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Processes and mechanisms of predatory interactions on newly settled reef fish

Thesis submitted by

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Statement on the contribution of others

This thesis includes some collaborative work with my supervisors Dr. Mark McCormick. While undertaking these collaborations, I was responsible for the project concept and design, data collection, analysis and interpretation and final synthesis of results into a format suitable for publication. My co-author provided intellectual guidance, financial support, technical instruction and editorial assistance.

Financial support for the project was provided by my supervisor Dr. Mark McCormick, the Lizard Island Doctoral Fellowship Program, the Ecological Society of Australia, the Linnean Society of NSW and James Cook University, and Stipend support was provided by an Australian Postgraduate Award.

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General Abstract

Predation is generally thought to be one of the major processes influencing the size of populations and the structure of ecological communities. As such, the mechanisms of prey survival during predatory interactions will play a large role in determining those characteristics and traits that are passed on to later life stages. These mechanisms will be particularly important during periods of high mortality, such as transitional periods between life history stages for organisms with complex, bi-partite life cycles. One such period is that of settlement from the pelagic larval phase to the more benthic associated juvenile phase in many coral reef fishes.

This project examines the mechanisms influencing survival during interactions with small reef fish predators over this early post-settlement period. The focus is split between two distinct ecological areas that are thought to play a major role in determining survival during transitional life stages: the phenotypic and performance characteristics of predator and prey; and the behavioural responses of prey to potential predation threats. The chapters of this thesis addresses the following questions: 1) how selective is predation with respect to three key prey characteristics: body size, body weight and burst swimming speed; 2) how does predator size and identity influence the nature of size selection; 3) how do behavioural characteristics associated with body size influence size-selective patterns; 4) what is the role of chemical alarm cues in anti-predator responses and predator identification; and 5) how do anti-predator responses to both visual and chemical predation cues differ with a changing level of threat.

The common Ambon damselfish, *Pomacentrus amboinensis,* was used as the model prey species throughout all experiments. These were collected during settlement pulses using light traps, so as to keep them naïve to all reef-based processes. All experiments examining the selective nature of predation were conducted in aquaria. Individual predators (*Pseudochromis fuscus*) were offered a choice of prey, differing in either body size, body weight or burst swimming speed. Predation by this species was found to be highly selective towards larger body size at the time of settlement. In contrast, there was no evidence of selection with regards to either prey body weight or burst swimming speed. These patterns were found to differ from those observed in field based trials, where prey were open to multiple predator communities. These results indicate that body size may be the most important prey characteristic influencing prey survival during predatory encounters over this early period. Further, the discrepancy between single and multiple species trials suggest that the nature of selection towards this trait may differ between predator species and sizes.

Closer examination of this hypothesis using further aquarium trials showed that the intensity and direction of size selectivity differed significantly between four of the key predatory fish species (the dottyback, *Pseudochromis fuscus*; the moonwrasse *Thalassoma lunare*; the lizardfish *Synodus variegatus*; and the rockcod, *Cephalopholis microprion*). Some species preferentially removed smaller individuals (*T. lunare, S. variegatus*), while others removed larger individuals (*P. fuscus*) or were non-selective (*C. microprion*). However, these patterns of selectivity were not found to differ with predator size. These results suggest that no specific expression of a phenotypic trait holds a definitive survival advantage during all encounters. Instead, prey survival may in part be determined by the behavioural characteristics of different sized prey within a

hierarchy, and how this influences vulnerability to predation by different ‘modes’ of predation

Size associated differences in prey behaviour within simple hierarchical groups were examined in both aquaria and on small patch reefs constructed immediately adjacent to shallow lagoonal habitat. Small and large individuals were paired and assessed for five behavioural traits. Large individuals were found to make more aggressive strikes on conspecifics and had higher feeding rates than their smaller counterparts. We suggest that the dominant behaviours displayed by larger individuals in a group could result in increased vulnerability of smaller individuals to opportunistic predation, leading to the patterns of predation observed in the previous chapter (with the exception of *P. fuscus*).

How species react to predation threats and acquire knowledge of them in previously novel habitats will have a large influence on survival during transitional periods. To examine the role that visual and chemical cues play in this process, fishes were assessed for behavioural responses to potential visual predation cues and chemical alarm cues released from injured fishes. Additionally, fish were assessed to determine whether they could use chemical alarm cues to associate novel predator scents with danger. Fish were found to respond to conspecific chemical alarm cues only by reducing their feeding rate. Individuals were able to use these alarm cues to associate a previously novel predator scent with danger, after only a single previous exposure to the paired conspecific alarm/novel scent cue. In contrast, responses to visual cues were more widespread but diffuse, and fish were unable to distinguish between predatory and non-predatory cues. These results indicate the important role that chemical cues in particular play in both threat detection and learned predator recognition during the early post-

settlement period in coral reef fishes. Although visual cues also play a role, their utility appears limited whilst still naïve to reef based processes, due to a lack of innate recognition of predator identity.

When tested across a range of predation threat levels (by manipulating chemical cue concentration and distance from visual cue), behavioural responses were found to be threat dependent in nature. Although significant changes were observed, responses to visual cues were again inconsistent, whilst responses to extremely low chemical cue concentrations were marginal, indicating a possible threshold lower limit. This demonstrates the ability of newly settled fish to assess the level of predation risk using both visual and chemical cues, and respond appropriately.

This project provides us with a detailed insight into the mechanisms and processes of survival during a potentially critical life history period for coral reef fishes. In doing so, it shows how both phenotypic characteristics, predator identity and behavioural changes associated with threat detection and predator learning may influence the outcome of predatory interactions during this early period.

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Chapter 1: General Introduction

Predation is generally thought to be one of the major processes influencing the size of populations and the structure of ecological communities (Sih 1987; Beukers and Jones 1997). As such, it has the potential to be a strong selective force over evolutionary time, and has long been recognised as important in the evolution of adaptations (Lima and Dill 1990). Over shorter time frames, the selective nature of predation has been widely implicated in determining the traits that will be passed on to successive life stages. Those characteristics and behaviours that decrease vulnerability will be retained, whilst those that do not will be lost.

The effects of these traits on the outcome of predation events may be particularly important during periods of high mortality. Such periods are common during both environmental bottlenecks, where resources are a limiting factor (e.g. food, habitat; Payne and Wilson 1999; Finstad et al. 2009), and transitional periods between life stages for organisms with complex life-history cycles (e.g. amphibians; Werner 1986, insects; Fuester and Taylor 1996, marine invertebrate; Moloney et al. 1994, marine teleosts; McCormick et al. 2002; Leis and McCormick 2002). Due to the intense mortality pressures during these periods (much of which commonly comes directly from predation), the factors that influence survival will play a disproportionately large role, as individuals struggle to gain some advantage that may increase their probability of passing through to the next life stage. Although environmental characteristics (e.g. habitat complexity; Babbitt and Tanner 1998; Ray-Culp et al. 1999; light levels and visibility; Rilov et al. 2007) also play a major role, the key intrinsic factors influencing survival during these periods are generally thought to be the phenotypic (Litvak and Leggett 1992; Dorner and Wagner 2003), performance (Husak 2006) and behavioural characteristics (Alvarez and Nicieza 2006) of predator and prey.

Phenotypic and performance characteristics that may influence survival during predatory encounters relate directly to the morphological and physiological state of an individual. On the other hand, behavioural characteristics can be said to be any behaviour that will affect the susceptibility of prey to capture at some point during the predation process. This can incorporate a wide range of behavioural processes as potential prey seek to avoid detection and vulnerability early in the predation sequence (Nilsson and Forsman 2003; Titelman and Kiorboe 2003; Berger and Gotthard 2008), whilst also increasing their ability to escape if engaged by a predator further into the sequence of events (Andrade and Lopez 2005; Paglianti and Domenici 2006). During transitional periods between life stages (which commonly involve transitions to new habitats; Barriga and Battini 2009), one area that will greatly influence survival is an individual’s ability to gain knowledge of the new system and apply this in the context of predation events (e.g. learning the identity of relevant predators; Wisenden et al. 1997; Mirza et al. 2006; Gonzalo et al. 2007).

Phenotypic and performance characteristics

The idea that phenotypic and performance characteristics influence an individual’s probability of survival has received considerable attention in the literature. From a prey’s prespective, body size (Allen 2008; Sakamoto and Hanazoto 2008), overall condition (Husseman et al. 2003; Grorud-Colvert and Sponaugle 2006; Figueira et al.

2008), growth rate (Takasuka et al. 2003; Sponaugle and Grorud-Colvert 2006; Urban 2007), sensory development (Poling & Fuiman 1997) and escape speed (Brana 2003) have all been implicated in determining the outcome of predatory events. Of these, body size is widely considered to be the most important (Cohen et al. 1993; Wellborn 1994; Sogard 1997; Wang et al. 2007). The most common theory regarding this characteristic is that larger size conveys a survival advantage during predatory interactions, through enhanced competitive abilities and an increased ability to escape predators (bigger-is-better hypothesis; Litvak and Leggett 1992). However, due to the level of covariance commonly associated with body size and other phenotypic and performance characteristics (i.e. larger body size for a given age is commonly associated with higher condition and growth rate, increased sensory development, and faster escape speed; McCormick and Molony 1993; Miles et al. 1995; Kerrigan 1996), it is often difficult to elucidate the true mechanisms underlying this relationship. To date however, this potentially confounding factor is frequently overlooked in many studies examining the dynamics of predator-prey interactions.

When considering the influence of phenotypic and performance characteristics on predator-prey interactions, the characteristics of the predator must also be considered. Optimal foraging theory (OFT) predicts that predators preferentially prey on an optimal prey phenotype in order to maximise the net rate of energy intake (MacArthur and Pianka 1966; Hughes 1980). Differentiating somewhat from the ‘bigger-is-better’ hypothesis, this suggests that both larger and smaller prey sizes convey a survival advantage, as selective profiles of predators tend to be dome shaped (Rice et al. 1997). As predator size increases with ontogeny, this theory additionally predicts that their optimal prey phenotype will shift with it (i.e. preferred prey size will increase with predator size; Hughes 1980). As such, in interactions involving single species of predator and prey, the size distribution of the predator may well determine the size range of prey eaten (Woodward et al. 2005; Urban 2007).

Behavioural Mechanisms

In comparison to morphological and physiological characteristics, the influence of behavioural processes in determining the outcome of predatory events has received relatively little attention. For prey, the level of shelter use, foraging rate, space use (Stauffer and Semlitsch 1993; Chivers and Smith 1998; Griffiths et al. 1998; Grorud-Colvert and Sponaugle 2006) and even the level of boldness (Sih et al. 2004; Stamps 2007) are all behaviours that may determine how vulnerable individuals are at different stages in the predation sequence. Predator behaviour will also play a role, determining the suite of prey characteristics that are most susceptible to capture. This is largely a product of the behavioural traits that define predation modes, with certain prey characteristics being more vulnerable to specific predation types (e.g. individuals who spend long periods away from shelter may be more susceptible to opportunistic predators). Therefore, who is lost to predation may well be decided by the behavioural characteristics of both prey and surrounding predators.

For individuals who have recently transitioned to a new habitat, experience gained from previous, unsuccessful, predation events has been shown to greatly increase the probability of survival (Mathis et al. 2003; McCormick and Holmes 2006). This experience is thought to come from the association of previously novel cues with a threat, resulting in changes to behavioural patterns (e.g. reduction in foraging activity) that subsequently reduce the vulnerability to predation when the same cue is again encountered (Woody and Mathis 1998). Methods of detecting these threats vary between systems and species, but includes the use of visual (Lohrey et al. 2009; McPhee et al. 2009), acoustic (Durant 2000; Blumstein et al. 2008), olfactory (Gonzalo et al. 2008; Roth et al. 2008) and seismic cues (Warkentin et al. 2007; Lohrey et al. 2009). In aquatic systems, the two key sensory techniques are thought to be vision and olfaction (Mathis and Vincent 2000; Chivers et al. 2001; Kim et al. 2009). Although each may play a slightly different role in the acquisition of threat information (i.e. olfactory cues may be more important earlier in the predation sequence), both are considered important for both detection and learning purposes (Brown and Magnavacca 2003).

The study system

In many coral reef fishes, the period of settlement to the reef environment involves a rapid transition from the pelagic environment to the reef habitat (Leis and McCormick 2002). This period is commonly characterised by significant changes in morphology and behaviour, as fish metamorphose from larvae into more benthic-associated forms (McCormick and Makey 1997; McCormick et al. 2002). Not surprisingly, individuals passing through this stage experience extremely high levels of mortality, as fish adapt to life in a habitat in which they have no prior experience. Upwards of 60% of individuals have been shown to be lost within the first two days post-settlement (Doherty et al. 2004; Almany and Webster 2006), and much of this is thought to be attributed to the actions of small site-associated fish predators (Carr and Hixon

1995; Holbrook and Schmitt 2002). As such, the processes and mechanisms influencing prey survival during interactions with these predatory fishes will have a disproportionate role in determining those individuals, and characteristics, that are passed into juvenile and adult populations.

Despite the potential importance of this period to population dynamics, to date we know very little about how individuals maximise their probability of survival whilst passing through it. Ecological theory (ie. ‘bigger-is-better’ hypothesis) would suggest that larger body size, and its covariates (e.g. increased condition, performance), should convey a survival advantage. However, studies examining this in the marine environment are commonly confounded by inter-related variables, and generalisations are frequently made across communities and systems without considering the potential for differences between species-specific interactions. Additionally, an individual’s ability to detect and respond appropriately to relevant threats during this period of intense predation pressure is predicted to play a substantial role in determining who survives through to the next life stage. Yet, we currently know nothing about methods of threat detection, modes of response, or mechanisms of learning in juvenile reef fish.

Study Species

The present study uses a single species of newly-settled coral reef fish as a model prey species. The Ambon damselfish (*Pomacentrus amboinensis*; Fig. 1.1) is common amongst coral reef fish communities within the Indo-Pacific, particularly in the central Great Barrier Reef. It settles to a wide variety of habitats, but is found in highest densities associated with small reef patches at the base of shallow reefs. The species has a pelagic larval phase of between 15 – 23 days and settles at 10.3 – 15.1 mm standard length (Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site-attached, making it an ideal species for experimental manipulation. They recruit in substantial numbers at Lizard Island around the new moon during the summer months (October – January), and are easily collected at the time of settlement with light traps (Milicich and Doherty 1994). This life cycle, body plan and approximate size is common to a large number of damselfish (Pomacentridae) species.

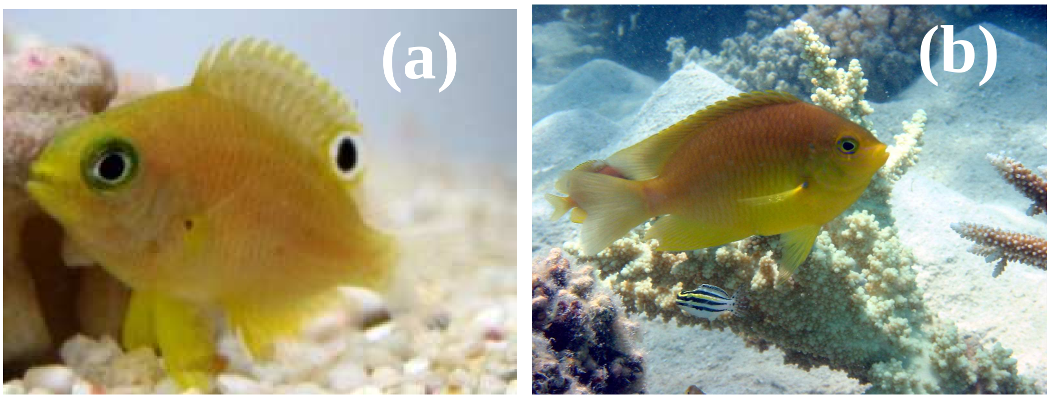

Figure 1.1: (a) Newly-settled, and (b) adult *Pomacentrus amboinensis* (Pomacentridae).

Four species of small site-associated fishes were used as predator species throughout experiments (Fig. 1.2): the brown dottyback, *Pseudochromis fuscus* (Pseudochromidae); the moonwrasse, *Thalassoma lunare* (Labridae); the sand lizardfish, *Synodus dermatogenys* (Synodontidae); and the freckled rockcod, *Cephalopholis microprion* (Serranidae). Each is known to prey heavily upon newly-settled and juvenile fish during settlement periods (Martin 1994, TH Holmes and MI McCormick *personal observations*), and is common on shallow reefs throughout much of the West Pacific and Indian Oceans. *P. fuscus* is a small (max size 72.4 mm SL), solitary cryptic pursuit predator commonly found on small coral bommies or along reef edges. *T. lunare* is a highly active opportunistic predator (max size 200 mm SL), generally found higher in the water column in haremic groups across a range of reef habitats. *S. dermatogenys* is a cryptic ambush predator (max size 210 mm SL) commonly found on sandy substrata immediately adjacent to the reef base or amongst small bommies and coral rubble. *C. microprion* is another cryptic predator (max size 210 mm SL), generally found in caves or beneath ledges in both coral and rubble habitats.

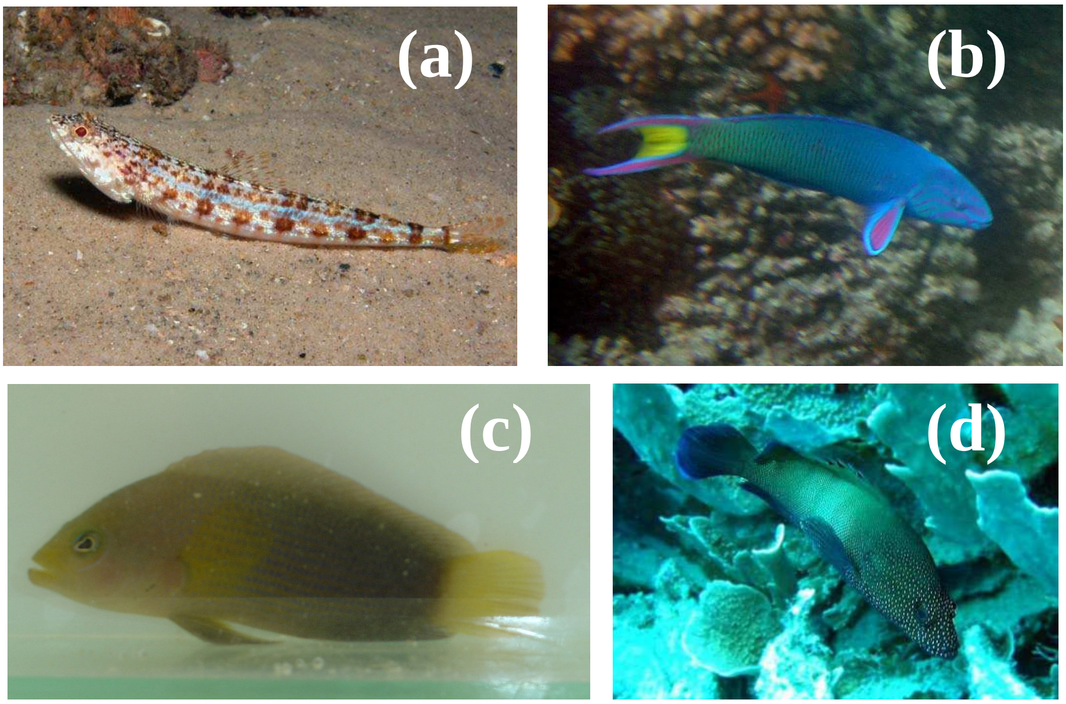

Figure 1.2: Common fish predators of newly-settled reef fish at Lizard Island: (a) the sand lizardfish, *Synodus dermatogenys* (Synodontidae); the moonwrasse, *Thalassoma lunare* (Labridae); the brown dottyback, *Pseudochromis fuscus* (Pseudochromidae); and the freckled rockcod, *Cephalopholis microprion* (Serranidae).

Aims and Thesis Outline

This study examines the mechanisms influencing survival in newly-settled coral reef fish during interactions with small reef fish predators. The focus is split between two distinct ecological areas that are thought to play a major role in determining survival during transitional life stages: the phenotypic and performance characteristics of predator and prey; and the behavioural responses of prey to potential predation threats. Using a series of aquarium and field-based experiments, this study addresses the following questions: 1) how selective is predation with respect to a number of prey body and performance characteristics; 2) how does predator size and identity influence the nature of size selection; 3) what is the role of chemical alarm cues in anti-predator responses and predator identification; and 4) how do anti-predator responses to both visual and chemical predation cues differ with a changing level of threat.

These questions are examined in five separate studies, each corresponding to one of the chapters outlined below. **Chapter 2** assesses the selectivity of predation by a single common predatory species with respect to three key prey characteristics: body size, body weight and burst swimming speed. Body size was standardised during body weight and swimming speed trials to avoid confounding the results, whilst relationships between eight body and performance characteristics at the time of settlement are examined to elucidate co-variation between traits. **Chapter 3** draws on this work and focuses more directly on the effects of body size on the outcome of predatory interactions. Specifically, how predator size and identity (species) influence the nature of size selection is examined for both the early post-settlement and juvenile prey. **Chapter 4** examines the behavioural characteristics of large and small prey within simple hierarchical groups, and infers the role that these may play in determining the size-selective patterns observed in Chapter 2. **Chapter 5** begins to assess the behavioural responses of prey to potential predation threats, by looking at the role of chemical alarm cues in both threat identification and the learning of predator identity. Finally, **Chapter 6** follows on from the previous chapter, examining how behavioural responses to both visual and chemical predation cues change with varying levels of perceived threat.

Although each chapter has been purposefully written as a stand-alone paper (currently in varying stages of publication; see Appendix 1), the progression of the dissertation follows a logical sequence, with all chapters encompassed within the central theme of the key mechanisms influencing early post-settlement survival. In doing so, it tests existing tenets of ecological theory that are widely considered to underlie survival probability in aquatic communities.

Chapter 2: Influence of prey body characteristics and performance on predator selection

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2.1. Introduction

Body characteristics and performance attributes are generally thought to play a large role in determining an individual's probability of survival throughout its lifetime. Such traits have been widely linked to influencing survival during a range of events, including predatory (Litvak and Leggett 1992; Janzen 1993; Twombly and Tisch 2000; Dorner and Wagner 2003; Hoey and McCormick 2004; Alvarez and Nicieza 2006; Husak 2006) and competitive interactions (Smith 1990; Marshall et al. 2006; Persson and De Roos 2006; Van Buskirk 2007; Zedrosser et al. 2007), and threat of starvation and disease (Biro et al. 2004; Lyons et al. 2004; Bystrom et al. 2006; Reim et al. 2006; Hall et al. 2007; Smith et al. 2007). In recent years, the importance of these prey characteristics during predatory interactions has received considerable attention, as predation is widely thought to be one of the major processes influencing the size of populations and the structure of ecological communities (Sih 1987). The selective nature of predation means that prey characteristics that decrease an individual's vulnerability to predators will be retained within a population, whilst those that increase vulnerability will be selectively lost. The extent to which predation is selective will be dependent on the preferences and selective profiles of those predators within the community.

Predator selectivity may be of particular importance during periods of high mortality. Such cases are common during transitional periods between life history stages for organisms with complex life-cycles (e.g. bi-partite life cycle of many amphibians, and marine invertebrates and fishes; Werner 1986; Gosselin and Qian 1997; Hunt and Scheibling 1997; McCormick et al. 2002; Leis and McCormick 2002). If predation is selective during such periods, then it may have a disproportionate influence on those traits that are passed into successive life-stages. If, however predation is not selective, then high mortality alone does not necessarily indicate a critical life stage for population regulation or for life history evolution (e.g. Crouse et al. 1987).

Coral reef fishes are an ideal group on which to study the selective nature of predation. At the time of settlement to the reef environment, many species undergo a transitional period between a planktonic larval stage and a more ‘benthic-associated’ adult/juvenile stage, often marked by rapid morphological and physiological changes (McCormick and Makey 1997; McCormick et al. 2002). This period is characterized by high levels of mortality, with upwards of 50% of individuals being lost within the first 1-2 days post-settlement (Doherty et al. 2004; Almany and Webster 2006). Much of this mortality has been attributed to predation by small ‘reef associated’ fish predators (Carr and Hixon 1995; Holbrook and Schmitt 2002). In addition, at the time of settlement individuals generally possess moderate to high levels of variability in a number of traits known to influence survival during a predatory encounter (McCormick and Molony 1993; Hoey and McCormick 2004).

Prey body size is one morphological characteristic that is generally thought to play a large role in influencing the outcome of such encounters (Sogard 1997; Schmitt and Holbrook 1999; Brunton and Booth 2003; McCormick and Hoey 2004; Holmes and McCormick 2006). One common theory (the ‘Bigger-is-Better’ hypothesis) suggests that from a prey's perspective, being larger at a given life-history stage results in a survival advantage, through reduced predation rates (Rice et al. 1993; Takasuka et al. 2003), enhanced competitive abilities and decreased susceptibility to starvation (Sogard 1997; Schmitt and Holbrock 1999). Thus, as prey size increases, vulnerability to predation is predicted to decrease. An alternate ecological theory, known as optimal foraging theory (OFT), predicts that predators preferentially prey on an optimal prey size in order to maximise the net rate of energy intake (MacArthur and Pianka 1966; Hughes 1980). This theory predicts that, from a prey's perspective both large and small size conveys a survival advantage during a predatory encounter. The characteristics of the prey that are targeted are contingent on the selectivity profile of the predator, which tend to be dome-shaped (e.g. Rice et al. 1997).

Prey body condition has also been shown to have important implications for survival during the early post-settlement period (Mesa et al. 1994; Booth and Hixon 1999; Booth and Beretta 2004; Hoey and McCormick 2004; Sponaugle and Grorud-Colvert 2006; Figueira et al. 2008). However, its direct influence on the outcome of predator-prey relationships remains largely untested. Condition of a fish may be measured in a number of different ways, including growth, lipid content, liver hepatosomatic indices, body robustness and developmental state (McCormick and Molony 1993; McCormick 1998; Ferron and Leggett 1994; Hoey et al. 2007). From a prey's point of view, a larger, fatter individual may be able to escape or survive a predatory attack more often than a thinner counterpart. Similarly, it would be expected that further developed individuals with a faster escape response would also have an advantage. Although a number of studies have shown evidence of predator selectivity with respect to prey body characteristics (Sogard 1997; Booth and Hixon 1999; Vigliola and Meekan 2002; Holmes and McCormick 2006; McCormick and Meekan 2007), to date no study has directly examined the mechanisms underlying such selection during this early period.

At the time of settlement, body characteristics generally show poor levels of correlation among themselves when compared for the same individuals (McCormick and Molony 1993; Kerrigan 1996; Hoey and McCormick 2004). However, to date only two studies have attempted to incorporate either pre-settlement growth or a measure of performance (ie. burst/escape speed) into these comparisons (see McCormick and Molony 1993; Grorud-Colvert and Sponaugle 2006). Since many of these traits are driven by similar or inter-related growth processes, it is important to understand how all these characteristics covary to elucidate the mechanism underlying selective loss.

The present study focuses on individual and community level predator selectivity on a common Indo-Pacific coral reef fish (*Pomacentrus amboinensis*) during the early post-settlement period. Experiments were conducted with respect to three "non-destructive" body and performance attributes known to show moderate levels of variability at the time of settlement: body size, body weight and burst/escape swimming speed. Specifically, the aims of the study were: (1) to examine the interrelationships between body characteristics and performance attributes, including pre-settlement growth and burst/escape swimming speed; (2) to determine whether predation by the common predator *Pseudochromis fuscus,* was selective with respect to prey body size, body weight and burst swimming speed; and (3) to determine whether predation by a natural multi-species predator community was selective, and how it differed from the selectivity regime demonstrated by *P. fuscus*.

2.2. Materials and Methods

Study site

This study was conducted at Lizard Island (14°40’S, 145°28’E), northern Great Barrier Reef (GBR), Australia during November and December of 2005 and 2006. The flow through salt water aquarium system at Lizard Island Research Station was used to conduct the aquarium trials, whilst the surrounding shallow lagoonal reefs and sand flats were used for the patch reef trials.

Study species

The ubiquitous damselfish *Pomacentrus amboinensis* was used as the prey species for all experimental trials. This species is common within coral reef fish communities within the Indo-Pacific, particularly in the central GBR. They settle to a wide variety of habitats on the northern GBR, but are found in highest densities associated with small reef patches at the base of shallow reefs. *P. amboinensis* has a pelagic larval phase of between 15 – 23 days and settles at 10.3 – 15.1 mm standard length (Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site attached, making it an ideal species for experimental manipulation. They recruit in substantial numbers at Lizard Island around the new moon during the austral months (October – January), and are easily collected at the time of settlement with light traps (Milicich and Doherty 1994).

The brown dottyback, *Pseudochromis fuscus,* was used as the model predator species for the aquarium trials. *P. fuscus* is a small (max size 72.4 mm SL), site attached predator common on shallow reefs throughout the Indo-Pacific. They are known to consume newly-settled and juvenile fishes in both the laboratory and field (Holmes and McCormick 2006; Almany et al. 2007).

In the natural system, newly-settled reef fishes are subject to a range of resident and transient predators. At Lizard Island the most common predators have been identified as the brown-barred rock cod (*Cephalopholis boenak*), moonwrasse (*Thalassoma lunare*), two species of lizardfish (*Synodus variegatus* and *S. dermatogenys*), and the brown dottyback (*Pseudochromis fuscus*; Martin 1994; Beukers and Jones 1997; Holmes and McCormick 2006).

Fish Collection

Settlement stage *Pomacentrus amboinensis* were collected using light traps moored overnight close to the reef crest, and transported back to the Lizard Island Research Station at dawn. Fish were maintained in 25L flow-through aquaria systems for ~24 hours, and fed newly hatched *Artemia* sp. twice per day ad libitum to allow for recovery from the stress of capture. Growth during this period was minimal.

Adult *Pseudochromis fuscus* (38 – 71.4mm SL) were collected from surrounding reefs using clove oil and hand nets. All fishes were maintained in individual 57L flow-through aquaria systems for 48 hours before use in aquarium trials. Fish were not fed during this period to standardize for satiation, and to avoid handler-associated learning.

Correlation of prey condition/performance measures

Ninety-four *Pomacentrus amboinensis* were randomly selected from a single days light trap catch and measured for eight potential indicators of body condition and performance: standard length, maximum burst speed, mean burst speed, wet weight, pre-settlement growth, dry weight, lipid content and overall body condition. To obtain standard length individual fish were placed into small clip-seal polyethylene bags with a small amount of sea water and measured using calipers (± 0.1mm).

To measure maximum and mean burst speed fish were placed individually into a narrow aquarium (10 x 150 x 200mm) filled to 30mm depth with fresh seawater. The narrow shape of the aquarium effectively forced the fish to move in two dimensions, minimising errors associated with movement away from the viewing plane. Escape bursts in this species were generally observed to occur within this 'side on' viewing plane (McCormick and Molony 1993), as opposed to the 'top down' viewing plane used in previous burst speed studies of Red Drum larvae (Fuiman and Cowan 2003; Fuiman et al. 2006) A 5 x 5 mm reference grid was positioned on the back of the aquarium. Fish were maneuvered to one end of the aquarium and a rubber ball pendulum was dropped from a 45° angle against the glass end of the aquarium immediately behind the fish, to induce the burst response. The fish was allowed to recover from stress before being maneuvered back to the end of the aquarium. This process was repeated until either five reliable bursts were recorded or the fish became too stressed to produce reliable bursts. Only those fish that recorded two or more successful bursts were included in the analysis. A digital camera (frame speed 0.04 sec), positioned facing the front of the aquarium, was used to record each burst. These recordings were analysed, and the 5 x 5 mm grid was used to determine the distance traveled over the first two frames (0.08 sec) of each burst. Only the first two frames were assessed as this initial stage of the burst sequence is thought to be most important when determining the ability of an individual to escape a predation event. From these measurements, maximum and mean burst speeds were calculated for each fish.

Fish were lightly blotted dry and weighed to the nearest 1 mg using a mass balance (wet weight). Euthanised fish were then placed into a freeze drier for 24 hours before being weighed to the nearest 1 µg using a mass balance to obtain a dry weight. Pre-settlement growth was determined by examining the microstructural increments deposited within the sagittal otolith. Otoliths were ground to produce a thin transverse section (as per Wilson and McCormick 1999) and increment widths were measured along the longest axis, the most sensitive axis to growth changes recorded in the otolith profile. The mean width of the outer 7 increments was used as a relative measure of pre-settlement growth. The assumptions that the frequency of increment formation is daily and the distance between consecutive increments is proportional to fish growth have been validated for *P. amboinensis* juveniles by Pitcher (1988) and Hoey (1999).

To determine total lipid content each fish was homogenized in 1 ml of distilled water immediately after freeze drying. A 300µm aliquot of each homogenate sample was analysed for lipid content by first extracting the lipid material using chloroform-methanol extraction (Mann and Gallager 1985). This material was subsequently analysed using the phosphosulphovanillin method, as described by Barnes and Blackstock (1973). The method uses a cholesterol standard calibrated against gravimetric values to convert chlorometric values to total lipids. Chlorometric values were obtained using a spectrophotometer (Labsystems iEMS Reader MF) and associated computer software (Genesis 3.04^{TM}), at a wavelength of 520nm. The cholesterol standard was mixed at concentrations of 0, 2.5, 5, 7.5 and 10 mg ml^{-1} and run through the same extraction process as the homogenate samples, to produce a calibration line between chlorometric values and cholesterol concentration. Once completed, a known ratio of 4:5 (cholesterol:lipid) was used to convert the resulting values from cholesterol to lipid concentration (mg ml^{-1}). The initial dry weight obtained for each sample was then used to express the total lipid content as mg g^{-1} dry weight.

A measure of overall body condition was obtained using residual regression analysis (Koops et al. 2004). This method uses the residuals of a standard length/wet weight regression as an index of relative condition.

Experiment 1 - Laboratory trials

P. amboinensis were taken from light trap catches and sorted into groups of two (weight or burst speed trials) or three (size trials), for each of three measures of body and performance attributes: prey body size, body weight, and burst swimming speed.

For trials testing prey body size, individual *P. amboinensis* were first placed in a clip-seal plastic bag containing a small amount of aerated seawater and measured for standard length (SL) using calipers (± 0.1mm). Fish were placed into 'groups' of three, such that one individual of each of 3 size classes was present. These size classes were set at 10.8 – 11.5mm, 11.9 – 12.1mm, and 12.5 – 13mm SL. The classes were chosen to span the entire size range of individuals at the time of settlement. The size difference of individuals between classes for all trials was always at least 0.5 mm. Overall, the size of individuals caught in light traps during the course of the experiment ranged between 10.8 and 13 mm SL, with a mean of 11.94 mm. Thus, a difference of 0.5 mm between size classes represents ~4.2% of the mean prey size.

To test the influence of prey body weight, *P. amboinensis* were randomly selected and placed into one of two identical 25 L aquaria with flowing seawater. Fish in one aquaria were fed ad libitum (high feed treatment) with *Artemia* sp. nauplii, whilst those in the other aquaria were fed 1/5 ad libitum (low feed treatment). The different feed trials were used in order to accentuate the level of variability in standardized body weight amongst individuals. After two days, the fish were removed from both aquaria, measured for SL (± 0.1 mm using calipers) and weighed (±1 mg). In order to decrease fish stress during the weighing process, fish were first anaesthetized using MS-222 (0.1mg ml^{-1} sea water). *P. amboinensis* from the high feed treatment and of heavier weight ('heavy' individual) were paired with those from the low feed treatment and of lower weight ('light' individual), but equal standard length. A standardized weight difference of 5 – 10 mg was maintained between individuals within a pair throughout the experiment. Individuals within the pair were tagged with either a red or black subcutaneous fluorescent elastomer tattoo using a 27-gauge hypodermic needle for the purpose of individual identification, as per Hoey and McCormick (2006). Tag colours were alternated between replicate trials to avoid the possibility of predators selecting prey based on tag colour. Hoey & McCormick (2006) found that the tagging technique had no influence on survival or growth of *P. amboinensis* in aquaria over a 2 wk period. The wet weight of individuals used for trials during the experiment ranged between 22 – 74 mg, with a mean of 52.04 mg. Thus, a difference of 5 – 10 mg between weight classes represents ~9.6 – 19.2% of the mean prey weight. The mean SL of prey was 12.2 mm.

In order to test the influence of prey burst speed, individual *P. amboinensis* were firstly transferred to a 25 L flow through aquarium for 24 hours. After this period, fish were removed and measured for SL using calipers (± 0.1mm). Individuals were then measured for burst swimming speed using the same technique previously described in this methods section. In order to reduce stress on the fish, only three reliable bursts were obtained per individual. *P. amboinensis* with a high burst speed ('fast') were paired with those with a low burst speed ('slow'), but equal standard length. Individuals were tagged for identification purposes in the same manner as above. The difference in burst speed between 'fast' and 'slow' individuals in a pair ranged from 110 – 280 mm sec^{-1}. The burst speed of all individuals measured during the course of the experiment ranged between 201 – 825 mm sec^{-1}, with a mean of 423.98 mm sec^{-1}. Therefore, the difference of 110 – 280 mm sec^{-1} between individuals in a pair represents ~25.9 – 66% of the mean prey burst speed. The mean SL of prey was 11.98 mm.

Eighteen identical flow-through aquaria were constructed, as per Almany et al. (2007). Each aquarium had an internal volume of 57.4L (600x255x375mm). Aquaria were divided into two equal sized sections by a removable opaque perspex partition. A 15cm length of 105mm diameter PVC pipe cut in half was placed into one section of the aquarium as a predator shelter. A single, artificial (white moulded resin) branching coral (item no. 21505; Wardleys/TFH, Sydney; dimensions: 140 x 115 x 50 mm) was placed in the other section as prey shelter. Aquaria were surrounded by black plastic to visually isolate them from each other and other external disturbances. A small hole was cut in one side of the plastic to allow observation of the trials.

At the commencement of each trial, aquaria were divided in half with the opaque partition. A single *P. amboinensis* group/pair was placed into one half, along with the artificial branching coral. A single predator (*Pseudochromis fuscus*) was allowed to acclimate in the opposite section of the aquaria for 48 hours prior to the trials. Prey were acclimated for 1 hour before the partition was removed and the trial started. Prey abundance was continuously monitored for the first 20 min and every 10 min thereafter. When 1 or more of the prey individuals were found to be missing, the trial was ended. Any survivors were either re-measured for SL (body size trials), or their tag colour was recorded (body weight and burst speed trials) to determine the identity of the missing individual(s). If more than one prey were found to missing, the trial was discarded. The mean SL of predators over all trials was 55.89 mm. At the termination of each trial, the predator and all remaining prey were released at the point of capture. Predator individuals were used only once to maintain independence between trials. Thirty-four successful trials were run testing prey body size, whilst 25 successful trials were run to test both prey body weight and burst swimming speed.

Experiment 2 - Field trial

P. amboinensis were taken from light trap catches and sorted into pairs as per the protocol set out in the prey body weight trials in experiment 1. Each pair consisted of one ‘light’ and one ‘heavy’ individual for a standardised body length. The wet weight of individuals weighed for trials during the course of the experiment ranged between 46 –

74 mg, with a mean of 59.04 mg. The weight difference of 5 – 10 mg between weight classes represents ~8.5 – 16.9% of the mean prey weight. The mean SL of prey was 12.61 mm.

A series of small patch reefs (20 x 20 x 20 cm) were constructed on the sand flat immediately adjacent to the edge of a shallow lagoonal reef. Patches were arranged approximately 2 m from the reef base and approximately 3 to 4 m apart within a 50 m wide section of reef edge. Each patch consisted of a combination of live and dead *Pocillopora damicornis* (a bushy scleractinian). Such patch reefs are common settlement sites for this species (McCormick and Hoey 2004). All patches were open to the full array of reef-based and transient predators at each site.

Prior to releasing a tagged pair, the patch reef was cleared of all resident fishes and large invertebrates using small hand nets. A single *Pomacentrus amboinensis* pair was then placed onto patch reefs and shielded by a diver from predators for 5 to 10 min until acclimated to the new environment. Within 30 s of release, fish were observed feeding on food items from the water column, which suggested a rapid acclimatization to their new environment.

Survival of each of the experimental pairs was monitored 3 times per day (morning, mid-day, evening) by visual census. Each replicate trial ended when one or both of the tagged fish were found to be missing from a patch, at which point the identity of the remaining fish was recorded and the surrounding reef area was searched to determine whether the missing individual had emigrated. If a result was not obtained within 24 h, the *P. amboinensis* pair was removed and the trial was abandoned. Trials where both individuals were found to be missing were discarded from the replicate group.

Where a result occurred, surrounding reef habitat was searched for evidence of migration away from the patches (with migrants clearly visible due to tag). No evidence of migration away from patches after the acclimation period was recorded during the study. A total of 23 successful trials were completed.

Analysis

Pearson's correlations were used to examine relationships among the eight condition measures. The coefficient of variation (CV) was calculated for the burst speed of each individual, using replicate recordings obtained from the Correlation of prey condition/performance measures. This was used to obtain an estimate of performance consistency among replicate bursts for individuals. CV's were calculated using the number of successful bursts, which varied between 2 and 5.

Tests of significance for Pearson's correlations were not corrected for multiple tests, due to the exploratory nature of the analysis. The frequency of first mortality between treatments, within each set of predation trials, was compared using a Chi-squared Goodness of Fit test. With the exception of the size-based aquarium trials, Yates' correction was incorporated in all analyses to correct for d.f. = 1.

Projected survival over the 24 hr period of predation trials, incorporating censored data previously excluded in Chi-squared tests, was analysed using survival analysis. Projected survival curves of each treatment within a trial set were calculated and plotted using the Kaplan-Meier Product-Limit method. Projected survival between treatments within trial sets were compared using a Cox-Mantel test (weight and burst speed based laboratory trials, weight based field trial) or a Chi-squared test for multiple groups (size based laboratory trials).

2.3. Results

Correlation of prey condition/performance measures

The eight measures of condition/performance of *P. amboinensis* at settlement displayed markedly different levels of variability among fish (Table 2.1). Maximum and mean burst speed were the most variable (CV = 25.9% and 23.2% respectively), followed by lipid content (CV = 17.4%). Wet weight (CV = 14.3%) and dry weight (CV = 13.2%) displayed moderate levels of variation, whilst standard length (CV = 3.1%) and pre-settlement growth rate (CV = 8.1%) displayed the lowest levels. An accurate estimate of variability for overall body condition (obtained during Residual Regression Analysis) was not possible, due to the positive and negative expression of the variable measures (range = 0.01348 to -0.01349, standard deviation = 0.00525)

Table 2.1: Statistical summary of the eight measures of condition/performance obtained from newly settled *Pomacentrus amboinensis* collected from light traps (n = 94).

Condition Measure	**n**	**Mean**	**Range**	**CV**
Standard Length (mm)	94	12.3	13.3 – 11.4	3.1
Wet Weight (g)	94	0.05	0.069 – 0.035	14.3
Dry Weight (g)	93	0.01	0.016 – 0.009	13.2
Body Condition Index	94	-0.00028	0.01348 – -0.01398	N/A
Lipid Content (mg/g)	87	102.9	150.3 – 60.9	17.4
Max.Burst Speed (mm/sec)	89	491.3	800 – 125	25.9
Mean Burst Speed (mm/sec)	89	423.8	608.8 – 125	23.2
Pre-settlement Otolith Growth Rate (µm/day)	74	16.9	19.9 – 13.3	8.1

Correlations between the eight measures were generally poor (Table 2.2). The morphological measures were the general exception, with standard length, wet weight and dry weight all displaying strong positive correlations. Wet weight and body condition, and maximum and mean burst speed also displayed strong positive relationships, whilst lipid content and dry wet weight showed a relatively strong negative relationship. Weaker correlations also existed between standard length and lipid content (negative), dry weight and body condition (positive), standard length and pre-settlement growth (positive), and dry weight and pre settlement growth (positive). Correlations between the performance (maximum or mean burst speed) and condition measures were poor overall, with standard length being the only condition measure correlated with maximum burst speed, albeit it only weekly (r=0.252, Table 2.2).

Table 2.2: Correlations among eight measures of condition/performance of newly settled Pomacentrus amboinensis collected from light traps (n = 94). Pearson correlation coefficients are given.
* p < 0.05; ** p < 0.01; *** p < 0.001.

	Standard Length	Wet Weight	Dry Weight	Body Condition	Lipid Content	Max. Burst Speed	Mean Burst Speed	Pre-settlement Growth
Standard Length	1.00	0.6472 ***	0.8087 ***	0.001 ns	-0.3379 **	0.2518 *	0.2074 ns	0.2997 **
Wet Weight		1.00	0.7353 ***	0.761 ***	-0.167 ns	0.0889 ns	0.1118 ns	0.2651 *
Dry Weight			1.00	0.271 **	-0.3511 ***	0.0735 ns	0.0648 ns	0.361 **
Body Condition				1.00	0.059 ns	-0.086 ns	-0.021 ns	0.106 ns
Lipid Content					1.00	0.051 ns	0.1127 ns	-0.1265 ns
Max. Burst Speed						1.00	0.924 ***	-0.051 ns
Mean Burst Speed							1.00	-0.01 ns

The consistency of burst speed within individuals was moderate, with a mean CV of 14.38% (±0.95% SE) and a median CV of 15.44% for the 89 individuals measured.

Experiment 1 – Laboratory

During aquarium trials, where prey from three size classes were exposed to the predator *Pseudochromis fuscus*, prey fish from the largest size class (12.5 – 13 mm SL) were found to be selected first significantly more often than those of the small and medium size classes ($\chi^2_{\text{df 2}} = 24.772$, $p < 0.001$; Fig. 2.1). This result occurred in 73.5% of the trials run, whilst small and medium prey sizes were selected first in only 14.7% and 11.8% of trials respectively. A similar pattern was found in the projected survival schedules of the three different size classes, with survival analysis showing a significant difference over the 24 hour duration of the trials ($\chi^2_{\text{df 2}} = 17.258$, $p = 0.0002$; Fig. 2.2). Projected mortality of large individuals was initially high, with 50% mortality occurring within 0.3 hours and 80% mortality occurring within 1.3 hours. Survival then became relatively stable for the remainder of the trial period. Projected mortality of both small and medium individuals was comparatively more constant over the duration of the trials, with ~50% mortality occurring at 3.5 and 5.7 hours respectively. 100% mortality was predicted for all groups 19.6 hours after the commencement of trials

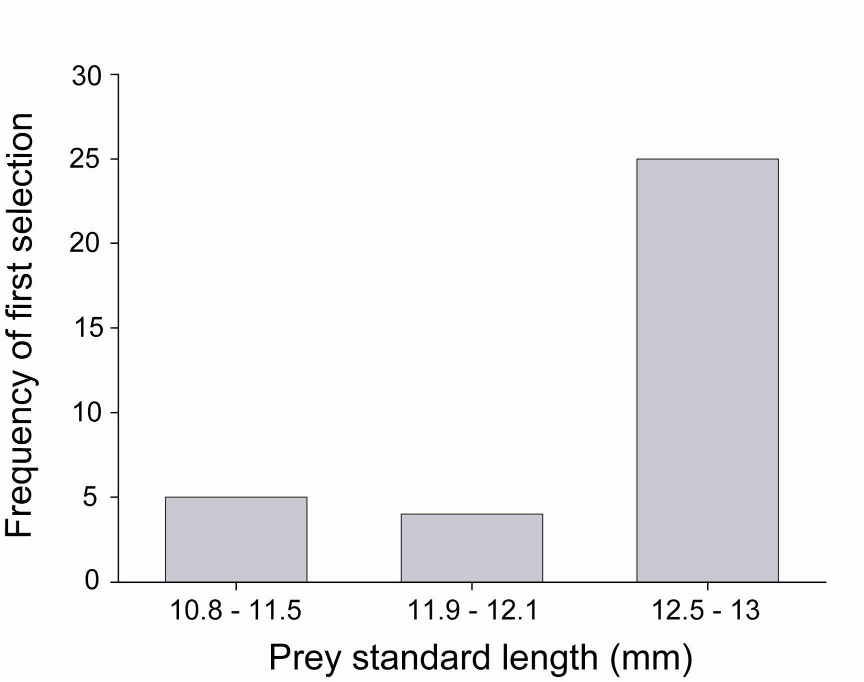

Figure 2.1: Frequency of selection of newly settled *Pomacentrus amboinensis* by *Pseudochromis fuscus* during size-based aquarium trials.

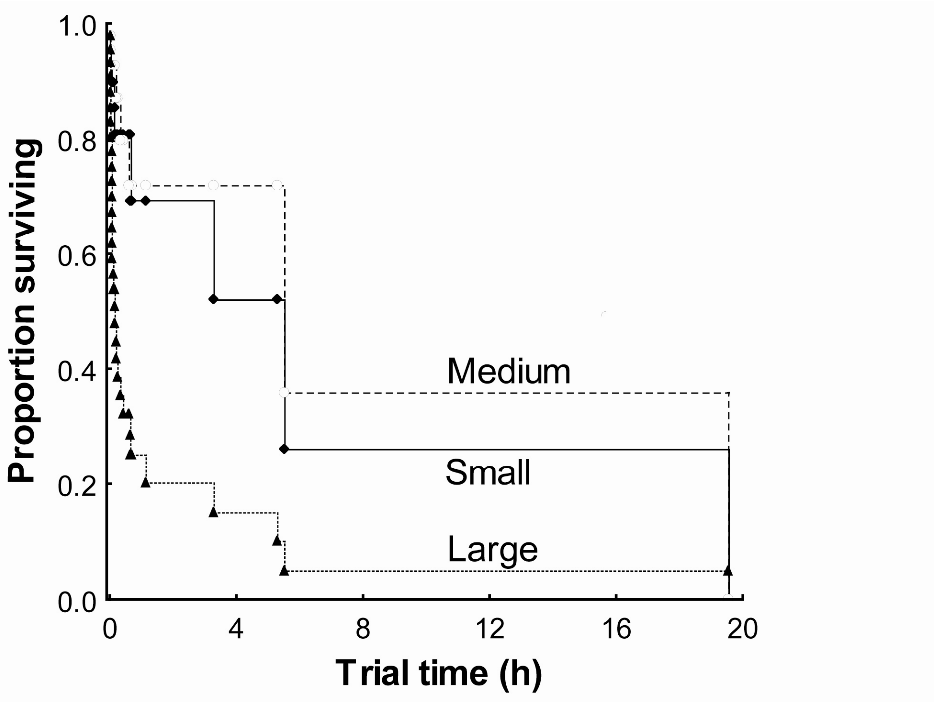

Figure 2.2: Kaplain-Meier Product-Limit plot for predicted survival of small, medium and large size classes of prey during size-based predation trials with *Pseudochromis fuscus*.

No difference in predator choice was detected in either the weight-based or burst speed-based trials during the aquarium experiments (both $\chi^2_{\text{df } 1} = 0.00$, p = 1.00). The mortality of both treatments within a pair was almost identical in both cases, with lighter and slower individuals being selected marginally more frequently (both chosen first in 52% of trials) than their heavier and faster counterparts during respective trial sets. Similarly, no difference was found between the projected survival schedules of either treatment in both the weight-based and burst speed-based trials over the 24 hour trial period (Cox-Mantel$_{38,\ 38}$ = 0.000, p = 1.000; and Cox-Mantel$_{26,\ 26}$ = 0.000, p = 1.000 respectively). Projected mortality was initially high during the weight-based trials, with ~70% mortality occurring within 0.7 and 0.9 hours for light and heavy individuals respectively. Mortality then eased, until 100% mortality occurred for both groups at ~4.4

hours. For the burst speed-based trials, projected mortality remained extremely high for the duration of the trials, with 50% mortality occurring at 0.09 and 0.14 hours for slow and fast individuals respectively, and 100% mortality occurring at 0.58 hours for both groups.

Experiment 2 – Field trials

There was a significant difference in survival between 'heavy' and 'light' individuals during the weight-based predation trials on open patch reefs, with the 'heavier' individuals found to be missing more frequently than their 'lighter' counterparts ($\chi^2_{\text{df } 1} = 4.348$, $p = 0.037$; Fig. 2.3). This result occurred in 73.9% of the 23 trials. However, the difference between the projected mortality curves of the two treatments was non-significant over the 24 hour trial period when analysed using survival analysis (Cox-Mantel$_{34, 56}$ = 1.662, $p = 0.096$; Fig. 2.4). Due to the fact that trials were started in the late afternoon, and censuses could not be completed overnight, the first recordings of mortality did not occur until 16 – 18 hours into the trial. Any mortality that occurred overnight was therefore recorded in the 16 – 18 hour period. As such, projected survival of light individuals decreased from 100% at 16 hours to ~70% at 18 hours, before stabilizing to reach ~55% survival at the end of the trial period (24 hours). Projected survival was lower during the 16 -18 hour period for heavy individuals, with a drop from 100% to ~55%. The mortality rate eased over the following 6 hours, leaving ~35% survival at the end of the 24 hour trial period.

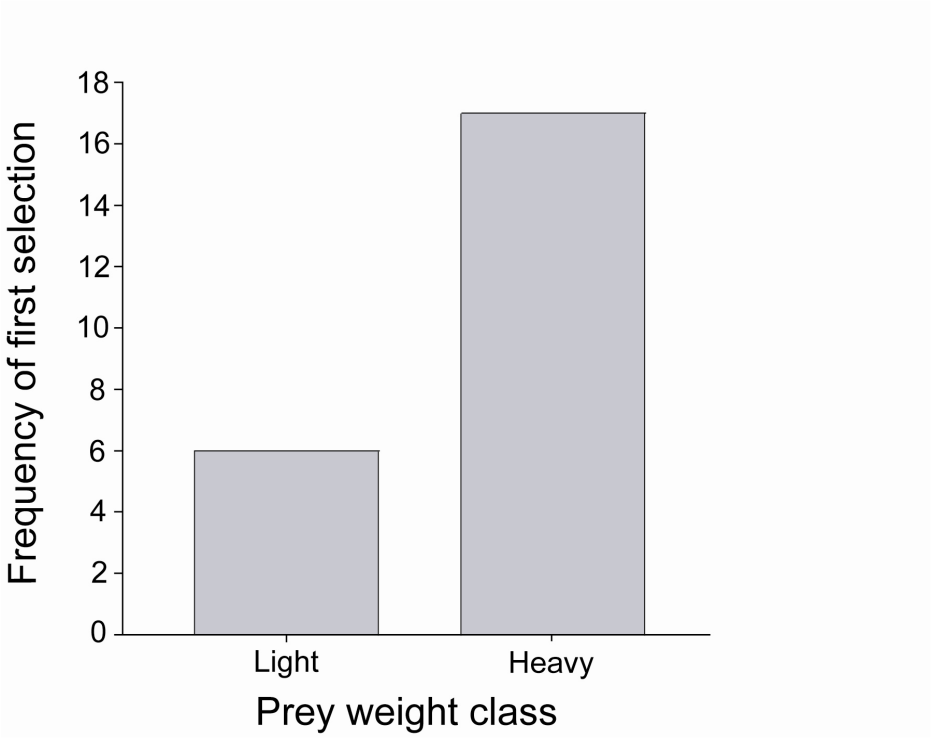

Figure 2.3: Frequency of selection of newly settled *Pomacentrus amboinensis* during weight-based trials on open patch reefs.

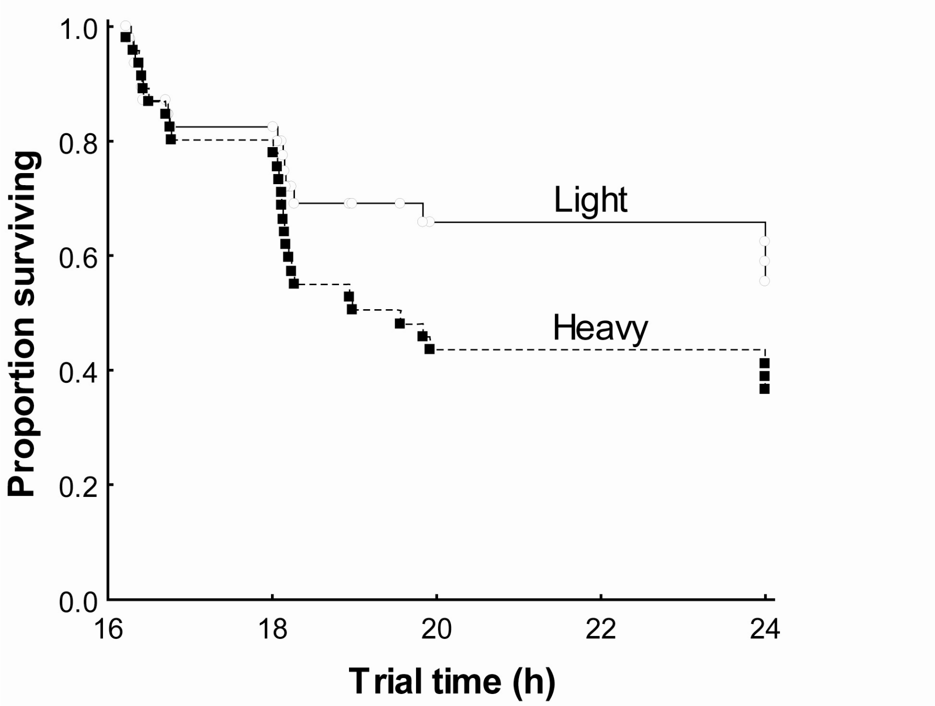

Figure 2.4: Kaplain-Meier Product-Limit plot for predicted survival of light and heavy standardized weight classes of prey during weight-based predation trials on open patch reefs.

As there was no evidence of emigration away from patches during the experiment and all prey individuals were released in good condition, any mortality during the trials on open patches was directly attributed to localised predation by fish predators.

To increase interpretability, a summary of all laboratory and field trials conducted can be found in Table 2.3.

Table 2.3: Summary of all laboratory and field trials conducted during study. Preferential prey type indicates the type of prey preferentially (statistically significant) chosen by the predator during the trials.

Trial Location	Prey Characteristic Tested	Prey Type	Proportion of First Selection	Preferential Prey Type
Laboratory	Body Size	Small	14.7%	Large
		Medium	11.8%	
		Large	73.5%	
	Weight	Light	52%	None
		Heavy	48%	
	Burst swimming speed	Slow	52%	None
		Fast	48%	
Field	Weight	Light	26.1%	Heavy
		Heavy	73.9%	

2.4. Discussion

The general selective nature of mortality has been well documented in the literature in recent times (Gosselin and Qian 1997; Sogard 1997; Blanckenhorn 1998; Meekan et al. 2006; Anderson et al. 2008). The causes of such selective loss have varied, with examples of starvation, disease, competition and predation all being shown in a range of different systems. There is now also increasing evidence to suggest the selective nature of predation on coral reef fishes at the time of settlement (e.g. Booth and Hixon 1999; Booth and Beretta 2004; Hoey and McCormick 2004; McCormick and Hoey 2004;

Holmes and McCormick 2006; Gagliano et al. 2007). The relative importance of this to the structure of future populations may well be amplified by the high, Type III mortality characteristic of this period (Almany and Webster 2006). Our study contributes significantly to this body of literature, and provides some of the first direct evidence of size-selective predation by a single predator species during this period for any aquatic system. The dottyback *Pseudochromis fuscus*, a common predator of small reef fishes (Beukers and Jones 1997), was found to be highly selective towards larger sized individuals when tested in an aquarium system. This finding confirms the suggestion of positive size selection by the dottyback from a previous field experiment (McCormick and Meekan 2007). Interestingly, the same predatory species was found to be non-selective towards both prey body weight and burst/escape swimming speed when the confounding influence of variable fish size was experimentally removed.

With the exception of the morphological characteristics (i.e. standard length, wet weight, dry weight), correlations between measures of body condition were generally found to be poor. This result is similar to the findings of other studies by McCormick and Molony (1993), Kerrigan (1996) and Hoey and McCormick (2004) who consequently suggested that selection with respect to one trait has little influence on the patterns of variability in other traits. Both standard length and dry weight were found to be correlated with lipid content, displaying a fairly strong negative correlation in both cases. Interestingly, similar relationships were obtained between standard length and lipid content in studies by Kerrigan (1996) and Hoey and McCormick (2004), indicating that lipid content may decrease with increasing size during this life-history stage. This relationship may be the result of trade-offs carried over from the larval life stage. For example, fast growth during the larval period may result in smaller size at settlement as well as less time in which to amass lipids. However, if larval growth is slower (due to water temperatures, poorer environmental conditions, or just as a phenotypic trait), fish may settle older, larger and with less lipids (Meekan et al. 2003; Hoey and McCormick 2004). Pre-settlement growth rate was also found to be positively correlated with standard length and dry weight, indicating that events occurring late in the larval stage may be important to subsequent survival during the early post-settlement period (Searcy and Sponaugle 2001).

Burst swimming speed was generally not found to be well correlated with morphological measures. This outcome conflicts with a number of past studies that have previously found burst speed to be positively related with fish length (Bailey 1984; Fuiman 1986). Indeed the positive relationship between fish length and burst speed has been widely integrated into fish ecology through the ‘Bigger-is-Better’ hypothesis, which often associates increased speed as a characteristic of larger size (Fuiman 1989; Paradis et al. 1999). However, the results presented in this study are not the only evidence to suggest otherwise. McCormick and Molony (1993) made a similar conclusion in a study of newly settled goatfish, *Upeneus tragula*. Using an experimental design similar to that used in this study, the results indicated that burst swimming speed was poorly correlated with standard methods of assessing condition. The difference between these and other studies that find a relationship between size and performance may be because other studies have integrated findings over the whole of the larval phase, thereby encompassing a wide range of different developmental stages and performance capabilities. Meanwhile, the present study and that of McCormick and Molony (1993) examined the relationship at a specific developmental stage (metamorphosis and settlement). The finding of a poor relationship between size and performance at a particular developmental stage has important ramifications for the determinants of survival within replenishment cohorts.

According to the 'bigger-is-better' hypothesis, larger size should convey a survival advantage for prey during such predatory encounters (Rice et al. 1993). This however, does not appear to be the case when settlement stage individuals encounter *P. fuscus*. This result is consistent with a recent field study by McCormick and Meekan (2007), who found that the removal of *P. fuscus* from territories resulted in a shift in the direction of local juvenile size selection from negative (i.e. selection of larger individuals) to random. The exact mechanism underlying this positive size selection is unclear, but there are a number of possibilities that could account for it. It may well be that predators are making an active choice to select the prey size that provides the highest energy return. This would be contingent with optimal foraging theory, which predicts that predators should prey upon those individuals that will maximize the energy return per unit of handling time (MacArthur and Pianka 1966; Hughes 1980). Given that predation by *P. fuscus* was not found to be selective with respect to the variability in escape/burst swimming speed present during this period (i.e., faster individuals are selected equally as often as slower individuals), correlations between escape/burst speed and body size were generally poor, and energy return increases with prey body size (Holmes and McCormick *unpublished data*), it is reasonable to suggest that predators may be actively selecting larger prey due to the associated higher energy return.

Alternately, patterns of prey body size selection by *P. fuscus* could be explained by behavioural differences between large and small prey. Huntingford (2007) discussed the idea that an individual's behaviour can be classified as either 'proactive' (bold) or 'reactive' (timid), in relation to how they react in a given situation. She further suggests that how aggressive an individual is to a conspecific is often correlated with its readiness to take risks in other contexts. Arguments in favour of such 'behavioural syndromes' have recently gained significant momentum, and have now been documented in a range of animal groups, including insects (Johnson and Sih 2007), freshwater fishes (Bell and Sih 2007; Wilson and McLaughlin 2007), birds (Dingemanse et al. 2004), lizards (Stapley and Keogh 2005) and mammals (Dochtermann and Jenkins 2007). Given that size – based dominance hierarchies are commonly observed within reef fish communities during the early post-settlement period (personal observations), larger more aggressive individuals may be proactive in their behavioural decisions, making them more susceptible to certain forms of predation.

Relative visibility of prey may also play a role in determining susceptibility to predation. Smaller body size has previously been associated with a survival advantage during the larval period of marine fish due to decreased encounter rates with visual predators (Fuiman 1989; Litvak and Leggett 1992). For larval phase fish it has been argued that this may be the result of changes in morphology with size and developmental stage (e.g. transparency and lack of body pigmentation in smaller individuals; O'Brien 1979; Fuiman 1989). This however, seems an unlikely explanation within this system, given the relatively homogeneous pigmentation of individuals tested in the trials.

Whatever the underlying mechanism, this experiment has shown that in relation to the characteristics tested in this study, prey body size is the most important of the three in influencing prey survival during an encounter with *P. fuscus*. This contradicts the findings of two previous studies of selective mortality on early post-settlement coral reef fishes, which found evidence to suggest that prey body weight also significantly influences survival (Booth and Hixon 1999; Searcy and Sponaugle 2001). However, both of these studies failed to standardize for prey body length and hence the findings are somewhat confounded.

Despite the relatively high level of variation in burst speed between individuals, and the generalized idea that prey with a faster escape response should have a survival advantage over slower counterparts, the fact that burst/escape speed also had no influence on survival was not entirely unexpected. In one of the only other known studies directly examining the influence of prey performance on survival during predatory interactions for any aquatic system, Fuiman et al. (2006) came to a similar conclusion, finding that burst/escape speed did not influence the survival of red drum larvae (*Sciaenops ocellatus*) during predatory encounters with the longnose killifish (*Fundulus similis*). This outcome, combined with the moderate level of variability in burst speed across repeated bursts for an individual, suggests that escape ability may have a random factor that is intrinsic to the prey and their state at the time of escaping a predator. If this were the case, then survival may actually be more related to the ‘proactive’ or ‘reactive’ coping styles of individuals, rather than a set measure of individual performance (see Sih et al. 2004a, b; Bell 2007; Huntingford 2007; Stamps 2007).

Alternately, burst/escape speed may not relate to the probability of capture by a predator at settlement because they do not know when to use it, since they have not yet learnt to identify predators within the new environment. Holmes and McCormick (2006) suggested that the new recruits have to learn the identity of predators before an escape response can be initiated. Research suggests that juveniles of many aquatic organisms with complex life cycles need to learn the identity of predators to efficiently escape encounters with them (Mathis et al. 1996; Brown and Laland 2003). If this hypothesis is correct, fish that have experimentally learnt the identity of a predator could be expected to display the predicted negative relationship between burst speed and capture success by a predator.

The results of this study show that the patterns of selectivity displayed by a single predator species under controlled aquarium conditions were different from the selective signature of the multi-species predator community on open patch reefs, with respect to prey body weight. The pattern of body weight selection became highly significant, with individuals of lower standardised weight having a higher probability of survival. This may relate to the higher predation pressures placed on the prey within the confines of the aquarium trials, as displayed by the Kaplan-Meier Product-Limit plots. However, a more likely explanation lies within the feeding ecology of different predator species and the interaction with their prey-selectivity profiles.

According to a recent study by Holmes and McCormick (2006), the cause of this discrepancy could be a result of predators within the local community having different selectivity fields. *P. fuscus* is only one of a number of fish species, all with differing morphologies and predation modes, known to prey upon juvenile fishes in this system (Martin 1994). Such differences in selective preferences have previously been described in temperate marine fishes by Scharf et al. (2000), as well as in a range of invertebrate predators and their amphibious prey by Toledo et al. (2007). The selective patterns described in this study could combine with local environmental conditions, such as habitat complexity and water current speed, to alter the selectivity functions produced in the single predator system.

Recent studies suggest that higher measures of body condition (ie. lipid content, standardized weight) positively influence the survival of prey individuals during the early post-settlement period of coral reef fishes (Booth and Hixon 1999; Searcy and Sponaugle 2001; Grorud-Colvert and Sponaugle 2006). Our study however, suggests otherwise, with individuals of lower standardised weight showing a higher probability of survival than those of a higher standardised weight. As movement away from the patches was not detected, it can therefore be assumed that predators were actively selecting the ‘heavy’ individuals over their ‘light’ counterparts. The findings are similar to those of Hoey and McCormick (2004) who, by using a gross manipulation of predator access to prey, concluded that predation was selective for *Pomacentrus amboinensis* recruits of a high standardised weight. These results are surprising given that standardised weight (commonly expressed as Fulton’s K) is starvation-dependent, with higher values thought to represent fish in better condition (Suthers 1998). Fish in better condition have also been found to take fewer risks, and are hence less exposed to predators (Giaquinto and Volpato 2001; Grorud-Colvert and Sponaugle 2006). The inconsistency in selective direction reported between studies may well be a product of differing growth forms between the study species, exposure to differing predator suites, or the actions of selective predation targeted towards a trait negatively correlated with standardized weight.

In comparison to other terrestrial and aquatic systems, we currently know very little about predation on tropical reef fishes. In addressing this issue, the present study has provided us with some of the most direct evidence of the selective processes underlying predation during a period potentially critical to adult population dynamics. Our results suggest that under controlled conditions, larger size at the time of settlement may actually be a distinct disadvantage to prey during interactions with some predator species. However, these relationships appear to be more complex under natural conditions, where the expression of prey characteristics, the selectivity fields of a number of different predators, their relative abundance, and the action of external environmental characteristics, may all influence which individuals survive. A greater knowledge of these interactions and their underlying mechanisms is crucial for the management of fisheries and conservation of tropical marine ecosystems. It is only by understanding predator-prey dynamics that we can predict how prey may respond to changing predator populations or vice-versa.

Chapter 3: How selective are reef fish predators for prey size?

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3.1. Introduction

Understanding the processes underlying population dynamics is one of the key issues confronting ecologists working in complex systems. An intimate knowledge of these processes, and how they interact, is essential before broad scale predictions can be made at community and population levels. Predation is generally thought to play a major role in determining the size of populations and the structure of terrestrial and aquatic communities (Wilbur et al. 1983; Sih et al. 1985; Risbey et al. 2000). However, due to its speed and decisive nature, predation is notoriously difficult to study. The selectivity of predation over ecological time frames (i.e. phenotypic selection) has been widely implicated in determining those character traits that are passed into successive life stages. These selective forces may act on a number of different body and performance characteristics, all of which can influence survival in a variety of situations. Such characteristics include body size (Allen 2008; Sakamoto and Hanazoto 2008), overall condition (Husseman et al. 2003; Penteriani et al. 2008), growth rate (Takasuka et al. 2003; Sponaugle and Grorud-Colvert 2006; Urban 2007), sensory development (Poling and Fuiman 1997) and escape speed (Brana 2003). Body size has by far received the most attention in the literature, and is generally regarded as one of the major characteristics linked to survival during predatory encounters (Cohen et al. 1993; Wellborn 1994; Sogard 1997; Wang et al. 2007).

One common theory (the ‘Bigger-is-Better’ hypothesis) suggests that from a prey’s perspective, larger size at a given life-history stage results in a survival advantage, through lower predation rates (Rice et al. 1993; Congdon et al. 1999; Wang et al. 2007), enhanced competitive abilities and a lower susceptibility to starvation (Stuart-Smith et al. 2007). Thus, as prey size increases, vulnerability to predation is predicted to decrease. In contrast, optimal foraging theory (OFT) predicts that predators preferentially prey on an optimal prey size in order to maximise the net rate of energy intake (MacArthur and Pianka 1966; Hughes 1980). This theory predicts that both large and small size may convey a survival advantage during a predatory encounter. The characteristics of the prey that are targeted are contingent on the selective preferences of the predator, which tend to be dome-shaped (e.g. Rice et al. 1993).

To understand the influence of body size on the outcome of predatory encounters the relative sizes of predator and prey need to be considered. Prey selected by a predator depends on the characteristics expressed by both predator and prey, and how these interact (Cohen et al. 1993; Woodward et al. 2005; Urban 2007). For many piscivores, the upper limit of potential prey sizes is set by morphological constraints imposed by mouth width, or gape size (a mechanism known as ‘gape-limitation’; Persson et al. 1996; Slaughter and Jacobson 2008). According to OFT, as predator size increases, the optimal prey size on which to feed should also increase (Hughes 1980). Thus, as predator size increases, individuals are predicted to preferentially select larger prey (Rice et al. 1993; Scharf et al. 2000). This increase in preferred prey size has been attributed to ontogenetic increases in mouth gape, visual acuity, digestive capacity and locomotive performance. As a result of these underlying mechanisms, in relationships involving single species of predator and prey, the size distribution of the predator will determine the size range of prey eaten. The size range of prey available will then determine the nature of size selection.

At the time of settlement to the reef environment coral reef fishes are subjected to extremely high levels of mortality, with upwards of 60% of individuals being lost within 48 hours of settlement (Doherty et al. 2004; Almany and Webster 2006). Much of this mortality is attributed to the actions of small reef-associated predatory fish (Carr and Hixon 1995; Holbrook and Schmitt 2002). As a result, there is the potential for predator selection during this period to have a large influence on those traits that are passed on to successive life stages. The gape-limitation imposed on many predatory fish means that the key relationship underlying predator-prey interactions in this system may well be predator mouth width versus the prey body depth (Werner 1974; Werner and Gilliam 1984; Hill et al. 2005). However, given the diversity of predator morphologies, behaviours and attack modes amongst predatory reef fish, it is reasonable to suggest that different species may exploit the size range of prey in different ways (Holmes and McCormick 2006). To date, there is little data on the species-specific selectivity of predators within communities for any system. If we are to predict how prey populations may respond to changing predator communities, and vice-versa, it is essential that we gain an understanding of how selective patterns differ between predators within a system.

This study examines the nature of size selection by predators on the common Indo-Pacific damselfish *Pomacentrus amboinensis*, during the early post-settlement period. To investigate the changing dynamic of this process, the selectivity of predators on naïve newly metamorphosed individuals is compared to experienced juveniles.

Previous research indicates that experience obtained by individuals in the days immediately following settlement increases survival during predatory encounters (McCormick and Holmes 2006), and hence may also influence selective patterns. Ecological theory suggests that, from a predator's perspective, selective preferences towards a particular prey trait may differ between predator species. However, from a prey's perspective, conventional theory would suggest that a particular expression of that trait provides a generalised survival advantage during all interactions (e.g. bigger-is-better). We addressed these tenets in a series of aquarium experiments using four predator species known to be responsible for a majority of predation on juvenile reef fish on shallow lagoonal reefs throughout the Indo-Pacific. Specifically, we assessed: (1) whether different predator species will differ in their size-selective preferences during predatory interactions with newly metamorphosed and early juvenile prey individuals, (2) if, in keeping with Optimal Foraging Theory, preferred prey size increases with increasing predator size, and (3) the role of predator gape size in predator-prey relationships between four important predator species and juvenile and settlement stage prey.

3.2. Materials and Methods

Study Site and Species

This study was conducted at Lizard Island (14°40'S, 145°28'E), northern Great Barrier Reef (GBR), Australia during November and December of 2006 and 2007. The flow through salt water aquarium system at Lizard Island Research Station was used to conduct the aquarium trials, whilst all fish collections were made from the surrounding shallow lagoonal reefs and sand flats.

The common damselfish *Pomacentrus amboinensis* was used as the model prey species for all experimental trials. This species is common within coral reef fish communities within the Indo-Pacific, particularly in the central GBR. They settle to a wide variety of habitats on the northern GBR, but are found in highest densities associated with small reef patches at the base of shallow reefs. The species has a pelagic larval phase of between 15 – 23 days and settles at 10.3 – 15.1 mm standard length (Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site-attached, making it an ideal species for experimental manipulation. They recruit in substantial numbers at Lizard Island around the new moon during the summer months (October – January), and are easily collected at the time of settlement with light traps (Milicich and Doherty 1994). This life cycle, body plan and approximate size is common to a large number of damselfish (Pomacentridae) species. Hence, any selective processes found to be operating on *P. amboinensis* in this study may be generalised to a wide range of Pomacentrid species.

Four species of small site-associated reef fish were used as predators during aquarium trials: the brown dottyback, *Pseudochromis fuscus* (Pseudochromidae); the moonwrasse, *Thalassoma lunare* (Labridae); the sand lizardfish, *Synodus dermatogenys* (Synodontidae); and the freckled rockcod, *Cephalopholis microprion* (Serranidae). All species are common on shallow reefs throughout much of the West Pacific and Indian Oceans, and are generally thought to be responsible for a majority of predation on newly settled reef fishes in these habitats (Martin 1994; TH Holmes and MI McCormick *personal observations*). *P. fuscus* is a small (max size 72.4 mm SL), solitary cryptic pursuit predator commonly found on small coral bommies or along reef edges. *T. lunare* is a highly active opportunistic predator (max size 200 mm SL), generally found higher in the water column in haremic groups across a range of reef habitats. *S. dermatogenys* is a cryptic ambush predator (max size 210 mm SL) commonly found on sandy substrata immediately adjacent to the reef base or amongst small bommies and coral rubble. *C. microprion* is another cryptic predator (max size 210 mm SL), generally found in caves or beneath ledges in both coral and rubble habitats.

Fish Collection

Settlement stage *Pomacentrus amboinensis* were collected overnight using light traps moored at the back of the reef, and fish were transported to the Lizard Island Research Station at dawn. Juvenile *P. amboinensis* were collected from the base of shallow reefs approximately one week after the settlement peak, using the anesthetic Clove Oil and hand nets. Many of these fish were settlers from the previous month and were thus assumed to be approximately 3-4 weeks pos-settlement. All fish were maintained in 25L flow-through aquaria systems for ~24 hours, and fed ad libitum newly hatched *Artemia* sp. twice per day to facilitate recovery from the stress of capture. Growth during this period was minimal.

Adult *P. fuscus, T. lunare, S. dermatogenys* and *C. microprion* were collected from surrounding reefs using a combination of anesthetic (clove oil/sea water mix), hand nets, barrier nets and baited hand lines. Immediately following collection, all fish were maintained in individual 57L flow-through aquaria systems for 48 hours before use in aquarium trials. Fish were not fed during this period to standardize for satiation, and to avoid handler-associated learning. This level of food deprivation is not thought to be unusual in the wild, given that available information suggest a high degree of gut emptiness for piscivores and generally slow through put rates through the digestive system (Martin 1994; Sweatman 1984).

Experiment 1: Predator selectivity at time of settlement

Settlement stage *P. amboinensis* were placed into a clip-seal bag of aerated water and measured for standard length (SL ±0.1 mm) using calipers. Fish were then placed into one of three size classes: small (10.8-11.5 mm SL), medium (11.9-12.1 mm SL) and large (12.5-13.0 mm SL). Size classes were chosen to represent the entire range of sizes present at the time of settlement. Although the total range may be considered slightly conservative for this species, the size range present in individual light trap catches can vary considerably between days. The chosen classes allowed trials to be run over consecutive days whilst still maintaining the highest possible proportion of the total species' size range.

Predatory fish were also measured for SL prior to the beginning of trials. The size range of all four species used in trials was as follows: *Pseudochromis fuscus* (40.0-71.4 mm SL), *Thalassoma lunare* (52.0-167.6 mm SL), *Synodus dermatogenys* (39.0-102.0 mm SL), and *Cephalopholis microprion* (79 -155.0 mm SL). Although larger individuals of *T. lunare* and *S. dermatogenys* were caught, they were not used in trials due to difficulties associated with acclimation in aquaria. All species were then divided into three separate size classes to examine changes in size-selection with ontogeny (*P. fuscus*:

small 40-51mm, medium 52-60mm, large 61-72mm SL; *T. lunare*: small 52-88mm, medium 89-110mm, large 111-168mm SL; *S. dermatogenys*: small 39-62mm, medium 63-79mm, large 80-102mm SL; *C. microprion*: small 79-115mm, medium 116-127mm, large 128-145mm SL).

Eighteen identical flow-through aquaria were constructed, as per Almany et al. (2007). Each aquarium had an internal volume of 57.4L (600 x 255 x 375 mm). Aquaria were divided into two equal sized sections by a removable opaque perspex partition. A 15cm length of 105mm diameter PVC pipe cut in half was placed into one section of the aquarium as predator shelter. A single, artificial (white moulded resin) branching coral (item no. 21505; Wardleys/TFH, Sydney; dimensions: 140 x 115 x 50 mm) was placed in the other section as prey shelter. Aquaria were surrounded by black plastic to visually isolate them from each other and other external disturbances. A small hole was cut in one side of the plastic to allow observation of the trials. All aquaria were maintained under natural lighting regimes (ie. regular daylight hours), with experimental trials commencing between 10:00 and 14:00 of each day.

At the commencement of each trial, aquaria were divided in half with the opaque partition. Three settlement stage *P. amboinensis* (one from each of the three size classes) were placed into one half along with the artificial branching coral. The size difference of individuals between size classes within a trial was always at least 0.5 mm. This corresponds to a body depth difference of approximately 0.31 mm. Given that the mean body depth of individuals caught in light traps during the course of the experiment was 4.87. mm, this difference of 0.31 mm represents ~6.4.% of the mean prey body depth. A single predator of known species and SL was allowed to acclimate in the opposite section of the aquaria for 48 hours prior to the trials. Prey were acclimated for 1 hour before the partition was removed and the trial started. Prey abundance was continuously monitored for the first 20 min and every 10 min thereafter. When one or more of the prey were found to be missing, the trial was ended. Any survivors were re-measured for SL to determine the identity of the missing individual(s). If all three prey were found to be missing, the trial was discarded. 40 successful trials (13 small, 14 medium, 13 large predators) were completed using *P. fuscus* as the predator, 46 (15 small, 16 medium, 15 large predators) using *T. lunare*, 43 (14 small, 15 medium, 14 large predators) using *S. dermatogenys*, and 48 (16 small, 16 medium, 16 large predators) using *C. microprion*. Predatory fish were each used in one trial only (whether successful or not) and were released at their point of capture when trials were completed.

Experiment 2: Predator selectivity during early juvenile period

Juvenile *P. amboinensis* were measured as per the previous experiment. Fish were subsequently placed into one of five size categories according to their SL: 11.8-13.0 mm, 13.1-15.0 mm, 15.1-17.0 mm, 17.1-19.0 mm, and 19.1-22.0 mm. These classes were chosen so as to cover a size range from the time of settlement to approximately 3-4 weeks post-settlement. Two predator species only were used in trials with juvenile prey: *Pseudochromis fuscus* and *Cephalopholis microprion*. Predator SL was measured, with the size range of the two species as follows; *P. fuscus* (43-70mm SL), and *C. microprion* (79-145mm SL). As in the previous experiment, predators were then divided into three separate size classes to examine changes in size-selection with ontogeny (*P. fuscus*: small

40-51mm, medium 52-60mm, large 61-72mm SL; *C. microprion*: small 79-115mm, medium 116-127mm, large 128-145mm SL).

The same experimental aquaria and protocol was used as in experiment 1, the only difference being that five prey (one from each of the five size classes) were placed into one half of the aquaria along with the artificial branching coral. The size difference between classes within a trial ranged from 1.4-3.3 mm, representing ~ 8.7-20.5% of the mean prey SL (16.1 mm). When 1 or more of the prey were found to be missing, the trial was ended. Any survivors were re-measured for SL to determine the identity of the missing individual(s). If more than two prey were missing, the trial was discarded. 69 successful trials (23 small, 23 medium, 23 large predators) were completed using *P. fuscus* as the predator, whilst 45 (15 small, 15 medium, 15 large predators) were completed using *C.microprion*.

Predator gape limitation

Before predators were released, a measure of maximum gape size was taken for all four species, in order to obtain body length/gape size relationships. Maximum gape size was taken as the maximum internal horizontal distance within the fish’s mouth without visible distortion. This was obtained by extending pincer calipers within the mouth (at the axial point between the upper and lower jaws) until the point of resistance.

Body depth and SL measurements were taken from a number of settlement stage (collected from light traps) and juvenile (collected from surrounding reefs) *P. amboinensis* to calculate body length/depth relationships. Body depth was obtained using calipers and taken as the widest vertical distance along the fish’s length. Dorsal and ventral fins were not included in this measurement due to their delicate and non-rigid nature in this species at this point in development. Both measurements were taken on the observation that prey were almost always ingested by the predator tail first and orientated on their side. Thus, any gape limitation will be driven by the maximum width of the predator's gape in comparison to the maximum body depth of the prey.

Body length/gape size ratios of the four predator species and body length/depth ratios of *P. amboinensis* were then compared to determine the extent to which predator-prey relationships were potentially limited by predator gape size.

Analysis

The number of times each size class was selected first was totaled for comparison within and between predator species and sizes for both experiments. In cases where two prey were taken before the trial was ended, each of the missing size classes were assigned a half count (0.5) and included in the analysis. The selection counts of the three prey size classes were compared for each predator species (total) and predator size class, using a chi-squared goodness of fit test. Selective profiles were compared between predator species and size class using a generalized linear model incorporating an ordinal multinomial distribution. This method was deemed the most appropriate form of analysis due to the categorical nature of the multinomial variables (size classes; Ambrosius 2007). Assumptions of homogeneity of variance and normality were examined prior to analysis.

In order to determine the potential for gape limitation within interactions, predator body length/gape size relationships were plotted for each of the four species. This plot was then overlaid with the range of body depths of both the settlement stage and juvenile prey individuals used in trials. The resulting figure allows for the visual comparison of predator sizes that are potentially constrained by maximum gape during interactions with settlement stage and juvenile prey. Prey body length/depth relationships were then used in conjunction with predator body length/gape size relationships to estimate the number of times predators fed above their maximum gape during predation trials.

3.3. Results

Experiment 1: Selectivity at settlement

The size-selection profiles for settlement stage *Pomacentrus amboinensis* differed significantly among the four predator species (Wald Statistic $_{df\ 3}$ = 20.375, p = 0.0001; Fig. 3.1). *Pseudochromis fuscus* selected large prey (68.75% of cases) significantly more often than small or medium prey sizes (17.50% and 13.75% of cases respectively; $\chi^2_{df\ 2} = 22.663$, $p < 0.0001$). In contrast, both *Thalassoma lunare* and *Synodus dermatogenys* displayed a non-significant trend to select the smallest of the three size classes (47.83% and 45.35% of cases respectively) compared to the medium and large *P. amboinensis* (35.87% and 20.65% for *T. lunare*, $\chi^2_{df\ 2} = 4.906$, $p = 0.0860$; and 30.23% and 24.42% for *S. dermatogenys*, $\chi^2_{df\ 2} = 0.656$, $p = 0.8438$). *Cephalopholis microprion* showed no clear preference between prey sizes, with small, medium and large size classes being selected relatively evenly ($\chi^2_{df\ 2} = 0.656$, $p = 0.8438$).

There was no difference in size-selection between predator sizes within all four of the predator species (*P. fuscus*, Wald Statistic $_{df\ 2}$ = 0.368, p = 0.832; *T. lunare*, Wald Statistic $_{df\ 2}$ = 1.037, p = 0.595; *S. dermatogenys*, Wald Statistic $_{df\ 2}$ = 0.145, p = 0.93; and *C. microprion*, Wald Statistic $_{df\ 2}$ = 0.143, p = 0.931).

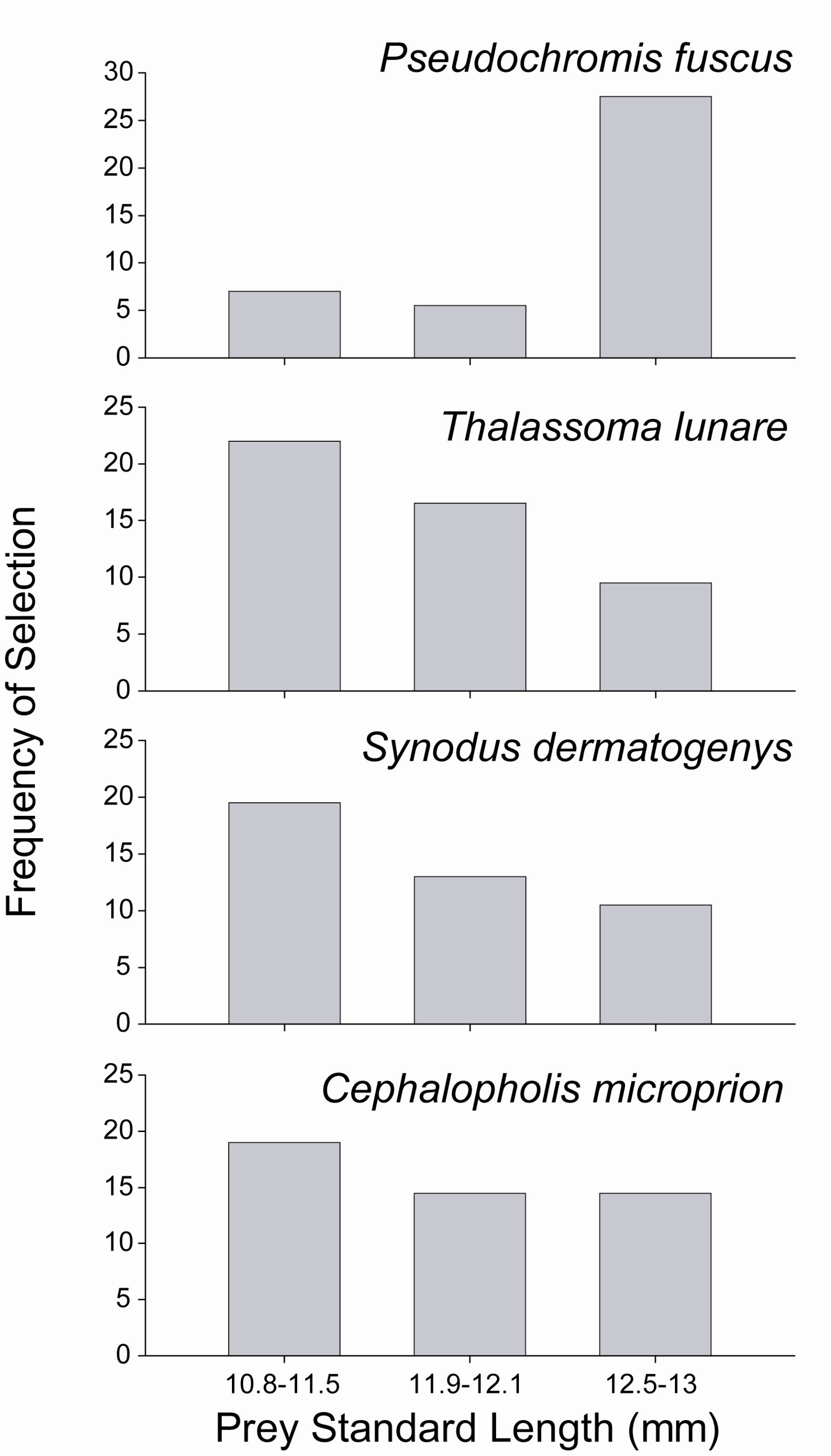

Figure 3.1: Frequency of first selection of settlement stage *Pomacentrus amboinensis* during aquarium based predation trials with four important predatory fish species.

Experiment 2: Selectivity during early juvenile stage

There was a significant difference in the size-selective profiles of the two predator species during trials with early juvenile *P. amboinensis* (Wald Statistic $_{\text{df }1}$ = 17.764, $p <$ 0.0001; Fig. 3.2). *P. fuscus* selected the smallest prey size significantly more often (54.95% of cases) than the four other size classes (24.72%, 12.64%, 7.69% and 0% of cases respectively; $\chi^2_{\text{df }4} = 59.297$, $p < 0.0001$). The largest size class (19-22 mm SL) was not selected by *P. fuscus* during any trials throughout the experiment. Interestingly, although the direction of selection changed between experiments, the size class targeted by *P. fuscus* in this experiment (smallest) roughly corresponds to the size range of the size class targeted in experiment 1 (largest). *C. microprion* showed no detectable preference for prey size during interactions with juveniles ($\chi^2_{\text{df }4} = 2.222$, $p = 0.6950$), with the five size classes have a similar probability of selection.

Predator size had no effect on size-selective preference within species, with no significant difference being detected between the three size classes within both *P. fuscus* (Wald Statistic $_{\text{df }2}$ = 1.735, $p = 0.4200$) and *C. microprion* (Wald Statistic $_{\text{df }2}$ = 1.494, p = 0.4740).

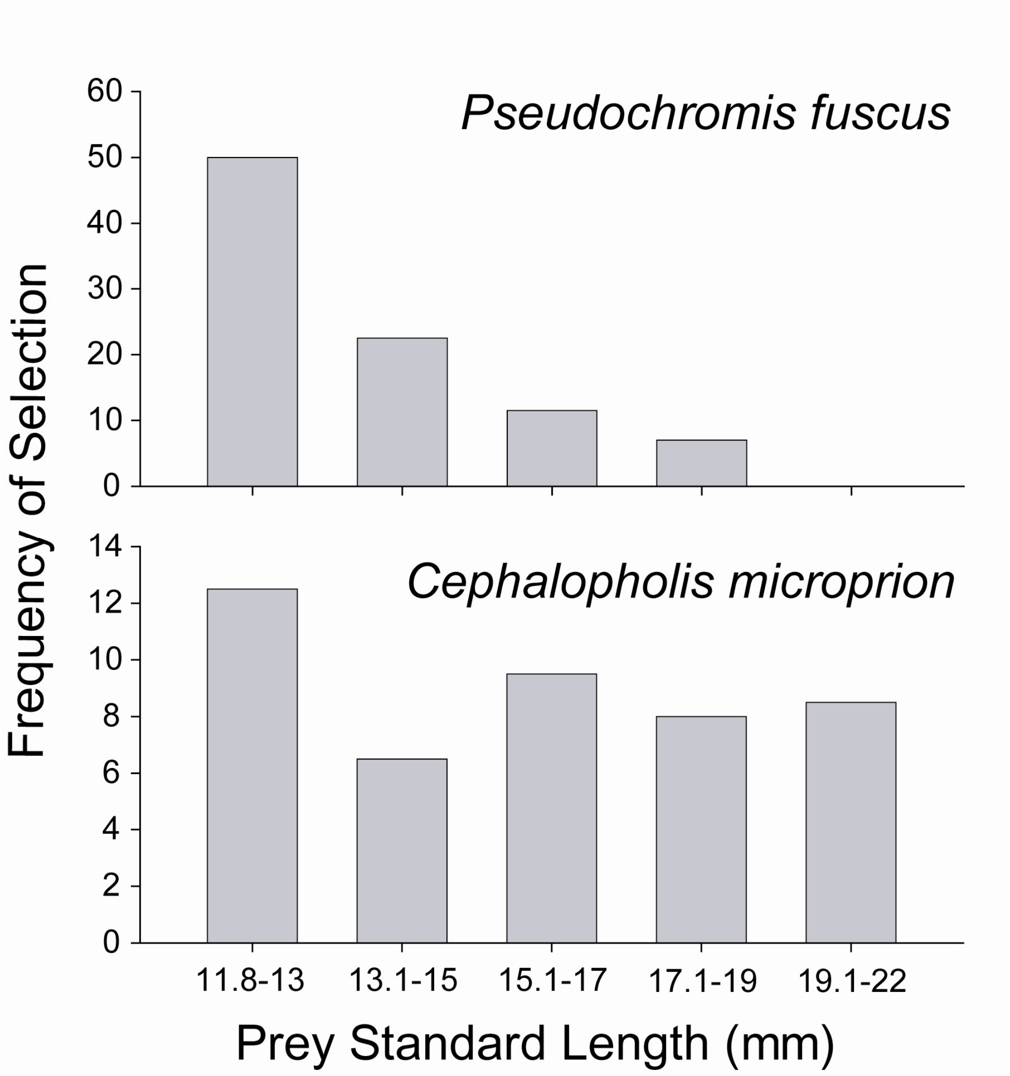

Figure 3.2: Frequency of first selection of early juvenile *Pomacentrus amboinensis* during aquarium based predation trials with two important predatory fish species.

Predator gape limitation

Prey body length/depth relationships were characterised by positive regressions for both settlement stage ($y = 0.505x - 1.0761$, $R^2 = 0.819$) and juvenile *P. amboinensis* ($y = 0.564x - 1.235$, $R^2 = 0.963$). Settlement stage individuals had lower length/depth limits of 10.6/3.9 mm and upper limits of 13.0/5.7 mm, whilst juveniles used in this study had lower length/depth limits of 11.8/5.1mm and upper limits of 21.1/10.4mm.

Constraints imposed by predator gape size have the potential to greatly influence the outcome of interactions between both *P. fuscus* and *T. lunare*, and juvenile/settlement stage *P. amboinensis* (Fig. 3.3). Given that the determinants of maximum prey size are predator gape width and prey body depth, the relationship suggests that all sizes of *P. fuscus* measured during this study were limited to varying extents by maximum gape during interactions with prey > 21 mm SL. As the maximum size at settlement for *P. amboinensis* is ~13.3 mm SL, gape limitation will play a role in determining the capture probabilities of settlement stage *P. amboinensis* for any *P. fuscus* below ~44 mm SL. In the case of *T. lunare*, any fish below 144 mm SL will potentially have limitations imposed by gape size during interactions with juvenile prey > 21 mm SL. Any *T. lunare* less than 91 mm SL may also be gape limited during interactions with settlement stage prey.

Neither *S. dermatogenys* nor *C. microprion*, of the sizes collected and measured in this study, appear to be constrained by maximum gape size during interactions with juvenile or settlement stage *P. amboinensis* (Fig. 3.3). Interpolation suggests that *S. dermatogenys* may potentially have limitations imposed at sizes below 47.7 mm SL, whilst *C. microprion* may be limited at sizes below 56.8 mm SL.

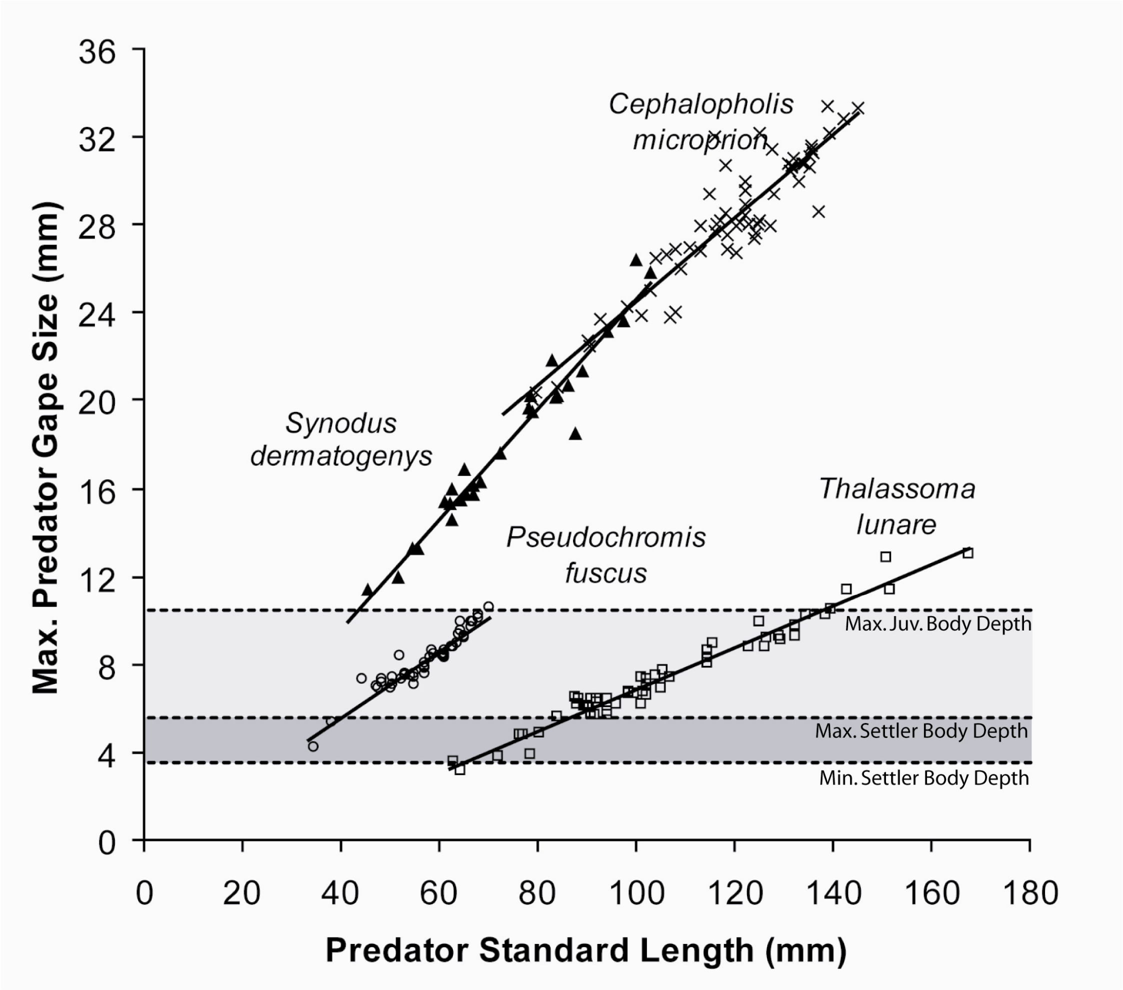

Figure 3.3: Potential for gape limitation in four important predator species (*Pseudochromis fuscus* , *Thalassoma lunare, Synodus dermatogenys* and *Cephalopholis microprion*) during interactions with settlement stage and juvenile *Pomacentrus amboinensis*. Prey (*P. amboinensis*) standard length/body depth relationships have been overlaid on predator standard length/gape size relationships to ascertain the identity and size of those predators potentially constrained by gape size during predatory interactions (i.e. those individuals below or within the prey body depth ranges). The dark shade of grey refers to the body depth range for settlement stage individuals, whilst the lighter shade of grey refers to the body depth range for juvenile individuals.

Using the calculated predator body length/gape size and prey body length/depth relationships as a guide (Fig. 3.3), it was determined that of the 99 trials in which *P. fuscus* were provided a choice including prey sizes above their predicted maximum, individuals selected above this maximum on 8 occasions (8.1% of trials). In comparison, of the 20 trials in which *T. lunare* were offered a choice of prey including sizes above their predicted maximum, individuals selected above this maximum on 16 occasions (80% of trials). Measurement error was ruled out as a cause of this unexpected result due to the magnitude of the discrepancy, with prey body depth often exceeding maximum predator gape by up to 2.5 times. Due to the proportionately larger gapes on both *S. dermatogenys* and *C. microprion*, no trials were conducted on either species that involved a choice of prey above their predicted maximum. Of additional interest is the position and gradient of the body length/gape size relationship of *T. lunare* ($y = 10.136x + 30.67$) in comparison to that of *P. fuscus*, *S. dermatogenys* and *C. microprion* ($y = 5.9923x + 8.2031$, $y = 3.7987x + 5.2222$, and $y = 4.4714x - 6.8009$ respectively), indicating that gape is significantly smaller for a given size in this species and that it increases with ontogeny at a slower rate than the others.

3.4. Discussion

For juvenile coral reef fishes the direction and intensity of selection can differ over very small spatial scales (Holmes and McCormick 2006; McCormick and Meekan 2007; Samhouri et al. 2009). This variation is thought to be partially due to differences in the composition of predator assemblages between sites and their underlying selective preferences. However, this conclusion is based on inference due to the almost complete lack of information on species specific predator selectivity within marine systems, and ecosystems in general (for exceptions, see Scharf et al. 2000; Allen 2008). This study presents one of the first cases of species specific size selectivity for a number of important predators within an ecosystem. The results show that different predator species have different size-selective preferences during interactions with the settlement and early juvenile stages of a common coral reef fish species. While this gross finding of species differences matches the predictions of foraging theory, the details of the selection of prey by particular species does not conform well to predictions. Evidence suggests that at the time of settlement in the life of a coral reef fish, no particular body size confers a definitive survival advantage during predatory encounters. Instead, prey survival may in part be determined by the suite of predators present at the location of settlement, and how they interact with one another.

The idea that prey body size has a large influence on the outcome of predator-prey encounters is not new to the field of ecology (Janzen 1993; Litvak and Legget 1992; Mathis et al. 2003). The most common view amongst ecologists is that larger prey size conveys a universal survival advantage during such events (i.e. bigger is better; Rice et al. 1993; Takasuka et al. 2003; Wang et al. 2007). However, previous studies by Sogard (1997) and Allen (2008) have both found evidence to suggest otherwise, concluding that although larger prey size conveyed a survival advantage in a majority of species specific interactions (negative size selection), there were a number of cases in which it was also selected against (positive size selection). Our study describes a similar pattern of mixed selective direction amongst a number of predator species on settlement stage prey, within a single system and under identical experimental conditions. Incidentally, a recent field study by McCormick and Meekan (2007) also produced evidence to suggest similar patterns of contrasting selective preference in *T. lunare* and *P. fuscus*. Although this outcome was largely implied due to difficulties associated with controlling external factors in field experiments, it shows that the results obtained from aquarium trials in this study hold true in a field context. This further reinforces the potential for interspecific differences in selective preference amongst predators, and in conjunction with our study, suggests that the role of prey body size in determining the outcome of predatory interactions should be considered on a case by case basis, rather than in general terms.

So why may a particular expression of the body length trait not provide a uniform survival advantage across all interactions, as predicted by ‘bigger-is-better’ theory? The answer to this is likely to lie within variation in the predation mode and morphology between predator species (Keast and Webb 1966; Labropoulou and Eleftheriou 1997; Gaughan and Potter 1997). Such differences may potentially allow each predator species to exploit the available population in a different way, so as to optimize energy return. For example, the mobile and vigilant nature of *P. fuscus* may enable it to choose and target optimally sized prey, whilst the opportunistic (*T. lunare*) and ambush nature (*S. dermatogenys* and *C. microprion*) of predation by the other three species means that they may target anything that becomes vulnerable or comes within striking range. Such a suggestion has previously been made by Scharf et al. (2000) in which they concluded that size-based feeding strategies were related to predator foraging tactics, habitat overlap with prey, and morphological specializations that are particularly suited to specific habitats and/or prey types. Given the wide range of predator morphologies and feeding modes present amongst tropical reef fish communities (Hixon 1991) it is reasonable to suggest that the same may apply to this system.

With the exception of *P. fuscus*, and to a lesser extent *T. lunare* (only the smaller sizes are potentially constrained), gape size appears to play a minimal role in determining selective preferences amongst important predatory fish during interactions with newly settled and juvenile *P. amboinensis*. In the case of *T. lunare*, the evidence against gape limitation during these relationships is further heightened by the apparent lack of constraint imposed by maximum gape size, with fish regularly recorded feeding well above their predicted maximum prey size. This however is not necessarily surprising, given the opportunistic nature of *T. lunare*'s predation mode and the observation that gape size of the species is relatively small for a given size, and increases at a relatively slow rate with ontogeny. This indicates that gape size may not necessarily be a limiting factor in prey choice for this species. Alternately, this lack of constraint based on conventional morphological measurements could highlight the importance of other facets of jaw functional morphology (i.e. biomechanics) in influencing strike speed, jaw closure speed and bite force for this species (Grubich et al. 2008; Anderson and Westneat 2009). Despite both *C. microprion* and *S. dermatogenys* being identified as important predators of juvenile reef fish, only the smallest sizes of each have the potential to be gape limited in this system. However, no individuals of this size were trialed in this study, due entirely to problems associated with locating and catching fish of such a small size for these species. The lack of gape limitation was particularly obvious in *C. microprion*, and this large gape size in comparison to the size range of the prey, as well as the 'engulfing' nature of their attacks, helps to explain the lack of selectivity observed for this species.

Despite its absence in the other three species, gape limitation was apparent in *P. fuscus* during interactions with juvenile prey, with individuals rarely feeding above their predicted maximum size. However, during interactions with both settlement stage and juvenile prey, fish were generally observed to feed well below their predicted maximum, indicating that the observed selective patterns were more a result of behaviour rather than morphological constraints. Overall, this general lack of a gape constraint for predators feeding on newly settled *P. amboinensis* suggests that an important determinant of size selectivity during this period may be the behavioural traits exhibited by predator and prey (Gaughan and Potter 1997).

Evidence for the involvement of prey behaviour in influencing susceptibility to predation has grown in recent years (Biro et al. 2004, 2006; Sih et al. 2004; Stamps 2007; Biro and Stamps 2008). In the context of coral reef ecosystems, a recent field study by Meekan et al. (in press) found that *Pomacentrus amboinensis* of larger standard length spent more time foraging, were more aggressive towards smaller conspecifics, and swam greater distances than their smaller counterparts. These differences have also been shown to apply in an aquarium situation, with similar behavioural patterns occurring on the coral mould habitat used in this study (Chapter 4). It is possible that such differences in behaviour and space use between prey sizes may interact with predator feeding strategies and habitat niches to cause the observed selective patterns.

Despite our results, there is currently a significant body of literature suggesting that larger prey body size generally provides a survival advantage during the early post-settlement period in coral reef fishes (e.g. Schmitt and Holbrook 1999). Where these studies differ from the current research is in the duration over which selection is measured, which tends to be over days to weeks. This latter assessment has the potential to mask selective forces acting within the first 24 hours post-settlement, when individuals are most naïve to reef based predators and hence most susceptible to predation. For those individuals that make it through this period, the probability of survival during future encounters has been shown to increase as a result of their experiences (McCormick and Holmes 2006). This is presumably caused by behavioural changes within the newly settled individuals, which may in turn also affect the selective nature of predation.

The rapid change in the dynamics between predator and prey and its influence on prey selection has recently been highlighted by Meekan et al. (in press) in their study of behaviourally mediated mortality on open patch reefs. They found mortality to be selective towards larger individuals at the time of settlement, and towards smaller individuals a month following settlement. A similar pattern was observed in this study during trials with *P. fuscus*, with prey selection acting against larger individuals at the time of settlement, and against smaller individuals during the early juvenile period. This selection occurred despite the choice of prey sizes largely falling within gape constraints. One explanation for this may be that this prey size (which was the same in both experiments, despite the different size range of classes around it) represents the optimal choice for this particular predator species, with each experiment detecting one end only of the resulting dome shaped curve (Rice et al. 1993). An alternate explanation may lie within prey behaviour. At the upper end of this prey size spectrum, gape limitation will indeed play a role for this predator species. However, for the smaller prey sizes it is possible that experiences acquired by prey individuals during early settled life resulted in behavioural changes that make larger body size, and its associated covariates, distinctly advantageous. If such patterns are common, then it is important to distinguish between these two periods when assessing selective loss.

According to optimal foraging theory, preferred prey size should increase with increasing predator size (MacArthur and Pianka 1966; Hughes 1980; Rice et al. 1993).

However, this was not the case in our study, with predator body size failing to influence prey size selection for any of the predator species, during either the settlement or early juvenile trials. It may be that the size range of prey at the time of settlement was simply insufficient to cause a difference in selective preference, particularly in the case of the non gape-limited predators (i.e. *S. dermatogenys* and *C. microprion*). During the juvenile trials, all sizes of *P. fuscus* were largely constrained by gape size to the smaller size classes, limiting the potential for selective shifts, whilst the large gape size of *C. microprion* effectively negated any change. Alternately, the lack of change in prey size selection may be related to prey behaviour, in that specific prey sizes may be more vulnerable to predation by specific feeding strategies, which may not change greatly over the size range of predators used in this study (Bilcke et al. 2007; Chapter 4). Whatever the underlying mechanisms, in relation to the predator species used in this study, predator body size appears to play a relatively minor role in determining the outcome of predatory interactions during early settled life.

The importance of selective processes in structuring community dynamics of organisms with complex life histories has received much attention in recent years (Congdon et al. 1999; Chivers et al. 2001; Searcy and Sponaugle 2001; Allen 2008). Although caution should be observed when extrapolating these results to the wider prey community (due to the potential for morphological and behavioural differences between prey species), this study adds significantly to this literature, and provide us with further insight into the patterns of predator induced selectivity operating during a critical life stage for coral reef fishes. In doing so, we have shown that there is great potential for such selective processes to differ between locations, based on the composition of the predator community alone. In addition, this work highlights the importance of distinguishing between the settlement and early juvenile periods when assessing the mechanisms underlying selective loss. However, how these patterns hold in multi-species situations remains to be seen, due to the possibility of synergistic and antagonistic relationships between predatory and other non-predatory species altering individual preferences (e.g. McCormick and Meekan 2007; Samhouri et al. 2009). Such factors must be considered before any assessment of selective patterns in natural systems.

Chapter 4: Behaviour as a mechanism underlying size-based differences in vulnerability to predation

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4.1. Introduction

The behavioural characteristics of an individual can have a large impact on the probability of survival throughout its lifetime. These traits may directly or indirectly affect survival through a range of processes, including competitive (Kozak et al. 2007; Zuberogoitia et al. 2008) and predatory interactions (Nannini and Belk 2006; Kesavaraju et al. 2007; May et al. 2008). One important influence of behaviour is on the vulnerability to predation, as this is widely regarded as one of the most important processes structuring ecological communities (Sih et al. 1985; Wellborn et al. 1996; Banks et al. 2008). Specific behaviours, such as foraging rate, aggression towards conspecifics and habitat usage can influence this vulnerability, and play a role in determining which individuals may be lost from a population.

Size-based hierarchies within small groups have frequently been attributed to behavioural variation amongst individuals (Abbott and Dill 1989; Hunt 1992; Rychlik and Zwolak 2006; Ahvenharju and Ruohonen 2007). The common finding is that larger size within a group generally corresponds with hierarchical dominance, providing a competitive advantage over smaller individuals and allowing greater access to food resources and preferred habitat (Dou et al. 2004; Fisher and Cockburn 2006; Gherardi 2006). For smaller, subordinate individuals these dominance hierarchies can result in decreased growth rates (Johnson 2003; Montero et al. 2009), sub-optimal habitat usage

(Dickman et al. 1991; Trejo and Guthman 2003; May et al. 2008) and increased stress from aggressive conspecific attacks (Gilmour et al. 2005; Montero et al. 2009); all of which can potentially increase vulnerability to predation. However, a number of other studies have reported the opposite, with larger body size providing no competitive advantage, or even a disadvantage, during hierarchical interactions (Cote 2000; Schulte-Hostedde and Millar 2002). This indicates that the relationship may vary between systems, or that dominant behaviours are reliant on some other intrinsic factor (e.g. 'personality traits,' Sih et al. 2004; Huntingford 2007).

The potential importance of behavioural traits in influencing an individual's susceptibility to predatory attacks may have particular relevance during periods of high mortality. For organisms with complex life cycles, such as many invertebrates, amphibians and fishes, mortality is highest during the larval phase and around metamorphosis when organisms transition between environments (e.g. Wilbur 1980; Hunt and Scheibling 1997). In coral reef fishes, the period immediately following larval settlement to the reef environment is characterised by such high mortality (upwards of 60% within 48 hours post-settlement; Doherty et al. 2004; Almany and Webster 2006), with much of this loss being attributed to predation by small fish predators (Holbrook and Schmitt 2002). Given that many fish species often form small conspecific groups at settlement (e.g. McCormick and Meekan 2007), the interactions and behavioural characteristics relative to others in the group will greatly influence which individuals survive through to the juvenile and adult life stages (Errington 1946, 1956; Grorud-Colvert and Sponaugle 2006). Although hierarchical systems have been documented in a number of coral reef fish groups (e.g. Forrester 1991; Booth 1995; Whiteman and Cote

2004), to date only a single study has examined behavioural differences among individuals within a simple group during the early post-settlement period (Meekan et al. 2010), although these observations were solely field based.

The present study examines the behaviour of a common, newly-settled damselfish, *Pomacentrus amboinensis*, within a simple paired hierarchical system. Consistent differences in behaviour may lead to ecological advantages, such as enhanced growth and survival. In the present study we explored the behavioural differences associated with differing body size between individuals within a group. Because predation experiments are often conducted in aquaria (e.g. Beukers and Jones 1997; Almany et al. 2007; Figueira et al. 2008) it is important to determine whether fish behave similarly within laboratory and field trials. Therefore, observational trials were conducted both in the field and aquaria to examine the consistency of behaviour traits exhibited between situations. It was predicted that behavioural characteristics of individuals would differ according to relative size, with larger individuals being dominant. Additionally, we expected no difference in overall behaviour between aquarium and field trials.

4.2. Material and methods

Study site and species

This study was conducted at Lizard Island (14°40’S, 145°28’E), northern Great Barrier Reef (GBR), Australia during November and December of 2007. The flow through salt water aquarium system at Lizard Island Research Station was used to conduct all aquarium trials, while field trials were conducted on a nearby shallow reef system.

Settlement stage *Pomacentrus amboinensis,* a common damselfish on Indo-Pacific coral reefs, were used as the model species for all experimental trials. This species settles to a wide variety of habitats on the northern GBR, but are found in highest densities associated with small reef patches at the base of shallow reefs. *P. amboinensis* has a pelagic larval phase of between 15 – 23 days and settles at 10.3 – 15.1 mm standard length (Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site attached, making it an ideal species for experimental manipulation and observation experiments. They recruit in substantial numbers at Lizard Island around the new moon during the austral months (October – January), and are easily collected at the time of settlement with light traps (Milicich and Doherty 1994).

Fish collection and preparation

Settlement stage *Pomacentrus amboinensis* were collected using light traps (see Meekan et al. 2001 for design) moored overnight close to the reef crest, and fish were transported to the Lizard Island Research Station at dawn. All fish were maintained in 25 L flow-through aquaria systems for ~24 hours, and fed newly hatched *Artemia* sp. twice per day ad libitum to allow for recovery from the stress of capture. Growth during this period was minimal.

Immediately following the 24 hour acclimation period, individual *P. amboinensis* were removed from aquaria, placed into plastic clip seal bags filled with aerated and measured for standard length (SL, ± 0.01 mm) with the aid of a magnifying glass using Vernier calipers. Fish were then paired such that a larger sized individual (mean = 11.72

mm SL ± 0.04 SE) was paired with a smaller sized conspecific (mean = 10.84 mm SL ± 0.03 SE). A standardized size difference of 0.7-1.0 mm SL was maintained between individuals within a pair throughout all trials. For identification purposes, individuals within the pair were tagged with either a red or black subcutaneous fluorescent elastomer tattoo (Northwest Marine Technologies) using a 27-gauge hypodermic needle, as per Hoey and McCormick (2006). Tag colours were alternated between replicate trials to avoid any potential behavioural biases. Hoey and McCormick (2006) found that the tagging technique had no influence on growth or survival of *P. amboinensis* in aquaria over a 2 wk period.

Aquarium trials

Aquarium trials were conducted in 57 L (600x255x375mm) glass aquaria, surrounded on three sides by black plastic. One longitudinal side was left open to allow observation. Two artificial (white moulded resin) branching corals (item no. 21505; Wardleys/TFH, Sydney; dimensions: 140 x 115 x 50 mm) were placed at one end of the tank against the glass, to act as prey shelter. The horizontal distance away from the coral shelter (along the longitudinal axis) was then marked at 50 mm intervals for reference purposes, with the start point being the outer edge of the coral mould branches. A vertical line was drawn on the outside of the aquaria at each of these 50 mm intervals, effectively dividing the horizontal scale into a series of zones. The vertical distance above the substrate was also marked at 50 mm intervals for reference purposes, with the top of the coral moulds being at 140 mm.

A single *P. amboinensis* pair (1 large, 1 small individual) was placed into each aquarium, along with 30 ml of newly hatched *Artemia* sp., and left to acclimate for two hours. All individuals were observed to have settled to the coral moulds and were feeding within the water column after 15 minutes. At the end of the acclimation, and immediately prior to the observation period, a further 10 ml of newly hatched *Artemia* sp. was added to the aquaria to stimulate feeding.

Behavioural observation periods lasted 15 minutes, with recordings being made by two observers sitting behind black plastic sheeting to avoid influencing fish behaviour. Each observer focused on the behaviour of a single fish only during this period. Distance from the coral shelter and vertical height in the water column, was recorded every 30 seconds. If an individual was within the shelter at the time of the recording, the distance from shelter was said to be 0. In addition, aggressive strikes on conspecific and movement between horizontal zones were recorded whenever they were observed during the observation period. An individual was said to have moved between zones whenever they crossed a border at one of the 50 mm intervals, including the 0 mm interval at the edge of the coral shelter. At the end of the original 15 minute period, a further 5 minutes was used to record feeding rate. This was recorded as the number of feeding strikes made by each individual during this period, with feeding strikes on *Artemia* sp. being easily visible against the black background.

Field trials

Field trials were conducted on a series of small patch reefs, created on sand substrate immediately adjacent to a shallow lagoonal reef. Each patch consisted of a 200 x 200 x 200 mm *Pocillopora damicornis* (a bushy hard coral) coral head, separated from the main reef by approximately 5 m. Small pegs were placed radially outwards from the coral head in 50 mm increments, with the start point being the outer edge of the coral branches. A thin piece of string was then used to join each of the pegs at specific increment widths, dividing the horizontal distance away from shelter into 50 mm zones.

A single *P. amboinensis* pair (1 large, 1 small individual) was placed on to each patch, and immediately covered with a 5mm wire mesh cage (300 x 300 x 300 mm) to prevent predator access during a 2 hour acclimation period. All individuals were observed to be settled to the coral head and feeding within the water column within 30 minutes. At the end of the acclimation, and immediately prior to the observation period, the mesh cage was removed, and divers remained beside the patch for 10 minutes to allow fish to acclimate to diver presence.

The observational protocol followed that of the aquarium trials, with a 15 minute behavioural period (recording distance from shelter, height in water column, aggressive strikes on conspecific, and movement between zones) followed by a 5 minute feeding strike recording period. As in the aquarium trials, recordings were made by two observers, with each observer focusing on a single fish only. Observations were made from a distance of approximately 1.5 metres, with a magnifying glass used to assist in recording the number of feeding strikes. Divers were present approximately 5 minutes before the commencement of observational periods, and care was taken to avoid any sudden movements so as to reduce diver effects on fish behaviour.

Analysis

Mean values of each of the five behaviours were calculated for both large and small individuals, within both the aquarium and field trials. Overall behavioural differences between fish sizes (2 levels: large, small) and trial type (2 levels: field, laboratory) were then compared using a two-way multivariate analysis of variance (MANOVA). The data was $\log_{10}$ transformed in order to meet the assumptions of this test. A canonical discriminant analysis (CDA) was performed to display how each of the five behaviours differed between fish sizes and trial types. Trends in the behavioural variables are represented as vectors which are plotted on the first two canonical axes, together with treatment centroids and their 95% confidence clouds (Seber 1984). In order to statistically analyse these differences, two-way analyses of variance (ANOVA's) were performed on each of the five behaviours. The data was once again, $\log_{10}$ transformed in order to meet the assumptions of the test. Bonferroni corrections were used to correct for multiple ANOVA's on potentially interrelated variables within a single dataset, with a revised alpha level of 0.01.

4.3. Results

There was a significant difference in overall behaviour of individuals between both the trial types (aquarium and field; Pillai's $\text{trace}_{5,\,74} = 0.3638$, $p < 0.0001$) and fish sizes (large and small; Pillai's $\text{trace}_{5,\,74} = 0.4016$, $p < 0.0001$). No interaction effect was found between trial type and fish size (Pillai's $\text{trace}_{5,\,74} = 0.0223$, $p = 0.8886$).

The CDA displays how the five behavioural measurements influenced the difference between both the large and small sizes, within both the aquarium and field trials. The first two canonical variates explained 76.49 and 23.27% of the overall variation respectively. There was a clear separation of individuals in the aquarium trials, from those in the field trials (Fig. 4.1). This relationship is driven by the number of feeding strikes, the number of movements between zones and the distance from shelter, with aquarium fish showing higher feeding rates, higher movement and greater distances from shelter. In the field fish tended to be higher in the water column than when they were within an aquarium. There was also a pronounced separation of small individuals from large individuals in the aquaria trials (Fig. 4.1). This pattern is largely driven by the number of aggressive strikes on conspecifics, and to a lesser extent the number of feeding strikes, with larger individuals being both more aggressive and feeding more frequently. Although small and large individuals were not clearly separated in the field trials, there was differentiation along the ‘aggressive strike on conspecific’ axis, which suggests that this behaviour is mostly responsible for any difference between the two.

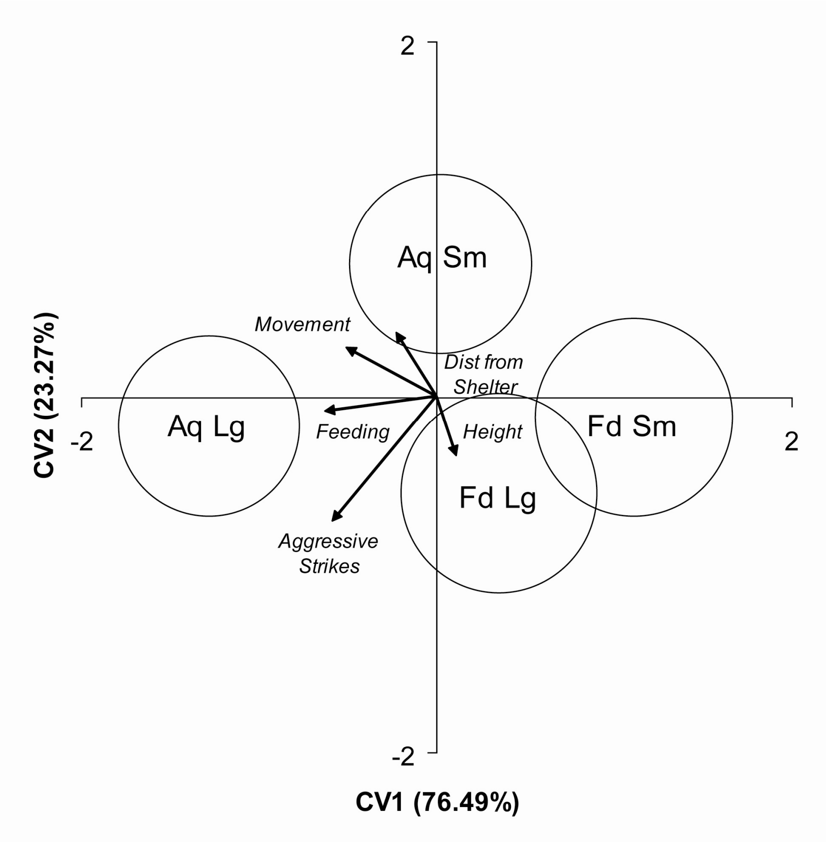

Figure 4.1: A canonical discriminant analysis comparing the behavioural characteristics of large (Lg) and small (Sm) newly-settled *Pomacentrus amboinensis* within simple paired groups, during field (Fd) and aquarium (Aq) trials. The circles represent 95% confidence intervals

The results from the ANOVA's statistically confirmed the visual representation of the behavioural differences between groups in the CDA. Larger individuals were found to make significantly more aggressive strikes on smaller conspecifics across both sets of trials (81.18 and 98.15% more during field and aquarium trials, respectively; $F_{1,\ 78}$ = 19.224, $p < 0.0001$; Fig. 4.2). Likewise, large fish exhibited more feeding strikes than smaller individuals across both sets of trials (26.1 and 26.31% more during field and aquarium trials respectively; $F_{1,\ 78}$ = 10.995, p = 0.0014). At the trial level, fish from the aquarium trials made significantly more feeding strikes ($F_{1,\ 78}$ = 9.596, p = 0.0027) and made significantly more movements between horizontal zones ($F_{1,\ 78}$ = 6.8746, $p < 0.0001$) than fish tested in field trials. This difference was particularly marked in the number of movements made between zones, with 76.5 and 78.91% more movements being made by large and small individuals respectively, in aquarium trials than in field trials. Despite this, the pattern between sizes remained largely the same, with larger individuals making marginally more movements in both sets of trials.

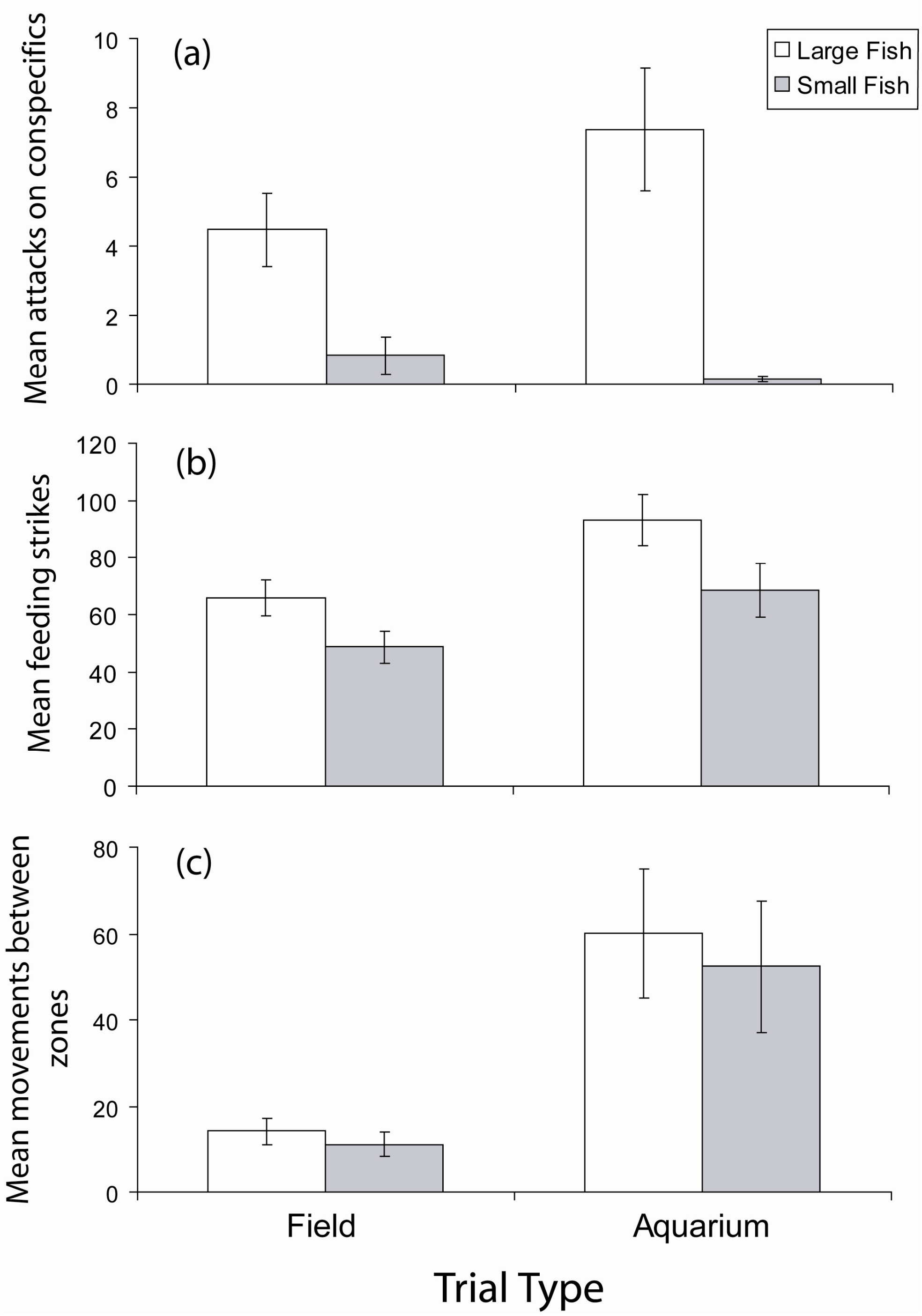

Figure 4.2: Mean differences in (a) aggressive attacks on conspecifics, (b) number of feeding strikes, and (c) number of movements between horizontal zones, between large and small newly-settled *Pomacentrus amboinensis* in simple paired groups, during field and aquarium trials

4.4. Discussion

In conventional predator-prey scenarios involving a single predator and prey, the outcome will largely be determined by the prey's phenotype and how it fits into the predator's selective profile (Hare and Cowen 1997; Rice et al. 1997). However, relationships can become more complex in multi-prey scenarios, where behavioural interactions between prey individuals may work to modify the influence of predator selection and determine the eventual outcome (Koivula et. al. 1994). For prey species with significant grouping tendencies during part or all of their life history, these behavioural interactions amongst individuals will consequently modify the effect that a predator may have on the prey population. This study shows how interactions between prey individuals can alter relative behaviour, in a species known to commonly recruit in small conspecific groups. We found significant differences in behaviour between individuals that differed in relative size in both aquarium and field trials. In both situations large fish were behaviourally dominant over smaller fish within simple paired groups, exhibiting higher feeding rates and more aggression towards the conspecific. Findings indicate that the strict hierarchical system in these coral reef fish is based on body size, and suggest that the associated behavioural differences could alter prey vulnerability and influence predator selection at the time of settlement.

The social interactions within the pairs of fish naïve to reef based-predators highlighted that larger individuals within the pairs made more feeding strikes and were more aggressive towards conspecifics than their smaller counterparts. The findings reinforce a common pattern in the literature (e.g. Gilmour et al. 2005; Xiaowei et al. 2007; Montero et al. 2009) and emphasise the size-based nature of the dominance hierarchy during this critical period in the coral reef fish life cycle. These size-related behavioural differences could have implications for relative growth rates within a group, with larger individuals gaining a further size advantage by securing a greater share of food resources and maximizing growth (Cubitt et al. 2008). This also highlights the potential for differences in vulnerability to predation based on prey behavioural characteristics associated with relative body size and its phenotypic covariates (see Kerrigan 1996). Although this does not entirely discount the role of 'personality traits' in influencing hierarchical position, it does suggest that intrinsic behavioural factors may be closely related to body size during this particular life stage.

The predominant theory in the literature is that larger, dominant individuals are less susceptible to predation, decreasing their vulnerability by occupying optimal habitat and aggressively forcing smaller subordinate individuals into more 'risky' sub-optimal habitats or behaviours (Gilmour et al. 2005; Murray et al. 2007). For example, in a study of feeding site selection by Willow Tits (*Parus montanus*), Koivula et al. (1994) found that socially subordinate individuals were frequently forced to feed in more 'risky' locations due to competitive interference from dominants. If this mechanism were operating in our system, it would be expected that subordinate smaller fish would be forced to feed further away from more 'optimal' coral habitat occupied by the larger conspecifics (given that a greater distance from shelter is assumed to be associated with increased vulnerability to predation). Although our recordings produced no evidence of this in either of our experiments, it may well be that such differences in risk taking behaviour are not evident unless subjected to a direct predation threat. In a study of the influence of body condition on the behaviour and survival potential of newly settled

Thalassoma bifasciatum (Bluehead wrasse), Grorud-Colvert and Sponaugle (2006) found individuals of higher body condition to exhibit less risk taking behaviour when exposed to a simulated predation threat. This indicates that the effects of hierarchical dominance within a group may indirectly influence vulnerability by forcing subordinate individuals to take higher risks in the presence of a predation threat, in order to maximize their share of the available resource.

Vulnerability to predation may also be indirectly related to lower feeding rates experienced by smaller individuals as a result of aggression from dominant conspecifics. In order to secure adequate resources, this deprivation of feeding activity could induce ‘risky’ behaviour in subordinate individuals during suboptimal situations (e.g. when under threat of predation; Vehanen 2003; Borcherding and Magnhagen 2008). Although the tendency of fish to take risks was not assessed in this study, this is a potential source of differential vulnerability in this system given that larger individuals were found to have significantly higher feeding rates and were significantly more aggressive towards conspecifics. In Chapter 3, smaller individuals were selectively removed by two species of opportunistic fish predator (*Thalassoma lunare* and *Synodus dermatogenys*) when given a choice of prey sizes. Although smaller prey were selected, both predator species were morphologically capable of ingesting much larger sized prey. Rather than this pattern being a result of direct predator preference towards smaller individuals, it is more likely that behavioural differences such as those described above, make smaller individuals in a group more vulnerable to this form of predation.

An assumption underlying many laboratory experiments is that the balance of processes influencing survival will be the same as in the natural field situation, so results from the laboratory may be able to inform us about the processes that generate patterns in the natural world (Geiser et al. 2007). Seldom is this assumption tested. We found significant differences in fish behaviour between trials run on open patch reefs in the field and on coral habitat within aquaria. Feeding rate and horizontal movement were both found to be significantly higher in aquarium trials than in the field. Although differences in feeding rate can be largely discounted due to the inability to standardise the amount of food available between trial scenarios, the differences in movement could have significant ramifications for the results of predation trials run within the confined spaces of aquaria. The expected result would be an overall increase in predation rate, with the increased movement making fish more susceptible to predator attacks in general. Such a mechanism would help to explain the elevated predation rates recently found in Chapter 2. Predation pressure in laboratory experiments was much higher than that experienced by fish on field-based patch reefs. This enhanced predation potentially had the effect of masking selective processes that may have been underlying the predatory interactions during these experiments.

Alternatively, the decreased movement of fish found in the field may be an experimental artifact resulting from the presence of scuba divers. In order to accurately record feeding strikes divers were positioned approximately 1.5 m from the coral head shelter, well within the field of view of the experimental fish. In contrast, during the aquarium trials observers were obscured from the fish’s view behind plastic sheeting. Although open observational methods have previously been used in field based assessments of fish behaviour (Mero 2009; Meekan et al. *in press*), this is a potential bias that is difficult to completely discount. Despite this, the results of this study highlight the potential for certain behavioural differences between field and aquarium experiments in this system, and care should be taken when attempting to extrapolate aquarium based findings into a natural context (e.g. Chapter 2).

The present study shows how behavioural characteristics within newly settled coral reef fishes can differ predictably between individuals within a group, potentially influencing which fish are most susceptible to specific types of predation. The strictness of the size-based dominance hierarchy in these and many other organisms (Fisher and Cockburn 2006; Gherardi 2006) means that dominance is closely related to relative body size within a group. Although we did not assess how these size based differences are directly linked to vulnerability, a number of recent studies of this species in the same system have shown that mortality is often strongly negatively size related (Holmes and McCormick 2006; McCormick and Meekan 2007; Chapter 2). This study suggests that this size selective loss may be largely due to the behavioural interaction between conspecific within a dominance hierarchy rather than the selective profiles of common predators.

Chapter 5: Smell, learn and live: the role of chemical alarm cues in predator learning during early life history in a marine fish

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5.1. Introduction

The ability of individuals to detect and respond to a perceived threat will have a large influence on who is lost from a population. This may be particularly important during encounters with potential predators, when the adoption of certain behaviours (e.g. decreased foraging, increase in shelter use) may significantly decrease vulnerability (Sih 1986; Spieler and Linsenmair 1999; Blumstein et al. 2002; Hunter et al. 2007). As such, the capacity to detect and react appropriately to a predation threat early in the predation process will presumably be advantageous to survival (Downes 2002). The use of anti-predator behaviour by prey individuals can however be energetically costly, with time spent avoiding predation reducing the amount of time spent on foraging and reproductive activities (Sih 1980; Lind and Cresswell 2005; Fievet et al. 2008). From a fitness perspective, it is therefore also advantageous to be able to distinguish a ‘possible’ threat from an ‘actual’ threat, so as to reduce the time spent reacting to false alarms.

One mechanism for early detection and recognition of a predation threat is through the use of alarm cues (Sih 1986; Martin and Lopez 2005; McCormick and Manassa 2008). In aquatic organisms, chemical cues produced by damaged individuals have been shown to provide an early warning to conspecifics of a predation threat (Chivers and Smith 1998; Gonzalo et al. 2007; Wisenden 2000; Mirza and Chivers 2002). By recognising such cues and adopting anti-predator behaviours, others in the general vicinity or within the group will greatly increase their chance of survival. One major limitation to this mechanism however, is that the production of the cue is secondary to an event having already occurred. If group sizes are small, this means that there is still a relatively high probability of being the target of the primary predation event. As a result, a more definitive survival advantage would be gained from the ability to detect and recognise a potential predator cue before the initial attack.

The use of alarm cues to facilitate learning of primary predator cues has been well documented in a range of different animal taxa (e.g. flatworms, Wisenden and Millard 2001; insects, Wisenden et al. 1997; amphibians, Woody and Mathis 1998; Gonzalo et al. 2007; mammals, Herzog and Hopf 1984; and fish, Chivers et al. 1995). This is particularly so in the case of fishes, with conspecific alarm cues and predator scents known to play a role in teaching naïve prey the identity of potential predators (Magurran 1989; Chivers and Smith 1994, 1995; Brown et al. 1997; Mirza and Chivers 2000; Smith et al. 2008). In making the direct connection between the predator cue and danger, prey are able to adopt anti-predator behaviours whenever the predator is within detectable distance, increasing an individuals probability of surviving an encounter. The importance of this learning process may be particularly heightened during periods of high mortality, when the speed with which the connection is made by prey may determine who survives (Spieler and Linsenmair 1999).

In comparison to freshwater systems, we currently know very little about the use of chemical alarm cues amongst marine fishes. What little published work has been done has focused entirely on adult life stages (Smith and Smith 1989; Smith 2000; Larson and McCormick 2005; McCormick and Larson 2007; McCormick and Larson 2008;

McCormick and Manassa 2008), with no attention being given to earlier or transitional life stages when predation pressures are significantly higher. Certainly, there is scope for the use of olfactory senses in predator avoidance during these early periods, given that olfaction has been shown to assist larval reef fish in homing in on appropriate reef habitat during the settlement process (Arvedlund et al. 1999; Lara 2008). The utility of alarm cues in predator avoidance and learning processes is particularly high during the period immediately following settlement to the reef environment in coral reef fishes. During this stage, 'reef naïve' individuals are subjected to extremely high levels of predation (Doherty et al. 2004; Almany and Webster 2006) and must rapidly learn to recognise and react appropriately to predation threats in a completely novel system. A recent study by McCormick and Holmes (2006) has shown that individuals are able to acquire knowledge from prior predation experiences during this period and translate this into a survival advantage during future predatory encounters. However, the mechanism underlying this acquired learning is currently not known. Although it is possible that the coupling of chemical alarm cues plays some role in this process due to the mechanism's prior documentation in later life stages, it is presently unknown at what stage in development the mechanism becomes functional.

This study investigated the use of chemical alarm cues in the anti-predator response of a common coral reef damselfish, *Pomacentrus amboinensis*, during the period immediately following settlement. Using aquarium-based observation trials, we examined whether chemical cues released by damaged conspecifics and heterospecifics elicited an anti-predator response amongst individuals, and if so, how the behavioural response was characterised. Additionally, we examined whether conspecific alarm cues were a mechanism by which fish could learn the scent of novel predators. Due to the extreme predation pressures placed on fish during this life history period, and the ecological importance of a rapid learning mechanism, individuals were given a single exposure to a paired alarm/predator scent cue, before being retested with the predator scent alone the following day.

5.2. Materials and Methods

Study site and species

This study was conducted at Lizard Island (14°40’S, 145°28’E), northern Great Barrier Reef (GBR), Australia during November and December of 2006 and 2007. The laboratories and flow through salt water aquarium system at Lizard Island Research Station was used to conduct all experiments, while fish were collected from surrounding waters.

The common damselfish *Pomacentrus amboinensis* was used as the prey species for all experimental trials. This species is common within coral reef fish communities within the Indo-Pacific, particularly in the central GBR. They settle to a wide variety of habitats on the northern GBR, but are found in highest densities associated with small reef patches at the base of shallow reefs. *P. amboinensis* has a pelagic larval phase of between 15 – 23 days and settles at 10.3 – 15.1 mm standard length (Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site attached, making it an ideal species for experimental manipulation. They recruit in substantial numbers at Lizard Island around the new moon during the austral months (October – January), and are easily collected at the time of settlement with light traps (Milicich and Doherty 1994).

Fish collection

Settlement stage *P. amboinensis* were collected using light traps (see Meekan et al. 2001 for design) moored overnight close to the reef crest, and transported back to the Lizard Island Research Station at dawn. Fish were collected using this method so as to keep them naïve to all reef-based processes and influences. All fish were maintained in 25L flow-through aquaria systems for ~24 hours, and fed newly hatched *Artemia* sp. twice per day ad libitum to allow for recovery from the stress of capture. Growth during this period was negligible.

Experimental aquaria

Experiments were conducted in transparent 12L (320 x 230 x 200 mm) aquaria with flow through sea water. Two artificial branching *Acropora* corals (moulded resin; item no. 21505; Wardleys/TFH, Sydney; dimensions: 14 x 11.5 x 5 cm) were placed against one end of the tank for shelter, and a single airstone was placed at the other end. A 1.5 metre length of plastic tubing was attached to the airline to allow for the injection of potential chemical cues into the aquaria without disturbance. The end of the tubing was attached just above the airstone, enabling rapid dispersal of the extracts throughout the aquaria. All aquaria were maintained under approximately 12L :12D photoperiod, with a constant flow of seawater until the commencement of trials. Observations were conducted from behind a black plastic barrier to avoid human disturbance.

Stimulus preparation

Potential conspecific chemical alarm cues were prepared using skin extracts taken from settlement stage *P. amboinensis* collected in light traps. Potential heterospecific alarm cues were prepared using skin extracts taken from adult *Apogon cyanosoma* (Apogonidae), collected using clove oil from surrounding reefs. All collected *A. cyanosoma* were maintained in aquaria for 24 hours prior to experiments to remove any residue clove oil. This extract was used to test behavioural changes that may result from exposure to an extract of any injured fish. *A. cyanosoma* live in similar reef habitat to *P. amboinensis*, but are ecologically and phylogenetically distinct. The donor fish were sacrificed by cold shock and placed into a clean petri dish. A fresh scalpel blade was used to make seven superficial vertical incisions along each flank. Incisions damaged the skin but caused minimal flesh damage. Extracts were standardized by body area, meaning that two sacrificed *P. amboinensis* were rinsed in 15 ml of seawater for each conspecific extract, while a single *A. cyanosoma* was rinsed in the same amount of seawater for each apogonid extract. Extracts were then filtered through filter paper (125mm Ø, qualitative 1) to remove any solid particles (e.g. scales), and drawn into a disposable 60 ml syringe for injection into the experimental aquaria. All extracts were used within 20 minutes of preparation, as they may loose potency if frozen or stored for long periods (Smith 1989).

Novel predator scents were prepared using adult dottybacks, *Pseudochromis fuscus*, collected from surrounding reefs. *P. fuscus* is a known predator of *P. amboinensis* once settled to the reef habitat. However, at the point of capture in this study, *P. amboinensis* are yet to settle, and hence are assumed to be completely naïve to any cues or potential stimuli produced by *P. fuscus*. Immediately following collection, *P. fuscus* were placed into acclimation aquaria for 36 hours so that any fecal matter could pass through the system and prevent contamination of the final stimulus. Fish were subsequently placed into individual 6L aquaria with fresh seawater for four days. The seawater was not changed during this period, but aquaria were provided with constant aeration. Fish were not fed throughout the entire process, again to prevent fecal matter from contaminating the final stimulus. At the end of this period, the water was drawn from the aquaria and frozen in 60 ml portions until used in experiments. At the end of the experimental period *P. fuscus* were fed ad libitum a commercial fish diet and released at the point of capture two days later.

Experiment 1 – Presence of conspecific alarm cue

The behavioural response of fish to three different stimulus treatments was tested: a conspecific skin extract, a heterospecific skin extract, and a salt water control. The heterospecific extract was used to determine whether there were any generalized behavioural responses to any injured fish, while the salt water stimulus allowed us to control for any behavioural changes resulting from the injection of any stimulus into the experimental aquaria. Trials consisted of a 10 minute pre-stimulus and a 10 minute post-stimulus observational period.

Individual *P. amboinensis* were placed into experimental aquaria and left to acclimate for 24 hours. Flowing seawater and constant aeration were provided during this period, and fish were fed twice daily with newly hatched *Artemia* sp. Immediately prior to the trial period, the water flow was turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding. 60 ml of salt water was slowly drawn out of the stimuli injection tubing using a disposable syringe and discarded to remove any stagnant water from within the line. An additional 60 ml of salt water was drawn out and kept within the disposable syringe.

The behaviour of the single fish within each aquaria was first recorded for a 10 minute pre-stimulus period. At completion, 15 ml of the experimental stimulus (conspecific skin extract, heterospecific skin extract or saltwater control) was injected into the tank via the injection tubing, immediately followed by the 60 ml of saltwater previously removed from the aquaria to flush the stimulus into the aquaria. The behaviour of the fish was then recorded for a further 10 minute period. Dye trails (using food colouring) showed that it took a mean of 58 seconds (± 4.2 seconds) for the stimulus to disperse evenly throughout the aquaria. Behavioural observations were carried out by two observers sitting behind a black plastic barrier, so as to avoid disturbing fish during the trials.

A total of seven different behavioural traits were recorded for each fish during each observational period: (1) number of feeding strikes; (2) number of ‘bobs’; (3) time spent in shelter; (4) number of movements into/out of shelter; (5) amount of horizontal movement outside of shelter; (6) distance from shelter; and (7) vertical position in water column. The number of feeding strikes was recorded as the total number of strikes (successful or unsuccessful) made by an individual during each 10 minute observation period. The number of ‘bobs’ was recorded as the total number of defined upward or downward bobbing movements by an individual within the period. Although its function is unknown, juvenile *P. amboinensis* undertake a bobbing behaviour, consisting of short, sharp vertical movements in the water column. It is possible the behaviour serves a purpose in either predator detection or avoidance and as such, it was recorded in this study.

Every 15 seconds the fish's horizontal distance from shelter and vertical position in the water column was recorded. If the fish was within the confines of the branched shelter, its horizontal position was recorded as 0. From this data, measures of time spent within shelter, average distance from shelter and average vertical position within the water column were obtained. To obtain an estimate of space use, in the form of movement in and out of shelter and horizontal movement outside of shelter, the experimental aquaria was divided up into a series of horizontal zones. The first zone was set at the outside edge of the branching coral shelter, and they continued at 50 mm intervals along the horizontal axis, away from the shelter. The number of times a test fish moved both in and out of shelter and between horizontal zones outside of the shelter was recorded during each observational period.

A new fish was used for each separate observation trial and water was changed in each aquaria after the completion of each trial. A total of 45 observation trials were conducted over the duration of the experiment, 15 from each of the three stimulus treatments.

Experiment 2 – Learning of a novel predator scent

The second experiment aimed to identify whether naïve fish were able to learn to associate a novel scent with danger, after previously being exposed to a potential alarm cue paired with the novel scent. This experiment used new fish collected in light traps and consisted of three separate observational periods conducted over three days. On the first day, fish were exposed to a single paired presentation of both a skin extract (fish were conditioned with either a conspecific or heterospecific stimulus treatment) and a novel predator scent (i.e. the *P. fuscus* tank water, not previously associated with danger). On the second day, fish from both condition treatments were exposed to the novel predator scent only, to determine whether they had learned to associate the cue with potential danger after a single exposure on the previous day. On the third day, fish were exposed to a salt water control, to determine whether any behavioural changes were a result of a learned response to the injection process (Mathis and Smith 1993).

As in experiment 1, individual *P. amboinensis* were placed into experimental aquaria and left to acclimate for 24 hours prior to the first trial day. The maintenance, feeding and experimental regime followed that of experiment 1, with the same 10 minute pre-stimulus and 10 minute post-stimulus observation protocol used on each day. Seven behavioural traits (number of feeding strikes, number of 'bobs,' time spent in shelter, number of movements into/out of shelter, amount of horizontal movement outside of shelter, distance from shelter, and vertical position in water column) were recorded during these observation periods, as in the previous experiment.

Immediately prior to the observation period on each day, 60 ml of salt water was drawn out of the stimuli injection tubing using a disposable syringe, and discarded to remove any stagnant water from within the line. An additional 60 ml of salt water was drawn out and kept within the disposable syringe. The water flow was again turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding.

The chemical stimulus injected into the aquaria between the pre and post-stimulus observation periods, differed on each of the three observation days. During trials conducted on the first day, fish were exposed to one of the following pairs of stimuli: 15 ml conspecific skin extract (potential alarm cue) and 15 ml *P. fuscus* scent (novel cue) – “Conspecific Paired Cue”; or 15 ml heterospecific skin extract (control for alarm cue) and 15 ml *P. fuscus* scent – “Heterospecific Paired Cue.” On the second day of trials, fish from both the “Conspecific-” and “Heterospecific Paired Cue” treatments were exposed to 15 ml of the predator scent only – “Conspecific + Predator Cue” and “Heterospecific + Predator Cue.” During trials conducted on the third day, all fish were exposed to 15 ml of salt water control only – “Conspecific Control Cue” and “Heterospecific Control Cue.” On each occasion, the injection of the stimulus was immediately followed by the injection of the 60 ml of salt water previously retained, in order to flush the stimulus through the injection line. Between trial days all fish were fed twice daily on *Artemia* sp., and water flow was returned to all aquaria to ensure that all residue stimulus was flushed from the system.

The three day trial process was conducted on a total of 30 separate fish over the duration of the experiment, 15 from each of the two original conditioning treatments (i.e. conspecific and heterospecific).

Analyses

Changes in the seven prey behaviours were calculated between pre and post-stimulus observation periods. These values were compared between treatments within each experiment using a multivariate analysis of variance (MANOVA). Canonical discriminant analyses (CDA’s) were performed for each experiment to determine how fishes differed between treatments with respect to changes in the seven behavioural traits. Trends in the behavioural variables are represented as vectors which are plotted on the first two canonical axes, together with treatment centroids and their 95% confidence clouds (Seber 1984). The assumption of multivariate normality was examined before the analysis. To further explore the differences between treatments one-way ANOVA's (Experiment 1) and repeated measures ANOVA's (Experiment 2) were used to identify significant differences within individual behaviours of interest. Any differences were further examined using Tukey's HSD means comparison tests. Residual analysis was used to examine the assumptions of normality and heterogeneity of variances.

5.3. Results

Experiment 1– Presence of conspecific alarm cue

There was a significant difference in the overall change in behaviour in response to the chemical stimulus among the three alarm cue treatments (conspecific, heterospecific and saltwater control; Pillai's $\text{Trace}_{14,\ 70} = 0.6531$, $p = 0.0078$). The CDA visually shows a clear separation of the three treatments into two distinct groups with respect to the seven behavioural measurements, with the conspecific treatment being separate from the heterospecific and saltwater control treatments (Fig. 5.1). This differentiation is largely due to differences in the number of feeding strikes, with a suggestion that the number of movements in/out of shelter may also play a minor role. These two behaviours were analysed individually using ANOVA's, and the sugestion was statistically confirmed, with exposure to the conspecific alarm cue causing a significantly greater change in the number of feeding strikes than the heterospecific and saltwater controls ($F_{2,\ 40} = 10.839$, $p = 0.0002$; Table 5.1). The introduction of the conspecific skin extract caused a large reduction in the number of feeding strikes (mean 27.21 strikes 10 min^{-1} less), whilst both the heterospecific and saltwater control cues caused only minor reductions (mean 4.27 and 6.5 strikes 10 min^{-1} less, respectively; Fig. 5.2). There was however no significant difference in the change in number of movements in/out of shelter between treatments ($F_{2, 42} = 1.983$, $p = 0.1504$).

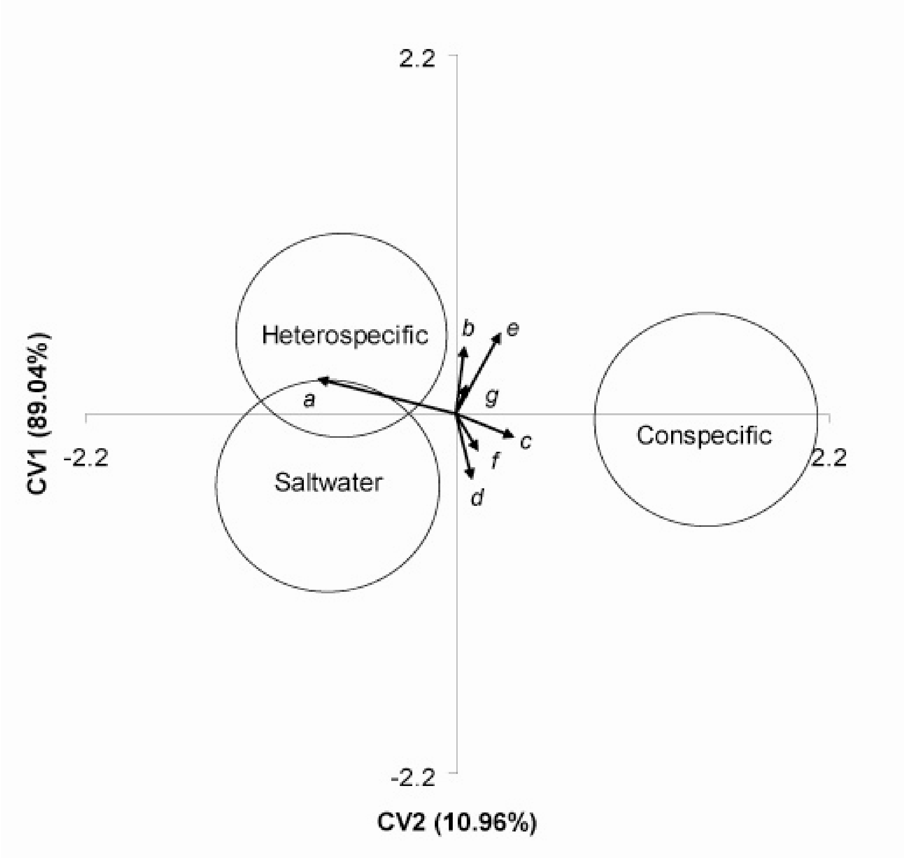

Figure 5.1: The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to conspecific, heterospecific and control alarm cues. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the three chemical cue treatments tested in Experiment 1: Conspecific skin extracts, Heterospecific skin extracts and Saltwater Control. Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (*a*), bobs (*b*), movement in/out shelter (*c*), movement between zones (*d*), time in shelter (*e*), distance from shelter (*f*), position in water column (*g*). The circles represent 95% confidence intervals.

Table 5.1: Summary of one-way ANOVA's comparing changes in behaviour for each of the three chemical cue treatments (conspecific, heterospecific and saltwater control) during Experiment 1. * denotes significance at p = 0.025 (incorporating Bonferroni correction).

Behaviour	Source	DF	MS	F	p
Feeding	Treatment	2	2275.954	10.839	0.0002*
	Error	40	209.97		
Movement (in/out shelter)	Treatment	2	115.467	1.983	0.1504
	Error	42	58.235		

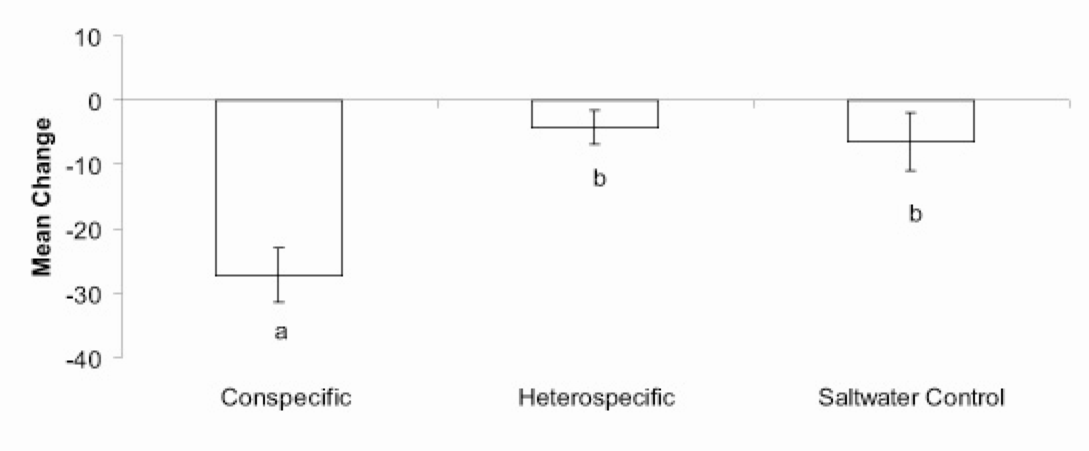

Figure 5.2: Comparison of the mean change (± SE) in the number of feeding strikes between pre and post-stimulus periods made by *Pomacentrus amboinensis* tested in each of the three chemical cue treatments (Conspecific, Heterospecific and Saltwater Control) in Experiment 1. Alphabetic notations (a, b) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

Experiment 2– Learning of a novel predator scent

We found a significant difference between the overall pre and post-stimulus behaviour between the six chemical cue treatments (Pillai's Trace$_{35,\ 380}$ = 0.6175, p = 0.0307). The CDA shows the chemical cue treatments to be split into two groups with respect to the seven behavioural measurements, with the "Conspecific Paired Cue," and the "Conspecific + Predator Cue" from the conspecific treatment separated from the other four (Fig. 5.3). This treatment separation indicates three important results. Firstly, the introduction of conspecific skin extracts had changed the fish's behaviour, with a reduction in activity. Secondly, that once fish had been exposed to the novel predator scent and a conspecific skin extract together, they reacted to the predator scent on its own with a similar reduction in activity. Thirdly, that fish exposed to the novel predator scent without being conditioned with the conspecific skin extract did not change their behaviour. The significant differentiation between groups again appears to be largely due to differences in the number of feeding strikes between treatments. Differentiation between treatments for this behaviour was further examined using a repeated measures ANOVA. This confirmed the suggestions obtained from the CDA, with a significant difference being shown in the change in number of feeding strikes between treatments ($F_{2,\ 52} = 3.81049$, $p < 0.0289$). In this case, individuals used in both the 'conspecific-paired cue' and the 'conspecific-predator cue' trials from the conspecific treatment had significantly fewer feeding strikes after being exposed to the stimulus (mean 46.53 and 47.8 strikes 10 mins^{-1} less, respectively), than those used in the other four treatments (Fig. 5.4). Changes in the number of feeding strikes in response to the 'saltwater cue' from the conspecific treatment, and the heterospecific 'paired cue,' 'predator scent only cue' and 'saltwater cue' treatments were negligible.

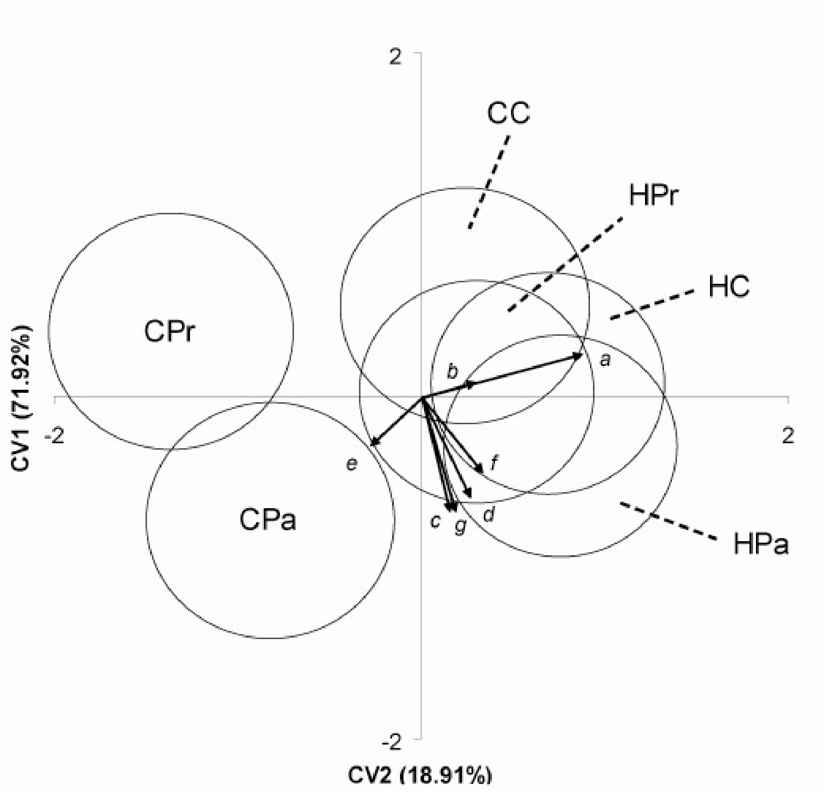

Figure 5.3: Importance of chemical alarm cues to learning predator scents. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the six chemical cue treatments tested in Experiment 2: Conspecific-Paired (CPa: day 1 conspecific cue + predator scent), Conspecific-Predator (CPr: day 1 conspecific cue + predator scent, day 2 predator cue), Conspecific-Control (CC: day 1 conspecific cue + predator scent, day 2 predator cue, day 3 saltwater control), Heterospecific-Paired (HP: day 1 heterospecific cue + predator scent), Heterospecific-Predator (HPr: day 1 heterospecific cue + predator scent, day 2 predator cue) and Heterospecific-Control (HC: day 1 heterospecific cue + predator scent, day 2 predator cue, day 3 saltwater control) (see methods for detailed description). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (*a*), bobs (*b*), movement in/out shelter (*c*), movement between zones (*d*), time in shelter (*e*), distance from shelter (*f*), position in water column (*g*). The circles represent 95% confidence intervals.

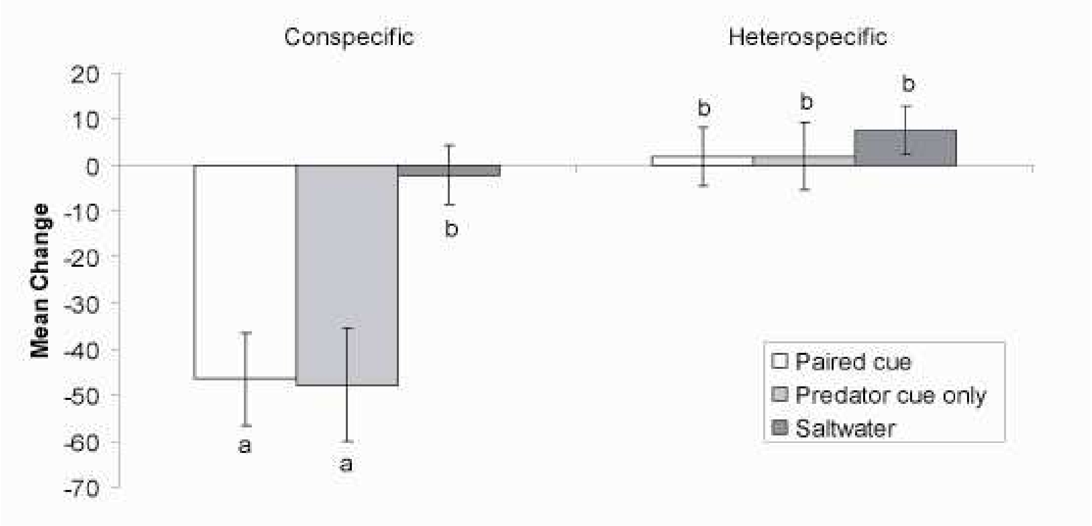

Figure 5.4: Mean change (± SE) in the number of feeding strikes between pre and post-stimulus periods made by *Pomacentrus amboinensis* tested in each of the six chemical cue treatments (Conspecific Paired, Conspecific Predator, Conspecific Control, Heterospecific Paired, Heterospecific Predator and Heterospecific Control) in Experiment 2. Alphabetic notations (a, b) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

5.4. Discussion

The ability to recognise and alter behaviour in response to a predation threat will greatly increase an individual's probability of survival (Sih 1986; Brown and Laland 2003). Although anti-predator responses can vary between groups, species and ecosystems, they typically involve decreases in foraging rate and space use, grouping/schooling behaviour, and/or an increase in shelter usage (Stauffer and Semlitsch 1993; Chivers and Smith 1998; Griffiths et al. 1998). The results of this study show that the newly settled reef fish *Pomacentrus amboinensis,* significantly alters its behaviour in response to a conspecific chemical alarm cue, by decreasing its feeding rate. In addition, individuals were able to learn and respond to a previously novel predator cue by pairing it with the conspecific alarm cue. After only a single exposure to the novel predator cue pared with a conspecific cue, fish showed a behavioural change consistent with the first experiment (reduction in feeding rate) when retested with the predator cue only. The lack of behavioural change in response to the novel predator scent during the heterospecific treatments indicate that fish had no innate response to the novel scent on its own. Our findings show that alarm cues may play a key role in the learning process of naïve recruits during the hazardous early post-settlement period in coral reef fishes.

A recent study found that experience acquired through exposure to predation during the early post-settlement period significantly increased an individual's probability of survival in the field (McCormick and Holmes 2006). The results of the current study suggest that this process of acquired learning may be largely due to the pairing of conspecific alarm cues with a predator scent. The pairing of this cue potentially allows prey to identify when a predator is in the general vicinity, through olfactory reception, and initiate anti-predator behaviours at an earlier stage in the predation process. Although this relationship has been identified during the larval stage in amphibians (Kiesecker et al. 1996; Gonzalo et al. 2007), as well as in later life stages in a number of freshwater (e.g. Brown et al. 1997; Mirza and Chivers 2000; Smith et al. 2008) and one marine fish (Larson and McCormick 2005), this is the first time that it has been identified at such an early and critical life stage in the marine environment.

The presence of this learning mechanism during this early juvenile stage is of particular significance for a number of reasons. The period immediately after settlement from the larval life stage is characterised by extremely high levels of mortality for organisms with complex life histories, with estimates of mortality of over 56% within the first 2 days (Gosselin and Qian 1997; Almany and Webster 2006). For coral reef fishes, much of this mortality is caused by small site attached fish predators (Carr and Hixon 1995; Holbrook and Schmitt 2002; McCormick and Meekan 2007). The speed with which individuals are able to acquire knowledge of these predators, and react accordingly, will have a large influence on who is lost and who persists within a population (Wisenden et al. 1997; Brown and Chivers 2006). Our study indicates that newly settled fish are able to make this link after a single exposure to the paired chemical cue, suggesting that this mechanism provides an extremely effective method of knowledge acquisition during this early period. The fact that this knowledge is acquired, rather than hereditary, is also advantageous, as it allows individuals to mould their anti-predator responses to the particular suite of predators around them. Given that many coral reef fish possess a highly dispersive pelagic larval stage, and that the composition of predatory communities can differ greatly over both local and regional scales (Holmes and McCormick 2006), the predator community into which individuals of the same species settle has the potential to differ considerably. The ability to adapt to variation in predation threat through a single exposure to the predator cue, rather than be constrained by genetically inherited anti-predator responses, would be advantageous to both fitness and survival (Brown and Chivers 2006).

The response of newly settled *P. amboinensis* to conspecific skin extracts involved a substantial and significant reduction in feeding rate. However, it did not affect shelter use or movement patterns, two other behavioural changes commonly linked to anti-predator responses. For example, in a study of the behaviour of western toad (*Bufo boreas*) tadpoles, Kiesecker et al. (1996) found a reduction in activity and an increase in shelter use in response to olfactory predation cues. Similarly, Hirvonen et al. (2007) noted a decrease in activity and increase in shelter use by the noble crayfish (*Astacus astacus*) when in the presence of the odour from the predatory European eel (*Anguilla anguilla*). This lack of change with respect to these other behaviours in our study may be related to the newly metamorphosed state of the fish that are not only naïve to reef-based predators, but also to their benthic environment. It is perhaps not surprising therefore that newly metamorphosed *P. amboinensis* do not show significant retreat to shelter within a foreign and novel 3-dimensional environment.

There will be strong selective pressure on settling larvae of all organisms to learn to identify and avoid the predators in their new environment; individuals who survive initial, unsuccessful predation attempts and learn from this experience will have a survival advantage (Kristensen and Closs 2004; McCormick and Holmes 2006). Detailed field observations on the ontogenetic behavioural shifts that occur immediately around settlement have shown that many species go through a transition period of hours to weeks while they adopt behaviours displayed by juveniles (McCormick and Makey 1997). More extensive antipredator responses are predicted to develop with greater experience with the benthic habitat and community. In an investigation of variation in the response of fathead minnow (*Pimephales promelas*) to alarm cues, Pollock et al. (2006) found behavioural reactions to differ with ontogeny. When exposed to the alarm cue, larger/older individuals exhibited significantly increased anti-predator responses in comparison to smaller/younger minnows during the non-breeding season. This same relationship may be present in the marine system, with younger newly settled individuals displaying a weaker anti-predator response in comparison to older individuals of the same species. As experience of predatory events increases with age, so to will the knowledge of appropriate anti-predator behaviours.

Within aquatic systems, chemical cues have been shown to play a large role in both knowledge acquisition and knowledge transfer amongst individuals within close social groups (e.g. social learning; Brown and Laland 2003; Ferrari et al. 2007). Given the rapidity and efficiency with which knowledge of a predation threat was acquired by individuals through chemical means in this study, it is reasonable to suggest that chemical signals may play a wider role within the community ecology of newly settled fish outside of this function (e.g. social learning, habitat selection, foraging). However, to date this remains largely unstudied in marine systems. Our study adds significantly to our current understanding and provides an insight into the mechanisms of knowledge acquisition and predator recognition during a high mortality period in marine fishes.

Chapter 6: Response across a gradient: behavioural reactions of newly settled fish to predation cues

Accepted at *Animal Behaviour*

6.1. Introduction

The manner and intensity with which an individual responds to a perceived predation threat can greatly influence the probability of survival (Rhoades and Blumstein 2007). The effects on survival can be both direct, through behaviourally induced changes in vulnerability to the threat (Sih 1986; Spieler and Linsenmair 1999; Blumstein et al. 2002; Hunter et al. 2007), and indirect, through changes in body condition arising from associated behavioural trade-offs (e.g. decrease in foraging time; Amo et al. 2007; Sunardi et al. 2007; Cresswell 2008; Heithaus et al. 2008). If individuals react too strongly to a weak or irrelevant threat, the cost may be an unnecessary loss of overall fitness. On the other hand, if individuals react too weakly, or not at all, to a strong threat, the cost may well be death. It is therefore advantageous to detect the relevance and strength of a predation threat before choosing an appropriate response (Helfman 1989; Godin 1997).

There are a number of methods that can be used in the detection and recognition of predation threats. These include visual (Amo et al. 2006; Collier et al. 2008; Lohrey et al. 2009; McPhee et al. 2009), acoustic (Durant 2000; Blumstein et al. 2008), olfactory (Amo et al. 2006; Gonzalo et al. 2008; Roth et al. 2008) and seismic means (Warkentin et al. 2007; Lohrey et al. 2009). In aquatic organisms, olfactory, or chemical, cues are a key source of threat information (Sih 1986; Mathis and Vincent 2000; Chivers et al. 2001;

McCormick and Manassa 2008; Kim et al. 2009). Olfactory cues have the advantage over other threat cues because they are available early in the predator-prey interaction sequence, often well before an initial encounter with a predator has occurred. However, their utility later in the predation process (i.e. once prey have been visually engaged by the predator) is arguable, due largely to the relatively slow rate at which these cues travel in an aqueous environment. Once the predator has started the capture sequence, visual cues may play a larger role in the threat detection process, particularly during interactions with non-cryptic predators (Brown and Magnavacca 2003).

With respect to olfactory and visual cues, the level of threat posed will be a function of whether the cue is a recognised predation threat, and its relative strength (assessed as cue concentration or source distance, respectively). Although the identification of relevant cues is often innate (Semlitsch and Gavasso 1992; Kiesecker and Blaustein 1997; Hawkins et al. 2007), or can be acquired through experience (Chivers et al. 1995; Woody and Mathis 1998; Larson and McCormick 2005), how individuals respond to different levels of these may vary. In some circumstances, the intensity of the response may be proportionate to the level of the threat detected ('threat-sensitive' response; Mathis and Vincent 2000; Chivers et al. 2001; Mathot et al. 2009). Alternately, individuals may choose to respond to a threat only when it is above a certain threshold level ('threshold' response; Mirza and Chivers 2003; Roh et al. 2004; Brown et al. 2006). In this case, the lower levels are not deemed threatening enough to warrant any investment in a response (Brown et al. 2004). However, although some focus has been given to the response of prey to differing predation threat levels in freshwater systems, to date this has received little attention in the marine environment (see Helfman 1989 for exception).

In coral reef fishes, the period immediately following settlement to the reef environment is characterised by extremely high levels of mortality (Doherty et al. 2004; Almany and Webster 2006). The intense predation pressures present during this stage mean that individuals must quickly learn to respond appropriately to authentic predation threats or face death. We already know that the innate recognition of olfactory alarm cues released by damaged conspecifics plays at least some role in this process (Chapter 5). However, the function of visual cues in threat recognition during this early period is largely unknown. Given the generally high clarity of water on coral reefs, and the degree of development of visual acuity in settlement stage fish (at least over short distances; Losey et al. 2003; Siebeck et al. 2008), it is reasonable to suggest that it may play a role at some point in the predation process. It is only through understanding how individuals identify and respond to potential predation threats that we can fully comprehend the mechanisms of survival during this potentially critical life stage.

This study examines the behavioural response of the newly settled coral reef damselfish, *Pomacentrus amboinensis*, to varying levels of olfactory and visual predation threat cues. Previous work indicates that this species is able to recognise conspecific chemical alarm cues during this early life stage, and responds accordingly by decreasing its feeding rate (Chapter 5). However, it is unknown how fish respond to potential visual alarm cues (i.e. presence of a potential predator within the prey's visual field) or whether they possess an innate recognition of predator identity in this unfamiliar reef system. We used controlled aquarium experiments to manipulate the exposure of reef-naïve individuals to both a range of concentrations of conspecific chemical alarm cue, and potential visual predation cues over a range of distances. Specifically, we aimed to: 1) identify the behavioural response of a naïve newly settled individual to a potential visual predation cue, and whether this differs in response to predatory and non-predatory individuals; and 2) identify how behavioural responses to both visual cues and conspecific chemical alarm cues change across a concentration/distance gradient.

6.2. Materials and Methods

Study site and species

This study was conducted at Lizard Island (14°40’S, 145°28’E), northern Great Barrier Reef (GBR), Australia during November and December of 2006 and 2007. The laboratories and flow through salt water aquarium system at Lizard Island Research Station was used to conduct all experiments, while fish were collected from surrounding waters.

The common damselfish *Pomacentrus amboinensis* was used as the prey species for all experimental trials. This species is common within coral reef fish communities within the Indo-Pacific, particularly in the central GBR. They settle to a wide variety of habitats on the northern GBR, but are found in highest densities associated with small reef patches at the base of shallow reefs. *P. amboinensis* has a pelagic larval phase of between 15 – 23 days and settles at 10.3 – 15.1 mm standard length (Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site attached, making it an ideal species for experimental manipulation. They recruit in substantial numbers at Lizard Island around the new moon during the austral months (October – January), and are easily collected at the time of settlement with light traps (Milicich and Doherty 1994).

During the visual stimulus trials, the brown dottyback (*Pseudochromis fuscus*) was used as the predator species, whilst the blue green damselfish (*Chromis viridis*) was used as the control 'non-predator' species. *P. fuscus* is a small (max size 72.4 mm SL) cryptic solitary pursuit predator commonly found on small coral bommies or along reef edges. It is common on shallow reefs throughout the West Pacific and Indian Oceans and is known to feed heavily on newly settled reef fishes in these habitats (Martin 1994; personal observations). *C. viridis* is a small reef associated planktivore commonly found in large aggregations around live coral heads in the West Pacific and Indian Oceans. Although frequently found in similar habitat and in close proximity to many newly settled fish species they are not known to prey upon them.

Fish collection

Settlement stage *P. amboinensis* were collected using light traps moored overnight close to the reef crest, and transported back to the Lizard Island Research Station at dawn. All fish were maintained in 25L flow-through aquaria systems for ~24 hours, and fed newly hatched *Artemia* sp. twice per day ad libitum to allow for recovery from the stress of capture. Growth during this period was minimal.

Experimental aquaria

Visual stimulus experiments (Experiments 1 and 2) were conducted in 24 L (280 x 255 x 350mm) glass aquaria, with flow-through sea water ('prey aquaria'). Two of the aquarium sides were covered in black plastic to reduce external stimulus. A third side was placed against a similar sized second glass aquaria ('predator aquaria') maintained on a separate flow-through seawater line. Glass sides of both aquaria were transparent, allowing vision between the two tanks. However a removable opaque partition could be placed between the two tanks to prevent vision. The fourth side of the prey aquarium was left open to allow for behavioural observations. Two artificial branching coral were placed against one end of the tank for shelter. The predator aquarium was divided into three chambers (of approximately the same size), such that chambers were a minimum of 0, 100 and 200mm away from the edge of the prey tank, respectively. Given that the artificial branching coral was positioned against the opposite side of the prey aquaria and that it was approximately 55mm in width, this meant that chambers were 200 (minimum), 300 (medium) and 400mm (maximum) from the edge of the prey shelter. Chambers were separated by clear perspex, allowing vision between the 'prey aquaria' and all predator chambers.

Chemical stimulus experiments (Experiment 3) were conducted in transparent 12L (320 x 230 x 200 mm) aquaria with flow-through sea water. Three sides were covered in black plastic to reduce external stimulus, with the fourth being left open to allow behavioural observations. Two artificial branching coral were placed against one end of the tank for shelter, and a single airstone was placed at the other end. A 1.5 metre length of plastic tubing was attached to the airline to allow for the injection of extracts into the aquaria without disturbance. The end of the tubing was attached just above the airstone, enabling rapid dispersal of the extracts throughout the aquaria.

All aquaria were maintained under approximately 12L :12D photoperiod, with a constant flow of seawater until the commencement of trials. Observations were conducted from behind a black plastic barrier to avoid human disturbance

Behavioural Observations

Ten minute observation periods were conducted both before and after exposure to the test stimulus. During each period, 7 behaviours were quantified: number of feeding strikes, number of 'bobs,' time spent in shelter, number of movements into/out of shelter, amount of horizontal movement outside of shelter, distance from shelter, and vertical position in water column. The number of feeding strikes was recorded as the total number of strikes (successful or unsuccessful) made by an individual during each 10 minute observation period. Similarly, the number of 'bobs' was recorded as the total number of defined upward or downward bobbing movements by an individual within the period. Although its function is unknown in this species, juvenile *P. amboinensis* possess a distinctive bobbing behaviour, consisting of short, sharp vertical movements in the water column. It is possible the behaviour serves a purpose in either predator vigilance or avoidance.

Every 15 seconds, a recording of the fish's horizontal distance from shelter and vertical position in the water column was taken. If the fish was within the confines of the branched shelter, its horizontal position was recorded as 0. From these data, approximate measures of time spent within shelter, average distance from shelter and average vertical position within the water column were obtained. To obtain rough estimates of space use, in the form of movement in and out of shelter and horizontal movement outside of shelter, the experimental aquaria was divided up into a series of horizontal zones. The first zone was set at the outside edge of the branching coral shelter, and they continued at 50 mm intervals along the horizontal axis, away from the shelter. The number of times a test fish moved both in and out of shelter and between horizontal zones outside of the shelter was recorded during each observational period.

Experiment 1 - Response to visual stimulus

Before fish were added to the experimental system, the removable partition was placed between the predator and prey aquaria. Individual *P. amboinensis* were then placed into the prey aquaria, while either a single adult *P. fuscus* (known predator) or adult *C. viridis* (non-predator) were placed into the closest chamber (0 mm distance from prey aquaria) of the predator aquaria. Fish were left to acclimate for 24 hours before the commencement of trials. Flowing seawater and constant aeration were provided during this period, and fish were fed twice daily with newly hatched *Artemia* sp. Immediately prior to the trial period, the water flow was turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding.

The behaviour of the single *P. amboinensis* was first recorded for the 10 minute pre-stimulus period. At the completion of this time, the removable partition between the predator and prey aquaria was removed and the prey was exposed to the visual cue of either the single *P. fuscus, C. viridis* or an empty chamber control. The behaviour of the prey was then recorded for the 10 minute post-stimulus period.

A total of 15 prey individuals were run for each of the three predator visual treatments.

Experiment 2 – Response to changing visual stimulus distance

As in Experiment 1, the removable partition was placed between the predator and prey aquaria before any fish were added to the experimental system. A single *P. amboinensis* was once again placed into the prey aquaria, while a single adult *P. fuscus* was placed into one of the three predator aquaria chambers. Fish were left to acclimate for 24 hours before the commencement of trials. The experimental protocol followed that of Experiment 1, except that prey fish were exposed to the visual *P. fuscus* cue at distances of either 200, 300 or 400mm from the prey shelter. An empty chamber control was also included in the analysis.

A total of 15 prey individuals were run for each of the four visual stimulus distance treatments.

Experiment 3 – Response to changing chemical alarm concentration

Skin extracts were prepared using settlement stage *P. amboinensis*, collected in light traps. The donor fish were sacrificed by cold shock and placed into a clean Petri dish. A clean scalpel blade was used to make seven superficial vertical incisions along each flank. Incisions damaged the skin but caused minimal flesh damage. Sacrificed fish were rinsed in 15ml of seawater, filtered through filter paper (125mm Ø, qualitative 1) to remove any solid particles, and drawn into a disposable 60 ml syringe for injection into the experimental aquaria. Extracts were prepared in 3 different concentrations, according to the number of sacrificed fish rinsed in the 15ml of seawater: low (1 fish), medium (2 fish) and high concentration (4 fish). All extracts were used within 20 minutes of preparation, as they have been show to lose potency if frozen or stored for long periods (Smith 1989).

Individual *P. amboinensis* were placed into experimental aquaria and left to acclimate for 24 hours. Flowing seawater and constant aeration were provided during this period, and fish were fed twice daily with newly hatched *Artemia* sp. Immediately prior to the trial period, the water flow was turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding. 60 ml of salt water was drawn out of the stimuli injection tubing using a disposable syringe, and discarded to remove any stagnant water from within the line. An additional 60 ml of salt water was drawn out and kept within the disposable syringe.

The behaviour of the single fish within each aquaria was first recorded for the 10 minute pre-stimulus period. At the completion of this time, 15 ml of the experimental stimulus (low concentration, medium concentration, high concentration or saltwater control) was injected into the tank via the injection tubing, immediately followed by the 60 ml of saltwater previously removed from the aquaria. The behaviour of the fish was then recorded for the 10 minute post-stimulus period. Food colour trials showed that it took approximately 58 seconds (± 4.2 seconds) for the stimulus to disperse evenly throughout the aquaria.

A total of 15 individuals were tested in each of the four chemical stimulus treatments.

Analysis

Changes in the seven prey behaviours were calculated between pre and post-stimulus observation periods. These values were compared between treatments within each experiment using a multivariate analysis of variance (MANOVA). Canonical discriminant analyses (CDA's) were performed for each experiment to determine how fishes differed between treatments with respect to changes in the seven behavioural traits. Trends in the behavioural variables are represented as vectors which are plotted on the first two canonical axes, together with treatment centroids and their 95% confidence clouds (Seber 1984). The assumption of multivariate normality was examined before the analysis. To further explore the differences between treatments, one-way ANOVA's were used to identify significant differences within individual behaviours of interest. Bonferroni corrections were used to correct for multiple ANOVA's on potentially interrelated variables within a single dataset. Any differences were further examined using Tukey's HSD means comparison tests. Residual analysis was to examine the assumptions of normality and heterogeneity of variances.

6.3. Results

Experiment 1 - Response to visual stimulus

There was a significant difference in the overall change in behaviour between the three visual cue treatments (Pillai's Trace$_{14,\,74}$ = 0.7197, p = 0.0012). The CDA shows this separation between treatments clearly, with a majority of the variation seemingly due to differences in a combination of the number of bobs, the mean distance from shelter, mean height in the water column, the number of horizontal movements between zones, and the number of feeding strikes (Fig. 6.1). These five behavioural characters were further examined using ANOVA's, resulting in a revised alpha level of 0.01. Individuals displayed a significantly greater increase in the number of bobs in response to the predator stimulus than to the non-predator and control stimuli ($F_{2,\ 42}$ = 6.4561, p = 0.00036; Fig. 6.2), while fish exposed to both the predator and non-predator cues decreased their mean distance from shelter significantly more than those responding to the control stimulus ($F_{2,\ 42}$ = 5.9532, p = 0.0053). Although there was no significant difference between treatments with regards to the other three behaviours, individuals exposed to the predator and non-predator stimuli generally had a greater decline in mean height in the water column, horizontal movements between zones, and number of feeding strikes than those exposed to the control stimulus (Fig. 6.2).

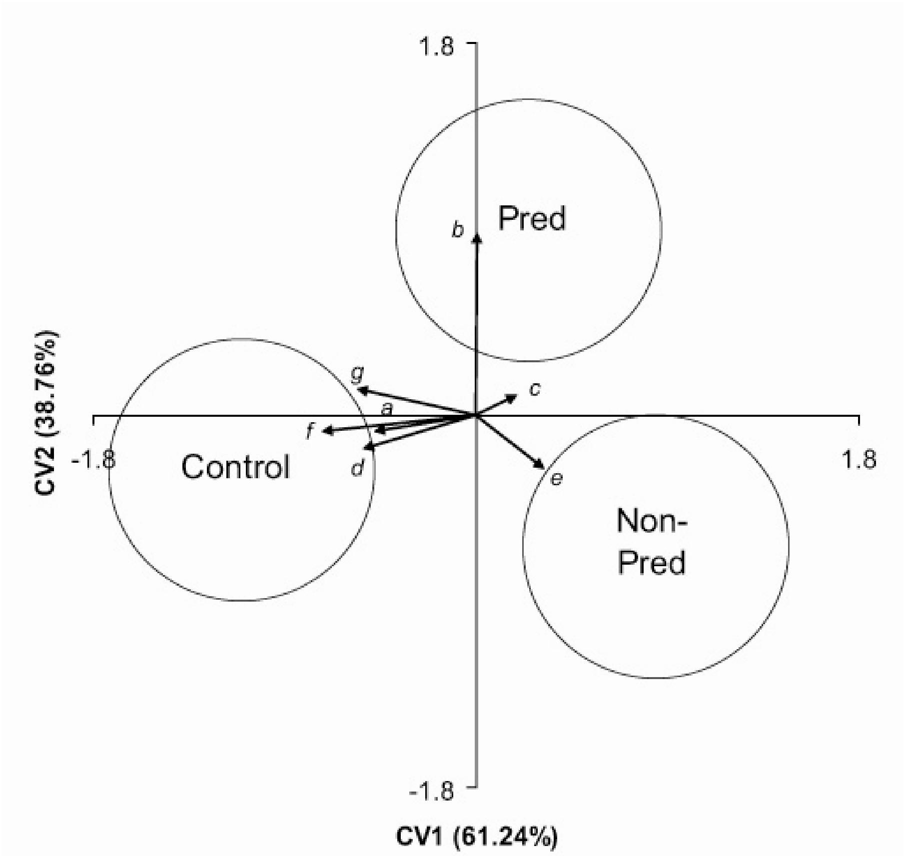

Figure 6.1: The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to visual predation cues. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the three visual cue treatments tested in Experiment 1: Predator Stimulus (Pred) , Non-Predator Stimulus (Non-Pred) and Empty Tank Control Stimulus (Control). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (*a*), bobs (*b*), movement in/out shelter (*c*), horizontal movement between zones (*d*), time in shelter (*e*), distance from shelter (*f*), height in the water column (*g*). The circles represent 95% confidence intervals.

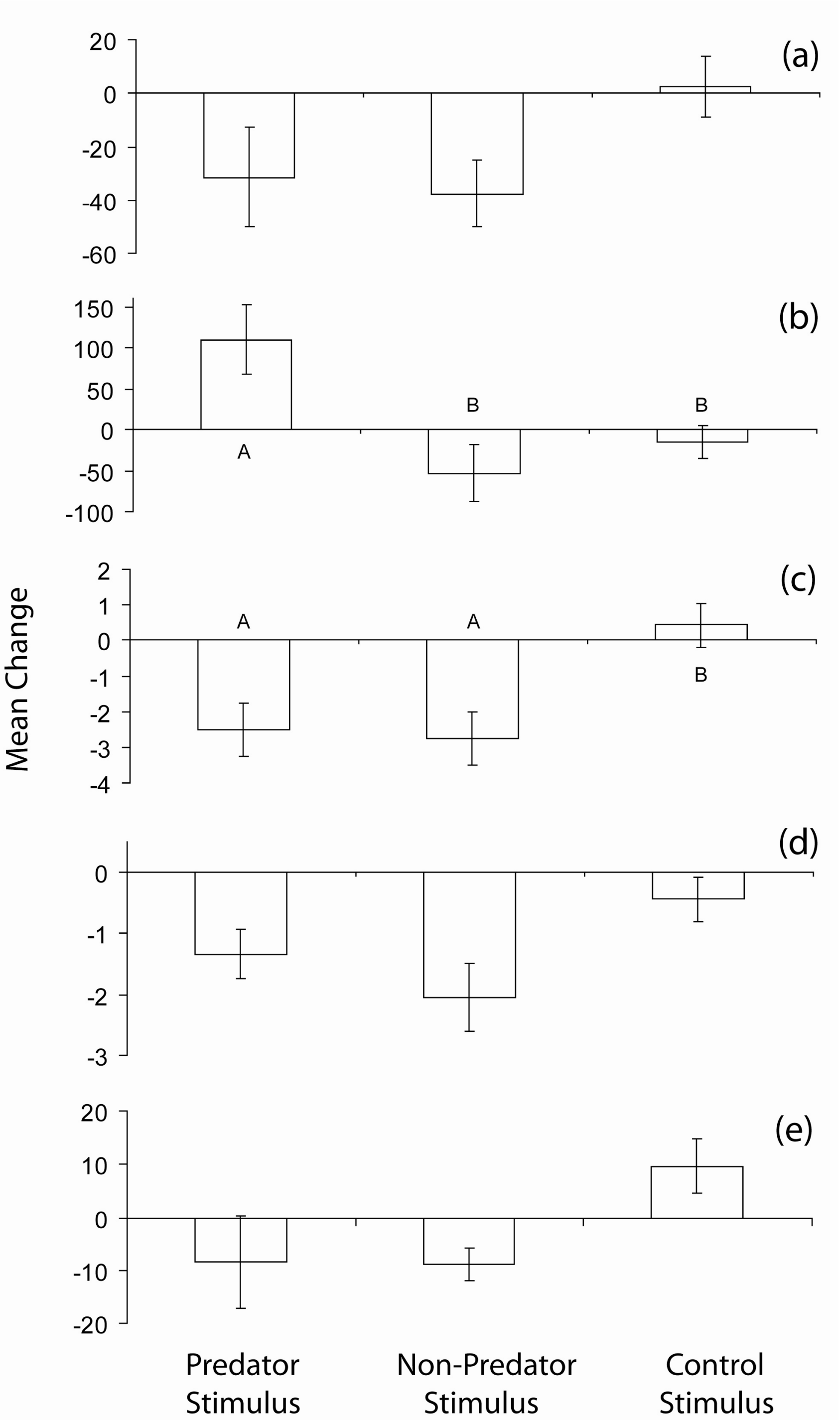

Figure 6.2: Mean change (± SD) in (a) feeding strikes, (b) bobs, (c) distance from shelter, (d) height in water column, and (e) horizontal movement between zones, between pre and post-stimulus periods by newly metamorphosed *Pomacentrus amboinensis* tested in each of the three visual cue treatments (Predator Stimulus, Non-Predator Stimulus, Empty Tank Control Stimulus) in Experiment 1. Alphabetic notations (A, B) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

Experiment 2 – Response to changing visual stimulus distance

There was a significant difference in the overall behavioural change between the four visual stimulus treatments (Pillai's $\text{Trace}_{21,\ 156} = 0.6572$, $p = 0.0059$). The CDA shows distinct separation between the three visual distance stimuli (minimum, medium and maximum; Fig. 6.3). However, the behavioural changes in response to the control stimulus are relatively similar to those observed in the maximum stimulus distance trials. Differentiation between groups appears to be largely a result of variation in the number of feeding strikes, the number of bobs, the mean distance from shelter and the number of horizontal movements between zones. These four behavioural characteristics were further examined using ANOVA's, resulting in a revised alpha level of 0.0125. This showed a significant difference in both the change in number of feeding strikes ($F_{3,\ 56} = 4.2890$, $p = 0.0086$) and mean distance from shelter between treatments ($F_{3,\ 56} = 4.1001$, $p = 0.0106$). The largest behavioural changes were observed in the minimum distance treatment, with a large decrease in both the number of feeding strikes and distance from shelter in response to the visual cue being recorded (Fig. 6.4). The magnitude of the change in feeding strikes decreased as the distance from the predator stimulus increased (Fig. 6.4a). Although there was no statistical difference between treatments, it is interesting to note that a similar trend was evident in the change in the number of bobs, with the magnitude of mean change decreasing with increasing distance (Fig. 6.4b). In contrast, mean distance from shelter significantly decreased only for the minimum distance treatment (Tukey's HSD; Fig. 6.4c).

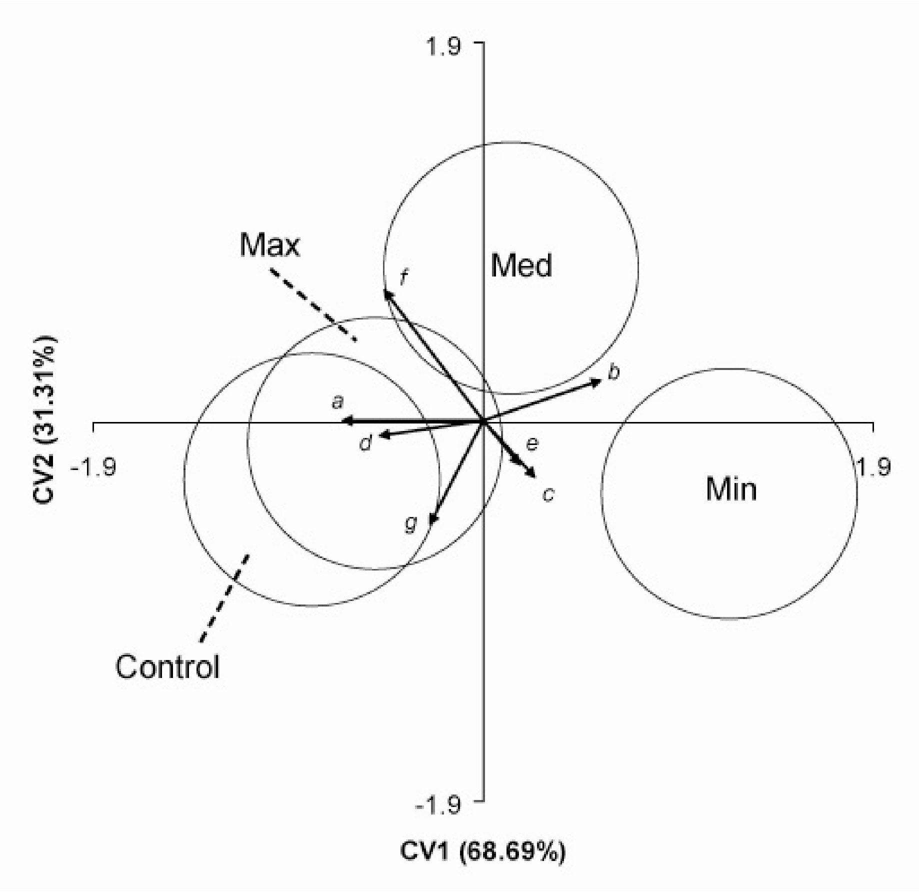

Figure 6.3: The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to visual predation cues over different distances. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the four visual cue treatments tested in Experiment 2: Minimum Distance (Min), Medium Distance (Med), Maximum Distance (Max) and Empty Tank Control (Control). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (*a*), bobs (*b*), movement in/out shelter (*c*), horizontal movement between zones (*d*), time in shelter (*e*), distance from shelter (*f*), height in water column (*g*). The circles represent 95% confidence intervals.

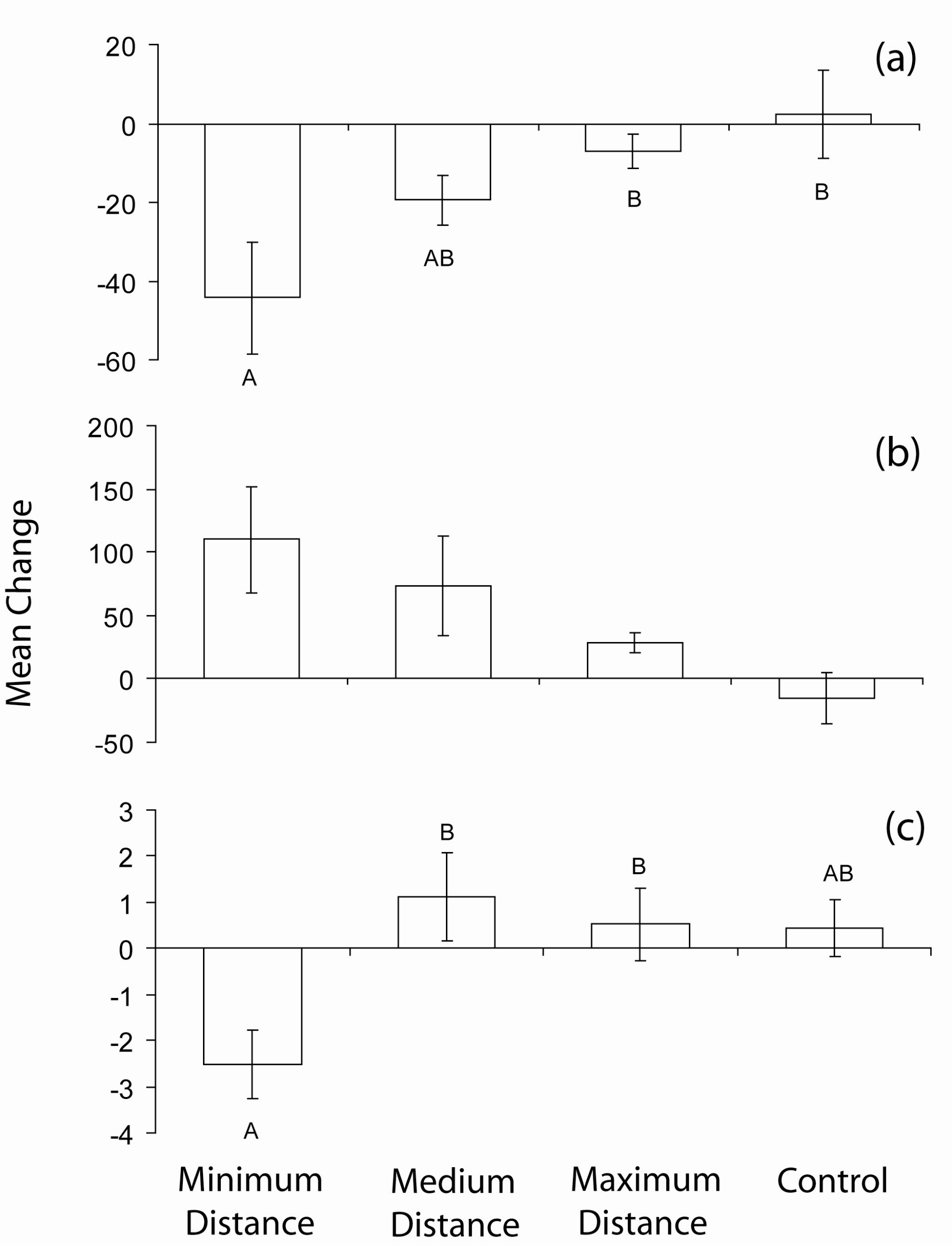

Figure 6.4: Mean change (± SD) in (a) feeding strikes, (b) bobs, and (c) distance from shelter, between pre and post-stimulus periods by newly metamorphosed *Pomacentrus amboinensis* tested in each of the four visual cue distance treatments (Minimum Distance, Medium Distance, Maximum Distance, Empty Tank Control) in Experiment 2. Alphabetic notations (A, B) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

Experiment 3 – Response to changing chemical alarm concentration

There was no significant difference in the overall behavioural change between the four chemical stimulus treatments (Pillai's $\text{Trace}_{21,\,150}$ = 0.39495, p = 0.3721). The CDA clearly shows this overlap in behaviour between the treatments (Fig. 6.5). Despite this lack of difference, an examination of the CDA showed one vector (i.e. change in the number of feeding strikes) to be responsible for a disproportionate amount of the overall variation. It was decided to further examine changes in this trait using an ANOVA. This showed there to be a significant difference in the change in the number of feeding strikes between treatments ($F_{3,\,54}$ = 4.217, p = 0.0094). Those individuals exposed to the high concentration treatment had the largest decrease in the number of feeding strikes, with those exposed to the medium concentration treatment showing a slightly smaller decrease (Fig. 6.6). It is interesting to note that there is a large amount of variability in the mean change in feeding strikes in the high concentration treatment. Individuals in both the low concentration and control treatments showed little change in response to the visual cue.

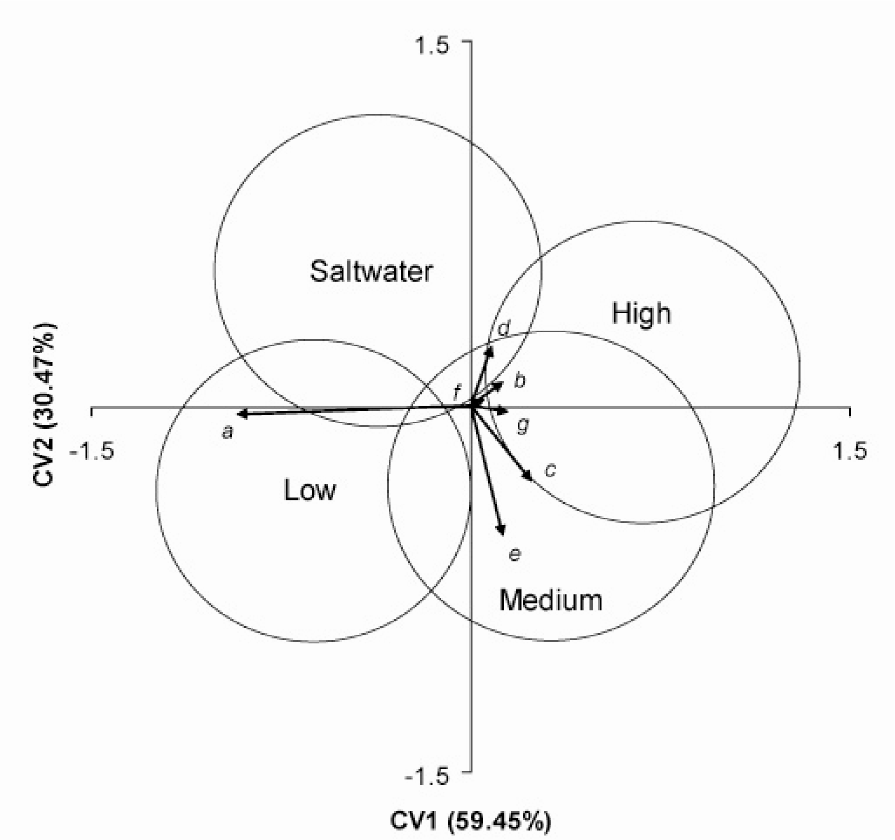

Figure 6.5: The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to different concentrations of conspecific chemical alarm cue. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the three visual cue treatments tested in Experiment 3: High Concentration (High), Medium Concentration (Medium), Low Concentration (Low) and Saltwater Control (Saltwater). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (*a*), bobs (*b*), movement in/out shelter (*c*), horizontal movement between zones (*d*), time in shelter (*e*), distance from shelter (*f*), height in water column (*g*). The circles represent 95% confidence intervals.

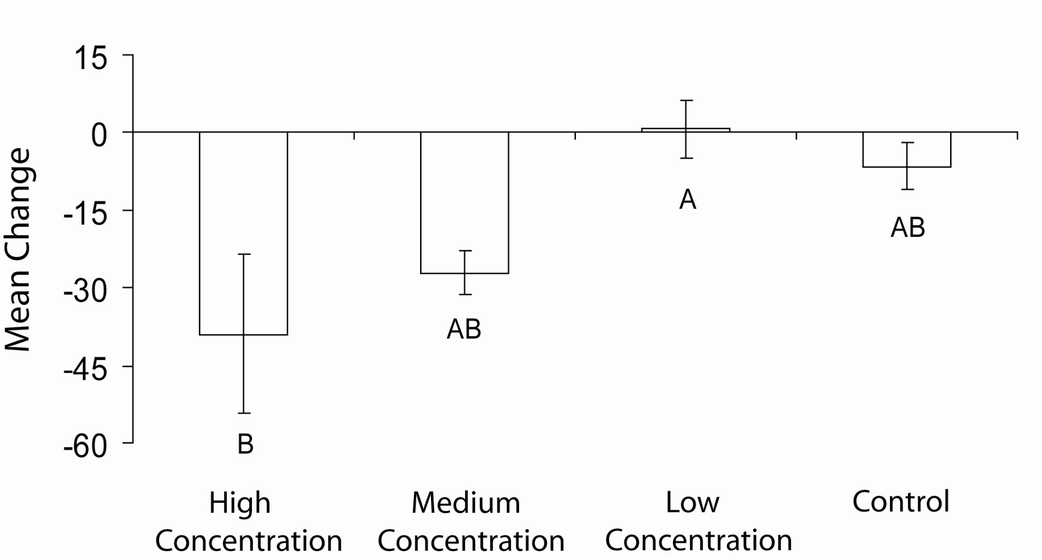

Figure 6.6: Mean change (± SD) in feeding strikes between pre and post-stimulus periods by newly metamorphosed *Pomacentrus amboinensis* tested in each of the four conspecific chemical cue concentration treatments (High Concentration, Medium Concentration, Low Concentration and Saltwater Control) in Experiment 3. Alphabetic notations (A, B) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

6.4. Discussion

Both olfaction and vision are thought to play important roles in threat detection in a wide range of systems, and have previously been demonstrated to operate in tandem in both freshwater (Mathis and Vincent 2000; Brown and Magnavacca 2003) and marine environments (McCormick and Manassa 2008). Their utility over other sensory systems in aquatic environments is largely a result of the aqueous medium in which they function, allowing in particular, for the effective transmission of chemical cues over relatively large areas (Wisenden 2000). However, how each of these sensory cues may be used by animals transitioning to previously unknown habitats is largely unknown. Our results show that the reef fish *Pomacentrus amboinensis* was able to detect and respond to both visual and chemical cues during their settlement transition. The intensity of the behavioural responses to both sensory cues varied with the level of threat detected, with a higher degree of perceived threat eliciting more intense responses. However, the type and nature of the behavioural responses differed markedly between the two sensory cues. Additionally, individuals were not able to distinguish between predatory and non-predatory visual cues whilst still naive to the reef environment. This indicates that the mechanisms underlying predator avoidance and the learning of predator identity immediately following settlement will be a complex interaction between multiple sensory cues.

The nature of anti-predator responses can differ greatly between systems, species and even life history stages (Ferrari et al. 2009). However, generally these responses are characterised by one or more of a decrease in foraging activity, a decrease in overall movement and an increase in shelter use (Stauffer and Semlitsch 1993; Chivers and Smith 1998; Griffiths et al. 1998). The behavioural response to conspecific chemical alarm cues across all concentrations in this study was characterised by a decrease in feeding rate only (although differing in intensity among treatments). Although this response is perhaps not as diverse as those found in other studies of adult marine fishes (see Smith and Smith 1989; Larson and McCormick 2005; McCormick and Manassa 2008), it is consistent with the only other study examining the response to conspecific alarm cues during the early post-settlement period (Chapter 5). This consistency in the nature of behavioural change both between experiments and between concentration levels in this study highlights the important role that this innate cue appears to play in the identification of relevant predation threats during this early transitional period.

In contrast to the narrow nature of responses to relevant chemical cues, responses to visual cues affected more facets of behaviour and were more consistent in effect. In general, the nature and direction of response was most consistent with regards to feeding rate, distance from shelter (which both tended to decrease with exposure to visual cues) and the number of bobs (which tended to increase with exposure to visual cues). This reaction is similar to that found by McCormick and Manassa (2008) in a recent study on the response of the marine goby, *Asteropteryx semipunctatus*, to visual and chemical cues. However, although variable in direction and intensity, our study also recorded behavioural changes with regard to the mean height in the water column and horizontal movement. This diverse, yet variable response indicates two things: that visual cues may elicit a more diverse behavioural response due to the more immediate nature of a visual threat; and, that there appears to be a degree of uncertainty as to the identity of relevant predation threats without prior knowledge of reef systems, based on visual cues alone.

The uncertainty in the visual identification of relevant predators during the early post-settlement period is further highlighted by the inability of naïve individuals to differentiate between predatory and non-predatory fishes. With the exception of the change in bobbing behaviour, all other behavioural traits that either showed significant (distance from shelter) or non-significant trends in change (feeding rate, height in water column and horizontal movement) recorded similar responses to both predatory (*Pseudochromis fuscus*) and non-predatory (*Chromis viridis*) cues. Similar patterns of non-differentiation during transitional early life history stages have also been found by Mathis and Vincent (2000) and Kiesecker et al. (1996) during studies of predator recognition and avoidance in amphibian tadpoles. Given that a number of studies have demonstrated that later stage aquatic organisms can visually distinguish between relevant and non-relevant threats (Gerlai 1993; Kelley and Magurran 2003; Ferris and Rudolf 2007; McCormick and Manassa 2008), this demonstrates that the visual identity of relevant predators is not innate and suggests that such knowledge must be acquired through experience in the new habitat.

Threat-sensitive responses to predator cues have been well documented amongst freshwater organisms (Mirza et al. 2006; Kesavaraju et al. 2007; Ferrari et al. 2008; Brown et al 2009). This study presents a similar pattern of threat-sensitive behavioural responses to both visual and chemical predator cues for a newly settled reef fish. Although differences between concentration/distance treatments were not always statistically significant, the general pattern amongst the characteristic behavioural responses (with the exception of change in distance from shelter in response to visual cues) was of decreasing intensity with decreasing chemical cue concentration and increasing distance from visual cue. However, there is also some evidence to suggest that threshold response levels may exist at the lower levels of these graded responses, with individuals showing no change in the distance from shelter in response to visual cues at either the medium or maximum distances. Additionally, there was little to no change in feeding rate at the lowest chemical alarm concentration, indicating that individuals either cannot detect, or choose not to respond to this type of alarm cue at extremely low levels.

This is not the first example of a graded anti-predator response in the marine environment. Helfman (1989) previously demonstrated a threat-sensitive response to visual predation cues in the three-spot damselfish (*Stegastes planifrons*), whilst Legault and Himmelman (1993) found multiple bivalve, gastropod and holothuroid species respond according to the level of threat posed by varying predators. The novelty of our findings lies in the stage in development at which the response occurs in the study species. The early post-settlement period for coral reef fish is characterised by extremely high levels of predation. As such, there is significant pressure placed on individuals to both directly avoid predatory encounters, as well as maximise growth to decrease the amount of time vulnerable to gape limited predators (Chapter 3). Our results show that reef fish possess an unexpectedly complex sensory system for such an early period of physiological development; being able to use cues from multiple sensory sources to acquire information on local predation risk, and respond accordingly. In doing so, they are able to effectively balance between minimising predation risk whilst still maximizing the time spent foraging for food.

Although it is clear that naïve newly settled fish possess the ability to detect and react to predatory cues from multiple sources, the nature of both the responses and the sensory cues themselves suggest that independent of one another their utility may be limited. What may be most critical is the ability of naïve individuals to couple these cues together into knowledge that can be directly applied to the new system. In a study of threat-sensitive predator avoidance by slimy sculpins (*Cottus cognaus*), Chivers et al. (2001) concluded that sensory cues may perform very different roles, with chemical cues functioning to warn that a predator is in the vicinity, and visual cues used to accurately assess the predation risk. Although such a function is also possible in coral reef fishes, it is more likely that the pairing of cues during this early period works to fine tune the visual identification of relevant predatory species. This theory is supported by McCormick and Manassa (2008) who found that a species of marine goby (*Asteropteryx* *semipunctatus*) had the ability to independently differentiate between predatory and non-predatory visual cues during later life stages. In making this link, our study adds considerably to the current lack of information regarding the mechanisms of threat detection, and the role or predatory cues during transitional periods.

Chapter 7: General Discussion

For organisms with complex life history cycles, the processes and mechanisms influencing survival during high mortality transitional periods will play a disproportionately important role in determining those traits that are passed on to later life stages. The present study adds considerably to our knowledge of these processes and mechanisms during just such a transitional period in coral reef fishes. In doing so, the study highlights the complex nature of predatory interactions on reef fishes immediately following settlement to the reef environment, and stresses the potential for both phenotypic and behavioural mechanisms to play key roles in influencing prey survival.

During interactions with a common predatory fish species (*Pseudochromis fuscus*), body size was found to be the most important prey phenotypic or performance trait influencing survival at the time of settlement (Chapter 2). However, contrary to conventional ecological theory (i.e. the bigger-is-better hypothesis), larger body size was preferentially selected by this predatory species. When tested in a field context, the selectivity of predation towards prey body weight shifted towards heavy individuals, indicating that the selective patterns may be modified by either external environmental factors or differential selective preferences of other predatory species.

Chapter 3 reinforced the potential for selective preferences to differ between species within the same system, with patterns of size selectivity on settlement stage and juvenile *Pomacentrus amboinensis* found to differ significantly between four important predatory species. Although larger body size became more advantageous once individuals grew into more experienced juvenile stages, this was not always the case at the time of settlement, with the direction and intensity of selection differing greatly. I argue that differences in prey selection between predatory species can in part be explained by variation in prey behaviour between different sized individuals within a group (Chapter 4). This behavioural variation may allow predatory species to exploit the prey size distribution in different ways (Scharf et al. 2000), with smaller individuals being more vulnerable to opportunistic predation modes, and larger sizes being more susceptible to highly selective pursuit predators.

Experience obtained during early settled life influenced the probability of survival during future predation events. Experiments showed that prior exposure to conspecific alarm cues significantly decreased foraging rates amongst newly settled *P. amboinensis*. Such a behavioural change is commonly associated with anti-predator behaviour (Stauffer and Semlitsch 1993; Chivers and Smith 1998; Griffiths et al. 1998), directly decreasing the time spent vulnerable to predation during risky foraging activities. Individuals were also able to use these alarm cues to associate a previously novel predator scent with danger, indicating that chemical cues play a large role in the acquisition of knowledge during this early stage. In comparison, responses to visual predation cues were much more diffuse and variable, suggesting that independently they may be of limited use without prior knowledge of relevant predators (Kiesecker et al. 1996; Mathis and Vincent 2000). I argue that such cues possibly play a complementary role during this period, working together to provide the most useful information to prey individuals (Chivers et al. 2001).

Behavioural responses to both visual and chemical cues were threat sensitive in nature when tested across a range of predation threat levels. This suggests an unexpectedly complex level of development in sensory systems during such a transitionary period. However, in its presence this mechanism will allow prey individuals to effectively balance the importance of reducing predation risk, whilst still maximizing growth and competitive advantages through a graded behavioural response (Rhoades and Blumstein 2007).

Significance

One of the fundamental goals of ecological research is to understand the processes that structure populations and communities. When considering these processes, predation is widely thought to play one of the more important roles, and as such has received considerable attention in the literature (Glasser 1979; Sih et al. 1985; Hixon 1991). Although some form of predation pressure is generally present throughout an animal's entire life, its effects are commonly concentrated in specific periods when individuals are most vulnerable (e.g. transitional periods between life-history stages or habitats; Wilbur 1980; Gosselin and Qian 1997). However, despite the potential importance of the mechanisms influencing survival during such periods, we currently know very little about them. This study is significant in that it examines the processes and mechanisms influencing prey survival during predatory interactions over just such a high mortality period in a common coral reef fish. Where this work differs from other studies during similar periods is in the timeframe over which it is examined. While others investigate the effects of predatory interactions over extended periods (e.g. weeks to months following transition; Searcy and Sponaugle 2001; Vigliola and Meekan 2002; Hoey and McCormick 2004; Raventos and MacPherson 2005; Sponaugle and Grorud-Colvert 2006;

Gagliano et al. 2007; Robert et al. 2007), this study focuses specifically on the point at which predation is highest (in this case, the hours immediately following transition). Given that this study found predation to be selective during this period of highest mortality, it indicates that this relatively short period following settlement may be disproportionately important to the structure of adult populations for coral reef fishes. Phenotypic selectivity does not necessarily diminish outside of this initial period. However assessing the impacts of predation over greater periods in the juvenile life phase may lead to masking or diluting its effects, due to the potential for changes in the nature of phenotypic selection and intensity (Chapter 3; Meekan et al. *in press*).

These findings have implications for any organism with complex life history characteristics, and highlight the necessity to focus on the correct timeframes when examining the dynamics of transitionary periods. For instance, amphibians are generally characterised by bi-partite life cycles in which aquatic larval stage are followed by more amphibious adult stages (e.g. frogs, toads and salamanders; Werner 1986; Scott et al. 2007; Walsh et al. 2008). In order to examine those processes most influencing adult life stages, focus must be placed on those specific periods in which mortality is both intense and selective (Crouse et al. 1987).

This research also highlights the variability in the nature of ecological mechanisms underlying survival that may occur at the species and life history level. The principles of ecological theories are commonly examined within communities or populations as a whole, with little focus being given to potential differences that may occur with species specific interactions or at particular points in an organism's life history (e.g. bigger is better hypothesis). While this may give us an understanding of the overall picture, it fails to provide an insight into the true mechanisms influencing survival. By examining predation processes at a more detailed level, we are able to examine how prey populations may directly respond to changing community structures, and vice versa. Given that trophic links are often specific to species and life-history stages, such information allows us to more accurately predict how changes at certain levels will affect others within the community food chain. This type of knowledge is particularly relevant in more recent times, given the predicted and documented effects of human pressures (ie. climate change, habitat degradation, over-fishing) on fish community structure in coral reef systems (McClanahan 1994; Jennings and Polunin 1997; Booth and Beretta 2002; Graham et al. 2007). Oddly however, this is a factor rarely considered in ecological studies.

In addition to the above, this is also the first study to examine the mechanisms of prey learning and threat detection during such an early period in coral reef fishes. Given the intense pressure placed on individuals to identify relevant threats during this transitional stage, the methods by which information is obtained and how they respond to this information will be disproportionately important. This study highlights the role that olfactory cues play during this early period, and stresses its importance in the acquisition of relevant knowledge. Although similar mechanisms have been found in amphibians (Woody and Mathis 1998), freshwater fishes (Brown 2003) and in later life stages of marine fishes (Larson and McCormick 2005; McCormick and Manassa 2008), the novelty of the finding is in the timeframe in which it operates in this coral reef system. Recent research has shown that prior experience obtained over the days immediately following settlement has a large influence on the probability of survival during future predatory encounters (McCormick and Holmes 2006). This research isolates potentially the most important mechanisms driving this learning process and shows how these mechanisms are modified with changing levels of predation threat.

Future Directions

The results and conclusions of this project pose a number of further interesting questions. Chapter 3 identifies the selective profiles of four important predatory species under controlled conditions, when preying on newly settled *P. amboinensis*. While these patterns are a necessary first step in understanding the mechanisms underlying selective loss during this early period, how they relate to selective patterns in natural, multispecies communities remains largely unknown. In a study of social facilitation of selective mortality in the same system, McCormick and Meekan (2007) found indirect evidence to suggest that the preferences of both *P. fuscus* and *Thalassoma lunare* in the natural system were similar to those found in this study. This indicates that the results may have some relevance in natural systems. However, in order to fully understand these relationships in natural systems, particularly in multi-species predator communities, we must understand how predators and potential competitors interact with one another and their environment. Synergistic or antagonistic relationships between individuals have the potential to significantly alter selective patterns (Berger and Gese 2007), by influencing either the level of access that each has to the prey source, or the level of vulnerability to the predation source (Figueira et al. 2008). Furthermore, external environmental characteristics such as habitat complexity (Beukers and Jones 1997; Rilov et al. 2007), water movement (Gagnon et al. 2003) and presence of coral bleaching (Coker et al. 2009, McCormick 2009) may also play a role in influencing existing preferences.

Both prey body size and prior experience of predation events have individually been shown to greatly influence survival during the period immediately following settlement (Chapter 2; McCormick and Holmes 2006). However, which of these plays the most definitive role during this potentially critical period is not known. If the effects of prey experience are superior, then body size can effectively be negated when comparing the survival probabilities of newly-settled naïve individuals with those settled even 24 hours prior. On the other hand, if body size overrides prior experience then it would be expected that the probability of survival would not greatly increase until individuals have grown above the size ranges most vulnerable to predation (Chapter 2). What is most likely however is that the mechanisms interact with one another to form a complex relationship, with the acquisition of knowledge resulting in a change in the nature or intensity of size selection. Chapter 3 suggests just such a relationship during interactions between *P. fuscus* and juvenile *P. amboinensis*. Further research examining how these factors interact is required to further elucidate the true dynamics of survival during early settled life.

Chapter 4 makes the suggestion that behavioural differences between different sized individuals within a group may influence the vulnerability of each to predation events. Further, McCormick (2009) demonstrated that the way individuals interacted to influence this vulnerability was affected by habitat. Although these studies were able to isolate what these behavioural differences were, how each of these directly affected vulnerability was only inferred. In order to confidently identify the true mechanisms underlying the size-selective preferences of predatory individuals, it is essential to understand the balance between this socially controlled vulnerability and predator selection. What would be interesting would be to manipulate behavioural traits in isolation and directly expose them to predation pressures. By doing so we would be able to gauge exactly which behavioural traits directly influence vulnerability to certain types of predation. Such a finding could be applied beyond the scope of hierarchical systems, and be further used to interpret the implications of changes in behaviour associated with prey learning (Chapters 5 and 6).

Concluding Remarks

The rapid and decisive nature of predation makes it an extremely difficult process to study and understand. This project significantly increases our knowledge of the process of predation during a high mortality period in the life of a coral reef fish, and provides us with a mechanistic understanding of how these processes function. My findings have implications not only for coral reef fishes, but for organisms with complex life-histories in general, in that it highlights two critical factors influencing survival for organisms transitioning to new habitats: the importance of body size, and knowledge acquired from prior experiences. In doing so, it offers insight into the mechanisms underlying survival during this period, and provides a necessary step forward in the study of population dynamics in coral reef fishes.

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Appendix 1: Published Papers

Chapter 2 published as:

Holmes TH, McCormick MI (2009) Influence of prey body characteristics and performance on predator selection. Oecologia 159: 401-413

Chapter 3 publised as:

Holmes TH, McCormick MI (2010) Size-selectivity of predatory reef fish on juvenile prey. Marine Ecology Progress Series 399: 273-283

Chapter 4 in review as:

Holmes TH, McCormick (in review) Behaviour as a mechanism underlying size-based differences in vulnerability to predation. Environmental Biology of Fishes

Chapter 5 published as:

Holmes TH, McCormick MI (2010) Smell, learn and live: the role of chemical alarm cues in predator learning during early life history in a marine fish. Behavioural Processes 83: 299-305

Chapter 6 accepted as:

Holmes TH, McCormick (accepted) Response across a gradient: behavioural reactions of newly settled fish to predation cues. Animal Behaviour