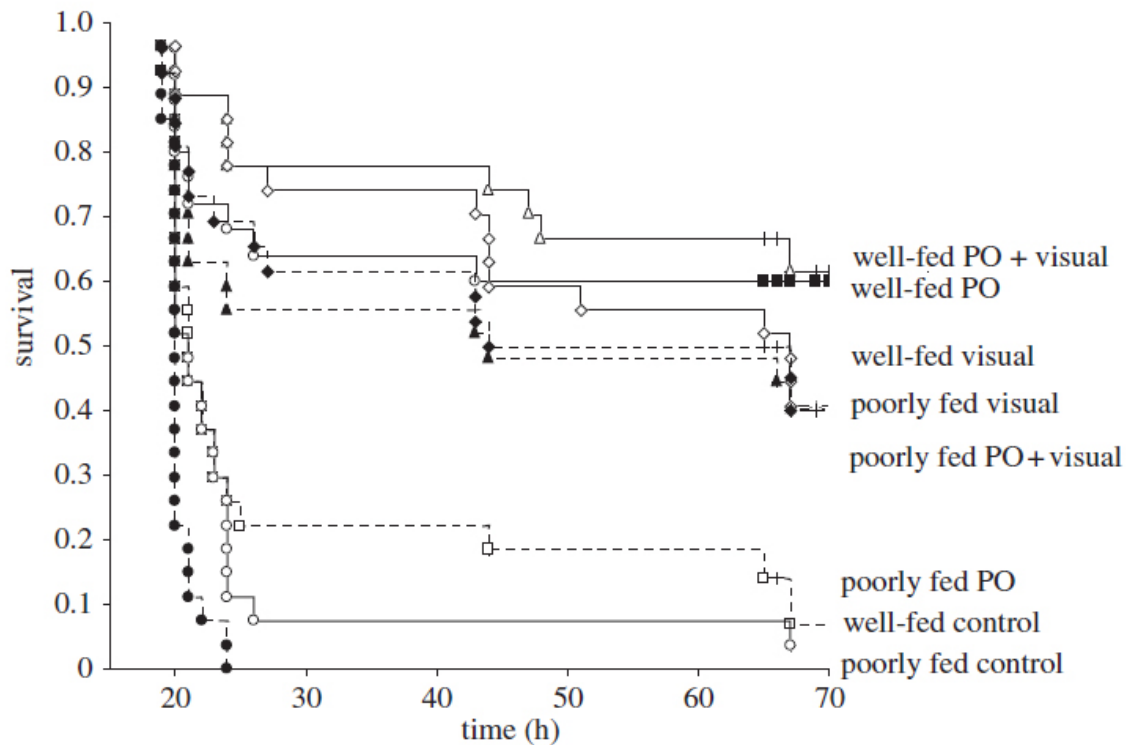
There was an interaction in how chemical and visual cues affected fish behaviour (Pillai's Trace:  $F_{4,194} = 0.075$ ,  $P = 0.004$ ; Fig. 2.1b,d,f). This was driven by a strong interaction in maximum distance ventured ( $F_{1,197} = 11.597$ ,  $P = 0.0008$ ) that was caused by the saltwater control differing from all the other three treatment levels, which did not differ from one another (Fig. 2.1d). This suggests that pre-exposure to visual or olfactory cues of key predators both cause prey fish to act conservatively once in the field context. One source of information did not appear to outweigh the importance of the other. The same trend was



**Figure 2.1.** Comparison of behaviour in the field of poorly fed or well-fed *Pomacentrus wardi* (A, C, E) that had been exposed to the odour (plus a conspecific skin extract; chemical) or the visual presence of two common predator species (visual), or a combination of both (B, D, F). Controls were not pre-exposed to predator cues. Behaviours are (A, B) total distance moved in 3 min, (C, D) maximum distance ventured from the coral patch in 3 min and (E, F) boldness (recorded on a 3-point continuous scale, where 0 is shy and 3 is bold). Error bars are s.e. Letters above the bars represent unequal-sample Tukey's HSD groupings. Number of replicates between feeding levels is 106–107 fish; number of replicates for treatments pooled over feeding levels is 50–52 fish.

displayed in fish boldness, with fish pre-exposed to seawater (i.e. controls) being the boldest and fish exposed to chemical, visual or a combination of cues being equally shy, however, this was not significant at the adjusted alpha level ( $F_{1,196} = 4.540$ ,  $P = 0.034$ ; Fig. 2.1e).

Survival was affected by predator experience and feeding history ( $\chi^2_{7,0.05} = 70.0$ ,  $P < 0.0001$ ; Fig. 2.2). The main pattern of survival among treatments was established within the first 24h from release. Survival analyses found two groupings of treatments. A group of three treatments had the lowest and similar survival and included both seawater controls (i.e. either well or poorly fed fish that had received no predator conditioning) and the poorly-fed-predator-odour treatment (Fig. 2.2). The second group contained the remaining 5 treatments.



**Figure 2.2.** Survival curves (Kaplan–Meier plot) of poorly fed or well-fed *Pomacentrus wardi* that had been exposed to the odour (plus a conspecific skin extract; PO) or the visual presence (visual) of two common predator species, or a combination of both. Controls were not pre-exposed to predator cues (control). Fish were placed on small patch reefs along the edge of a shallow reef and survivorship monitored over 3–4 days. The time variable represents hours from release (12.00–14.00 h).  $n = 25–27$ .

## 2.5 Discussion

Experience with a predator threat, whether olfactory or visual, was found to increase the likelihood of surviving in the natural environment. As predicted, feeding history influenced the likelihood that fish would take risks, especially when they had no visual experience of common predators in the local area. The mechanism underlying survivorship appeared to be behavioural; experience and a better history of feeding reduced the propensity to take risk, as shown by reduced activity levels and reluctance to venture far from shelter. While other studies have shown that exposure to chemical cues of predators coupled with chemicals released from damaged conspecifics leads to risk-averse behaviour by prey, this is the first study to demonstrate that both olfactory and visual cues can play an equally important role in affecting survival of prey in the field.

Our study expands the understanding of the significance of feeding history and experience for the survival of fish at the critical life-history transition between pelagic and benthic habitats. Fish that had experience with the two common predators, whether through olfactory cues or visual presence, exhibited less risky behaviours when in the field than fish without this experience. Earlier work has shown that the survival of another tropical damselfish, *Pomacentrus amboinensis*, in size-matched trials was influenced by experience with two common predators (McCormick & Holmes 2006). However, the relative importance of experience from visual, olfactory or mechanical (e.g. vibration or impact) cues could not be determined. Moreover, Holmes & McCormick (2006) found that only experience influenced survival at the one of two study sites where mortality (and selection) was highest. Combined with our results, such observations stress the importance of having fast and efficient ways of learning the identity of predators, and the context-specific nature of this information.

Laboratory studies have found that chemical information about the identity of predators may increase the survival of prey in predator encounters. Experiments with northern pike (*Esox lucius*) have shown that conditioning with a skin extract from conspecifics and predator odour increased the survival time of juvenile rainbow trout (*Oncorhynchus mykiss*) when placed together with the pike predator in an aquarium (Mirza & Chivers 2003). Zhao *et al.* (2006) found that goldfish (*Carassius auratus*) responded in a threat sensitive way to varying concentrations of odour from a pike that had previously consumed goldfish. Those that had been exposed to high concentrations of the odour prior to interaction with predators in staged interactions within a tank had higher survival than those fish exposed to lower concentrations.

Our study strongly suggests that associative learning was the mechanism underlying the survival benefit of the olfactory treatment, although this result must be treated with caution, because logistical constraints prevented the implementation of all possible treatment combinations in our study that would have conclusively demonstrated the survival benefits of associative learning of predator smells. An increase in antipredator behaviour after exposure to skin extracts from a damaged conspecific is an innate response common to many freshwater and marine fishes, as well as invertebrates (Chivers & Smith 1998; Larson & McCormick 2005; Ferrari *et al.* 2010b; Ferrari *et al.* 2011a). The fish used in our field experiment were the sole occupants of patch reefs and could only have been potentially exposed to dilute alarm cues from predatory activity within the general area. Moreover, laboratory experiments have shown that prey do not increase their antipredator behaviour in response to the odour of unknown predators (Holmes & McCormick 2010b). Thus, the important difference between the sea water control and olfactory treatment in our study was the co-occurrence of the alarm cue with odours from key predators prior to release of fish in

the treatment. Although further experiments are required, our study strongly suggests that cataloguing predators through smell benefits survival of young fish at a key life-history stage.

Visual presence of a predator in the absence of damage-released chemicals from prey also enhanced survival of young fish by making their behaviours more risk-averse. In nature, this may occur through social learning, whereby one individual learns a behavioural response to a predator through watching the antipredator behaviour of another and mimicking that behaviour in subsequent independent encounters with the predator. Although there are as yet few marine examples, social learning has been shown to occur in a diverse range of animals, from tammar wallabies (*Macropus eugenii*; Chivers *et al.* 2001) to freshwater fishes (Brown & Laland 2003) and insects (Leadbeater & Chittka 2006). The ability to associate an image with danger may also occur through the co-occurrence of damage-released chemicals with the visual presence of the animal (Brown & Smith 1996), or through direct experience with a predator strike or unsuccessful capture attempt (McCormick & Holmes 2006). Mechanisms to learn the identity of potential predators that do not involve olfactory cues should be expected to evolve in most aquatic systems because, simply due to chance, 50 per cent of all first encounters by prey with novel predators will occur from a down-current direction, where chemical cues will not be available to prey.

Prey conditioned with either visual or olfactory predator cues in isolation displayed levels of risk-taking behaviour in the field that did not differ from prey that had been conditioned with olfactory and visual cues in combination. This similarity between the effects of visual and olfactory experience may be because predators seldom occurred close to the experimental reefs when divers were in the vicinity for behavioural observations, so visual cues of predators for the focal damselfish would have been minimal. The risk-averse behaviour of visually experienced damselfish suggests that conditioning led to behaviour that was inherently conservative. This appears counterintuitive because it would lead to reduced

feeding opportunities, access to a smaller variety of food items and a lower energy intake. A possible reason for this finding is that, at this vulnerable life stage, experienced prey generalize any visual presence with danger, and the human observer was therefore seen as a threat. Regardless of the underlying reason, our study suggests that visual experience with two key predators enhanced survival of young fish.

Feeding history had a dramatic influence on behaviour and survival in a predator-rich environment. We showed that fish from the well-fed treatment were more risk-averse in their behaviours than poorly fed fish, a result that parallels a recent finding that naïve recruit fish that were in lower body condition exhibited riskier behaviour in the laboratory than fish in good body condition (Lönnerstedt & McCormick 2011a). In our study, poorly fed fish that had just been exposed to the olfactory cues of predators, coupled with damage-released skin extracts of conspecifics, were bolder and had a lower survival than those exposed to visual cues or a combination of visual and chemical experience. This reinforces the idea, suggested by others (Chivers *et al.* 2001; Holmes & McCormick 2011), that olfactory cues forewarn fish of potential danger and this reaction can be moderated by visual information, which acts as a more direct indication of impending threat. Interestingly, it was experience with a predator (regardless of the cue) rather than feeding history that dominated the differences in survival among groups, suggesting that mortality is not simply explained by behavioural vulnerability alone.

Mortality of newly settled coral reef fishes tends to be size-selective, with positive and negative selection for a particular size range dependent upon predator selection profiles in the vicinity of the settlement site (Holmes & McCormick 2006; Holmes & McCormick 2010b; McCormick & Meekan 2007). However, in our study, size differences between well-fed and poorly fed treatments were very small (0.2 mm for an approx. 14 mm SL fish), and much less than the size differences on which selection has typically acted in field



experiments. Various aspects of body morphology and body condition covary with feeding history (McCormick & Molony 1992), and have been shown to be selected by predators. For instance, Holmes & McCormick (2009) found selection by a common predator, *Pseudochromis fuscus*, acted on variation in body weight of a damselfish of a standardized size. Moreover, Gagliano *et al.* (2007b) found that there were marked carry-over effects associated with previous growth history that influenced survival later in life, but the present-day traits under actual selection (which covaried with previous growth history) could not be identified (McCormick & Meekan 2010). In our study, it appears that size plays a more minor role than experience in influencing survival, at least during the first few days after settlement.

Early experience of a predation threat appears to be crucial in determining the survival of juveniles during the transition between pelagic and settled life stages. Although feeding history influenced space use and activity, it did not appear to affect survival as much as whether individuals had been forewarned of the identity of common predators or not. Many fishes undergo ontogenetic habitat shifts; for example, juveniles moving from nursery grounds to adult habitats (Helfman *et al.* 1982; Werner & Gilliam 1984; Sheaves 1995). Retention of predator learning and recognition mechanisms, such as visual and olfactory labelling (Mitchell *et al.* 2011b), will be critical for the rapid cataloguing of novel predators. Given the commonality of the bipartite life cycle in reef fishes, it is likely that our findings of the importance of predator recognition systems are general to all coral reef fishes. Indeed, previous laboratory studies suggest that it is a mechanism that occurs among diverse taxa (Ferrari *et al.* 2010a). With increasing pressures on coral reefs from harvest (Hughes *et al.* 2007), some species are being restocked onto reefs in the hope of supplementing depleted populations (Bell *et al.* 2009; Heenan *et al.* 2009). Teaching fishes key predators prior to juvenile release may greatly enhance the early survival of stocked fish and improve stocking efficacy.

## Chapter 3

### Predator-induced changes in the growth of eyes and false eyespots \*

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#### 3.1 Synopsis

The animal world is full of brilliant colours and striking patterns that serve to hide individuals or attract the attention of others. False eyespots are pervasive across a variety of animal taxa and are among nature's most conspicuous markings. Understanding the adaptive significance of eyespots has long fascinated evolutionary ecologists. Here we show for the first time that the size of eyespots is plastic and increases upon exposure to predators. Associated with the growth of eyespots there is a corresponding reduction in growth of eyes in juvenile Ambon damselfish, *Pomacentrus amboinensis*. These morphological changes likely direct attacks away from the head region. Exposure to predators also induced changes in prey behaviour and morphology. Such changes could prevent or deter attacks and increase burst speed, aiding in escape. Damselfish exposed to predators had drastically higher survival suffering only 10% mortality while controls suffered 60% mortality 72 h after release.

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### 3.2 Introduction

Colour patterns are often adaptations to ecological pressures, and the sheer diversity of patterns represents an important form of morphological evolution in animals (Endler 1986). Many terrestrial insects, especially lepidopterans, as well as marine and freshwater fishes are often characterized by one or several conspicuous eyespots present on less essential regions of the body (Brakefield *et al.* 1996; Stevens 2005). False eyespots are large, dark circles surrounded by a lightly coloured ring thought to represent an iris around a pupil, mimicking the appearance of a vertebrate eye. The adaptive significance of false eyespots in prey has long been debated among ecologists. Decades of research have led to four hypotheses regarding their function, and their presence has been attributed to - deterring predators (intimidation hypothesis; Blest 1957), as a diversion technique drawing the attacks of predators to non-vital regions of the body (deflective hypothesis; Neudecker 1989), a form of status signalling (i.e., status signalling hypothesis; Lyon & Montgomerie 1986) or simply as an evolutionary remnant no longer utilized (Gagliano 2008).

Due to the widespread occurrence of eyespots in a variety of unrelated taxa, these ‘false eyes’ are believed to have evolved in response to selective pressures (Stevens 2005). Powell (1982) found that the conspicuous black tail tip (thought to mimic an eye) on long-tailed white weasels (*Mustela frenata*) reduces predation by avian predators. Hawks attacking white weasel models in snowy environments were more likely to become confused and attack the conspicuous tail tip, often missing their target. Similarly, Blest (1957) and Smith (1976) found that predators were more likely to direct their attacks toward conspicuous eyespots that had been painted on insect prey. It appears as if colour patterns that mimic eyes may be an effective deflection mark for many different prey species, although the adaptive significance of this has yet to be tested. Predators have been found to trigger striking changes in growth

and morphology in a variety of prey (e.g. body depth; Brönmark & Miner 1992; Schoeppner & Relyea 2009), but whether presence of predators influence the development of prey eyespots has never been tested.

In addition to triggering morphological defences cues from predators and/or injured conspecifics also affect prey behaviour. The presence of consumers induce ‘anti-predator behaviours’ in prey, such as reduced foraging, lowered activity and increased refuge use (Chapter 2). These behavioural defences will ultimately influence the prey’s success by altering the balance between defensive behaviours and other activities that promote fitness. The relative importance of predator cues in influencing behaviour and survival of prey has received attention in a number of studies (Chivers *et al.* 2001; Chapter 2) but few studies have looked at how different predation cues simultaneously affect prey development, colour patterns and behaviour over an extended period of time (but see Relyea & Hoverman 2011). Reducing predation through behavioural and physiological means could potentially increase short-term survival but may also result in lowered overall fitness and reduced survival in the long-term.

Juvenile damselfish have lightly coloured bodies and a conspicuous eyespots on the rear dorsal fin, which fades away as individuals approach maturation. Damselfish are an abundant component of the Great Barrier Reef fish community, with high vulnerability to predation during recruitment (Almany & Webster 2006), and represent a useful organism with which to explore how growth and colour patterns are affected by the continuous exposure to predators, and how these changes may confer a survival advantage to individuals in their natural environment. The current study therefore explored how threat cues from a common predator, *Pseudochromis fuscus*, indirectly affected development and performance of a juvenile damselfish, *Pomacentrus amboinensis*. Specifically, we tested how the continuous exposure of individual prey to a predator affected prey morphology (body depth,

BD; standard length, SL), eyespot size (total diameter), total visible size of the eye and behaviour over a 6-week period, after which survival patterns in the field were monitored. Usually, *P. amboinensis* will lose their eyespots as they age (Gagliano 2008), but we hypothesized that if eyespots evolved as a defence against consumers then the continuous exposure of prey to predators would result in the continued growth of the eyespot.

### 3.3 Materials and Methods

#### *Study organisms and collections*

The study was conducted from October through to December 2010 in the laboratory facilities and reefs around Lizard Island Research Station (14°38'S, 145°28'E) on the northern Great Barrier Reef, Australia. Settlement stage damselfish (family Pomacentridae) were collected from light traps that had been deployed overnight about 50m from the reef edge. The study species, *Pomacentrus amboinensis*, is an abundant damselfish species that settles on the reefs during the summer months after a pelagic larval phase of 15-23 days (Kerrigan 1996). Light traps catch the fish at the end of their larval phase, as they are entering the reefs at night to settle, therefore ensuring fish are naïve to reef-based, bottom-dwelling predators. Within 6 hours of settlement *P. amboinensis* will metamorphose and lose the transparent colour typical of the pelagic larval stage and gain the bright yellow body coloration and conspicuous black dorsal eye spot representing the juvenile stage of this species (McCormick *et al.* 2002). The predator used as the stimulus was the dusky dottyback, *Pseudochromis fuscus*, which is one of the most abundant meso-predators on the shallow reefs throughout the Indo-Pacific (Beukers & Jones 1998). This particular species is responsible for consuming a large amount of the newly settled and juvenile damselfish during the summer recruitment season (Feeney *et al.* 2012), and is found in areas where *P. amboinensis* settle. A herbivorous goby,

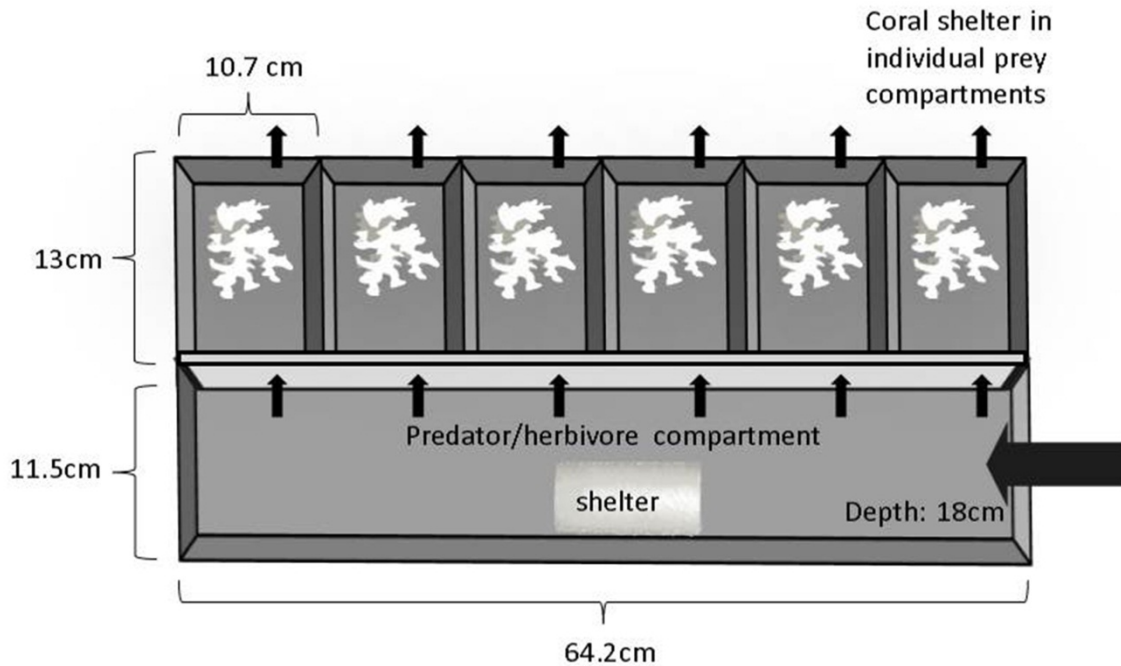
*Amblygobius phalanea*, was used as an experimental control to test for the effect of exposing *P. amboinensis* to visual and chemical cues of any heterospecific fish (Chapter 4). This fish has a similar body shape and size to the predatory dottyback and is often found in areas of the reef where recruits settle. Both species were caught on the reefs surrounding Lizard using a dilute clove oil anaesthetic and a handnet. Research was conducted under James Cook University ethics approval A1593 and A1720.

### ***Laboratory study and experimental design***

Individual *P. amboinensis* were exposed to a combination of olfactory and visual cues of a predator (*P. fuscus*), a non-predator (*A. phalanea*) or a blank control (receiving no cue sources). The growth, development and behaviour of *P. amboinensis* were assessed over a 6 week period. Naïve prey fish that had been collected with light traps (were brought back to the laboratory and placed in 60-L flow-through tanks (density: 50fish/tank) over a period of 10 days and fed *Artemia nauplii* ad libitum 3 times per day (ensuring all fish used in the experiment had an analogous baseline body condition at the start of the experiment). All *P. amboinensis* individuals were then conditioned to recognize the sight and olfactory cues of *P. fuscus* by placing the predator inside a transparent plastic bag in their tank for 30 minutes, while simultaneously injecting previously collected odour cues of the predator and skin extract cues of *P. amboinensis*. This is a training procedure found to increase the probability of survival in the ambon damselfish (McCormick & Holmes 2006), and is necessary to make sure that prey can recognise the cues of the predator species. It also ensured that all fish had the same baseline predator experience before the commencement of the study.

Individual prey then had their morphology and shape photographically recorded against a scale before being transferred into a series of specially-designed 18-L PVC

predator–prey tanks (64.2 x 11.5 x 18cm; Fig. 3.1). The tanks had a 7.5-L main section (containing either a predator or a herbivore) and 6 individually isolated prey compartments (1.5-L: 10.7x13x18cm). The main compartment was separated from each of the 6 prey compartments by transparent Perspex that contained a series of small holes. The fish in the six prey compartments were visually isolated from each other using grey PVC partitions. Water flowed from the main predator/herbivore compartment to each of the prey compartments and then out the side of each of the prey compartments. This arrangement ensured that the prey fish in each of the six compartments were also chemically isolated from one another. The bottom of both the predator/herbivore compartment and the prey compartment was covered by a 1.5cm layer of sand and the predator/ herbivore section had one plastic tube (12x5cm) placed in the centre to provide shelter. A small coral skeleton (*Pocillopora sp.* ~4x5x5cm) was placed at the back of each prey compartment to provide a refuge. The tanks were situated outside to ensure that animals received all natural temporal cues and the water was supplied by a flow through system from the ocean so organisms were given all the same environmental cues as that of fish residing in the wild. This design ensured that the individual prey in each compartment received all the olfactory diet cues as well as visual cues from the main section, ensuring all prey could both smell and see either the predator or herbivore ( $n=36$  fish/treatment), but that the prey could not see or smell each other. The chemical and visual isolation allowed us to consider the fish in each compartment as independent samples.



**Figure 3.1.** Design of the combined senses tank where prey received both olfactory and visual cues. Prey fish were placed in individual compartments containing a coral skeleton (shelter). These individual prey compartments were adjacent to one longer compartment containing a small shelter and either a predator or a herbivore (or no fish). The partitioning dividing the small compartments from the large one is transparent Perspex with small holes to allow the water through. Arrows indicate direction of water flow; the main section received the flowing water while small holes in the side of the compartments ensured the water flowed into the individual prey compartments and out through holes in each individual room (ensuring that each compartment received olfactory diet cues from the main section, and that the prey compartments were chemically isolated from each other). Individual prey compartments were separated from one another by opaque grey PVC barriers that were sealed in place (ensuring prey were visually isolated from one another).

Prey were fed twice daily with a standardized amount of boosted (DHA Selco) *Artemia sp.* nauplii (5ml with ~550 *Artemia*/ ml) while predators were fed two damselfish individuals morning and night, which is an accurate representations of what *P. fuscus* consume in their natural environment<sup>31</sup> ensuring that the cue stimulus provided to *P.*



*amboinensis* was realistic. Gobies were given a combination of dry fish food pellets (INVE Aquaculture Nutrition NRD pellets; containing no fish products) and small crustaceans. Predators and herbivores were replaced every two weeks, ensuring that significant effects could not be attributed to individual predators/herbivores. In addition to this there was an experimental control where individual prey were placed in separate 1.5-L compartments (10.7x13x18cm) that received no cue sources ( $n = 21$ ). After 6 weeks individual *P. amboinensis* were removed from their compartments and photographed against a scale (1x1cm) for morphological measurements. Shape and size of fish were analysed from digital photographs using the software Optimas 6.5. Five variables were measured: standard length, body depth, total area of ocellus, diameter of ocellus (black and white), and entire diameter of the visible eye.

### ***Monitoring prey behaviour***

One week after the commencement of the experiment, a mirror (80 x 40 cm) was suspended over each tank at 45° so that focal fish could be observed undisturbed from above. A wire grid (2x2 cm) was also placed on the top of each chamber so that movement and location of individuals could be accurately quantified as the number of times fish crossed a line on the grid. Water flow was stopped and individual *P. amboinensis* were fed *Artemia sp.* nauplii. One minute later the fish had their behaviour assessed for a 2 min period. The mirror and grid were then removed. This procedure was repeated after 5 weeks for all treatments. The behaviour of individual fish in each of the 7 experimental treatments was quantified by recording: total number of feeding strikes (successful or otherwise), activity (quantified as the number of times a fish crossed a line on the grid that had been suspended over the tank), and % time spent within shelter (defined as being inside the branches of the coral shelter).

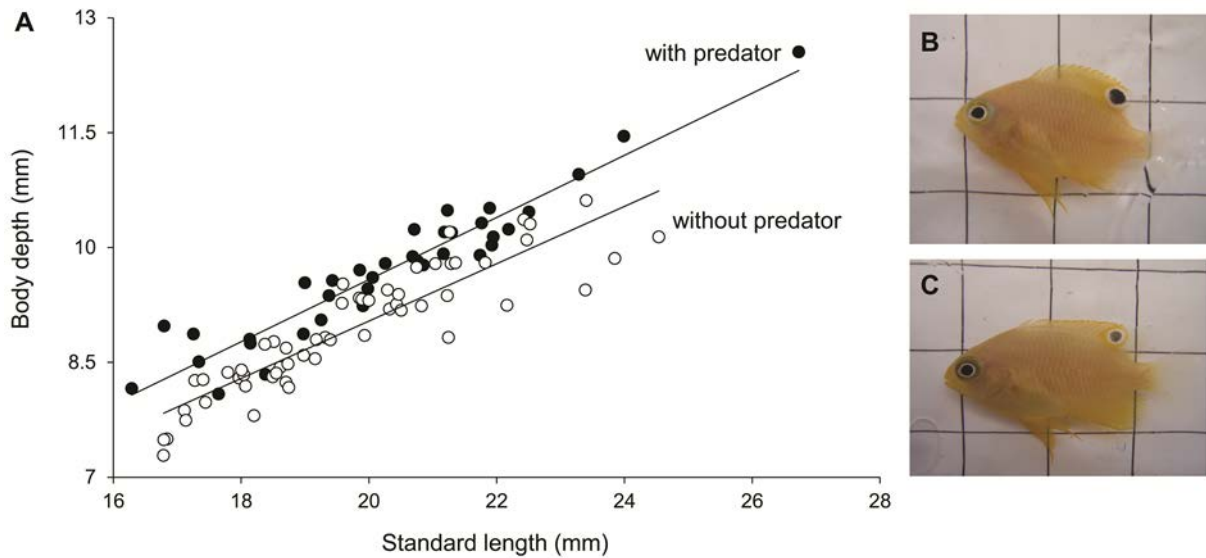
***Field survival***

After being photographed prey fish from each treatment were transferred onto individual patch reefs in the field. Patch reefs (25x15x20cm) were placed 2 meters away from the main reef and 3 metres apart and were made up of healthy *Pocillopora damicornis* colonies (a hard bushy coral), which is the preferred settlement site for *P. amboinensis*. Individual fish were transferred onto separate patch reefs and left to acclimate with a cage on top for 1 h, before having the cage removed (sample size ranged from 14-27 per treatment). Following the acclimation time, individual fish had their survival monitored twice a day (morning and afternoon) for 4 days after release by SCUBA divers (as per Chapter 2). Fish were assumed to be caught by a predator when missing from the patch reef. Cage controls that allowed fish to swim away found that there was no movement from patches, suggesting that when a fish was missing it was due to predation rather than migration.

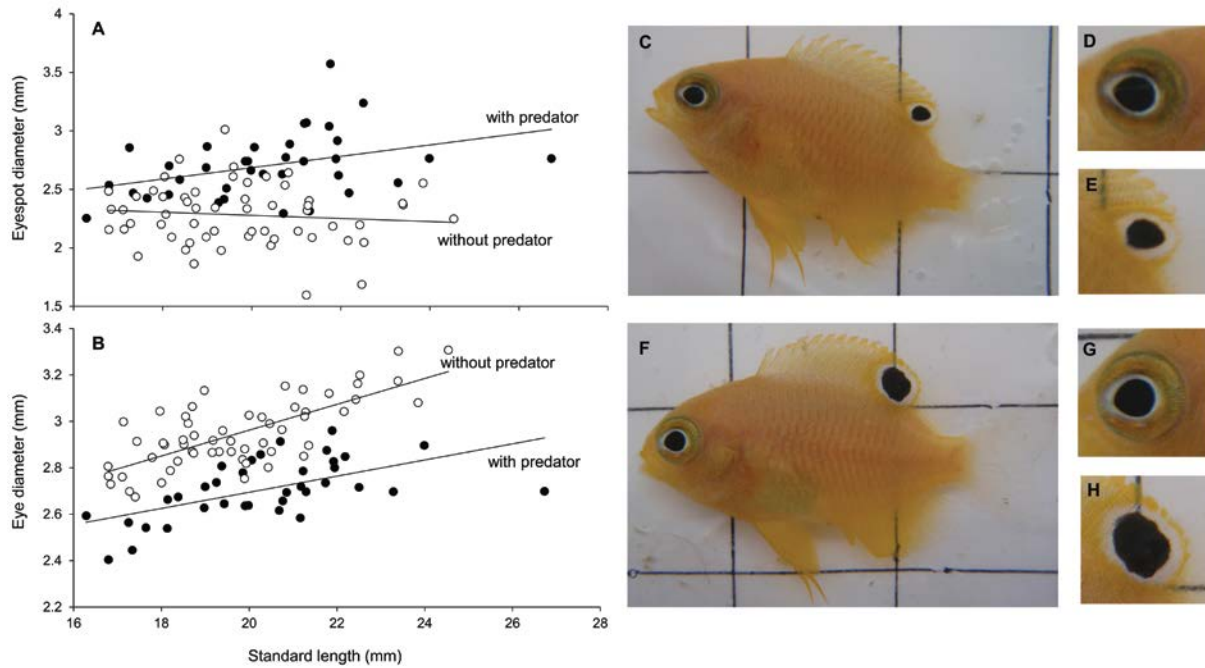
**3.4 Results*****Differences in morphology among treatments***

At week 0 there was no difference in morphological measurements (ANCOVA with standard length as covariate; body depth  $F_{2, 89} = 0.93$ ,  $P = 0.09$ ; size of ocellus  $F_{2, 89} = 0.47$ ,  $P = 0.63$ ; eye diameter  $F_{2, 89} = 0.65$ ,  $P = 0.52$ ) among fish from the three different treatments. After 6-weeks, prey that had been exposed to predator cues had significantly deeper bodies for any given length than fish from the two control treatments ( $F_{2, 89} = 33.14$ ,  $P < 0.001$ ; Fig. 3.2a-c) which, in turn, did not differ from one another ( $F_{1, 54} = 2.24$ ,  $P = 0.14$ ). Eyespot size (total diameter) was significantly different among treatments after a 6-week period, with prey exposed to predator cues having significantly larger eyespots for any given body length

compared to the control treatments (SL:  $F_{2,89} = 25.67$ ,  $P < 0.001$ ; Fig. 3.3a), which did not differ from one another ( $F_{1,54} = 0.19$ ,  $P = 0.077$ ). The visible part of the eye was also significantly different depending on treatment, with prey from predator treatments having significantly smaller eyes than individuals from the control treatments ( $F_{2,89} = 70.67$ ,  $P < 0.001$ ; Fig. 3.3b). There was no difference in eye size between the 2 control treatments ( $F_{1,54} = 0.13$ ,  $P = 0.72$ ).



**Figure 3.2.** Comparison of depth to length ratio. The relationship between standard length (SL) and body depth (BD) of *Pomacentrus amboinensis* when in the presence and absence of predators (A). Fish had significantly deeper bodies when exposed to predator cues (B) compared to the shallow bodied controls (C).

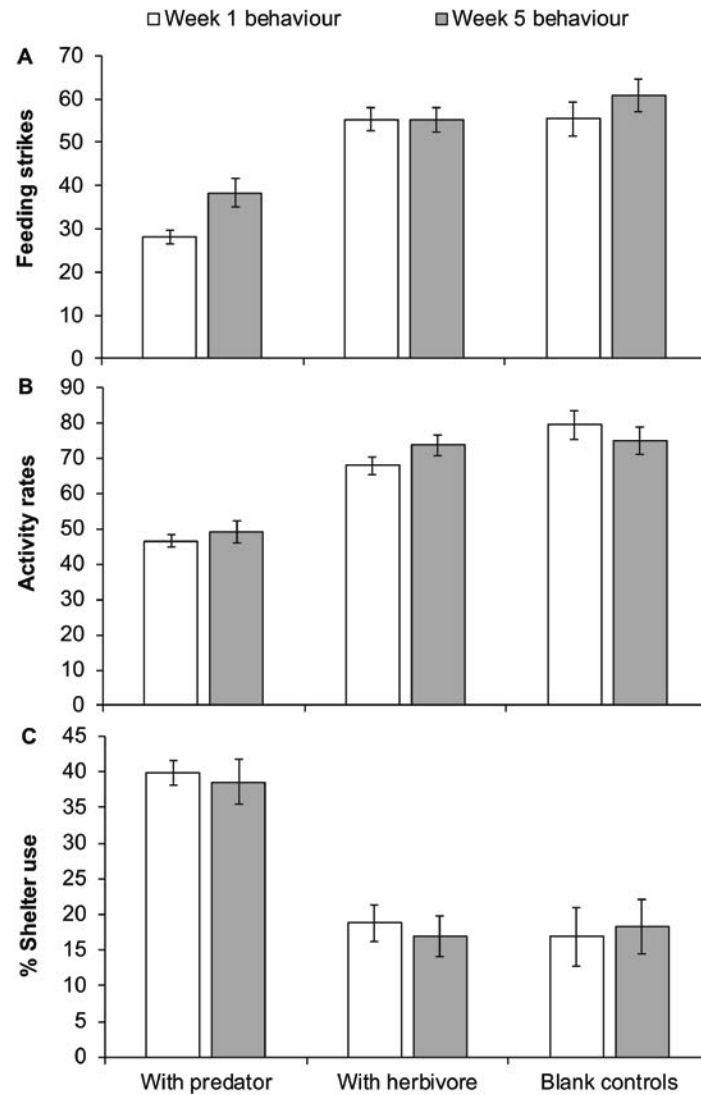


**Figure 3.3.** Relationships between eyespot size and eyeball size and body length. The relationship between standard length and eyespot diameter (A) and standard length and eye diameter (B) in *Pomacentrus amboinensis* in the presence and absence of predators. All prey fish exposed to predator cues over a 6 week period had significantly larger eyespots (F,H) and smaller eyes (F,G) than fish from the control treatments (C–E).

### *Differences in behaviour among treatments*

The multivariate analysis of variance revealed significant overall differences in behaviour depending on treatment after 1 week (MANOVA,  $F_{3,88} = 12.41$ ,  $P < 0.0001$ ). Univariate ANOVAs demonstrated that fish from the predator treatment foraged significantly less ( $F_{2,90} = 38.36$ ,  $P < 0.000$ ; Fig. 3.4a), were less active ( $F_{2,90} = 19.58$ ,  $P < 0.0001$ ; Fig. 3.4b) and spent more time in shelter ( $F_{2,90} = 29.10$ ,  $P < 0.000$ ; Fig. 3.4c) compared to individuals in the herbivore treatment and the seawater control after 1 week. After 5-weeks there was still a significant difference in overall behaviour of fish (MANOVA,  $F_{3,88} = 5.67$ ,  $P < 0.001$ ). Bite rate ( $F_{2,90} = 12.6$ ,  $P < 0.0001$ ; Fig. 3.4a) and activity ( $F_{2,90} = 12.09$ ,  $P < 0.0001$ ; Fig. 3.4b)

were significantly lower and time in shelter was significantly higher ( $F_{2,90} = 16.49$ ,  $P < 0.0001$ ; Fig. 3.4c) in fish exposed to predators than in fish exposed to herbivores or isolated.

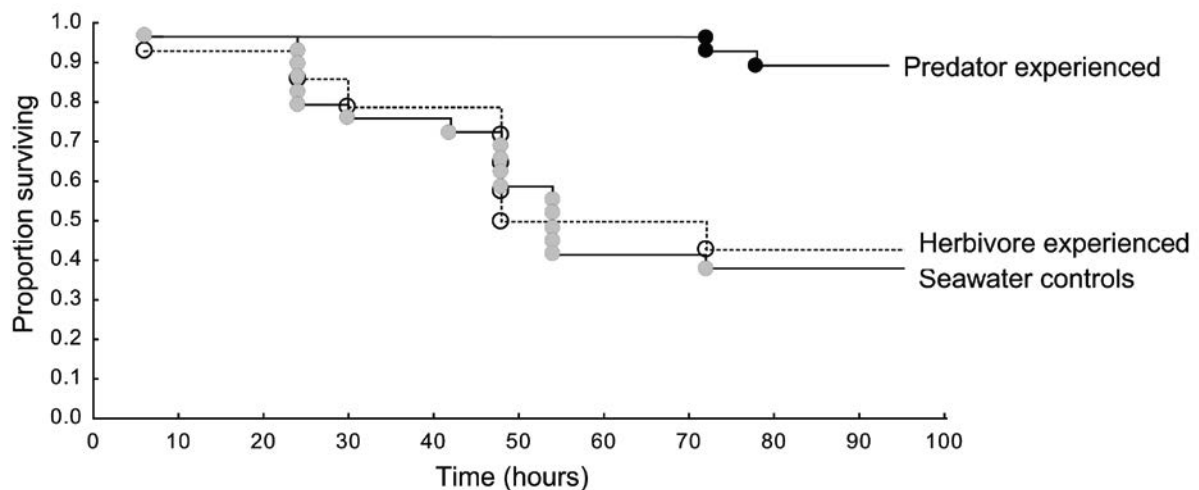


**Figure 3.4.** Predator presence influences prey behaviour. Fish exposed to predators foraged significantly less (A), displayed lower activity rates (B) and a significant increase in shelter use (C) compared to fish from the two control treatments after 1 week in the tanks. This pattern remained similar after 5 weeks. Bars are the standard errors around the mean from behavioural variables.

***Differences in survival among treatments***

Survival of prey when released in the field was affected by treatment ( $\chi^2_{2,0.05} = 19.88$ ,  $P < 0.001$ ; Fig. 4). Patterns of survival were established within the first 48h after release.

Treatments split into two groups, with one group containing fish that had experienced the herbivores for 6-weeks (40% were consumed within 48 hours) and fish from the seawater treatments (50% were consumed within 48 hours), all with similar and low survival. The second group contained fish that had experienced predators for 6 weeks, with high survival rates following release (no fish had been consumed after 72 hours and 89% of fish were still alive after 96 hours).



**Figure 3.5.** Survival patterns of fish from the three treatments. Survival curves (Kaplan Meier plot) of *P. amboinensis* in the field after laboratory exposure to predator cues, herbivore cues or no cues for a 6-week period. Fish were placed on small patch reefs along the edge of a reef and their survivorship was monitored 2 times a day for 4 days. Fish from predator treatment had the highest and similar survival.

### 3.5 Discussion

Here we show that the presence of a predator induced significant changes in morphology, colour patterns and behaviour in a juvenile damselfish. Prey exposed to predators for 6-weeks grew deeper bodies, developed larger eyespots and exhibited stunted eye growth compared to prey exposed to herbivores or those that were isolated from other fish. The increase in body depth has been found in previous studies and is considered a common prey response to gape limited predators in a multitude of freshwater taxa (Brönmark & Miner 1992; Relyea & Hovermann 2003; Chivers *et al.* 2008; Schoeppner & Relyea 2009). What is intriguing is the finding that juvenile prey grow larger eyespots and display smaller eyes when continuously exposed to predators. The large eyespot in the caudal area of prey taken together with the smaller eye in the head region give an impression of the true eye being present in the posterior end of the body, potentially confusing predators about the orientation of prey. Predators anticipate the direction prey will move as an attack is initiated, and a false eyespot may aid prey by causing the predator to misjudge the direction of the prey's escape (Powell 1982). Also, a prey attacked at the invulnerable caudal area can escape and survive (McPhail 1977; Powell 1982) however an attack on the head would damage vital parts allowing almost no chance of survival. McPhail (1977) demonstrated that caudal spots in a characid fish (*Hyphessobrycon panamensis*) deflect the aim of the characinoid predator *Ctenolucius beani*, as the majority of predators focused their attacks on the caudal area of prey fish that had an artificial caudal spot drawn on compared to fish with no spot. Clearly, prey with artificial eye spots escape predators more frequently than prey with no eyespots (Blest 1957; Smith 1976; McPhail 1977).

Findings from the current study suggest that false eyespots may be a direct short-term adaptation to the presence of predators, functioning to misdirect predator strikes and/ or

protect the head region from fatal attacks. If the increased growth of a larger false eyespot is associated with a cost such as the development of smaller eyes (and possibly poorer vision) it would only be advantageous to develop this type of anti-predator mechanism in certain circumstances, such as in predator rich environments. Flexibility and degeneration in eye growth has been found in other teleost fishes (Wallman & Winaver 2004), most notably in the Mexican cavefish *Astyanax mexicanus* (Avisé & Selander 1972; Yamamoto & Jeffrey 2000). This species has 2 morphological variations, a surface-dweller with pigmented eyes, and several different eyeless and depigmented cave-dwellers (Avisé & Selander 1972). It is evident that eye development is plastic and can evolve to suit certain environmental conditions, indeed in many young animals it is the visual stimuli received that influences eye growth patterns (Wallman & Winaver 2004). Ours is the first study to document predator-induced changes in the size of eyes and eye-spots in prey animals, however, others have documented that predation can result in selection for reduced eye pigments. For example, when comparing eye diameters in populations of the cladoceran, *Bosmina longirostris*, Zaret and Kerfoot (1975) found that prey living in areas associated with predators had significantly smaller eye-pigmentation diameter than *B. longirostris* from non-predation areas. They argue that fish predators select prey based on eye pigmentation area, and prey found in predator rich areas have evolved smaller eyes to minimize the probability of being caught.

Prey exposed to predators displayed more conservative behaviours, which included lower foraging rates, more time spent in shelter and reduced activity. Cautious behaviours remained largely intact even after 5 weeks in the predator treatment. The unchanged behaviours highlight the ecological relevance and importance of the predator stimulus. Reduced activity levels increases prey survival by making the prey less conspicuous to the predator (Werner & Anholt 1993). Reduced activity also saves energy, allowing individuals to allocate more into growth and/or development of predator-induced morphological defences



(Bordeau & Johansson 2012). The mere presence of predators is enough to suppress activity of prey, and it has recently been suggested that this lowered activity is responsible for the increased growth of fish as the energy conserved in the presence of predators is allocated to growth (Johansson & Andersson 2009).

Predator experience and subsequent morphological changes confer a survival advantage to prey in their natural environment, as predator experienced prey with larger eyespots and deeper bodies had drastically higher survival when stocked in the wild with control treatments suffering a 5-fold increase in mortality after 72 h on the reef. Results emphasize the importance of experience with predators to prey survival early on in life. The behavioural anti-predator response allows reduced detection by predators and the morphological defence and changed colour patterns may allow an improved ability to escape an attack. Deep bodies not only protect prey fish from gape-limited predators by deterring attacks (Brönmark & Miner 1992) but have also been found to improve speed, acceleration and manoeuvrability in both fish and amphibians (Dayton *et al.* 2005; Domenici *et al.* 2005). This is the first study to provide direct empirical evidence that eyespot size is increased upon exposure to predators. Predators also stunt eye growth, as there is reduction in the relative eye diameter over time. These morphological changes likely direct attacks away from the head region, protecting the more vulnerable regions of the body. Our results illustrate how phenotypically plastic development in prey morphology and coloration as well as conservative behaviours can result in dramatic increases in survival.

## Chapter 4

### **Lionfish have evolved to circumvent prey risk assessment abilities\***

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#### **4.1 Synopsis**

Invasive species cause catastrophic alterations to communities worldwide by changing the trophic balance within ecosystems. Ever since their introduction in the mid 1980's common red lionfish, *Pterois volitans*, are having dramatic impacts on the Caribbean ecosystem by displacing native species and disrupting food webs. Introduced lionfish capture prey at extraordinary rates, altering the composition of benthic communities. Here we demonstrate that the extraordinary success of the introduced lionfish lies in its capacity to circumvent prey risk assessment abilities as it is virtually undetectable by prey species in its native range. While experienced prey damselfish, *Chromis viridis*, respond with typical antipredator behaviours when exposed to a common predatory rock cod (*Cephalopholis microprion*) they fail to visibly react to either the scent or visual presentation of the red lionfish, and responded only to the scent (not the visual cue) of a lionfish of a different genus, *Dendrochirus zebra*. Experienced prey also had much higher survival when exposed to the two non-invasive predators compared to *P. volitans*. The cryptic nature of the red lionfish has enabled it to be destructive as a predator and a highly successful invasive species.

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\* This chapter appears in the journal PLOS ONE: Lönnstedt OM, McCormick MI (2013): Ultimate Predators: lionfish have evolved to circumvent prey risk assessment abilities. *PLOS ONE* 8 (10): e75781

## 4.2 Introduction

Invasive species are recognised as one of the greatest threats to marine biodiversity worldwide (Sala *et al.* 2000; Molnar *et al.* 2008), and have been found to cause catastrophic alterations to communities by changing the trophic balance within ecosystems (Cox & Lima 2006; Salo *et al.* 2007). Many of the invasive species that cause the most dramatic effects are predators. Release from their natural enemies and improper anti-predator behaviours by native prey can exacerbate the negative effects of the invasive species (Diamond & Case 1986; Colautti *et al.* 2004). Whether prey will react appropriately to an alien predator partly depends on the functional similarity and cues of the new predator to ones that are native to the system. This determines the establishment and spread of the invader and the level of impact on the unwitting community. Understanding the underlying aspects of the encounter between a non-native predator and its prey is key to understanding the success and impact of invaders (Sih *et al.* 2010). However, for many non-native predator species the reasons underlying their success are unclear because of the lack of information concerning the mechanisms that underlie their performance in their native communities.

Responding appropriately to predators requires prey to obtain accurate information on the trophic identity and intention of the predator (Chapter 2). Innate information can assist in the identification of predators and is most useful when the range of likely predators is small. Learned information augments innate knowledge and many studies have found that prey possess a variety of sophisticated anti-predator mechanisms whereby they can catalogue predators, reinforce memories or de-emphasise ('forget') information that is no longer relevant (Lima & Dill 1990; Griffin *et al.* 2000; Dixon *et al.* 2010; Ferrari *et al.* 2010a). Aquatic organisms in particular have been shown to have well developed mechanisms of identifying and assigning appropriate levels of risk to predator cues that operate through the olfactory and visual systems (Chivers & Smith 1998). When damage released skin extract

cues are coupled with the smell or sight of a novel predator, the subsequent smell or sight of the predator alone will elicit an antipredator response, through a process known as associative learning (Ferrari *et al.* 2010a). It is unclear whether or how non-native predators manage to circumvent this extremely efficient and rapid learning mechanism.

In the marine environment there are few examples of predator invasions that have been as destructive to the native marine fauna as introduction of the common lionfish, *Pterois volitans*, to the tropical and subtropical east coast of the United States and Caribbean basin. Native to the Indian and Western Pacific Oceans, the lionfish was introduced to Florida in the mid 1980's (Ruttenberg *et al.* 2012) and has become widespread throughout the Western Atlantic from Florida Keys to Cape Hatteras and throughout the Caribbean basin (Schofield 2009; Hines *et al.* 2011). The effects of the introduced lionfish are reverberating through the ecosystem, as these hyper-successful nuisance invaders have already altered recruitment patterns, abundance and species composition on many of the invaded reefs (Albins & Hixon 2011; Betancur 2011). While many aspects of the trophic ecology of the invading populations have recently come under intense scrutiny (Côté & Maljković 2010; Green *et al.* 2010; Munoz 2011; Cure *et al.* 2012; Jud & Layman 2012; Layman & Allgeier 2012), little is known of the ecology of the species in its native habitat. By obtaining a detailed understanding of the encounter between the lionfish predator and its native prey we can better understand why these predators may have become so successful in their novel system.

In this study we examined how experienced and naïve prey individuals (juvenile damselfish, *Chromis viridis*, hereafter *Chromis*) responded to different cues that signify the presence of three different predators. In a series of three experiments we tested whether *Chromis* were able to learn that the chemical cues, visual cues or combined cues of the red lionfish, *P. volitans*, represented a threat. Responses were compared to those prey that had been exposed to cues from a common predatory rockcod (*Cephalopholis microprius*) or a

lionfish of a different genus (zebra lionfish, *Dendrochirus zebra*). To determine the role learning plays in influencing survival, naïve and experienced *Chromis* were placed together with one of the three predators for 48h and monitored for survival. We show that the predatory success of the red lionfish lies in its capacity to circumvent prey risk assessment abilities as it is virtually undetectable by a common prey species in its native range. The effectiveness of this ability to block innate antipredator responses of prey has most likely contributed to the ecological success of *P. volitans* in invaded regions.

### 4.3 Materials and Methods

#### *Study Species and Sampling*

The experimental study was conducted at Lizard Island Research Station (14°40'S, 145°28'E), on the northern Great Barrier Reef, Australia during September-December 2012. The blue-green Chromis, *Chromis viridis* (Pomacentridae), is a site-faithful damselfish that is very common on the shallow reefs of the Indo-Pacific. Juvenile *Chromis* are subject to a variety of resident and transient predators. Individuals ( $12.7 \pm 0.4$  mm mean standard length  $SL \pm SE$ ) were collected as newly settled juveniles from the reef on SCUBA and maintained (in groups of 20 individuals) in 35L flow-through aquaria with shelter and fed *Artemia* nauplii twice a day. Common lionfish, *Pterois volitans* ( $129.4 \pm 3.9$  mm SL), zebra lionfish, *Dendrochirus zebra* ( $126.9 \pm 3.2$  mm SL) and the brown rockcod, *Cephalophalis microprius* ( $129.8 \pm 4.6$  mm SL) were collected from the fringing reefs surrounding the island and brought back to the research station. *Cephalophalis microprius* is a common predator along the Great Barrier Reef, often found feeding on juvenile damselfish (Beukers-Stewart & Jones 2004). *Dendrochirus zebra* is a much less abundant component of the reef community than other small predators, but is nonetheless more common in shallow reef areas than other members of the family Scorpaenidae (Caley 1993). The least abundant of the three predators

is *P. volitans* which is native to the GBR, but rarely seen. All predators were maintained individually in 15L flow through aquaria and fed juvenile fish of the family Apogoniidae. Other studies have shown that Apogoniids do not have damage-released alarm cues that are responded to by damselfishes (Lönnerstedt & McCormick 2011a,b). The research was carried out in accordance with the Australian Code of Practice for the care and use of animals for scientific purposes. This work was conducted with the approval and under the supervision of Lizard Island Research Station and James Cook University ethics guidelines (Permit Number: A1593). All procedures were conducted with care to avoid any pain or suffering in animal subjects.

### ***General Experimental Design***

When the epidermis of damselfish is damaged they release a species-specific chemical (a chemical alarm cue) that elicits an antipredator response in conspecifics (Chivers & Smith 1998; Chapter 2). When this skin extract cue is coupled with the smell or sight of a novel predator, the subsequent smell or sight of the predator alone will elicit an antipredator response, through a process known as associative learning (Chivers & Smith 1994; Ferrari *et al.* 2010a). Using associative learning *Chromis* were taught to recognize chemical, visual or a combination of chemical and visual cues of three predators. To test the idea that associative learning plays an important role in responding to and subsequently surviving predator encounters half (random allocation) of the *Chromis* juveniles were exposed to the chemical, visual or a combination of visual and chemical threat cues paired with conspecific skin extracts (true conditioning resulting in experienced individuals), while the other half were given the threat cue paired with seawater (false conditioning resulting in inexperienced individuals). The experimental procedure was therefore a two-step process that first involved a conditioning phase where fish were exposed to cues of injured conspecifics (true

conditioning) or seawater (pseudo conditioning) paired with those of a predator and second, a testing phase, where fish were exposed to the appropriate cue and had their behaviour assessed. The study was conducted as a series of three experiments.

Following conditioning *Chromis* were placed individually into 15 L aquaria (38x27x24 cm) and allowed to acclimate overnight. The basic tank set up included a 2 cm depth of coral sand and a small piece of healthy live hard coral (*Pocillopora damicornis*) for shelter, while a single air-tube was placed at the other end. A second tube was fixed to the aeration tube and allowed the introduction of *Artemia* food or chemical cues. The air facilitated the distribution of the cues throughout the tank; dye trials showed it took  $31.4 \pm 0.9$  s. Prior to the start of the trial, the water flow was stopped and 5ml of *Artemia* sp (~ 800) nauplii were added to the aquaria to stimulate feeding. The behaviour of a single *Chromis* was recorded for a 4 min pre-stimulus period. Immediately following the pre-stimulus period, a further 5ml of *Artemia* was added and fish were exposed to the appropriate cue treatment. The behavioural response to experimental treatments was quantified by recording: total number of feeding strikes (successful or otherwise), activity (quantified as the number of times a fish crossed a line on the grid (3 x 3cm) suspended over the tank), and total time (s) spent within the branches of the coral shelter. Data were analysed as the difference between the magnitude of behaviours before an experimental stimulus and after exposure to a stimulus (post-pre). Owing to the interdependency of the three behaviours, we analysed the three variables together using a one-way MANOVA, followed by univariate ANOVAs for each behavioural variable. Subsequent Tukey's post hoc tests were performed to assess the differences in behavioural responses between the different treatments.

***Learning to recognize predator cues***

Our first experiment investigated the ability of juvenile *Chromis* to learn to respond to predator odour alone following the conditioning phase. *Chromis* were conditioned with 20ml of the odour of either *P. volitans*, *D. zebra*, or *Ce. microprion*, paired with either 10 ml of seawater (pseudo-conditioning) or 10 ml of conspecific skin extract cues (true conditioning) (Chivers & Smith 1994; Bosiger *et al.* 2012). Predator odour was obtained by leaving individual fish predators in separate 68-L aerated flow-through plastic holding tanks filled with 30-L of aerated seawater. Two pairs of each predator was placed on staggered alternating cycles of 12 h water flow on and approximately 56 h water flow off, to ensure that predator odour was consistently available for experimental use, and stress was reduced. Following the cessation of water flow for 56 h, predator odour was prepared by drawing up the predator water into a syringe. Predator water was drawn from each predator tank within a pair to avoid intraspecific predator variability effects (a protocol used previously; Bosiger *et al.* 2012). Skin extracts were prepared following methods of Lönnstedt *et al.* 2013a; Chapter 5). The following day *Chromis* were exposed to the predator odour that they had been conditioned with on the previous day and their behaviour was assessed.

The second experiment examined how well *Chromis* learned to respond to the visual stimuli of the three different predators (*P. volitans*, *D. zebra* or *Ce. microprion*). Individual predators were placed in clear ziplock bags (20 x 20 cm) with aerated seawater and placed in 15L aquaria containing groups of prey fish (2-4 individuals). Bags were large enough to allow the predators to move around freely (and extend their pectoral fins) and they often attempted to strike at prey through the bag. *Chromis* were either pseudo-conditioned with seawater or genuinely conditioned with cues from injured conspecifics to recognize one of the three predators. The next day, fish that had been conditioned in groups were placed individually in aquaria and tested for a response to the exposure of the relevant predator.



Predators were placed individually in clear zip-locks bag containing water and a thin layer of gravel (ensuring bags settled on the bottom of the tank) and gently introduced at the end of the tank on the opposite side of the coral shelter. The bag was oriented such that the side of the predator was facing the *Chromis*.

Lastly, we tested responses of *Chromis* to the combination of chemical and visual cues of the three predators. Here, juvenile prey were placed in groups of 2-4 individuals in 15-L tanks and exposed to 20ml of predator odour and the predator inside of a zip-lock bag paired with either 10ml of seawater or 10ml of conspecific skin extract. After conditioning individual *Chromis* were acclimated overnight in experimental aquaria and tested for a response to the simultaneous exposure of the appropriate predator odour and visual stimuli the following day.

### ***Survival trials of prey***

The mortality rates were compared among *Chromis* from the six conditioning treatments [three predators (*P. volitans*, *D. zebra* or *Ce. microprion*) by two conditioning treatments (pseudo and true)]. Following conditioning with the pairing of olfactory and visual cues of the relevant predator, 4-6 randomly chosen individuals from the same conditioning treatment were placed in flow-through mesocosm pools (111 cm diameter, 45 cm high, 368-L). Mesocosms were set up as natural habitats containing a 2-cm deep layer of coral sand substrate, four air-stones, and a 30x30x20cm coral shelter (hard bushy coral; *Pocillopora damicornis*) in the centre. Sea water was pumped directly from the ocean so it followed natural temperature fluctuations. After one hour a predator (either *P. volitans*, *D. zebra* or *Ce. microprion*), present in a standing acclimation tube since the initiation of the trial, was released into the aquarium and survival of prey fish was monitored every 3 hrs for 48 hrs. Survival (up to 48 h) of fish was compared using multiple-sample survival analysis using a

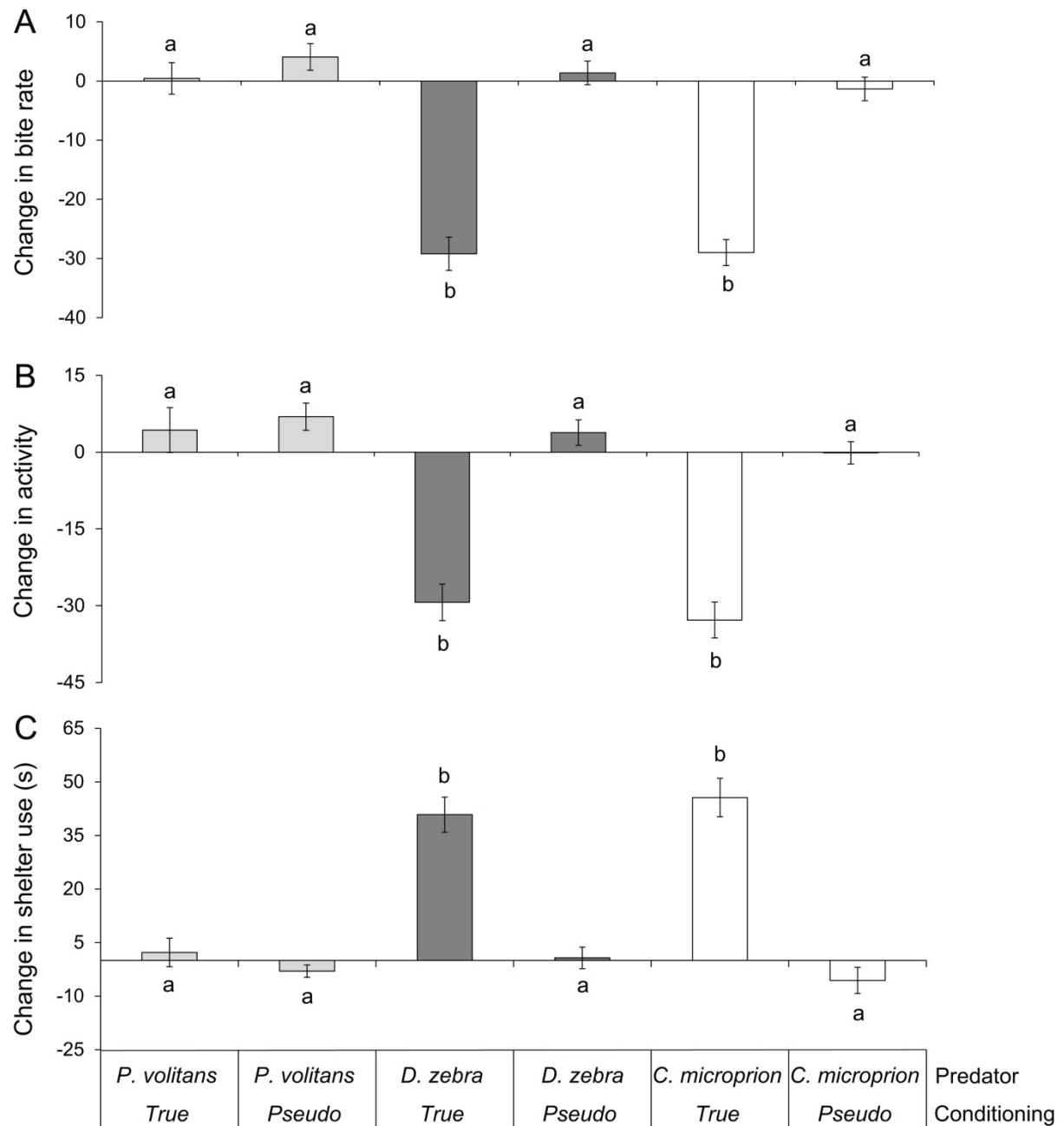
Cox's proportional hazard model (STATISTICA v. 10.0). Survival curves of experienced and inexperienced *Chromis* exposed to the three predators were calculated and plotted using the Kaplan–Meier product–limit method.

### 4.3 Results

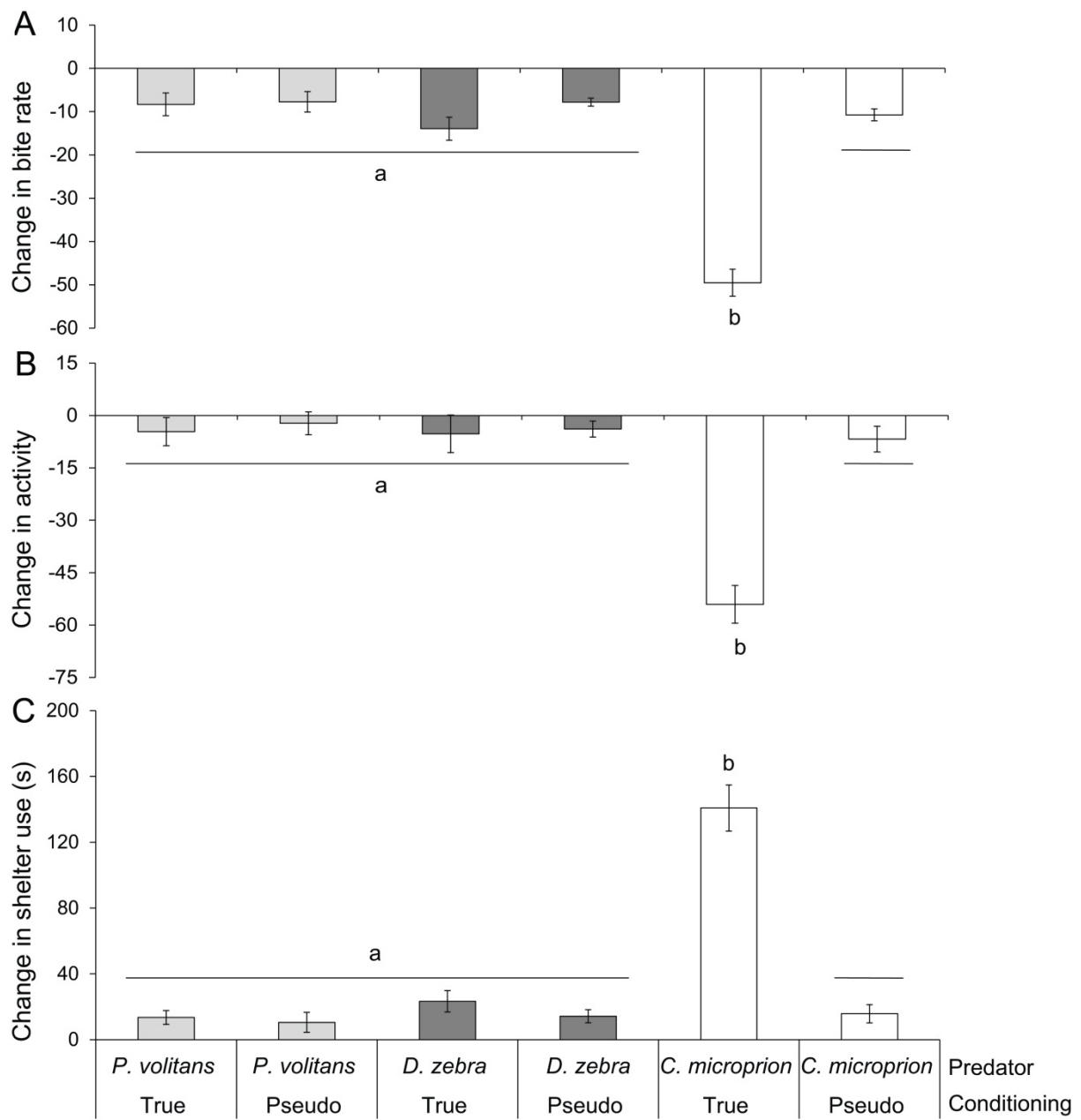
Behavioural responses of *Chromis* to predators differed significantly depending on both type of cue and the species of predator they were exposed to (MANOVA: Olfactory, Pillai's  $\text{trace}_{6,174} = 0.5$ ,  $P < 0.0001$ ; Visual, Pillai's  $\text{trace}_{6,178} = 0.6$ ,  $P < 0.0001$ ; Combination, Pillai's  $\text{trace}_{6,180} = 0.5$ ,  $P < 0.0001$ ). *Chromis* that had been conditioned to learn *Ce. microprion* cues displayed strong anti-predator responses upon presentation of all threat cues associated with this predator, with the strongest responses seen when prey were exposed to chemical and visual cues simultaneously (Fig. 4.1-3). When exposed to any *Ce. microprion* cue, experienced prey foraged less (MANOVA: Olfactory  $F_{2,88} = 20.4$ ,  $P < 0.0001$ ; Visual  $F_{2,90} = 39.4$ ,  $P < 0.0001$ ; Combination  $F_{2,91} = 31.9$ ,  $P < 0.0001$ ), reduced activity levels (Olfactory  $F_{2,88} = 14.9$ ,  $P < 0.0001$ ; Visual  $F_{2,90} = 19.5$ ,  $P < 0.0001$ ; Combination  $F_{2,91} = 16.3$ ,  $P < 0.0001$ ) and spent more time in shelter (Olfactory  $F_{2,88} = 18.8$ ,  $P < 0.0001$ ; Visual  $F_{2,90} = 43.2$ ,  $P < 0.0001$ ; Combination  $F_{2,91} = 38$ ,  $P < 0.0001$ ) compared with *Chromis* that had no prior experience of the *Ce. microprion* (Fig. 4.1-3).

*Chromis* with prior experience of *D. zebra* responded to the odour of the predator with reduced activity and feeding, as well as an increase in shelter use compared with inexperienced prey (Tukey's HSD test:  $P < 0.0001$ ; Fig. 4.1). There was no response to the visual appearance of *D. zebra* regardless of experience (Tukey's HSD:  $P > 0.05$ ; Fig. 4.2). The simultaneous presentation of *D. zebra* scent and visual cue resulted in a similar anti-predator response in experienced prey compared to the response to olfactory and visual cues alone (Tukey's HSD:  $P > 0.05$ ; Fig. 4.3). Regardless of experience, there was no response of

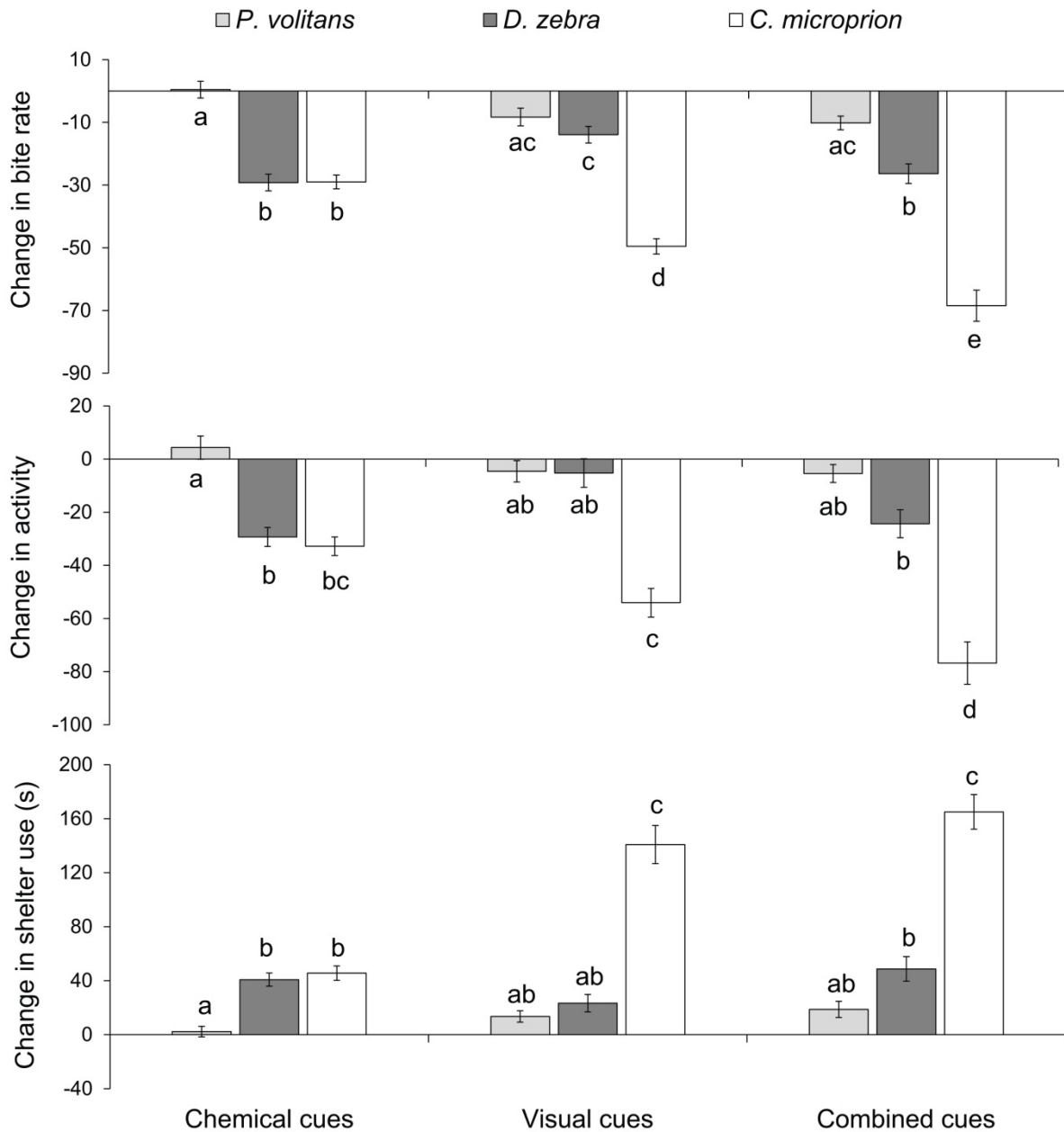
prey to any predator cue associated with the common lionfish, *P. volitans* (Tukey's HSD:  $P > 0.05$ ; Fig. 4.1-3). When exposed to *P. volitans* scent, visual presence or the combination of these cues prey did not appear to visibly react; they continued foraging at a similar rate as pre-exposure.



**Figure 4.1.** Behavioural responses of inexperienced and experienced juvenile *Chromis viridis* to olfactory cues of three different predators. Experienced prey fed less (A), lowered activity rates (B) and increased shelter use (C) when exposed to olfactory cues of *Dendrochirus zebra* and *Cephalopholis microprion* ( $n=16-19$ ). Letters indicate significant groupings.

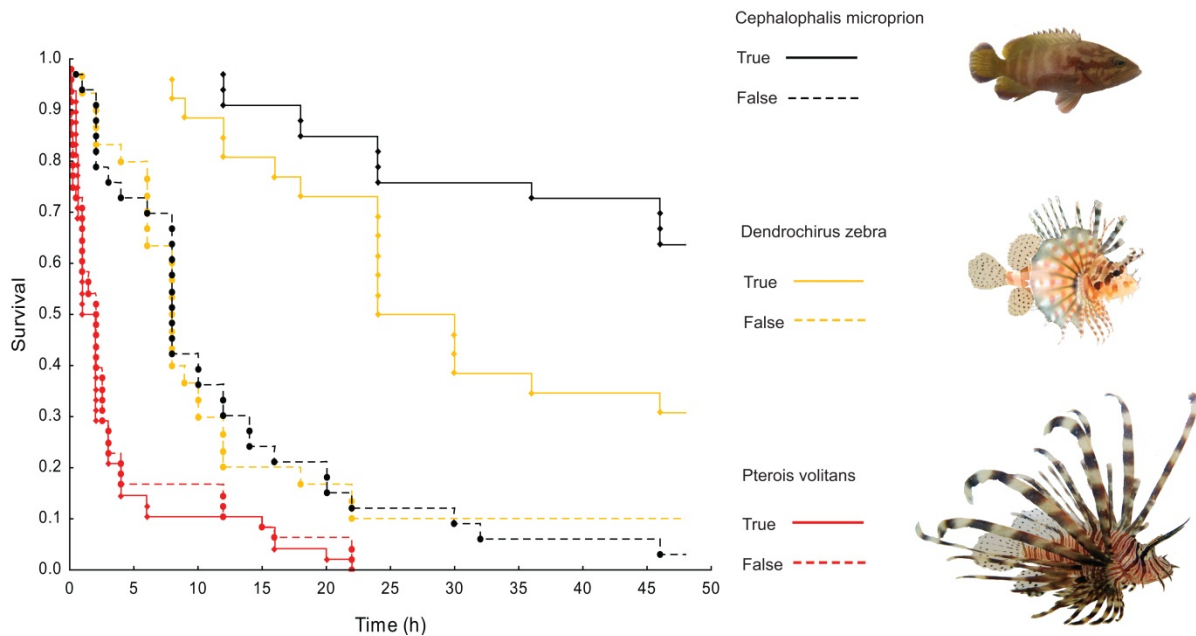


**Figure 4.2.** Behavioural responses of inexperienced and experienced juvenile *Chromis viridis* to the visual presentation of three different predators ( $n= 16-18$ ). Antipredator responses were only seen in experienced prey exposed to *Cephalopholis microprion*. Prey reduced foraging (A), lowered activity rates (B) and increased shelter use (C). Letters indicate significant groupings.



**Figure 4.3.** Behavioural responses of experienced juvenile *Chromis viridis* to the exposure of olfactory, visual and a combination of visual and olfactory cues of three different predators ( $n=16-19$ ). Prey did not respond with antipredator behaviours when exposed to any threat cues from *Pterois volitans*. Antipredator responses were seen when prey were exposed to olfactory cues of *Dendrochirus zebra*, but not to visual cues alone. When exposed to olfactory and visual threat cues of *Cephalopholis microprion* prey responded with reduced foraging (A), activity (B) and increased shelter use (C), and there was an additive effect when both cue sources were present. Letters indicate significant groupings.

Survival trials of *Chromis* revealed a strong influence of both experience and type of predator (Kaplan-Meier survival plot  $\chi^2_5 = 133$ ,  $P < 0.0001$ ; Fig. 4.4). Regardless of experience, all prey exposed to *P. volitans* were consumed within 24 hours after release with the majority (true conditioning = 79%, N=49; false conditioning = 77%, N=48) being eaten within the first 3 hours. Experienced prey placed together with *Ce. microprion* had a significantly higher survival with only 33% of individuals being consumed after 48 hours, while 94% of the inexperienced prey were eaten after 48 hours (N=33). Experienced *Chromis* exposed to *D. zebra* displayed an intermediate survival pattern with 43% uncaught after 24 hours and close to 30% still alive after 48 hours (N=36) while only 7% of the inexperienced prey ( $n=39$ ) remained uncaught after 48 hours.



**Figure 4.4.** Survival curves (Kaplan–Meier plot) of experienced (true conditioned; exposed to the combination of predator visual presence, odour and conspecific skin extracts) and inexperienced (false conditioned; exposed to the combination of predator visual presence, odour and seawater) *Chromis viridis* to three different predator species ( $n=33-49$ ).

## 4.4 Discussion

Our results show that the response of damselfish prey to three different predators greatly differs depending on predator species, threat signal (olfactory, visual or a combination of both) as well as previous experience. Experienced prey will respond strongly to a rockcod threat regardless of the cue, while the physical appearances of the two lionfish species prevented prey from detecting their presence, instead labelling them as non-threatening animals. In fact, irrespective of previous experience, damselfish prey did not respond to any signals, be they visual or chemical cues from the common lionfish, *P. volitans*. Survival patterns of prey emphasized the importance of behavioural responses, as damselfish with previous experience of *C. micropirion* had learnt to evade the predator, displaying significantly higher survival rates than inexperienced prey or those exposed to either lionfish species. Prey placed together with *P. volitans* did not survive long regardless of experience, highlighting the efficiency of the highly cryptic nature of the common lionfish. Experienced prey placed together with *D. zebra* displayed intermediate survival patterns, suggesting that at least some prey individuals are able to learn to avoid the predator through olfactory cues alone and/or a combination of olfactory and visual predator cues.

This study demonstrates that *P. volitans* have evolved into highly successful predators, with a common damselfish prey unable to recognize body-shape, coloration or scent of red lionfish in their native ranges. The ecological importance of *P. volitans*' ability to circumvent prey risk assessment can be seen in the successful invasion of this species in the Caribbean. This strategy of preventing prey detection, together with life history characteristics such as high reproductive output, rapid range expansions into many different habitats as well as lack of natural predators and/or parasites, helps explain their extraordinary success in colonising new habitats and in devastating native prey populations (Cox & Lima 2006; Sol *et al.* 2012). A similar pattern can be seen in another highly successful invader, the

ctenophore *Mnemiopsis leidyi*, a planktonic predator that is endemic to Atlantic coasts of North and South America. It has invaded several different regions from the Black Sea in the early 1980s through to the fairly recent invasion in the Baltic and North Sea (Colin *et al.* 2012), where it has altered the ecosystems by decimating zooplankton stocks, often followed by trophic cascades (Kideys 2002). Its ecological success is attributed to its highly efficient feeding technique whereby it generates a hydrodynamically silent current that entrains and transports prey while remaining undetected (Colin *et al.* 2012). *Mnemiopsis leidyi*, like *P. volitans*, is a large, slow swimming predator that greatly benefits from remaining concealed until after encountering prey, allowing them to become hyper-successful nuisance predators in introduced regions. Furthermore, alien predators that are more generalised in their feeding habits can exert keystone effects because of their complex roles in community dynamics. Lionfish prey upon fishes from a variety of functional groups (herbivores, detritivores and small predators alike) as well as numerous invertebrates, so their impact spans multiple trophic levels therefore having particularly widespread and detrimental effects on the communities they invade (Caley 1993; Green *et al.* 2010).

Our results illustrate the importance of prey detecting and appropriately responding to predator cues, as the predators responsible for the highest prey removal rates were visually and chemically concealed from prey. The appearance of lionfish differ from most other fish predators in that they have an extravagant body shape characterized by long dorsal spines, greatly expanded pectoral fins, as well as several filamentous appendages above and below their eyes and mouth. Taken together with their disruptive body markings (bright white spots throughout, horizontal stripes on body and vertical stripes on fins), the general outline of lionfish may function to continually confuse and lure prey as they are unable to detect and/ or recognize the lionfish as a predator (Ruxton *et al.* 2004). In terrestrial carnivores, vertical and horizontal stripes provide camouflage by background matching thus allowing the predator to



hunt prey undetected (Ortolani 1999). The lack of prey responses to *P. volitans*' olfactory cues may be due to chemical camouflage, where the predator gives off a scent that labels it as non-threatening. Many terrestrial insects display this type of mimicry, which allows them to enter prey territories undetected (Vander Meer & Wojkic 1982) or hide from their natural enemies (Akino *et al.* 2004). In many cases the chemically cryptic organisms secrete specific substances that hide their presence either through passive (Vander Meer & Wojkic 1982) or active mechanisms (Fishlyn & Phillips 1980). A less likely explanation is that *P. volitans* is odourless, having a chemically insignificant profile that allows them to merge with the background environment. Whatever the mechanism of olfactory crypsis, the technique is highly effective at allowing these predators to get very close to their prey. This coupled with the visual crypsis and toxic spines make them a dangerous and skilful predator adept at invading new regions.

While the novel predator-crypsis found in the present study may explain in part why red lionfish are so successful as predators, it does not explain their large population sizes as invasive species in the Caribbean ecosystem (Schofield 2009; Ruttenberg *et al.* 2012). There is very little information on the ecology, behaviour and life history of *P. volitans* in their native range that can assist us in understanding their extraordinary success in invaded regions. This lack of information is partly because these fish are highly cryptic when in low densities, with crepuscular or nocturnal activity patterns, and are therefore difficult to observe (Cure *et al.* 2012; Layman & Allgeier 2012). Currently, we can only speculate as to the underlying causes of the rarity of red lionfish in their native distribution. Possible causes include a release from their natural enemies, or environmental and biological conditions that influences their reproductive ecology or larval survival. Like most teleost fishes, lionfish are highly fecund (Albins & Hixon 2011), however recruit surveys that are conducted along the Great Barrier Reef hardly ever record red lionfish juveniles. This suggests that population

sizes may be constrained by processes that affect some aspect of the early life history from gamete viability and embryo development through to larval growth and survival. As red lionfish continue to invade the Caribbean it is important that invasion and evolutionary ecologists maximize their efforts in understanding lionfish ecology in their native ranges.

Our findings suggest that lionfish are one of the definitive fish predators. Their feeding success is not achieved through speed and surprise, but through a unique form of crypsis that circumvents the well-established mechanism whereby prey fishes learn about their predators (e.g. Ferrari *et al.* 2010a). The generality of these risk assessment mechanism (Chivers & Smith 1998; Ferrari *et al.* 2010a) suggests that the results should be broadly applicable to most fish prey species. Further research is warranted on how lionfish achieve this crypsis. Informed management and conservation strategies require a better understanding of how their efficient feeding strategy has promoted invasion through the interrelationship between foraging success and other aspects of their ecology, such as enhanced fecundity and offspring survival.

## Chapter 5

### **Degraded environments alter prey risk assessment\***

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#### **5.1 Synopsis**

Elevated water temperatures, a decrease in ocean pH, and an increasing prevalence of severe storms have led to bleaching and death of the hard corals that underpin coral reef ecosystems. As coral cover declines, fish diversity and abundance declines. How degradation of coral reefs affects behaviour of reef inhabitants is unknown. Here, we demonstrate that risk assessment behaviours of prey are severely affected by coral degradation. Juvenile damselfish were exposed to visual and olfactory indicators of predation risk in healthy live, thermally bleached, and dead coral in a series of laboratory and field experiments. While fish still responded to visual cues in all habitats, they did not respond to olfactory indicators of risk in dead coral habitats, likely as a result of alteration or degradation of chemical cues. These cues are critical for learning and avoiding predators, and a failure to respond can have dramatic repercussions for survival and recruitment.

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## 5.2 Introduction

Global Environmental Change (GEC) is having major impacts on all of the world's ecosystems and is viewed as one of the biggest threats to the natural world (Meehl *et al.* 2007). The earth's climate is warming at a far greater rate than at any time during the past 10,000 years, in part due to greatly increased emissions of atmospheric CO<sub>2</sub> (Walther *et al.* 2002). On a population level, GEC is expected to reduce both species abundance and diversity, in some cases resulting in local or even global extinctions (Hughes 2000; Williams *et al.* 2003; Munday 2004; Parmesan 2006). In addition to human induced threats, animals are continually exposed to a broad array of risks and dangers in their natural environment. The number of dangers an animal will face throughout its life are numerous and varied (e.g. parasites, bacterial infections, con- and hetero-specifics), but one threat that may end in instant death if ignored is predation (Sih 1984; Kavaliers & Choleris 2001). It is the decisions that individuals make under the threat of predation that decide their fate and the genes they hold, in this way indirectly shaping prey community composition (Abrams 2000). Predators and their prey must continuously react and adapt to their environment, but in today's changing world we know very little about how climate induced habitat change will affect the intricate, and at times subtle, relationships between predators and their prey (Ferrari *et al.* 2011a).

Impacts of GEC on the marine ecosystem include rising sea surface temperatures, changing hydrodynamic regimes and altered ocean chemistry (Munday *et al.* 2009; Roessig *et al.* 2004). In the ocean, coral reefs are among those habitats that are most likely to be adversely affected by climate change (Hughes *et al.* 2003; Hoegh-Guldberg *et al.* 2007). Coral reef environments represent one of the world's most biologically diverse ecosystems, however, very little is known of the interactions between predators and their prey that have

shaped this astonishing biodiversity. Although these habitats have become popular systems for examining various aspects of the effect of climate change on behavioural interactions, the subject is still very much in its infancy (e.g. McCormick 2009; Dixon *et al.* 2010; Munday *et al.* 2010; Ferrari *et al.* 2011a,b). Decreases in ocean pH along with increases in water temperatures and the prevalence of severe storms have led to bleaching and death of the live hard corals that underpin coral reef ecosystems (Hughes *et al.* 2003). As coral reefs degrade from live healthy coral to rubble, fish diversity and abundance declines (Graham *et al.* 2006). The majority of adult reef fishes are not directly dependent on live corals for survival (Pratchett *et al.* 2008). Despite this, whole fish communities have seen dramatic changes and declines following loss of coral cover suggesting a widespread reliance on the coral reef habitat (Jones *et al.* 2004). The wider effects of coral bleaching on fish communities, and in particular on the complex interrelationships between predators and prey remain poorly understood and research is required to identify the underlying behavioural processes that are driving the declines in abundance of fishes.

Coral reef fishes have complex life histories incorporating a widely dispersive larval phase, lasting from weeks to months, followed by settlement to the benthic reef environment. During this larval-juvenile transition, mortality rates are extremely high, primarily driven by predation (more than 50% are eaten in the first 48 hours; Almany & Webster 2006). Successful identification of predators requires the newly settled larvae to detect olfactory and visual signs of danger all within a highly complex environment containing numerous different stimuli. Olfaction is particularly important at night when the larvae settle and in the highly complex habitats of coral reefs that limit visual abilities and assist cryptic predators (McCormick 2009, Vail & McCormick 2011). At this time chemical alarm cues from the damaged skin of prey play an important role in the identification and avoidance of predators (Leduc *et al.* 2010, Chapter 2). Recent studies have suggested that GEC is threatening to

perturb the delicate balance between predators and their prey (Ferrari *et al.* 2011b). Munday *et al.* (2010) found that newly settled damselfish (*Pomacentrus wardi*) that had their olfactory sense disrupted through exposure to increased CO<sub>2</sub> levels had a 5 to 9 fold increase in mortality compared to control fish when placed on the reef. Similarly, Ferrari *et al.* (2011b) showed a 5 to 7 fold increase in mortality for another damselfish (*P. chrysurus*) exposed to elevated CO<sub>2</sub>. Furthermore, it has been suggested that coral dwelling damselfish (family Pomacentridae) are more susceptible to predation in bleached coral as the ability of prey fish to camouflage is diminished due to the increased perception of colorful prey fishes against the white background of the coral (Coker *et al.* 2009; McCormick 2009, 2012).

The goal of the present study was to determine how predator risk assessment abilities of a naïve coral reef fish prey (*Pomacentrus amboinensis*) were affected by three different coral reef habitats, which represent a cline from healthy to degraded coral. Specifically, we undertook laboratory and field experiments to examine whether three different stages of coral (live healthy, thermally bleached, or degraded algae-covered dead coral) affected prey responses to: (1) conspecific damage-released chemical cues, (2) visual cues of a predator, (3) a combination of visual and chemical cues. Further experiments addressed the mechanisms responsible for the impaired chemosensory responses in degraded coral habitats. Evidence suggests that the process of coral degradation will not only affect prey directly through changes in their resource base, but indirectly through modifications of the cues they use to assess predation risk.

### **5.3 Materials and Methods**

#### ***Study species and collections***

Experiments were conducted at Lizard Island (14°40'S, 145°28'E), northern Great Barrier Reef (GBR), Australia from October to November 2010. The ambon damselfish, *Pomacentrus amboinensis*, were used as a model prey species in all experimental trials. *P. amboinensis* is a common fish within coral reef fish communities in the Indo-Pacific (especially on the GBR) and settle to a wide range of habitats but are found in highest densities in shallow sandy areas on live corals (McCormick *et al.* 2010). Their pelagic larval phase lasts between 15-23 days and the new recruits are readily collected overnight with light traps that have been moored just outside the reef (see Meekan *et al.* 2001 for design). Fish used for the present studies were all caught in light traps and brought back to the Lizard island research station at dawn and placed in 60 L flow-through seawater holding tanks (densities of ~50 fish/ tank). Fish were fed twice daily with newly hatched *Artemia* sp. nauplii ad libitum to allow for recovery from the stress of capture. Juvenile *Apogon doederleini* were used as control fish for adding the skin extract cue of a heterospecific fish into the aquarium. These fish are phylogenetically and ecologically distant from *P. amboinensis* thus being an ideal control fish. Apogonids were collected on the reef using hand nets.

One of the most common and abundant predators on new settlers during the recruitment season is the dottyback *Pseudochromis fuscus* (Feeney *et al.* 2012). As naïve prey fish have been found to have an innate fright reaction to the sight of this predator (unpublished data) it was used a model predator species to expose fish to in the various habitats. As a control fish for the visual cues we used the herbivorous goby (*Amblygobius phalanea*), which are of similar size and shape as adult dottybacks. Both of these species are found in large numbers around Lizard Island and were collected using hand nets and a dilute solution of clove oil anaesthetic. Gobies and dottybacks were brought back to the research station and placed individually in 13 L aquaria and fed daily with fish food pellets.

Live healthy and dead-algae-covered hard coral (*Pocillopora damicornis*) were collected from the fringing reefs around Lizard Island and placed in well aerated 500 L flow through seawater holding tanks. The process of bleaching involves the expulsion of symbiotic zooxanthellae algae when the coral is under stress. This can happen when water temperatures reached  $> 1^{\circ}\text{C}$  above the summer maximum (Anthony *et al.* 2007). *Pocillopora damicornis* colonies bleach in about 10 days and will die in 2 – 3 weeks if the temperature remains consistently high, after which they get rapidly colonized by various algal and invertebrate species. In the current study, healthy colonies were thermally bleached over a 12 day period using the protocol of McCormick *et al.* (2010). After colonies had expelled their zooxanthellae and were visibly bleached but not dead, temperatures were once again lowered to the ambient  $28^{\circ}\text{C}$ .

### ***Experimental outline***

We conducted three separate experiments, two in the laboratory and one in the field. All experiments were designed to test the effects of coral degradation on anti-predator response of fishes to predation cues. The first experiment, conducted in the laboratory, examined responses of damselfish to visual, chemical and combined visual and chemical cues that indicate risk. The second experiment, conducted in the field, focused solely on responses to chemical information and was undertaken to determine the extent to which the findings of the first study were pertinent to natural populations. The final laboratory experiment tested if seawater that contained, or had been in contact with, dead algae-covered coral caused a modification (alteration or degradation) of conspecific chemical alarm cues or simply masked (i.e. overwhelmed) alarm cues from being detected.



***Design of laboratory experiments***

All behavioural observations were conducted in transparent 15-L aquaria (38x24x27cm) with a constant flow of seawater until the commencement of trials. The tanks were set up so they were continuously fed seawater from three separate reservoirs (60-L) that either contained 4 coral heads (10x15x12 cm) of live healthy, live thermally-bleached or dead-algae-covered coral habitat of the common bushy hard coral *Pocillopora damicornis*. One of the three types of coral habitat (live healthy, live bleached, or dead coral) was placed along the short side of the aquaria creating vertical shelters (18x20x4 cm). All corals were replaced every 2 days and used coral was returned to the field. Naïve *P. amboinensis* (n=15-17/treatment) were placed individually in the aquaria and allowed to acclimate overnight. Prior to the start of the trial, the water flow was stopped and 5 ml of *Artemia* sp (~ 550 *Artemia*) were added to the aquaria to stimulate feeding. The behaviour of a single *P. amboinensis* was recorded for a 4 min pre-stimulus period. Immediately following the pre-stimulus period, a further 5ml of *Artemia* was added and fish were exposed to the relevant cue treatment and the behaviour of the fish was then recorded for a further 4 min.

To prepare the damage-released cues, we sacrificed one recruit per trial using cold shock. The flank of each recruit was then superficially cut 6 times. The total cue area was rinsed with 10 ml of seawater that had been collected from the test aquaria and was then filtered through filter paper (47 mm Ø) prior to being used in the experiment. The behavioral response to experimental treatments was quantified by recording: total number of successful feeding strikes, total time spent inside of shelter (s), and activity (quantified as the number of times a fish crossed a line on the grid (3 x 3cm) that had been drawn on the vertical side of the tank).

***Experiment 1: Does coral degradation influence prey risk assessment in the laboratory?***

Naïve fish placed individually within aquaria containing one of three coral habitats (live healthy, bleached, or dead algae-covered coral) were exposed to one of 7 different cue treatments and their behaviour was recorded as above ( $n=15-16$ ). Chemical cue treatments included: (1) damage-released chemical cue of injured conspecifics; (2) control cues from injured heterospecifics, *A. doederleini* and (3) saltwater control. Visual treatments included: (4) a transparent bag filled with water; (5) a transparent bag that contained a herbivorous goby, *A. phalanea*; (6) a transparent bag that contained a predatory dottyback, *P. fuscus*. The seventh treatment included a combination of a pairing of treatment 1 and 6, as we reasoned that fish would have a stronger response when both sources of risk cues were available (e.g. Lima & Steury 2005, McCormick & Manassa 2008).

***Experiment 2: Does coral degradation influence the antipredator response to chemical indicators of risk in the field?***

Our laboratory studies indicated that coral degradation influences the responses of damselfish to chemical cues that indicate risk. This experiment aimed to determine whether there was evidence of environmental masking or alteration of damage-released cues in the field under natural conditions. All experimental trials were conducted within a sand patch surrounded by hard coral reef (composed of a typical diversity of live and dead coral habitats) using SCUBA at depths between 4 and 8 m. Small patch reefs (25x15x20cm) of either live healthy *P. damicornis*, thermally bleached *P. damicornis*, or dead algal-covered *P. damicornis* were assembled in the sandy area adjacent to the reef. To avoid any contamination between patch reefs, there was a minimum of 3 meters between patches and we moved in an up-current direction when doing the experiment. A single juvenile *P. amboinensis* was placed onto each

patch reef and allowed to acclimate for a minimum of 30 min before behavioural observations commenced. A 2 m plastic tube was attached up-current at the edge of the patch reef using metal skewers. The behavioural response of naïve *P. amboinensis* to 3 different treatments was tested: 1) skin extracts from damaged conspecifics; 2) skin extracts from damaged heterospecifics; and 3) saltwater (blank control) (n=15). The behaviour of focal fish was quantified for 3 min before (pre-stimulus period) and 3 min after (post-stimulus period) the addition of a stimulus (skin extract or saltwater).

To prepare skin extracts underwater, light trap caught *P. amboinensis* fish were brought underwater in 75x125 mm click seal bags which were filled with ~ 40 ml of sea water. Fish were euthanized by a quick blow to the brain case and the epidermis of the fish was lightly scratched using a scalpel blade that had been placed in the bag. A disposable syringe equipped with a fine needle was used to perforate the bag and extract 30 ml of the prepared stimulus. Behaviour of the fish was assessed by a SCUBA diver positioned at least 1.5 m away from the patch reef. Four aspects of activity and behaviour were estimated for each 3min sampling period: bite rate (successful and unsuccessful strikes), average distance from shelter (cm), maximum distance from shelter (cm) and time spent in shelter (s). Three minutes has previously been found to be sufficient to obtain a representative estimate of an individual's behaviour (bite rate) which also relates strongly to survival in the wild at this life stage (McCormick & Meekan 2010). Distance from shelter for these recently settled fishes has also been found to be closely related to survival in the first few days after settlement to the reef (McCormick 2009; 2012; McCormick & Meekan 2010; Munday *et al.* 2010).

***Experiment 3: Does dead coral mask or modify chemical indicators of predation risk?***

Here we attempted to identify a possible mechanism responsible for the impaired responses that we observed for fish exposed to alarm cues in dead coral habitats. Specifically, we tested whether the impaired chemosensory responses in dead coral likely resulted from (1) a chemical alteration/degradation of the cue (i.e, a structural change in the chemical cues that are not reversible) or (2) odour masking, whereby the lack of a behavioural response in dead coral occurs as a result of a high level of background odour that overwhelms the fish's olfactory sense making the cues hard to discern. To accomplish this individual naïve fish (n=16-19) were placed in tanks containing one of two habitats (live or dead hard coral) and left to acclimate. Fish in each habitat were then exposed to conspecific skin extracts that had been prepared (as above) with water from two different sources: 1) water that had flown past dead corals (from a 60-L flow-through tank containing 4 dead, algae-covered colonies of *P. damicornis* (10x15x12cm); or 2) water that had flown past live healthy *P. damicornis* (4 colonies in a 60-L tank). Their behaviour was recorded before and after the injection of the stimulus as above. In accordance with the previous experiments, we predicted impairment in behavioural responses for fish exposed to alarm cues prepared in healthy coral water but tested in dead coral habitats, and for fish exposed to alarm cues prepared in dead coral water and tested in dead coral habitats. We predicted fish exposed to alarm cues prepared from healthy coral water and then tested in healthy coral habitat would display antipredator responses. If alarm cues are altered/degraded by chemicals released from the dead coral and these changes are not reversible, then fish tested in healthy-live coral environments should fail to respond to alarm cues prepared in dead coral water. In contrast, if fish exposed to alarm cues prepared in dead coral water and tested in the presence of live coral, respond normally, then this would be regarded as evidence that the dead coral water simply masks the odour of the alarm cues, as the effect is reversible with dilution into the tank.

### *Statistics*

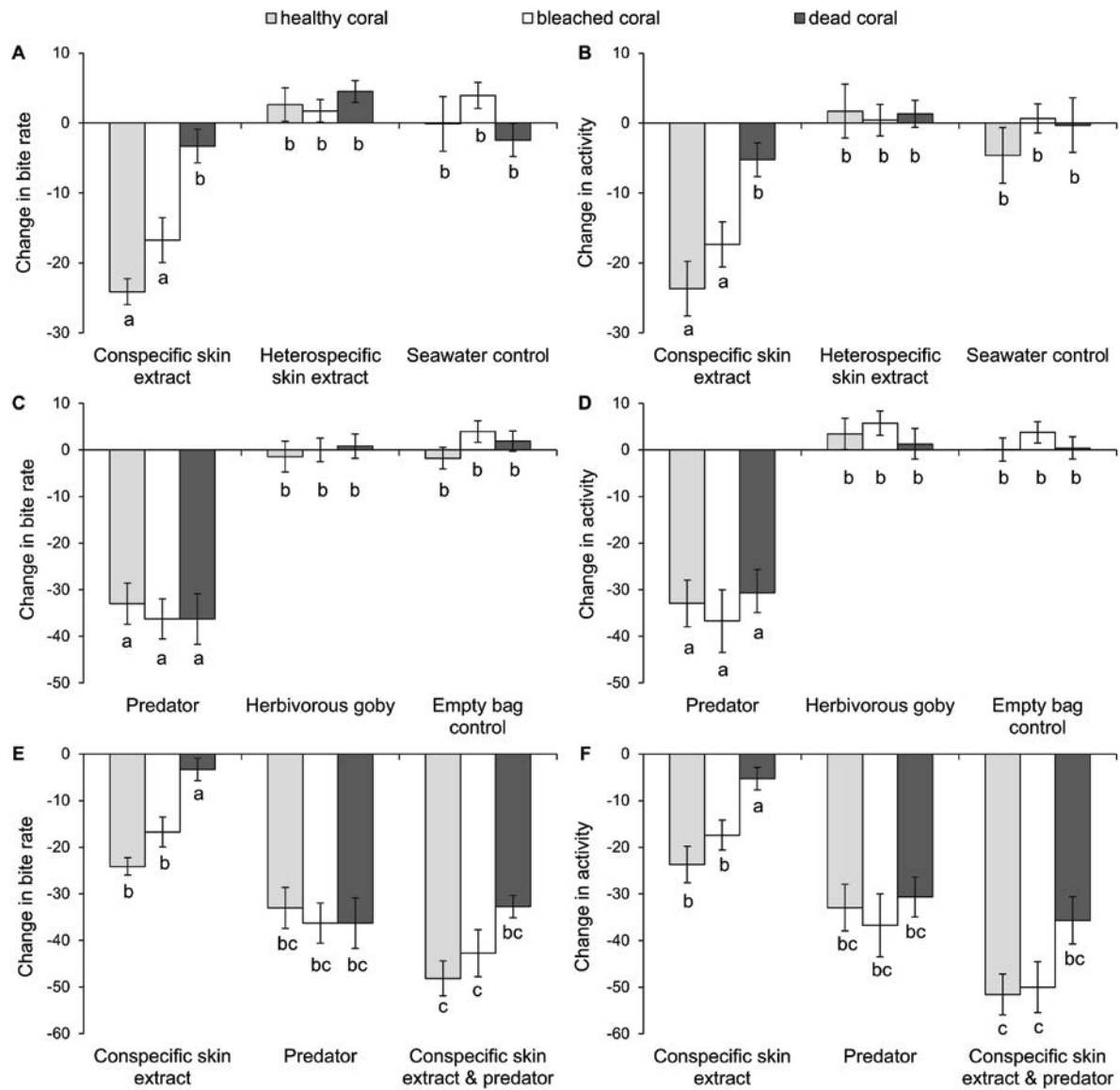
To test whether the behaviour of fish differed (in both the field and the laboratory) among the three different habitats (healthy, bleached and dead coral), and whether fish had been given olfactory indicators of risk (conspecific skin extract, heterospecific skin extract or a saltwater control), visual indicators of risk (visual predator, visual herbivore or none), or a combination of visual (predator) and chemical indicators of risk (conspecific chemical alarm cue) a MANOVA was employed. A two-way MANOVA tested whether the behaviour of fish differed between the background habitat (live or dead coral) or how the skin extract cue had been prepared (mixed with water in that had been in contact with live healthy coral or dead coral) and whether behaviour was affected by the interaction between these two factors. All data was analysed as the difference between the magnitude of behaviours before an experimental stimulus and after exposure to a stimulus (post-pre). Variables included in the analysis were: bite rate, activity level, distance from shelter and time spent in shelter. Time spent in shelter was  $\log_{10}(x + 1)$  transformed to meet assumptions of normality. Univariate ANOVA's were employed to examine the nature of the significant difference found by MANOVAs. Significant ANOVA's were further explored using unequal sample Tukey's HSD tests. A reduction in activity and foraging and movement into or close to the shelter are common anti-predator responses of damselfish to risk in both the laboratory and field (Chapter 2).

## **5.4 Results**

The way fish changed their behaviour in response to conspecific damage-released cues differed among habitats in the laboratory compared to the two controls (MANOVA: Pillai's Trace=0.19,  $df = 8, 266$ ,  $P < 0.001$ ). Fish exhibited a significant decrease in bite rate when

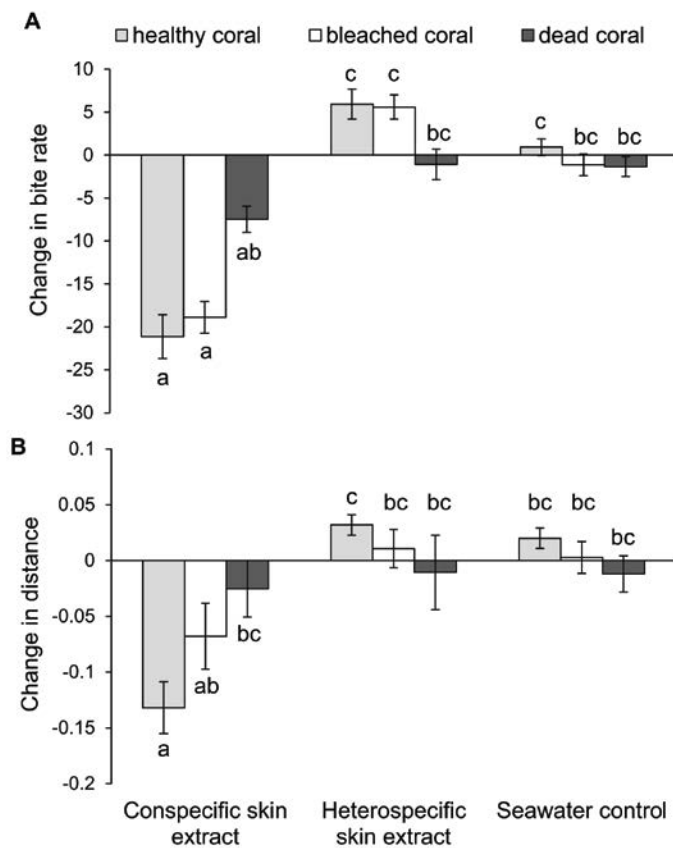
exposed to chemical cues in both the healthy and bleached coral habitats (Tukey's HSD tests:  $P < 0.05$ ; Fig. 5.1a). Fish in the dead coral habitat did not significantly change their bite rate compared to the controls when exposed to damage-released chemical cues (Tukey's HSD tests:  $P > 0.05$ ; Fig. 5.1a). Fish decreased foraging and activity in the three different habitats when exposed to the sight of a predator compared to the two visual controls (MANOVA: Pillai's Trace=0.17,  $df = 8, 266$ ,  $P < 0.0001$ ; Fig. 5.1c,d).

Habitat type strongly influenced the response of fish to chemical (conspecific skin extract), visual (visual predator) or a combination of chemical and visual predator cues in the laboratory (Pillai's Trace=0.19,  $df = 8, 266$ ,  $P < 0.005$ ). Univariate ANOVAs that examined the change in behaviour after exposure to the various threat cues showed that there was a significant difference in bite rate, activity, and time spent in shelter depending on which habitat the fish occupied ( $P < 0.01$ ; Fig. 5.1e,f). Fish in healthy and bleached habitats strongly reduced both activity levels and bite rates to visual and chemical threat cues and there was an additive effect when both cue sources were present. Prey fish did not respond stronger to the simultaneous exposure of both sources of risk in the dead coral compared to both the live and bleached habitats ( $P > 0.05$ ; Fig. 5.1e,f).



**Figure 5.1.** Coral degradation affects assessment of predation risk by prey in the laboratory; with the column on the left showing the mean change in bite rate, and the column on the right displays the mean change in activity of fish to the different treatments. Change in behavioural responses of coral reef fish exposed to chemical alarm cues (A, B), a predator (C, D), and a pairing of the two (E, F). Bite rates and activity levels are significantly decreased when exposed to threat cues in both healthy and bleached coral habitats. When exposed to the visual sight of a predator activity, levels and bite rates were strongly reduced regardless of the background habitat. In the healthy and bleached habitats, these behaviors were intensified when fish were presented with the sight of a predator paired with a chemical cue. Letters above or below bars represent Tukey's HSD grouping of means ( $\alpha = 0.05$ ).

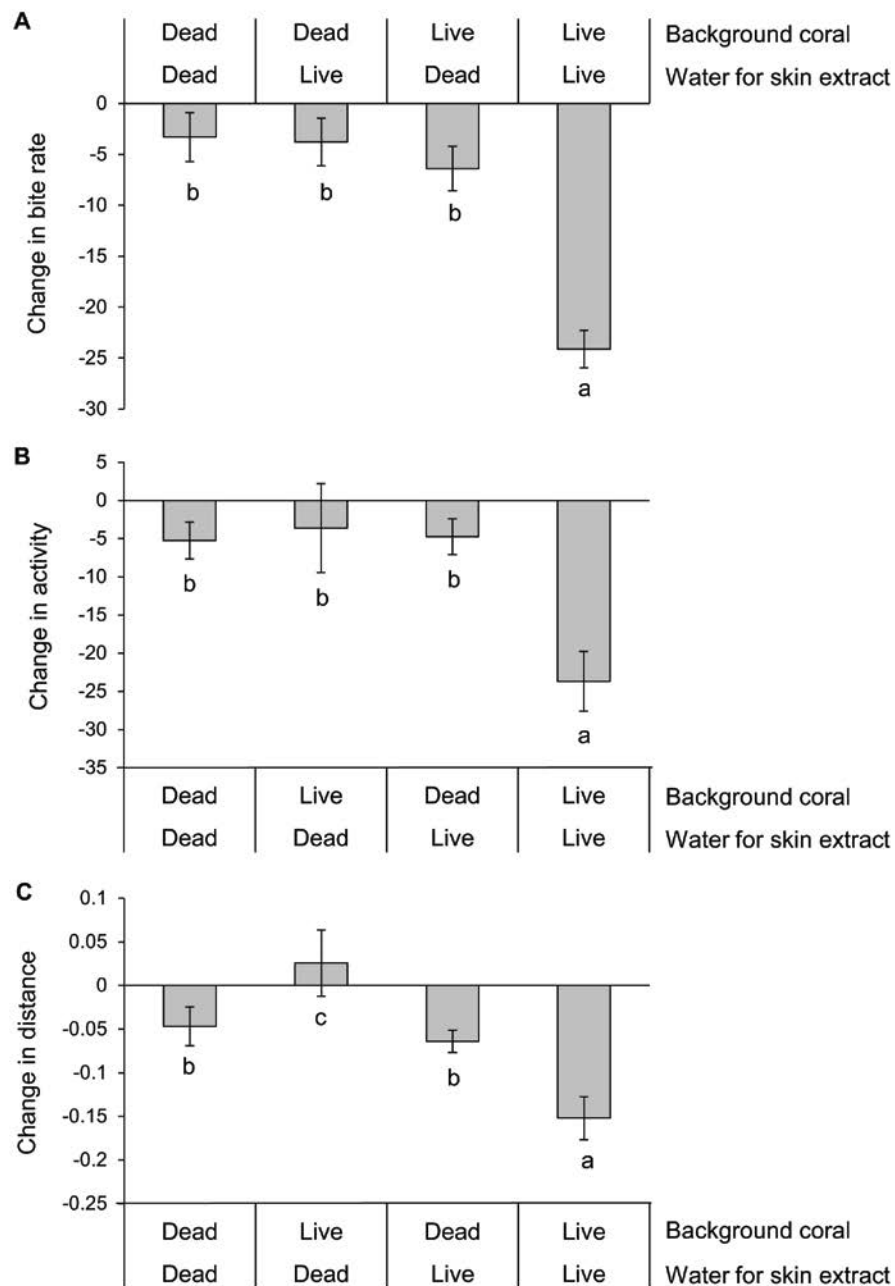
The habitat fish were on affected their response to damage released chemical alarm cues in the field (MANOVA: Pillai's Trace=0.35,  $df = 4,123$ ,  $P < 0.001$ ; Fig. 5.2). Univariate statistics indicate that prey fish were negatively impacted in dead coral habitats when assessing predation risk by olfaction. In the healthy habitats fish responded to chemical cues by retreating to shelter and reducing their foraging compared to the controls (Tukey's HSD tests:  $P < 0.05$ ; Fig. 5.2). Although fish responded to damage-released cues when in the bleached coral and fish spent less time inside the habitat, their behaviour did not significantly differ from the two controls (Fig. 5.2b;  $P < 0.05$ ). In the dead coral habitat, fish did not significantly change their behavior when exposed to damage-released cues compared to the controls (Tukey's HSD tests:  $P > 0.05$ ; Fig. 5.2).



**Figure 5.2.** Mean change of naïve fish when exposed to various olfactory cues in the field. (A) Bite rate is strongly reduced in both healthy and bleached coral when exposed to conspecific skin extracts while not when exposed to heterospecific skin extracts or a seawater control. (B) When exposed to chemical alarm cues of conspecifics, fish strongly reduced their distance from shelter in the live healthy coral, but tended to retire to shelter less in both bleached and dead coral habitats. Letters above or below bars represent Tukey's HSD grouping of means ( $\alpha = 0.05$ ).



There was a strong interactive effect of background habitat and the type of water that the cue was prepared with on the behaviour of naïve fish (Pillai's Trace=0.4,  $df=3,59$ ,  $P < 0.001$ ; fig. 5.3a,b). This was caused by the combination of a live healthy coral background and skin extract cues prepared with water that had been in contact with live coral differing from all the other treatments, which in turn, did not differ from one another (Fig. 5.3a,b). Univariate ANOVAs on each behavioural variable revealed that naïve fish in tanks with a background of healthy live coral responded with a reduction in activity, bite rate, and distance from shelter ( $F_{1,61}=17.7$ ,  $P < 0.001$ ;  $F_{1,61}=11.9$ ,  $P \leq 0.001$ ;  $F_{1,61}=18.7$ ,  $P < 0.001$ ) when exposed to conspecific skin extracts that had been prepared with seawater that had only been in contact with live healthy coral (Fig. 5.3a,b). Contrastingly, fish with a background habitat consisting of dead, algae-covered coral did not respond to any skin extracts (regardless of how they had been prepared). Similarly, fish in live healthy coral habitats did not respond to conspecifics skin extracts prepared with water that had been in contact with degraded coral habitats. It appears as though seawater that is, or has been in contact with, dead algae-covered coral may alter the structure of conspecific chemical alarm cues.



**Figure 5.3.** Comparison of the behavior of *Pomacentrus amboinensis* in the laboratory that had been exposed to conspecific skin extracts prepared with water containing either live healthy coral or dead algae-covered coral in one of two background habitats (live healthy or dead algae-covered). Behaviours are the change between the 4-min pre- and post-stimulus period in (A) bite rate, (B) activity level, and (C) average distance from the coral shelter. Letters above or below bars represent unequal Tukey's HSD grouping of means ( $\alpha = 0.05$ ).

## 5.5 Discussion

We showed that coral degradation had a profound influence on the behavioural responses of fish to cues that indicate predation risk. Fish in live healthy and bleached coral fed above the colony and reduced swimming, ignored food and sought refuge when exposed to either chemical or visual indicators of risk. Prey in the dead, algae-covered coral habitat showed a similar antipredator response when exposed to the sight of a predator, but when presented with damage released alarm cues of conspecifics they did not visibly change their behaviour in either the laboratory or the field. While a pairing of olfactory and visual threat cues had an additive effect on the prey response in live coral habitats, prey occupying degraded habitats did not show a stronger response when given the combined cue sources. Failing to respond to an olfactory indicator of risk greatly increases the likelihood of being preyed upon (Munday *et al.* 2010; Ferrari *et al.* 2011b). Fish with impaired olfactory abilities are also less likely to find a suitable settlement sites and potential mates (Curtis *et al.* 2001; Dixon *et al.* 2010; Munday *et al.* 2009; Munday *et al.* 2010; Devine *et al.* 2012a).

We know from previous studies that small bodied coral dwelling damselfish (family Pomacentridae) decline in abundance following coral bleaching and reef degradation (Wilson *et al.* 2006; McCormick 2009). Since they are not obligate corallivores it has been unclear why they exhibit such strong reductions in abundance following large scale bleaching events. It was initially believed to be due to a decline in coral cover and the subsequent reduction in the structural complexity of the coral reef framework (making them more susceptible to predators), but bleaching does not necessarily equate to a loss in habitat structure in the short term (Pratchett *et al.* 2008). During bleaching the density of zooxanthellae (photosynthetic algae within the coral tissue) are reduced either through the expulsion or death of the minute algal cells, thus not affecting the structure but only the pigmentation of the coral. It is the

subsequent death and erosion that results in the loss of coral structure (Booth & Beretta 2002). Hence, it is the live coral in itself that offers some sort of advantage to fish. The current study demonstrates that fish appear unwilling to retreat back into bleached or degraded coral when exposed to threat cues, spending less time in shelter compared to the when occupying the live healthy coral colonies. McCormick (2009) suggested that the smell of dying tissue may force recruit stage fish away from bleached coral, leading to higher vulnerability. Our results suggest that the mechanism underlying the move away from degraded coral habitats may be their reduced ability to identify the olfactory cues that are innately associated with predation threat (the chemical alarm cues). The information on which they base their decision has changed, affecting their perception of the where they should best sit along the axis of risk from shelter (and reduced foraging opportunities) to open water (and increased foraging opportunities).

The relative context in which a threat stimulus is received can influence both the quality and effectiveness of a signal as certain environmental conditions, or ‘background noise’, can alter the signals perceived form (Endler 1992). The phenomenon of odor masking has been well-studied in terrestrial environments (for a comprehensive review see Schroder & Hilker 2008), but the focus in this literature is often background odours masking resource indicating cues. For instance, certain plants produce an odour that repels insects, or hides the odours of their host plants (Mauchline *et al.* 2005). They benefit the plants by allowing them to effectively hide from consumers in a complex chemical environment. In our study we tested whether the background odour of dead coral masked or modified the scent of alarm cues, reducing the response of prey to threats. Once a coral is dead and overgrown by algae a whole new community settles into it and all these different life forms (together with the algae) may overpower other odours in the environment (such as the scent of wounded conspecifics). However, we found no evidence for odour masking, as fish exposed to alarm

cues prepared in dead coral water did not elicit a response in water containing healthy coral. We prepared the alarm cue in 10 ml of water and injected the cue into a tank containing 15 L of water. Despite this huge dilution effect, the “unmasked cue” did not elicit a fright response in the fish. Fish have been shown to have a remarkable ability to differentiate between threat cues even when presented together (Mitchell *et al.* 2011a), which also suggests that odour masking is unlikely. As an alternative to odour masking, our results support the hypothesis that dead coral rapidly alters or degrades the chemical alarm cue. Whenever the alarm cues were in contact with dead coral (either prepared in dead coral water or injected into a tank containing dead algae-covered coral) fish failed to elicit normal anti-predator responses. As such, our results resemble the responses of salmonid fishes in freshwater systems, whereby the alarm cues are rendered inactive when the pH drops to 6.0 (Leduc *et al.* 2004). The proximate chemical mechanism responsible for this change in our system remains unknown, but likely is not a result of a pH change as this was not altered in the study systems given that marine systems do not show large changes in pH (Gagliano *et al.* 2010).

The impact on the olfactory sense due to degraded habitat is different from the recently documented impacts of elevated dissolved CO<sub>2</sub> on the olfactory sense. Dissolved CO<sub>2</sub> elevated above 900 µatm has been shown to alter the function of neurotransmitters in fish (Nilsson *et al.* 2012), leading to the reduced discrimination of pertinent sensory cues (Dixon *et al.* 2010; Ferrari *et al.* 2012b) and the negation of learning processes associated with the correct identification of chemical alarm cues (Ferrari *et al.* 2012). Luckily there may be sufficient variability in the physiological response at low CO<sub>2</sub> concentrations (700 µatm) within populations for fish to adapt to this CO<sub>2</sub> rich world through ecological selection (Munday *et al.* 2012a,b). In contrast, the mechanism described in the present study is external to the animal, and involves the modification of the cue such that it is either not recognized or inappropriately categorized. Our data suggest that all individuals were similarly impacted,

suggesting a limited ability to adapt to the loss of this important sensory cue. As coral death and degradation becomes increasingly prevalent (Wilkinson 2004), further research is required to determine the extent to which the risk assessment of other species may be affected by the same mechanism and the community wide repercussions.

Due to GEC, coral reefs all over the world are declining in health and what once were fields of live coral are now low lying rubble beds. Fish living within these changed environments are more likely to become stressed as the coral degrades, both as a result of the loss of refuge space as well as from a change in their olfactory environment. Is it plausible that the fish are so stressed by their new surroundings that they fail to respond to predator threats? This seems very unlikely as the fish still responded to the sight of a predator when in the dead coral. Predation is one of the most important processes shaping coral reef fish communities. Our current findings suggest that coral bleaching and coral death will impact the crucial interactions between fish predators and their prey. Bleached and dead coral patches appear to interfere with olfactory cues critical for the assessment of risk by prey. Without detecting the olfactory signposts of risk, prey are unable to identify the early signs of danger and are more likely to fall prey to hungry predators (McCormick 2009; Munday *et al.* 2010). Biologists and managers wishing to predict the long term consequences of global environmental change on reef fish assemblages will need to understand the repercussions of this crucial developmental bottleneck (Chapter 2; Ferrari *et al.* 2012).

## Chapter 6

### **Ocean acidification and impaired responses to predation cues: can sensory compensation reduce the apparent impacts of elevated CO<sub>2</sub> on fish?**

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#### **6.1 Synopsis**

Carbon dioxide (CO<sub>2</sub>) levels in the atmosphere and surface ocean are rising at an unprecedented rate due to sustained and accelerating anthropogenic CO<sub>2</sub> emissions. While it is known that elevated CO<sub>2</sub> levels impairs the ability of prey fish to detect olfactory signposts of risk, it is unknown whether visual information may compensate for the lack of olfactory abilities. This study examined whether sensory compensation could reduce the impacts of ocean acidification on anti-predator responses. While the visual response to a predator was affected by high CO<sub>2</sub>, it was not entirely lost. Fish exposed to elevated CO<sub>2</sub>, spent less time in shelter than current-day controls and did not exhibit anti-predator signalling behaviour (bobbing) when multiple predator cues were present. They did, however, reduce feeding rate and activity levels to the same level as controls. Fish subjected to elevated CO<sub>2</sub> levels and exposed to chemical and visual predation cues simultaneously, responded with the same intensity as controls exposed to visual cues alone. Consequently, visual cues improve anti-predator behaviour of CO<sub>2</sub> exposed fish, but do not fully compensate for the loss of response to chemical threat cues. The reduced ability to correctly respond to a predator will have ramifications for survival in encounters with predators in the field, which could have repercussions for population replenishment in acidified oceans.

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## 5.2 Introduction

The concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere is rising at a rate unprecedented for millions of years, due to the release of CO<sub>2</sub> from fossil fuel burning, cement production and land-use changes by humans (Doney *et al.* 2009). Atmospheric CO<sub>2</sub> is now 400ppm (Dlugokencky & Tans 2013), higher than any time in the past 800,000 years (Luthi *et al.* 2008), and could exceed 900ppm by the end of the century if the current emissions trajectory is maintained (Meinshausen *et al.* 2011, Peters *et al.* 2012). One of the consequences of rising atmospheric CO<sub>2</sub> concentrations is the increased absorption of CO<sub>2</sub> in the ocean. Here, it reacts with seawater, causing a reduction in the concentration of carbonate ions and lowering seawater pH, a process known as ocean acidification (Raven *et al.* 2005). The partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ) in the ocean also increases with increasing atmospheric CO<sub>2</sub>, because the ocean surface is at approximate gas-equilibrium with the atmosphere (Doney 2010). These changes to ocean chemistry have been shown to affect fundamental biological processes, such as metabolism, growth, calcification, reproduction and behaviour, in a wide range of marine organisms (Fabry *et al.* 2008; Widdicombe & Spicer 2008; Doney *et al.* 2009; Kroeker *et al.* 2010; Briffa *et al.* 2012). However, the potentially interacting effects of ocean acidification on multiple biological traits, and the effects on ecological interactions among organisms, remain poorly understood (Fabry *et al.* 2008; Hendriks *et al.* 2010; Kroeker *et al.* 2012). Anticipating the responses of marine organisms to rising CO<sub>2</sub> levels and ocean acidification is a crucial test case for evolutionary ecologists.

Recent studies show that exposure to elevated CO<sub>2</sub> causes fish to fail to respond to ecologically important chemical cues including homing odours (Munday *et al.* 2009) and predation cues (Dixon *et al.* 2010; Ferrari *et al.* 2011b). Munday *et al.* (2010) and Ferrari *et al.* (2011a) both found that larval fish (*Pomacentris wardi* and *P. chrysurus*) raised in



seawater enriched with levels of CO<sub>2</sub> predicted for the end of this century dramatically altered their behaviour and displayed higher mortality compared to fish raised in current-day seawater. Fish exposed to elevated CO<sub>2</sub> levels have also been found to have impaired auditory abilities (Simpson *et al.* 2011) and reduced behavioural lateralisation (Domenici *et al.* 2011) providing evidence that high CO<sub>2</sub> directly affects brain function in juvenile fish (Nilsson *et al.* 2012). One study has demonstrated that ocean acidification will also affect recognition or cognitive processing of visual information. Ferrari *et al.* (2012b) found that larval damselfish raised in high CO<sub>2</sub> seawater responded differently to the sight of a large non-predatory fish (a spiny Chromis, *Acanthochromis polyacanthus*) to which the prey was unfamiliar. Fish exposed to current day levels of CO<sub>2</sub> reacted to *A. polyacanthus* with anti-predator behaviours indicating that the prey may show neophobic responses to any large fish, regardless of whether they pose a threat (Brown *et al.* 2013). Whether ocean acidification will impair visual recognition of prey to common predators is currently unknown.

A recent study by Devine *et al.* (2012b) found that there were differential effects of CO<sub>2</sub> on different senses and that one sense might overcome the impairment of another sense. Such sensory redundancy could reduce the effects of high CO<sub>2</sub>. In Devine *et al.*'s (2012b) study three species of damselfish that depend on both olfactory and visual cues to find appropriate settlement habitats retained the ability to select their preferred habitat although their olfactory sense had been impaired. This suggests that at least some fish will rely on other senses to compensate for the loss of one sense. Here, we investigated the effects of elevated CO<sub>2</sub> on responses of fishes to visual and chemical predator cues, separately or together. Specifically, we were interested in experimentally testing whether appropriate responses to visual information could compensate for impaired olfactory anti-predator senses that are commonly reported in damselfish.

The early life stages of many animals, including fishes, are extremely vulnerable to predation (Almany & Webster 2006). Due to their development in the plankton, settlement stage reef fish arrive to habitat patches that contain predators never before encountered. At this time, individuals would benefit from possessing a pre-programmed (i.e. innate) sensory response to a predation threat, be it visual or olfactory. There are many cases of fishes displaying innate responses to skin extracts of injured conspecifics (Chivers & Smith 1998; Lönnstedt & McCormick 2011a) and odour cues emitted by their natural predators (Hawkins *et al.* 2004; Dixon *et al.* 2010). However, limited research has investigated if naïve prey fish display an innate response to the visual cues of predators (for an exception see Coss 1979). Highly diverse environments, such as coral reefs, contain vast numbers of fish of different colours, sizes and body shapes (Marshall *et al.* 2003). Here, the ability to visually discriminate between predators and non-predators should be of crucial importance for naïve prey. While it is known that ocean acidification impairs the ability of prey fish to detect olfactory signposts of risk, it is unknown whether visual information may partially compensate for the lack of olfactory abilities, and thus help maintain population replenishment in acidified oceans.

The current study aimed to determine how prey fish (ambon damselfish, *Pomacentrus amboinensis*) respond to the separate and combined effects of olfactory and visual cues of predators when exposed to a CO<sub>2</sub> level (880 µatm CO<sub>2</sub>) projected for the surface ocean by the end of this century (Donet 2010, Meinshausen *et al.* 2011). We specifically chose to study ambon damselfish, because they appear to be the most sensitive of the 4 species of damselfishes to CO<sub>2</sub> induced impairment of their olfactory sense (Ferrari *et al.* 2011a). In order to test whether appropriate responses to visual cues could compensate for the loss of response to chemical cues, we conducted three experiments. The first experiment was designed to test how naïve (with no prior experience of predators) fish exposed to elevated

$p\text{CO}_2$  (880  $\mu\text{atm}$ ) respond to damage released skin extracts of conspecifics. Secondly, we tested whether responses to visual cues of a common predator were impaired in response to elevated  $p\text{CO}_2$ . The final experiment examined if fish retained an anti-predator response when exposed to visual and chemical indicators of risk simultaneously, testing whether the visual sense could overcome impairment of the olfactory sense.

### 5.3 Materials and methods

#### *Study species and sampling*

All experiments were carried out at Lizard Island Research Station (14°40'S, 145°28'E), northern GBR, Australia in October-November 2010. Ambon damselfish *Pomacentrus amboinensis* are a common component of reef fish communities around the Indo-Pacific. The predatory dottyback, *Pseudochromis fuscus*, is known to specialise and feed on newly settled fish during the recruitment season (Feeney *et al.* 2012) and was therefore used as the model predator for all experiments. *P. fuscus* is common cryptic predator on coral reefs and is found in habitats occupied by *P. amboinensis*. All fish were collected at the end of their larval phase (while naïve to reef-based, bottom-dwelling predators) using light traps that had been moored overnight off the reefs at Lizard Island. Fish were brought back to the research station and transferred into 35-L flow through seawater aquaria (in groups of 20-30 fish) maintained at one of two  $\text{CO}_2$  concentrations for four consecutive days (12L:12D photoperiod). Previous studies have shown that exposing fish to elevated  $\text{CO}_2$  for 4 days leads to identical behavioural impairment as fish that have been exposed to high  $\text{CO}_2$  since hatching (Munday *et al.* 2010). Furthermore, longer term exposure does not produce any acclimation of behavioural responses, with the behaviour of juveniles similarly impaired after 4 days and 4 weeks (Munday *et al.* 2013). Fish were fed ad libitum three times daily with newly hatched

brineshrimp, *Artemia sp.* *Pseudochromis fuscus* were collected on the fringing reefs around Lizard Island on SCUBA using a dilute clove oil anaesthetic and hand nets. Captured fish were placed in 10-L plastic bags and transported to the research station where they were held in 30-L flow through seawater tanks. Each plastic holding tank had a 2 cm layer of sand at the bottom and contained several plastic tubes that served as shelters.

### ***Ocean acidification system***

*P. amboinensis* were held a minimum of 96 hours in replicate aquariums supplied with a constant flow of either control seawater (440  $\mu\text{atm CO}_2$ ) or water enriched with carbon dioxide (880  $\mu\text{atm CO}_2$ ) (Table 6.1). *P. amboinensis* were kept in treatment for a minimum of 96 hours as previous studies have demonstrated that this is sufficient time for juvenile fish to be behaviourally affected by elevated  $\text{CO}_2$  and longer exposure does not further alter the behavioural changes associated with  $\text{CO}_2$  treatment (Munday *et al.* 2010), as described above.  $\text{CO}_2$  treatments were maintained by  $\text{CO}_2$  dosing to a set  $\text{pH}_{\text{NBS}}$ . Seawater was pumped from the ocean into 2 x 60 L sumps where it was diffused with ambient air or  $\text{CO}_2$  to achieve a pH of  $\sim 8.15$  (current day), or  $\sim 7.89$  (a value which is expected to be reached by the end of this century should  $\text{CO}_2$  emissions continue along the current trajectory). To maintain pH at the desired level a pH-controller (Tunze Aquarientechnik, Germany) was attached to the  $\text{CO}_2$  treated sump. A solenoid injected a slow stream of  $\text{CO}_2$  into a powerhead at the bottom of the sump whenever the pH of the seawater rose above the set point. The powerhead dissolved  $\text{CO}_2$  into the seawater while simultaneously serving as a vigorous stirrer. Equilibrated seawater from each sump was supplied at a rate of  $\sim 500\text{ml min}^{-1}$  to four replicate 35 L aquariums, each housing a group of larval fishes. To maintain oxygen levels and the required  $\text{pCO}_2$  levels, aquaria were individually aerated with unmanipulated air or  $\text{CO}_2$ -enriched air

(~880 ppm). The concentration of CO<sub>2</sub>-enriched air was controlled by a scientific-grade pressure regulator and precision needle valve and measured continuously with an infrared CO<sub>2</sub> probe (VaisalaGM70). Temperature and pH<sub>NBS</sub> of each aquarium was measured each morning and afternoon using a HQ40d pH meter (Hach, Colorado, USA) calibrated with fresh buffers. Total alkalinity of seawater was estimated by Gran titration from water samples taken twice weekly from each CO<sub>2</sub> treatment. Alkalinity standardizations performed before processing each batch achieved accuracy within 1% of certified reference material from Prof. A. Dickson (Scripps Institution of Oceanography). Average seawater *p*CO<sub>2</sub> was calculated using these parameters in the program CO2SYS and using the constants of Mehrbach *et al.* (1973) refit by Dickson & Millero (1987). Estimated seawater parameters are shown in Table 6.1.

**Table 6.1.** Mean (±SD) seawater parameters in the experimental system. Temperature, pH salinity, and total alkalinity (TA) were measured directly. *p*CO<sub>2</sub> was estimated from these parameters using CO2SYS.

pH <sub>NBS</sub>	Temp °C	Salinity ppt	TA μmol.kg <sup>-1</sup> SW	<i>p</i> CO <sub>2</sub> μatm
8.15 (0.04)	27.66 (0.98)	35	2269.66 (15.01)	440.53 (44.46)
7.89 (0.06)	27.74 (0.99)	35	2261.23 (14.92)	879.95 (140.64)

### ***Experimental protocol***

The experimental protocol is described as three separate experiments because a number of different controls were required to experimentally examine the response of fish from the elevated CO<sub>2</sub> treatment to chemical or visual stimuli. All experiments were conducted on random subsets of *P. amboinensis* which had been collected at a similar time and location, making comparisons across experiments valid. The same number of fish from both the control and CO<sub>2</sub> treatments was tested on a given day, and the order of testing was randomized. Following the CO<sub>2</sub> conditioning, individual *P. amboinensis* were transferred into transparent 15 L aquaria (38x24x27cm) with a constant flow of fresh seawater and allowed to

acclimate overnight. Juvenile damselfish are known retain their impaired behavioural response for a period of 48 hours after being returned to ambient seawater, and this response is no different to fish tested in elevated CO<sub>2</sub> water within the 48 hour window (Munday *et al.* 2010, Nilsson *et al.* 2012). Each aquarium was covered on 3 sides by black plastic with one long side having a 3x3 cm grid drawn on it. A single airstone was placed at the back corner of each tank with two 1.5 metre long plastic tubes fixed to the airline (one for the injection of food, and one for the injection of the experimental stimuli) allowing for rapid dispersal of extracts in the aquaria. Each tank contained a 2 cm layer of coral sand on the bottom and a live coral habitat (bushy hard coral, *Pocillopora damicornis*) along the short side of the aquaria creating a vertical shelter. Prior to the start of the trial, the water flow was stopped and 5 ml of *Artemia sp.* (approximately 550 *Artemia*) were added to the aquaria to stimulate feeding. The behaviour of a single *P. amboinensis* was recorded for a 4 min pre-stimulus period. Immediately following the pre-stimulus period food was injected again followed by one of the 7 different stimuli (depending on the experiment as described below), and the fish's behaviour was recorded for a further 4 min. Three behaviours were categorised and recorded: foraging, activity, and shelter use. Foraging was recorded as the total number of feeding strikes, activity level was quantified as the number of times a fish crossed a line on the grid, and shelter use was recorded as the total amount of time a fish spent in shelter.

### ***Experiment 1: Does elevated CO<sub>2</sub> impair responses to chemical alarm cues?***

Control and elevated-CO<sub>2</sub> treated *P. amboinensis* were tested to determine whether they respond to chemical cues released from damaged conspecifics. We also tested for a behavioural response to extracts from damaged heterospecifics (*Apogon doederlini*) and a saltwater control. The heterospecific skin extract allowed us to establish whether juvenile *P.*

*amboinensis* have a generalized behavioural response to any injured fish, while the saltwater stimulus served as an additional disturbance control. To prepare the alarm cues, we sacrificed the donor fish using cold shock. The flank of each recruit was then superficially cut 6 times. The total cue area was rinsed with 10 ml of saltwater and filtered through filter paper (47 mm Ø) prior to being used in the experiment.

### ***Experiment 2: Does elevated CO<sub>2</sub> impair responses to the sight of a predator?***

To test whether naïve *P. amboinensis* respond to the visual stimuli of a predator we assessed the change in behaviour of control and elevated-CO<sub>2</sub> treated fish upon presentation of a predator (*P. fuscus*). During each trial we introduced 5 ml of *Artemia sp.* and then quantified the behaviour of the fish for 4 minutes as in experiment 1. Following the pre-stimulus period a watertight plastic bag (15x23cm) containing the predator was carefully lowered into the aquaria on the opposite side of the coral shelter. After a 30-sec stimulus introduction period, a further 5 ml of *Artemia sp.* was added to the aquarium and the behaviour of the focal fish was quantified for a further 4 min. Fish were also exposed to bags containing (1) a non-predatory fish (*Amblygobius phalanea*) and (2) an empty bag controlling for changes resulting from the experimental procedure. To control for a response to the visual stimulus of any fish, we used the herbivorous goby, as it is a similar size and shape to *P. fuscus* but non-predatory.

### ***Experiment 3: Does sensory redundancy reduce the apparent impact of elevated CO<sub>2</sub>?***

In this experiment, control and elevated CO<sub>2</sub> treated *P. amboinensis* were exposed to (1) chemical alarm cues alone, (2) visual predator cues alone or (3) a combination of visual and chemical cues. The magnitude of the response of *P. amboinensis* to the paired chemical and

visual stimuli was then compared to the magnitude of response to chemical and visual cues in isolation. In this last experiment, we added a fifth behavioural measure; bobbing. Bobbing is a common antipredator behaviour in juvenile damselfish and consists of raising the anterior portion of the body, followed by a rapid descent, which is repeated several times (Smith & Smith 1989; Ferrari *et al.* 2012b).

### ***Statistical analysis***

For all of the experiments, the difference in behaviour between the pre-stimulus and post-stimulus periods was calculated and used for analysis. Differences between the control and elevated CO<sub>2</sub> treatment were examined using a one-way MANOVA. A two-factor MANOVA was then employed to examine whether the behaviour differed between fish from the two CO<sub>2</sub> treatments in response to the cue stimulus type (olfactory, visual or a combination of the two). The behavioural variables included in the analysis were feeding strikes, activity level and time spent in shelter (s). To further explore the nature of significant differences found by the MANOVA, univariate ANOVAs were used and significant differences were further examined using Tukey's HSD means comparison tests. The assumptions were examined and time spent in shelter was  $\text{Log}_{10}(x+1)$  transformed.

Log-linear models were used to examine how threat sources (conspecific skin extract, visual predator, or both) and CO<sub>2</sub> treatment (elevated and current-day) affected the occurrence of bobbing behaviour in the third experiment. Models were constructed to test five specific hypotheses concerning bobbing frequency: 1) independent of treatment or cue; 2) dependent on cue; 3) dependent on treatment; 4) dependent on both treatment and cue; 5) dependent on an interaction between treatment and cue. The models were fitted to the observed data in increasing order of complexity until there was no significant improvement in

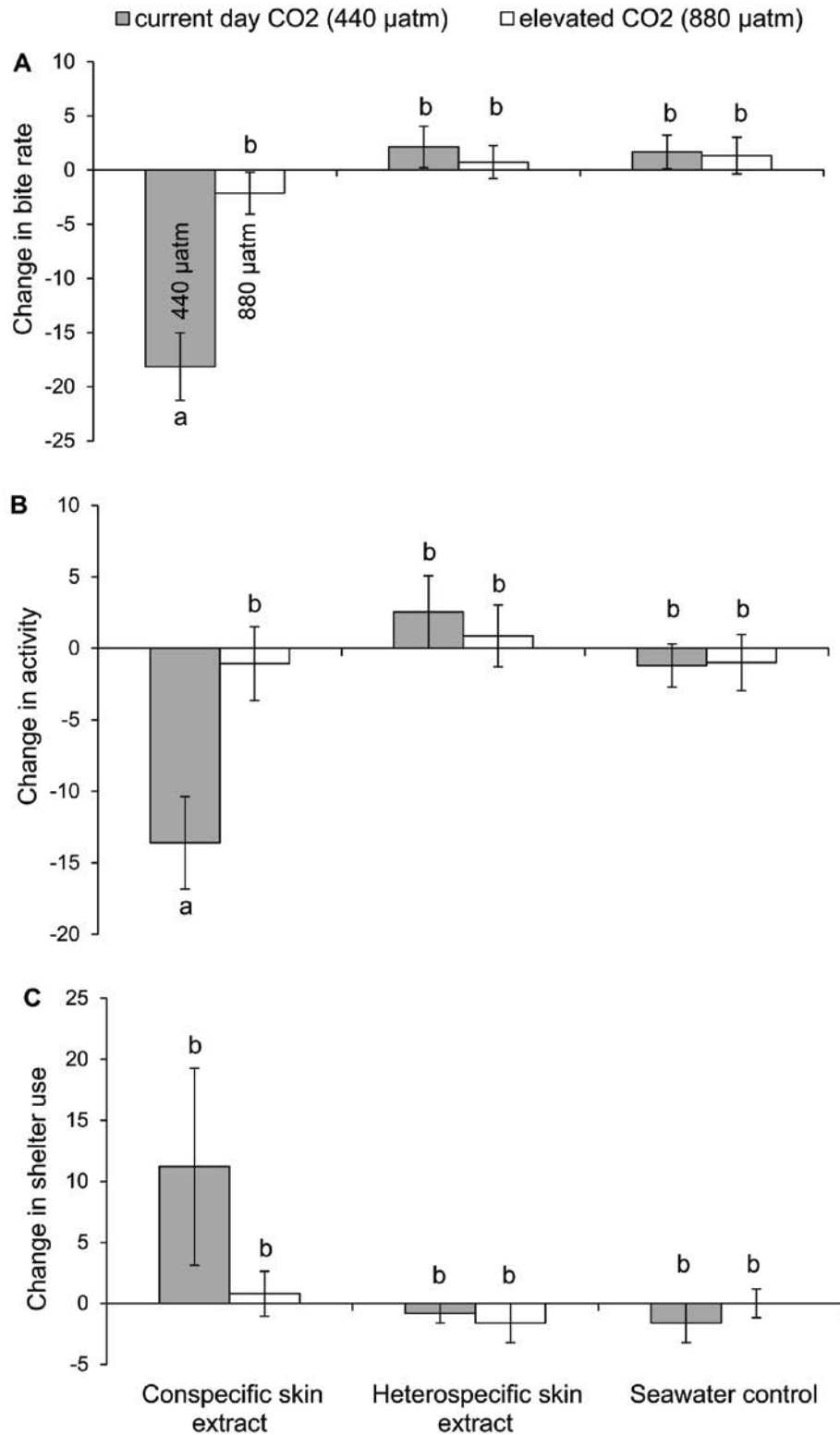


the goodness-of-fit statistic (likelihood ratio chi-square) from one model to the next. All statistics were undertaken using Statistica (v 10).

## 6.4 Results

### *Experiment 1: Does elevated CO<sub>2</sub> impair responses to chemical alarm cues?*

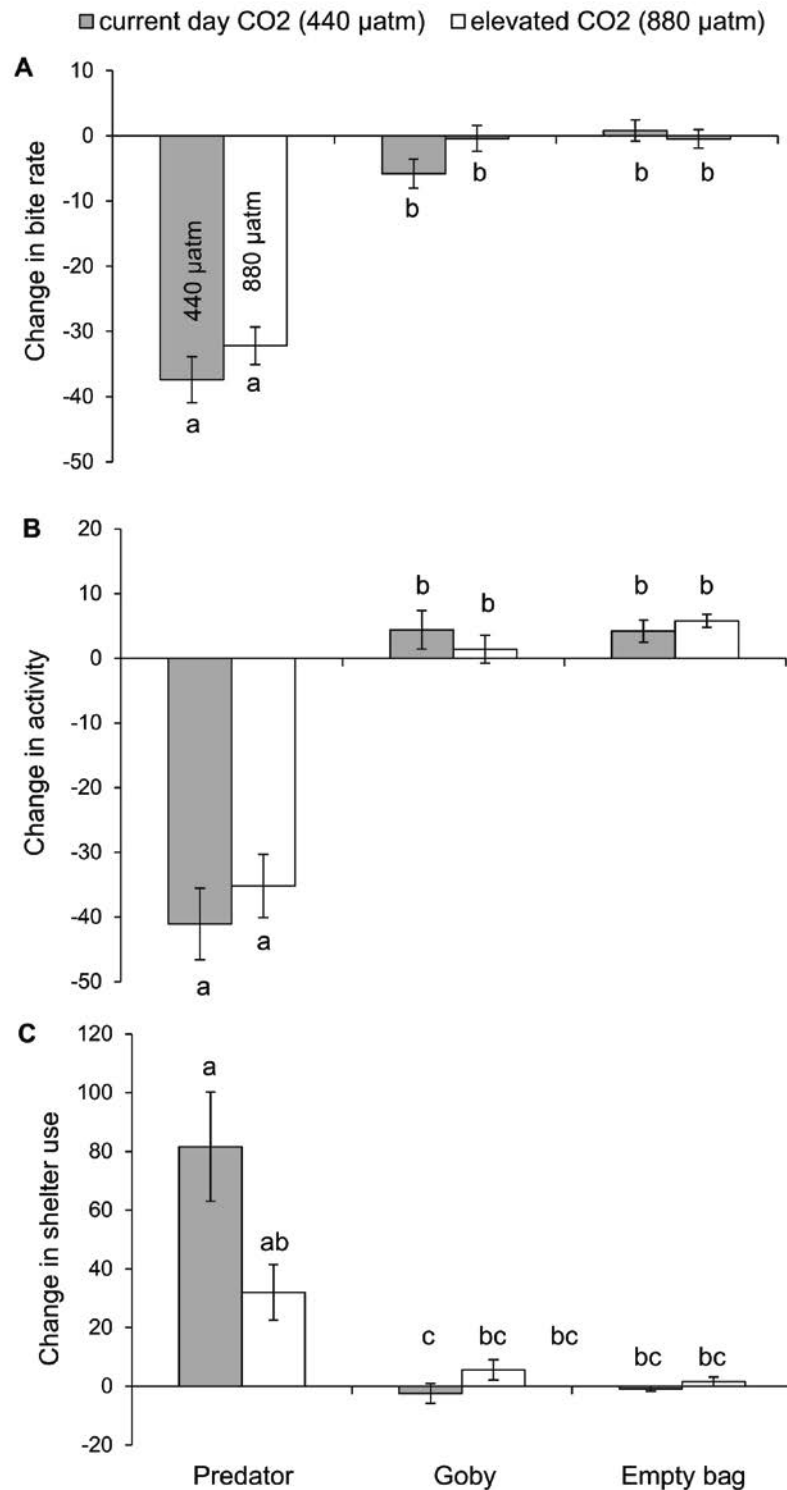
There was no effect of elevated CO<sub>2</sub> levels on the behaviour of fish during the pre-stimulus period (Pillai's Trace:  $F_{6,166} = 0.08$ ,  $P > 0.5$ ). However, there was a strong influence of elevated CO<sub>2</sub> on the response of fish to conspecific skin extracts (Pillai's Trace:  $F_{6,166} = 0.23$ ,  $P = 0.002$ ; Fig. 6.1). *Pomacentrus amboinensis* from the elevated CO<sub>2</sub> treatment did not elicit an antipredator response when exposed to the conspecific skin extracts (Fig. 6.1a,b). In contrast, prey exposed to current-day CO<sub>2</sub> treatment displayed a typical threat response to conspecific chemical alarm cues, greatly reducing both foraging ( $F_{2,84}=11.5$ ,  $P < 0.001$ ; Fig. 6.1a) and activity rates ( $F_{2,84} = 5.2$ ,  $P = 0.007$ ; Fig. 6.1b) compared to those exposed to heterospecific skin extracts or to saltwater control. Fish exposed to either elevated or current-day CO<sub>2</sub> treatments did not increase time spent in shelter upon the injection of conspecific skin extracts ( $F_{2,84} = 1.6$ ,  $P = 0.2$ ; Fig. 6.1c). Although there was a tendency for fish from the current-day CO<sub>2</sub> treatment to spend more time in shelter following injection of conspecific skin extracts, this was not statistically significant due to the large variation in responses among individuals (Fig. 6.1c).



**Figure 6.1.** Mean change (±SE) of behavior in (A) feeding strikes, (B) activity level, and (C) time spent in shelter (s) by naïve *Pomacentrus amboinensis* when exposed to conspecific skin extracts, heterospecific skin extracts, or a saltwater control between the pre- and post stimulus period for fish exposed to two different CO<sub>2</sub> concentrations. Letters above or below bars represent Tukey's HSD groupings of means.

***Experiment 2: Does elevated CO<sub>2</sub> impair responses to the sight of a predator?***

There was no effect of elevated CO<sub>2</sub> levels on the behaviour of fish during the pre-stimulus period (Pillai's Trace:  $F_{6,166} = 0.43$ ,  $P > 0.5$ ). Overall there was no effect of elevated CO<sub>2</sub> on the visual response of prey to the predator compared to the two controls (Pillai's Trace:  $F_{3,164} = 0.14$ ,  $P = 0.4$ ; Fig. 2). Univariate ANOVAs revealed that there was a significant reduction in feeding rate ( $F_{2,84} = 1.3$ ,  $P = 0.3$ ; Fig. 6.2a) and activity ( $F_{2,84} = 0.9$ ,  $P = 0.4$ ; Fig. 6.2b) when *P. amboinensis* juveniles were exposed to the sight of a predator irrespective of the CO<sub>2</sub> treatment. Time spent in shelter increased in fish exposed to the visual sight of a predator compared with the two experimental controls ( $F_{2,84} = 4.6$ ,  $P = 0.01$ ; Fig. 6.2c), however the response was not identical between CO<sub>2</sub> treatments. Fish from the current-day treatment significantly increased shelter use upon being presented with the visual cue of a predator control, whereas there was no significant difference in shelter use in the fish exposed to elevated CO<sub>2</sub> levels and the two controls (heterospecific skin extract and saltwater). The mean change in shelter time of fish from the elevated CO<sub>2</sub> treatment was intermediate to the fish from the current-day treatments and the two controls, suggesting that there were some effect of the high CO<sub>2</sub> on visual response to the predator (Fig. 6.2c).

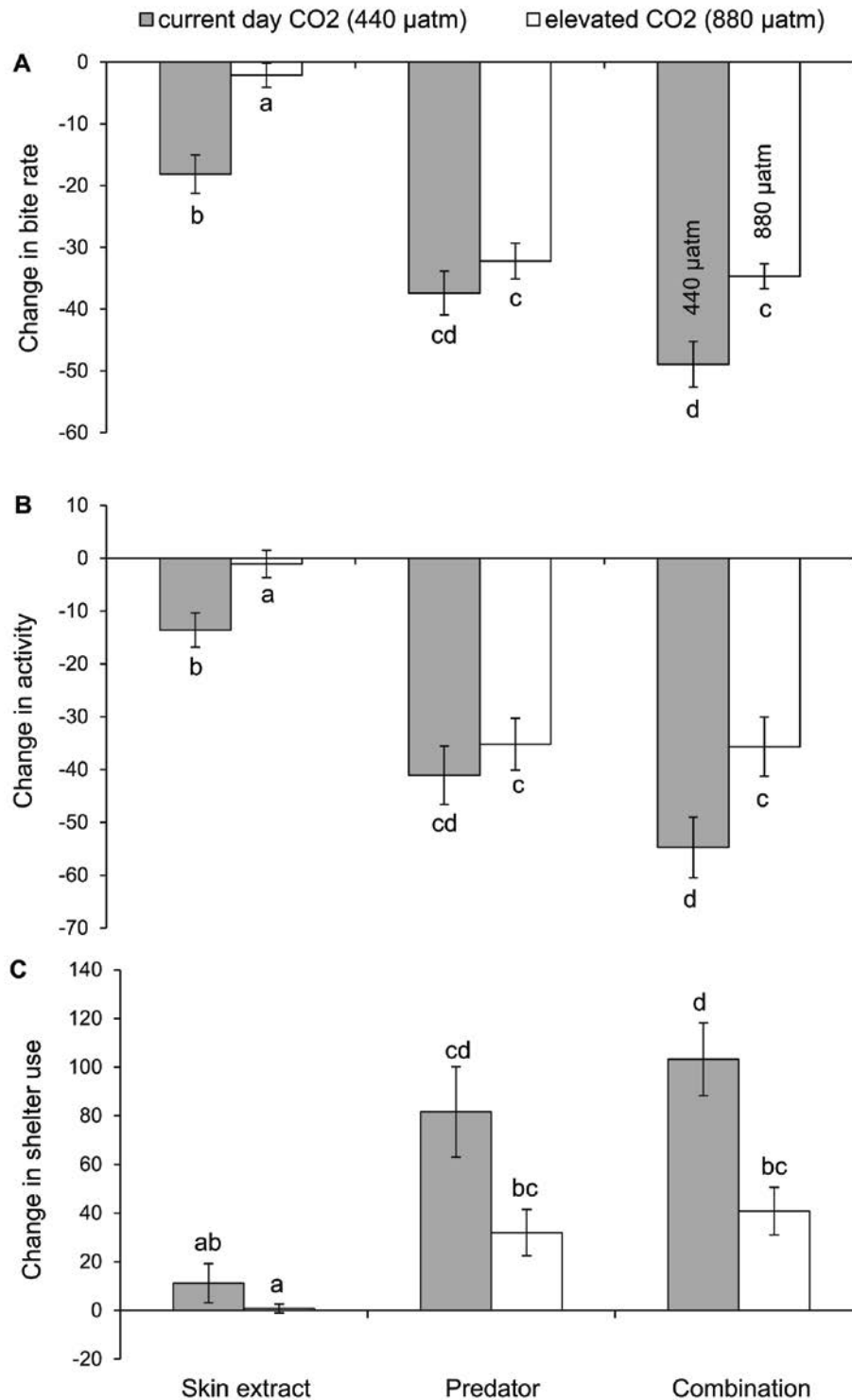


**Figure 6.2.** Mean change (±SE) in behavior in (A) feeding strikes, (B) activity level, and (C) time spent in shelter (s) by naïve *Pomacentrus amboinensis* when exposed to the sight of a common predator, *Pseudochromis fuscus*, a herbivorous goby (*Amblygobius phalanea*) or an empty bag control between the pre- and post stimulus period for fish exposed to two different CO<sub>2</sub> concentrations. Letters above or below bars represent Tukey's HSD groupings of means.

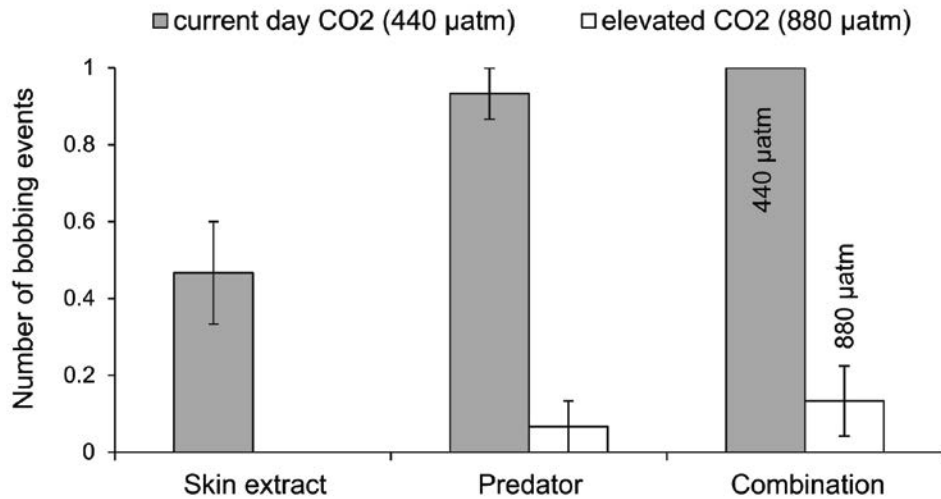
***Experiment 3: Does sensory redundancy reduce the apparent impact of elevated CO<sub>2</sub>?***

As observed in the previous two experiments, there was no effect of elevated CO<sub>2</sub> levels on the behaviour of fish during the pre-stimulus period (Pillai's Trace:  $F_{6,166} = 0.48$ ,  $P > 0.5$ ). The MANOVA revealed that there was an interaction between CO<sub>2</sub> treatment and cue source on the different antipredator responses of fish (Pillai's Trace:  $F_{3,164} = 0.69$ ,  $P = 0.001$ ; Fig. 6.3). In fish exposed to current-day conditions, the combined cue sources gave the strongest threat responses while olfactory cues alone gave the weakest reaction (Fig. 6.3). Fish exposed to elevated CO<sub>2</sub> concentrations did not respond to skin extracts, and the magnitude of their response to combined cue sources did not differ from that elicited when exposed to visual cues alone. Post-hoc tests revealed that prey from the current-day treatment exposed to both cue sources significantly reduced foraging and activity and increased time spent in shelter compared to the CO<sub>2</sub> treated fish (Tukeys HSD:  $P < 0.05$ ; Fig. 6.3).

CO<sub>2</sub> treatment and cue exposure significantly affected the outcome of frequency of bobbing behaviour by *P. amboinensis* (Table 2; model 4 was the best fit; Fig. 6.4). The inclusion of CO<sub>2</sub> treatment in the model resulted in the greatest improvement in the fit of the log-linear model (Table 2; models 1 vs. 3) compared to the inclusion of cue in the model (Table 2; models 1 vs. 2). Therefore, although the outcomes of trials were dependent on both treatment and cue type, CO<sub>2</sub> treatment had the greatest influence on frequency of bobs in *P. amboinensis*. Fish in the control treatment always responded with bobbing behaviour to the simultaneous exposure of both cue sources, whereas fish in the elevated CO<sub>2</sub> treatment bobbed significantly less than control fish, with only 2 out of 15 fish displaying this type of antipredator behaviour (Fig. 6.4).



**Figure 6.3.** Mean change (±SE) in behavior in (A) feeding strikes, (B) activity level, and (C) time spent in shelter (s) by naïve *Pomacentrus amboinensis* when exposed to conspecific skin extracts, the sight of a common predator, *Pseudochromis fuscus*, or a combination of the two between the pre- and post stimulus period for fish exposed to two different CO<sub>2</sub> concentrations. Letters above or below bars represent Tukey's HSD groupings of means.



**Figure 6.4.** Mean number of times that *Pomacentrus amboinensis* displayed a bobbing event ( $\pm$ SE) when naïve fish from each of the two CO<sub>2</sub> treatments were exposed to either conspecific skin extracts, the visual sight of a common predator, *Pseudochromis fuscus*, or a combination of the two. Fish exposed to the higher treatment (880 µatm) showed a significant decrease in the occurrence of this behaviour as compared to fish exposed to current-day CO<sub>2</sub> levels with all fish in this treatment responding to combined cues by bobbing (440 µatm).

**Table 6.2.** Determinant of bobbing frequency of *Pomacentrus amboinensis* when exposed to two CO<sub>2</sub> treatments (two levels) and predation risk cues (three levels). T = CO<sub>2</sub> treatment (elevated, present day), C = cue (skin extract, visual, both), R = reaction (bobs, no bobs).  $n = 15$

Model	Likelihood ratio Chi-square	df	Hypothesis: Bobbing frequency is	df	Difference between models
1) TxC + R	68.29 ***	6	independent of treatment or cue		
2) TxC + CxR	59.41***	3	dependent on cue	3	1 and 2, 8.88*
3) TxC + TxR	15.5**	4	dependent on treatment	2	1 and 3, 52.79***
<b>4)TxC + TxR + CxR</b>	<b>0.63 NS</b>	<b>2</b>	<b>Dependence on both treatment and cue</b>	<b>2</b>	<b>3 and 4, 14.87***</b>
5) TxCxR	0	0	Dependent on an interaction between treatment and cue	2	4 and 5, 0.63NS

## 6.5 Discussion

This study suggests that both the visual and chemical anti-predator systems of naïve prey are sensitive to changes in ocean acidification, however, the visually-based behavioural responses are less affected than chemically-based responses. Fish exposed to elevated CO<sub>2</sub> completely failed to respond chemical alarm cues. While still responding to the sight of a common predator with reduced foraging and movement, *P. amboinensis* exposed to high CO<sub>2</sub> displayed a delayed response to the piscivore spending less time in shelter. Furthermore, CO<sub>2</sub> treated fish did not display the typical ‘bobbing’ response common to damselfish when faced with a threat. It has been suggested that this ‘bobbing’ behaviour evolved as a means of pursuit deterrence; prey signal to the predator that they are aware of its presence, indicating to the predator that it is less likely to be successful in a strike (Smith & Smith 1989; Feeney *et al.* 2012). The lack of bobbing in fish exposed to elevated CO<sub>2</sub> suggests that prey are capable of visually detecting a large shape, responding with increased vigilance, but may not label the shape as a predator. Alternatively, the fish could well recognize the predator, but choose not to initiate bobbing if this is an energetically costly behaviour. Bobbing behaviour is probably similar to that of stotting behaviour in gazelles. Stotting is an honest signal of escape ability and only initiated by individuals that have the ability to escape from the predator (Caro 1986). The lack of a response to the herbivorous goby (visual control) in both the high and low CO<sub>2</sub> treatments suggests that naïve fish are in fact able to visually discriminate between a threatening and non-threatening fish. The response of the naïve prey exposed to acidified waters may simply represent a conservative neophobic response rather than an innate anti-predator response. This apparent lack of ability to correctly categorise a predator, and thus assess risk, will have ramifications for survival in encounters with predators in the field (e.g. Munday *et al.* 2010).



In marine environments, visual and chemical cues are the key sources of information for assessing predation risk (Brown & Chivers 2006; Chapter 2). Olfaction is often the first sense to alert a prey to the presence of a potential predator and once the prey is in the direct vicinity of the stimulus source vision takes over as the primary mode of predator detection (Brown & Chivers 2006; McCormick & Lönnstedt 2013). Visual cues are more reliable as they are fast, highly directional and provide accurate information on which informed behavioural decisions can be made, including predator size, speed of movement and direction, as well as the likelihood of attack (Coss 1979; Helfman 1989). However, to accurately assess the level of threat that a predator poses, prey will often use multiple sensory cues since visual and chemical information provide complimentary information (Lima & Steury 2005; McCormick & Manassa 2008). Despite this, only a few studies have compared the relative importance and balance of more than one stimulus. Given that predator avoidance behaviour is modified based on the magnitude of threat, visual information may maintain anti-predator behaviour, even while olfaction is impaired. Although naïve prey exposed to current day CO<sub>2</sub> levels responded slightly more strongly to the simultaneous exposure to both sensory cues compared to the fish maintained in the elevated CO<sub>2</sub> levels, there was no significant difference in the magnitude of responses in two common anti-predator behaviours (activity and foraging) compared to the experimental controls when fish from both treatments were exposed to the sight of a predator. This suggests that the visual system of prey fish may be able to help mitigate some of the effects of the loss of the olfactory anti-predator system, thus decreasing prey vulnerability to predators in acidified oceans.

Animals have been found to rely more strongly on one type of cue in environments where other cues necessary for predator detection are lacking. Fish have been found to rely more heavily on their chemical sense in situations where visual cues are limited. Stronger antipredator responses are found at night (Leduc *et al.* 2010), in turbid waters (Hartman &

Abrahams 2000; Leahy *et al.* 2011) and in topographically complex habitats (McCormick & Lönnstedt 2013). Similarly, Chivers *et al.* (2001) found that in high visibility environments fish only respond to chemical cues if given in conjunction with the visual cue of a predator. In this study we found that visual cues may be able to help compensate for the inability of fish to recognize threatening olfactory cues as fish still responded to the sight of *P. fuscus* with reduced foraging and movement. In the high clarity waters of coral reefs, it is crucial for prey fish to retain a visual response as they are surrounded by a multitude of different predators.

Our findings suggest that response of naïve prey to the predator *P. fuscus* is innate. Few studies have investigated the innate recognition of prey fish to the visual cue of piscivores. Coss (1979) found that naïve (reared in isolation; having received no previous predator cues) African jewel fish fry (*Hemichromis bimaculatus*) elicit evasive antipredator behaviours when exposed to the sight of a model with 2 front facing eyes (believed to be a widespread signal of danger) compared to a model with no eye-spots. *Pseudochromis fuscus* similarly has two frontally placed eyes, likely to label the fish as dangerous to new settlers. Coss's (1979) study taken together with the current study suggests that the ability of at least some fish to recognize predators appears to be predetermined. Katzenstein & Goren (2006) found that damselfish juveniles classify line-drawings with "smiley faces" as non-threatening and line drawings with 'sad' faces as predators. It appears that fish are good at categorisation of visual stimuli, thus it is possible that juveniles can generalise from their experiences with predators in the pelagic environment to predators on the reef. However, this visual recognition system appears to be impaired by ocean acidification as two of the crucial anti-predator behaviours (bobbing and hiding) were absent in fish exposed to high CO<sub>2</sub> seawater. The lack of bobbing in naïve *P. amboinensis* exposed to acidified seawater suggests that the prey fail to recognize the predator as a threat, alternatively failing to initiate an appropriate

anti-predator response despite recognising the piscivore as a threat, consequently spending less time in the security of shelter. This response could be attributed to CO<sub>2</sub> interfering with the nervous system of fish. An elegant study carried out by Nilsson *et al.* (2012) found that similar CO<sub>2</sub> levels (~900 µatm) inhibits the sensory system of fish by interfering with GABA-A neurotransmitters, thereby affecting chemosensory, auditory and visual abilities. If settlement stage fish are unable to determine the degree of possible threat due to reduced cognitive abilities, they may act in a cautious manner (as they would to any new stimulus) but not with the same intensity as to the presence of a known predator, as was found in this study.

Our results imply that some anti-predator behaviours of fish to chemical and visual threats will be affected by ocean acidification. While the visual response is not entirely lost, the sense is affected by rising CO<sub>2</sub> levels. Naïve prey exposed to higher CO<sub>2</sub> concentrations did respond to the presence of a predator, but with a lower intensity than control fish, failing to retire to the safety of shelter. Their lack of appropriate behavioural responses to piscivores could pose a major problem when crepuscular and nocturnal predators are active, as vision is even more limited during these hours. This is the time of the day when the majority of mortality occurs, and a well-developed visual sense along with the olfactory sense plays a key role in the identification and avoidance of predators. The cost of missing a sign of a nearby predator can be fatal, as was seen in Munday *et al.* (2010) where larval fish (*P. wardi*) raised in seawater enriched with levels of CO<sub>2</sub> predicted for the end of this century (~850 µatm) displayed up to 9 times higher mortality compared to fish raised in current day seawater. These fish had access to all naturally available predation cues, indicating that a partially functioning visual system was insufficient to prevent dramatic increases in predation of high CO<sub>2</sub> exposed fish. Additional field experiments are needed to determine whether the same would hold true for ambon damselfish.

Our study and previous studies have come to the same conclusion; the sensory systems and behavioural responses of fish will most likely be severely affected in future acidified oceans. A key question is whether marine organisms will be able to adapt to the changing pH of the world's oceans (Kelly & Hoffman 2012, Munday *et al.* 2012a). We already know that some animals are no longer able to cope with environments they have spent thousands of generations specifically adapting to (Walther *et al.* 2002). And it is a cause for concern when the nervous system and instinctive behaviours of naïve prey are damaged or lost as a consequence of rising CO<sub>2</sub> concentrations. Nevertheless, some studies have detected reduced impacts when several generations are exposed to the same high CO<sub>2</sub> environment (Parker *et al.* 2012, Miller *et al.* 2012) and there could be the potential for selection of CO<sub>2</sub> tolerant genotypes to occur over coming generations (Munday *et al.* 2012b). Whether differences in the severity of impacts to different sensory systems increases the potential for adaptation to a high CO<sub>2</sub> environment remains to be seen, but should be a priority area for future research.

## Chapter 7: General Discussion

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Predation is one of the fundamental processes that govern the lives of prey individuals, populations and communities over ecological and evolutionary timescales. Predators not only influence who lives and dies but also where to live, when and where to forage, how to behave and grow, and when to mature and reproduce (Sih 1980; Lima & Dill 1990; Brown 2003). Despite the overwhelming importance of predation, there is a paucity of information on how predators interact with and impact prey populations, and behavioural mechanisms that underlie these interactions. Predation affects the structure and complex dynamics of reef fish populations, and any increases in predation rates could have significant impacts on prey populations as well as overall trophodynamics of coral reef ecosystems. By furthering our understanding of the highly complex dynamics of predator-prey interactions we strengthen our ability to interpret the processes that regulate communities and can begin to understand how changes in our natural world will affect these crucial ecological processes. My dissertation explored how global environmental change affects basic predator-prey interactions at a key life history stage in coral reef fish. I found that changes in the biological and physical environment can have immediate and major consequences for how prey assess risk. Behavioural interactions and, more importantly, crucial sensory systems of naïve prey are affected by a number of biological and physical disturbances, which could have profound consequences for reef replenishment and recruitment.

### *Importance of visual and chemical sensory cues*

How predators and prey detect one another is dependent upon the sensory cues received whether auditory, chemical, mechanical or visual (Blumstein *et al.* 2000; Wisenden *et al.* 2008; Ferrari *et al.* 2010a). The importance and response to a predator cue varies among

different environments and is context dependent, differing with features of the site or habitat (McCormick & Lönnstedt 2013; Lönnstedt *et al.* 2014). In aquatic prey the most commonly used cues are visual (Helfman 1989; Smith & Belk 2001) and chemical stimuli (Brown 2003; Brown & Chivers 2006). While chemical compounds are sufficient to induce defences in freshwater systems where visibility is low and prey rely heavily on chemical cues (Brown 2003; Ferrari *et al.* 2010a), coral reefs are clear water environments with a myriad of predators of different colours and sizes (Chapter 2-6). Here, good visual abilities are particularly important to vulnerable new settlers (Chapter 2). In fact, the well-developed eye sight of many prey has prompted the majority of predators to hunt at twilight, when vision becomes less reliable and the odds of making successful strikes turn in the predators favour (Bosiger *et al.* 2012; Marshall *et al.* 2003).

However in aquatic environments, chemicals are available all the time while visual cues are only available during daytime and in visually-unrestricted habitats highlighting the need and importance of olfactory threat cues in coral reefs (McCormick & Lönnstedt 2013). When the olfactory sense is impaired (Chapter 4-6), visual cues may act to maintain anti-predator responses, however elevated mortality levels in fish with weakened olfactory abilities suggests that the visual system does not outweigh the loss of the olfactory response (McCormick 2009; Munday *et al.* 2010; Lönnstedt *et al.* 2014; Chapter 4). The strongest behavioural responses are seen in fish exposed to both visual and chemical predation cues (Chapter 2-6), as is more likely to be the case in natural field conditions. This highlights the importance of incorporating both sensory cues in experiments examining risk assessment mechanisms in naïve prey. Not surprisingly, prey are extremely vulnerable to predation-related mortality when both the visual and chemical senses are impaired (Chapter 4).

### ***Importance of associative learning***

Settlement is a critical life history transition for the majority of coral reef fishes, also representing a severe bottleneck in the population, as predator driven mortality can cause over 60% loss of a cohort in a single night (Almany & Webster 2006). The use of olfactory cues in general and injured conspecific cues in particular, is crucial for settlement stage prey to be able to respond adaptively to and minimize predation risk during this time (Chapter 2, 3). In particular, the simultaneous detection of injured conspecific cues and threat cues (like the smell or sight of a novel predator) leads to a quick learned association, allowing prey to recognize predators as threats during subsequent encounters (Brown & Chivers 2006; Mitchell *et al.* 2011a; Chapter 2). I found that pre-release teaching of settlement-stage damselfish to two main reef predators led to a ~65% survival after 3 days, while predator-naïve fish had <10% survival during the same period of time (Chapter 2).

Surprisingly, there is a predatory fish native to the Great Barrier Reef that has evolved to circumvent this learning mechanism in prey (Chapter 4). No other fish has been found to be able to circumvent the sophisticated threat-learning mechanism that fishes possess, and the current findings could be one of the reasons that *Pterois volitans* is such a successful invader in the Caribbean reef ecosystem. If prey are unable to detect and/or learn that a dangerous predator is a threat, that predator would be able to modify whole communities by the overconsumption of small prey species. Moreover, both ocean acidification and habitat degradation render conspecific skin extract cues useless (Chapter 5, 6) with severe consequences for settlement stage prey that rely on these cues for learning a new catalogue of predators that await them on the reef. What is excruciatingly clear is that learned predator responses are crucial as they greatly enhance survival rates for newly settled prey (Chapter 2). Thus, skin extract cues are mediating adaptations crucial to survival, and any factors affecting these cues are consequently affecting the outcome of predator-prey interactions.

***Importance of predators***

Cues from predators and injured conspecifics facilitate a number of behavioural, morphological and life-history adaptations (Chapter 2, 3). Fish exposed to predator cues early on in life displayed inherently more cautious behaviours than naïve fish, which had a strong influence on survival rates (Chapter 2-4). Interestingly, prey exposed to predators for 6 weeks grew deeper bodies and developed larger false eyespots and smaller eyes than fish exposed to herbivores or isolated, despite the same resource levels being given to all treatments (Chapter 3). The gape-limitation imposed on many predators means that one of the key relationships underlying catch success may well be predator mouth width versus prey body depth (Werner 1974, Werner & Gilliam 1984, Hill *et al.* 2005). *Pseudochromis fuscus* have been found to be highly selective, preferring juvenile prey of a certain size and phenotype (Holmes & McCormick 2010a). As a result of the highly selective nature, there is the potential for predator selection to have a large influence on the traits that are passed on to successive life stages. In Chapter 3 I show as a world first, that successful juvenile prey have evolved phenotypic plasticity as a direct response to the presence of a consumer in a coral reef ecosystem.

Although common mesopredators influenced behaviour, development and survival in prey, there appears to be a ‘predator effect’ whereby prey fish do not have a uniform response to all predator species (Chapter 4). Experience did not matter in prey exposed to red lionfish as small prey fish did not display overt behavioural responses regardless of learning in subsequent encounters with lionfish, compared to those prey exposed to two other, more common mesopredators. As biotic invasions are becoming a common global phenomenon, it is crucial that we try to understand the underlying causes driving the success of alien species.



### ***Importance of environment***

Whether it is from over-harvest, degradation of habitats, biotic invasions, pollution or the more topical issues of climate change, coral reefs have not escaped the ability of humans to modify the landscape of the planet (Hughes *et al.* 2007). Along with these anthropogenic stressors come changes to the habitats upon which fish depend. As the importance and response to a predator stimulus varies among different environments and is context dependent, the changing habitats may evoke careless or rash behaviours in response to predators (Stankowich & Blumstein 2005). While only a small number of fishes are coral obligates, many use live coral as a nursery habitat (Pratchett *et al.* 2008). Chapter 5 examined how risk assessment mechanisms in new settlers were affected by habitat degradation. In a series of experiments it was demonstrated that juveniles on dead coral display risk-prone behaviours, compared to those on living coral. In addition, they did not seem to display any overt antipredator response to injured conspecific cues in dead algal covered habitats. In whatever way it is affected, a reduced ability to correctly respond to predator cues can have serious ramifications for survival in encounters with predators in the field (Chapter 2, 4, 5). The results provide the first of many potential mechanisms through which habitat degradation can impact the relationship between prey and predators in the coral reef ecosystem. As the proportion of dead coral increases, the recruitment and replenishment of coral reef fishes will be threatened, and so will the level of diversity in these biodiversity hotspots.

Habitat degradation is not the only threat to the behavioural sensory systems used by prey. Ocean acidification is viewed as one of the major threats facing coral reef organisms today (Munday *et al.* 2012). We found that fish exposed to 850  $\mu\text{atm}$  showed reduced antipredator responses, however, contrary to our expectations, exposure to  $\text{CO}_2$  did not fully impair responses of the prey to the sight of the predator. Fish raised in elevated  $\text{CO}_2$  and exposed to both chemical and visual predation cues combined responded with the same

intensity as those exposed only to visual cues. Thus responses of fishes to visual cues may at least partially compensate for the lack of responses to olfactory cues (Dixon *et al.* 2010; Munday *et al.* 2010; Chapter 6). The key question is whether this ‘sensory compensation’ will allow prey fish to survive predation attacks in acidified oceans. Chapter 6 provides us with a glimmer of hope that fish can adapt and survive through selection, in an otherwise very disturbed ecosystem.

### ***Concluding remarks***

The current body of work has provided us with a wider understanding of how biological and physical habitat disturbances can affect species interactions and crucial behavioural processes in a severely stressed system. Everything within an ecosystem is connected, and if one player is removed and/or added to the ecological stage, or a background element is changed there can be serious repercussions for that ecosystem as a whole (Chapter 4-6). Interactions between predators and prey are not only responsible for a significant amount of today’s evolutionary diversity, but also drive energy transfer within food webs and the functioning of ecosystems. If these relationships are disrupted, there can be major repercussions for overall ecosystem stability. In a series of novel and innovative experiments, I have demonstrated how some of the complex dynamics of coral reef systems will change as these environments continue to be put under anthropogenic induced stressors. Accurate predictions of species’ responses to changing habitats are imperative if we want to conserve and protect ecosystems, which necessitates a detailed understanding of the complexity of how prey interact with predators and the role that physical and biological background habitat plays in these crucial interactions.

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