White, James Ryan (2015) ***The role of boldness and other personality traits in the ecology of juvenile marine fishes.*** **PhD thesis, James Cook University.**

The role of boldness and other personality traits in the ecology of juvenile marine fishes

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For the degree of
Doctor of Philosophy
College of Marine and Environmental Sciences
James Cook University
Townsville, Queensland
November 2015

Statement of the Contribution of Others

Financial Support

James Cook University International Postgraduate Scholarship
College of Marine and Environmental Sciences
ARC Centre of Excellence for Coral Reef Studies
AIMS@JCU

Statistical and Analytical Support

Prof. Mark McCormick
Dr. Maud Ferrari
Dr. Rabiul Beg
Dr. Niels Dingemanse
Dr. Ned Dochtermann

Supervision

Prof. Mark McCormick
Dr. Mark Meekan

Editorial Support

Prof. Mark McCormick
Dr. Mark Meekan

Declaration on Ethics

This research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Queensland Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the JCU Animal Ethics Committee Approval Numbers # A1067 & A1720.

Acknowledgements

Numerous people have provided help and support throughout my thesis. First, I would like to thank my primary supervisor, Mark McCormick. Mark was always quick to return drafts of my papers, with useful comments and insights with larger ecological perspective. He always allowed me the independence I needed to work freely and supported my numerous trips to Lizard Island for my own work and for collaborations with his contacts. My secondary supervisor, Mark Meekan, provided very thorough edits on my manuscripts and constant direct support in the field. Mark was always available to provide life and career advice, as well as help me drink any beer that would otherwise have been left behind in the field. I would also like to thank Maud Ferrari and Shaun Killen for their guidance with statistical approaches and experimental design, as well as a lot of great laughs and memories at Lizard Island.

I've had a lot of great volunteers in the field over the course of my thesis. Taylor Bodine, Caterina Schlott, Miwa Takahashi, Dylan Simonson, Marta Bertrand, Jon Smart and Kate Schoenrock all dedicated their time, effort and sanity working with me over long days at Lizard. Thanks guys for putting up with my sense of humor and tests of your patience!

I would not have gotten through the trials and tribulations of my thesis without the emotional support and laughter provided by my friends over the years. Rohan Brooker, Bridie Allan, Cecilia Villacorta Rath, Matt Mitchell, Ian McLeod, Jackie Davies, Jennifer Atherton, Jon Smart, Luke Pedini, Matt Jankowski, Melanie Trapon, Rahel Zemoi, Richard Duffy, Samantha Munroe (my zombie-apocalypse partner), Tom Roberts, Kate Schoenrock, Daniel Cooper, Stephen Staples, Jennifer Hodge, Scott Harte, Crystal Neligh, Daniel Dixson, Donald Warren, Eva McClure, Terry Efird, Moorea Efird, Chris Reeves and Tory Chase all helped make my time here wonderful and memorable and supported me from near and afar.

Ceci and Matt were especially supportive and valuable during my early years. They will be lifelong friends. Bridie single-handedly altered my perspective on the capabilities of human beings. I watched her grow from a relatively self-conscious, doubtful and inexperienced field scientist to a BAMF who kicks ass every day. Somehow she manages to be a full-time mom of two, wife, cook, PhD student, part-time research assistant and super-hero all at the same time. Knowing her makes me want to be a better person and shows me what we are all truly capable of with hard work and dedication. Bridie, your friendship made my time here worthwhile and is something I will always cherish.

My lab mates have always been a source of amusement and support. Thanks to Katy Korkill, Lucy Holmes, Ryan Ramasamy, Govinda Liénart, Justin Rizzari, Davina Poulos, Maria Palacios and Lauren Nadler.

Also, a special thanks to the staff at Lizard Island Research Station; Anne Hogget, Lyle Vail, Maryanne Pearce and Lance Pearce. They made Lizard Island such an easy, well-designed, and wonderful place to work.

I would like to acknowledge the financial support from James Cook University, AIMS@JCU, the ARC CoE, and the College of Marine and Environmental Science. Without their support, my work wouldn’t have been possible.

Finally, I would like to thank my family. My beautiful mother Susie, thank you for believing me and supporting my dreams in whatever way you could. Tim, you taught me what it means to be a strong man who supports his family. To Jeff, you are more than my twin brother, you are a part of me and I am a part of you. To Tyler, Troy, Beth and Savannah, when you guys showed up it changed my life dramatically, suddenly, and for the better. Watching you all grow up has taught me how to love unconditionally. And last but definitely not least, I would like to thank my Aunt Gail. She taught me to love the ocean, travelling, good food, and life in general. She has always been a personal

cheerleader, guidance counselor, financial supporter and advocate in my continued education and life goals. I would not be the man I am today without your love and encouragement.

Abstract

The theory of animal personality focuses on quantifying variation in behavior within and among individual organisms and attempts to account for the maintenance of differences in behavior that occur in a consistent manner among individuals. Personality has potentially important ecological consequences (e.g. behavioral tradeoffs) and can be shaped by population dynamics through selective mortality. Flexibility in behavior is advantageous for organisms that transition between stages of a complex life history. However, various constraints can set limits on plasticity, giving rise to the existence of personalities that have associated costs and benefits. One particularly important behavioral trait, boldness, is defined as the propensity of an animal to engage in risky behavior. Many variations of novel-object or novel-environment tests have been used to quantify the boldness of animals, although the relationship between test outcomes has rarely been investigated. Furthermore, the relationship of boldness measures to any ecological aspect of fitness is generally assumed, rather than measured directly. Understanding the costs and benefits of different behavioral phenotypes requires a greater understanding of structure and temporal consistency of intra-individual behaviors. More research is necessary for identifying the traits with potential fitness costs or showing how any trade-offs are manifested. This study therefore investigated the situational and temporal consistency of behavior, appropriateness of various boldness measures, and the relationships between different behavioral traits in order to better understand how coral reef fishes balance trade-offs related to risk.

To understand the stability of fish behavior across various field and laboratory settings, there is a need to understand the behavioral structure throughout different situations. **Chapter 2** tested for any evidence in consistency of behavior across situations in juveniles of a common damselfish, *Pomacentrus amboinensis* (Pomacentridae) at the transition between larval habitats in the plankton and juvenile habitats on the reef.

Naïve fish leaving the pelagic phase to settle on reefs were caught by light traps and their behaviors observed using similar methods across three different situations (small aquaria, large aquaria, field setting); all of which represent low risk and well-sheltered environments. Seven behavioral traits were compared within and among individuals across situations to determine if consistent behavioral syndromes existed. No consistency was found in any single or combination of behavioral traits for individuals across all situations. We suggest that high behavioral flexibility is likely beneficial for newly-settled fishes at this ontogenetic transition and it is possible that consistent behavioral syndromes are unlikely to emerge in juveniles until environmental experience is gained or certain combinations of behaviors are favored by selective mortality.

Despite the lack of evidence for behavioral syndromes, individual juvenile coral reef fish are likely to show behavioral repeatability within a single situation, over time (i.e. personality). **Chapter 3** documented a field and laboratory experiment that examined the consistency of measures of boldness, activity, and aggressive behavior in young *P. amboinensis* immediately following their transition between pelagic larval and benthic juvenile habitats. Newly-settled fish were observed in aquaria and in the field on replicated patches of natural habitat cleared of resident fishes. Seven behavioral traits representing aspects of boldness, activity and aggression were monitored directly and via video camera over short (minutes), medium (hours), and longer (3 days) time scales. With the exception of aggression, these behaviors were found to be moderately or highly consistent over all time scales in both laboratory and field settings, implying that these fish show stable personalities within various settings.

The various operational definitions and employed methodology for studying ‘boldness’ in animals confounds comparisons among behavioral studies. Also, little is known how these various techniques compare in an ecologically meaningful way. **Chapter 4** compared how the outcomes of the same test of boldness differed among observers and how different tests of boldness related to the survival of individuals in the field. Newly-metamorphosed lemon damselfish, *P. moluccensis*, were placed onto replicate patches of natural habitat. Individual behavior was quantified using four tests (composed of a total of 12 different measures of behavior): latency to enter a novel environment, activity in a novel environment and reactions to threatening and benign novel objects. After behavior was quantified, survival was monitored for two days during which time fish were exposed to natural predators. Variation in estimates of behavior among observers was low for most of the 12 measures, except distance moved and the threat test (reaction to probe thrust), which displayed unacceptable amounts of inter-observer variation. Body size and distance ventured from shelter were the only variables that had a direct and positive relationship with survival. Overall, the results of the behavioral tests suggested that novel environment and novel object tests quantified similar behaviors, yet these behavioral measures were not interchangeable.

Being more bold or shy is likely to produce a trade-off with other important facets of an individual animal's behavioral phenotype. **Chapter 5** used a laboratory experiment to examine the link between boldness and learning in juveniles of *P. amboinensis*. Newly-metamorphosed fish were ranked individually on a boldness-shyness axis on the basis of their willingness to emerge into a novel environment in an aquarium. Each fish was then given a simple task four times, which involved learning how to navigate a maze to reach a food source. A greater number of fish ranked with high boldness successfully navigated the maze compared to shy ranked fish. This result suggests that boldness is likely to be closely linked with learning appropriate behaviors while exploring new habitats. Although a higher level of boldness is inherently risky in a habitat where animals are subject to high rates of predation, the potential for increased rewards associated with this trait may explain why boldness persists as a behavior in natural populations.

This study is among the first to examine the consistency of behaviors in both field and laboratory settings in over various time scales at a critically important phase during the life cycle of a coral reef fish. Multiple measures of behavior within the context of novel environment were the most robust way to assess boldness, and these measures have a complex relationship with survivorship of young fish in the field. The persistence of multiple alternative behavioral phenotypes despite strong selective pressure from predation may reflect the balance between foraging and predator vigilance. Shy individuals may allocate more attention to exploring and searching environments in greater detail, since their inherent shyness means that they are naturally under lower predation threat than bolder individuals. Conversely, bolder individuals may allocate less attention to searching because of the need to have a greater degree of predator vigilance. If such a relationship exists, this would predict that greater numbers of bold individuals should occur within stable (e.g., consistent predator distribution and abundance) compared to variable environments. Thus, the ratio of bold to shy individuals of adult populations of coral reef fish might be influenced by the stability of the local environment they experienced as juveniles.

List of Publications from this Research

White JR, McCormick MI, Meekan MG (2013a) Syndromes or Flexibility: Behavior during a Life History Transition of a Coral Reef Fish. PLoS ONE **8**:e84262. doi: 10.1371/journal.pone.0084262

White JR, Meekan MG, McCormick MI, Ferrari MCO (2013b) A comparison of measures of boldness and their relationships to survival in young fish. PLoS ONE **8**:e68900. doi: 10.1371/journal.pone.0068900

White JR, Meekan MG, McCormick MI (2015) Individual consistency in the behaviors of newly-settled reef fish. PeerJ **3**:e961. doi: 10.7717/peerj.961

White JR, Meekan MG, McCormick MI (2015) Differences in spatial learning linked to boldness in fish. Ethology, Ecology & Evolution (in review).

Conference and Meeting Presentations

White, J.R., McCormick, M.I., Meekan, M.G. (2013) The relationship between boldness and learning in a tropical reef fish. Oral presentation at the 9th Indo-Pacific Fish Conference. Okinawa, Japan.

White, J.R., McCormick, M.I., Meekan, M.G. (2013) The relationship between boldness and learning in a tropical reef fish. Oral presentation at the annual JCU Marine and Tropical Biology Student's Conference. Townsville, Australia.

White, J.R., Meekan, M.G., McCormick, M.I., Ferrari, M.C.O. (2012) A comparison of field methods for assessing boldness in fishes. Oral presentation at the 12th International Coral Reef Symposium. Cairns, Australia.

White, J.R., Meekan, M.G., McCormick, M.I., Ferrari, M.C.O. (2011) A comparison of field methods for assessing boldness in fishes. Oral presentation at the annual AIMS@JCU Student's Conference. Townsville, Australia (Best oral presentation winner).

White, J.R., Meekan, M.G., McCormick, M.I., Ferrari, M.C.O. (2011) A comparison of field methods for assessing boldness in fishes. Oral presentation at the annual JCU Marine and Tropical Biology Student's Conference. Townsville, Australia (Best oral presentation winner).

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Chapter 1: General Introduction

After watching animals closely, researchers have long noted that individuals exhibit consistent and distinct behavioral profiles, or “personalities” (Francis 1990; Groothuis and Carere 2005). However, because these subtle differences in individual behavior were not easily characterized into the existing knowledge base of animal studies, initial literature on animal personality was anecdotal and there was little development of relevant theory on ecological and evolutionary implications of these behavioral observations (Francis 1990). Instead, individual differences were dismissed as minor variations around the average behavior of a species (Francis 1990; Wilson et al. 1994; Careau et al. 2008) that were a result of inaccurate measurements or alternative phenotypes (Groothuis and Carere 2005). This contrasts with the ready acceptance of the idea that variation in individual human behaviors was indicative of differences in personality or temperament (Groothuis and Carere 2005). In part, the reluctance to acknowledge animal personalities by researchers was also due to an apprehension by behavioral biologists of the use of labels and methods derived from studies of human psychology that might give the appearance of the introduction of an anthropomorphic bias (Gosling 2001; Groothuis and Carere 2005). Nevertheless, initial studies attempted to translate behavioral observations of animals of various species into the equivalent classification of personalities using the five axis model of psychology and psychologists (see Table 1, Gosling and John 1999). These initial studies generally looked towards research on animal behavior to gain insight into the development of human personality (Wilson et al. 1994; Gosling and John 1999; Gosling 2001).

Recent studies by ethologists and behavioral ecologists have noted consistent, individual differences in the behaviors of a taxonomically diverse range of more than 60 species (including primates, bears, felines, canines, cetaceans, amphibians, reptiles, insects, cephalopods, birds and fishes) under many different situations and contexts (Gosling 2001; Sih et al. 2004a; Frost et al. 2007; Stamps 2007). Key behavioral traits that that have been monitored include boldness, aggressiveness, activity levels, reactivity, sociability, fearfulness and exploration (Dall et al. 2004; Réale et al. 2007b).

Variation in consistent behavioral patterns among individuals have been interchangeably termed as: 'behavioral syndromes', 'temperament', 'personality', and 'coping styles' (Dall et al. 2004; Réale et al. 2007b; Dingemanse et al. 2010b; Sih et al. 2012); however, recent researchers have argued for a more restrictive use of terminology (Bell et al. 2009; Garamszegi and Herczeg 2012). Here, we adopt the definitions of Garamszegi and Herczeg (2012), where consistency in single behaviors (e.g. individuals that display repeatedly higher or lower levels of boldness, exploration, or aggression than others in the population) are described as displaying 'personality', and consistency in the relationship between two or more functionally different behaviors within the same individual is defined as a 'behavioral syndrome'.

Because personality traits now appear to be so widespread among animals, understanding the variation and flexibility in these traits has important implications for both ecology and evolutionary theory (Wilson et al. 1994; Wilson 1998; Dall et al. 2004; Sih et al. 2004a; Careau et al. 2008) and for the prediction of how animals might respond to environmental and ecological shifts (Sih et al. 2004a; Réale et al. 2007b). Furthermore, as knowledge of behavior contributes to a more holistic view of any particular animal (Sih et al. 2004a; Bell 2005), studies of personality have the potential to improve experimental design and interpretations of future research. These important implications of studies of behavioral profiles or personality have encouraged researchers from population biology (Bolnick et al. 2003), epidemiology (Lloyd-Smith et al. 2005), endocrinology (Williams 2008), behavioral ecology (Wilson et al. 1994; Sih et al. 2004a;

Réale et al. 2007b), and physiology (Bennett 1987; Careau et al. 2008) to integrate studies of inter-individual variation in behavior into their various ecological sub-disciplines (Careau et al. 2008). The resulting exponential growth in research on animal personality over the last decade has caused a major shift in the framework of behavioral ecology, whereby the previous standard "golden mean" of behaviors observed in populations (shifts in average behavior in response to environmental variation) has now been replaced with studies that emphasize individual differences (Wilson et al. 1993).

Behavioral traits that make up the personality of an individual animal are important for everyday ecological challenges. Behavioral decisions about foraging needs, predator avoidance, or reproduction have been shown to change during ontogeny, which can cause animals to shift habitats in order to meet their changing requirements (Werner and Gilliam 1984; McNamara and Houston 1986; Werner 1988; Ludwig and Rowe 1990). Age or size are likely to influence behavioral traits in animals because such aspects of environmental interactions change as they grow (van Gestel and van Broeckhoven 2003; Brown et al. 2005). Experience gained as individuals grow will also prompt them to either change their behavior to adjust to changing conditions (behavioral flexibility) or be eliminated from the population (selective mortality) (Brown et al. 2005). In particular, consistent within-individual behavioral traits are important to population ecology through limiting distribution and abundance, affecting species interactions, population dynamics (Sih et al. 2012), ecological invasions (Conrad et al. 2011; Sih et al. 2012) and responses to environmental and ecological shifts (Réale et al. 2007b; Sih et al. 2012). How the environment shapes and maintains behavioral traits over large (evolutionary) and small (ontogenetic) time-frames has until recently, received little attention from behavioral ecologists (Budaev et al. 1999; Brown et al. 2005).

Today, most research on animal personality is comprised of studies of behavioral traits (Gosling 2001) from either an evolutionary or ecological perspective. Research has overwhelmingly focused on measuring some aspect of a boldness-shyness axis (Bell and Sih 2007), with fewer studies focusing on animal activity or aggression. Past studies have shown high correlations across a range of behavioral traits, with bold individuals being more active, aggressive, taking more risks, and learning quickly (Sneddon 2003), while shy individuals (low boldness) displaying lower levels of activity, taking fewer risks, being slower to learn, and tending to be socially subordinate (Wilson et al. 1993; Koolhaas et al. 1999; Carere et al. 2005). However, it is dangerous to assume these behaviors are always correlated, and multiple tests of each one of these traits need to be made across multiple contexts. A bold, active, and aggressive individual is unlikely to be the better competitor or show higher fitness than its shy counterpart in all situations because otherwise, natural selection over time would result in no observable variation in behavioral traits among individuals (Dall et al. 2004). The observed variety of consistent differences in behavioral traits therefore means that both behavioral types must have a pay-off in some situations in order to persist in populations (Frost et al. 2007). For example, if predation risk is high, it would be beneficial for an animal to reduce foraging; conversely, if energy reserves are low, it may be worth the risk to obtain food. If these behaviors were fixed, then the higher exploratory character of the bold individual would be adaptive in a low-risk context, and maladaptive in a high-risk context (Dingemanse et al. 2007; Frost et al. 2007). Such flexibility may be of particular importance through ontogenetic changes or at times of key ecological transitions (McCormick and Meekan 2010).

Stable correlations between behaviors that are taxonomically widespread suggests a link between proximate factors (i.e. physiology, genetics, hormones) and behavior (Sih et al. 2012). Indeed, evidence has shown that personality traits are heritable (van Oers et al.

2005a; Réale et al. 2007b), have a partial genetic basis (Norton et al. 2011) and influence fitness (Dingemanse and Réale 2005; Smith and Blumstein 2008a) through their effects of predation rates (Réale and Festa-Bianchet 2003; Bell and Sih 2007), competition for females and food (Dingemanse et al. 2004), or reactions to social challenges (Dingemanse and Réale 2005; Sinn et al. 2006). However, heritable variation in behavioral phenotypes seems to remain robust despite the limiting effects of selection pressures (Penke et al. 2007; Réale et al. 2007b).

How an individual responds in one situation may be suggestive of its response in a very different situation (Careau et al. 2008). Adopting a certain behavioral syndrome can have both costs and benefits, for example, highly aggressive female fishing spiders (*Dolomedes triton*) are more successful at acquiring food in a competitive environment, but this aggression can be detrimental in another context such as when it leads to precopulatory sexual cannibalism (Johnson and Sih 2005). Likewise, farmed fish stocks live in an environment of high competition and zero predation pressure, and are often bolder, more aggressive, and take more risks than their wild counterparts (Sundström et al. 2004). Thus, consistent patterns in behavior among individuals can lead to trade-offs in aspects of fitness, which can ultimately influence population dynamics, community structure, and species diversity (Pruitt et al. 2013; Mittelbach et al. 2014). While intra-species genetic variation in behavioral phenotype can reduce success in some environments due temporal or situational trade-offs (where some phenotypes are less appropriate), this variation should increase overall long-term fitness and reduce likelihood of local extinction. Thus, species or populations that are comprised of a range of behavioral phenotypes are more robust to environmental perturbation (Sih et al. 2012). Meekan et al. (2010) recently investigated the relationship between mortality and growth rate mediated by behavior in the damselfish, *Pomacentrus ambonensis*. They found selective mortality for larger individuals, but no behavioral differences between size classes at settlement. However, 1-month-old fish showed the opposite pattern, with the smallest individuals having higher mortality and significantly different behavioral traits than the larger size class, thus demonstrating that behavioral variation and its mortality trade-offs can change with ontogeny (Meekan et al. 2010). Similarly, McCormick and Meekan (2010) showed evidence of links between mortality trade-offs and the growth rates and behaviors of juvenile *P. wardi* immediately after settlement (a time of high mortality and a key transition between life history phases). The persistence of alternative behavioral phenotypes within a species reinforces the idea of the evolutionary benefits of variation in behavior. Having variation in individual behavioral phenotypes within a species diminishes the trade-offs that might hinder performance associated with a given phenotype (Sih et al. 2012).

Overall, animal behavioral studies have many knowledge gaps to fill, especially concerning marine fishes. Most fish studies are conducted in the laboratory, with inferences made about behaviors under natural conditions (Brown et al. 2005; Toms et al. 2010). Little is known about the stability of behaviors in newly settled fish. Comparisons of behavioral studies are currently hampered by the inconsistent use and subtle differences amongst terminologies and methodologies (Bell et al. 2009; Toms et al. 2010; Garamszegi and Herczeg 2012). Also, potential ecological trade-offs associated with different behavioral strategies is not well understood for marine or juvenile fishes.

Aims and outline

This study explores the role of boldness and other personality traits in the ecology of settlement-stage coral reef fishes. **Chapters 2 and 3** explore the stability of behaviors in a newly-settled reef fish, *Pomacentrus amboinensis*. **Chapter 2** examines if relationships between behaviors are stable across different situations (i.e. presence of behavioral syndromes). Seven behavioral traits were compared within and among individuals across three situations in both field and laboratory settings. **Chapter 3** examines evidence for the presence of personalities (i.e. consistent behaviors within a single context). Here, I assess consistency of seven behavioral traits encompassing boldness, activity, and aggression using direct and video observation techniques in both the field and laboratory over various short-term (hours to days) time scales. **Chapter 4** aims to provide an objective analysis to improve the framework of personality studies by standardizing the current terminology and methodology. I objectively compared different methods of testing boldness against a measure of fitness by quantifying individual behavior of newly settled *P. moluccensis* using novel object and novel environment tests, then monitored survival for two days in the field under natural conditions. In conclusion, **Chapter 5** examines a potential ecological trade-off associated with a high risk or low risk behavioral trait (boldness-shyness). Using juvenile *P. amboinensis*, I investigate the link between boldness (i.e. propensity to take risks) and spatial learning. Learning to effectively use the environment to maximize foraging while minimizing predator capture is likely to be an important balance of a behavioral trade-off for juvenile reef fish.

Chapter 2: Syndromes or flexibility: Behavior during a life history transition of a coral reef fish

This chapter was published in PLoS ONE. DOI: 10.1371/journal.pone.0084262.

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2.1 Summary

The theory of behavioral syndromes focuses on quantifying variation in behavior within and among individual organisms and attempts to account for the maintenance of differences in behavior that occur in a consistent manner among individuals. Behavioral syndromes have potentially important ecological consequences (e.g. survivorship tradeoffs) and can be shaped by population dynamics through selective mortality. Here, we search for any evidence for correlations of behavior across situations in juveniles of a common damselfish, *Pomacentrus amboinensis* (Pomacentridae) at the transition between larval habitats in the plankton and juvenile habitats on the reef. Naïve fish leaving the pelagic phase to settle on reefs were caught by light traps and their behaviors observed using similar methods across three different situations (small aquaria, large aquaria, field setting); all of which represent low risk and well-sheltered environments. Seven behavioral traits were compared within and among individuals across situations to determine if consistent behavioral syndromes existed. No consistency was found in any single or combination of behavioral traits for individuals across all situations. We suggest that high behavioral flexibility is likely beneficial for newly-settled fish at this ontogenetic transition and it is possible that consistent behavioral syndromes are unlikely to emerge in juveniles until environmental experience is gained or certain combinations of behaviors are favored by selective mortality.

2.2 Introduction

The study of individual consistency in animal behavior has attracted recent attention from researchers because of the potential for this variability to reflect underlying processes influencing an animal's responses to a range of situations and for it to have significant effects on fitness (Sih et al. 2004a; Stamps 2007). Because behavioral syndromes seem to be so widespread taxonomically, an understanding of flexibility in these traits has important implications for the ecology and evolution of different species (Wilson et al. 1994; Wilson 1998; Dall et al. 2004; Sih et al. 2004a; Careau et al. 2008) and for predictions of how they will respond to environmental and ecological shifts (Sih et al. 2004a; Réale et al. 2007b).

Garamszegi and Herczeg (2012) define personality as occurring where consistency in single behaviors (e.g. individuals that display repeatedly higher or lower levels of boldness, exploration, or aggression than others in the population), and consistency in the relationship between two or more functionally different behaviors within the same individual is defined as a 'behavioral syndrome'. For example, a behavioral syndrome is evident in the correlation between boldness and aggression documented within individual sticklebacks (*Gasterosteus aculeatus*) (Bell 2005) and funnel-web spiders (*Agelenopsis aperta*) (Riechert and Hedrick 1993).

Most previous research has focused on explaining why individual variation in behavioral traits exists and why there should be consistency within an individual over time. The former question deals with factors maintaining variation in behavior among individuals from the same population, while the latter focuses on factors that maintain stability in behavioral traits (Stamps 2007). However, inconsistent use of terminology and methodology in previous work has led to similar behavioral traits being defined differently or distinct traits defined as equivalent across various animal behavioral studies, making comparisons difficult (Gosling 2001; Toms et al. 2010). Furthermore, there is a lack of fish studies that demonstrate consistent patterns of individual behavior by showing that multiple behavioral traits are correlated across multiple situations (especially in laboratory vs. natural settings). Additionally, few studies have used identical measures of behavior across situations. Perhaps unsurprisingly, these studies conclude that their target species showed little consistency in behavioral traits (Sinn and Moltschaniwskyj 2005; Wilson and Stevens 2005). Finally, a majority of fish behavioral studies have been conducted in the laboratory (Toms et al. 2010) on captive or captive-bred populations (Adriaenssens and Johnsson 2011). Although this is done to control for factors that could potentially confound results, it is extraordinarily difficult to create environments that approximate natural situations in the laboratory. Consequently, such studies assume that behavior of an animal in an artificial setting will be representative of its natural state. This assumption is rarely tested in the field (Brown et al. 2005).

Behavior can be influenced selectively by a wide variety of abiotic (temperature, illumination, habitat; (Brown et al. 2005; Frost et al. 2007; Biro et al. 2010) or biotic factors (hunger, thirst, stress; (Brown et al. 2005; Frost et al. 2007)) that can vary significantly through ontogeny (Bell 2005). These factors, along with experience gained as an individual grows, can lead to situations where it is beneficial for an individual to change their behavior to adjust to conditions (behavioral flexibility) or risk elimination from the population (selective mortality) (Brown et al. 2005). Group behavior also can influence changes in individual behavior through learning or conformity. For example, bold rainbow trout (*Onchorhyncus mykiss*) reduced boldness after observing shy conspecifics (Frost et al. 2007). The outcomes of research on associations between behavioral traits and behavioral flexibility (Adriaenssens and Johnsson 2011) have been inconsistent, although some studies have suggested that a tendency to display bold behavior increases an individual's ability to solve novel tasks (Dugatkin and Alfieri 2003;

Sneddon 2003), while others have shown that individuals that are more shy and unaggressive have more behavioral flexibility (Koolhaas et al. 1999).

Maintaining a certain degree of behavioral flexibility to suit changing environmental conditions is likely to be necessary for animals with complex life cycles that undergo life history transitions. The transition between these planktonic and benthic environments is a major developmental landmark for most coral reef fishes and makes these ideal organisms on which to investigate the relationship between adaptive behavioral traits and biotic factors. Furthermore, young reef fish arriving from the plankton into benthic habitats have no experience of their new environment. As such they make a useful model organism because these naïve juveniles enable researchers to control for learning behaviors through experience and examine behavioral consistency precisely at the time of settlement, which is a critical ontogenetic boundary and mortality bottleneck (Fuiman et al. 2010; Holmes and McCormick 2010; McCormick and Meekan 2010; Meekan et al. 2010; Lönnstedt et al. 2012). In this phase of their life cycle, reef fishes typically experience high mortality (Almany and Webster 2006), with rates within the first 48 hours averaging 57% (Doherty et al. 2004a; Almany and Webster 2006) but can be >90% (Gosselin and Qian 1997; McCormick and Meekan 2010). Behavioral decisions at early settlement can thus influence survival and possibly the structure of reef communities (McCormick and Meekan 2010; Lönnstedt et al. 2012). Thus, we expect juvenile reef fish to quickly adopt consistency in behaviors that are likely to influence survival at this life stage (e.g. boldness and aggression; (Fuiman et al. 2010)).

Our study examines whether naïve juveniles of the Ambon damselfish (*Pomacentrus amboinensis*) display consistent behavioral traits across three low-risk situations (i.e. whether they possessed behavioral syndromes). Because the same behavioral measures are used across different-sized laboratory arenas and within the field, this is one of the first studies to compare behaviors of fish observed in the laboratory with those in a natural setting. Furthermore, we investigated the role of an environmental factor (temperature) in influencing flexibility in behavioral traits. The relationships among behavioral traits across situations were also examined. If newly-settled fish display behavioral syndromes, then theory would predict that individuals should maintain a similar ranking of behavioral types (e.g. boldness) among situations.

2.3 Methods

Ethics Statement

This study was carried out in strict accordance with the recommendations under James Cook University (JCU) ethics protocols and approved by the JCU Animal Ethics Committee (Permit Number: A1067). All efforts were made to minimize animal handling and stress. Fish and coral collection was permitted by the Great Barrier Reef Marine Park Authority (Permit Number: G10/33784.1). Fish were collected using light traps and kept in flow through aquaria for the duration of the study where they were observed visually and subsequently returned to the field upon completion.

Study site and Species

Field experiments were conducted in the shallow coral habitats (2-4 m depths) at the back-reef of Lizard Island in the northern Great Barrier Reef (GBR) (14°40’S, 145°28’E). Juvenile *P. amboinensis* settle from the plankton at night to a variety of habitats in the northern GBR (Pitcher 1988) with the greatest densities found on small reef patches at the base of shallow (<10 m depth) reefs. *P. amboinensis* has a pelagic larval duration of 15-23 days and settles from 10.3-15.1 mm standard length (Kerrigan 1996). The juvenile body is mostly complete at settlement; however fish go through a rapid change in body pigmentation in less than 12 hours after settlement (McCormick et al. 2002). Previous studies have shown *P. amboinensis* is relatively site-attached (McCormick and Makey 1997) and moves only small distances (<1 m) during the first few months after settlement. Also, these damselfish can be collected immediately prior to the end of their larval phase before settling on the reef and thus are largely naïve to reef-based predators and behaviors learned after settlement. As experience can influence the behavioral phenotypes a fish will exhibit, using reef-naïve individuals reduces the variability that may result from markedly different experience histories. Due to its high abundance, small size, rapid development, and sedentary nature after settlement, *P. amboinensis* is an ideal model species for field observations and laboratory manipulations (Meekan et al. 2010).

Similar studies with *P. amboinensis* and *P. wardi* found individuals displayed consistent behaviors over multiple sampling periods across short time scales after settlement in the field (McCormick and Meekan 2010; White et al. 2015). This study examines if the behavioral consistency remains when individuals are observed within different situations.

Collection

We collected late-stage pelagic larvae of *P. amboinensis* using moored light traps (see small light trap in Figure 1 of (Meekan et al. 2001) for design) during January 2010. Fish caught in similar traps have been used in numerous published behavioral studies and individuals characteristically display considerable among-individual variability in behavioral traits such as boldness (Fuiman et al. 2010). Traps were anchored approximately 100 m from the nearest reef in ~16 m of water at dusk and left overnight. Catches were emptied from the traps the next morning at 0730-0800 hours. Fish collected from the traps were transported to the laboratory where *P. amboinensis* was separated from all other species and maintained in a 30 L aquarium of aerated seawater. Individual fish were then assigned a label and placed in separate small aquaria (13 L) for 24 h to acclimatize to laboratory conditions before experiments began during which time they were fed *Artemia* nauplii twice during daylight hours (1.5 mL^{1}; ~ 768 nauplii per fish per feeding). Fish were fasted for 12 h prior to initial observations. Seawater was unfiltered, therefore potentially contained food, although no feeding behaviors were observed outside of standard feeding times.

Situations

Standard behavioral observations (below) were conducted on each individual fish under three different environmental situations: small aquaria (13 L, 20 cm water depth), large aquaria (285 L, 25 cm water depth) and shallow reef (field). All were established so they represented a simple habitat in a low-risk environment with no competitors.

Firstly, behaviors of focal fish were observed in small aquaria (13 L) after 24 h acclimatization. Short pieces of PVC tubing (approx. 4cm diameter) in each tank provided a refuge for each fish. Observers viewed fish through slits cut into a blind (black plastic sheet) to reduce any effect of observer presence. Barriers of black plastic sheeting prevented individuals from seeing fish in neighboring tanks.

After behavioral observations in the small tanks, individual fish were then transferred to the center of large circular tanks (285 L) and given at least a 20 min acclimation period before another series of behavioral observations. A short piece of PVC tubing (approx. 4 cm diameter) and replica low complexity artificial coral (white molded resin branching coral, item no. 21505; Wardleys/TFH, Sydney; dimensions 14 x 11.5 x 5 cm) provided refuge for each fish. Observations were made from a distance of at least 1.5 m from the tank and fish took no apparent notice of observers.

A final series of observations of the same fish was made in the field. Each *P. amboinensis* was placed into a labeled 2 L clip-seal plastic bag containing aerated seawater and transported to the field. One fish was released onto each reef of an array of small (30 x 30 x 30 cm) patch reefs of live bushy hard coral (*Pocillopora damicornis*) on a shallow sand flat. Reefs were positioned 5 m apart and ~20 m from the nearest area of natural reef to avoid the re-dispersal of fish among reefs or colonization from natural reef. *P. amboinensis* naturally settles to this habitat. Prior to release of focal fish, patch reefs were cleared of all resident fishes using hand nets. Once fish were released, small wire cages (about 40 x 40 x 40 cm, 12 mm mesh size) were placed over the patch to allow the fish to acclimate to the new surroundings while being protected from predation. Cages were left 20 min and removed (carefully lifted with slow movements) immediately before observations. Divers conducted observations with the aid of a 2x magnifying glass from at least 1 m away to minimize any observer affects that may have been caused by close proximity to the target fish. This protocol has been used in other behavioral studies and has not been found to disturb fish or alter their behavior in any significant way (McCormick 2009; McCormick and Meekan 2010; Meekan et al. 2010; Fuiman et al. 2010).

While there were differences in habitat (e.g. variation in refuge size in different-sized arenas) and acclimation times among situations (small and large tanks and the field) these were mostly unavoidable trade-offs associated with logistical and efficiency issues typical of any empirical study. Once given time to habituate after initial capture, the juvenile fish are generally quick to adapt to new situations, hence the shorter acclimation times for the large tank and field situations. Each situation resulted in different conditions for the subject, but all provided a living space, shelter, and an absence of competition and larger predators. Also, the same testing stimuli were presented to the focal fish in all settings. The way the fish responds to the stimulus will be a product of how it behaves in its environment (its perception of risk is likely to be different between situations) and this will influence how the fish responds to the stimulus. There are many things that are associated with the situations that are different that may affect fish behavior. However, the main aim of the study is to look for consistency in the ranks of each behavior. This allows for plasticity to occur across situations based on the fishes' perception of risk.

In addition to behavioral data, water temperature in the field and laboratory was recorded every 20 min using calibrated data loggers (32K StowAway Tidbit).

Behavioral observations

Identical measurements of behavior were made on each fish under the three settings (small & large aquaria, field). Fish were fasted for 12 hours before initial observations and were observed in all three situations on the same day in order to minimize the behavioral influence of individual metabolism or food availability. We recorded: bite rate (the number of strikes towards objects floating in the water column during 3 min); distance moved (the total distance covered in cm during 3 min); distance ventured as the percentage of time spent at various distances from refuge (e.g. 90% of time spent 2 cm away, 10% of time 5 cm away etc.); height from substrate (an estimate of percentage of time spent in either the upper, middle, or lower third of the coral patch); aggression recorded as mirror strike rate (after the initial 3 min observation a mirror was carefully placed in front of the fish and the combined number of strikes, tail whips, or aggressive displays made toward their reflection over 3 min was recorded (Gerlai 2003; Marks et al. 2005); boldness (see below); latency (see below) to emerge from shelter after stimulus by a novel object (see below). The initial 7 behaviors were recorded during a 3 min observation, aggression was recorded in a separate 3 min trial, while boldness and latency were recorded in a separate 10 minute trial.

Boldness was defined as a continuous variable on a 0-3 scale, where 0 was hiding in a refuge before or immediately after introduction of a novel object and seldom emerging afterwards; 1 was retreating to refuge after a threat and taking more than 5 sec to re-emerge, then tentatively striking at food; 2 was retreating to refuge after a threat but emerging within 5 sec and tenaciously striking at food; 3 was not hiding but continuing to explore or strike at food aggressively. This scale was similar to other measures of boldness used in earlier studies and has been shown to be consistent over short time periods (hours) within individuals, normally distributed, and related to survival (McCormick 2009; McCormick and Meekan 2010; Meekan et al. 2010; Fuiman et al. 2010). The boldness score basically establishes a spectrum of risk perception to a stimulus and records where an individual lies within this spectrum.

Latency to emerge from hiding was recorded as the amount of time it took the fish to leave their refuge after introduction of a novel object. This variable was limited to a 10 min observation time. In both boldness and latency measures, the novel object was a lead bean sinker weight that was tied to clear fishing line and dropped from 1 m above the refuge in each setting. In the small and large aquariums, short sections of PVC pipe were suspended above each tank in order to consistently guide the lead weights over the center of each refuge. In the field setting, a PVC frame held a guiding section of pipe, to standardize and center the weight to a 1 m drop over the patch reef. The length of each line was calibrated to prevent the weight from hitting the substrate in order to reduce auditory or vibration cues. In the field setting, the weight was dropped while underwater, so it lacked the auditory and visual cues from the weight breaking the surface of the water. However, all fish in the field responded in some way to the weight drop. Observers triggered the release from either behind a blind (small aquaria setting) or from at least 1.5 m away (large aquaria and field settings).

Distances were estimated visually. A pilot study revealed visual estimates were within 10% of actual distances measured with a ruler. Two observers collected the data used in this study; both were trained by a more experienced observer. During training, inter-observer variation was less than 10% for all behavioral measures. Observations of behavior were aided by the use of a magnifying glass (2x). An observation time of 3 min was used to assess behavior since McCormick and Meekan (2010) found that this period produced low coefficients of variation (0-0.15) in behavioral observations (McCormick and Meekan 2010). Their study demonstrated consistency in behaviors among three consecutive 3 min observations, and White et al. (2015) demonstrated behavioral consistency of individuals (using the variables measured in our study) in a field situation over 2-3 days.

Data analysis

Height from substrate was recorded as the percentage of time an individual fish spent on the bottom (B), middle (M), or top (T) portions within or around the coral or PVC refuge. These values were transformed to a single, continuous mean variable using the formula: (B x 0/100) + (M x 5/100) + (T x 10/100). Similarly, distance ventured was recorded as the percentage of time spent 0 (A), 2 (B), 5 (C), or 10 (D) cm away from refuge. Data was transformed to a mean distance ventured using the formula: (A x 0/100) + (B x 2/100) + (C x 5/100) + (D x 10/100).

To examine whether individual fish changed behaviors across situations, but did so consistently, individuals were ranked and plotted for each behavioral trait and situation. Person's product-moment correlations were compared across situations for each trait and were also used to identify relationships between specific traits, as well as behavioral traits and water temperature for each situation.

Principal components analysis (PCA) was used to examine the inter-relationships of behaviors (bite rate, distance moved, distance ventured, height, boldness, latency, and aggression) and individual behavioral consistency across each situation. Parallel analysis was used to determine the number of factors to be extracted (using permutations of 1000 parallel generated datasets) (Budaev 2010). With the correct number of factors determined by the parallel analysis, principal component loadings were calculated using a correlation matrix with Direct Oblimin rotation (Budaev 2010). Hierarchical agglomerative cluster analysis (Quinn and Keough 2002) for the 7 behavioral variables was overlaid with the PCA in order to determine if fish behaved similarly within each situation. Euclidean distance and unweighted pair-group method using arithmetic averages (UPGMA) were used to calculate clusters. For the PCA and Pearson's product-moment correlations, the seven traits were $\log_{10}(x+1)$ transformed to improve normality. Analyses used SPSS (2011) software.

2.4 Results

A pilot study revealed that fish in the field began to explore their environment and feed within 1 minute of release onto patch reefs. Fish released into aquaria needed 20 mins to a few hours before exhibiting similar behavior (large and small aquaria respectively). The quicker acclimation time suggested that fish were less stressed and naturally inclined to start exhibiting “normal” behaviors in the field. There was no effect of time of day or observer on observed behavioral measures. All behavioral traits were highly variable both within and among individuals among settings.

Consistency among situations

There was no clear pattern of rankings of individuals for any of the behaviors such as boldness, distance moved and bite rate across situations (Fig. 2.1). A fish ranked highly for these traits in the small tank was just as likely to be medium or low ranked in the large tank and field situations. There was a significant positive correlation in height scores between small tanks and the field (r = 0.48, p = 0.004).

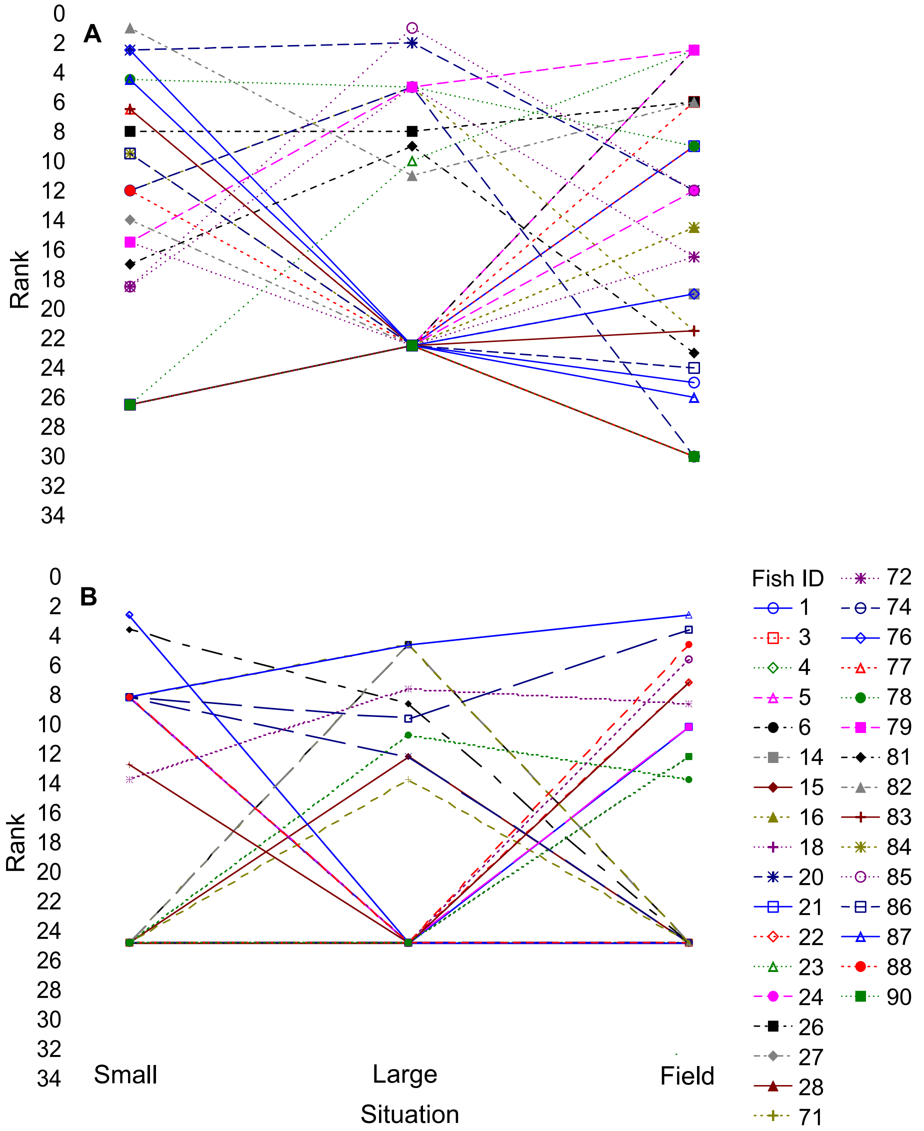

Figure 2.1. Rank order of boldness and height across situations. A) Ranking of boldness and B) ranking of height across small tanks, large tanks, and field site). Each line and unique symbol represents individual fish (N = 33). Individuals were ranked sequentially according to the individual's observed behavioral traits (1 = highest recorded value). Average ranking scores were assigned when multiple fish had a tie in values.

Situation-dependent relationships among behaviors

Principal component analysis showed distance moved and bite rate had similar loadings across PC1 for each situation (Fig. 2.2; Table 2.1). Other relationships between behavioral traits differed among situations (Figs 2.2 & 2.3). In small tanks, boldness and latency had nearly opposite loadings over PC2 (Fig. 2.2a), which differed across situations. In large tanks, distance moved, distance ventured, and boldness scores had similar correlations with PC1. Height rank and latency also showed similar loadings over PC2 (Fig. 2.2b). In the field, boldness and distance ventured had similar loadings on both PC1 and PC2 (Fig. 2.2c). Height rank and latency had similar loadings, yet with different strengths of correlation with PC1 and PC2 for observations within large tanks and in the field (Figs. 2.2b & c).

Hierarchical cluster analysis created overlapping groups when superimposed on the principal components analysis. Groups did not separate clearly by situation (Fig. 2.3a). We did not find any consistency in behavioral traits when comparing a single situation at a time (Fig. 2.2) or across all situations at once (Fig. 2.3b). Correlation analysis showed a pattern of an increasing number of significant correlations among measured behavioral traits from small tanks, field site, to the large tanks (Table 2.2). In the small tank and field situations, there were strong significant correlations between bite rate and distance moved. In large tanks, distance moved was correlated with distance ventured. Also, boldness was positively correlated with distance ventured. While in the field, bite rate was positively related to distance moved. Fish which ventured a greater distance also tended to have higher scores of boldness.

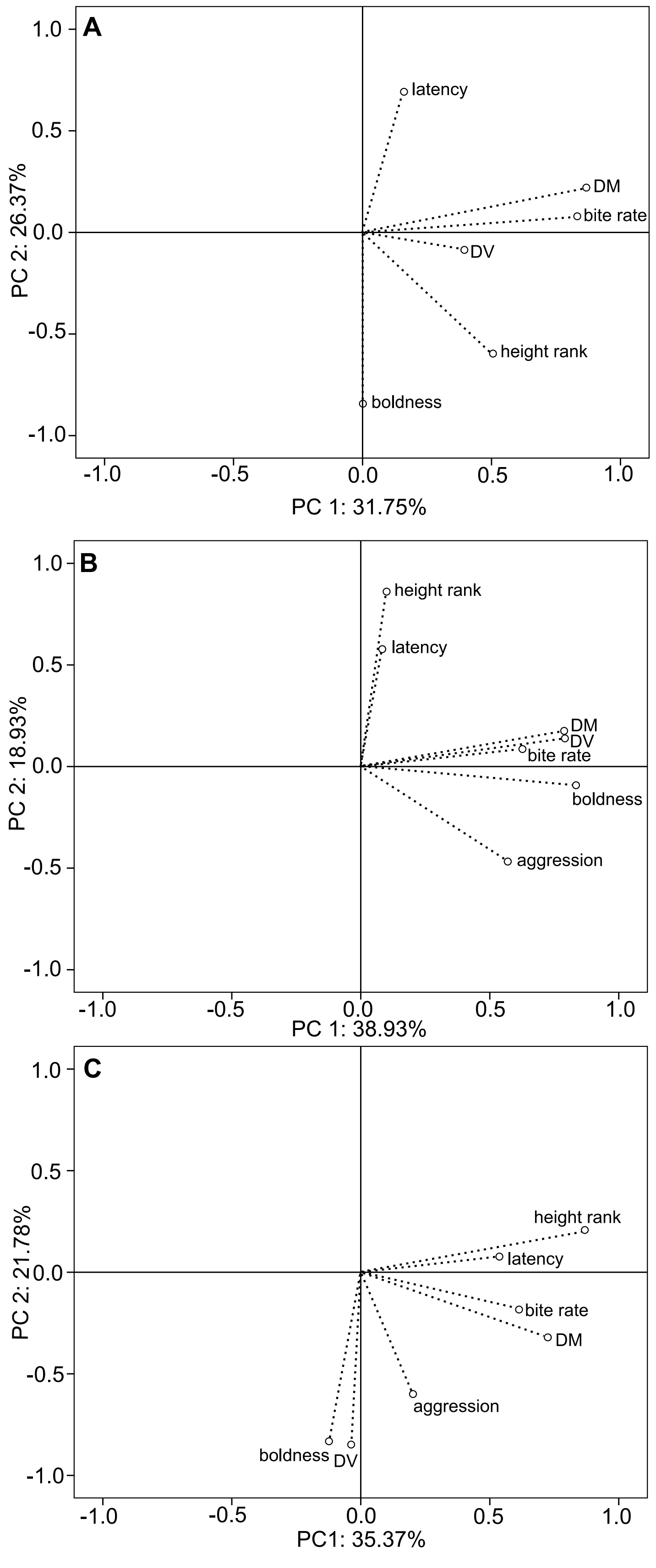

Figure 2.2. Principal component analysis of relationships between 7 behavioral traits. Traits include: bite rate, distance moved, distance ventured, height, boldness, latency and aggression in *P. amboinensis* in A) small tanks, B) large tanks and C) field situations.

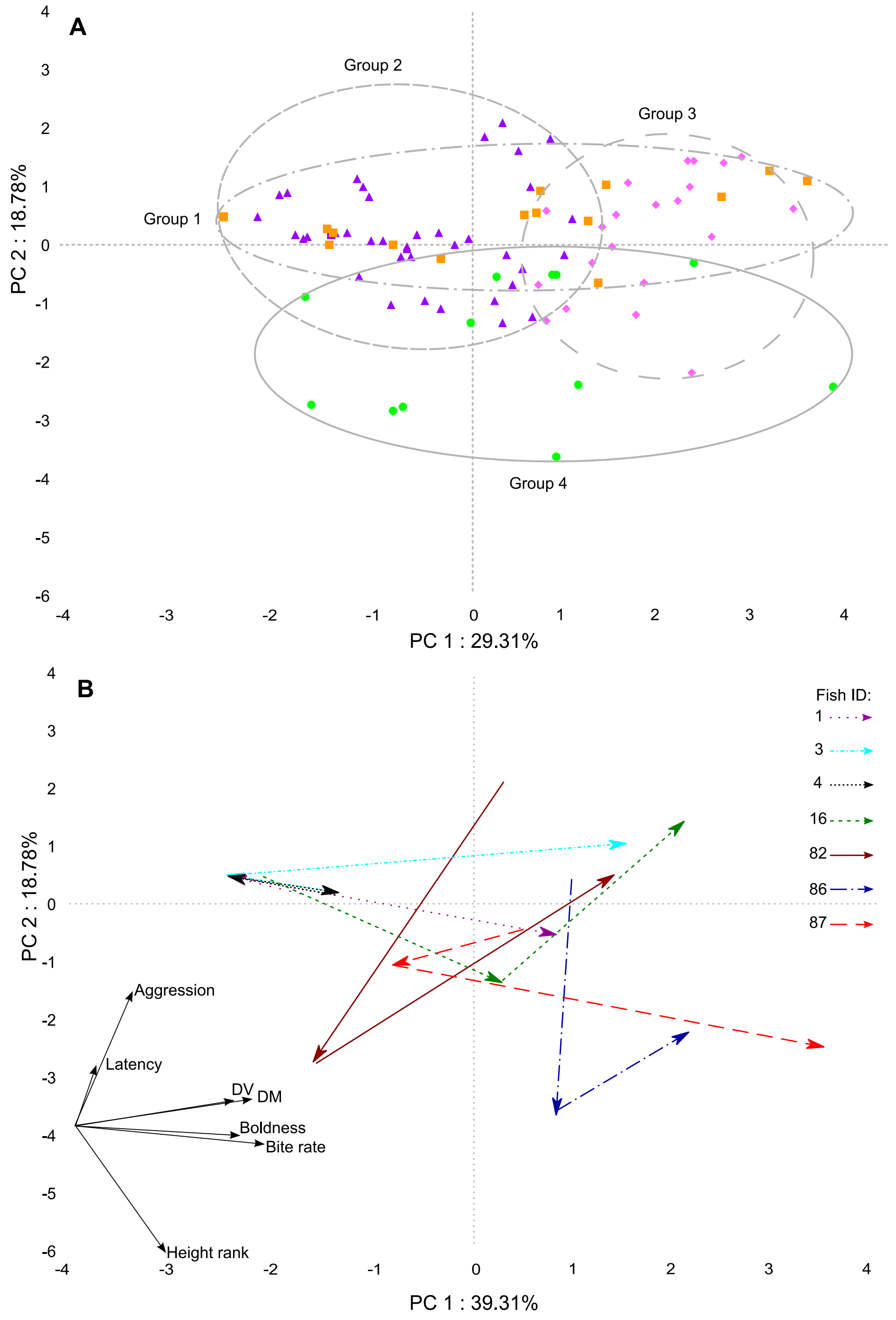

Figure 2.3. Principal component analysis of 7 behavioral traits for individual fish. A) PCA traits include: bite rate, distance moved, distance ventured, height, boldness, latency and aggression for individual fish (N = 33) across small tanks, large tanks, and field site (Total N = 99). Factor loadings of these traits represented by arrows in lower left corner. Ovals represent groupings created by clustering analysis. 67% of replicates in group 1 (square symbol) were from observations made in large tanks. 56% of replicates in group 2 (triangle symbol) were from small tanks. Group 3 (diamond symbol) is comprised of 76% of large tank observations. Group 4 (circle symbol) is composed of 42% large tank observations. Group 4 and the combination of groups 1, 2, and 3 represent the first split in the hierarchy. B) Identical principal components analysis with fish plots removed. Arrows represent factor loading patterns for seven randomly chosen fish from small tanks, large tanks, and field site.

Table 2.1. Factor components from PCA of behaviors in each situation.

	Component	
	1	**2**
Small tank		
Bite rate	0.832	0.079
Distance moved	0.868	0.220
Distance ventured	0.395	-0.086
Height rank	0.506	-0.596
Boldness	0.002	-0.843
Latency	0.161	0.692
Large tank		
Bite rate	0.627	0.086
Distance moved	0.788	0.175
Distance ventured	0.791	0.139
Height rank	0.100	0.861
Boldness	0.834	-0.092
Latency	0.083	0.578
Aggression	0.570	-0.468
Field		
Bite rate	0.614	-0.184
Distance moved	0.725	-0.321
Distance ventured	-0.037	-0.848
Height rank	0.869	0.208
Boldness	-0.123	-0.832
Latency	0.537	0.077
Aggression	0.203	-0.600

Table 2.2. Correlations between 8 behavioral traits of *P. amboinensis* in a) small tanks, b) large tanks, and c) field site. Only significant values are presented (p < 0.05). DM = distance moved, DV = distance ventured.

† Values of P do not control for multiple testing of the same data (*P<0.05; **P<0.01; ***P<0.001). Only values printed in bold are significant after Holm's sequential Bonferonni adjustment of experimental error rates (Quinn and Keough 2002).

a) †Small	Bite rate	DM	DV	Height	Boldness	Latency	Aggression
Bite rate		**0.54****					
DM							
DV							
Height rank					0.44*		
Boldness							
Latency							
Aggression							

b) †Large	Bite rate	DM	DV	Height	Boldness	Latency	Aggression
Bite rate		0.49**	0.38*		0.41*		
DM			**0.61*****		0.44*		
DV					**0.64*****		
Height rank							
Boldness							0.45**
Latency							
Aggression							

c) †Field site	Bite rate	DM	DV	Height	Boldness	Latency	Aggression
Bite rate		**0.52****		0.44**			
DM				0.42*			0.50**
DV					**0.56*****		0.39*
Height rank						0.35*	
Boldness							
Latency							
Aggression							

Table 2.3. Average temperature ranges during behavioral assessments within each situation (small tank, large tank, field situation).

	Temperature (°C)			
Situation	**Mean**	**Min.**	**Max.**	**Range**
Small tank	29.1	24.1	33.0	8.9
Large tank	29.4	24.6	33.6	9.0
Field	29.0	28.6	30.5	1.9

Effects of temperature

There were no consistent correlations across situations for the relationship between behavioral traits and temperature. There was a positive relationship between temperature and bite rate, and height rank in the small tanks. Aggression was negatively related to water temperature in the large tanks while distance ventured and maximum distance ventured were negatively correlated in the field. However, none of the relationships were significant after Holm's sequential Bonferonni adjustment (Quinn and Keough 2002). Overall, temperatures averaged 29°C, ranging between 24-33.6°C, however this varied slightly among situations (Table 2.3).

2.5 Discussion

A behavioral syndrome across individuals can appear as consistent trends in the direction of loadings in a principal component analysis and can also be demonstrated among individuals by multiple significant correlations among the same behavioral traits across situations (Sih et al. 2004a). *P. amboinensis* did not show any evidence of a behavioral syndrome (i.e. a suite of correlated behaviors across situations) based on these analyses, although the lack of behavioral stability is not necessarily surprising.

An important result of our study was the lack of consistency in the rank order of all behavioral traits for individual *P. amboinensis* across the three situations (small tanks, large tanks, field site). The definition of behavioral syndromes accounts for this type of flexibility across situations (Sih and Bell 2008) yet the premise of behavioral syndromes suggests some limitation of flexibility of behavioral responses (Conrad et al. 2011). Our results suggest that at this life history stage it is advantageous to remain highly flexible (Sih et al. 2004a; McCormick and Meekan 2010) in behavior, rather than to develop syndromes. Young fish at settlement undergo high rates of mortality (averaging ~ 60% within 48 hours, (Almany and Webster 2006)) due to their small size and relatively poor competitive abilities (Munday et al. 2001) and they must be prepared to adapt rapidly to novel conditions. For these reasons, the ability to alter behavior to suit the new challenges they face may be key to survival.

White et al. (2015) recorded consistent individual rankings in scores distance moved and occupancy height of reefs for newly-settled *P. amboinensis* over a three day period in the field. This finding suggests that behavioral patterns can be highly variable across different situations, yet at the same time show consistency within a single situation. Similarly, Coleman and Wilson (1998) found consistent individual boldness scores in two different contexts, but no correlation across contexts in juvenile pumpkinseed sunfish (*Lepomis gibbosus*) (Coleman and Wilson 1998). This implies that behavioral studies may have limited predictive ability when expanded to other situations; a finding that may be particularly relevant to laboratory-based work. Artificial environments can introduce variation in behavior due to confounding factors such as handling stress or experiences gained from life in captivity (Brown et al. 2005). For example, farmed fish that live in an environment of high competition and no predation pressure are often bolder, more aggressive and take more risks than their wild counterparts (Sundström et al. 2004; Dingemanse and Réale 2005). This idea was also supported by Wilson et al. (1993) who found individual boldness to be stable in nature but absent in the laboratory for juvenile pumpkinseed sunfish (*L. gibbosus*). They argued local environmental conditions maintain differences between individual behavioral phenotypes (Wilson et al. 1993). Our pilot study revealed that fish in the field have a reduced acclimation time compared to those held in aquaria, suggesting they are less stressed and naturally inclined to start exhibiting “normal” behaviors more quickly in the field. Studies in the field also have the added benefit of incorporating realistic environmental and ecological factors that may influence behavior (e.g. quantifying the ecological trade-offs of individual variation in behavior).

There were no significant correlations between behavioral traits and temperature for each of the three situations after correcting for multiple comparisons. Thus, despite large fluctuations in daily water temperature (up to 9°C and 2°C in the lab and field respectively), temperature did not meaningfully affect behavioral traits in any consistent manner. In a laboratory study using 6 L tanks filled halfway, Biro *et al.* (2010) found average values for activity, boldness and aggressiveness to increase by a factor of 2.5 to 6 in two species of damselfish in response to daily water temperature fluctuations of 3°C or less (Biro et al. 2010). McCormick and Meekan (2010) also found a significant positive relationship between activity and temperature in the field for *P. amboinensis* (McCormick and Meekan 2010). Metabolic rate has been shown to increase exponentially with temperature in other ectotherms (Clarke and Johnston 1999), and individual differences in metabolism are thought to contribute to individual differences in behavioral traits for these animals (Biro and Stamps 2008; Careau et al. 2008). In our study, bite rate showed a positive correlation (however this was not significant after Bonferonni adjustment) with temperature within small tanks (over twice as large as the aquaria used by Biro *et al.* 2010), which would seem to agree with Biro *et al*.'s (2010) findings. Perhaps if behavioral observations in the present study were conducted following the protocol of Biro *et al.* (2010) (only one situation, in very small aquaria), our results might concur. In any event, it is clear that correlations developed from laboratory studies require validation in field environments in order to confirm that they have real ecological meaning.

The most consistent relationships among behaviors across situations were the close positive relationship between bite rate and distance moved across both the principal components (PC1 & PC2). Similarly, the correlation analysis showed positive correlations between bite rate and distance moved across small tank and field situations. These relationships are intuitive because individuals move more often and at greater distances when in pursuit of planktonic food. These relationships probably reflect the active foraging style of damselfish. All other relationships between behavioral traits were inconsistent across all situations. This result agrees with previous work on sticklebacks, which found no consistency in boldness, aggression, and activity across different situations between two populations (Bell 2005). This suggests behavioral syndromes do not always fit within the theory of the “constraint hypothesis” (Stamps 1991) which states behavioral syndromes are derived from a shared link between behaviors and assumes that the decoupling mechanisms underlying correlated behavioral traits do not evolve readily, because they would require changes in hormonal machinery (Bell 2005). The different relationships of behavioral traits among situations suggests either that there are biotic or abiotic factors that influence certain traits in different situations (Bell 2005) or that the traits themselves can represent different things in different situations (e.g. the same measurement of boldness, or propensity to take risks, across different situations may in fact represent different traits due to how the individual perceives risk across the different situations).

It has been assumed that consistent individual differences in behavioral traits can occur due to differences in underlying physiological, behavioral, or morphological characteristics (i.e. state variables;(Houston and McNamara 1999; Stamps 2007)) and that these variables establish the efficiency of certain types of behavior (Dall et al. 2004). For example, if predation risk is a function of body size, and since body size is stable over short time scales (daily), animals of different body sizes should differ consistently with respect to their tendency to take risks while foraging. Therefore, theory predicts behavioral patterns related to body size should also be stable over the same time frame (Ambrose and Strimling 2006; Stamps 2007). The same logic applies to any other behavioral pattern linked to underlying state variables that are stable over time but vary among individuals (Dall et al. 2004) such as metabolic rate or stress responses. While state variables are likely important in establishing stable behaviors, individual fish can rapidly respond to environmental factors that influence the behavioral patterns displayed. Thus, environmental factors are likely to be just as important in developing or maintaining stable behavioral syndromes. Behavioral flexibility is likely necessary for fish to quickly adjust to a completely different environment once they leave their pelagic larval phase. Bell and Sih (2007) found only populations of sticklebacks raised under strong predation pressure developed a correlative relationship between aggressiveness and boldness (Bell and Sih 2007), demonstrating selective mortality and/or experience can help shape and establish behavioral patterns. Coral reef fishes may need some exposure to predators and environmental experience before developing a consistent behavioral syndrome.

In this study, naïve newly settled juveniles of *P. amboinensis* were found to lack consistency in: i) the rank order of behaviors across situations; ii) the relationship between behaviors and water temperature; iii) correlations in behaviors across situations. Given our results, it is interesting to note the lack of multi-situation or setting comparisons in previous studies that are often cited as evidence for the existence of behavioral syndromes. The vast majority of fish studies only measure one behavioral trait, or show correlation under a single situation/context. Evidence for behavioral syndromes and personality in fish is weakened by the lack of multi-situation or multi-context comparisons. For example, boldness is defined as risk-taking and aggression as agonistic interactions between individuals and correlations between these two traits are often cited as a demonstration of behavioral syndromes. However, the operational definition and methods used to assess boldness are quite varied (White et al. 2013b). In some instances, aggressive interactions could also be considered risky. In some previous studies, this relationship could be an artifact of overlapping behavioral traits, where boldness and aggression are characteristics of a single behavioral trait rather than two distinct traits, or alternatively inadequate measures, if methods used to assess boldness actually assess both boldness and aggression. These issues cannot be resolved from observations made in a single situation. Comparisons across multiple situations (ideally including a natural setting) are necessary to establish personality or behavioral syndromes with some degree of situation or context independence.

Chapter 3: Individual consistency in the behaviors of newly-settled reef fish

This chapter was published in Peer J. DOI: 10.7717/peerj.961.

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3.1 Summary

Flexibility in behavior is advantageous for organisms that transition between stages of a complex life history. However, various constraints can set limits on plasticity, giving rise to the existence of personalities that have associated costs and benefits. Here, we document a field and laboratory experiment that examines the consistency of measures of boldness, activity, and aggressive behavior in the young of a tropical reef fish, *Pomacentrus amboinensis* (Pomacentridae) immediately following their transition between pelagic larval and benthic juvenile habitats. Newly-settled fish were observed in aquaria and in the field on replicated patches of natural habitat cleared of resident fishes. Seven behavioral traits representing aspects of boldness, activity and aggression were monitored directly and via video camera over short (minutes), medium (hours), and long (3 days) time scales. With the exception of aggression, these behaviors were found to be moderately or highly consistent over all time scales in both laboratory and field settings, implying that these fish show stable personalities within various settings. Our study is the first to examine the temporal constancy of behaviors in both field and laboratory settings in over various time scales at a critically important phase during the life cycle of a reef fish.

3.2 Introduction

There has been considerable interest in, and evidence for, consistent patterns in the behaviors of individual animals within a species over the last decade (Dall et al. 2004; Sih et al. 2004a; Sih et al. 2004b; Dingemanse and Réale 2005; Bell 2007; Réale et al. 2007b; Smith and Blumstein 2008a; Bell et al. 2009). Differences in the amount of aggressive, exploratory and bold behaviors among individuals have been shown to be widespread and heritable (Boake 1994; Stirling et al. 2002; Kolliker 2005; van Oers et al. 2005a; Réale et al. 2007b) across a diverse array of taxa (Dingemanse and Réale 2005; Smith and Blumstein 2008a) and to influence survival (Downes 2002; Dingemanse et al. 2004), reproductive success (Both et al. 2005; Sih and Watters 2005; Pruitt and Ferrari 2011), resource acquisition (Webster et al. 2009) and growth (Biro et al. 2006; Meekan et al. 2010). Adopting a certain behavioral phenotype can have both costs and benefits, for example, highly aggressive female fishing spiders (*Dolomedes triton*) are more successful at acquiring food in a competitive environment, but this aggression can be detrimental in another context such as when it leads to precopulatory sexual cannibalism (Johnson and Sih 2005). Thus, consistent patterns in behavior among individuals can lead to trade-offs in aspects of fitness, which can ultimately influence population dynamics, community structure, and species diversity (Pruitt et al. 2013; Mittelbach et al. 2014).

Variation in consistent behavioral patterns among individuals have been variously (and interchangeably) termed ‘behavioral syndromes’, ‘temperament’, ‘personality’, and ‘coping styles’ (Dall et al. 2004; Réale et al. 2007b; Dingemanse et al. 2010b; Sih et al. 2012), although some authors have argued for a more restrictive use of terminology (Bell et al. 2009; Garamszegi and Herczeg 2012). Here, we adopt the definitions of Garamszegi and Herczeg (2012), where consistency in single behaviors (e.g. individuals that display repeatedly higher or lower levels of boldness, exploration, or aggression than others in the population) are described as displaying ‘personality’, and consistency in the relationship between two or more functionally different behaviors within the same individual is defined as a ‘behavioral syndrome’. For example, a behavioral syndrome is evident in the correlation between boldness and aggression documented within individual sticklebacks (*Gasterosteus aculeatus*) (Bell 2005) and funnel-web spiders (*Agelenopsis aperta*) (Riechert and Hedrick 1993).

Although the ability to alter behavior to suit changing environmental conditions is likely to be advantageous (Kelley et al. 2013), behavior is not infinitely plastic (DeWitt et al. 1998). If a single optimal behavioral phenotype existed, natural selection should reduce genotypic variation over generations (Réale et al. 2007b). Because behavioral phenotypes show heritable variation not eroded by selection (Penke et al. 2007; Réale et al. 2007b), different behavioral strategies are likely to have different associated costs and benefits (Kelley et al. 2013). For example, larger, bolder and faster-growing phenotypes of rainbow trout (*Oncorhynchus mykiss*) are more likely to be captured by fishing gears (Biro and Post 2008).

Estimating the consistency of a behavioral trait is necessary for measuring the repeatable characteristics of a focal organism, quantifying trait plasticity and determining trait heritability (Nakagawa et al. 2007). Historically, personality studies using a single assay were common, but it has been recently suggested that repeated tests are essential for any personality study (Réale et al. 2007b) and the strength of behavioral syndromes are likely underestimated when based upon single assays of varying traits (Adolph and Hardin 2007; Beckmann and Biro 2013; White et al. 2013b; White et al. 2013a).

Clearly, there is a need to determine the consistency of behaviors before examinations of personality, behavioral syndromes and associated trade-offs of alternative behavioral strategies can be attempted. Here, we examine evidence for personalities in a juvenile tropical reef fish, the Ambon damselfish (*Pomacentrus amboinensis*), by establishing the consistency of commonly-used field and laboratory assays of activity, aggression and boldness over time scales ranging from minutes to days following settlement. Similar to many reef fishes, young of this species can be collected at the end of their larval phase immediately prior to settlement on the reef, when they are naïve to reef-based predators and behaviors learned after settlement (Meekan et al. 2010). In this immediate post-settlement phase of their life cycle, reef fishes typically experience very high mortality (Almany and Webster 2006), with rates within the first 48 hours of benthic life averaging 57% (Doherty et al. 2004a; Almany and Webster 2006). Because experience can influence behavioral phenotypes (Budaev 1997; Bell and Sih 2007; Dingemanse et al. 2009), the use of naïve study organisms allows us to control for variation and consistency in behavior associated with experience and to examine ecologically important behavioral traits at a critical ontogenetic boundary (McCormick and Meekan 2010; Poulos and McCormick 2014). Because field measurements are made directly by an observer on SCUBA (where visual and auditory presence is not easily concealed), we tested for an effect of observer presence by comparing observed behaviors to those recorded by video-camera. Specifically, we aimed to determine if juvenile damselfish behaviors were: 1) significantly altered by observer presence; 2) consistent over various time scales (minutes, hours, days) relevant to their major mortality bottleneck (first 48 hours following settlement); 3) consistent in an aquarium setting; and 4) correlated between field and lab-based measurements. Based on our anecdotal previous experience with this system and study species, we predicted all behaviors to be moderately consistent in the field and laboratory.

3.3 Methods

Ethics statement

Fish collection locations/activities and handling protocols were approved by the Great Barrier Reef Marine Park Authority (Permit Number: G10/33784.1) and JCU Animal

Ethics Committee (Permit Number: A1720). All efforts were made to minimize animal handling and stress.

Study site and species

This study was conducted on the shallow reef (2-4 m depth) offshore from the Lizard Island Research Station (14°40’S, 145°28’E) on the northern Great Barrier Reef, Australia. Our study species, the Ambon damsel, *P. amboinensis*, is common on Indo-Pacific coral reefs (Beukers and Jones 1998). After approximately 20 days as pelagic larvae and at about 11 mm standard length (Wellington and Victor 1989), young fish settle from the plankton at night to reefs (Pitcher 1988). These fish preferentially choose to settle on live coral (McCormick and Weaver 2012) and settlement occurs predominantly between October and January around the time of the new moon (Meekan et al. 1993). Newly settled fish are found as solitary individuals associated with conspecific adults and sub-adults (McCormick and Makey 1997). *P. amboinensis* has a relatively small home range (Brunton and Booth 2003), moving only small distances (<1 m) during the first few months after settlement (McCormick and Makey 1997). Due to its high abundance, small size, rapid development, and sedentary nature, *P. amboinensis* is an ideal model organism for field and laboratory based behavioral studies (Meekan et al. 2010).

Experimental design

Collection

We collected newly-metamorphosed juveniles of *P. amboinensis* (McCormick and Makey 1997) using moored light traps (see small light trap of Figure 1 in Meekan et al. 2001 for design) during the October recruitment pulse. Different cohorts of fish were used for the different experiments. Traps were anchored approximately 100 m from the nearest reef in ~10 m of water at dusk and left overnight. Catches were emptied from the traps the next morning between 05:30-07:00 h. All fish collected from the traps were transported to the laboratory where *P. amboinensis* was separated from all other species and maintained in a 25 L aquarium (at densities < 100 individuals/25 L) of aerated seawater for 24 h to acclimatize to local conditions and reduce handling stress before experiments began. Fish were fed *Artemia* nauplii twice daily while in captivity. For field experiments, each acclimated *P. amboinensis* was transported to the field in individually-labeled clip-seal plastic bag. After final observations, study organisms were released unharmed on nearby natural habitat.

Observational protocol

Behavioral consistency in the field

All behavioral observations were made on individual fish in the field or aquaria in the laboratory using separate groups of fish for each assessment. Each *P. amboinensis* was placed into a labeled 2 L clip-seal plastic bag containing aerated seawater and transported to the field. Divers released an individual fish onto a small patch reef (30 x 30 x 30 cm) constructed from live and dead pieces of the bushy hard coral *Pocillopora damicornis* on the shallow (3-4 m water depth) sand flat. *P. amboinensis* recruits occur naturally in this habitat. Reefs were deployed in a single row, approximately 3 m apart, parallel to and 5 m from the nearest area of natural reef. Means and ranges of temperatures did not vary among reefs or among aquaria (M. McCormick unpubl. data) and care was taken in reef construction to ensure that patch reefs had only very minor differences in habitat structure. Previous studies have shown that such minor variation in topographic complexity of patch reefs has no effect on behavior of young fish (McCormick and Meekan 2010; Meekan et al. 2010). Before introduction of the study fish, patch reefs were cleared of any resident fishes using hand nets. These were released on nearby natural reef far enough away to prevent their return (approx. 10 m).

Individual study fish were then released onto their respective patch reefs and the first behavioral variable (latency to enter a novel environment; see description below) was recorded. Immediately afterwards, small wire cages (about 30 x 30 x 30 cm, 12 mm mesh size) were placed over the patch to allow the fish to acclimate to the new surroundings while being protected from predation. Cages were left a minimum of 20 min and carefully removed immediately before observations. Following established protocols outlined below (McCormick and Meekan 2010; Meekan et al. 2010; White et al. 2013b), divers conducted observations from at least 1 m away (with the aid of a 2 x magnifying glass) to avoid any effects that may have been caused by the proximity of the observer to the target fish.

Short term consistency

Three behavioral measures of activity were recorded simultaneously over a 3 min observation interval for each fish (n = 18) during October 2009: bite rate (number of feeding strikes towards objects floating in the water column); distance ventured (DV; the maximum distance in centimeters fish moved away from their patch reef) and; height on the reef (categorized as a cumulative proportion of the time spent at varying heights over the 3 min observation period, with the top of the patch taken as height of 1, middle of the patch a height of 0.5, and bottom a height of 0). Relative height on the patch was summarized as a cumulative proportion of the time spent at varying heights over the 3 min observation period, calculated from the sum of the proportions multiplied by the height categories (0, 0.5, or 1). Following the 3 min interval, a 30 x 30 cm acrylic mirror (mounted on a 1 m PVC pole) was gently placed 10 cm in front of the focal fish. After a 1 min acclimation period, two scores of aggression were recorded as latency until first strike (‘attack latency’) and ‘mirror strike rate’ (combined number of strikes or tail whips) made toward their reflection over 3 min was recorded (Gerlai 2003;

Marks et al. 2005). To examine the level of behavioral consistency over a 2 hour period, the entire suite of behavioral assays were repeated three times with 30 min between observations over a single day.

Consistency over multiple days in field

A separate sample of fish (n = 21) was used to assess behavior over multiple days in October 2012. Observations were made 3 times each day (at 9:00, 12:00, 16:00 h) for each of 3 days giving a total of 9 repeated observations per individual. During each observation, activity (bite rates, distance ventured (DV), and height) was recorded as described earlier.

Observer vs. video

To assess if there were any effects of observer presence, behaviors were recorded with a GoPro Hero 2™ high definition video camera (720p resolution) and compared against observer scores (n = 29) using fish collected in October 2012. The camera was placed 30 cm from focal fish and left to record for 10 min. The first observation was a 3 min period of the behaviors recorded by the observer (1 m away) and camera simultaneously. The second observation was the last recorded 3 min of video (without an observer present). For analysis, this provided three data sets for every fish: 'observer', the 'simultaneous video' recorded at the same time as the direct observation, and the 'video' recording without observer presence. Because of the difficulty in discerning distance in the video, only bite rates and height (see below) were recorded and observations in which fish moved out of view of the camera for more than 20 sec in total were discarded. Although the recording of observations (observer, simultaneous video and video) in the same order could have potentially introduced a habituation effect, we followed this protocol because it minimized disturbance to fish.

Behavioral consistency in the laboratory

Short term consistency

Individual fish (n = 10) were assessed for boldness during the 2012 field season using a variation of a common test, latency to emerge from a shelter (Budaev 1997; Fraser et al. 2001; Brown et al. 2005; Chang et al. 2012). Each fish was gently transferred via hand net into an opaque ~162 cm^3 plastic holding chamber within an aquaria (13L, 20 cm water depth) that also contained a small refuge of live *Pocillopora damicornis* at the opposite end and allowed to acclimatize for 30 min. The holding chamber was believed to be of adequate size because the fish displayed no apparent signs of confinement stress. The sides of each aquarium were blacked out with plastic sheeting to isolate them from neighboring tanks. After acclimation, observers standing behind a blind (black plastic sheeting) gently revealed the opening to the holding chamber. Time to emerge ('latency to emerge'; defined as more than half of the body length outside of the holding chamber), was recorded for each fish with a cut-off time for the observation of 180 s. Location (categorized as a cumulative proportion of the time spent in various sections of the aquaria, with the third of the aquaria with the chamber given a value of 1, middle third of the aquaria a value of 0.5, and the third with coral refuge a value of 0) was recorded in the 5 minutes following emergence. A location score was calculated from the sum of the proportions multiplied by the location categories. Here, a lower location score represents a bolder fish. To get to the coral refuge they must exit the chamber and swim across the length of the aquaria, while a shyer fish would not risk leaving the chamber. Aggression was tested by gently placing an acrylic mirror (30 x 15 cm) upright on the back wall of the aquaria, with the aquaria orientated lengthwise to the observer. Traits of aggression were measured in the same manner as in the field, as outlined earlier. Water flow was shut off during the acclimation period and behavioral observations to reduce auditory disturbance, but a gentle air flow through air stones was maintained to ensure adequate dissolved oxygen levels. Fish were fasted for 12 h before trials and fed *Artemia* upon completion to prevent varying hunger levels of individual fish potentially confounding behaviors. Assays were repeated 3 times over a 2 h period throughout a single day.

Field vs. laboratory

One sample of fish (n = 32) was compared across field and aquaria settings in 2012. In the morning (9:00) *P. amboinensis* within 2 d of capture by light traps were assessed for boldness (latency to emerge and location) and aggression (attack latency and strikes) in aquaria as described above. If fish did not emerge from the chamber, it would be unlikely to approach a mirror, potentially cofounding these measures. However, this was only an issue for one fish which was removed from data analysis. Later that afternoon (13:00) they were assessed for release latency, bite rate, distance ventured, height, and aggression (attack latency and mirror strike rate) in the field as described earlier. After resident fish were cleared from the patch reefs, each damselfish was carefully released from the plastic bag onto the sand 10 cm from the patch reef. Latency to emerge was the amount of time it took for the fish to move onto refuge of the patch reef and was timed from the moment the fish exited the bag, to the instant it reached the edge of the reef shelter. In both the field and lab measures, fish are seeking out coral refuge after emerging from a plastic bag or PVC shelter, respectively.

Data analysis

For all fish (total n = 110), consistency was calculated with a repeatability score (R), defined as the intra-class correlation coefficient (ICC), representing the fraction of total variation in a set of measurements attributable to the variance among individuals (Wolak et al. 2012). R was calculated by constructing a general linear mixed model with individual (ID) included as a random factor in a one-way analysis of variance (ANOVA) model, with the transformed behavioral score as the dependent variable. All scores were $\log_{10}$ (x+1) transformed to meet the assumption of normality and linearity. The ratio of variance explained by among-individual variance to total variance calculated from an ANOVA represents a common measure of repeatability of each behavior (Lessells and Boag 1987). Confidence intervals (CI) around each repeatability estimate were calculated using the exact confidence limit equation in Searle (1971), which has been shown to be precise for this type of dataset (Donner and Wells 1986; Wolak et al. 2012). The R value indicates the strength of repeatability and ranges from 0 to 1, with values approaching one indicating high repeatability (Briffa and Greenaway 2011). The p-value associated with the ANOVA is then used to determine if repeatability is significantly greater than zero (Lessells and Boag 1987).

Relationships between behavioral traits observed in the field and aquaria were analyzed using Pearson's product-moment correlation. All scores were $\log_{10}$ (x+1) transformed to improve normality. Statistical analysis used SPSS version 20.0 (SPSS Inc., Chicago, IL, U.S.A.).

3.4 Results

Short term consistency in the field

In the field, activity measurements (bite rate, DV, and reef height) were highly repeatable, with repeatability scores between 0.52 and 0.69 (n = 18, Table 3.1). The aggression measures (attack latency and mirror strike rate) decreased over time and were not significantly repeatable. By the third observation, fish did not respond to their reflection aggressively at all, suggesting that they became habituated to the mirror.

Consistency over multiple days in field

Fish sampled three times a day for 3 days also displayed activity (bite rate, DV, and height) behaviors that were moderately to highly consistent (n = 21, R = 0.33 to 0.77; Table 3.1).

Observer vs. video

Observer and simultaneously collected video data were very consistent (n = 29, R = 0.46 bite rate, 0.76 reef height: Table 3.1), as were the two video observations (n = 29, R = 0.69 bite rate, 0.89 reef height; Table 3.1).

Short-term consistency in the laboratory

The measure of boldness (i.e., latency to emerge) and location after emergence were moderately consistent (n = 10, R = 0.38 and 0.54 respectively; Table 3.1).

Table 3.1. Repeatability (R) values with 95% confidence intervals (CI) for various measures of boldness and activity for juvenile Ambon Damselfish (*Pomacentrus amboinensis*). For the observer vs. video section, the human observation is labeled 'observer,' the simultaneous video camera recording 'simultaneous video,' and the independent video recording 'video.'

Trait	**R**	**p**	**R CI Low**	**R CI High**
Field				
Short term consistency (n = 18)				
Bite rate	0.64	<0.001	0.39	0.83
Distance ventured	0.69	<0.001	0.46	0.86
Reef height	0.52	<0.001	0.24	0.76
Aggression latency	0.20	NS	0.07	0.52
Aggression strikes	0.20	NS	0.07	0.52
Multiple days (n = 21)				
Bite rate	0.77	<0.001	0.64	0.88
Distance ventured	0.62	<0.001	0.45	0.79
Reef height	0.33	<0.001	0.16	0.55
Observer vs. video (n = 29)				
Observer vs. simultaneous video				
Bite rate	0.46	0.005	0.13	0.71
Reef height	0.76	<0.001	0.56	0.88
Simultaneous video vs. video				
Bite rate	0.69	<0.001	0.45	0.84
Reef height	0.89	<0.001	0.79	0.95
Laboratory				
Short term consistency (n = 10)				
Latency to emerge	0.38	0.026	-0.004	0.76
Location	0.54	0.003	0.16	0.84

Field vs. laboratory

There were only two significant correlations between field and laboratory-based measurements of behavior, with a moderate positive correlation between latency to emerge values in the field and the lab (n = 32, r = 0.35, p = 0.049; Table 3.2) and between field and lab measures of aggression latency (n = 32, r = -0.385, p = 0.030; Table 3.2). The other variables (i.e. measures of location and aggression) showed no evidence of consistency between laboratory and field measurements, suggesting that the behaviors are context dependent and laboratory measures have little relevance to field studies.

Table 3.2. Pearson's product-moment correlations between field and laboratory measures of boldness and aggression for juvenile Ambon damselfish (*Pomacentrus amboinensis*). All data (n =32) was $\log_{10}$ (x+1) transformed.

Trait	**Field L**	**Field BR**	**Field DV**	**Field height**	**Field AL**	**Field ASR**
Lab L	0.350*	0.169	0.110	-0.102	0.027	0.202
Lab Location	0.189	0.094	-0.147	0.044	0.156	-0.272
Lab AL	0.144	-0.079	0.067	0.227	-0.385*	-0.090
Lab ASR	-0.088	-0.051	-0.016	0.172	-0.262	-0.037

Note. L = latency, BR = Bite rate, DV = Distance ventured, H = Height, AL = Aggression latency, ASR = Aggression strike rate, * = statistically significant at $p < 0.05$ level.

3.5 Discussion

Our study is one of the most detailed assessments of behavioral consistency of a marine organism to date. It shows that shortly after entering a new habitat at the end of their larval phase fish approximately three weeks old already have a complex repertoire of behaviors that are displayed in a consistent way through time, indicative of the existence of individual personalities. Moreover, this personality appears to be established prior to or immediately upon metamorphosis and settlement. Factors that are likely to favor consistent over conditional behavior, and thus give rise to individual personalities are diverse and include: genetic, physiological or developmental limits, costs of flexibility, costs and availability of information acquisition, metabolism, body size, or constraints on behavioral plasticity (Sih et al. 2004a; Bergmuller et al. 2010; Briffa and Greenaway 2011). Stable behavioral states are hypothesized to be created when positive feedback loops form between state variables such as size, competitive ability, or condition and state-dependent behavioral decisions (Dall et al. 2004; Sih and

Bell 2008). For example, individuals with higher body condition may be more cooperative compared to those in poorer condition because they can afford the energy expenditure. If cooperative behavior then led to increased energy gains, this feedback loop would maintain higher body condition (Bergmuller et al. 2010). Naïve juvenile reef fish exhibiting personalities at settlement suggests a genetic component and strong trade-offs related to adopting alternative personalities. High mortality rates at this phase of their life cycle could provide very strong selective force and are most likely to be involved (McCormick and Meekan 2010).

Generally, our study found moderate to highly repeatable behavioral scores for almost all behavioral measures. These ranged from 0.33 (height on the habitat patch across multiple days) to 0.89 (height across camera observations), values well within the range recorded by earlier studies. A recent meta-analysis by Bell et al. (2009) reported an average repeatability value of 0.37 in various behavioral traits across 114 studies and 98 species. They found mating, habitat selection and aggression to be the most repeatable traits; while activity, mate preference, and migration were the least repeatable. Consistency was generally higher for behaviors measured at closer time intervals, juveniles compared to adults and field studies versus laboratory settings (Bell et al. 2009). Approximately 70% of this distribution was between 0.1 and 0.6 (see Fig. 1, Bell et al. 2009). An additional 11 studies published more recently (Réale et al. 2000a; Smith and Blumstein 2008a; Briffa and Greenaway 2011; Marras et al. 2011; Couchoux and Cresswell 2012; Carter et al. 2012; Beckmann and Biro 2013; Neumann et al. 2013; Pruitt et al. 2013; Kelley et al. 2013; Burtka and Grindstaff 2013) reported repeatability scores ranging from as low as 0.14 for a measure of aggression in male crested macaques (*Macaca nigra*) (Neumann et al. 2013) to as high as 0.92 for a measure of escape response in European sea bass (*Dicentrarchus labrax*) (Marras et al. 2011). Despite the wide range in these scores, they were cited as evidence of the consistency of behaviors and therefore personalities. On this basis, the repeatability scores we obtained suggest evidence for personality in the 3-week old damselfish that were the subjects of our study.

Large confidence intervals around a repeatability estimate suggest significant within-individual variation in behavior (Jones and Godin 2009). While juvenile damselfish are known to adopt a wide range of behavioral strategies (White et al. 2013b; White et al. 2013a), some of the variation we recorded may be due to plasticity in the amount of habituation to the experimental protocol (Martin and Réale 2008). Across repeated trials, an environment or test may become less novel and individuals may habituate to novelty in itself (Réale et al. 2007b; Edwards et al. 2013), or alternatively become less responsive or sensitized (Budaev 1997; Martin and Réale 2008; Kelley et al. 2013). In our study, the tests that involved an experimental set-up, such as laboratory-based measurements of boldness (e.g. latency to emerge), have some of the largest confident intervals. However, given our significant repeatability estimates, we are confident all the measures reported are reliable measures of an individual’s behavior within these contexts.

Variables that originated from the aggression assay (strike latency and mirror strike rate) were the only measurements found not to be repeatable through time or context. This suggests the moderate negative correlation found between field and laboratory measures of aggression strike latency is likely to be ecologically irrelevant. While a commonly-used test (Gerlai 2003; Marks et al. 2005), these measures may be susceptible to the habituation effect discussed above. A closely-related species, *P. moluccensis*, has been shown to recognize threats after a single exposure (Mitchell et al. 2011). Perhaps *P. amboinensis* similarly learns to ignore the false threat of their reflection after repeated exposures.

Observations repeated over short time scales (4 min apart, simultaneous video vs. video observations) had the highest repeatability scores. Measures conducted over longer (30 minutes apart and 3 times daily over 3 days) time periods had similar, but lower scores. This agrees with results from a meta-analysis, which showed higher estimates of repeatability for behaviors measured at shorter time intervals (Bell et al. 2009). Our results suggest juvenile damselfish quickly adopt stable behavioral phenotypes regarding foraging and activity rates following settlement and remain consistent throughout the intense predation pressure experienced during the first few days on the reef.

There was a trend for repeatability estimates obtained in the laboratory to be lower compared to field-based measurements. This same pattern was found in Bell et al.'s (2009) meta-analysis. If there are advantages to behaving consistently (Dall et al. 2004; McElreath and Strimling 2006), then the greater environmental variance in the field might create micro-niches, increasing repeatability by allowing individual expression of behavioral variations (Bell et al. 2009). Also, because juveniles are exposed to innately higher predation pressure in the field, this could act as a directional or stabilizing selection on behavior (Bell et al. 2009). However, in this study fish are initially naïve and neophobic upon introduction to the field (Meekan et al. 2010; Chivers et al. 2014; Ferrari et al. 2015), so perhaps the greater sensory input in the field environment is enough to act as a stabilizing influence. For example, three-spined sticklebacks (*Gasterosteus aculeatus*) adopted stable boldness-aggressiveness correlations once exposed to predators (Bell and Sih 2007). Juvenile damselfish quickly learn about predators (Mitchell et al. 2011) and are likely to swiftly adopt a consistent behavioral phenotype when faced with the variations and challenges of their natural habitat. Given the few and weak correlations found between field and laboratory measures, and lower consistency for laboratory studies suggests inferences about natural behaviors in the field derived from laboratory studies need to be made cautiously (White et al. 2013a). The lack of predators and increased novelty of the laboratory environment may enable juvenile damselfish to exhibit a great variability of behaviors or prompt different behavioral responses that have little bearing on likely behavior under natural conditions. This implies using laboratory measures to predict behaviors in the field must be done cautiously (White et al. 2013b; White et al. 2013a).

Interestingly, Beckmann and Biro (2013) reported repeatability values almost identical to ours for the same laboratory-based boldness measure. They tested two species of juvenile damselfish (*P. wardi* and *P. amboinensis*) and showed repeatability in the emergence latency test in home tanks (R = 0.42 for *P. amboinensis* on the third observation), but no correlations when compared against the same and different behavioral tests in different contexts. Others have also argued for the use of multiple measures of boldness in order to obtain an ecologically relevant assessment of this behavioral trait (White et al. 2013b), and have also found a lack of behavioral consistency across situations (White et al. 2013a) for juvenile damselfish. While Beckmann and Biro (2013) argue the lack of correlation across contexts means this assay is inadequate to measure boldness, their study likely had issues with habituation (Edwards et al. 2013). In contrast, we found latency to emerge behavior to be significantly repeatable within a single context and moderately positively correlated with an emergence test in the field.

Another important result of our study was that the presence of observers seemed to have no significant impact on fish behavior. While fishes are the focus of much behavioral research, they are rarely observed in their natural environments (Réale et al. 2000a; Bell et al. 2009). Typically, observations in a field situation would be conducted from behind a blind (Martin and Bateson 2007), a luxury not afforded to a noisy bubbleblowing SCUBA diver. While the simultaneous observer and video observations had slightly lower repeatability scores for bite rate and height compared to the comparison of the two video scores (difference of 0.23 and 0.13, respectively), this is most likely an artifact of the difficulties associated with observing detailed behavior via camera. Even with high resolution video, it was difficult to distinguish between feeding strikes and the natural stop-start swimming of these fish. Also, fish leaving the field of view of the camera for a short duration was not an issue for the diver who could maintain visual contact with the target fish at all times. Overall, discrepancies between the methods of observation may have resulted in a slight over-counting of bite rates in the video. This suggests video data is less useful for subjects such as these small damselfish that are quick moving and very mobile. As long as slow, deliberate movements are employed and the observer remains a least a meter away, juvenile damselfish seem indifferent to human presence thus diver observations provide useful records of behavior.

In summary, our results demonstrate that measures of boldness and activity, both in the field and the laboratory, are highly repeatable over time scales relevant to this species during a key period of their life history. These stable behaviors indicate that these 3-week old juvenile fish already have personalities. From a methodological perspective, our results indicate that an initial 3 min assessment of their behavior provides a useful record of an individual's personality. However, caution is required when comparing field and laboratory based behaviors (White et al. 2013b). Future studies with this species can reasonably use a single (i.e. unrepeated) assay to reduce animal stress, which can then be correlated with physical measures of performance and success to determine how individual characteristics combine to affect fitness. Future research will investigate if adult *P. amboinensis* retain this behavioral consistency through ontogeny.

Chapter 4: A comparison of measures of boldness and their relationships to survival in young fish

This chapter was published in PLoS ONE. DOI: 10.1371/journal.pone.0068900.

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4.1 Summary

Boldness is the propensity of an animal to engage in risky behavior. Many variations of novel-object or novel-environment tests have been used to quantify the boldness of animals, although the relationship between test outcomes has rarely been investigated. Furthermore, the relationship of outcomes to any ecological aspect of fitness is generally assumed, rather than measured directly. Our study is the first to compare how the outcomes of the same test of boldness differ among observers and how different tests of boldness relate to the survival of individuals in the field. Newly-metamorphosed lemon damselfish, *Pomacentrus moluccensis*, were placed onto replicate patches of natural habitat. Individual behavior was quantified using four tests (composed of a total of 12 different measures of behavior): latency to enter a novel environment, activity in a novel environment and reactions to threatening and benign novel objects. After behavior was quantified, survival was monitored for two days during which time fish were exposed to natural predators. Variation among observers was low for most of the 12 measures, except distance moved and the threat test (reaction to probe thrust), which displayed unacceptable amounts of inter-observer variation (average difference of 12 cm and 1 point of a 3 point scale, respectively). Overall, the results of the behavioral tests suggested that novel environment and novel object tests quantified similar behaviors, yet these behavioral measures were not interchangeable. Multiple measures of behavior within the context of novel environment or object tests were the most robust way to assess boldness and these measures have a complex relationship

with survivorship of young fish in the field. Body size and distance ventured from shelter were the only variables that had a direct and positive correlation with survival.

4.2 Introduction

The propensity of an animal to take a risk is often described along an axis of boldness and shyness, where high likelihood of risk-taking is defined as boldness and low likelihood is defined as shyness. This behavior is important on both ecological and evolutionary time scales. Individuals can display various levels of boldness or shyness that can influence the outcome of everyday ecological challenges, such as competition for females (Dugatkin and Alfieri 2003) or food (Dingemanse et al. 2004), foraging under predation pressure (Dugatkin 1992; Biro et al. 2006; Stamps 2007) and habitat selection (Wilson et al. 1993; Budaev 1997). Consequently, boldness and shyness can influence reproduction, survival and thus ultimately affect fitness (Smith and Blumstein 2008a). Boldness may have underlying physiological components and may be heritable (Boissy 1995; Koolhaas et al. 1999; Brown et al. 2007a), so can be subject to evolution following natural selection in subsequent generations (Réale and Festa-Bianchet 2003). Measurements of boldness dominates research on animal behavior (Toms et al. 2010). Garamszegi and Herczeg (2012) define personality as occurring where consistency in single behaviors (e.g. individuals that display repeatedly higher or lower levels of boldness, exploration, or aggression than others in the population), and consistency in the relationship between two or more functionally different behaviors within the same individual is defined as a ‘behavioral syndrome’. Unfortunately, attempts to generalize the results of this work are hampered by a lack of common language and methodology (Gosling 2001; Toms et al. 2010). For instance, some studies have defined boldness as the tendency of an individual to move through or explore an unfamiliar space (i.e. a novel environment) (Fraser et al. 2001; Wilson et al. 1994; Budaev 1997), while others consider it the propensity to forage under predation risk (Budaev and Brown 2011) or alternatively, reaction to a novel object (Wright et al. 2006). Additionally, researchers have used a variety of behavioral attributes to measure boldness (Budaev and Brown 2011), such as latency to emerge into a novel environment, frequency of predator inspection (Dugatkin 1992; Budaev et al. 1999), propensity to enter traps (Wilson et al. 1994), or flight response to a novel object (Wilson et al. 1994; Frost et al. 2007). These measures may have some relation to one another (i.e. correlated behavioral measures within or across certain contexts), but do not necessarily quantify the same behavioral trait (Réale et al. 2007b). Recent attempts have been made to address this issue with proposed standardized terminology (Réale et al. 2007a; Budaev and Brown 2011), however this has yet to be adopted universally.

The techniques used to measure boldness are almost as numerous as the studies that have assessed this trait in different taxa. Some researchers have argued that boldness should be tested in familiar, rather than novel environments (Réale et al. 2007a) and to date, only a few studies have attempted to quantify behavior using multiple tests of boldness among individuals. For example, Wilson and Goden (2009) assessed individual differences in exploratory behavior, activity, and anti-predator behavior of juvenile sunfish using novel object and environment tests in the laboratory (Wilson and Godin 2009), while an earlier aquaria study by Brown et al. (2007) found a strong correlation between two independent assays of boldness (time to emerge into a novel environment and propensity to inspect a novel object) in a peociliid fish (Brown et al. 2007b).

Due to the great variety of techniques used to quantify boldness, it remains unclear how studies compare in terms of the trait that they actually measure. Additionally, given that few assessments of behavioral syndromes have been conducted within an organism’s natural environment, it is also difficult to determine how the results of these tests predict the likelihood of real ecological consequences for the subject animals.

Clearly, there is a need to clarify the relationships among the various measures of and tests for behavior on the boldness-shyness axis on subject animals in the field. Here, we focus on this task using a tropical reef fish model. Young reef fish can be collected at the end of their larval phase immediately prior to settlement on the reef, when they are naïve to reef-based predators and behaviors learned after settlement (Lonnstedt et al. 2012). Also, by collecting fish from a single recruitment pulse, we control for gross variations in size and age (Kerrigan 1996). In this phase of their life cycle, reef fishes typically experience high mortality (Almany and Webster 2006), with rates within the first 48 hours of benthic life averaging 57% (Doherty et al. 2004a; Almany and Webster 2006) but sometimes >90% (Gosselin and Qian 1997). The distributions that are established through differential mortality often set the pattern for abundances of juveniles and later life stages. Because experience can influence behavioral phenotypes (Budaev 1997; Bell and Sih 2007; Dingemanse et al. 2009), the use of naïve study organisms allows us to control for variation and consistency in behavior and to examine ecologically important behavioral traits at a critical ontogenetic boundary (McCormick and Meekan 2010). Here, we use short-term (48 hours) survival as a measure of the ecological consequences of differences in boldness, assayed using a variety of techniques. For juvenile coral reef fish, short-term survival immediately following settlement is a critical selective bottleneck for populations and is relatively straightforward to measure, making it ideal for use in our study. While our survival estimate is just one of a number of possible estimates of fitness that are ecologically relevant, because of the magnitude of mortality at this stage, the trait of survivorship is likely to be very important. For these young reef fish, we aimed to determine: 1) if different types of boldness measurements quantified a similar behavioral trait, 2) which of the commonly-used methods of assessing boldness (variants of novel object and novel environment tests) was the most closely correlated with an ecological outcome (survival), and 3) which behavioral measures were easiest to conduct *in situ* with low variability among multiple observers. Based on our previous experience with this system and study species, we predicted that novel object and environment tests would not covary in how they quantified boldness, with novel environment activity measures more likely to predict survivorship. We expected that correlations among behaviors would show that bold fish tended to be larger overall, spend more time actively foraging in ways that left them more exposed to predators, while being less reactive to any sort of novel object test than shy fish.

4.3 Methods

Ethics statement

This study was carried out in strict accordance with the recommendations under James Cook University (JCU) ethics protocols and approved by the JCU Animal Ethics Committee (Permit Number: A1067). All efforts were made to minimize animal handling and stress.

Study site and species

This study was conducted on the shallow reef (2-4 m depth) offshore from the Lizard Island Research Station (14°40’S, 145°28’E) on the northern Great Barrier Reef, Australia. Our study species, the lemon damsel, *P. moluccensis*, is common on Indo-Pacific coral reefs (Beukers and Jones 1998). Juveniles settle from the plankton at night (Pitcher 1988), between October and January around the time of the new moon (Meekan et al. 1993), preferentially settling on live coral (McCormick and Weaver 2012). Larvae recruit onto the reef after approximately 20 days in the plankton, at about 11 mm standard length (Wellington and Victor 1989). *P. moluccensis* has a relatively small home range (Brunton and Booth 2003), moving only small distances (<1 m) during the first few months after settlement (McCormick and Weaver 2012). Due to its high abundance, small size, rapid development, and sedentary nature, *P. moluccensis* is an ideal model organism for field and laboratory based behavioral studies (Meekan et al. 2010).

Experimental design

Collection

We collected newly-metamorphosed juveniles of *P. moluccensis* using moored light traps (see small light trap of Figure 1 in Meekan et al. 2001 for design) during November 2010. Traps were anchored approximately 100 m from the nearest reef in ~10 m of water at dusk and left overnight. Catches were emptied from the traps the next morning between 05:30-07:00 h. Fish collected from the traps were transported to the laboratory where *P. moluccensis* was separated from all other species and maintained in a 25 L aquarium of aerated seawater for at least 24 h to acclimatize to local conditions and reduce handling stress before experiments began. Fish were fed *Artemia* nauplii twice daily while in captivity. After acclimation, each *P. moluccensis* was placed into a clip-seal polyethylene bag containing aerated seawater and were measured for total length (to the nearest mm) with calipers, photographed, and then transported to the field in individually-labeled plastic bags. After final observations, study organisms were released unharmed on nearby natural habitat. Fish collection locations/activities and handling protocols were approved by the Great Barrier Reef Marine Park Authority (Permit Number: G10/33784.1) and JCU Animal Ethics Committee (Permit Number: A1067).

Observational protocol

All behavioral observations were made on individual fish in the field. Divers released a single fish onto a small patch reef (30 x 30 x 30 cm) haphazardly chosen from 35 that were constructed from live and dead pieces of the bushy hard coral *Pocillopora damicornis* on the shallow (3-4 m water depth) sand flat. *P. moluccensis* recruits occur naturally in this habitat. Reefs were deployed in rows, 5 m apart and approximately 10 m from the nearest area of natural reef. Means and ranges of temperatures did not vary among reefs (M. McCormick unpubl. data) and care was taken in reef construction to ensure that patch reefs had only very minor differences in habitat structure. Previous studies have shown that such minor variation in topographic complexity of patch reefs has no effect on behavior of young fish (McCormick and Meekan 2010; Meekan et al. 2010). Before introduction of the study fish, patch reefs were cleared of any resident fishes using hand nets. These were released on nearby natural reef far enough away to prevent their return (approx. 10 m). Individual study fish were then released onto their respective patch reefs and the first behavioral variable (latency to enter a novel environment; see description below) was recorded. Immediately afterwards, small wire cages (about 40 x 40 x 40 cm, 12 mm mesh size) were placed over the patch to allow the fish to acclimate to the new surroundings while being protected from predation. Cages were left a minimum of 20 min and carefully removed immediately before observations. Following established protocols, divers conducted observations from at least 1.5 m away (with the aid of a 2 x magnifying glass) to avoid any effects that may have been caused by the proximity of the observer to the target fish (McCormick and Meekan 2010; Meekan et al. 2010). A pilot study where estimates of distance were checked against a ruler found these estimates to be within 10% of the true value.

Behavioral traits were measured for a total of 92 fish during eight periods of observation spread over 5 days. The first six of these periods (n = 59 fish) were conducted by three experienced observers, each assessing the same fish simultaneously to quantify variance in measures among observers. All subsequent observations were conducted by JRW and MGM. Data from all observation periods were used for comparisons of behavioral traits among fish and data collected by three observers was used for a comparison of variability in estimates of behavior among observers. Each behavioral test was only trialed once with individual fish because *P. moluccensis* has been shown to recognize threats after a single exposure (Mitchell et al. 2011), which could have altered the outcomes of some boldness measures. In general, the behavioral responses of individuals have been shown to be stable at least over the time of our relatively short experiments (McCormick and Meekan 2010; White et al. 2015). In both a pilot study and this experiment, we found no relationship between observed behaviors of individual fish and specific patch reefs or time of day. This suggests differences in local environmental conditions such as minor variations in habitat, light conditions and food abundance across patch reefs did not noticeably influence behaviors.

The behavior of each fish was assessed using variations of two novel-object and two novel-environment tests that were composed of 12 behavioral measures:

1) Novel environment: release

After resident fish were cleared from the patch reefs, each damselfish was carefully released from the plastic bag onto the sand 10 cm from the patch reef. The amount of time it took for the fish to move onto refuge of the patch reef was termed 'latency at release'. This was timed from the moment the fish exited the bag, to the instant it reached the edge of the reef shelter. If the individual took more than 60 seconds to move to the reef, observations were discontinued and individuals were assigned a top value (~10% of fish).

2) Novel environment: overall activity

Six behavioral measures were recorded simultaneously over a 3 min observation interval for each fish: bite rate (number of feeding strikes towards objects floating in the water column); distance moved (total distance covered (cm) during 3 min); distance ventured (the maximum distance (cm) fish moved away from their patch reef; the distance ventured from the patch (categorized as % of time spent within 0, 2, 5, or 10 cm away from the patch); and position on the reef (categorized as a cumulative proportion of the time spent at varying heights over the 3 min observation period, with the top of the patch taken as height of 1, middle of the patch a height of 0.5, and bottom a height of 0). Mean distance ventured was calculated from the sum of the proportions of time spent in each of the distance categories multiplied by the distance that each category represented. Relative height on the patch was summarized as a cumulative proportion of the time spent at varying heights over the 3 min observation period, calculated from the sum of the proportions multiplied by the height categories (0, 0.5, or 1). Estimated distances were verified with a ruler after the 3 min observation period was completed.

3) Novel object: benign

Each fish was presented with a novel object (2.4x2.1x1.6 cm consistent assortment of blue and yellow Lego™ blocks, with the same blocks used for each fish) that was gently placed 10 cm away from its location. Fish were not obviously disturbed by this action. Over a 60 s observational period, minimum approach distance (cm) and a visual estimate of mean approach distance (cm) were recorded.

4) Novel object: threat

The reaction of each damselfish to the thrust (~120 cm/s over 20 cm) of an observer’s probe (pencil 13 cm long) towards them was recorded as the minimum distance from the tip of the probe (cm) before fleeing, the maximum distance traveled (cm) by the fish after the presentation of the threat, and the latency (seconds) of the fish to leave shelter of a particular part of the coral patch and return to its original location. Latency was limited to a 60 s observation time. A reaction score was quantified as a continuous variable on a 0-3 scale with 0.1 unit increments, where: **0**- hiding in refuge before or immediately after thrust and seldom emerging afterwards; **1**- retreating to refuge when scared and taking more than 5 s to re-emerge, then tentatively striking at food; **2**- retreating to refuge when scared but emerging quickly and striking at food; **3**- not hiding but continuing to explore or strike at food aggressively. The reaction score summarized the combination of overall individual behavior during the 3 min observation and reaction to the probe thrust.

Survival

The presence of fish on reefs was monitored twice daily (between 10:00-11:00 and 15:00-16:00 h) over two days (mean 44.9 h). Previous studies have shown that any migration of newly-settled fish from patch reefs in this location is negligible (approximately 1% of 300 tagged fish in 3 days) so that the absence of fish from a reefs can most likely be attributed to predation (Hoey and McCormick 2004).

Data analysis

The overall variability of each behavioral measure was quantified using a coefficient of variation. The coefficient of variation and comparison of behavioral traits with survival were calculated using one score (from the most experienced observer, MGM) per fish. Behavioral responses were z-transformed to standardize differences in mean and variance while maintaining patterns of covariance.

In order to compare observers, the range of values (maximum-minimum scores) for each trait recorded by the three observers was compared across six observation periods (n = 59). Because the range values did not meet assumptions of normality, a Friedman test was used as a nonparametric alternative to one-way repeated measures ANOVA.

The influence of a single behavioral trait on survival was determined with Kaplan-Meier survival analysis and its significance with Cox's F-Test using multiple single-predictor models. In order to highlight the influence of behaviors at either high or low extremes, the twenty highest and twenty lowest scoring fish of each trait were compared. Traits identified as significant by the Kaplan-Meier test were further compared using phenotypic selection gradient analysis (Lande and Arnold 1983) as a more explicit test of the relationships between single and combinations of traits on fitness. This test was used to identify behavioral traits that best predicted survivorship, while accounting for direct and indirect selection. First, behavioral variables were z-transformed (standardized). Then, logistic regression was used to regress the standardized values, their squared terms, and the cross-products of the pairwise combinations on relative fitness (whether an individual lived or died, divided by average fitness of the population) to estimate directional, stabilizing, and correlation selection gradients, respectively (Lande and Arnold 1983; Bell and Sih 2007).

Relationships between behavioral traits were analyzed using Pearson's product moment correlation. The statistical effect value (r) associated with these correlations are simply used as potential indicators of the strength of relationships rather than indicators of biological significance. However, sequential Bonferroni adjustments are included to account for multiple testing (Type I) errors.

Confirmatory factor analysis, a form of structural equation modeling (Grace 2006), was used to determine the structure of a combination of behavioral measures used to assess boldness for the population during a 48 hr post-settlement period. We followed the proposed framework established by Dingemanse et al. (2010) for using structural equation modeling (SEM) to compare hypothesized patterns of behavioral covariance. Eight alternative models formulated *a priori* (as described below) for boldness syndrome structure were separately assessed and the relative fit of each model was compared. Models were compared using Akaike's Information Criterion (AIC), which was calculated from model discrepancies (Ĉ) estimated by maximum likelihood using Bollen-Stine bootstrapping (2000 bootstraps). AIC values compare the fit of a model to data while rewarding parsimony, with lower values indicating greater model support (Akaike 1973; Dingemanse et al. 2010a). Models were compared by AIC differences (ΔAIC) relative to the model with the lowest AIC value, with ΔAIC values greater than two suggesting less support (Burnham and Anderson 2002). The maximum convergence limit for data to fit to models was set at 50 iterations.

In order to increase parsimony of the structural equation models, the most similar behavior responses were combined into composite variables by extracting their factor scores using factor analysis. Distance ventured and maximum distance ventured were combined into a new variable termed 'Exposure'. Minimum and average distances to Lego blocks were combined to form the new variable 'Benign response', while minimum and maximum distances to the threatening object (probe) formed the new variable 'Flight response'. Rather than the traditional method of using factors with eigenvalues greater than one, parallel analysis was used to determine the number of factors to be extracted (using permutations of 1000 parallel generated datasets) as outlined in Budaev (2010). With the correct number of factors determined by the parallel analysis, factor scores were calculated using principle axis factoring with Varimax rotation and the regression method (Budaev 2010).

Prior to SEM analysis, Bartlett's test of sphericity and the Kaiser-Meyer-Olkin (KMO) index were calculated for the dataset. The sphericity test determined if the behavioral variance-covariance matrix differed from random (Dingemanse et al. 2010a), while the KMO index compared observed correlations and partial correlations among original variables (Budaev 2010). In our data, the matrices differed from random ($\chi^2_{28} = 118.40$, $P < 0.001$). The KMO values were above the 0.5 acceptable threshold (Budaev 2010) with KMO = 0.52. However, the efficacy of the KMO test for a confirmatory factor analysis with a single latent factor (as used in this study) is unknown (Dingemanse et al. 2010a) and both tests are unlikely to be necessary for simple models with few observed variables (Dochtermann and Jenkins 2011).

Eight *a priori* hypotheses of boldness structure were considered based on the different types of boldness tests in behavioral syndrome literature (models 1-8, Fig. 4.1). Model 1 was the null model, where there was an absence of covariance and behavioral responses varied independently (Coleman and Wilson 1998). Model 2 represented a domain-general model of boldness structure, where all types of novel environment and novel object tests were linked via an underlying factor. Models 3 and 4 represented a domain-general model where size and latency at release, respectively, were considered contextually different from the rest of the behavioral responses. Model 5 considered foraging and height contextually different. Model 6 removed the benign response from the other boldness measures. Model 7 removed the threatening novel object measures: flight response and latency to threat. Model 8 considered bite rate contextually different from other activity, novel object, and novel environment tests.

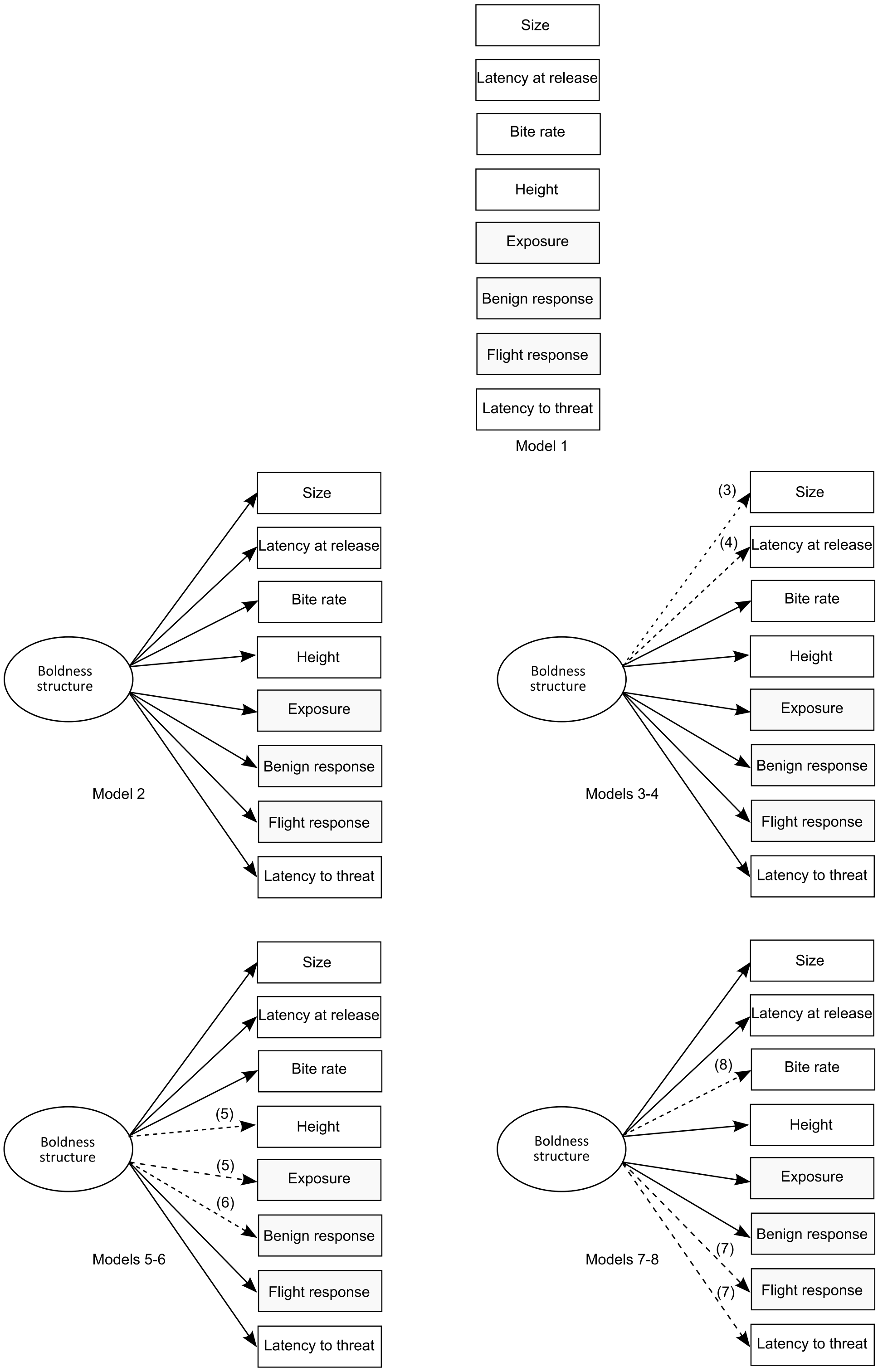

Figure 4.1. Eight models of boldness syndrome structure developed based on *a priori* hypotheses of boldness structure. Model 1 represents behavioral independence. Model 2 represents a domain-general model of syndrome structure while models 3-8 are more constrained, representing different types of boldness tests. The measured behaviors are represented in rectangular boxes, with shaded boxes representing composite variables. Underlying causal connections (latent variables) resulting in boldness structure are represented in ovals. In order to save space, multiple models are presented with alternative structures denoted by dashed lines labeled with model number (e.g. model 3 excluded size, as denoted with a dashed line labeled 3).

Because models were built on *a priori* hypotheses, models 2-8 were compared against the model of no boldness syndrome structure (model 1) to quantify the amount of variation explained by the different models. This was done by calculating D_x, which represented the proportion of variation in the behavioral variance-covariance matrix explained by each model, relative to the null model (Stamps et al. 2005; Dingemanse et al. 2010a). D_x was calculated as: $D_x = 1- \hat{C}_x/\hat{C}_{null}$ where $\hat{C}_{null}$ was the discrepancy for the null model (i.e. model 1, Fig. 4.2) and $\hat{C}_x$ was the discrepancy for other hypothesized models (i.e. models 2-8, Fig. 2). D_x is interpreted similarly to an R^2 value (Dingemanse et al. 2010a).

Statistical analyses used SPSS version 20.0 (SPSS Inc., Chicago, IL, U.S.A.). Structural equation models were constructed using AMOS version 20.0 (SPSS, Inc.).

4.4 Results

Variability of behaviors

Most traits showed high variability among individuals (Table 4.1), which allowed one or more traits to affect post-settlement mortality. Coefficients of variation ranged between 8-82% for most measures, with the exceptions of latency at release, time budget and escape latency to a probe thrust, which all had CVs over 100% of mean values. Latency at release had the highest CV (167%), but this was skewed due to a small number of fish (9 of 92 fish) that did not move to patch reefs within the 60 s observation period. The CV reduced to 102% when these slow-to-respond fish were excluded from the data set. The time budget had high CVs since few fish remained motionless or did activities other than feeding. Some fish (7 of 92) remained hidden within the refuge of the patch reefs after the probe thrust, skewing the CV for this measure.

Mortality was monitored for at least two nights in the field (mean 44.9 h). A total of 41.8% of all fish disappeared from reefs and were assumed to have died (Table 4.2). Of these, 84% died within the first 24 h, typically at sometime between the last observation in the afternoon and the next observation the following morning.

Table 4.1. Summary statistics for various measures of novel object or novel environment tests of the Lemon damsel (*Pomacentrus moluccensis*).

Variable	**N**	**Mean**	**SD**	**CV (%)**	**Mean Inter-Observer SD/Equivalent in units**
Physical character					
Size (cm)	92	1.3	0.1	8	N/A
Novel environment: release					
Latency at release (s)	92	17.1	28.5	167	1.0/2
Novel environment: activity					
Bite rate	92	26.7	15.1	56	8.1/16
Distance moved (cm)	92	17.5	14.4	82	8.4/15
Distance ventured (% time index)	92	1.8	0.9	52	0.6/1
Max. distance ventured (cm)	92	3.3	1.8	55	1.8/3
Position on reef (height index)	92	2.5	0.7	26	0.1/0.2
Novel object: benign					
Minimum distance to Legos (cm)	92	4.2	1.8	44	1.0/2
Mean distance to Legos (cm)	92	7.2	2.7	37	1.2/2
Novel object: threat					
Minimum distance to threat (cm)	92	3.2	2.2	69	0.7/2
Max. distance travelled from threat (cm)	92	5.3	1.8	34	1.6/3
Latency to threat (s)	92	14.2	18.3	129	2.4/4
Threat test (0-3 score)	92	1.7	0.6	37	0.4/0.8

Table 4.2. Survival (%) of newly settled Lemon damsel (*Pomacentrus moluccensis*) on patch reefs.

Field trial	**Trial duration (h)**	**N**	**Survival (%)**
1	47	9	60
2	47	7	57
3	42.5	9	67
4	42.5	12	67
5	42.5	7	43
6	42.5	14	43
7	48	14	64
8	47	20	65
Mean or total	44.9	92	58

Variability among observers

The threat test was the only measure that showed significant variability among observers across trials ($\chi^2(5) = 12.72$, $p = 0.026$). There was no pattern of improvement in observer consistency over time, with the variability in trials 1, 4, and 6 lower on average than trials 2, 3, and 5. The variability in threat test scores among observers ranged from a 0.5 to 1.0 difference (on 0-3 scale).

Other behavioral measures did not differ significantly among trials and observers, suggesting no major improvement or decline in observer consistency. Most variables had a low level of observer variance (Table 4.1), with a difference of only 1-3 cm or 1-3 seconds (~3% of maximum observation time). However, distance moved had a relatively large variance, with observers disagreeing by an average of 12 cm in most trials, although this improved to 5 cm by the end of the study. Estimates of bite rate were moderately variable but improved with time, with the average difference ranging between 6-22 strikes. Because of the high and inconsistent inter-observer variability in measures of the threat test and distance moved, these measures were omitted from subsequent analysis.

Individual behavioral traits and survival

The ability to discriminate survivors from non-survivors on the basis of a single behavioral trait was poor. However, Kaplan-Meier survival analysis showed maximum distance ventured ($F_{12,22}= 2.42$, $p = 0.035$) and initial size ($F_{8,22}= 3.72$, $p = 0.007$) were good predictors of survival (Figures 4.2 a & b). Larger fish and those willing to venture further from the reef had better survival rates. Bag latency at release was suggestive of a trend ($F_{16,28}= 1.97$, $p = 0.056$), with fish that quickly moved to the patch reef having lower average mortality.

The phenotypic selection analysis showed a significant relationship between the behavioral traits and relative fitness ($\chi^2 = 9.304$, df = 3), however this model accounted for a relatively low amount of the variation with Cox & Snell $R^2 = 0.096$ (Table 4.3). Overall, larger fish survived better, with size as the only variable identified as significant directional ($\beta = 0.469$, $p < 0.05$) and stabilizing ($\beta = 0.234$, $p < 0.05$) selection gradients, even though the size range was only 1.1 – 1.6 mm total length. No other directional, stabilizing, or correlational selection gradients were found to be significant. The model was adequate and predicted 63% of the responses correctly.

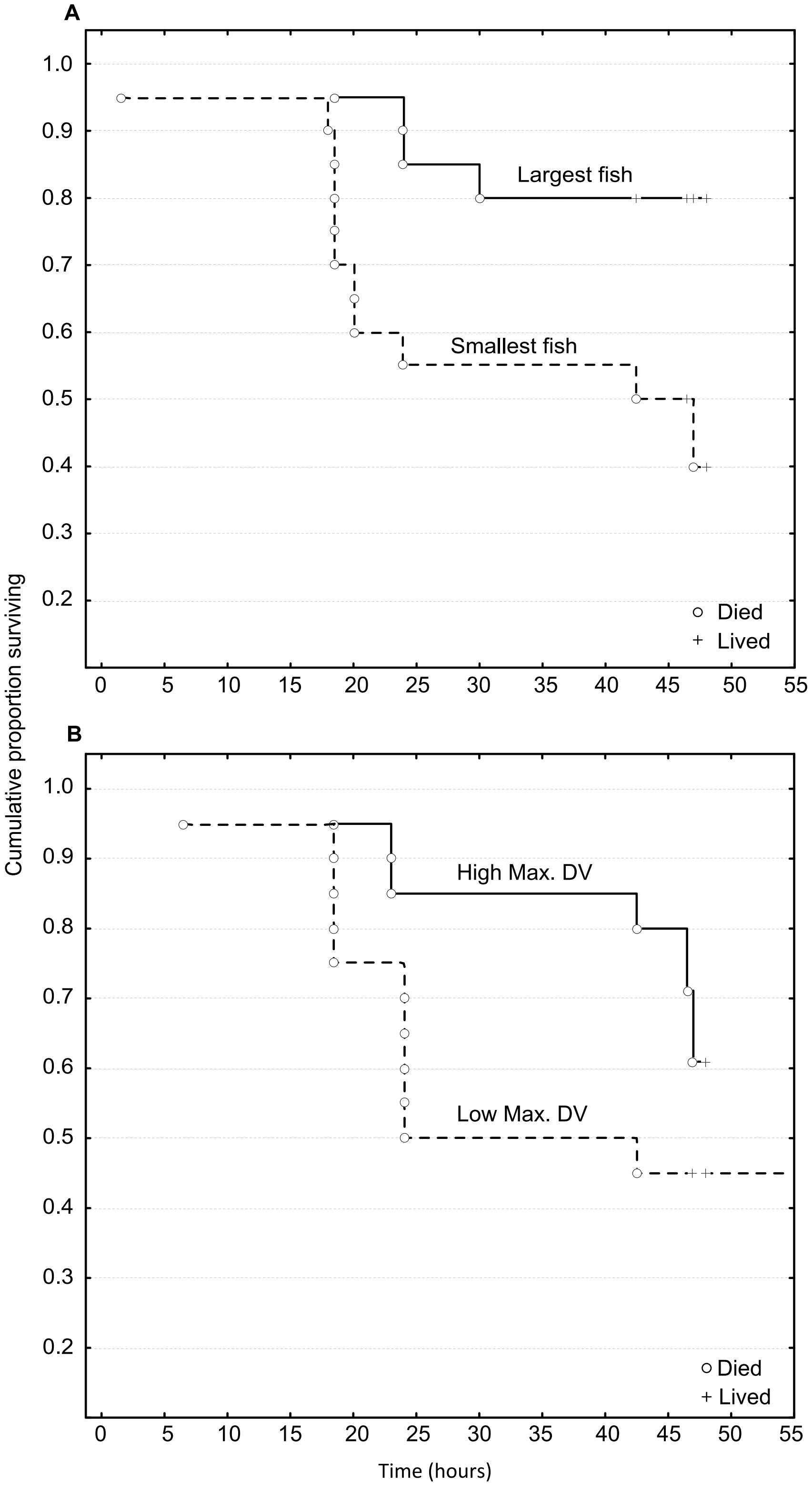

Figure 4.2. Survival over two nights in the field. Kaplan-Meier survival analysis with respect to: a) maximum distance ventured (DV) from shelter and b) size (TL) of juvenile *P. moluccensis* on patch reefs in the field. Fish were sequentially ranked for their scores on each trait and two groups (high and low ranked) of twenty fish (21.7% of total) were compared. Solid lines and dashed lines represent the two groups of highest and lowest ranked fish, respectively. Symbols represent presence or absence of individual fish during subsequent mortality surveys.

Table 4.3. Directional, stabilizing and correlational standardized selection gradients (β) from logistic regression.

	β	SE	*P*- value	β *avggrad*
Size	0.469	0.240	0.050	0.170
Latency at release	-0.418	0.234	0.074	-0.152
Max. DV	0.072	0.229	0.754	0.026
$Size^2$	0.234	0.120	0.050	0.085
Latency at $release^2$	-0.209	0.117	0.074	-0.076
Max. DV^2	0.036	0.115	0.754	0.013
Size * Latency at release	0.256	0.280	0.360	0.093
Size * Max. DV	0.100	0.230	0.665	0.036
Latency at release * Max. DV	0.232	0.206	0.261	0.084

Model χ^2=9.304, df=3, *P*= 0.026, Cox & Snell R^2= 0.096

Correlations among behavioral traits

There were two significantly correlated relationships between behavioral traits (Table 4.4). Bite rate had a high positive correlation with exposure. Bite rate was also moderately negatively correlated with latency to a threat. This general lack of correlation suggests that each variable is quantifying a different aspect of behavior or space use.

Table 4.4. Phenotypic correlations between seven behavioral traits for Lemon damselfish.

†Behavior	Relative fitness	Size	Latency at release	Bite rate	Height	Exposure	Benign response	Flight response	Latency to threat
Relative fitness	-	0.25*	-0.24*	0.00	0.04	0.18	0.09	-0.05	-0.12
Size		-	-0.22*	0.11	0.21*	-0.26	-0.05	0.10	0.08
Latency at release			-	-0.13	-0.15	0.01	0.14	-0.09	0.20
Bite rate				-	0.16	**0.61***	-0.12	-0.01	**-0.35***
Height					-	-0.05	-0.31**	0.19	-0.16
Exposure						-	0.11	0.13	-0.24*
Benign response							-	0.25	0.30**
Flight response								-	0.15
Latency to threat									-

† Values of P do not control for multiple testing of the same data (*P<0.05; **P<0.01; ***P<0.001). Only values printed in bold are significant after Holm's sequential Bonferroni adjustment of experimental error rates [70].

Structure of multiple behavioral traits

There was equal support for models in which response to the benign novel object (model 6, ΔAIC = 0; Table 4.5), size (model 3, ΔAIC = 0.10; Table 4.5), latency at release (model 4, ΔAIC = 0.29; Table 4.5) varied independently of other behavioral measures and also for the model in which all measures were included (model 2, ΔAIC = 0.85; Table 4.5). These models explained approximately 51% of the variance-covariance matrix variation in behavior (Table 4.5). In summary, four models fit the data equally well and accounted for about half the total variation.

The behavioral patterns were best explained by models that showed a similar pattern in variable loadings. Path coefficients for the best fit models (models 2-4, 6) all had negative loadings for bite rate, exposure, size and height and positive loadings for latency at release, latency to threat, benign novel object and flight responses (Fig. 4.3). Loadings with the same sign imply an unknown proximate factor or factors that affect the expression of behaviors in the same manner (Dingemanse et al. 2010a). The SEM structure explained a high amount of variance in data sets for bite rate and exposure behaviors, suggesting these measures were better suited to assess boldness of juvenile fish in the field.

Table 4.5. Model comparison results for confirmatory factor analysis.

Model (x)	$\hat{C}$ (discrepancy)	k	AIC	ΔAIC	D_x
6	58.76	15	88.76	0	0.51
3	58.87	15	88.87	0.10	0.50
4	59.05	15	89.05	0.29	0.50
2	57.61	16	89.61	0.85	0.52
5	92.00	14	119.99	31.23	0.23
8	91.66	15	121.66	32.89	0.23
1	118.8	8	134.8	46.04	0

Structural equation models (SEMs) were evaluated based on difference in Akaike's information criterion (AIC) values. Small values represent an increased parsimony-informed fit to the data. AIC values were calculated based on the discrepancy between the statistical model for a hypothesis ($\hat{C}$) and the number of parameters (k). D_x values represent the proportion of the variance explained by the focal model relative to null expectations of no boldness structure. D_x can be interpreted as analogous to R^2. Unlisted models were those where the data did not converge within 50 iterations.

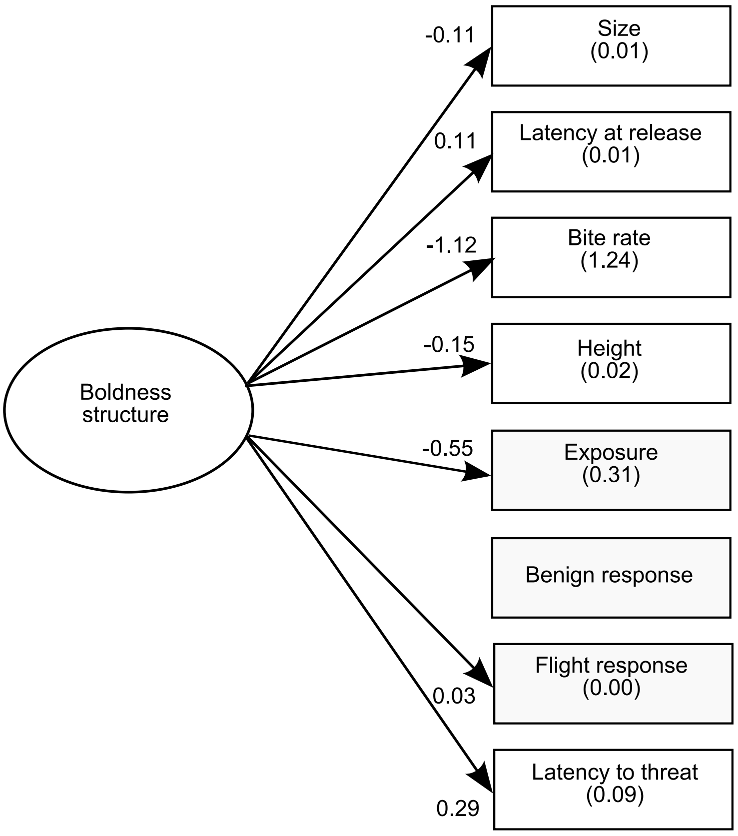

Figure 4.3. Best fitting structural equation model (SEM). This SEM shows how behaviors were related within the best fitting model for damselfish. Numbers in parentheses are variances of the different behaviors explained by the SEM structure (R^2) for 'model 6' (see Fig. 4.2). Numbers associated with arrows are standardized factor loadings for the effects of the underlying boldness structure on a particular behavior. These represent how behavioral responses are predicted to change based on changes to the underlying boldness structure (e.g. a shift of 1 SD along the distribution of boldness structure for the population would result in a 0.15 SD decrease in height).

4.5 Discussion

Individual behavioral traits and survival

Single behavioral traits had limited ability to predict survivorship for our model species. Those fish that were larger or were willing to venture further from the edge of patch reefs had greater survivorship during this critical phase of the life cycle; a conclusion supported by studies of intra- and inter-specific behavioral interactions at this life stage (McCormick 2009; McCormick 2012). There was a strong (though non-significant at $p = 0.056$) trend for fish that moved quickly to patch reefs when released to survive better than those that were slow to travel to the reef. Phenotypic selection analysis suggested only size had a significant effect on survivorship and that combinations of behavioral measures did not influence survival. Size and condition at settlement has previously been shown to be important for survival (Vigliola and Meekan 2002; Gagliano et al. 2007), with larger fish often having greater survivorship (McCormick and Hoey 2004). However, this pattern is not consistent at all times and places, with some studies showing that newly-settled individuals that were larger suffered higher mortality than smaller fish in some cases (McCormick and Meekan 2007; Meekan et al. 2010). Additionally, earlier work has found no links between foraging behaviors and selective mortality at settlement (Meekan et al. 2010), or a positive correlation between distance ventured from reefs and mortality (McCormick and Meekan 2010). Such differences in outcomes of studies may simply be a reflection of the temporal or spatial variability in predator/prey abundance (Holmes and McCormick 2006; Fuiman et al. 2010) or a predator's individual preference of prey species (Smith and Blumstein 2010). These complex relationships between predator/prey abundance and predator behaviors could be a major driving force in shaping individual variation in the prey’s behavior and ultimately, survival in the population. For example, Holmes & McCormick (2009) have shown that one of the major predators on newly-settled damselfish, *Pseudochromis fuscus*, which is common in shallow reefs adjacent to our patch reefs (McCormick and Meekan 2007; Holmes and McCormick 2009), preferentially targets larger recruiting fishes. If *P. fuscus* was more abundant in previous years, or selectively targets certain species (Almany et al. 2007), then spatial and temporal differences in the relationship of size or behavioral traits with mortality would be expected.

We used short-term (over 2 nights) survival as an ecologically relevant measure of the consequences of behavioral decisions although other measures of fitness (e.g. long term survival, reproductive output, offspring quality, etc.) or some other aspect of an animal's ecology could be used as an equally valid trait against which behaviors could be compared. Indeed, the different measures of boldness might vary in relevance depending on the trait against which they are measured and ontogenetic stage (Dingemanse et al. 2004). The high and selective mortality that normally occurs during the settlement transition for organisms with complex life cycles such as fishes makes the short term mortality measured in the present study, and the behavioral correlations explored, ecologically relevant.

Correlations among behavioral traits

The limited number of correlations among behaviors found in our study suggests that the behavioral variables we assessed measured slightly different aspects of boldness and were not interchangeable. The positive relationship between the composite variable 'Exposure' and bite rate was expected because juvenile fish tend to actively swim and explore the vicinity of their habitat while foraging. Fish that had higher bite rates also tended to quickly resume feeding after being threatened with a probe. With size being the principal predictor of short-term survival, one viable strategy would be for these fish to prioritize behaviors that maximized growth rates. By growing quickly, juveniles would escape gape-limited predators and better compete for space and resources. In this case, it would be advantageous for juvenile pomacentrids to quickly learn to recognize and ignore false threats, a trait that is a feature of these fishes (Mitchell et al. 2011).

Structure of multiple behavioral traits

Multiple SEM models could be fitted to the data for juvenile lemon damselfish. This suggests that there was considerable variability in the expression of boldness among individuals at the same life stage, in this case within the first few days of settling to the coral reef environment. Having a relatively adaptable expression of boldness at this time may allow individuals to properly assess and deal with the risks associated with the large assortment of predators that preferentially target fish recruits.

The use of a wild-caught population of juvenile fish rather than laboratory-bred individuals may account for a lower value for overall model fit ($D_x = 0.51$) compared to similar studies (Dingemanse et al. 2010a). Previous work has shown similar species of juvenile damselfish are highly flexible in their behavioral responses across different situations (White et al. in review). Relatively large individuals also had relatively high bite rates and spent more time near the top of the reef (greater height) while being relatively quick to exit the bag at release, were more exposed, and less reactive to novel objects. This was in agreement with our predictions on how boldness would be structured. However, contrary to our predictions, novel object and novel environment tests did not vary independently, with the fit of the data lending equal support to the unrestricted domain general model (model 2). All measures were considered to be behavioral responses that were contextually similar in regards to boldness structure. In other words, all measures accounted for the structure of boldness.

Variability among observers

Variability among observers measuring the same trait did not decline or increase over time for most behaviors, with the exception of the threat test. Variation in this measure increased during the study, probably reflecting the subjective nature of the measure, at least when multiple observers were involved in the work. Measures of bite rate, escape distance from a probe thrust and minimum distance from a probe thrust all showed some signs of reduced variation among observers over time. Observer variation in observed bite rate was initially high, but was reduced to acceptable levels (< 10 strikes) after limited training. Overall, generation of consistent and accurate measures of distance moved and reaction to the threat test proved difficult when multiple observers were involved, however the recording of behavior using high resolution cameras may offer a means to further reduce this source of variation in these measurements.

Conclusion

Although we measured 12 behavioral variables, only one (distance from shelter) predicted short-term survival. Fish size (a physical character) was the most influential in determining survival. In the past, most studies have considered boldness as a binary trait that was that could be quantified with a single variable. However, our study suggested that multiple measures of behavior and habitat use were necessary to adequately quantify boldness in our study species, because all quantified slightly different and largely uncorrelated aspects of behavior. Additionally, our multivariate analysis suggested that both novel object and environment tests were related via some underlying causal factor to boldness structure, but the lack of correlations suggested that these behavioral measures were not interchangeable. For our study animal, a tropical reef fish, we argue that most of the behavioral variables measured that required little to no interaction with the study subject gave a good overall insight into boldness structure. Boldness measures that involve interaction (e.g. presentation of novel objects), while correlated with another measure (bite rate), provided only a small amount of additional predictive value with regards to boldness structure of the fish. Also, due to the ability of *P. moluccensis* (Mitchell et al. 2011) and other juvenile fishes (Colgan et al. 1991; Kieffer and Colgan 1992) to learn rapidly, novel object tests may be less repeatable once fish have acclimated toward the stimuli (Wilson and Godin 2009). We suggest that novel object tests may engender responses that have little relevance to the environments in which naïve young fish find themselves after settlement, so that the results may have no bearing on the likely behavior of individuals in response to natural predators, at least in the first few days after settlement. While our results show novel environment and object tests both give insight into boldness structure, the repeatability and ecological relevance should be considered when selecting the most appropriate boldness measure for a study organism.

Chapter 5: Differences in spatial learning linked to boldness in fish

This chapter is in review with Ecology, Ethology, and Evolution.

Authors: J. R. White, M. G. Meekan, and M. I. McCormick

5.1 Summary

Boldness is a key personality trait involving the propensity to take risks and explore new environments. Although theory suggests that for juvenile organisms undergoing intense predation pressure, there will be ecological trade-offs associated with their position on the boldness-shyness axis, it is not clear what traits might be involved or how any trade-offs could be manifested. Here, we use a laboratory experiment to examine the link between boldness and learning in juveniles of a common tropical reef damselfish, Pomacentrus amboinensis (Pomacentridae). Newly-metamorphosed fish were ranked individually on a boldness-shyness axis on the basis of their willingness to emerge into a novel environment in an aquarium. Each fish was then given a simple task four times, which involved learning how to navigate a maze to reach a food source. A greater number of fish ranked with high boldness were successful at navigating the maze than shy ranked fish. This result suggests that boldness is likely to be linked with learning appropriate behaviors while exploring new habitats. Although a higher level of boldness is inherently risky, the potential for increased rewards associated with this trait may explain why boldness persists as a behavior in natural populations.

5.2 Introduction

The adoption of behaviors appropriate to overcome ecological challenges is necessary for the day-to-day survival of an organism. For animals with a bipartite life cycle such as coral reef fishes, the transition between larval and juvenile environments is a critical period, often characterized by a very high mortality rate (Gosselin and Qian 1997; Almany and Webster 2006; Doherty et al. 2004b). At this time, inexperience and reduced caution makes individuals more vulnerable to predation (Olla et al. 1998; Lönnstedt et al. 2012) and the rapid adoption of context-appropriate behaviors can greatly influence the selective mortality (McCormick and Holmes 2006) that establishes the patterns of distribution and abundance of juveniles and adults. Given that some behaviors have a heritable basis (Boissy 1995; Koolhaas et al. 1999; van Oers et al. 2005b; Brown et al. 2007a; Réale et al. 2007b), it is likely that any ability that increases an individual's fitness during this mortality bottleneck will influence behavioral phenotypes in future populations.

Boldness, or the propensity to take risks, is one of the most important and well-studied behaviors of animals. This trait plays an important role in decisions made by animals in response to a variety of ecological challenges (Frost et al. 2007). For example, an individual's ranking on the boldness-shyness axis is thought to affect reactions to novel situations, avoidance of predators and investments in reproduction and behavior in social contexts (Réale et al. 2000b). However, the relationship between boldness and other behavioral traits and their associated ecological trade-offs (especially in regards to aspects of fitness) is complex. A meta-analysis of 31 publications on the fitness consequences of boldness, exploration and/or aggression found boldness to be positively associated with reproductive success, but have negative effects on survival, while exploration had a positive effect on survival, but not for males or wild animals (Smith and Blumstein 2008b). However, for juvenile damselfish, the relationship between boldness and survival can vary between species and across years (McCormick and Meekan 2010; Meekan et al. 2010; White et al. 2013b). Also, in male rainbowfish (*Melanotaenia duboulayi*), dominant individuals were more aggressive, bold and active compared to subordinates, even though there were no direct relationships between aggressiveness and boldness or activity levels (Colléter and Brown 2011). This pattern could be formed by traits sharing proximate mechanisms that are difficult to decouple (Sih 2004; Bell and Sih 2007; Wolf et al. 2007; Budaev and Brown 2011), a phenomenon demonstrated recently by a study that found that a single gene simultaneously modulated the expression of aggression, boldness and exploratory behavior in zebrafish (*Danio rerio*) (Norton et al. 2011).

Boldness has been linked to learning ability, or speed of acquiring a task in some fishes. For example, male (Dugatkin and Alfieri 2003) and female (Trompf and Brown 2014) guppies (*Poecilia reticulata*) that learned to associate a cue with food were bolder than those that did not (Dugatkin and Alfieri 2003), while Sneddon (2003) found bold rainbow trout (*Oncorhynchus mykiss*) learned a simple conditioning task more quickly than shy fish. Alternatively, convincing arguments have been made for a link between personality types and cognitive style (the way individual animals acquire and use information), separate from cognitive ability (Sih and Del Giudice 2012). Boldness has also been linked to more thorough exploration (Verbeek et al. 1994; Réale et al. 2007b; Carere and Locurto 2011; Sih and Del Giudice 2012; Griffin et al. 2015). Exploration is important because it enables animals to discover locations of food and refuge and familiarity with surroundings may influence outcomes of competitive interactions (Sandell and Smith 1991; Verbeek et al. 1994) and because it is inherently risky. Such a link should be very important in a species such as a coral reef fish that must encounter an entirely novel habitat on the transition from a pelagic larval environment to a juvenile benthic habitat.

In the present study we investigated the link between boldness and learning, which is likely to be important to survival in juveniles of a coral reef damselfish the Ambon damsel, *Pomacentrus amboinensis.* We asked whether the bold/shy behavior types were associated with their ability to learn to maximize food rewards in a new environment.

This question is particularly relevant to this life-history transition stage as reef fishes typically experience very high mortality (average 57%) within the first 48 hours of benthic life (Almany and Webster 2006). Because experience can influence behavioral phenotypes (Budaev 1997; Bell and Sih 2007; Dingemanse et al. 2009), the use of naïve study organisms that were collected immediately prior to their settlement to the reef enabled us to control for variation and consistency in any behaviors adopted from varying experiences at earlier times (Poulos and McCormick 2014) and to examine ecologically important behavioral traits at a critical ontogenetic boundary (McCormick and Meekan 2010).

5.3 Methods

Study site and species

This experiment was conducted at the Lizard Island Research Station in the northern Great Barrier Reef (GBR) (14°40’S, 145°28’E). Juvenile *P. amboinensis* settle from the plankton at night to a variety of habitats in the northern GBR (McCormick and Weaver 2012) with the greatest densities found on small reef patches at the base of shallow (<10 m depth) coral reefs. *P. amboinensis* is a protogynous species (Gagliano and Depczynski 2013) and has a pelagic larval duration of 15-23 days and is between 10.3 - 15.1 mm standard length at settlement (Kerrigan 1996). The juvenile body is mostly complete at settlement; however fish go through a rapid change in body pigmentation in less than 12 hours after settlement (McCormick et al. 2002). Previous studies have shown *P. amboinensis* is relatively site-attached (McCormick and Makey 1997) and moves only small distances (<1 m) during the first few months after settlement. Also, these damselfish can be collected immediately prior to the end of their larval phase before settling on the reef and thus are largely naïve to reef-based predators and behaviors learned after settlement.

Experimental design

Collection

We collected newly-metamorphosed juveniles of *P. amboinensis* using moored light traps (see small light trap design in Figure 1 of Meekan et al. 2001) during November 2012. Traps were anchored in different locations around the island approximately 100 m from the nearest reef in ~10 m of water at dusk and left overnight. Catches were emptied from the traps the next morning between 05:30-07:00 h. Fish collected from the traps were transported in 30 L plastic bins with aerated seawater (approximately 500 fish per container) to the laboratory. Water was changed every 10 minutes to keep it aerated. Traps were approximately 500 m from shore and boat transport took a maximum of 30 mins. Less than 5% of fish died during transport and sorting. Daily catches from light traps varied, ranging from <100 fish to ~ 2000. In the laboratory *P. amboinensis* was separated from all other species during 2 hours by a team of researchers using plastic trays with aerated seawater and small hand nets. Other species were transferred to 25 L aquaria of aerated seawater, fed and released back to the nearest reef approx. 500 m from shore (on SCUBA) in the afternoon. *P. amboinensis* were maintained in a 25 L aquarium of aerated seawater for 24 h to acclimatize to local conditions and reduce handling stress before experiments began. Fish were stocked at <200 individuals per 25 L aquaria with various artificial refuges provided to reduce stress. Aquaria were kept at natural 12 h light:12 h dark regimes and the flow through water supply kept aquaria at natural ocean temperatures, ranging from 27 - 32° C. Aquaria were opaque to prevent visual disturbance and fish were handled as little as possible to reduce stress while in captivity. This acclimation period offered an opportunity for individual fish to recover from being caught in a light trap, brought back to the field station, sorted, and transferred to aquaria. After this acclimation period, fish displayed similar behaviors as in the wild, actively foraging and exploring their nearby vicinity, suggesting they were much less stressed than those observed within the first 24 h of being caught in light traps. Fish were fed *Artemia* nauplii twice daily while in captivity. At the completion of the experiment, fish were released unharmed in nearby natural habitat in the field. Fish collection locations/activities and handling protocols were approved by the Great Barrier Reef Marine Park Authority (Permit Number: G10/33784.1) and JCU Animal Ethics Committee (Permit Number: A1067).

Observational protocol

Boldness test

On Day 1 of experimentation, individual fish were assessed for boldness by measuring their latency to emerge from a shelter. This is a common test for boldness in fish (Budaev 1997; Fraser et al. 2001; Brown et al. 2005; Chang et al. 2012; White et al. 2013a) and is consistent for this species over the time frame used in this study (White et al. 2015). Each fish was gently transferred via hand net into an opaque ~162 cm^3 plastic holding chamber within an aquaria (13L, 20cm water depth) containing a small refuge of live *Pocillopora damicornis* at the opposite end (Fig. 5.1A) and allowed to acclimatize for 30 minutes. The holding chamber was believed to be of adequate size because there were no apparent signs of confinement stress by the fish. The sides of each aquarium were blacked out with plastic sheeting to isolate them from neighboring tanks. After acclimation, observers standing behind a blind (black plastic sheeting) gently revealed the opening to the chamber. Time (i.e. latency) to emerge (defined as more than half of the body length outside of the holding chamber), was recorded for each fish with a cut-off of 180 seconds. If fish did not emerge before this time they were given a ceiling value of 180 seconds. Water flow was shut off during the acclimation period and behavioral observations to reduce auditory disturbance, but a gentle air flow through air stones was maintained to ensure adequate dissolved oxygen levels. Fish were fasted for 12 hours before trials and fed upon completion.

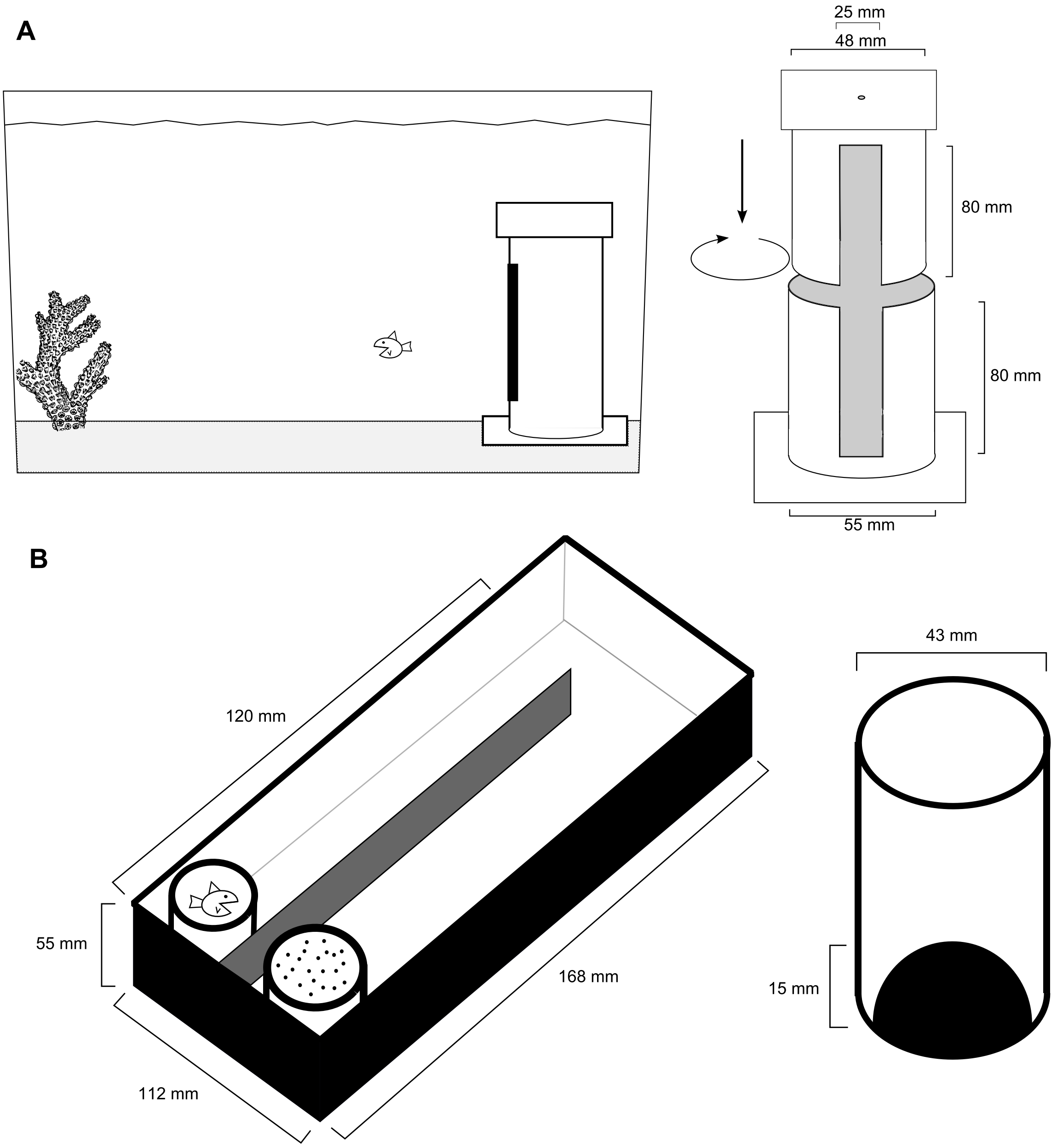

Figure 5.1. Testing arenas for boldness and learning trials. A) Boldness trials were conducted in a ~13L aquaria with a course layer of sand on the bottom and filled to 20cm water depth. A small piece of live *P. damicornis* provided refuge on the opposite end from the release chambers. Release chambers were constructed of nested PVC pipes which gave an approximate internal volume of 162 cm3. B) Maze trials were conducted in a simple U-shaped maze with a volume of approximately 1 L. The center partition extended ~70% of the total length of the aquaria. Fish were acclimated in a small PVC pipe on the left end of the maze and *Artemia* nauplii were introduced into a feeding arena on the opposite end of the maze.

Maze trial

On Day 2, fish were tested for their learning abilities using a simple maze (Fig. 5.1B). This is a simplified version of a common spatial-learning test (t-maze) others have used to assess learning in zebrafish (Darland and Dowling 2001) and mangrove killifish (Chang et al. 2012). In our maze, fish have the choice to remain stationary, advance, turn around, move around a 180° bend, enter or not enter a feeding tube, or any combination of the above. Fish were never forced to move forward, unlike some t-maze tests. Individual fish were gently released into a cylindrical holding area (34 mm diameter) at the beginning of the maze to acclimate for 30 minutes. The holding area was believed to be of adequate size because there were no apparent signs of confinement stress by the fish. Immediately before release, observers gently introduced 1 mL of *Artemia* solution (at approximately 300 nauplii/mL density) into an opaque feeding tube (43 mm diameter with 15 mm radius half circle at the bottom) that restricted the presence of this food source to the opposite end of the maze. *Artemia* remained in the feeding tube throughout the duration of the trial and were only visible to the fish upon entering the tube. Fish were fasted for 12 hours before maze trials and a pilot study showed this amount of food to be enough to provide an adequate reward, but not enough to reach satiation even after multiple trials. After *Artemia* introduction, the acclimation tube was gently removed by hand (carefully held from the sides to prevent visual cues) to release the target damselfish. All efforts were made to minimize sudden disturbance and any confounding effects of observer presence were reduced by conducting observations from behind a black plastic blind. Feeding latency was recorded from the moment of release until fish took their first feeding strike within the feeding tube (with a 600 sec. cut-off time). If fish did not feed within the cut-off time, they were assigned a maximum value of 600 seconds and allowed to remain in the maze until they eventually fed. Some previous studies have suggested these types of food-reward tests could be confounded by fish learning to follow the olfactory cue (i.e., *Artemia* odor) rather than learning the spatial location (i.e. location of *Artemia*) (Lucon-Xiccato and Bisazza 2014; Mamuneas et al. 2015). However, this was unlikely a factor in our study because water was changed between trials, *Artemia* was introduced only seconds before fish were released in the maze, and the water was stagnant during the short trials.

After the first maze trial, fish were gently transferred to individual holding aquaria and allowed to rest before being retested in subsequent maze trials three more times in the same day, with 2 hours rest between trials. Water in the mazes was changed between each trial.

Statistical analyses

A pilot study gave us a frequency distribution of latency of a separate group of 148 (58 bold, 70 shy, 20 intermediate) *P. amboinensis* to emerge from the holding chamber in the boldness test (Fig. 5.2). We used the extreme values of this distribution to focus on the most "Bold" or "Shy" fish in the present study. We classified bold fish as those who emerged in ≤10 seconds (n = 28) while shy fish took ≥100 seconds (n = 39). Each fish was assigned a learning score based on how quickly they began feeding on the *Artemia* (≤ 20 seconds in trials 2-4). Another pilot study showed a similar dichotomy existed in feeding latency (time to feed). If fish fed within ≤20 seconds, they travelled directly to the food source, without hesitation or exploration. Therefore, we classified fish to have completed the maze successfully if feeding latency scores were ≤20 seconds. We chose this cut-off value for analysis because we were comparing boldness at either end of the spectrum (very bold vs. very shy). A repeated measures ANOVA was not conducted because it would not answer the aims of the study.

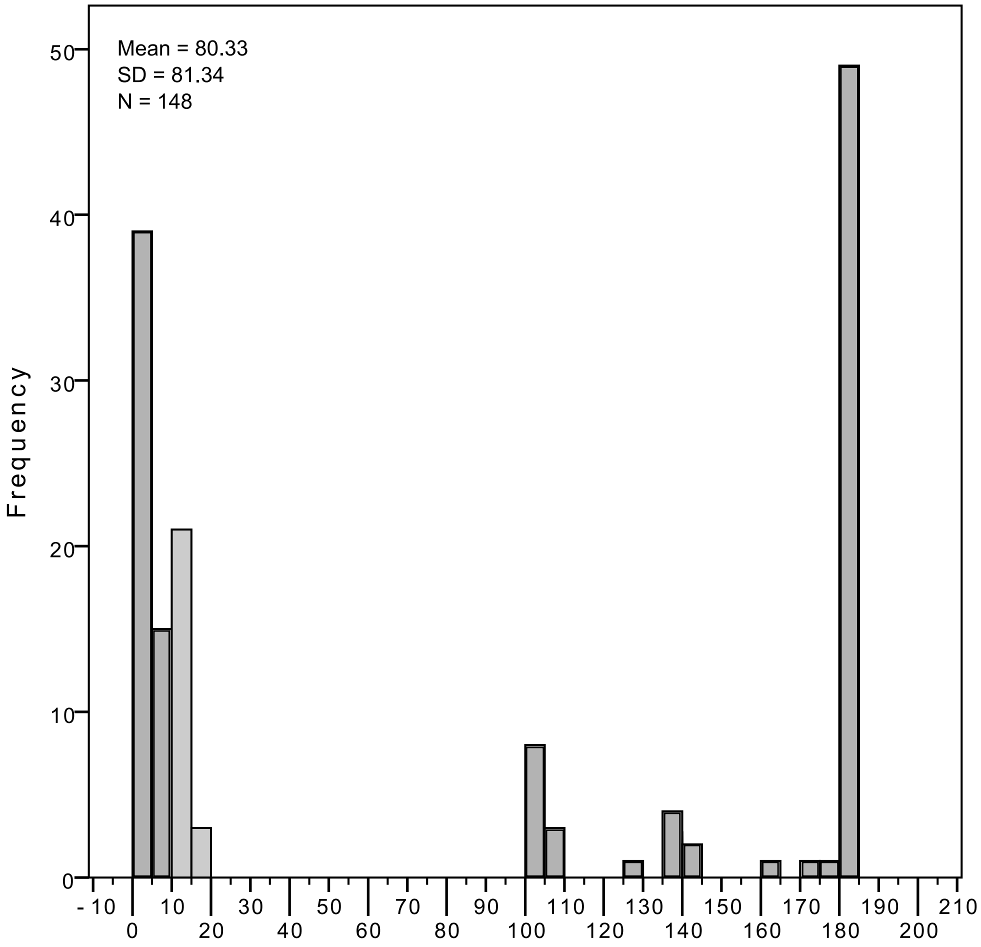

Figure 5.2. Frequency distribution of latencies to emerge into a novel environment (boldness test) of newly metamorphosed *P. amboinensis* in a separate pilot study. Values were selected as cut-off points for assigning fish a categorical rank of either 'bold' (≤10 sec.) or 'shy' (≥100 sec.). This selection criterion assigns a 'bold' or 'shy' classification to 39% and 47% of the sampled population, respectively.

The first trial was considered to be training and the learning scores were based on completions of the maze in multiple subsequent trials. The score was assigned to the first successful trial if it was successful again in a later trial (possible scores were "2", "3" or "5"). For example, if a fish received a feeding latency of ≤20 sec. during the second trial and again in the fourth, then it was assigned a score of "2". If fish completed the maze successfully only once of the four trials, it was assigned an unsuccessful value of "5".

The distribution of learning scores was compared across bold and shy fish using Chi-squared tests of independence. Statistical analyses used SPSS version 20.0 (SPSS Inc., Chicago, IL, U.S.A.).

5.4 Results

Bold fish tended to be quicker to learn how to navigate the maze successfully (Chi-square test: $\chi^2_2 = 6.51$, n = 67, p= 0.039; Fig. 5.3). Bold fish achieved successful learning scores (2 or 3) 42.8% and 130.7% higher than expected, respectively. Shy fish received the same scores 31.0% and 100% lower than expected, respectively.

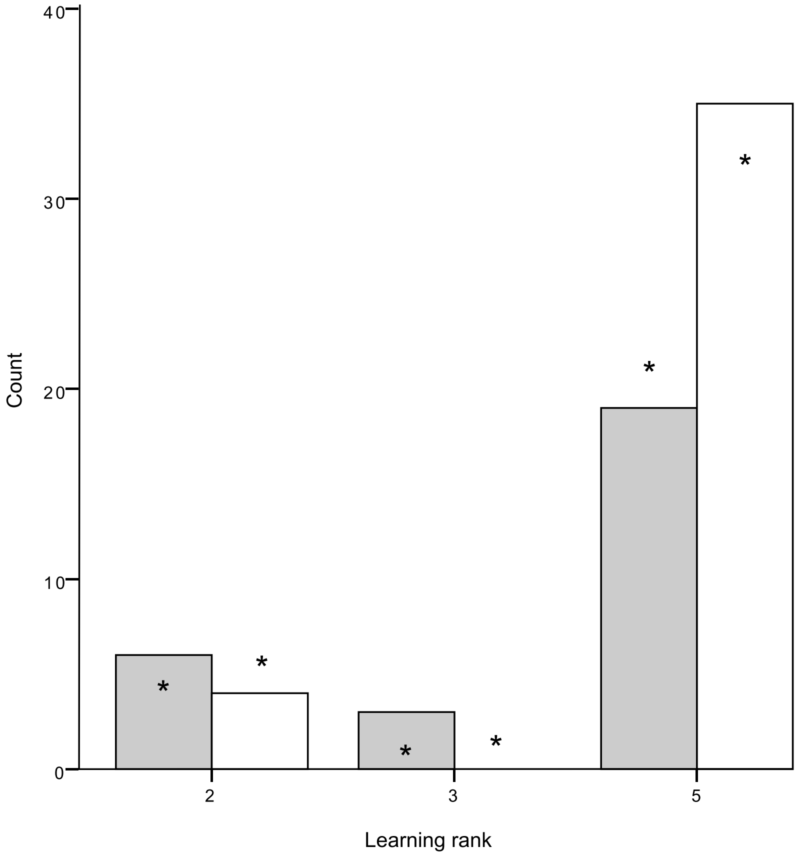

Figure 5.3. Frequency distribution of learning scores for bold and shy *P. amboinensis.* Grey bars represent bold fish (n = 28), white bars represent shy fish (n = 39). Stars represent expected values.

5.5 Discussion

Bold *P. amboinensis* learned to repeatedly navigate a novel environment for a food reward more rapidly than shy fish. Our work is the first to demonstrate such a positive relationship between boldness and learning for a marine animal and joins a limited number of studies from different systems that show a similar relationship in these behavioral traits (Dugatkin and Alfieri 2003; Sneddon 2003). More work is necessary to determine if this relationship originates during the juvenile stage and persists through ontogeny.

The definition of boldness (i.e. a propensity to take risks) implies that there are negative trade-offs associated with this trait. For many taxa, bold individuals experience higher mortality (Smith and Blumstein 2008b), so natural selection should have removed this behavioral trait from populations unless there was some counter-posing advantage bestowed by boldness (Dugatkin and Alfieri 2003; Frost et al. 2007). In this wider context, variations in behavioral phenotypes are likely maintained by the cost-benefits of a single trait (or a suite of correlated traits that are intrinsically linked) under different ecological circumstances. For example, bold male guppies (*Poeciliia reticulata*) were found to be more attractive to females than their shy counterparts (Godin and Dugatkin 1997), but while being bold during the mating period may be beneficial, this may not necessarily be the case when feeding in a predator-rich environment. Boldness can vary with the local predation risk (Budaev and Brown 2011; Brown et al. 2014). Similarly, the propensity to explore a novel environment more rapidly may not necessarily be advantageous in all situations. For example, Verbeek et al. (1994) found that shy great tits (*Parus major*) explored environments more slowly, but more thoroughly than bold birds. Consequently, these shy individuals were able to respond more rapidly to changes in the environment than bold birds (Verbeek et al. 1994). Again, this relationship may represent yet another trade-off (speed/accuracy) whereby shy individuals are able to allocate more attention to exploring and searching environments in greater detail, since their inherent shyness means that they are naturally under lower predation threat than bolder individuals. Conversely, bolder individuals may allocate less attention to searching because of the need to have a greater degree of predator vigilance (Krause and Godin 1996; Dukas and Kamil 2000; Dukas and Kamil 2001; Clark and Dukas 2003; Kaby and Lind 2003; Fernández-Juricic et al. 2004). If such relationships exist, this would predict that greater numbers of bold individuals should occur within stable environments than those that are variable. Thus, the ratio of bold to shy individuals of adult populations of coral reef fish might be influenced by the stability of the local environment they experienced as juveniles.

One hypothesis argues traits that are adaptive in one context but non-adaptive in another should be dynamically conditional among different contexts; a certain degree of behavioral plasticity will facilitate a more adaptive response (Dall et al. 2004). There is some evidence that this is particularly the case in juvenile or naïve individuals. For example, three-spined sticklebacks (*Gasterosteus aculeatus*) adopted more fixed behaviors after exposure to predators (Bell and Sih 2007) and naïve Arctic charr (*Salvelinus alpinus*) displayed more appropriate anti-predator responses when placed with experienced conspecifics (Vilhunen et al. 2004). Likewise, juvenile damselfish have high variation in the same behaviors across different testing arenas (White et al. 2013a). If boldness was consistently linked to the ability to learn rapidly, then this could explain why boldness may be a beneficial trait in certain contexts, despite the inherent risks associated with the behavior. The ability to balance speed and accuracy of predator recognition in regard to balancing risk is likely to be beneficial to animals during particularly vulnerable phases of their life history (Chivers et al. 2014).

One question this study prompts is: are individual fish bolder because they are fast learners, or do they learn faster because they are bold? Some argue in order to understand the ecological cost-benefits (i.e., adaptive value or fitness-affecting properties) of behavioral phenotypes we need to understand the underlying mechanisms, not the behaviors they produce (Stevens 2008). This would suggest that individual differences in how animals learn or respond to changes in environment may underlie the variation in boldness and other personality traits (Dingemanse et al. 2010b; Fawcett et al. 2012). This might be due to a stable polymorphism of learning rules within a population or variations of parameter values (e.g., interpreting past events differently or with varying degrees of sensitivity) to a shared basic learning rule, both of which would be maintained by frequency-dependent selection (Fawcett et al. 2012). Behavioral ecologists have generally not considered this hypothesis or integrated it with a functional assessment of behavior (Brunner et al. 1996; Stephens 2002). Alternatively, if boldness and other traits are determined by underlying physiology (e.g. stress responses or metabolic rates), then these traits are likely to drive learning. Further investigation of the underlying causes of learning is warranted due to the potentially useful framework it provides for the study of intraspecific variation in animal behavior.

Chapter 6: General Discussion

During the transition from their planktonic stage in the open ocean to settlement on coral reefs, fish are opportunistically targeted by a suite of predators (Beukers and Jones 1997; Beukers-Stewart and Jones 2004; Almany and Webster 2006). As these young reef fish have no experience in this new environment, they make useful model organisms for studies of personality, because these naïve juveniles enable us to control for learned behaviors and examine behavioral consistency precisely at the time of settlement, which is a critical ontogenetic boundary and mortality bottleneck (Fuiman et al. 2010; Holmes and McCormick 2010; McCormick and Meekan 2010; Meekan et al. 2010; Lönnstedt et al. 2012). The behavioral decisions made by these fish at settlement are crucial for their successful response to ecological challenges, such as interactions with competitors and predators. Thus behavior during this transition from larval to juvenile habitats plays an influential role in survival and possibly the structure of future reef communities (McCormick and Meekan 2010; Lönnstedt et al. 2012).

Differences in the expression of aggressive, exploratory and bold behaviors among individuals have been shown to be widespread and heritable (Boake 1994; Stirling et al. 2002; Kolliker 2005; van Oers et al. 2005a; Réale et al. 2007b) across a diverse array of taxa (Dingemanse and Réale 2005; Smith and Blumstein 2008a) and to influence survival (Downes 2002; Dingemanse et al. 2004), reproductive success (Both et al. 2005; Sih and Watters 2005; Pruitt and Ferrari 2011), resource acquisition (Webster et al. 2009) and growth (Biro et al. 2006; Meekan et al. 2010). Adopting a consistent behavioral phenotype (Chapter 3) can lead to trade-offs (Chapter 5), which can ultimately influence population dynamics, community structure, and species diversity (Pruitt et al. 2013; Mittelbach et al. 2014). Boldness, or the propensity to take risks, is one of the most important and well-studied behaviors of animals, but different measures of this trait account for different aspects of boldness, and multiple measures are needed to get a comprehensive assessment (Chapter 4). An individual's ranking on the boldness-shyness axis is thought to affect reactions to novel situations, avoidance of predators and investments in reproduction and behavior in social contexts (Réale et al. 2000b). However, the relationship between boldness and other behavioral traits and their associated ecological trade-offs (especially in regards to aspects of fitness) is complex (Chapter 4).

Problematically, few studies have used identical measures of boldness which hampers comparison (Chapter 4). Additionally, there is a lack of studies that demonstrate consistent patterns of individual behavior or show that multiple behavioral traits are correlated across laboratory and field settings. Most behavioral studies of fishes have been conducted in the laboratory (Toms et al. 2010) on captive or captive-bred populations (Adriaenssens and Johnsson 2011). This is done to control for potentially confounding factors and recreating realistic natural situations in the laboratory is extraordinarily difficult. Consequently, such studies assume that behavior of an animal in an artificial setting will be representative of its natural state, which is rarely tested in the field (Brown et al. 2005). Fish have demonstrated consistency in individual behaviors within a single situation, yet are more variable across different situations in the field or laboratory (Coleman and Wilson 1998; Chapter 2; Chapter 3). While laboratory and field comparisons for juvenile pomacentrids were remarkably consistent (Chapter 2; Chapter 3), this is not universally applicable, so behavioral studies may have limited predictive ability when expanded to other situations. Assumptions about natural behaviors in the field made under laboratory-based settings should be done cautiously (Chapter 2; Chapter 4). Artificial environments can introduce variation in behavior due to confounding factors such as handling stress or experiences gained from life in captivity

(Wilson et al. 1993; Sundström et al. 2004; Brown et al. 2005; Dingemanse and Réale 2005). Studies in the field also have the added benefit of incorporating realistic environmental and ecological factors that may influence behavior (Chapter 2; Chapter 4). If there are advantages to behaving consistently (Dall et al. 2004; McElreath and Strimling 2006), then the greater environmental variance and sensory input in the field might create micro-niches, which act as directional or stabilizing forces, increasing consistency by allowing individual expression of behavioral variations (Bell et al. 2009).

Overall, most short-term assessments of behaviors in the field and laboratory were found to be adequate for juvenile coral reef fish (Chapter 3; Chapter 4). Only a few studies have compared alternative methods of assessing the same behavioral traits (Brown et al. 2007b; Wilson and Godin 2009; Chapter 4). However, multiple types of measures of behavior are likely necessary to comprehensively quantify boldness, due to alternative measures quantifying slightly different and largely uncorrelated aspects of overall boldness structure (Chapter 4). Additionally, both novel object and environment tests were related via some underlying causal factor to boldness structure, but these measures were not interchangeable (Chapter 4). For coral reef fish, novel environment tests in the field gave a good overall insight into behavioral structure, while the specific variables of ‘distance moved’, ‘reaction score’ and mirror aggression were inadequate due to issues with inter-observer reliability and test acclimation, respectively (Chapter 3; Chapter 4).

Coral reefs are highly complex environments with an abundance of predators of damselfishes, which is likely to produce situations where it is beneficial for an individual to change their behavior to adjust to conditions (Chapter 2) or risk elimination from the population (selective mortality) (Brown et al. 2005). The outcomes of research on associations between behavioral traits and behavioral flexibility (Adriaenssens and

Johnsson 2011; Chapter 2; Chapter 3) have been inconsistent, although studies have suggested that a tendency to display bold behavior increases an individual's ability to solve novel tasks (Dugatkin and Alfieri 2003; Sneddon 2003; Chapter 5), while others have shown that individuals that are more shy and unaggressive have more behavioral flexibility (Koolhaas et al. 1999). The definition of behavioral syndromes allows for for this type of flexibility across situations (Sih and Bell 2008) yet the premise of the term suggests some limitation of flexibility of behavioral response (DeWitt et al. 1998; Conrad et al. 2011), explored in the literature as 'reaction norms' (Dingemanse et al. 2010b). Young fish at settlement undergo high rates of mortality and it is advantageous to remain highly flexible in behavior (Sih et al. 2004a; McCormick and Meekan 2010; Kelley et al. 2013; Chapter 2), rather than to develop syndromes.

The finding that naïve juvenile reef fish exhibit personalities at settlement (Chapter 3) suggests a genetic component and strong trade-offs related to adopting alternative personalities. Stable behavioral phenotypes are thought to be created when positive feedback loops form between underlying proximate factors (i.e., physiological, genetic, morphological characteristics) such as size, competitive ability, or condition and state-dependent behavioral decisions (Houston and McNamara 1999; Dall et al. 2004; Stamps 2007; Sih and Bell 2008) and that these variables establish the efficiency of certain types of behavior (Dall et al. 2004). For example, if predation risk is a function of body size, and since body size is stable over short time scales (daily), animals of different body sizes should differ consistently with respect to their tendency to take risks while foraging. Therefore, theory predicts behavioral patterns related to body size should also be stable over the same time frame (Ambrose and Strimling 2006; Stamps 2007). However, if only a single optimal behavioral phenotype existed, natural selection should reduce genotypic variation over generations (Réale et al. 2007b).

Since behavioral phenotypes show heritable variation not eroded by selection (Penke et al. 2007; Réale et al. 2007b), different behavioral strategies are likely to have different associated trade-offs (Kelley et al. 2013) across environments. The definition of boldness (i.e., propensity to take risks) implies that there are negative trade-offs associated with this trait. In many taxa, bold individuals experience higher mortality (Smith and Blumstein 2008b), so natural selection should have removed this behavioral trait from populations unless there was some counter-posing advantage bestowed by boldness (Dugatkin and Alfieri 2003; Frost et al. 2007). One possible advantage of greater boldness is a link to quicker learning ability (Chapter 5), or speed of gaining better access to resources in some fishes (Dugatkin and Alfieri 2003; Sneddon 2003; Trompf and Brown 2014). If behavioral traits are dynamically conditional among different contexts (i.e., adaptive in one context but non-adaptive in another), a certain degree of behavioral flexibility (Chapter 2) will facilitate a more adaptive response (Dall et al. 2004). From an evolutionary perspective, this also suggests how population level variability guards against environmental stochasticity. There is evidence that this is particularly the case in juvenile or naïve individuals (Vilhunen et al. 2004; Bell and Sih 2007; Chapter 2). With boldness linked to rapid learning ability (Chapter 5), this could explain why boldness may be a beneficial trait in certain contexts, despite the inherent risks. The ability to quickly and accurately learn to recognize predators and effectively use the environment to evade capture while balancing risk is likely to be beneficial to animals during particularly vulnerable phases of their life history (Chivers et al. 2014). Likewise, boldness has been linked with more thorough exploration (Verbeek et al. 1994; Réale et al. 2007b; Carere and Locurto 2011; Sih and Del Giudice 2012; Griffin et al. 2015). While inherently risky, exploration is important because it enables animals to discover locations of food and refuge and familiarity with surroundings may influence outcomes of competitive interactions (Sandell and Smith 1991; Verbeek et al. 1994).

Such a link should be very important in a species such as a coral reef fish that must encounter an entirely novel habitat on the transition from a pelagic larval environment to a juvenile benthic habitat. In this wider context, variations in behavioral phenotypes are likely maintained by the cost/benefits of a trait or multiple linked traits under different ecological circumstances, such as boldness increasing mating success (Godin and Dugatkin 1997). Similarly, the propensity to explore a novel environment more rapidly may represent a trade-off if this trait is not advantageous in all situations. If shy individuals explore environments slowly, but more thoroughly than bold animals, then shy individuals would be able to respond more quickly to environmental changes (Verbeek et al. 1994). Then this relationship may represent a trade-off where shy individuals are able to allocate more attention to exploring and searching environments in greater detail, since their shyness means that they are actively avoiding predators more than bolder individuals. Conversely, bolder individuals may allocate less attention to searching because of the need to have a greater degree of predator vigilance (Krause and Godin 1996; Dukas and Kamil 2000; Dukas and Kamil 2001; Clark and Dukas 2003; Kaby and Lind 2003; Fernández-Juricic et al. 2004). If such relationships exist for coral reef fishes, this would predict that greater numbers of bold individuals should occur within stable compared to variable environments. Thus, the ratio of bold to shy individuals of adult populations of coral reef fish might be influenced by the stability of the local environment they experienced as juveniles.

While proximate factors are likely important in establishing stable behaviors, experience gained through ontogeny or exposure to predators can help shape and establish behavioral patterns (Bell and Sih 2007). Coral reef fishes may need some exposure to competitors (Poulos and McCormick 2014) or predators before developing a consistent behavioral syndrome. Only size (but not any other single or combinations of traits) predicted survivorship for juvenile damselfish (Chapter 4) during the mortality bottleneck at early settlement; a conclusion supported by studies of intra- and inter-specific behavioral interactions at this life stage (McCormick 2009; McCormick 2012). Small prey, such as juvenile coral reef fish, need to grow rapidly to avoid gape limitations of local predators (Anderson 1988; Arendt 1997; McCormick and Meekan 2007) and provide competitive dominance over conspecifics (Holmes and McCormick 2006; McCormick 2009). This theory is supported by research that has shown juvenile coral reef fishes grow faster compared to fish in other environments (Fonseca and Cabral 2007) and size and condition at settlement are important for survival (Vigliola and Meekan 2002; Gagliano et al. 2007), with larger fish often having greater survivorship (McCormick and Hoey 2004). However, this pattern is not consistent at all times and places, with some studies showing that newly-settled individuals that were larger suffered higher mortality than smaller fish in some cases (McCormick and Meekan 2007; Meekan et al. 2010). Additionally, earlier work has found no links between foraging behaviors or distance ventured and selective mortality at settlement (McCormick and Meekan 2010; Meekan et al. 2010). Such differences in outcomes of studies may simply be a reflection of the temporal or spatial variability in predator/prey abundance (Holmes and McCormick 2006; Fuiman et al. 2010) or a predator's individual preference of prey species (Holmes and McCormick 2010; Smith and Blumstein 2010). If abundance of predators varies across time and space (Stewart and Jones 2001), or predators selectively target certain species (Almany et al. 2007), then spatial and temporal differences in the relationship of behavioral traits with mortality would be expected. With size being the main predictor of short-term survival (Chapter 4), one viable strategy would be for these fish to prioritize behaviors that maximized growth rates. By growing quickly, juveniles would escape gape-limited predators and better compete for space and resources. In this case, it would be advantageous for juvenile pomacentrids to quickly learn to recognize and ignore false threats (e.g., aggression towards reflection in a mirror; Chapter 3), a trait that is a feature of these fishes (Mitchell et al. 2011). These complex relationships between predator/prey abundance and predator behaviors could be a major driving force in shaping individual variation in the prey's behavior and ultimately, survival in the population.

It is possible that the behavioral relationships in juvenile coral reef fish discussed here are unique to this stage of their life cycle. More research is necessary to determine if these relationships originating during the juvenile stage persist through ontogeny. Also, recent work suggests boldness and other traits are determined by underlying physiology (Killen et al. 2013) or modulated by genetics (Norton et al. 2011; Norton and Bally-Cuif 2012), and highlights the need for more investigations on the proximate causes of behavioral variation. Likewise, further development of the underlying causes of learning is warranted due to the potentially useful framework it provides for the study of intraspecific variation in animal behavior. This study has focused on individual, solitary behavior, however group behavior can also influence changes in individual behavior through social learning (Frost et al. 2007; Manassa and McCormick 2012; Manassa et al. 2013) or competitive interactions (McCormick and Weaver 2012; Poulos and McCormick 2014). Additionally, individual variation in predator behavior and learning abilities will likely be important in determining the outcome in predator-prey interactions. Studies of how social dynamics and predator interactions affect development and stability of behavioral phenotypes may prove to be very useful in the development of the theory of behavioral ecology.

This thesis demonstrates the importance of boldness and other behavioral traits to the ecology of juvenile reef fish, especially during a critical transitional phase in their life history. After settlement on coral reefs, relatively naïve fish rapidly develop personalities but retain flexibility in their behavioral responses across situations. Alternative behavioral phenotypes adopted by these fish are likely maintained across generations due to trade-offs related to competition and predation, such as learning ability. By integrating individual variation in behavior into studies of life histories and community dynamics, we can better understand mechanisms that drive population and community ecology.

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Appendices

Using insights from animal behaviour and behavioural ecology to inform marine conservation initiatives

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Abstract

The impacts of human activities on the natural world are becoming increasingly apparent, with rapid development and exploitation occurring at the expense of habitat quality and biodiversity. Declines are especially evident in the oceans, which hosts some of the most fragile and biologically rich ecosystems on the planet. These ecosystems hold intrinsic value due to their uniqueness; however, they also possess substantial sociological and economic importance, making their preservation critical for the wellbeing of much of the world's population. In response to recent declines in biodiversity, conservation initiatives incorporate interdisciplinary knowledge to create targeted conservation and management strategies. Here, we review how the incorporation of knowledge from the fields of Animal Behaviour and Behavioural Ecology has, and may further assist conservation initiatives in marine environments. We first discuss how consideration of larval ecology and the cues they use to navigate their environment can have far-reaching implications for the effectiveness of ecologically appropriate marine protected areas. Next, we look at patterns of habitat specialisation across marine organisms; consider how this affects a species vulnerability to habitat degradation, and how protection of key resources and species may affect community persistence. Harvesting and reintroduction techniques can inadvertently skew the behavioural phenotypes of populations, and we discuss how these techniques can be modified to lessen this skew to positively reinforce population persistence. Finally, the effects of climate change on marine ecosystems are almost ubiquitous, and an in-depth understanding of how species physiologically and behaviourally respond to a changing environment provides insights into areas of particular vulnerability for prioritized conservation attention. The complex nature of conservation programs inherently results in interdisciplinary responses, and the incorporation of knowledge from the fields of Animal Behaviour and Behavioural Ecology have, and may further increase their effectiveness at stemming biodiversity loss.

Keywords: Behaviour, Conservation, Coral Reefs, Marine Systems, Fisheries

Introduction

Human activities are rapidly reshaping the natural world (Vitousek et al. 1997; Butchart et al. 2010; Rands et al. 2010). The degradation of ecosystems through the extraction of resources, development of habitats, and exploitation of wild populations has a myriad of negative effects on associated organisms and can reduce population sizes to below viability, while simultaneously limiting their potential for recovery (Andrén 1994; Trombulak and Frissell 2000; Jackson et al. 2001; Fahrig 2003; Bellwood et al. 2004; Fabricius 2005). In addition, pollution produced as a by-product of human activity can not only affect individual species but also entire ecosystem dynamics by changing the climate and chemical state of the local environment (Munday et al. 2009; Dixson et al. 2010). The extent of human activity is sufficiently intense that it is changing the ecological condition of the entire planet (Orr et al. 2005; Seinfeld and Pandis 2006; Doney et al. 2012), which is having ubiquitous and profound effects on global biodiversity (Walther et al. 2002; Parmesan and Yohe 2003; Thomas et al. 2004; Rosenzweig et al. 2008; Bellard et al. 2012; Doney et al. 2012). As such, while efforts to stem the loss of biodiversity are delivering some successes (Butchart et al. 2006; Hoffmann et al. 2010) overall, global biodiversity continues to decline.

The field of conservation biology developed during the 1980s as a direct response to this biodiversity decline (Soulé 1985). With the primary aim of conserving biodiversity, it is transforming conservation from a practice that relies primarily on information obtained through personal experience and word of mouth, into an evidence-based practice that relies on systematically accumulated evidence and meta-analyses (Sutherland et al. 2004; Stewart et al. 2005; Waylen et al. 2010; Kareiva and Marvier 2012). Environmental problems are inherently complex, and the responses are inherently interdisciplinary. Consequently, viable conservation initiatives blend disparate fields such as anthropology, climate science, communication, conservation biology, economics, psychology, public health and public policy (Kareiva and Marvier 2012). Even within conservation biology, the complex nature of environmental problems has spurred the emergence of a variety of sub-disciplines, such as conservation genetics (Hedrick 2001), conservation physiology (Wikelski and Cooke 2006) and conservation behaviour (Sutherland 1998).

Conservation behaviour applies insights from animal behaviour and behavioural ecology to conservation initiatives (Sutherland 1998; Caro 1999; Buchholz 2007; Berger-Tal et al. 2011; Cooke et al. 2014). While understanding how an organism behaves is intuitively important for its conservation, the usefulness of conservation behaviour stems from an understanding of the proximate and ultimate underpinnings of behavioural processes, which can be viewed as a higher-order function of an organism’s genetics and physiology (Tinbergen 1963; Buchholz 2007). The usefulness of these methods for delivering effective conservation outcomes has been the subject of recent debate, primarily due to the inherently different scopes of conservation biology and behavioural ecology (e.g. Caro

2007); however, there are success stories. For example, the use of artificial settlement cues (conspecific vocalizations) has been used to establish new populations of black-capped vireos (*Viero atricapilla*) (Ward and Schlossberg 2004) and predator training appears to have a positive effect on post-release survival in captive-reared prairie dogs (*Cynomys ludovicianus*) (Shier and Owings 2006; Buchholz 2007). Examples like these suggest that this emerging field has the potential to be an important component of effective conservation efforts.

While great attention has been paid to the decline of terrestrial ecosystems, an increasing proportion of the global conservation effort is now directed towards the marine environment. These areas include some of the most biodiverse and biologically unique ecosystems on the planet, from shallow coastal coral reefs to deepwater seamounts. They are also amongst the most fragile, with habitats being degraded and species abundances in decline (Hoegh-Guldberg 2015). The uniqueness of these ecosystems confers intrinsic natural value, but they also carry huge economic value: as an asset, the world's ocean was recently valued at least at $24 trillion USD each year through its support of industries as diverse as fisheries, tourism and trade (Hughes 1994; Hughes et al. 2002; Roberts et al. 2002; Orr et al. 2005; Doney et al. 2009; Munday et al. 2009; Dixson et al. 2010; Hoegh-Guldberg 2015). This review will provide an overview of some key areas where insights from aspects of Animal Behaviour and Behavioural Ecology can contribute to effective conservation efforts in marine ecosystems, with a particular focus on the conservation of tropical fish assemblages and fisheries. In particular, we will focus on how 1) understanding larval behaviour can improve predictions of dispersal, 2) how examining behavioural flexibility could help identify extinction risk in resource specialists and inform targeted conservation efforts, 3) how determining behavioural variability within populations could assist fisheries management and practices, and 4) how determining the relationship between physiology and behaviour can aid predictions of climate change effects. Promising avenues for future research will be discussed throughout.

Identifying links between larval behaviour, habitat selection, and connectivity

Choosing where to settle is a critical decision that has both immediate and long-term consequences for survival and reproduction (Thorpe 1945; Klopfer 1963; Morris 2003; Radovic and Mikuska 2009; Schulte and Koehler 2010; Mestre and Lubin 2011). Juveniles may either remain close to natal sites, or disperse to other locations, leading to complex distributions that can span broad geographic areas (Robbins et al. 2009; Costello 2010; Gienapp and Merilae 2011; le Roux et al. 2011; Oro et al. 2011). Whether in search of home or a new habitat, a diverse range of sensory information is used to inform juvenile settlement decisions (Klopfer 1963; Wecker 1963). These behaviours can be learned, innate, or a compromise between the two (Dixson et al. 2014). Given the continued degradation and loss of natural habitats, the need to understand how settlement decisions are made is vital.

Marine species typically have a life history characterized by a pelagic larval stage followed by a relatively sedentary adult stage (Brothers et al. 1983; Leis 1991; Kingsford et al. 2002). The pelagic larval stage usually begins immediately after or just before embryogenesis (Petersen et al. 2001), lasting from a few days to months depending on the species. Until the late 1990s, it was thought that marine larval dispersal was a purely physical process, with larval transport dictated by hydrodynamic processes (Roberts and Hawkins 1997). As a result, it was assumed that dispersal and population connectivity over large spatial scales was determined by local and regional physical processes that “delivered” larvae to a location (Cowen and Sponaugle 2009). However, this paradigm has been repeatedly challenged by demonstrations of well-developed behavioural and sensory abilities in larvae, and patterns of dispersal that operate at smaller scales than would be expected from physical processes alone (Kingsford et al. 2002; Swearer et al. 2002; Leis et al. 2011; Buston et al. 2012). Determining the degree of connectivity between populations is critical for understanding population and community dynamics. As directly measuring connectivity remains impossible for most species on scales other than evolutionary gene flow, creating realistic models of dispersal potential is essential. Incorporating larval behaviour and sensory preferences into models has been shown to greatly increase their accuracy, providing invaluable information for management (Thorrold et al. 2002; Warner and Cowan 2002; Sale et al. 2005).

The larvae of many marine animals, such as fish, crustaceans, and corals, have well-developed auditory and olfactory systems that they use to inform settlement decisions (Myrberg and Fuiman 2002; Tolimieri et al. 2004; Vermeij et al. 2010; Dixson et al. 2014; Brooker and Dixson 2015). For instance, olfactory cues are used by larval fish to distinguish between microhabitats and locate areas with food resources, conspecifics, or predators (Sweatman 1983; Knutsen 1992; Døving et al. 1994; Batty and Hoyt 1995; Arvedlund et al. 1999; Ben-Tzvi et al. 2010). Olfactory cues are also used for orientation and navigation at greater spatial scales (Dixson et al. 2008; Dixson et al. 2011; Leis et al.

2011), with odors from native terrestrial vegetation shown to guide fishes to near-shore habitats over considerable distances (Dixson et al. 2008; Leis et al. 2011). Also important for many larvae, auditory cues can travel through water, irrespective of current and with little attenuation, over hundreds of meters (Simpson et al. 2005; Montgomery et al. 2006; Vermeij et al. 2010). As the soundscapes of different habitats are the result of a unique combination of biotic and abiotic factors, acoustic cues can provide larvae with critical information as to the type and relative quality of surrounding habitats (Piercy et al. 2014; Nedelec et al. 2015). Although the majority of research has examined the chemosensory and auditory abilities of larvae, it is likely that various other sensory systems play an equally important role, with cues used in tandem and varying in importance over temporal and spatial scales (Paris et al. 2013; Dixson et al. 2014; Lecchini et al. 2013).

While sensory cues appear to be a critical driver of habitat selection it is generally unclear whether larvae respond to a specific component of an overall cue i.e. a key chemical or sound frequency, or if a range of habitat-specific components are needed to invoke an attraction or avoidance. It is also likely that cue concentration and concentration gradients play a key role in stimulating orientation and locomotion. However, the concentration thresholds that are needed to invoke a behavioural response and critically, whether these responses are innate or flexible, are unknown. Therefore, research that attempts to isolate the nature of sensory cues is needed to predict settlement behaviour in degraded sensory landscapes (Dixson et al. 2014). For instance, while chemical cues from native vegetation can act as sensory indicators of fringing reefs (Dixson et al. 2008), areas of tropical coastal forest are rapidly being converted to agricultural crops such as sugar cane, mahogany, and palm oil. Examining behavioural responses to reduced native cue concentrations and novel cues could indicate whether larvae will continue to settle into these habitats. Likewise, declines in coral cover and associated reef biodiversity threatens to reduce reef noise below thresholds perceptible to larvae, with healthy reefs producing stronger auditory signals (Piercy et al. 2014). Determining the effects of habitat degradation and anthropogenic noise, and whether species can adapt to these new conditions (i.e. are larvae originating from degraded habitats more likely to accept degraded sites at settlement) is a critical next step. While marine conservation and management strategies often focus on single ecosystems, incorporating the ecological linkages between various systems will likely increase the success (Nagelkerken 2009). For instance, tropical coastal systems are often comprised of seagrass meadows and mangrove forests that can act as an important buffer between coral reefs and coastal developments. These coastal systems and coral reefs are also linked with regards to larval connectivity (see reviews by Cowen et al. 2006; Leis 2006), acting as important nursery areas for many of the recreational and commercial fisheries species that occur on coral reefs as adults. Thus, preservation of coral reefs as well as these supporting ecosystems, through the creation of marine protected areas (MPAs) or restricting adjacent development, will also play a key role in the maintenance of fisheries resources and biodiversity and preserving sensory environments.

Resource specialisation, vulnerability to habitat degradation, and minimising biodiversity loss

While an animal's habitat provides all the resources that are required for survival, it is likely to contain a wider range of exploitable resources than are actually used. As with habitat selection, the use of essential resources, such as food and shelter, is often the result of active behavioural decisions by the individual, with species existing somewhere along an axis from specialist, only selecting a limited range of items from those available, to generalist, exhibiting greater selective flexibility (MacNally 1995). Resource specialists evolve when organisms have stable access to preferred resources and often have physical or behavioural adaptations that allow them to maximise the efficiency with which they can exploit these resources (Futuyma and Moreno 1988; Forister et al. 2011). Therefore, when access to preferred resources is not limited, specialists can be expected to hold a competitive advantage over more generalist species occupying the same habitat (Caley and Munday 2003). However, these adaptations may disadvantage an animal if preferred resources become scarce and they reduce its ability to exploit alternatives. Under non-ideal conditions, specialists that can successfully switch to non-preferred resources may suffer sub-lethal effects, such as declines in condition that reduce their relative competitiveness (Berumen et al. 2005). However, if an animal is so specialised that it is unable to modify its behaviour and select alternative resources this could have lethal consequences at the individual, population, or even species level (Gaston and Blackburn 2000; Biesmeijer et al. 2006).

Resource specialists from a diverse range of taxa are found across the marine environment, from the polar oceans to shallow tropical seas (i.e. Stella et al. 2011; Pitman and Durban 2012), where they often fulfil important ecological or socioeconomic roles (Pratchett 2001; Bellwood et al. 2003; Grutter et al. 2003; Graham and Nash 2013). However, as the condition of marine habitats steadily declines specialist species are expected to face the greatest risk of extinction following changes in resource availability, with generalist species meanwhile increasing in abundance (Clavel et al. 2010). Despite this prediction, accurate assessment of species vulnerability is often hindered by a limited understanding of a) the exact type and breadth of resources used, and b) the degree of behavioural flexibility exhibited at the individual, population and species level. For example, many of the organisms found on coral reefs have a critical dependence on reef-building scleractinian corals, for food, shelter or during settlement processes (Cole et al. 2008; Stella et al. 2011; Bonin 2012), with declines in coral cover leading to declines in the abundance of many reef-associated species (Jones et al. 2004; Pratchett et al. 2006). While the importance of corals for the health, diversity, and socioeconomic viability of reef communities is well documented (Pratchett et al. 2008b; Graham et al. 2011; Graham 2014), an increasing body of research into the behaviour of coral-associated animals is revealing that many are far more specialised than previously thought, displaying selectivity between corals at the genus, species, or even intra-colony level (i.e. Munday 2004; Pratchett 2007; Rotjan and Lewis 2009; Gardiner and Jones 2010; Stella et al. 2011; Brooker et al. 2013b; Brooker et al. 2013a). Being this selective will directly increase extinction risk and suggests that measuring the vulnerability of coral reef species based on measures such as coral cover may be of limited value if other factors, such as benthic community composition and the differential susceptibility of corals to disturbance (Marshall and Baird 2000), are not also included. For example, Munday (2004) found that declines in the abundance in a guild of coral-dwelling gobies following coral loss varied inter-specifically relative to the breadth of corals used as habitat. Likewise, dramatic declines in the abundance of some coral-feeding fishes have been observed following catastrophic reductions in coral abundance (Kokita and Nakazono 2001; Brooker et al. 2014), while other species appear to be resilient to these changes (Pratchett et al. 2004). The magnitude of decline appears to reflect the behavioural inflexibility of some species, with local extinctions appearing to correlate with declines of key prey species, despite the presence of alternative coral prey (Brooker et al. 2014).

Like coral reefs, living organisms create habitat structure and diversity in many marine habitats, for example macroalgae within temperate reef ecosystems (Steneck et al. 2002) or deep-water sessile invertebrate communities (Rowden et al. 2010). Many habitat-forming species are themselves specialised with regards to resource requirements or abiotic tolerance (i.e. Smale and Wernberg 2013), and are also vulnerable to environmental degradation, with inter-specific differences in vulnerability likely to shift benthic composition towards low-diversity communities dominated by tolerant generalist species (Pratchett et al. 2011). Therefore, declines in total biodiversity may not only follow declines in total resource abundance, but also as a result of subtle compositional changes to benthic communities that reduce niche diversity. As a result, management strategies that preserve benthic habitat composition (i.e. by limiting practices such as dredging or creating habitat refuges) may play a critical role in preserving associated biodiversity. Inter-individual variation in prey or habitat selectivity may also offer a potential buffer against the decline of a specific resources and examining variation in resource use across gradients in availability (i.e. Lawton et al. 2012) could act as a proxy measure of behavioural flexibility, with selective congruence between disparate population indicating critical resources.

Aside from protecting specialised habitat forming species that promote biodiversity (i.e. *Acropora* corals, *Macrocystis* kelps), some specialised animals have a disproportionate affect on surrounding biodiversity, making targeted measures that also ensure their persistence particularly important. For instance, macroalgae is a pressing threat to the coral reef biodiversity as it can rapidly outcompete corals, reducing complex habitats into low-diversity systems (Hughes et al. 2007). While many coral reef fishes are herbivorous, only a limited number will selectively feed on the species that pose the greatest threat (Bellwood et al. 2006). Protection of functionally important species is therefore paramount, and could be achieved through the reduction of fishing pressure, via community education or the creation of MPAs. Likewise, the cleaner wrasse (*Labroides dimidiatus)* has a highly specialised diet consisting of parasites consumed during cleaning interactions. However, the removal of cleaner wrasse from reefs can result in massive declines in the diversity, abundance, and health of local fish communities (Grutter et al. 2003; Clague et al. 2011). As with many coral reef fishes, wild stocks of cleaner wrasse are often heavily exploited for the commercial aquarium trade. Simply reducing pressure on wild stock by shifting reliance onto the growing ornamental aquaculture industry may be an achievable method of limiting declines in fish diversity.

Incorporating behavioural variation into resource conservation and management

As discussed above, the capacity of marine species to adapt to predicted environmental changes is intimately tied to their behaviour. If the behaviour of individuals from within a population varies substantially, this can make it difficult to accurately predict species level responses (Norton et al. 2011; Archard et al. 2012). Consistent, inter-individual behavioural differences are almost ubiquitously recognised across taxa (reviewed in Gosling 2001), with individuals differing in behavioural traits such as boldness, aggressiveness, activity levels, reactivity, sociability, fearfulness and exploration (Dall et al. 2004; Réale et al. 2007). If an individual displays consistency in a single behaviour over time or contexts this is termed 'personality' whereas consistency in the relationship between two or more functionally different behaviours is defined as a 'behavioural syndrome' (Garamszegi and Herczeg 2012). The presence of either personality and behavioural syndromes implies that an individual can only exhibit a limited number of behavioural responses, creating cost-benefit trade-offs (Kelley et al. 2013) that can influence survival (Downes 2002; Dingemanse et al. 2004; Smith and Blumstein 2008), reproductive success, (Both et al. 2005; Sih and Watters 2005; Pruitt and Ferrari 2011) resource acquisition (Webster et al. 2009), and growth (Meekan et al. 2010). Determining how variable and flexible behavioural traits are, both within and between individuals, is critical for understanding the ecology and evolution of species, and how they will respond to ecological change (Wilson et al. 1994; Dall et al. 2004; Sih et al. 2004; Carter et al. 2013). For instance, the level of behavioural consistency within populations will directly influence distribution and abundance, inter-species interactions, population dynamics (Sih et al. 2012; Pruitt et al. 2013; Mittelbach et al. 2014), ecological invasions (Conrad et al. 2011; Sih et al. 2012) and responses to environmental and ecological shifts (Réale et al. 2007; Sih et al. 2012). If individuals within a population display a range of behavioural responses to ecological change this may increase that populations overall adaptability and ability to persist under novel conditions. It is therefore beneficial to have a spectrum of behavioural phenotypes within a population in order to cope with future selective pressures. Therefore, as with resource selectivity, determining variability within populations, and undertaking measures to preserve it, may be an important conservation tool (McDougall et al. 2006; Smith and Blumstein 2008; Powell and Gartner 2011).

Evolutionary changes are thought to happen quickly in populations with a large variation in behavioural phenotypes, thus depletion of behavioural variation may slow down rates of evolution **(**Wolf and Weissing 2012), and thus limit recovery potential (Hutchings and Reynolds 2004)., In marine ecosystems, individual fish and fish populations vary consistently in their behaviour and display differences in their behavioural heterogeneity (Biro et al. 2010; Carter and Feeney 2012). Selective pressures that limit variation in behavioural types may reduce the viability of these populations to respond or adapt to their environment, and this may already be occurring in some exploited populations. For example, commercial fisheries typically target the largest individuals, indirectly biasing populations towards phenotypes with slow growth and early maturation (Biro and Post 2008). Because fast growth phenotypes are on average more active, bold, and aggressive, this makes them more vulnerable to fishing compared to more slow-growing conspecifics (Biro and Post 2008; Biro and Dingemanse 2009; Wilson et al. 2011). Fisheries policy decisions that take catch size and behavioural diversity into account are therefore necessary to ensure population persistence and the ongoing viability of fisheries. Imposing maximum as well as minimum size catch restrictions, as well as selectively targeting species with naturally short life spans, might allow for a greater range of personality types to persist in wild populations and allow for a less biased removal of personality types.

Another commonly applied fisheries conservation method is species reintroductions and population enhancements (Wolf and Weissing 2012) with the success of these efforts potentially influenced by behavioural variability. For instance, current methods of hatchery-rearing fish stocks for release into the wild are largely unsuccessful with as little as 3% of released fish surviving until adulthood, largely due to behavioural deficits in released fishes (Brown and Day 2002). While hatchery-reared individuals are typically more bold and aggressive than their uncultivated counterparts (Sundström et al. 2004) they often have a limited ability to recognize, capture, and handle wild prey (Brown and Day 2002), and are inexperienced with predators (Jackson and Brown 2011) leading to the low rate of survival. It is possible that mortality could be reduced through pre-release behavioural training of juveniles, with different training regimes undertaken to increase the diversity of behavioural phenotypes in the released stock (Brown and Day 2002). This training could be accomplished via exposure to conspecifics that exhibit a desired behavioural trait. Fishes are capable of rapidly learning novel tasks or important anti-predator skills through social learning (Manassa and McCormick 2013; Manassa et al. 2013) therefore exposure to trained conspecifics could greatly improve the success rates of reintroduction programs. However, Brown et al. (2013) discuss how individual variation in retention of learned information can influence post-release survival. They suggest increasing the strength/frequency of conditioning, reducing time between conditioning and release, reducing food supply before release, conditioning with multiple predator cues and conditioning in an appropriate habitat (Brown et al. 2013). Clearly, there is a need for a greater understanding of variation in behavioural responses and for the factors that promote behavioural variability to be integrated into conservation management strategies. A greater understanding of how individuals within a population cope with changes to their direct environment will greatly improve our ability to predict and manage how populations as a whole may respond to the changes in the ecosystem. Specifically, classifying the behavioural phenotypes through ontogeny in a population of commercially harvested species would allow fisheries to avoid eliminating personality types from vulnerable species or age-classes. Also, more research is needed in individual variation of learning and associated factors of retaining ecologically important (i.e. foraging and anti-predator) behaviours necessary to improve post-release survival in reintroduction programs.

Identifying the role of physiology in behavioural responses to climate change

In addition to incorporating behavioural variation, effective conservation efforts should also consider the various external and internal stressors that drive these behavioural responses. While it is well known that habitat degradation or exploitation can affect behavioural patterns, even subtle changes in water biochemistry and temperature can cause equally dramatic physiological and behavioural changes in individuals and populations, ultimately leading to reductions in fitness and abundance even in seemingly healthy habitats (see Buckley et al. 2011; Cheung et al. 2013). Understanding the connection between physiology and behaviour, particularly the behavioural strategies that marine organisms typically use to alleviate physiological stressors can be invaluable for effective conservation strategies.

Since the majority of marine organisms are ectothermic, their capacity for physical and physiological performance is directly affected by the ambient water temperature (e.g. Pörtner and Farrell 2008; Pörtner et al. 2010). Rising temperatures increase the rate of biochemical and cellular processes, which increases the energetic cost of activity, growth, and reproduction (Pörtner et al. 2010; Hein and Keirsted 2012). In fishes, for example, energetic requirements generally rise 2-3 fold for every 10°C increase in temperature (Evans and Claiborne 2006), suggesting that the necessity to hunt prey or conserve energy can rise significantly during warmer summer months, particular in temperate regions with large seasonal temperature fluctuations. Similarly, if global warming causes a 3°C increase in mean ocean surface temperature by 2100 as predicted, this could increase the basal metabolic maintenance cost of affected fishes by 30-40% (i.e. the minimum energy required for survival: Evans and Claiborne 2006; Stocker et al. 2013). While increases in metabolic demand can cause significant reductions in swimming speeds and activity patterns of large predatory species, consistent with a strategy to conserve energy, acquisition of energy for growth and reproduction is critical for continued survival, and so reduced activity may be detrimental for species that require large home-ranges, travel vast distances to spawn, or depend on strong swimming performance to catch prey (Johansen et al. 2014). More importantly, it is unlikely that all trophic levels will increase productivity by the required 30-40% due to associated habitat loss and exploitation (see Jones et al. 2004; Pratchett et al. 2008a; Graham 2014), which may lead to increased top-down competition for a dwindling number of prey, leading to cascading changes in species compositions and abundance across ecosystems.

Shelter selection and within-habitat settlement patterns can also be predicted in conservation strategies through the inclusion of thermal physiological metrics such as aerobic scope. Aerobic scope is the difference between standard metabolic rate (SMR, i.e. the minimum oxygen uptake required for metabolic maintenance) and maximum metabolic rate (MMR), indicating the physiological capacity for critical activities at a given temperature. Aerobic scope has been related directly to hypoxia tolerance (Nilsson et al. 2010), swimming performance (Johansen and Jones 2011), competitive dominance (Killen et al. 2014) and reproductive output (Donelson et al. 2014). Of these, hypoxia tolerance is particularly important in coral reef fishes as many species seek shelter among coral branches where oxygen levels become depleted at night (Nilsson et al. 2010). As hypoxia tolerance in fishes is generally dictated by metabolic oxygen demand, elevated temperatures, that increase this demand can therefore force species to vacate established night-time shelters and relocate into open habitats with a greater risk of predation (Nilsson et al. 2010). Diminished aerobic scope may also compromise maximum oxygen delivery to tissues and muscles and hence the capacity to swim. In fact, elevated temperatures can reduce maximum swimming speeds to such an extent that fishes are no longer able to occupy high current habitats (Johansen and Jones 2011). As a result, conservation strategies aimed at safeguarding habitat structure or population composition may benefit from an understanding of the physiological parameters that can cause temporal changes to habitat shelter selection or distribution patterns within habitats.

Most current efforts to conserve heavily-fished species consist of bans on fishing during the spawning season, but this approach is not always effective. Gonadal development and reproduction of many species is regulated by photoperiod and temperature and often a long photoperiod is required for reproductive stability (e.g. Bapary et al. 2009). As a result, while blanket fishing bans may be effective for protecting species with limited distributions, fished species that have a large latitudinal distribution can exhibit differential spawning onset across latitudes and so may benefit from differentially timed fishing closures. Additionally, global warming is expected to change the onset and conclusion of the breeding season of numerous species by limiting reproduction to acceptable thermal windows and photoperiods during early spring and late summer (see Pankhurst and Munday 2011). The tight link between reproductive physiology and ambient biophysical conditions means that changes in reproductive behaviours, such as the timing of migratory movements to spawning grounds, can be predicted and anticipated in conservation strategies.

The environmental conditions found in some marine ecosystems are so physiologically incompatible with the biology of certain fishes, that these species are forced to either relocate or avoid these areas altogether. For instance, many coral reef fishes found in the Red Sea and Arabian peninsula are absent from reefs in the Arabian gulf where temperatures can reach >35°C during summer, indicating an upper limit to thermal adaptation (Al-Rashidi et al. 2009; Burt et al. 2011). Similarly, recent work on equatorial fish populations has revealed reduced capacities to tolerate the 3°C temperature increase expected by 2100 (Rummer et al. 2014), while populations at higher latitudes appear better able to tolerate similar conditions (Gardiner et al. 2010; Takahashi et al. 2012; McLeod et al. 2015). Consequently, incorporating behavioural mitigation patterns, such as the tendency of populations to relocate to cooler habitats, deeper habitats, or to higher latitudes, into conservation strategies is expected to become increasingly important as global climatic conditions change both in tropical and temperate regions (Dulvy et al. 2008; Booth et al. 2011; Burrows et al. 2011; Madin et al. 2012). Work by Schurmann et al. (1991) and Killen (2014) has shown that some marine teleosts will actively search for habitats with ambient water temperatures that increase physiological performance. This preferred temperature is tightly regulated by thermal physiological performance, as species are thought to actively avoid conditions of metabolic depression (Schurmann et al. 1991; Killen 2014). Therefore, efforts to conserve specific habitats should consider both how current conditions benefit the inhabiting species, and how these conditions may change over time.

There is increasing evidence that rising sea temperatures are changing the distributions of many marine species, with these range shifts expected to increase in magnitude over time. The species most likely to relocate deeper or pole-ward are those with larger body sizes and greater mobility (Feary et al. 2014), with low levels of parental care (i.e. broadcast spawners), and those with relatively wide latitudinal ranges (indicative of a tolerance to a greater thermal window). To date more than 360 species of tropical fishes spanning 55 different families have been recorded settling into cooler higher latitude regions (Feary et al. 2014), with no species demonstrating the opposite trend of range shrinkage to lower latitudes (Feary et al. 2014). However, evidence suggests that such changes in patterns of habitat-use may negatively impact the majority of affected populations. For instance, as previously mentioned specialized species will have a limited capacity for geographical shifts in habitat use compared to generalist species. Importantly, major changes in habitat structure and species distribution patterns are also expected to create novel ecosystems with completely different fish assemblages, dominated by habitat generalists (Graham 2014). Such changes will have unpredictable consequences for many species. From a conservation standpoint, localized extinctions are inevitable. However, given adequate insight into the physiological resilience and threshold tolerance levels of important species, effective mitigation and industry preparation may reduce the severity of such occurrences.

Conclusions

Conservation initiatives incorporate knowledge from disparate fields to create targeted responses. Animal Behaviour and Behavioural Ecology are intuitively important for the management of biodiversity; however, while success stories exist, there is also scepticism about the extent to which this information can contribute to programs that aim to stem the loss of biodiversity (e.g. Caro 2007). Throughout this review, we have highlighted how studies of the proximate and ultimate causes of animal behaviour has shed light on how and why animals in the tropical marine assemblages select habitats, occupy different ecological niches and exhibit differences in their behaviours as well as the physiological and behavioural changes that result from a changing environment. We discuss how this information could be useful for conservation initiatives; give specific examples of how changes in human activities could positives reinforce biodiversity and highlight important areas for future empirical and theoretical studies that could further inform conservation biology. The study of Animal Behaviour and Behavioural Ecology provides insights that can inform conservation efforts, and further research into areas of particular conservation importance facilitates opportunities to integrate these intuitively similar applied and fundamental fields.

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