This file is part of the following reference:


Access to this file is available from:

http://researchonline.jcu.edu.au/47533/

*The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owner of any third party copyright material included in this document. If you believe that this is not the case, please contact ResearchOnline@jcu.edu.au and quote http://researchonline.jcu.edu.au/47533/*
EFFECTS OF TEMPERATURE AND FOOD AVAILABILITY
ON THE ANTIPREDATOR BEHAVIOUR
OF JUVENILE CORAL REEF FISHES

Thesis submitted by
Govinda-Das Hugo, LIENART
(BSc, MSc)
in July 2016

for the degree of Doctor of Philosophy
College of Marine and Environmental Science
Centre of Excellence for Coral Reef Studies
James Cook University
Statement of contribution of others

This thesis includes collaborative work with my supervisors, Professor Mark McCormick and Associate Professor Maud Ferrari; as well as with Dr. Matthew Mitchell. While undertaking these collaborations, I was responsible for the research concepts and designs, data collection, analysis and interpretation of results. My co-authors provided intellectual guidance, editorial assistance and technical support throughout the present research project.

Financial support was provided by the Australian Research Council (Mark I. McCormick), the ARC Centre of Excellence for Coral Reef Studies (Mark I. McCormick) and James Cook University Graduate Research Funding Scheme (Govinda D. H., Lienart). A stipend was provided through the Australian Postgraduate Award scheme.


Chapter 5 has been submitted as Govinda D. H. Lienart, Maud C.O. Ferrari, Mark I. McCormick (2016). Thermal environment and nutritional condition affect the efficacy of chemical alarm cues by prey fish. *Environmental biology of fishes*. 
Declaration of ethics

The research presented in this thesis was conducted in accordance 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 research received and was conducted under the animal ethics approval from the JCU Animal Ethic Committee Approval number #A2080.
Acknowledgments

I guess the most obvious way to describe a journey towards the completion of a doctoral thesis is through the lens of climbing the highest peak on earth. Although I recognise that an 8.848m mountain and a PhD is not exactly the same, they both represent an immense challenge, and more importantly, they are optional and an inherently privileged pursuit in life. After a long journey of persistent climbing I did eventually reach the peak. However, the view from here is not exactly what I had expected; I am surrounded by dense clouds and it is hard to grasp that I am at the top. Perhaps this confirms what people say, it really is the journey and not the destination that you most remember. And hell yeah, what a bloody enriching adventure it has been! Looking back, I also realise that this achievement would simply not have been possible without an amazing number of souls that have supported and guided me along my ascent.

My foremost words of acknowledgement go to my main supervisor, Mark McCormick, who not only gave me this opportunity but also has been doing an amazing job in cautiously and patiently guiding me from the first until the very last step of this climb to the top. Thank you Mark, for the support, the trust and for giving me such an incredible amount of flexibility to mark out my own itinerary. Another person that has played a key role along the way, is my co-supervisor, Maud Ferrari. She is a tough and expert hiker that would not hesitate to call me back on track if necessary. Thank you Maud for being so exigent and upfront with me. The times you were pleased with the manner of which I was dealing with some of the obstacles I faced, were a true indicator for me, that I was heading in the right direction.

Then there are also the people that accompanied me during a more transitory timeframe along my path; however, without their support I wouldn’t be writing these words. I am especially thankful to Matthew Mitchell, who played a critical role at the very beginning of my journey, by pointing out which direction best for me to go up and by sharing critically important survival skills. I also need to mention the following individuals,
who significantly contributed to my work along the way: Cecilia Villacortez, Lucy Holmes, Amy Douglas, Christine Fernance, Miwa Takahashi, Sue Reilly, Bridie Allan, Lyle Vail, Anne Hogget and Rhondda Jones.

The doctoral path does, however, sometimes feel like an eternity of lonely scrambling and head-scratching. Yet, you do hear, in the here and in the there, other voices through the fog. It is then that you realise that you are not the only one trying to find a route to the summit of self-realisation. On occasion you cross paths with individuals - sharing experiences and sometimes set about finding a route that doesn’t demand too many difficult moves. In this context I would like to mention the following courageous and inspiring doctoral adventurers I met along the way: Ryan Ramasamy, Bridie Allen, Lauren Nadler, James White, Davina Poulos, Maria Del Mar Palacios, Justin Rizzari, Lisa Bostrom, Oona Lönnstedt, Giverny Rodgers, Ian McLeod, Megan Welch, Blake Spady, Rachel Manassa, Melina Rodriguez and Donny Warren.

I am also especially thankful to all my beloved friends who have added colour to my life and have helped to gently shape the moments that make up the journey. Among them are Pedrinio Pereira, Amanda Gois, Gerardo Martin, Rosaura Valdez, Salito Maldonado, Miwa-cha Takahashi, Peter Morse, Amaury Frankl (R), Hugo Pous, Bridie Allan (BB), Maria Del Mar Palacios, Tessa Hempson, Magali Poetje, Julian Galdino, Rafael Magris, Magui Libelula, Cindy Huchery, Zara Woef, Kimmke Woef, Jihyun Wankod, Magda Cor, Pollita Rodriguez, Skatie Van Latham, Ilke kleintje, Ceci Cabronita, Federico Hermano, Vidje douterlunghe, Karèl de Muynck, Meli Rodriguez, Priya Modi, Paul and Sondra Cracknell. These are just a few names among many others that have made my daily life so much more fascinating and enjoyable.

And then of course there is ‘la familia’ - without their tremendous emotional support, ascending the steep mounts that I faced may have become desperately more difficult, if not impossible. I am infinitely grateful to my mother (Fabienne De Wolf), my father (Baudouin Lienart), and my brother (Django, mon petit Canard), for their unconditional love and constant belief in me and my dreams. I am lucky to have such a large and beautiful family, including my cousin Thibaut de Wolf who is like a brother.
to me. Of course, I have to mention my grandfather (Daniel De Wolf) and finally my grandmother (Josette Lenoir). Grandma, I wish you didn’t have to go, but your smile is still here with me and always reminds me your sweetness. I want to dedicate this piece of work to you, bonne-maman.

Last but not least, I want to express my gratitude to all of the water-breathing animals that aided me in this study. I initially pursued this PhD-journey with the idea that I would study sharks; probably because since I was a child these animals represented the ultimate majesty of the ocean. I ended up studying baby coral reef fishes that were just over a few millimetres in size. However, over the years, the more I delved into their world, the more I became fascinated about the prodigious greatness that is encapsulated in these tiny creatures. This realization definitely sparkled in me an even deeper respect for all forms of nature and a formidable willing to contribute in finding ways in which we humans could live in a more harmonious cohabitation with all the other living beings on this lonely planet.
General abstract

All organisms must obtain energy to survive, grow and reproduce. For species in the middle of food chains, such as juvenile coral reef fishes, energy acquisition can be particularly risky because individuals are often more vulnerable to predators while foraging. Thus, prey are under continuous pressure to optimize trade-offs between the benefits of antipredator behaviour and foraging behaviour. In addition, environmental conditions can also further cause shifts on how prey balance the costs and benefits of foraging in the light of predation risk. In particular, temperature and food availability might be expected to affect antipredator behaviour of animals through the profound impact they can have on the animal’s physiology. However, the drivers and different pathways linking these environmental factors to changes in behaviour of prey animals when facing predation risk remain poorly investigated. The aim of this dissertation was to investigate the different pathways by which the thermal and feeding history of prey fish can impact antipredator behaviour. The series of laboratory-based studies use juvenile coral reef fishes (Pomacentridae) as model taxa.

The importance of a prey’s feeding history has commonly been highlighted as a decisive factor in determining how prey respond to predation risk, with hungry prey accepting higher risk of predation while foraging to avoid risk of starvation. Surprisingly, while rising temperature can also impose higher energy demands for ectotherms such as fishes, no attempt has been made to test whether temperature interacts with food availability to further affect antipredator responses in prey. I explored this research question in Chapter 2. My data indicated that temperature in isolation does not influence the propensity to take risk under predation threat in well-fed fish. However, prey animals reared in condition of restrained food availability appeared to take more risks under predation threat with increasing temperature. As such, the results show that poorly and well-fed fish at 27 °C still displayed a detectable antipredator response to predation threat. However, at 30 °C (a thermal regime equivalent to the regional maximum summer temperature), poorly-fed fish,
not well-fed foraged at a high rate even under the threat of predation. Interestingly, in **Chapter 5** I found, based on level of energy reserves in the liver, supporting evidence that such risk-prone behaviour is very likely to be caused by a temperature-mediated increase in metabolic rate and the need for food to fuel the associated higher energy demands.

A rise in energy demands can lead prey to favor foraging at the expense of increasing exposure to predation threat. However, it can be expected that there will be an offset in the extent to which such risk-prone behaviour may happen with increasing sources of sensory information on predation threat. My experiment, in **Chapter 3** is the first study to address this possibility. Although the observed behavioural patterns were not completely in line with the initial prediction, the results strongly suggest that additional sensory information on predation can lead to a further enhancement of the response, which is dependent on the prey’s feeding history. Poorly and moderately-fed fish responded to a single predator cue, but further enhanced the intensity of their response to multiple predator cues. In contrast, well-fed fish displayed a fully-developed antipredator responses to any threat cue regardless of the nature of the sensory source or number of senses that informed the threat. The high vigilance of well-fed individuals is best explained by the asset-protection principle, which predicts that the more an individual stands to lose (i.e., high body reserves and larger body size), the more cautiously it should behave.

While higher-asset individuals may be more cautious, higher assets in the form of higher condition can in certain context-specific circumstances also lead to increased risk-taking behaviour in prey. In particular, it may be expected that as prey grow they will reduce their level of vigilance to small gape-limited predators as an adaptive mechanism to avoid losing foraging opportunities. It has been further proposed that predator-related information acquired by prey would become outdated and lose its relevancy more quickly in prey animals under high growth rate, since they outgrow their predators sooner than slow-growing individuals. In **Chapter 4**, I empirically tested this prediction by rearing predator-conditioned prey fish under different temperature mediated growth
trajectories (27 °C vs. 30 °C) for 14 days and assessed weekly whether prey still responded to the initially acquired predator information. Although with time after the initial conditioning event all fish gradually decreased the intensity of their response to the learned predator cue, there were no significant differences in the decline of the response among the different growth trajectories. The temperature-mediated growth trajectories may have been too subtle to find detectable differences in behavioural patterns. However, these findings support the existence of an adaptive mechanism causing a decline in the informational value of a learned predator cue over time. This mechanism should give prey the ability to not persevere with maladaptive behaviour towards outdated or irrelevant information.

Temperature and food availability can also be expected to influence a prey’s behavioural decision through alteration of the quality and/or quantity of the available predation-related cues to which they will be exposed. Several studies have highlighted that chemical alarm cues obtained from a poorly-fed donor prey are less effective in eliciting an alarm response in conspecifics than those obtained from fish with a good feeding history. The experiment in Chapter 5 is the first study to test how food availability can, concurrently with temperature, affect the properties alarm cues produced by a donor prey. The results indicate that the alarm cues produced by donor prey triggered a stronger antipredator response in conspecifics as rearing temperature increased from 27 °C up to 30 °C, regardless of the feeding history or body condition of the donors. However, at 32 °C, alarm cues from poorly-fed donor fish do not elicit an antipredator response as effectively in conspecifics compared to those produced by well-fed fish. These results clearly show that food supply and the thermal environment in which an organism lives can impact the effectiveness of the produced alarm cues to trigger antipredator responses in conspecifics.

This dissertation embodies a number of studies that have empirically identified, quantified and examined a series of pathways by which food availability and temperature may affect cost-benefit trade-offs between foraging and predator avoidance. Trends in the data were best explained by the animals’ physiology, growth patterns and energy budget,
and by the underlying impact of these on threat-sensitive and state-dependent behavioural mechanisms. While the current work assessed the individual effects of these pathways, it is clear that in the wild, such pathways will simultaneously occur to further lead to counterbalancing effects on a particular behavioural pattern. The resulting change in the intensity of the antipredator response can be expected to have rapid, widespread and diverse consequences ecological consequences. While further work is required, the current body of work demonstrates that small ecologically-relevant changes in temperature and food supply can independently and interactively determine the strength of predator-prey interaction within tropical aquatic ecosystems.
Table of contents

Statement of contribution of others ................................................................. ii
Declaration of ethics ............................................................................................ iii
Acknowledgments ............................................................................................... iv
General abstract .................................................................................................... vii
Table of contents ................................................................................................. xi
List of figures ......................................................................................................... xiii
List of tables .......................................................................................................... xv
List of supplementary files .................................................................................. xvi

Chapter 1: General introduction .......................................................................... 1
  1.1 Temperature, food availability and ectothermic species ............................... 1
  1.2 State-dependent and threat-sensitive antipredator behaviour of prey. ........ 3
  1.3 Linking food, temperature and antipredator behaviour .............................. 6
  1.4 Aim of the study .......................................................................................... 10
  1.5 Study location and species ......................................................................... 11

Chapter 2: Temperature and food availability affect risk assessment in an ectotherm ........................................................................................................... 15
  2.1 Summary ..................................................................................................... 15
  2.2 Introduction .................................................................................................. 16
  2.3 Methods ...................................................................................................... 18
  2.3.1 Study site and species ............................................................................. 18
  2.3.2 Fish collection, housing and release ....................................................... 19
  2.3.3 Experimental aquaria ............................................................................ 20
  2.3.4 Stimulus preparation ............................................................................. 21
  2.3.5 Experimental setup ............................................................................... 21
  2.3.6 Statistical analyses ................................................................................ 23
  2.4 Results ......................................................................................................... 24
  2.5 Discussion ................................................................................................... 25

Chapter 3: The effect of food availability on sensory complementation of predator information by prey fish ................................................................. 30
  3.1 Summary ..................................................................................................... 30
  3.2 Introduction .................................................................................................. 31
  3.3 Methods ...................................................................................................... 34
  3.3.1 Study organisms and collection ............................................................. 34
  3.3.2 Experimental aquaria .......................................................................... 34
  3.3.3 Conditioning .......................................................................................... 36
  3.3.4 Observation tanks and recognition trials ............................................ 37
  3.3.5 Quantification of energy reserves ......................................................... 39
  3.3.6 Statistical analyses .............................................................................. 40
  3.4 Results ......................................................................................................... 41
  3.5 Discussion ................................................................................................... 47

Chapter 4: Retention of acquired predator recognition by a juvenile coral reef fish under different temperature-mediated growth trajectories ......................................................... 53
  4.1 Summary ..................................................................................................... 53
  4.2 Introduction .................................................................................................. 54
4.3 Methods ................................................................................................................................. 58
  4.3.1 Study site and species ........................................................................................................ 58
  4.3.2 Fish collection, housing, and release .............................................................................. 58
  4.3.3 Experimental tanks .......................................................................................................... 59
  4.3.4 Conditioning .................................................................................................................... 60
  4.3.5 Observation tanks and recognition trials ...................................................................... 62
  4.3.5 Statistical analyses ......................................................................................................... 63
4.4 Results ................................................................................................................................. 64
4.5 Discussion ............................................................................................................................. 70

Chapter 5: Thermal environment and nutritional condition affect the efficacy of chemical alarm cues produced by prey fish ............................................ 75
  5.1 Summary ............................................................................................................................. 75
  5.2 Introduction .......................................................................................................................... 75
  5.3 Methods ............................................................................................................................... 78
    5.3.1 Study species, collection and maintenance ................................................................. 78
    5.3.2 Experimental treatments .............................................................................................. 79
    5.3.3 Body measurements of donor fish ................................................................................. 81
    5.3.4 Stimulus preparation .................................................................................................... 81
    5.3.5 Behavioural trials ........................................................................................................ 82
    5.3.6 Statistical analysis ........................................................................................................ 83
  5.4 Results .................................................................................................................................. 84
  5.5 Discussion ............................................................................................................................ 89

Chapter 6: General discussion ................................................................................................. 94
  6.1 Food supply, temperature and starvation avoidance .......................................................... 94
  6.3 Size and context-specific adjustment of antipredator response ...................................... 98
  6.4 Temperature, food supply and efficacy of donor alarm cues ........................................... 99
  6.5 Concluding remarks .......................................................................................................... 101

Literature cited ......................................................................................................................... 103
Supplementary files ................................................................................................................. 126
List of figures

Figure 1.1 (a) Location of Lizard Island Group in Australia; (b) Satellite view of Lizard Island Group; (c) Areal view on fringing reef around Palfrey Island (front) and Lizard Island (back) (picture Maarten de Brauwer); (d) Laboratory at Lizard Island Research Station

Figure 1.2 (a) Pseudochromis fuscus eyeing on Pomacentrus sp. (picture Christopher Mirbach); (b) Pomacentrus chrysurus; (c) P. moluccensis; (d) P. coelestis (picture a, b & c; Mark McCormick)

Figure 2.1 Feeding rate (bites/min ± 1 SE) for juvenile Pomacentrus chrysurus maintained at either of two temperatures a) 27 °C and b) 30 °C and two food rations (300 and 1000 Artemia per liter twice a day) for 5 days. Time period 1, 2 and 3 represent respectively the behavioural response (feeding rate) of fish to nothing (first observation: baseline), water (second observation: control), and chemical alarm cue (third observation)

Figure 3.1 The influence of feeding history on body morphology. Mean (±SE) (a) standard length (cm), (b) body depth (cm) and (c) body mass (g) for poorly, moderately, and well-fed juvenile Pomacentrus moluccensis. Numbers with bars are replicates and letters represent Tukey’s HSD groupings of means among all treatments (p < 0.05)

Figure 3.2 The influence of feeding history on liver hepatocyte densities. Mean (±SE) number of hepatocytes for poorly, moderately, and well-fed juvenile Pomacentrus moluccensis. Numbers with bars are replicates and letters represents Tukey’s HSD groupings of means among all treatments (p < 0.05)

Figure 3.3 Mean (±SE) difference in feeding strikes between pre- and post-stimulus period for poorly, moderately, and well-fed juvenile Pomacentrus moluccensis exposed to control, predator odour, visual cue of the predator, or the co-occurrence of visual and chemical cues. Numbers within bars are replicates and letters represent Tukey’s HSD groupings of means within each food treatment (p < 0.05)

Figure 3.4 Mean (±SE) difference in line crosses between pre- and post-stimulus period for poorly, moderately, and well-fed juvenile Pomacentrus moluccensis maintained exposed to: control, predator odour, visual cue of the predator, or the co-occurrence of visual and chemical cues
Figure 4.1 Proportion change in feeding strikes (±SE) from the pre-stimulus baseline for juvenile *Pomacentrus coelestis* exposed to predator odour (PO) (grey bars) or saltwater (SW) (white bars). *Pomacentrus coelestis* were raised at either 27 °C or 30 °C and were tested for recognition of the predator odour 1 day, 7 and or 14 days post-conditioning. Letters above or below the bars represent Tukey’s significant difference groupings of means. From left to right number of replicates: 17, 18, 20, 18, 13, 13, 14, 14, 19, 17, 17, 16......................................................65

Figure 4.2 Mean (±SE) (a) standard length (cm) and (b) body depth (cm) for juvenile *Pomacentrus coelestis* after 1 day, 7 days and 14 days post-conditioning raised at either 27 °C (dark bars) or 30 °C (open bars). *Significant difference between 27 °C and 30 °C temperature treatments. From left to right number of replicates: (a) 36, 36, 31, 33, 34, 31; (b) 36, 36, 31, 33, 34, 31 .....................................................................................68

Figure 5.1 Seasonal water temperature at Lizard Island, northern Great Barrier Reef, Australia (temperature logger deployed at 0.6 m). Solid black line: mean monthly temperature; grey shaded area: minimum and maximum temperature range; horizontal black dotted lines represent the selected experimental temperature regimes (27 °C, 30 °C and 32 °C) .... 79

Figure 5.2 Mean (±SE) (a) standard length (cm), (b) body depth (cm) and (c) body mass (g) for poorly-fed and well-fed juvenile *Pomacentrus moluccensis* maintained at either 27 °C, 30 °C or 32 °C for 9 days. Numbers within bars are number of replicates, and letters represents Tukey’s HSD groupings of means among all treatments (p < 0.05) .......... 85

Figure 5.3 Mean (±SE) number of hepatocytes (per micrometer$^2$) for poorly-fed or well-fed juvenile *Pomacentrus moluccensis* maintained at either 27 °C, 30 °C or for 9 days. Numbers within bars are the number of replicates, and letters represent Tukey’s HSD groupings of means among all treatments (p < 0.05) .................................................................86

Figure 5.4 Mean (±SE) proportional change in feeding strikes for juvenile *Pomacentrus moluccensis* exposed to a saltwater control or to a chemical alarm solution obtained from poorly or well-fed juveniles maintained at either 27 °C, 30 °C or 32 °C for 9 days. Numbers above bars are the number of replicates. Minuscle and majuscule letters below bars represent Tukey’s HSD significant differences between groups among well-fed and poorly-fed fish, respectively. The asterisk (*) above bar of saltwater control indicate Tukey’s HSD a significant difference with all other groups.................................................................87
List of tables

Table 4.1 ANOVA results for the effect of temperature (27 °C versus 30 °C), time post-conditioning (1 day versus 7 days versus 14 days) and cue (saltwater versus predator odour) on behavioural response (change in feeding strikes) of *Pomacentrus coelestis* ................................................ 66

Table 4.2 ANOVA results for overall effect of temperature (27 °C versus 30 °C) and time post-conditioning (1 day versus 7 days versus 14 days) on morphometric traits of *Pomacentrus coelestis* ........................................ 67
List of supplementary files

S 3.1 MANOVA results (Pillai’s Trace) with dependent variables feeding strikes and line crosses of *Pomacentrus moluccensis* and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food) ................................................................. 129

S 3.2. ANOVA results across all food levels for dependent variables feeding strikes and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food) ......................... 129

S 3.3 ANOVA results at each food level for the dependent variable feeding strikes of *Pomacentrus moluccensis* and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food) 129

S 3.4 ANOVA results across all food levels for dependent variables line crosses and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food) ................................................ 130

S 4.1 Overview table of number of replicates used during behavioural assessment of *Pomacentrus coelestis* .................................................................................................................................................. 130

S 4.2 Overview table of number of replicates used during morphometric assessment of *Pomacentrus coelestis* .................................................................................................................................................. 130

S 5.1 Overall MANOVA results (Pillai’s Trace) with dependent variables standard length, body depth and body mass of *Pomacentrus moluccensis* and as independent factors Food (low versus high) and Temperature (27 °C, 30 °C or 32 °C) ........................................................................................................ 131

S 5.2 Univariate results for dependent variables standard length, body depth and body mass of *Pomacentrus moluccensis* with as independent factors Food (low versus high) and Temperature (27 °C, 30 °C or 32 °C) ........................................................................................................ 131

S 5.3 Univariate results for dependent variables pre-stimulus feeding strikes and proportional change in feeding strikes of *Pomacentrus moluccensis* with as independent factors Food (low versus high) and Temperature (27 °C, 30 °C or 32 °C) ........................................................................................................ 131
Chapter 1: General introduction

“Most organisms live under the fear of predation. Short-term responses to predation threat are typically translated into an increase in vigilance and a reduction of any conspicuous activities that would make prey vulnerable to predation (Houston et al., 1993; Ferrari et al., 2010). However, while predator avoidance decreases mortality rates, it will often be at the cost of future growth and reproductive investment because of reduced foraging activity. Thus, prey are under continuous pressure to seek a trade-off between the conflicting demands of foraging and predator avoidance (Lima & Dill, 1990; Houston et al., 1993). In addition, the extent to which prey will adjust these trade-offs can be further shaped by environmental factors (e.g. Krause et al., 2011; Elvidge et al., 2013). In particular, temperature and food availability can be expected to affect the antipredator behaviour of an individual through the impact that these factors can have on the animal’s physiology, growth patterns and energy budget (e.g. McLeod et al., 2010). However, the drivers and different pathways linking these environmental factors to changes in behaviour of prey animals when facing predation threat are seldom explored. Understanding how key environmental factors such as food availability and temperature can influence prey behaviour is critical, as any environmentally-driven change to predator-prey interactions is very likely to have cascading effects on ecosystem function (e.g. Rall, 2010).

1.1 Temperature, food availability and ectothermic species

Temperature has often been considered as an ecological master factor within biological systems (Brett, 1971), particularly for ectothermic organisms, such as fish (Green & Fisher, 2004), reptiles (Rhen et al., 2011), amphibians (Touchon et al., 2011) and invertebrates (Everatt et al., 2015), for which body temperature directly depends of the surrounding ambient temperature. Many ectothermic species, and in particular those that live within a limited spatial home range, can endure throughout their
lifetime considerable fluctuations in their thermal environment (Leichter et al., 2006; McCabe et al., 2010). Temperature can naturally fluctuate between years, seasons and even over the course of the day or tidal cycle (Kaplan et al., 2003; Chollett et al., 2012). However, evidence suggests that the increasing anthropogenic activities since the beginning of the industrial revolution have led to an abnormal yearly increase in global average temperatures. Following the business-as-usual climate change scenario, a further increase of 3-4 °C above current average temperature values can be expected within the next 50 to 100 years (Lough, 2012; IPCC, 2013). Such small increases in temperature have been shown to have effects on the biology of tropical species, as they evolved in a comparatively narrow range of seasonal temperatures compared to their temperate counterparts (Tewksbury et al., 2008; Rummer et al., 2014).

Projected climate change impacts on organismal up to ecosystem functioning, have led to a proliferation of research to better understand the sensitivity of organisms to elevated temperature. Thermal performance curves are commonly used as a unifying conceptual framework to study differences in the thermal sensitivity among organisms and assess the extent to which organisms may show plasticity to thermal environmental stress (e.g., Pörtner et al., 2001; Morley et al., 2012). Thermal performance curves of ectothermic species may, depending of the trait, have distinct shapes, but commonly it is represented as a skewed normal curve with a ‘rise’ phase, an optimum and a sharply declining ‘fall’ phase (Deere et al., 2006). The slope of the rising phase is thought to mainly be driven by a temperature-induced increase in the kinetic energy of rate-limiting enzymes and metabolism level (Brown et al., 2004b; Clarke et al., 2004), while the ‘falling’ phase of the curve has been related to dysfunctions at molecular, cellular and organismal level (Dell et al., 2001; Ratkowsky et al., 2005). Although there is still little consensus about the mechanisms responsible for such a complex relationship between temperature and performance, the temperature at which the thermal optimum of a particular trait is reached still provides critical information on the thermal tolerance of an organism.
Ectothermic species have typically been found to increase their growth rate with rising temperature within their thermal tolerance (e.g., clownfish, Green & Fisher, 2004). This is very likely to occur through a temperature-mediated enhancement of enzymatic reactions and associated rate at which ingested food is converted into biomass and growth (Clarke & Johnston, 1999; McConnachie & Alexander, 2004). Increased metabolic rate with rising temperature may lead to faster growth rate, but only if there is sufficient food available to fuel the higher metabolic demands (McLeod et al., 2013). However, food is seldom unlimited in the wild (Okamoto et al., 2012) and animals with a poor feeding history may not grow as fast with rising temperatures, owing to combined effects of exhausted energy reserves and increasing metabolic demands (Hayes et al., 2014). Thus, temperature and food availability can have profound effects on the energy budget and development of an organism. However, the extent to which these environmentally mediated changes in the intrinsic body characteristics of an organism may independently and interactively influence the behavioural traits of an organisms are poorly understood.

1.2 State-dependent and threat-sensitive antipredator behaviour of prey

Individuals often differ in behaviour because they differ in their intrinsic body characteristics, also referred as state, and adjust their behaviour in an adaptive fashion to these differences (Lima, 1985; Luttbeg & Sih, 2010; Mathot, 2011). In behavioural ecology, the state of an individual will typically involve labile characteristics of the individual such as its energy reserves, condition, physiology or size (Sih et al., 2015). Theoretical models, supported by some experimental studies, have indicated that the state of the prey can play a determining role on the extent to which prey may take risk when facing predation threat (e.g. Lima & Dill, 1990; Houston et al., 1993; Clark, 1994; Reinhardt 2002). For example, a prey animal with low energy reserves may decide to act in a risk-prone manner at the expense of increased exposure to predator attack in order to avoid
mortality through starvation (Smith, 1981; Caraco, 1990; Lännstedt & McCormick, 2011b). Thus, it can be expected that any thermal- and nutritional-mediated change in the state of the animal will have an effect on how prey adjust their response to predation risk. However, a prey individual will not only respond depending on its state, it will also aim at responding in a threat sensitive manner by reacting in accordance to the level of imposed predation threat (e.g. Mirza et al., 2006; Vavrek & Brown, 2009).

1.2.1 Gathering sensory information on predation threat

To assess the level of predation, prey animals need to gather sensory information on predation threat (Munoz & Blumstein, 2012). Prey have evolved a whole set of sensory modalities to detect and respond adequately to predation risk (Smith & Belk, 2001; Ward & Mehner, 2010). However, the main senses used for assessing predation in aquatic environments are very likely to be based on visual and chemical cues (Brown & Chivers, 2006; Blanchet et al., 2007; McCormick & Manassa, 2008). Both sensory systems have their benefits but also its own set of limitations (Brown & Cowan, 2000; Munoz & Blumstein, 2012). Visual cues are both spatially and temporally reliable, as they provide accurate information about the behaviour and location of the predator (Helfman, 1989; Smith & Belk, 2001). Nevertheless, prey needs to be within the visual range of the predator in order to obtain visual sensory information on predation threat, which may represent a high risk. In addition, visual cues may easily be manipulated by the predator or be obscured due to suboptimal visual conditions (Swanbrow Becker, 2012; Manassa et al., 2013a; Cortesi et al., 2015). In contrast with visual cues, chemical cues are much more dispersive, thus less accurate both spatially and temporally (Giske et al., 1998). However, chemoosensory information on predation threat are often carried over greater distances providing prey early warning on predation threat, thus less risky to gain than visual cues (Holmes & McCormick, 2011). Ideally, aquatic prey animals will aim at gathering as much sensory information on predation threat as possible,
but it is thought that the relative importance of each of the sensory modalities to be context-dependent (Elvidge et al., 2013). As such, in suboptimal visual conditions, for instance at night or in structurally complex systems such as coral reef habitats, prey will very likely rely more on chemosensory signalling than visual cues (Brown & Cowan, 2000; Lönnstedt et al., 2013b; Manassa et al., 2013b).

A whole range of chemicals may provide prey with information on predation threat. However, in aquatic habitats, predator odour and chemical alarm cues are very likely to play a predominant role in the chemosensory assessment of predation threat (Ferrari et al., 2010b; Leahy et al., 2011). In contrast with predator odours, which are cues that are released by the predator itself (Ferrari et al., 2006a; Smith et al., 2008), chemical alarm cues represent chemosensory information that originates from prey (Wisenden et al., 2004; McCormick & Larson, 2007). Chemical alarm cues are involuntary released upon damage of the epidermis during a predation event and have typically been found to elicit short term antipredator responses in both conspecifics and closely related guild members (Wisenden et al., 2003; Brown et al., 2004a). In addition, exposure of prey to chemical alarm cues can also elicit a suit of long term responses, including induction of morphological and life history changes (Chivers et al., 2008; Lönnstedt et al., 2013a) and facilitation of learned recognition (Larson & McCormick, 2005; Smith et al., 2008). While, sensory integration of chemical alarm cue may enhance fitness and the likelihood of survival of the signal-receiver, the cost-benefit of the sender to produce chemical alarm cue is still under heavy debate (Chivers et al., 2007a; Lönnstedt & McCormick, 2015).

1.2.2 Assessment of sensory information and behavioural decisions

Following the gathering of sensory information, prey will assess the level of predation risk (Munoz & Blumstein, 2012). The term ‘assessment of predation risk’ has sometimes been used as the process by which prey convert acquired predator information into an observable behavioural response. However, strictly taken, assessment may probably better been
described as the process whereby prey lends following assessment rules a ‘meaning’ or a ‘value’ to all perceived predator stimuli. This process has sometimes also been referred as the informational state (Blumstein & Bouskila, 1996). Subsequent to the assessment process, prey will go through a decision-making process whereby the informational state is coupled, following ‘decision’ or ‘transformational’ rules, to a behavioural response (Blumstein & Bouskila, 1996). During the process of assessment and behavioural decision prey will not only take into account information obtained on predation, but will also integrate other types of stimuli, including internal body cues informing the animal on its physical ‘state’ (Reinhardt & Healey, 1999). Ultimately, prey will translate the behavioural decision into a behavioural response. In aquatic prey animals, such as fish larvae and tadpoles, the nature of the behavioural response has commonly been observed as an adjustment in the intensity of the foraging rate and activity (e.g. Mirza et al., 2006; Lönnstedt & McCormick, 2011b).

1.3 Linking food, temperature and antipredator behaviour

There are a series of concepts relating to state-dependent and threat-sensitive antipredator behaviour that could potentially provide a viable and exciting framework to further test how temperature, food availability, and level of predation threat may affect the informational state and the associated behavioural responses of prey animals. The key concepts used in the present dissertation are: (1) body assets and predator avoidance; (2) body assets and availability of sensory predator information; (3) previous experience and informational value of learned information; and (4) body assets and properties of the produced chemical alarm cue.

1.3.1 Body assets and predator avoidance

Prey must continuously assess the risk of predation against the gains obtained through foraging and other fitness related activities (Lima & Dill, 1990). Foraging-optimization models, supported by empirical studies, have indicated that in order to avoid starvation prey with a low body condition
will typically maximize foraging at the expense of antipredator behaviours (Caraco, 1990; Lima & Dill, 1990; Houston et al., 1993; McCormick & Larson, 2008). In contrast, prey with a good feeding history and in better body condition are more likely to behave in a cautious manner (Smith, 1981; Lönnstedt & McCormick, 2011b). Such state-dependent behavioural decision have been integrated into a more general theoretical framework, referred to as the asset-protection principle, whereby as the value of the accrued body assets (energy reserves, body size, reproductive investment) increase, prey are expected to prioritize predator avoidance (Clark, 1994; Reinhardt, 2002; Sih et al., 2015). This is because a prey gains less from a given absolute increase in assets relative to what it would lose through a successful predation event (Reinhardt, 1999; 2002). While there is evidence that food availability can influence predator avoidance through changes in the body assets (e.g. Caraco, 1990; McCormick & Larson, 2008), there are few studies investigating how other environmental factors can influence the extent to which prey may shift the trade-off between predator avoidance and foraging. Temperature on its own and interactively with food availability may be expected to have a dramatic impact on the risk-taking behaviour of ectothermic prey animals through alteration of an organisms’ body assets (Killen & Brown, 2006; Pink & Abrahams, 2015). More specifically, it is expected that prey individuals with low body assets due to limited food availability will be more sensitive to increased temperature regimes and may therefore display a risk-prone behaviour to compensate for the high energy demands and requirements of growth. However, no studies have yet directly tested the extent to which the thermal environment of an animal may alter the influence of food availability on risk-taking behavior (objective chapter 2).

1.3.2 Body assets and availability of sensory predator information

An important factor that can further shape the informational state of a prey animal is the number of available modes of sensory information available with which to characterize the nature and magnitude of predation threat.
The availability of multiple sensory inputs to detect predators should improve risk assessment and behavioural decisions by decreasing uncertainty, thereby leading to finer-tuned threat-sensitive responses (Munoz & Blumstein, 2012; Elvidge et al., 2013). Thus, two or more sensory modes are unlikely to be completely redundant, and might provide complementary sources of information (Amo et al., 2004; Munoz & Blumstein, 2012). Empirical studies using fish as model species have indicated that prey exposed to visual and olfactory information on predation will typically combine the value of these cues in an additive manner and respond with an intensity that is proportionally stronger than to any of the cue alone (Munoz & Blumstein, 2012; Elvidge et al., 2013; Manassa et al., 2013a). While the willingness to take risk may depend on the level of predation threat, it can also be expected that prey may adjust its response depending on its body assets (Clark, 1994; Reinhardt, 2002; Sih et al., 2015). Studies have commonly investigated state-dependent behavioural decisions by exposing prey to a single predation-related sensory cue (e.g. Fraker et al., 2008; Krause et al., 2011). No studies have yet investigated the extent to which the complementarity of multiple sensory cues can be state-dependent. It can be expected that prey with a poor feeding history may decrease their willingness to intensify their antipredator in response to additional information on predation threats in order to satisfy their high energetic demands and necessity to increase their body assets (objective chapter 3).

1.3.3 Previous experience and informational value of learned information

Experience with a relevant predator threat is likely to be an important factor shaping an organisms’ informational state and associated behavioural decisions (Munoz & Blumstein, 2012). Experience comes via learning and many prey animals have been shown to predominantly learn through ‘associative learning’ (Mathis et al., 2008; Brown, 2003; Gonzalo et al., 2009). In particular, it is well-established that chemical alarm cues play a pivotal role in facilitating associative learning in many aquatic
animal species (Brown, 2003; Ferrari et al., 2010b). Any novel cue that coincides with the presence of chemical alarm cue can become associated with predation risk (Brown & Chivers, 2006; Lönnstedt et al., 2012a). Because prey animals, and particularly juveniles, are continuously exposed to a wide array of novel cues there is also a high likelihood that they will learn irrelevant information (Ferrari & Chivers, 2006; Mitchell et al., 2011b). However, there are ways of effectively forgetting irrelevant or outdated information. A number of studies have shown that prey conditioned to recognize a predator cue will, with time, gradually reduce the intensity of their response to a learned cue (e.g. Brown and Smith, 1998; Hazlett et al., 2002; Gonzalo et al., 2009; Ferrari et al., 2010c). These observations have led to the suggestion that in the absence of any reinforcement of the learned information, prey will decline the informational value of the learned cue to avoid wasting valuable time and energy responding to irrelevant information (Kraemer & Golding, 1997; Ferrari et al., 2012a; 2012b; Ferrari & Chivers, 2013;). Theoretical work, supported by a limited amount of empirical studies, have further proposed that the relevancy of a learned cue may also be dramatically affected as prey individuals grow (Brown et al., 2011; Ferrari et al., 2011a; Ferrari et al., 2012a). Indeed, size could potentially change the vulnerability of prey to gape-limited predators, thereby indirectly shaping the length of time prey should retain the acquired information about a specific predator (Ferrari et al., 2010c). A corollary to this is that any factor that influences the growth of prey that are exposed to gape-limited predator threats, such as environmental temperature, will affect the time that information should be retained; but this remains untested (objective chapter 4).

1.3.4 Body assets and properties of the produced chemical alarm cue

Studies have indicated that the quality and/or quantity of the chemical alarm cue to which a prey is exposed can also influence the informational state of a prey organism and associated intensity of its antipredator response (Roh et al., 2004; Ferrari et al., 2008a). In aquatic habitats, environmental factors such as solar radiation and pH have been shown to
influence the properties of chemical alarm once released by the signal-sender (Chivers, et al., 2013a), by influencing the rate at which the cue degrades. However, the properties of chemical alarm cue may also be altered by the signal sender (also referred as donor) before release into the environment (Brown et al., 2004a; Roh et al., 2004). In particular, studies have revealed that chemical alarm cues from donors with low body condition due to limited food availability do not elicit an alarm reaction in conspecific receivers as effectively as chemical alarm cue obtained from well-fed donors in good condition (Brown et al., 2004a; McCormick & Larson, 2008). Interestingly, no studies have yet considered how factors other than food availability and diet may affect the energy balance and growth of the sender and its associated production of chemical alarm cues. In particular, it can be expected that temperature and food-mediated changes in the energy balance and growth of prey may influence the production of chemical alarm cue (objective chapter 5).

1.4 Aim of the study

Through a series of four interconnected but separate studies, this dissertation examines the different pathway by which temperature and food availability can influence the antipredator behaviour of juvenile coral reef fish. Specifically, I addressed a series of four discrete research questions, which comprise the following chapters:

*Chapter 2* explores the extent to which temperature and food availability influence risk-taking behaviour of prey when exposed to chemical alarm cue;

*Chapter 3* further investigates whether the feeding history of prey influence sensory complementarity of multiple sources of information about the threat of predation;

*Chapter 4* examines whether temperature-mediated growth of prey may influence the retention of acquired predator information, through changes in the relevancy of the learned cue;
Chapter 5 explores the effect of food availability and temperature on the physical condition of prey and whether changes in body assets affects the efficacy of the chemical alarm cue they produce.

Few studies have investigated how key environmental factors, such as temperature and food availability, may independently and concurrently affect the antipredator behaviour of ectothermic water-breathing prey animals. Addressing this knowledge gap is critical, as any environmentally-mediated change in predator-prey interaction may be expected to have dramatic effects on the functioning of marine and freshwater ecosystems.

1.5 Study location and species

All experiments were conducted in controlled laboratory conditions at Lizard Island Research Station, northern Great Barrier Reef (GBR), Australia, during the summer months (Oct-Jan) of 2012-2014. Lizard Island comprises a main island and three smaller islands surrounded by fringing reef. It is situated on the mid-shelf of the great-barrier reef, 30 kilometres from the Australian mainland and 19 kilometres from the outer barrier reefs that line the edge of the continental shelf (Fig 1.1).

Studies within this dissertation used different species of juvenile damselfish species as prey (Family Pomacentridae): *P. chrysurus, P. coelestis and P. moluccensis*. All these coral reef fish species were collected around Lizard Island and are commonly found along the Great Barrier Reef and Indo-Pacific (Allen, 1991; Feeney et al., 2012) (Fig 1.2). The species of damselfish used in the present study where typically selected based on the abundance of the different species at the moment of sampling. This ensured that sufficient replicates were available to conduct a particular experiment. All the used species are phylogenetically speaking closely related and are therefore expected to have evolved similar physiological machineries and other biological responses (Rummer et al., 2013). Thus, although inter-specific variability can lead to subtle differences in the response to environmental change, it is reasonable to generalize the observed response patterns for a broader range of coral
reef fish species. Furthermore, it is very likely to observe similar responsive patterns for phylogenetically more distant taxa. This is especially true for tropical ectothermic species, who show particularly high vulnerability to small changes in thermal regimes (Rummer et al., 2014; Tewksbury et al., 2008).

Juvenile coral reef fish represent a particularly ideal model taxon to test the effects of temperature and food availability on predator avoidance. This taxon has a complex life cycle with a relatively short larval phase in the pelagic environment followed by a sedentary and site-attached adult stage in the reef (McCormick & Makey, 1997). During the pelagic phase, they are completely naïve to reef-based benthic predators (Mitchell et al., 2011b; Lönnstedt et al., 2012a), providing a unique opportunity to examine and modulate their behaviour without the confounding effects of previous experience with predators. Upon settlement juvenile tropical fishes are exposed to intense but variable predation pressure (Houde, 1989; Almany & Webster, 2006) and avoiding predators at this point is central to their chances of survival (Lönnstedt et al., 2012a). Studies in the last decade show that these juvenile have a sophisticated and efficient mechanism of learning and emphasizing/de-emphasizing information about predator threats, similar to that found for some freshwater fishes (Brown et al., 2006; Chivers & Brown, 2006; Ferrari & Chivers, 2006; Mitchell et al., 2011b).
The predators that threaten the newly settled fishes have a diversity of predation strategies from ambush to pursuit (Beukers-Stewart & Jones 2004). The model predator used in the current studies was a small (maximum 80mm TL) but vocacious piscivore, the dusky dottyback (Pseudochromidae: *Pseudochromis fuscus*). Behavioural research and metabolic estimates of this species at Lizard Island suggest it may eat in average 16 juvenile reef fishes over a 13h-day, making it one of the most effective predators on tropical reefs (Feeney et al. 2012).

**Figure 1.1** (a) Location of Lizard Island Group in Australia; (b) Satellite view of Lizard Island Group; (c) Areal view on fringing reef around Palfrey Island (front) and Lizard Island (back) (picture Maarten de Brauwer); (d) Laboratory at Lizard Island Research Station.
Like most tropical ectothermic organisms, juvenile coral reef fishes evolved under relatively stable thermal conditions and thus are expected to live naturally close to their thermal optimum. Research investigating the aerobic scope of juvenile coral reef fishes in the northern Great Barrier Reef have indicated that their thermal optima is typically about \( \sim 1 \, ^\circ C \) above the regional average maximum summer temperature in which they living (Rummer et al., 2014). Several studies on juvenile coral reef fishes have shown evidence that small increases in temperature can, due the animal’s narrow thermal tolerance, affect a wide range of fitness-related traits (Munday et al., 2008; Biro et al., 2010; Donelson et al., 2010; Johansen & Jones, 2011). In addition, the extent to which these traits may be affected by rising temperatures will strongly be dependent on the animals’ capacity to ingest sufficient food to fuel the higher temperature mediated energy requirements (Munday et al., 2008; Donelson et al., 2010; McLeod et al., 2013; Hayes et al., 2014). However, food availability and temperature are rarely constant factors in the wild and this is especially true for animals with limited spatial range such as juvenile coral reef fishes.
Chapter 2: Temperature and food availability affect risk assessment in an ectotherm


2.1 Summary

Risk assessment in ectotherms is strongly affected by an organism’s energy expenditure and acquisition because these will alter the motivation to feed, which is balanced against antipredator behaviours. Temperature and food availability are known to affect the physiological condition of ectotherms, but how interactions between these variables may influence predator-prey dynamics is still poorly understood. This study examined the interactive effects of food availability and temperature on the trade-offs between predator avoidance behaviour and foraging in juveniles of a marine damselfish, *Pomacentrus chrysurus*. Predator avoidance behaviour was tested by exposing fish to chemical alarm cues obtained from skin extract of conspecifics. When detected these cues elicit an antipredator response in fish, typically characterized by decreased foraging. Fish maintained under high food ration displayed distinct antipredator responses to chemical alarm cues, regardless of temperature. However, fish maintained in conditions of low food ration and 3 °C above ambient temperature did not display an antipredator response when exposed to chemical alarm cues, while those in ambient temperature did. These results suggest that individuals in low physiological condition due to limited food availability are more susceptible to increased temperature and may therefore take greater risks under predation threats to satisfy their energetic requirements.
2.2 Introduction

Predation is known to drive behavioural patterns associated with foraging, reproduction and other fitness related activities (Lima & Dill, 1990; Houston et al., 1993; Candolin, 1997). Decisions made by an organism under the risk of predation are often described as a trade-off between avoiding predation and obtaining resources (Abrams, 1993; Lima, 1998). Predator avoidance decreases mortality rates but often at the cost of future growth and reproductive investment because of reduced foraging activity (Abrahams & Pratt, 2000; Cressler et al., 2010). The extent to which individuals allocate their time to such activities depends on how an individual assesses the potential gains to overall fitness, given their current physiological state (McNamara & Houston, 1986; Lima & Dill, 1990; Houston et al., 1993; Mathot & Dall, 2013;). Consequently, these state dependent decisions can be heavily influenced by environmental parameters that impose an energetic cost, such as temperature (Caraco et al., 1990; Abrahams et al., 2007). While the importance of environmental parameters in determining antipredator strategies has been acknowledged, few studies have directly tested how interactions between different parameters impact risk assessment.

Theoretical and empirical studies have stressed the importance of an individual’s physiological state as a driving component that should influence the trade-off between foraging and avoiding predation. According to these studies, animals exposed to conditions of higher physiological demands should be willing to take greater risks in the presence of a predator (Mangel & Clark, 1986; Caraco et al., 1990; Lima & Dill, 1990; Houston et al., 1993). Killen et al. (2011) found that the combined effects of high metabolic rate and food deprivation on risk-taking during foraging led to an increased tendency for fish to ignore a visual threat. In keeping with this finding, feeding history has been shown to affect risk-taking behaviour, with hungry animals reducing their antipredator response when presented with conspecific alarm cues (Smith, 1981; Chivers et al., 2000; Giaquinto & Volpato, 2001; McCormick & Larson, 2008). While food availability and its effect on physiological condition has been shown to
influence behavioural decisions in fishes, there has been a lack of studies investigating how other environmental factors may further affect threat-sensitive trade-offs between the benefits of antipredator behaviour and foraging behaviour.

For most organisms temperature is one of the major environmental influences on life history processes. This is especially true for ectothermic species (such as amphibians (Touchon & Warkentin, 2011), reptiles (Rhen et al., 2011) and fishes (Green & Fisher, 2004), where changes in their thermal environment directly affect physiology. Temperature influences fundamental properties of their energy budgets, metabolic demands, digestion rates, assimilation efficiencies (Roessig et al., 2004; Clarke & Fraser, 2004; Englund et al., 2011) and associated behaviours (Biro et al., 2010; Nowicki et al., 2012). Indeed, the influence of temperature on physiological processes is so universally important that it has been described as an ‘abiotic master factor’ (Brett, 1971).

Temperature may also have an impact on risk assessment as many organisms are exposed to substantial changes in temperature on a range of temporal and spatial scales. At the spatial scale of an animal’s home range, key drivers of small scale fluctuations in temperature are season, time of day and microhabitat. Seasonal variability is largely driven by predictable variation in solar radiation (Leichter et al., 2006). On Heron Island, Great Barrier Reef, Australia, water surface temperature throughout the year has been reported to reach a minimum value of 20°C in the winter and peak to a maximum value of 29°C in the summer (Rummer et al., 2013). Additionally, for aquatic organisms, the flow of water through habitats along with tides or floods can lead to dramatic short-term changes in temperature over hours to days (Jimenez et al., 2011; Jimenez et al., 2012). For instance, diurnal changes in temperature in the lagoon of Lady Elliot Island, Great Barrier Reef, Australia, can range over 4°C - 8 °C, with peak changes over 12°C during summertime (McCabe et al., 2010). Consequently, variation in temperature over the short, medium and long term may significantly alter energy demand and risk assessment.
Temperature changes are also expected to have a more marked influence on ectotherms that live close to the equator, where organisms have evolved under relatively stable temperature conditions and live closer to their thermal maxima (Tewksbury et al., 2008; Rummer et al., 2013). The effect of temperature on threat sensitive behaviour may also be magnified in the transition between life stages, such as the transition from larval to postlarval life stage in organisms with complex life cycles, because the risk is often high due to unfamiliar predators (e.g., Lönnstedt et al. 2012b). During the settlement period tropical larvae fishes are exposed to exceedingly high levels of predation (Houde, 1989; Almany & Webster 2006) and avoiding predators at this point is central to their chances of survival (Lönnstedt et al., 2012b). Thus, tropical fishes at settlement stage are ideal organisms with which to examine the influence of temperature on risk assessment. Ambient water temperatures over the summer months in the study region (Lizard Island, Australia) can vary by 4-5°C and food availability can be highly variable and patchy (Rummer et al., 2013). Consequently, the local environment into which juvenile fishes settle may influence how they perceive and respond to predation risk. The aim of our study was to investigate the short-term (5 days) interactive effects of water temperature (27 °C vs. 30 °C) and food availability (low food vs. high food) on risk assessment in a model tropical ectotherm, the marine damselfish *Pomacentrus chrysurus*. We experimentally tested the hypothesis that fish subjected to higher energetic demands due to higher water temperature, coupled with limited food availability would decrease their antipredator response when exposed to a threat, as indicated by a conspecific chemical alarm cue.

2.3 Methods

2.3.1 Study site and species

This study was conducted at Lizard Island (145°27'E, 14°41' S), northern Great Barrier Reef, Australia between October and November 2012. The laboratories and flow through seawater aquarium system at Lizard Island
Research Station were used to conduct all experiments, and fish were collected from the shallow fringing reef.

We used juvenile *P. chrysurus* for all experimental trials. This small damselfish inhabits rocky outcrops in sandy areas and is commonly found in areas high in coral rubble, especially on shallow reef flats (Randall et al., 1997).

### 2.3.2 Fish collection, housing and release

*Pomacentrus chrysurus* were collected as newly metamorphosed juveniles using light traps (Meekan et al., 2001) deployed overnight, or as newly settled fish from the fringing reef using hand nets and a solution of anaesthetic clove oil mixed with alcohol and seawater. Fish were transported back to the research station (approximately 10 min boat trip) in plastic covered bins (65 cm x 41 cm x 40 cm deep). Each bin was filled with approximately 60-l seawater and contained a maximum of 200 juvenile fishes. The bins were aerated using portable oxygen air pumps in order to avoid asphyxiation of fishes during transport. Once at the Lizard island research station, all fish were maintained in 25-l flow-through aquaria systems for about 24 h, and fed newly hatched *Artemia* twice per day ad libitum to allow for recovery from the stress of capture. Aquaria were maintained under a 12:12 h light:dark regime. Flow-through aquaria systems were fed directly from surrounding lagoon waters so that water temperatures in aquaria mirrored that found in the natural environment (~27 °C – 30 °C). Fish were collected in batches and used in experiments within about 48 h of capture to avoid biases associated with ontogenetic development.

At the end of the experiment fishes from all treatments were maintained for a minimum of 48 hours and were fed ad libitum to allow for recovery prior to being released in their natural habitat. No mortality of fish was observed during capture and release of fish. Food availability but not temperature during maintenance of fish affected survival. All fish maintained at high food ration survived, however mortality for fish maintained at low food ration was approximately 5%. All research was
conducted under permits from the Great Barrier Reef Marine Park Authority and James Cook University Animal Ethics Committee (permit no. A1720).

2.3.3 Experimental aquaria

After collection, fish were allocated at random to 8 thermally insulated 18-l aquaria (40 cm x 30 cm x 15 cm deep) representing 2 tanks in each combination of the two feeding levels and two temperature treatments. The two feeding levels were either poorly fed (300 Artemia/l twice daily) or well-fed (1000 Artemia/l twice daily). These feeding levels were established based on treatments used in similar feeding experiment on congeneric species (Lönnstedt & McCormick, 2011b; Lönnstedt et al., 2012a). Fish from each feeding treatment were either exposed to ambient temperature (27 °C) or high temperature (30 °C), to match natural fluctuation in summer sea temperature during the recruitment period (e.g. McCormick & Molony, 1995). Electric batten heaters (300W) were used to control the temperature of seawater. Fish were acclimated to the high temperature by slowly raising the water temperature over a 48 h period. Fish were kept in the four treatment combinations for 5 days (under a 12:12 light:dark photoperiod) prior to being used in behavioural trials. Tanks had a slow flow-through seawater system and an air stone within each tank kept the Artemia in suspension and distributed throughout the tank, so all fish had similar access to food. As a result of constraints in time and tank availability during the observation trials, the total number of fish at one time needed to be staggered across different tanks and days. Each experimental aquaria contained approximately between 5-10 fishes. Not all fishes were tested for behavioural response, however, morphometric measures were taken for each individual. Sample size for behavioural trials and growth analysis were as follow: n = 15 and 35 (low food - 27 °C); n = 32 and 52 (high food - 27 °C); n = 24 and 46 (low food - 30 °C); n = 27 and 52 (high food - 30 °C).
2.3.4 Stimulus preparation

Antipredator responses were tested by exposing fish to chemical alarm cues obtained from conspecifics. These chemical alarm cues are found in the epidermis and elicit distinct antipredator behaviours upon detection by conspecifics and closely related species (Mitchell et al., 2012). The presence of such damage released chemical alarm cues have been demonstrated in a wide range of fish taxa and other aquatic organisms such as amphibians and invertebrates (Ferrari et al., 2010b). Skin extracts were prepared using *P. chrysurus* collected either from light traps or from the reef (one fish per trial). The donor fish were killed individually through thermal shock by completely immersing in ice slurry (in accordance with James Cook University Animal Ethics; permit no. A1067). Mortality in juvenile is usually identified as a lack of opercula movement, which generally occurred within 10 sec. However, fish were immersed in ice for the full 2 min to ensure complete brain death. Thermal shock was opted above other killing methods due to the speed of death and it prevents the release of potentially confounding body odours (blow to the head or a spike through the brain) or the introduction of foreign odours (e.g. anesthesia overdose). A clean scalpel blade was used to make 10 superficial vertical incisions along each flank. Fish were then rinsed with 20 ml of seawater, and the solution was filtered to remove any solid material. Skin extracts were prepared within 5 min of injection into the observation tank to avoid any time-related decrease in potency. For each behavioural trial, we injected chemical alarm cue obtained from one conspecific donor fish.

2.3.5 Experimental setup

Our design followed a 2 x 2 x 3 repeated-measures design, whereby fish maintained under 2 temperatures (27 °C vs. 30 °C) and under 2 food rations (low vs. high), were subsequently observed during 3 successive periods: during the pre-stimulus period (baseline), after an exposure to water (control stimulus for disturbance), and finally after an exposure to a
chemical alarm cue (experimental stimulus).

Observations of fish behaviour were conducted in 13-l flow-through aquaria (36 cm x 21 cm x 20 cm deep). Each tank had a 3 cm layer of sand and a small terracotta pot (5 cm diameter) for shelter at one end and an air stone at the opposite end. A feeding tube and stimulus injection tube were attached to the air stone tube with their ends placed just above the stone to aid rapid dispersal of the chemical stimuli. The injection tubes allowed the food and stimuli to be introduced with minimal disturbance to the fish. Each tank was surrounded on three sides with black plastic and insulation foam to isolate the fish visually and thermally. Fish were observed through small holes cut in a black plastic curtain that was hung in front of the tanks to minimize disturbance to the fish.

The behaviour of the fish was quantified by counting feeding strikes over a specific length of time (3 min in the present study). Many studies have shown that a decrease in foraging is a common behavioural response observed in animals facing a risky situation (Bishop & Brown, 1992; Lima, 1998; Williams & Brown, 1991; Killen et al., 2007), including larval damselfish (e.g. Mitchell et al., 2011). The foraging rate included all feeding strikes on *Artemia* irrespective of whether they were successful at capturing prey. *Artemia* has commonly been used in experiments on foraging and antipredator behaviour of fish, partly because feeding rate on these low calorie prey items is stable over a relatively extended period of time before fish attain satiation threshold (McCormick & Larson, 2008; Holmes & McCormick, 2010).

Prior to the start of the trial, the fish were given 2.5 ml of food to remove the ‘feeding frenzy’ effect associated with the sudden presentation of food in the tank. This pre-feeding phase consisted in injecting 2.5 ml of food (an *Artemia* solution containing 250 individuals per ml) in the tank, followed by 20 ml of seawater to completely flush the food into the tank, hence allowing the fish to reach a stable feeding rate before the trial. The trial started 3 min later.

Each trial consisted of a 3-min pre-stimulus observation period (first observation), another 3-min observation after the injection of water (second observation) and finally a 3-min post-stimulus observation after
the injection of alarm cues (third observation). The flow-through system was turned off during the trials. At the start of the first observation, 2.5 ml of food was introduced and flushed with 20 ml of saltwater. Subsequently, a second observation was initiated by injecting 2.5 ml of food followed by 20 mL saltwater and flushed with 20 ml of saltwater. At the start of the third observation, 2.5 ml of food was injected, followed by 20 ml of chemical alarm cue and flushed with 20 ml of saltwater. After observational trials, larvae were photographed in a lateral position on a 0.5 mm plastic grid. Standard length (SL) to the nearest 0.01 mm was estimated from each fish from the digital photograph using image analysis software (ImageJ version 1.45s, National Institute of Health, USA).

2.3.6 Statistical analyses

The manipulation of both temperature and food could have resulted in a difference in the size of the fish, which could potentially affect their antipredator response. Thus, we included fish size as a covariate in all analyses. We initially ran a 2-way repeated-measures ANCOVA, using temperature and food as fixed factors, our 3 observations as repeated factor, and size as a covariate. Given the difficulty in interpreting potential 4-way interactions, we split the analysis to address 3 simple questions: (1) do food and/or temperature affect the baseline activity of the fish? (2) do they affect the response of fish to disturbance (water) and (3) do they affect the response of the fish to risk (alarm cues)? For the first question, we used the feeding strikes from the first time period as raw data in the analysis. For the second and third questions, we computed the percentage change in feeding strikes (water - baseline)/baseline for question 2 and (alarm cues - water)/water for question 3 to take into account the change in pre-stimulus baseline. Assumptions of homoscedasticity were met, and we verified that no interaction existed between the covariate and any of the factors. We did not predict any differences in behaviour between the first and second observations (control vs. water – question 2). However, we predicted that fish able to display an antipredator response to risk
would decrease the number of strikes during the third observations (i.e., following an injection of alarm cues, question 3).

2.4 Results

Baseline activity: The 2-way ANCOVA revealed a significant interaction between food and temperature ($F_{1,93} = 14.84, P < 0.01$), but no effect of size ($F_{1,93} = 0.09, P = 0.76$) on the response of the fish. Tukey’s post-hoc tests revealed that basal feeding rate in poorly fed fish maintained at high temperature was significantly higher than that from poorly fed fish maintained at ambient temperature ($P < 0.01$), and from that of well fed fish maintained at ambient ($P < 0.01$) and high temperature ($P < 0.01$) (Fig. 2.1).

Response to disturbance: The 2-way ANCOVA revealed no effect of food ($F_{1,92} = 2.47, P = 0.12$), no effect of temperature ($F_{1,92} = 2.20, P = 0.14$) nor any interaction ($F_{1,92} = 0.00, P = 0.96$) on the behavioural response of the fish. In addition, the effect of size was not significant ($F_{1,92} = 3.76, P = 0.06$).

Response to risk: The 2-way ANCOVA revealed a significant interaction between food and temperature ($F_{1,93} = 16.60, P < 0.05$), but no effect of size ($F_{1,93} = 0.09, P = 0.77$) on the response of the fish. Tukey’s post-hoc tests revealed that the change in feeding rates for fish in the poorly fed, high temperature treatment was significantly different from poorly fed fish maintained at ambient temperature ($P < 0.01$) and well fed fish maintained at both ambient ($P < 0.01$) and high temperature ($P < 0.01$).

Size: Despite the absence of any significant effect of size as a covariate, we ensured that the fish did not differ in size among treatment. Two-way ANOVA indicated that standard length of fish were not significantly influenced by food availability and temperature (for food availability $F_{1,181} = 3.70, P = 0.06$; for temperature $F_{1,181} = 0.70, P = 0.40$; for interaction, $F_{1,181} = 0.54, P = 0.46$). Standard length (mean ± SD; range) of fish across all treatments was 13.75 ± 1.20 mm; range 11.60-18.20 mm).
Figure 2.1 Feeding rate (bites/min ± 1 SE) for juvenile *Pomacentrus chrysurus* maintained at either of two temperatures a) 27 °C and b) 30 °C and two food rations (300 and 1000 Artemia per liter twice a day) for 5 days. Time period 1, 2 and 3 represent respectively the behavioural response (feeding rate) of fish to nothing (first observation: baseline), water (second observation: control), and chemical alarm cue (third observation).

2.5 Discussion

Environmental variability in food availability and temperature are known to play major roles in the bioenergetics of ectotherms and has been shown to affect a wide range of traits including physiological condition, growth rate, reproduction and behaviour (Wildhaber & Crowder, 1990; Nicieza & Metcalfe, 1997; Donelson et al., 2010; McLeod et al., 2013). However, whether the interaction of these environmental variables may influence antipredatory behaviour in prey organisms and in particular the trade-offs
between foraging and predator avoidance remains poorly understood. The results of the present study clearly show that temperature and food level interact to have substantial effects on the energetic requirements of *P. chrysurus* and that this in turn influences the trade-off between predator avoidance and foraging. Fish maintained in the high temperature-low food treatment had a significantly higher baseline foraging rate than fish maintained in other temperature and food level combinations, suggesting these fish were subjected to significantly higher energetic demands. Consequently, these differences influenced decisions about how individuals responded to risky situations. Well fed fish maintained at either ambient or 3 °C above ambient temperature, and fish maintained under low food rations and ambient temperature, all responded with a typical antipredator response when exposed to chemical alarm cues. In contrast, fish maintained on low food rations and 3 °C above ambient temperature did not display a measurable antipredator response when exposed to chemical alarm cues. These results highlight how natural variation in environmental parameters may interact to have detrimental effects on the trade-off between satisfying energetic demands and avoiding predation.

Theoretical models, supported by some experimental work, suggest that animals increase their foraging behaviour as their energetic state declines, often at the cost of increased exposure to predators (Mangel & Clark, 1986; Caraco et al., 1990; Lima & Dill, 1990; Houston et al., 1993; Lönnstedt & McCormick, 2011b). For example, fish deprived of food exhibited significantly reduced alarm responses when presented with conspecific alarm substances (Smith, 1981; Chivers et al., 2000; Giaquinto & Volpato, 2001; McCormick & Larson, 2008; Lönnstedt & McCormick, 2011b). Our study indicate that environmental factors, such as temperature and food availability, may change the basal energetic cost of daily activities and alter the level of sustenance required for the individual to stay below the threshold where they are willing to increase risk. According to the foraging models developed by McNamara and Houston (1986) and Mangel and Clark (1986), the choice of actions made by an animal should be dependent upon its 'metabolic state'. Although many factors induce changes in metabolic rate, it has been suggested that body
size, food availability and temperature are key drivers of metabolic rates through their effects on growth rates (Parry, 1983). Increased energetic requirements in individuals with a higher metabolic demand require them to forage more often or take more risks to achieve a higher rate of food intake (Killen et al., 2011).

In isolation, temperature did not significantly affect risk assessment. Well fed juvenile fish reared at 27 °C showed reductions in foraging activity that were similar in magnitude to those observed at 30 °C. These findings are consistent with similar work done with juvenile temperate fish reared at 3 or 8 °C (Killen & Brown, 2006). Although water temperature seemed to have a direct impact on the lipid composition and energy storage abilities, newly hatched ocean pout *Macrozoarces americanus* showed a decrease in foraging activity similar in magnitude to those observed at lower temperature. This is surprising, as it was expected that a higher rearing temperature might have engendered continued foraging even while under the threat of predation. Interestingly, the results from the present study indicate that temperature in conjunction with food availability appeared to interactively influence risk assessment in *P. chrysurus*. A threshold towards a risk-prone foraging behaviour was attained for fish reared at 30 °C and in conditions of low food availability. This leads to the suggestion that individuals with a lower physiological condition due to limited food availability are more susceptible to increased temperature and may therefore take greater risks under predation threats to satisfy their energetic requirements. Killen and Brown (2006) may have failed to detect a ‘hunger’ response at higher temperature for ocean pout because they used a food ration that may still have been too high to induce an increase in their willingness to engage in risky behavior.

Although there is a lack of comparative data on threat sensitive behaviour across a latitudinal gradient, tropical fishes are expected to be more sensitive to elevated temperature because annual variation in water temperature experienced by these fishes is generally less than that experienced by temperate species (Addo-Bediako et al., 2000; Tewksbury et al., 2008). Many tropical ectotherms live much of the year in environments where body temperatures are near or above optimal
temperatures for performance (Nguyen et al., 2011; Sunday et al., 2011; Rummer et al., 2013). Some ectothermic species are able to behaviourally thermoregulate by selecting habitats with preferred temperatures (Gibson et al., 1998; Breau et al., 2011; Vickers et al., 2011). However, a broad range of tropical ectothermic species, including *P. chrysurus*, are relatively sedentary (McCormick & Makey, 1997), which make them particularly vulnerable to local environmental changes such as temperature and food availability. How species will be affected by spatial and temporal variability in temperature regimes will mainly depend on their capacity to thermally acclimate and the shape of the species’ thermal reaction norm for that geographic locality (Pörtner & Farrell, 2008; Niehaus et al., 2012). The extent to which individuals are pushed over the optimum of performance characteristics by temperature changes, and the frequency and duration of these occurrences, will determine how food and temperature regimes interact to affect the balance between vigilance and foraging. Future research should therefore further investigate sensitivity of species and populations to changes in temperature, as small increases of just a few degrees appear to have dramatic effects on behavioural and physiological traits of ectothermic species.

While natural variation in single environmental parameters might not have significant effect on risk assessment, when fish are simultaneously exposed to multiple environmental stressors antipredator behaviours can be compromised, as shown in the present study. Short-term changes in environmental parameters may therefore play a significant role in predator-prey dynamics, with ramifications for population dynamics. This may be particularly relevant for communities where supply-side processes play a major role in structuring communities e.g. coral reef fishes and larval amphibians. Both juvenile coral reef fishes and tadpoles must grow rapidly to pass through a predation bottleneck if they are to survive to become adults (Almany & Webster, 2006; Doherty et al., 2006; Ferrari et al., 2011a). During this time, short-term changes in the environment may significantly alter survival rates, as risk assessment plays a critical role in determining survival (Lönnstedt et al., 2012a).
Our results underscore the importance of understanding how the interactive effects of environmental conditions on physiological demands determine behavioural decisions. The balance of information from visual, olfactory and other senses, on which behavioural decisions are based, may also play a crucial role in predator risk assessment. McCormick & Manassa (2008) reported that fish can react with a similar magnitude of antipredator response to a strong visual cue or olfactory alarm cue. While the response of organisms to cues usually follows a graded response to cue intensity (e.g. Holmes & McCormick, 2011), studies have shown that cues from different sources with threat-relevant information tend be lead to an additive response when cues are well represented (e.g. Smith & Belk, 2001; McCormick & Manassa, 2008). It may therefore be expected that under conditions with high energetic requirements, the threshold towards a risk-prone foraging behaviour could be further postponed if additional sensory information is available. We therefore stress the need of further studies assessing cue-based sensitivity in risk taking while foraging, across a range of environmental conditions.
Chapter 3: The effect of food availability on sensory complementation of predator information by prey fish

This chapter has been submitted to Animal behaviour

3.1 Summary

Researchers have typically investigated the ability of animals to adjust their motivation to take risk in response to their feeding history by exposing prey to a single predator cue. However, at any given time, prey in their natural environment may gather information about predator threats through multiple sensory modes. Here, we tested whether complementarity of information on predation risk from two sensory modes (visual and olfactory) could be affected by the feeding history of prey. The lemon damselfish (Pomacentrus moluccensis) was used as a model prey species, while predator cues were obtained from a common coral reef mesopredator, the dusky dottyback (Pseudochromis fuscus). Foraging rate, but not activity, was threat-sensitive to olfactory and visual information, but responses were dependent on the body condition and size of the prey. Co-occurrence of visual with chemical information about the predator led to an additive decrease of foraging behaviour in poorly and moderately fed fish. In contrast, well-fed fish up-regulated vigilance to predation threat, whereby additional predator information did not lead to an enhancement of the antipredator response. A good history of feeding and growth may lead prey to prioritize vigilance over the relative benefits of energy intake under risk of predation. Our findings underscore the importance of considering the feeding history of prey when characterizing the extent to which sensory complementation of multiple predator cues may further intensify the antipredator response.
3.2 Introduction

Most organisms live under persistent predation threat (Lima & Dill, 1990). Failure to respond adequately to predators may be fatal, but over-responding to predation threat is counterproductive as it wastes valuable time and energy that could otherwise be used for activities such as foraging and reproduction (Houston, McNamara & Hutchinson, 1993; Lima & Dill, 1990; Candolin, 1997). One way to effectively balance this trade-off is through threat-sensitive predator avoidance, where prey respond to predatory threats with an intensity that is proportional to the degree of perceived predation threat (Helfman, 1989; Brown, Rive, Ferrari & Chivers, 2006). To provide the most accurate assessment of the risk posed, animals combine and cross-reference information on the threat of predation from a number of sensory modalities (Johnstone, 1996; Lima & Steury, 2005; Ward & Mehner, 2010; Munoz & Blumstein, 2012). Research on a variety of aquatic organisms have shown that chemical and visual cues of predators act as a key source of information for antipredator-related decisions by prey (Mikheev, Wanzenböck & Pasternak, 2006; Dalesman & Inchley, 2008; Holmes & McCormick, 2011). Co-occurrence of these sources of information can lead to an enhancement in the intensity of the response (Hazlett, 1999; Dalesman & Inchley, 2008; Kim et al., 2009; Manassa, Dixson, McCormick & Chivers, 2013a) but the reaction to risk might be strongly dependent on the prey’s level of motivation to take risk (Krause, Steinfartz & Caspers, 2011; Mathot & Dall, 2013; Katwaroo-Andersen et al., 2015).

The willingness to take risks while foraging has been shown to be linked to the feeding history of the animal (Lima, 1998; Killen, Marras & McKenzie, 2011). Food availability is known to be one of the major environmental variables in determining an organism’s fundamental intrinsic properties such as growth rate, energy budgets, and associated behaviours (Lima & Dill, 1990; McCormick & Molony, 1992; Copeland, Murphy & Ney, 2010; Hayes et al., 2014). Many aquatic heterotrophic organisms experience fluctuating resource availability, which can be unpredictable, scarce or even absent (McCormick & Molony, 1992;
Okamoto, Schmitt, Holbrook & Reed, 2012; Fraser & McCormick 2014). This natural variation in food resources is expected to affect temporal and spatial patterns in the motivation to forage (Brown, 1999; Hochman & Kotler, 2007). Prey with depleted energy storage due to low food availability are expected to adopt more risk-prone behaviour when facing predation risk to avoid imminent starvation (Houston & McNamara, 1999; Giaquinto & Volpato, 2001; McCormick & Larson, 2008). Likewise, prey that are far enough from the starvation threshold can behave in a cautious manner without a chance of imminent starvation (Krause, Steinfartz & Caspers, 2011). Such state-dependent behavioural decisions have been integrated into a more general theoretical framework (referred as the asset-protection principle – Clark, 1994; Reinhardt, 2002) whereby the increase in accrued assets (body size, energy reserves, reproductive investment) are predicted to lead to prey being more cautious (Clark, 1994; Reinhardt, 2002). This is because prey gain less from a given absolute increase in assets relative to what it would lose if caught by a predator (Clark, 1994; Reinhardt & Healey, 1999; Reinhardt, 2002).

Since the trade-off between foraging and vigilance is directly affected by the feeding history of the individual, we also expect feeding history to affect the way prey respond to the amount and type of information concerning the nature and intensity of threat. Information from various sensory modes are often used in a complementary way (Lima & Steury, 2005; Ward & Mehner, 2010), with olfaction warning aquatic prey that there is potential danger in the vicinity, and visual cues identifying the magnitude and nature of the threats (McCormick & Manassa, 2008; Holmes & McCormick, 2011). Olfactory and visual threat cues have commonly been found to act in an additive, threat-sensitive way, with combined cues leading to a stronger antipredator reaction than either cue on their own (Blanchet, Bernatchez & Dodson, 2007; McCormick & Manassa, 2008; Smith & Belk, 2011). However, as prey approach starvation, individuals will be more willing to take risks to capitalize on foraging opportunities and information from the different sensory modalities may not necessarily lead to an additive response because prey must take greater risks or die from starvation. In contrast, fish that have a
good feeding- or growth-history can afford to be highly risk averse and may, following the asset protection principle, up-regulate predator vigilance to increasing levels of predation threat (Clark, 1994; Reinhardt & Healey, 1999; Reinhardt, 2002).

The few studies that have investigated state-dependent antipredator behaviour in marine organisms have typically examined a prey’s response to predator-cues from one sensory modality (Smith, 1981; Giaquinto & Volpato, 2001; Lienart, Mitchell, Ferrari & McCormick, 2014). In their natural environment, prey may gather information about predation threat through different sensory modalities at any given point to maximize accuracy (Johnstone, 1996; Ward & Mehner, 2010; Munoz & Blumstein, 2012). While it has been shown that marine prey simultaneously exposed to visual and olfactory cues will typically undertake antipredator responses of greater intensity than from any single cue (McCormick & Manassa, 2008; Munoz & Blumstein, 2012), the extent to which such complementarity of multiple sensory cues is state-dependent is currently unknown. By manipulating feeding history, the present study examined how prey body condition affected the complementarity of information from two sensory modes (olfaction and vision) in the assessment of predation risk by a marine damselfish, *Pomacentrus moluccensis*. Damselfish were collected prior to settlement and exposed to three feeding levels for 10 days under laboratory conditions to influence their intrinsic body characteristics (body mass, body size, and energy reserves). Juveniles were then exposed to single or combined sensory cues from a known predator (the dottyback, *Pseudochromis fuscus*) and their change in activity and foraging observed. We predicted that with low food availability, prey would decrease their willingness to intensify their antipredator response to additional sensory information on predation threats in order to satisfy their high energetic demands and necessity to increase their body assets.
3.3 Methods

3.3.1 Study organisms and collection

This study was conducted at Lizard Island (145°27’E, 14°41’S), northern Great Barrier Reef, Australia, in November to December 2014. Fish were collected from the shallow fringing reef, and all experiments were conducted using the laboratories and flow-through seawater aquarium system of the Lizard Island Research Station. We used juvenile lemon damselfish, *P. moluccensis*, as prey species and the dottyback, *P. fuscus*, as predator species for the experimental trials.

*Pomacentrus moluccensis* is a planktivorous damselfish commonly found in association with hard branching corals throughout the Indo-Pacific region and the Great Barrier Reef, Australia (Hutchings, Kingsford & Hoegh-Guldberg, 2008). Newly metamorphosed juvenile fish were collected prior settling using light traps deployed overnight near the reef crest around Lizard Island. Fish were immediately transported back to the research station in a 60 L container of aerated seawater, carefully sorted to species level and transferred into 25 L tanks on a flow-through seawater system, where they were maintained for 24 h. Fish were fed *ad libitum* with newly hatched Artemia sp. twice a day to allow for recovery from the stress of capture.

*Pseudochromis fuscus* is a crypto-benthic predator common in shallow reefs throughout the Indo-Pacific. This species is known to exert strong predation pressure on newly settled and juvenile damselfish during the summer recruitment season (Feeney et al., 2012). *P. fuscus* were collected from the lagoon at Lizard Island using hand nets and anaesthetic solution of clove oil. Fish were maintained in 25 L aquaria and fed twice a day with bait squid.

3.3.2 Experimental aquaria

Twenty four hours after collection, *P. moluccensis* individuals were allocated at random into one of the 18 thermally insulated 11 L aquaria (29
Chapter 3

x 39 x 10 cm) and were reared under one of the three feeding levels: poorly fed (300 Artemia/L twice daily), moderately fed (750 Artemia/L twice daily) or well-fed (1875 Artemia/L twice daily). These feeding levels were established based on treatments used in similar experiments on juvenile tropical marine species (McCormick & Molony 1993; Lönnstedt & McCormick, 2011b; Lienart et al., 2014). During the experiment fish were maintained at a temperature of 30 °C, representing the regional average maximum temperature during the summer period (e.g. McCormick & Molony, 1995; Lienart et al., 2014). This temperature was chosen as a previous experiment had found marked differences in energy reserves and risk taking behaviour under different feeding regimes (Lienart et al., 2014; Lienart, unpublished data). Electric batten heaters (300 W) were used to control the temperature of the seawater. Fish were acclimated to the high temperature by slowly raising the water temperature (from ± 28 °C) over a 48 h period. Fish were kept in the three food treatment combinations for 10 days (under a 12:12 h light:dark photoperiod) prior to being used in behavioural trials. Each experimental aquarium (in total 18) contained approximately 15-20 individuals. Tanks had a slow flow-through seawater system, and an air stone within each tank kept the Artemia in suspension and distributed them throughout the tank to prevent individuals from dominating discrete foraging patches.

The experiment consisted of two phases: 1) a conditioning phase, where after 10 days all fish regardless of food treatment were taught to – simultaneously recognize the sight and the odour of P. fuscus as a threat, and 2) a testing phase, where each fish was tested for their antipredator response to the predator. Each fish was tested only once. Standard length of fish at the initiation of the experimental treatments was 11.54 mm ± 0.054 (mean ± SD; N = 43).

At the end of the experiment fish were maintained for a minimum of 48 h and were fed ad libitum to allow for recovery prior to being released in their natural habitat. No mortality of fish was observed during capture and release of fish. All fish maintained at the high and moderate food ration survived; however, mortality for fish maintained at the low food ration was approximately 3%. Alarm cue donors were euthanized.
individually through thermal shock by complete immersion in a slurry of ice and seawater (in accordance with James Cook University Animal Ethics; permit no. A2080). Death in juveniles is usually identified as a lack of opercula movement, which generally occurred within 10 s. However, fish were immersed in the ice slurry for the full 2 min to ensure complete brain death. Thermal shock was used rather than other methods because of the speed of death and because it prevents the release of potentially confounding body odours or the introduction of foreign odours (e.g., anaesthesia overdose).

### 3.3.3 Conditioning

To ensure that predator naive damselfish recognize the predator as a threat we conditioned prey fish by pairing chemical alarm cue obtained from conspecifics in combination with a visual cue of the predator, *P. fuscus* and its odour. Chemical alarm cues are known to play a key role forewarning prey of predation threat (Ferrari, Wisenden & Chivers, 2010b). These cues are released from a fish upon laceration of the epidermis and have been found to elicit antipredator behaviours in conspecifics and closely related taxa or guild members (Mitchell, Cowman & McCormick, 2012). When this reliable indicator of damage co-occurs with another cue such as a visual or an olfactory cue of a novel predator, then the new cue receives a threat label (Smith, 1992; Brown & Smith, 1998; Mitchell, McCormick, Ferrari & Chivers, 2011). This is a process known as associative learning and has been well documented in a broad range of taxa (Gonzalo, Lopez & Martin, 2009; Ferrari et al., 2010b).

Chemical alarm cues (Ferrari et al., 2010b) were prepared using donor *P. moluccensis* collected from light traps. Recruits were sacrificed individually (by cold-shock as above) and a clean scalpel blade was used to make five superficial vertical cuts along each flank of four individual fish (mean standard length: 1.306 cm ± 0.108 SD, n = 58) with a clean scalpel blade. The fish were then rinsed with 15 ml of seawater, yielding a total volume of 60 ml of alarm cues from the four fish. This solution was filtered through a filter paper to remove any solid material prior to use.
Predator odour was obtained from *P. fuscus* by leaving individual fish in separate 8 L holding tanks filled with aerated seawater. To ensure predator odour was consistently available during the whole experimental period, three *P. fuscus* (67.8; 67.17; and 69.0 mm standard length) were placed individually in their holding tanks on a 3 d rotating cycle: day 1 - fed twice/d in flowing seawater; day 2 – not fed in flowing seawater; day 3 – not fed, but water turned off to concentrate the odour cues. The water collected from the day 3 tank was used for prey conditioning and behavioural trials.

During the conditioning event prey fish were trained to recognize the odour and sight of the predator. Here, juvenile prey fish were directly conditioned within their treatment tanks. The water flow was turned off and juveniles were then exposed to 60 ml chemical alarm cue paired with 15 ml of predator odour. At the same time, a 1 L zip-lock bag containing a *P. fuscus* was carefully lowered against the side of the tank to provide the visual cue. Fish were left undisturbed for 10 min, then the predator was removed and water flow was turned on again to flush any olfactory cue from the holding tank. The day before trials were run prey fish were fed using their respective food-treatment and subsequently transferred to observation tanks in which they could acclimate overnight. The following day recognition trials were run within the observation tanks.

### 3.3.4 Observation tanks and recognition trials

All behavioural observations were undertaken in 15 L observation tanks (36 x 21 x 20 cm). Tanks contained a 3-cm deep substrate of sand, a small shelter (5 cm diameter PVC tube) at one end and an air stone at the opposite end. An additional piece of plastic tubing was attached to the airline with the end approximately 1 cm above the air stone. This allowed food or an olfactory cue to be introduced with minimal disturbance to the fish. The air facilitated the distribution of the cues and food throughout the tank. Every observation tank contained an empty small clear tank (2.5 L) for the introduction of the visual stimuli. The empty tub was used to minimize disturbance of fish when introducing the predator (within a 2 L
zip-lock bag of aerated seawater) into the observation tank. The zip-lock bag containing the predator (65-70 mm standard length) was large enough to allow the predator to move around freely. The empty tub was always positioned on the opposite side of the tank to the fish shelter such that the side of the predator was generally facing the prey. Temperature of the observation tanks was maintained at 30 °C. Each tank was surrounded on three sides with black plastic to avoid the focal fish observing adjacent observation tanks. Fish were observed through small holes cut in a black plastic curtain that was hung in front of the tanks to minimize disturbance to the fishes.

We used a well-established behavioural protocol to quantify the antipredator responses of fish larvae (e.g. Ferrari et al., 2011c; Mitchell et al., 2011a). The behaviour of the fish was observed during a 4-min pre- and 4-min post-stimulus period. We quantified two response variables: foraging rate and activity. The foraging rate included the total number of feeding strikes displayed by the fish, irrespective of whether they were successful at capturing prey (Artemia sp.). The activity was quantified as the total number of lines the fish crossed during the observation period, using a 4 x 4 cm grid drawn on the side of the tank. A line was counted as crossed when the entire body of the fish crossed a line.

Prior to the start of the trial, fish were fed to remove the ‘feeding frenzy’ effect associated with the sudden presentation of food in the tank. This feeding period consisted of injecting 2.5 ml of food (an Artemia solution containing 250 individuals per ml) in the tank, followed by 20 ml of saltwater to completely flush the food into the tank. Pre-stimulus observations began 4 min later, when another 2.5 ml of food was injected into the tank and flushed with 20 ml of saltwater. Following the pre-stimulus period, we initiated the post-stimulus period by injection of 2.5 ml of food, introduction of the experimental stimulus and flushing with 20 ml of saltwater.

The experimental stimulus was represented by either of the following cues: a) control (no predator odour or visual cue), b) predator odour alone, c) visual cue of the predator alone, or d) the odour and visual cue of the predator combined. These stimuli were respectively tested by:
injecting 20 ml saltwater and introducing a seawater containing zip-lock bag (control); injecting 20 ml predator odour and by introducing a water containing zip-lock bag (odour stimuli); injecting 20 ml salt water control and by introducing a zip-lock bag containing the predator (visual stimuli) or injecting 20 ml predator odour and by introducing a zip-lock bag containing the predator (co-occurrence odour and visual stimuli). Each fish was exposed only once to the stimuli.). While responses to visual or olfactory cues have been shown to be threat sensitive (i.e., dosage dependent), we have used one level of each olfactory and visual predator cue. The levels used were chosen to be ecologically relevant and represent significant predator threats in the near vicinity (i.e., within the tank). Number of replicate fish exposed to saltwater control, chemical cue, visual cue or both predator cues simultaneously were, respectively, within the poorly fed fish group: 19, 20, 20, 20; within the moderately fed fish group: 16,17,16,18; and within the well-fed fish group: 17,19,18,19.

After observational trials, juveniles were photographed in a lateral position on a 1-cm grid. Standard length and body depth was estimated to the nearest 0.01 mm from each fish from the digital photograph using image analysis software (ImageJ version 1.45s, National Institute of Health, U.S.A., http://rsbweb.nih.gov/ij/). We also measured body mass of fish to the nearest 0.0001 g. Number of replicate fish for standard length, body depth, and body mass, respectively, were for poorly fed fish: 69,62,62; moderately fed fish: 63,62,61 and well-fed fish: 78, 65,59.

3.3.5 Quantification of energy reserves

Energy reserves of *P. moluccensis* were estimated by quantifying hepatocyte density in the liver. Studies have shown that their density respond rapidly to variations in energy demands and diet. With increasing body condition, fish will contain higher glycogen and lipid stores (Green & McCormick, 1999). Ultimately, this will lead to higher level of vacuolation and consequently lower number of hepatocytes per liver area (e.g. Hoey, McCormick & Hoey, 2007; Storch & Juario, 1983). In the present study, all larval samples were individually fixed in FAAC (4% formaldehyde, 5%
acetic acid, 1.3% calcium chloride). Whole larvae were embedded in paraffin and serially sectioned along the sagittal plane (5 mm) with a microtome. The sections were then stained with Schiff’s reagent and counterstained with hematoxylin (Periodic Acid Schiff-Hematoxylin). Photographs of the slides were taken through the eyepiece of a dissecting microscope and subsequently analyzed using ImageJ (version 1.45s, National Institute of Health, U.S.A., http://rsbweb.nih.gov/ij/). Counts of hepatocyte within three quadrats (47.657 x 47.657 micrometer at 40x magnification) were undertaken for a randomly chosen liver section of each replicate fish. Number of replicate fish within the poorly-, moderately- and well-fed group was 11, 13 and 11, respectively.

3.3.6 Statistical analyses

Morphometric measures and hepatocyte density

Since standard length, body depth and body mass of fish are not independent of one another, we used a one-factor MANOVA to investigate the effect of food availability on overall morphology of fish (body mass, size and depth). We then performed ANOVAs to explore the nature of the significant difference found by MANOVA. Similarly, one-factor MANOVA was used to explore whether there was an effect of food availability on the number of hepatocytes and liver cell density. Tukey’s HSD post-hoc means comparisons were used to explore the differences found by ANOVA. Prior to analyses assumptions of normality and homoscedasticity were explored with residual analysis. Morphometric variables required log_{10} transformation to meet these assumptions.

Behavioural measures

A one-factor MANOVA, followed by univariate analysis, was used to test for equality of baseline levels in the number of feeding strikes and line crosses among food levels. To account for differences in pre-stimulus values among food levels, we computed the proportional change in
feeding strikes and line crosses from the pre-stimulus baseline \(((\text{post−pre})/\text{pre})\). We then used this metric as our behavioural response variable in subsequent analyses. This was followed by a series of analyses on the prey’s behavioural response testing the interactions of visual predator cues (2 levels: absence/presence) and chemical predator cues (2 levels: absence/presence).

We initially ran a three-factor MANOVA on feeding strikes and line crosses using food, visual and chemical cues as fixed factors. We subsequently analyzed the interaction between these factors on each of the behavioural traits separately using a three-factor ANOVA. To ease the interpretation of the three-factor interactions between food and visual and chemical cues on change in feeding strikes, we split up the analysis by food levels. We then ran separated two-factor ANOVAs with visual and chemical cues as fixed factors. Tukey’s HSD post-hoc tests were performed to investigate the nature of any significant differences found by ANOVA. Assumptions of normality and homoscedasticity were examined with residual analysis and found to be met.

We predicted no differences in behaviour when fish were exposed to the control (seawater) cue. We also predicted that fish who were able to display an antipredator response to risk would decrease their foraging rate and activity when exposed to singular or combined predation threat cue. Furthermore, if visual and chemical predation cues have additive effect we should find an interaction between both cues (i.e., the co-occurrence of chemical and visual cues will elicit an additive increase in the responses of prey).

3.4 Results

**Morphometric measures and hepatocyte density**

Feeding level had a significant effect on morphometric measurements (MANOVA: Pillai’s \(= 0.688, F_{6,348} = 30.4, P < 0.001\)). The univariate ANOVAs showed that food availability had a significant effect on standard length \(F_{2,192} = 70.6, P < 0.001\), body depth \(F_{2,183} = 87.6, P < 0.001\) and
body mass ($F_{2,199} = 99.9, P < 0.001$). Tukey’s post-hoc tests revealed that fish increased significantly in standard length and body depth with rising food availability (all $P < 0.001$). Poorly fed fish had significantly lower body mass than fish maintained in condition of moderate and high food availability (all $P < 0.001$). However, there was no significant difference in body mass between moderately fed and well-fed fish ($P > 0.366$) (Fig. 3.1).

Feeding levels also had a significant effect on the number of hepatocytes, and therefore body condition (ANOVA: $F_{2,32} = 20.9, P < 0.001$). Tukey’s post-hoc tests revealed that hepatocyte density in poorly fed fish differed significantly from moderately and well-fed fish (all $P < 0.001$). However, moderately fed fish and well-fed fish did not show a significant difference in hepatocyte density ($P = 0.720$) (Fig. 3.2).
Figure 3.1 The influence of feeding history on body morphology. Mean (±SE) (a) standard length (cm), (b) body depth (cm) and (c) body mass (g) for poorly, moderately, and well-fed juvenile *Pomacentrus moluccensis*. Numbers with bars are replicates and letters represents Tukey’s HSD groupings of means among all treatments (p < 0.05).
Figure 3.2 The influence of feeding history on liver hepatocyte densities. Mean (±SE) number of hepatocytes for poorly, moderately, and well-fed juvenile *Pomacentrus moluccensis*. Numbers with bars are replicates and letters represents Tukey’s HSD groupings of means among all treatments (p < 0.05).

**Behavioural measures**

**Pre-stimulus foraging**

Food availability had a significant effect on the behavioural traits of juvenile *P. moluccensis* measured during the pre-stimulus period (MANOVA: Pillai’s = 0.189, $F_{4,432} = 11.2$, $P < 0.001$). Univariate analyses indicated that food level had a significant effect on both number of feeding strikes ($F_{2,216} = 18.9$, $P < 0.001$) and line crosses ($F_{2,216} = 7.4$, $P < 0.001$). Tukey’s post-hoc tests for the pre-stimulus period showed that moderately fed fish showed significantly higher number of feeding strikes and were significantly more active compared with poorly fed (feeding strikes: $P = 0.001$; line crosses: $P = 0.001$) and well-fed fish (feeding strikes: $P < 0.001$; line crosses: $P = 0.007$). Post-hoc tests also indicated that poorly
fed fish showed similar number of line crosses ($P = 0.889$), but higher number of feeding strikes compared with well-fed fish ($P = 0.027$).

**Anti-predator behaviour**

A three-factor MANOVA on the dependent variables (feeding strikes and line crosses) showed there was a significant 3-factor interaction between food and visual and chemical cues ($\text{Pillai's } = 0.041$, $F_{4,414} = 2.2$, $P = 0.070$) (see supplementary file S 3.1 for overview of the MANOVA results). A three-factor ANOVA on change in feeding strikes indicated there was a significant three-factor interaction among Food, Chemical and Visual cues ($F_{2,207} = 4.1$, $P = 0.019$) (see supplementary file S 3.2 for overview of the ANOVA). Subsequent two-factor ANOVAs for each food level indicated that there was no significant two-way interaction between Visual and Chemical cues on change in feeding strikes in poorly ($F_{1,75} = 0.1$, $P = 0.719$) and moderately fed fish ($F_{1,63} = 2.1$, $P = 0.153$). However, there was an interaction between these factors for well-fed fish ($F_{1,69} = 7.0$; $P = 0.01$) (see supplementary file S 3.3 for overview of the ANOVA results within each food level).

Tukey’s post-hoc tests confirmed that fish at all food levels showed a decrease in the proportional change in feeding strikes when exposed to a chemical cue (all $P < 0.007$), a visual cue (all $P < 0.001$) or a combination of both predator cues (all $P < 0.001$). Tests also revealed that fish within each food level displayed similar decrease in the proportional change in feeding strikes when exposed to either a chemical or a visual predator cue (all $P > 0.187$). However, post-hoc tests showed that poorly and moderately fed fish responded with greater intensity to multiple predator cues compared to when only exposed to chemical (all $P < 0.001$) or visual cues (all $P < 0.012$). In contrast, tests indicated that well-fed fish responded with similar intensity of the response, regardless of whether they were exposed to a single or multiple predator cues (all $P > 0.063$) (Fig. 3.3).

A three-factor ANOVA found there was no interaction between food and chemical and visual cues with change in number of line crosses ($F_{2,207} = 0.207$, $P = 0.818$).
= 0.734; \( P = 0.481 \); see Fig. 3.4 & supplementary file S 3.4 for ANOVA results and figure).

**Figure 3.3** Mean (±SE) difference in feeding strikes between pre- and post-stimulus period for poorly, moderately, and well-fed juvenile *Pomacentrus moluccensis* exposed to control, predator odour, visual cue of the predator, or the co-occurrence of visual and chemical cues. Numbers within bars are replicates and letters represent Tukey’s HSD groupings of means within each food treatment (\( p < 0.05 \)).
Figure 3.4. Mean (±SE) difference in line crosses between pre- and post-stimulus period for poorly, moderately, and well-fed juvenile *Pomacentrus moluccensis* maintained exposed to: control, predator odour, visual cue of the predator, or the co-occurrence of visual and chemical cues.

3.5 Discussion

While research have shown evidence that animals possess the ability to adjust their behavioural decision in response to their intrinsic state and level of predation risk (e.g. Fraker, 2008; McCormick & Larson, 2008; Krause et al., 2011), few studies have addressed this topic from a multisensory perspective (but see Lönnstedt, McCormick, Meekan, Ferrari & Chivers, 2012). We investigated this question under controlled laboratory conditions using a common juvenile reef fish *P. moluccensis*. As expected, an increase in food availability yielded fish with higher growth rates and larger body size that possessed greater energy reserves.
by the end of the experiment. Foraging rate, but not activity, appeared to display state-dependency and threat-sensitivity in their response to one or more types of information concerning predation threat. Fish decreased their foraging rate in response to risk information from either olfactory or visual sources. Co-occurrence of these sensory modes led poorly- and moderately-fed fish to enhance the intensity of their response in an additive way. This contrasted with well-fed fish with better body assets, who did not display a reduction in their foraging rate in relation to an additional source of information concerning the immediate threat. These results provide evidence that the extent to which complementarity effects of multiple sensory cues on the response may occur and can be state-dependent.

The reduction in foraging of *P. moluccensis* when exposed to the odour or the sight of a predator is a common antipredator response observed in fish taxa (e.g. Mikheev et al., 2006; McCormick & Manassa, 2008; Rizzari, Frish, Hoey & McCormick, 2014). Visual or chemosensory modalities have their benefits but also its own set of limitations (Mikheev et al., 2006; Dalesman & Inchley, 2008; Holmes & McCormick, 2011; Munoz & Blumstein, 2012). In condition where transmission of visual information is reduced, for instance at night or in structurally highly complex habitats such as coral reef ecosystems, chemical cues are expected to be heavily relied upon during risk assessment (Manassa, McCormick, Chivers & Ferrari, 2013b; McCormick & Lönnstedt, 2013). However, owing to the relatively slow rate at which chemosensory information is diffused in an aqueous environment, they may not always be spatially and temporally reliable (Giske, Huse & Fiksen, 1998; Holmes & McCormick, 2011). In contrast, visual cues are transmitted quickly and provide more direct information on the predator’s level of motivations and threat (e.g. Helfman, 1989, Smith and Belk, 2001). Nevertheless, visual cues may easily be manipulated by the predator or be obscured due to suboptimal visual condition (e.g. Manassa, McCormick, Chivers & Ferrari, 2013a; Cortesi et al., 2015). Thus, risk assessment based on a singular sensory modality may lead prey to over- or underestimate predation threat (Munoz & Blumstein, 2012).
By using multiple sensory information simultaneously, animals can gather a greater amount of information and potentially cross-reference the different inputs to increase accuracy during risk assessment (Ward & Mehner, 2010). Prey simultaneously exposed to visual and chemical predator cues have been found to typically respond in an additive way to yield a graded response in the level of risk-sensitive behaviour (Blanchet et al., 2007; McCormick & Manassa, 2008; Smith & Belk, 2011). Similarly as has been found for other coral reef fish species (McCormick and Manassa, 2008; McCormick & Lönnstedt, 2013) and taxa thriving in different systems (e.g. Kim et al., 2009; Smith & Belk, 2011), *P. moluccensis* appear to have the ability to enhance their antipredator response in an additive manner to additional sensory information on predation threat. The novelty of our findings lies in the context specificity in which multiple inputs may lead to additive antipredator response. Here we show that the extent to which additivity to risk assessment cues may happen can strongly be influenced by a prey’s feeding history and associated changes in the perception of predation threat.

Animals are expected to forage differently when hungry than when satiated (Caraco, 1983; Godin & Crossman 1994). Theoretical models, supported by some experimental work, suggest that animals will often intensify foraging at the expense of vigilance as their energetic state declines (Caraco et al., 1990; Houston et al., 1993; Lima, 1998; Lönnstedt & McCormick, 2011b). For instance, Lienart et al. (2014) showed for another damselfish species, *Pomacentrus chrysurus*, that high physiological demands eliminated any behavioural response to alarm cues obtained from injured conspecifics. In the present study, fish with a poor feeding history displayed a well-developed threat-sensitive antipredator response to the odour or sight of the predator. Additionally, disregarding its relatively low body condition and requirement for food, the co-occurrence of chemical and visual risk cues led poorly fed fish to further decrease their foraging rate with a strongly additive signature. Such conservative foraging behaviour is surprising giving their poor growth and feeding history. In addition to a depressed growth rate, liver sections in poorly fed fish revealed highly compacted hepatocytes, indicative of low
levels of energy storage (Hoey et al., 2007). Interestingly, although moderately fed fish showed better growth history and nutritional conditions, they shared very similar antipredator responses to the poorly-fed group. Our study is not alone in its lack of a marked effect of hunger on the response of prey to predation threat. Other authors have suggested that the lower food treatment used may still have been too high to evoke a ‘hunger’ response (William & Brown, 1991; Killen & Brown, 2006). A similar phenomenon may have occurred in our study, in which, although poorly and moderately fed fish were ‘energetically stressed’, their body assets were not reduced to a level where they would prioritize foraging over an immediate threat. Alternatively, it may have been that poorly fed fish displayed a supra-threshold antipredator response. In other words, the used levels of predation risk were sufficiently high to shape the trade-off between resource acquisition and antipredator behaviours despite the low nutritional condition of the fish.

While no distinctive risk-prone behaviours towards the lower end of the hunger-satiation continuum were observed, fish raised in high food conditions displayed a strong antipredator response when exposed to predation threat. Well-fed fish showed similar low motivation to forage when facing any risk cue, independently whether they were exposed to single or paired predator cues. Such an increase in vigilance may have been driven by state-dependent mechanisms, where individuals with greater accumulated fitness assets invest more in anti-predator behaviour (Reinhardt & Healey, 1999; Reinhardt, 2002). In juvenile prey organisms, whose reproductive output is not yet a priority, assets protection is expected to be accentuated by a good feeding history (Dill & Fraser, 1984; Lönnstedt & McCormick, 2011b), high growth rate (Damsgård & Dill, 1998, Reinhardt & Healey, 1999) and large energy stores (Bull & Metcalfe, 1997). While well-fed fish in the present study showed similar body mass and level of energy reserves as moderately fed fish, they did appear to have the highest growth rate. Empirical studies on fish and other taxa have similarly found greater predation risk avoidance in relatively larger individuals (e.g. Hegner, 1985; Grant & Noakes 1987; Grand & Dill 1997; Lönnstedt & McCormick, 2011b). Larger individuals may have
accumulated more fitness-associated assets, progressively leading to a reduction of the gains obtained from a unit of food relative to the chance of being preyed upon (Reinhardt & Healey, 1999). However, both positive and inverse relationships between size and willingness to take risks have been reported in the literature (e.g. Johnsson, 1993; Fraker, 2008), including for congenerics of the current study species (Clark, 1994; Johnson & Hixon, 2010; Lönnstedt & McCormick, 2011b). This highlights the need for more studies to elucidate potential mechanisms underlying the relationship observed between a prey’s intrinsic state and its risk taking behaviour.

Our findings suggest that favorable nutritional conditions led well-fed fish to respond to any risk cue with a fully developed anti-predator response, which maximised vigilance. Interestingly, additional information on predation threat did not detectably lead to a further intensification of the antipredator response. This was surprising, as according to the complementarity hypothesis, prey organisms are expected to further increase their vigilance with increased certainty of predation risk (Lima & Steury, 2005; Ferrari et al., 2008b; Munoz & Blumstein, 2012). The lack of further increase in the response to additional predator information may be due to prey lacking a requirement to feed and so prioritizing vigilance based on a single piece of information concerning predation threat; any further reduction in the foraging in response to additional information may lead to suboptimal foraging strategies or maladaptive behaviours. This response pattern in well-fed fish to multiple cues indicates that prey do not always translate additional publically available information on predation risk into a simple linear additive or synergetic antipredator response. The extent to which the incorporation of multiple cues may lead to a greater response than a single cue is very likely driven by the feeding and growth history of the prey. Future studies should take into account the feeding history or physiological condition of prey when characterizing sensory complementation of predator information. Likewise, it can be expected that other state-variables affecting perception of acute risk (e.g., reproductive assets, age, gender, genetic relatedness with other individuals) may also lead to a context-specific threat-sensitive antipredator response of prey to

Because the antipredator response of prey could be a composite of many behaviours we also examined how prey fish from the different feeding treatments adjusted their activity when exposed to either the odour or the sight of the predator, or a combination of both predator cues. Interestingly, activity of prey fish, as defined by number of line crosses, was independent of the animal's feeding history and the amount or nature of predator related cues. The higher threat-sensitivity of foraging rate (i.e. number of feeding strikes) to increasing levels of predation threat, this compared with activity, may indicate that our study fish tempt to prioritize vigilance while foraging rather than avoiding absolute conspicuousness. There is in the literature other examples in which some behaviours may additively respond to predation threats while others may respond in a non-additive fashion (Blanchet et al., 2007). It has been suggested that additive and non-additive responses leading to potential fitness lost could be compensated by the adjustment of other behaviours (Ajie et al. 2007, Blanchet et al., 2007; Lind & Cresswell 2005). These contrasts in responsiveness between activity and foraging rate underscore the importance of a ‘multi-behaviour approach’.
Chapter 4: Retention of acquired predator recognition by a juvenile coral reef fish under different temperature-mediated growth trajectories

Govinda D. H. Lienart, Maud C.O. Ferrari, Mark I. McCormick
This chapter has been submitted to Behavioural Ecology & Sociobiology

4.1 Summary

While many studies have examined the way prey learn, little is known of how long prey should retain learned recognition of a predation threat. Recent studies suggest that factors affecting a prey’s intrinsic characteristics such as growth and size could potentially change its vulnerability to predation and therefore indirectly shape the length of time prey should retain acquired predator recognition. Here we tested whether there was a decline of the response to a learned predator cue with time and if ecologically-relevant changes in temperature may affect growth rate and size, thereby changing the length of time prey show acquired recognition of a predation risk. Juvenile coral reef fish, Pomacentrus coelestis, were conditioned to recognize a predator odour using a chemical alarm cue and reared in two temperature regimes (27 °C vs. 30 °C) for 2 weeks. Individuals were then tested weekly for a response to the predator odour. A 3 °C difference affected body size and growth rate over 14 days. However, differences in growth rate did not appear to affect the retention of predator information between treatments. Conditioned prey still responded to a learned predation cue after one week regardless of their temperature treatment. However, after 14 days post-conditioning, fish from both treatments failed to display overt antipredator responses when exposed to the learned predator odour. Results show that in the absence of reinforcement, prey retain learned predator recognition only for a limited time, regardless of environmental temperature or growth, which may be the result of a fitness-relevant adaptive mechanism contributing to survival and reproductive success.
4.2 Introduction

Prey must continually balance the conflicting pressures of avoiding predation while maximizing other fitness-related demands such as foraging and reproduction (Lima and Dill 1990; Houston et al. 1993; Candolin 1997). The innate ability to behaviourally respond to predator-related cues, which is supported by a finite set of genetic recognition templates of danger, can contribute to a prey’s survival, particularly at vulnerable juvenile stages (Berejikian et al. 2003; Epp and Gabor 2008; Ferrari et al. 2010c). However, predation risk can be highly variable over a range of temporal scales (Danilowicz and Sale 1999; Sih et al. 2000). For instance, it may vary with the physiological condition of the predator, which could depend on their satiation level or nutrient balance (Bence and Murdoch 1986; Mayntz et al. 2009). The level of threat can also change seasonally as predators move to spawning grounds or shift habitat preference (Werner and Hall 1988; Meyer et al. 2007). In addition to variability in the activity or presence of predators (Bosiger et al. 2012), vulnerability of prey may change with its ontogeny (Lingle et al. 2008). As prey grow, they may be less vulnerable to certain predators, but at the same time be confronted by new predation threats (Craig et al. 2006). Under such variable conditions, a mechanism that enables the rapid learning of risk can give prey an ability to dynamically adjust their responses to perceived predation threat (Riffell et al. 2008; Holmes and McCormick 2010; Lönnstedt et al. 2012a).

In aquatic organisms, one such rapid learning mechanism involves injured conspecific cues. In fishes, these cues (often referred to as ‘chemical alarm cues’) are contained within the epidermis of the prey and can elicit strong and immediate antipredator responses when detected by conspecifics (reviewed in Ferrari et al. 2010c). However, these cues can only be released in the water column upon mechanical damage of the skin, which usually occurs during a predation event. This makes them a reliable indicator of risk. An extensive number of studies have shown that many aquatic species can learn to recognize cues from novel predators (sight, smell, sound) as risky, when these cues are paired with injured
conspecific cues (Bouwma and Hazlett 2001; Mitchell et al. 2011a), in a form of associative learning or also often referred as Pavlovian learning (see Ferrari et al. 2010c for a review). However, little is known about the duration that prey should retain a learned response and whether extrinsic and intrinsic factors, such as temperature and growth rate, influence the retention of the acquired recognition of predation threat over time (Ferrari et al. 2010c; Brown et al. 2013a; 2013b).

Research on a variety of taxa has shown that the response to a learned stimulus will decline over time in the absence of reinforcement (Mirza and Chivers 2000; Hazlett et al. 2002; Gonzalo et al. 2009). However, whether the information is truly lost and forgotten, or is still present but ignored, is not well understood. Two recent studies, Ferrari and Chivers (2013) and Chivers and Ferrari (2013b), showed that information learned by tadpoles, which was subsequently ‘forgotten’, still had an effect on learning events that followed. This indicated that the information was stored and used in subsequent learning events, even though the individuals initially failed to display a response to those cues. Because of the lack of a well-established body of scientific knowledge on whether the information is actually retained or not, we preferred to be cautious in the use of information retention-related terms. In the present paper, we therefore opted to use the terms retention and eventual absence of the ‘response to’ and ‘recognition of’ a learned predation threat, without making any inference on whether the information per se is retained or forgotten.

Regardless of whether the acquired information is actually forgotten or not, studies indicate that the gradual decline in the intensity of the response to a learned stimulus may be the result of a fitness-relevant adaptive mechanism contributing to reproductive success and survival (Kraemer and Golding 1997; Ferrari et al. 2012b). For example, the gradual decrease in the acquired recognition of previously rich but now poor feeding sites will benefit individuals (van Bergen et al. 2004). Organisms are often confronted with environmental changes that could lead to obsolete behavioural responses: a response that was once appropriate might become inappropriate as the situation changes.
(Schlaepfer et al. 2002), and therefore the cost of responding to outdated information may exceed the benefits (McNamara and Houston 1987; Dall et al. 2005).

Models and empirical research on the adaptive waning of responses to a learned stimulus have primarily been developed within the context of foraging (McNamara and Houston 1987; Hirvonen et al. 1999). Only a small number of studies have integrated the adaptive waning of responses within the context of threat-sensitive antipredator responses of prey (e.g. Ferrari et al. 2010a; Brown et al. 2013a). Ferrari et al. (2010a) proposed a theoretical framework in which they identified a suite of extrinsic and intrinsic factors that could potentially affect the retention of information within the context of predation. They highlighted the fact that predation risk is likely to vary with an individual’s intrinsic characteristics and should therefore affect the length of retention of the acquired predator recognition. The corollary of this theory is that any environmental factor that affects an organism’s intrinsic characteristic, such as growth or size, can potentially change a prey’s vulnerability to a specific predation threat and indirectly shape the duration of the retention of the acquired predator recognition.

For most organisms, temperature is one of the major environmental influences on life-history processes (Brett 1971). This is especially true for ectothermic species, such as amphibians (Touchon and Warkentin 2011), reptiles (Rhen et al. 2011), fishes (Green and Fisher 2004) and invertebrates (Hayes et al. 2014), for which body temperature directly depends on their thermal environment. In tropical ecosystems, the temperature is more constant than that observed in temperate systems (Addo-Bediako et al. 2000). However, many species live close to their temperature optimum and small variations in temperature can have dramatic consequences on the life history of aquatic organisms (Tewksbury et al. 2008; Rummer et al. 2013). In particular, increases in temperature within the optimal range typically lead to an increased growth rate when food is unlimited (Handeland et al. 2008). Therefore, higher temperatures may cause prey to outgrow gape limits of some predators, while at the same time become the prey of choice for others (Urban 2007;
Holmes and McCormick 2010). As this happens, information acquired about the identity of predators will progressively lose in its relevancy. Consequently, there should be a link between temperature, growth rate/size and retention of acquired recognition of past threats. If temperature increases lead to altered growth rates and body size, it is expected that the duration of retention will also change.

To test these predictions, Ferrari et al. (2011a; 2012a) conducted a series of studies in which they evaluated the effect of changes in temperature-induced growth rate on retention of acquired predator recognition in tadpoles. Tadpoles raised on a slow-growth trajectory (i.e. under cold conditions) and subsequently taught to recognize a novel salamander as a threat, showed longer retention of the acquired predator recognition than tadpoles raised on a fast-growth trajectory (i.e. under higher temperatures). However, a tadpoles’ life history is such that they are often pushed towards a growth-maximizing strategy where they are prone to ignore risk, due to the fact that they need to obtain enough resources to metamorphose before the aquatic habitat in which they live dries out (Altwegg 2003; Ferrari et al. 2011a). Different species may show distinct plasticity in the length of the retention time of the acquired predator information due to a variety of factors including body and brain sizes, genetic predispositions, life history, and the environmental conditions in which they thrive (Ferrari et al. 2011a; Ferrari et al. 2012a).

The goal of the present study was to investigate the duration of retention of learned predator information in a juvenile reef fish (Pomacentrus coelestis), and examine whether intrinsic factors of the prey, such as growth rate and size, caused by small but ecologically-relevant variations in temperature affected the retention of acquired predator recognition. We predicted that prey fish conditioned to recognize the odour of the predator, Pseudochromis fuscus, should decrease their acquired recognition of the predator cue over time. We also predicted that prey with fast growth trajectories would rapidly outgrow the size window of threat by the gape-limited predator, and so retain predator recognition for a shorter time compared to fish that have slower growth. Here, conditioned fish were tested after being brought to different trajectories, not before, to
contrast negative results obtained in a similar study on fish (see Brown et al., 2011).

4.3 Methods

4.3.1 Study site and species

This study was conducted at Lizard Island (145°27'E, 14°41'S), northern Great Barrier Reef, Australia in January 2014. The laboratories and flow-through seawater aquarium system at Lizard Island Research Station were used to conduct all experiments, and fish were collected from the shallow fringing reef. We used juvenile neon damselfish *P. coelestis* (Pomacentridae) for all experimental trials. This small damselfish is often found in areas of shallow exposed reef with coral rubble (Allen 1991).

The predator used for this experiment was the dusky dottyback, *P. fuscus* (Pseudochromidae), a crypto-benthic predator common in shallow reefs throughout the Indo-Pacific. *Pseudochromis fuscus* is known to exert strong predation pressure on newly settled and juvenile damselfish during the summer recruitment season (Feeney et al. 2012). This species shows a pronounced gape-limitation during interaction with juvenile damselfish (Holmes and McCormick 2010; Feeney et al. 2012), which makes this predator only relevant to small juvenile fishes. It typically feeds on juvenile fishes with body depth under 0.51 cm and has an absolute gape limitation for body depths of 1.04 cm (Holmes and McCormick 2010). At the start of the current experiment, prey fish had an average body depth close to reported threshold in gap limitation (0.48 ± 0.05(SD) cm). It was therefore expected that prey with high growth trajectories would move quickly outside the predation window of *P. fuscus*.

4.3.2 Fish collection, housing, and release

*Pomacentrus coelestis* were collected as newly metamorphosed juveniles using light traps deployed overnight in the vicinity of fringing reef around Lizard Island. Fish were immediately transported back to the Lizard Island
Research Station (approximately 10 min boat trip) in 60-l containers (65 x 41 cm and 40 cm deep), which contained a maximum of 200 juvenile fishes. The bins were aerated using portable air pumps. Once at the laboratory, light trap catches were carefully sorted to species level and *P. coelestis* were transferred into 25-l tanks on a flow-through seawater system, where they were maintained for 24 h. Fish were fed with newly hatched *Artemia* twice per day ad libitum to facilitate recovery from the stress of capture. Only batches of fish collected within a 3-days interval were used for the experiment.

Four adult *P. fuscus* were collected using handnets and a dilute solution of anaesthetic clove oil from the shallow fringing reef around Lizard Island. Immediately after collection, fish were kept within separated plastic bags and transported back to the Lizard Island Research Station where they were housed separately in mesh breeding baskets within a 25-l aquarium to avoid aggressive interactions. Predators were fed twice a day with squid. All fish were maintained under a 12:12 h light:dark regime. Water was constantly provided via a flow-through system fed with water pumped from surrounding lagoon, ensuring that water temperature in holding aquaria mirrored that found in the natural environment. Once the experiment was completed, all experimental fishes from all treatments were maintained for a minimum of 48 h and were fed ad libitum to allow for recovery prior to being released to their natural habitat. No mortality of fish was observed during the capture and release of fish. Mortality for fish during the treatment period was approximately 4%. All research was conducted under permits from the Great Barrier Reef Marine Park Authority and James Cook University Animal Ethics Committee (permit no. A2080 and A2005).

4.3.3 Experimental tanks

Fish were about 1 week old at the initiation of the experiment and were allocated at random to 8 thermally insulated 18-l aquaria (40 x 30 cm and 15 cm deep) representing 4 tanks in each temperature treatment. Each tank contained a batch of 60 fish and were fed daily twice a day (3500
Chapter 4

*Artemia* (L). Fish were exposed to either low (i.e., ambient) temperature (27 ± 0.5 °C) or high temperature (30 ± 0.5 °C). Juvenile fish naturally experience these temperatures as they fall within the temperature range of a typical summer recruitment period at our study location (e.g., McCormick and Molony 1995). The low and high treatments were maintained using header tanks with chillers and heating units, respectively. These temperatures were chosen to represent the realistic temperature range on the northern Great Barrier Reef over the recruitment season. Fish were acclimated to the high temperature by slowly raising the water temperature over a 48-h period, and then temperatures were maintained in the 2 treatments for 4 days (under a 12:12 h light:dark photoperiod) prior to fish conditioning and behavioural trials. An airstone within each tank kept the *Artemia* in suspension and distributed them throughout the tank, so all fish had similar access to food. The experiment consisted of two phases: a conditioning phase, where predator-naïve fish maintained at 27 or 30 °C were taught to recognize *P. fuscus* odour as risky, and a testing phase, where the individuals were tested for their response to the predator odour. This testing phase took place at 1, 7 or 14 days after the conditioning phase. Each fish was only tested once.

### 4.3.4 Conditioning

All fish within the experiment were initially conditioned to recognize the odour of *P. fuscus* as a threat. Conditioning to the predator involved individual damselfish being simultaneously exposed to predator odour and a solution of chemical alarm cue collected from the damaged epidermis of juvenile *P. coelestis*. Based on logistical constrains and to avoid the excessive use of animals we did not explicitly test for an innate response to *P. fuscus* odour. Mitchell et al. (2011b) showed for a closely related species to our study species that individuals do not innately respond to *P. fuscus* odour. Furthermore, research has demonstrated that newly metamorphosed coral reef fishes that were not conditioned to recognize the odour of *P. fuscus* have dramatically lower survival rate on the reef compared with those fish that were conditioned to recognize the predator odour.
cue (Manassa and McCormick, 2012a). Together, these results provide strong evidence that naive coral reef fish do not innately respond to odours obtained from predator species (Ferrari et al. 2011b; Lönnstedt et al. 2012a).

Predator odour was prepared from the four *P. fuscus* described earlier (63.1, 61.3, 55.2, 61.3 mm standard length) housed in a 25-l aquaria. The evening prior to the trials, the water to the tank was turned off (leaving only the aeration) and left undisturbed overnight. This water (hereafter predator odour) was then used for conditioning and behavioural trials the following day.

Injured conspecific cues (i.e., chemical alarm cues; Ferrari et al. 2010c) were prepared using donor *P. coelestis* collected from light traps. Recruits were euthanized individually via cold shock (an ice-slurry in seawater; in accordance with James Cook University Animal Ethics; permit no. A2005). Death in juveniles is commonly identified as a sudden lack of opercula movement, which generally occurred within 10 sec. However, fish were immersed in an ice-slurry for the full 2 min to ensure complete brain death. Thermal shock was chosen above other killing methods because of the speed of death and it also prevents the release of potentially confounding body odours (e.g., blow to the head) or the introduction of foreign odours (e.g., anaesthesia overdose). A clean scalpel blade was used to make five superficial vertical incisions along each flank of five individual fish. The fish were then rinsed with 20 ml of seawater and the solution was filtered to remove any solid material (scales). We obtained a 100 ml chemical alarm cue solution (obtained from the five fishes), which was further diluted in 900 ml seawater in order to obtain 1 l of alarm cue solution.

Prior to conditioning, fish were randomly allocated to one of the two temperature treatments (as above). Individual fish were placed in plastic zip-lock bags (20 cm x 20 cm) containing aerated seawater. Bagged fish were then placed in their allocated temperature tank (either 27 °C or 30 °C) and left undisturbed for one hour. Fish were individually conditioned by gently injecting 40 ml of predator odour and 20 ml of alarm cue within each plastic bag, using 60-ml syringes. Fish were left undisturbed for a further
90 min then released from their bags into their holding tank of matching temperature.

As a result of constraints in time and tank availability during the observation trials, all fish could not be tested on the same day. We therefore conditioned on each day one batch of fish from each temperature treatment for four consecutive days. Consequently, the trails for each of the represented post-conditioning days (1, 7 and 14 days) were spread out over 4 days (see supplementary file S 4.1 for more details on number of replicates)

### 4.3.5 Observation tanks and recognition trials

Testing took place 1, 7 and 14 days following the conditioning event. Prior to each testing day, individual *P. coelestis* were placed in 13-l observation tanks to acclimate overnight. The temperature of the tank water matched the treatment temperature of the fish (27 °C or 30 °C). Tanks contained a 3-cm layer of sand, a small shelter (5-cm diameter PVC tube) at one end and an airstone at the opposite end. An injection tube was fixed to the aeration tube and allowed *Artemia* food or a stimulus cue to be introduced with minimal disturbance to the fish. The airstone facilitated the distribution of the cues throughout the tank. Each tank was surrounded on three sides with black plastic to prevent distractions to the focal fish. Fish were observed through small holes cut in a black plastic curtain that was hung in front of the tanks to minimize disturbance to the fish.

The behaviour of the fish was quantified by counting feeding strikes during a 4-min pre- and 4-min post-stimulus period. A broad range of studies has shown that a decrease in foraging is a common behavioural response observed in animals facing a risky situation (Williams and Brown 1991; Bishop and Brown 1992; Killen 2007), including larval damselfish (e.g. Mitchell et al. 2011a; Lienart et al. 2014). The foraging rate included all feeding strikes on *Artemia* irrespective of whether fish were successful at capturing prey.

Prior to the start of the trial, fish were fed to remove the ‘feeding frenzy’ effect associated with the sudden presentation of food in the tank.
This feeding period consisted of injecting 2.5 ml of food (an Artemia solution containing approximately 250 individuals per ml) in the tank, followed by 60 ml of saltwater to flush completely the food into the tank. Pre-stimulus observations began 4 min later, when another 2.5 ml of food was injected into the tank and flushed with 60 ml of saltwater. After the pre-stimulus period, we injected 2.5 ml of food, followed by 60 ml saltwater (control) or predator odour (experimental stimulus), flushed with 60 ml of saltwater. We assessed the behaviour of 13-20 fish in each of the 6 treatment groups (2 temperatures x 3 testing days; (see supplementary file S 4.1 for more details on number of replicates). The observer was blind with respect to the identity of the injected cue.

After observational trials, juveniles were photographed in a lateral position on a 1-cm grid. Standard length and body depth was estimated for each fish to the nearest 0.01 mm from digital photographs using image analysis software ImageJ. We measured morphometrics of 27-36 fish from each of the 6 treatment groups (2 temperatures x 3 testing days) (see supplementary file S 4.2 for more details on number of replicates).

4.3.5 Statistical analyses

Behavioural Measures

We used the pre- and post-stimulus data to compute a percent change in foraging activity from the pre-stimulus baseline ([post-pre]/pre) and used this metric as our response variable in subsequent analyses. We ran a four-way nested ANOVA to test the effect of temperature, cue and time on the behavioural response of fish. Here we nested each tank within its respective temperature treatment to account for undesired potential variance in behaviour due to tank condition within a treatment. We subsequently performed Tukey’s HSD post-hoc tests to investigate the nature of any significant differences found by ANOVA. Assumptions of normality and homoscedasticity were examined with residual analysis and found to be met.
Morphometric measures

Because body depth and standard length of the fish are not independent of one another, we used a three-way nested multivariate ANOVA (three-way MANOVA) to investigate the effect of temperature and time on the morphometry of the fish. We performed separate three-way nested ANOVAs to investigate the effect of temperature and time on standard length and body depth. Similarly as for morphometric analysis, each experimental tank was nested within its respective temperature treatment to account for tank effect. We subsequently performed Tukey’s HSD post-hoc tests to investigate the nature of any significant differences found by ANOVA, and assumptions of normality and homoscedasticity were examined with residual analysis and found to be met.

4.4 Results

Behavioural measurements

There was a significant two-way interaction between time post-conditioning and cue on the change in feeding strikes by fish when exposed to predation threat ($F_{2,182} = 45.459, P < 0.001$) (Table 4.1). Tukey’s post hoc tests on this interaction revealed that the behaviour of fish exposed to predator odour differed significantly from fish exposed to saltwater control, on 1 day post-conditioning ($P < 0.001$) and 7 days post-conditioning ($P < 0.001$). However, after 14 days post-conditioning, fish exposed to predator odour did not differ significantly from fish exposed to saltwater control ($P = 0.92$) (Fig. 4.1). Four-way nested ANOVA revealed there was no tank effect, as all terms involving tank were non-significant (all $P > 0.336$; Table 4.1).
Figure 4.1 Proportion change in feeding strikes (±SE) from the pre-stimulus baseline for juvenile *Pomacentrus coelestis* exposed to predator odour (PO) (grey bars) or saltwater (SW) (white bars). *Pomacentrus coelestis* were raised at either 27 °C or 30 °C and were tested for recognition of the predator odour 1 day, 7 and or 14 days post-conditioning. Letters above or below the bars represent Tukey’s significant difference groupings of means. From left to right number of replicates: 17, 18, 20, 18, 13, 13, 14, 14, 19, 17, 17, 16
Table 4.1 ANOVA results for the effect of temperature (27 °C versus 30 °C), time post-conditioning (1 day versus 7 days versus 14 days) and cue (saltwater versus predator odour) on behavioural response (change in feeding strikes) of *Pomacentrus coelestis*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.00003</td>
<td>0.008</td>
<td>0.931</td>
</tr>
<tr>
<td>Time post-conditioning</td>
<td>2</td>
<td>0.157</td>
<td>32.288</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cue</td>
<td>1</td>
<td>0.797</td>
<td>684.66</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Temperature * Time post-conditioning</td>
<td>2</td>
<td>0.005</td>
<td>1.054</td>
<td>0.378</td>
</tr>
<tr>
<td>Temperature * Cue</td>
<td>1</td>
<td>0.0004</td>
<td>0.373</td>
<td>0.563</td>
</tr>
<tr>
<td>Time Post-conditioning * Cue</td>
<td>2</td>
<td>0.171</td>
<td>45.459</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Temperature * Time post-conditioning * Cue</td>
<td>2</td>
<td>0.007</td>
<td>1.726</td>
<td>0.219</td>
</tr>
<tr>
<td>Tank (Temperature)</td>
<td>6</td>
<td>0.004</td>
<td>1.875</td>
<td>0.438</td>
</tr>
<tr>
<td>Time * Tank (Temperature)</td>
<td>12</td>
<td>0.005</td>
<td>1.285</td>
<td>0.336</td>
</tr>
<tr>
<td>Cue * Tank (Temperature)</td>
<td>6</td>
<td>0.001</td>
<td>0.305</td>
<td>0.922</td>
</tr>
<tr>
<td>Time * Cue * Tank (Temperature)</td>
<td>12</td>
<td>0.003</td>
<td>1.011</td>
<td>0.442</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>2</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>
### Table 4.2 ANOVA results for overall effect of temperature (27 °C versus 30 °C) and time post-conditioning (1 day versus 7 days versus 14 days) on standard length of *Pomacentrus coelestis*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.08</td>
<td>13.04</td>
<td>0.010</td>
</tr>
<tr>
<td>Time post-conditioning</td>
<td>2</td>
<td>0.204</td>
<td>25.91</td>
<td>0.001</td>
</tr>
<tr>
<td>Temperature * Time post-conditioning</td>
<td>2</td>
<td>0.018</td>
<td>2.33</td>
<td>0.137</td>
</tr>
<tr>
<td>Tank (Temperature)</td>
<td>6</td>
<td>0.006</td>
<td>0.78</td>
<td>0.600</td>
</tr>
<tr>
<td>Time * Tank (Temperature)</td>
<td>12</td>
<td>0.008</td>
<td>0.79</td>
<td>0.663</td>
</tr>
<tr>
<td>Error</td>
<td>17</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4.3 ANOVA results for overall effect of temperature (27 °C versus 30 °C) and time post-conditioning (1 day versus 7 days versus 14 days) on body depth of *Pomacentrus coelestis*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.029</td>
<td>21.42</td>
<td>0.003</td>
</tr>
<tr>
<td>Time post-conditioning</td>
<td>2</td>
<td>0.037</td>
<td>8.81</td>
<td>0.004</td>
</tr>
<tr>
<td>Temperature * Time post-conditioning</td>
<td>2</td>
<td>0.008</td>
<td>2.08</td>
<td>0.166</td>
</tr>
<tr>
<td>Tank (Temperature)</td>
<td>6</td>
<td>0.001</td>
<td>0.31</td>
<td>0.920</td>
</tr>
<tr>
<td>Time * Tank (Temperature)</td>
<td>12</td>
<td>0.004</td>
<td>1.34</td>
<td>0.198</td>
</tr>
<tr>
<td>Error</td>
<td>17</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Morphometric measurements

There was an overall difference in the size of fish between the two temperature treatments (MANOVA: Pillai’s = 0.062, $F_{2,176} = 5.80$, $P = 0.004$). Similarly, time post-conditioning had a significant effect on the size of fish (MANOVA: Pillai’s = 0.212, $F_{4,354} = 10.50$, $P < 0.001$). There was no effect due to the tanks within temperature treatments or its interaction with time (all $P > 0.207$). Four-way nested ANOVAs revealed there was no tank effect, as all terms involving tank were non-significant (standard length: all $P > 0.600$; body depth: all $P > 0.198$). Univariate analyses indicated that temperature and time post-conditioning had significant main effects on standard length (temperature: $F_{1,177} = 13.04$, $P = 0.010$; time: $F_{2,177} = 25.91$, $P < 0.001$) and body depth (temperature: $F_{1,177} = 21.42$, $P = 0.003$; time: $F_{2,177} = 8.81$, $P = 0.004$) (Table 4.2). Tukey’s post-hoc analysis revealed that at 14 days post-conditioning fish kept at 27 °C had significantly deeper body depth and larger standard length compared with fish maintained at 30 °C (all $P < 0.05$; Fig. 4.2a-b). Further Tukey’s post-hoc analysis also indicated that fish maintained at 27 °C increased in standard length with time post-conditioning (all $P < 0.021$; Fig. 4.2a), while body depth only increased significantly between 1 day and 7 days ($P = 0.003$) post-conditioning and between 1 day and 14 days post-conditioning ($P < 0.001$) (Fig. 4.2b). At 30 °C fish increased significantly in standard length between 1 and 14 days post-conditioning ($P < 0.005$; Fig. 4.2a). However, body depth at 30 °C did not increase significantly with time post-conditioning (body depth: all $P > 0.129$) (Fig. 4.2b)
Figure 4.2 Mean (±SE) (a) standard length (cm) and (b) body depth (cm) for juvenile *Pomacentrus coelestis* after 1 day, 7 days and 14 days post-conditioning raised at either 27 °C (dark bars) or 30 °C (open bars). *Significant difference between 27 °C and 30 °C temperature treatments. From left to right number of replicates: (a) 36,36,31,33,34,31; (b) 36,36,31,33,34,31
4.5 Discussion

Theoretical models, supported by a number of empirical studies, suggest that over time an organism should decrease its response to a learned predator cue if the cue is not reinforced. Additionally, factors influencing vulnerability of prey organisms to risk, such as growth rate and size, may also potentially affect the retention of acquired predation recognition (Ferrari et al. 2010a, 2011a; Ferrari et al. 2012a). In the present study, we found a gradual waning in the intensity of the response to a learned cue over time as predicted, but surprisingly, changes in body growth induced by different rearing temperatures had no detectable effect on the retention of acquired predator recognition over time.

Fishes are ectothermic and have limited capacity to maintain a body temperature independent of their environment (Green and Fisher 2004; Roessig et al. 2004). Small changes in ambient temperatures can consequently lead to dramatic effects on the physiological and behavioural traits of ectothermic species (Biro et al. 2010; Donelson et al. 2010). Raising temperature within the optimal range of the performance curve of such a species is expected to increase size and growth rate (Clarke and Fraser 2004; Zuo et al. 2012). We found that a 3 °C difference for two weeks did affect the size of the fish; fish raised at 27 °C were longer and deeper than those raised at 30 °C. Studies on congeneric species of *P. coelestis*, have shown that thermal optima is approximately 31 °C, suggesting that our experimental temperature range should not be responsible for the observed depressed growth rate (Rummer et al. 2013). However, other studies on congeneric species showed similar growth trends to those found in the present study, with fish decreasing their growth rate with rising temperatures (26, 28 and 31 °C) (Munday et al. 2008). At higher temperatures, growth rates of fish are expected to decrease as the thermal maximum for growth is approached (Jobling 1997). More research is needed to establish thermal optimum at the species level, and across a latitudinal gradient, to ascertain the extent to which rising temperature may affect growth rate across populations.

Regardless of the direction of the temperature-induced difference,
we found a size/growth rate difference between our two experimental groups. Thus, in keeping with the logic of Ferrari et al. (2010c), we predicted that individuals with higher growth rates (here, fish raised at 27 °C) would undergo a more rapid decline in the retention in the learned recognition of a predation threat than slower growing prey (here, fish raised at 30 °C). However, no significant difference in response to the learned predation threat was found between the 2 temperature treatments for any given testing day. We found that both groups responded equally 1 and 7 days after conditioning, and that both groups failed to show overt antipredator responses to *P. fuscus* odour after 14 days. These findings contrast with two similar experiments in which tadpoles were raised in warm conditions (20 °C) and cold conditions (11 °C), which produced tadpoles with high and slow growth trajectories respectively (Ferrari et al. 2011a; Ferrari et al. 2012a). In both experiments, Ferrari and colleagues found that rapidly growing tadpoles were faster to lose the learned response compared to tadpoles that grew more slowly. In the present study, we used a much narrower temperature range (27 °C vs. 30 °C), but one that is relevant to the study organism and their ecological scenario. While such narrow thermal range, together with the relatively short duration of our study (though once again, ecologically relevant), did affect growth rate, resulting body depth of fish at the end of the treatment period (range: 0.375 - 0.647 cm) was still well within the gape size limit of the predator (0.51-1.04 cm; Holmes & McCormick 2010). Thus, the used experimental conditions may not have been sufficient to push individuals into growth trajectories, and associated body dimensions, that would engender differences in vulnerability and differential retention of the acquired predator recognition.

An alternative explanation for our lack of differentiation in information-retention between temperature treatments may be that the differentiation occurred, but during the time window between test intervals (between day 8 and day 13 post-testing). Ferrari et al. (2011a; 2012a) have shown that both the growth before and after conditioning contribute to the variation in information-retention. In the present study, we manipulated growth rate only after conditioning, rather than before
conditioning, and the growth rate at the time of, or prior to conditioning may have been key to information-retention rather that the post-conditioning growth trajectory. Brown et al. (2011) also showed that growth rate before learning and not after learning influenced the retention of a predator cue by rainbow trout. Whether the observed differences in the retention of acquired predator recognition among studies is due to differences in factors used to manipulate growth/size, the growth rate at the time an organism is conditioned, or the result of interspecific variation requires further study.

The lack of response to a learned predation threat does not necessarily mean that fish did not retain the information. Chivers and Ferrari (2013b) showed that the characteristics of the first conditioning trial influenced the length of time that tadpoles recalled the information after a second conditioning event. This may indicate that the apparently lost information may still be retrieved, and that fish may have been differentially affected by the temperature treatments in this regard. The findings of the present study, in which retention of the odour of the predator completely waned in both treatments despite differences in growth and body depth, suggests it may be interesting to test and compare retention of acquired predator recognition that involve other sensory modalities in organisms.

Although the results of the present study indicate that a 3 °C difference may not be enough to affect the acquired predator recognition in juvenile P. coelestis, we still detected a general waning trend whereby prey stopped detectably responding to a threat cue 14 days after conditioning. Other research indicates that the period of retention of learned predator recognition varies widely among different aquatic taxa. For instance, juvenile hatchery-reared rainbow trout, Oncorhynchus mykiss, retained a learned response for up to 21 days (Brown and Smith, 1998), though the intensity of the response waned after approximately 10 days (Mirza and Chivers 2000). Likewise, Gonzalo et al. (2009) demonstrated that Iberian green frog, Pelophylax perezi, could retain the recognition of a predation threat for up to 9 days. Hazlett et al. (2002) conditioned four different species of crayfish and found variation in their
retention of learned predators, ranging from 1 day to 4 weeks, depending on the species. Chivers and Smith (1994) found that fathead minnows, *Pimephales promelas*, retained their learned response to a novel predator cue for at least two months. Although different species show distinct durations in retention time, they all seem to exhibit a decline in their perceived risk to the learned cue over time. Regardless of whether the waning and eventual absence of a response to a stimulus results from the dissipation or retrieval-suppression of the initially acquired information, it is likely that these observations reflect an adaptive mechanism allowing animals to enhance behavioral plasticity (Kraemer and Golding 1997; Ferrari et al. 2010c; Chivers and Ferrari 2013b).

In contrast to a more fixed/innate response, a flexible behaviour sustained by learning and adaptive waning can be particularly valuable for predator-inexperienced newly metamorphosed coral reef fishes settling on reefs. The dispersive larval phase of marine fishes, and their small home ranges (Sale 1978), means that it is very unlikely that settling juveniles will encounter the same composition and density of predators as their natal reef. Moreover, as they grow and develop, or move away from nursery habitats, they will encounter new predators. Coral reefs are known for their high biodiversity, and so juveniles will be exposed to olfactory cues from many species (probably hundreds) within a single hour on the reef, many of which will be coincident with alarm cues. Studies within this system, and other systems with lower diversity, have found that there are well-developed mechanisms that reinforce the importance of some smells (e.g., diet cues, Ferrari et al. 2010c; Manassa and McCormick 2012a), while de-emphasizing the importance of other smells (e.g., latent inhibition, Ferrari and Chivers 2011b; Mitchell et al. 2011b). The information content is also pro-rated by the phylogenetically relatedness of the alarm cue donor to the recipient, with an apparent graded response (Mitchell et al. 2012). Given this constant reworking of the landscape of perceived risk, it is perhaps surprising within this system that information on risk is retained without reinforcement from a one off exposure for at least 7 days. Growth, and the factors that influence growth in ectotherms such as environmental temperature, may affect the way retained information is prioritized, but in
the current study the effect may have been too subtle to be detectable. It may be that in systems such as coral reefs where the potential predators come from a large and changing pool of species, that the processes of information reinforcement and de-emphasis play a much greater role in the prioritization of risks across a threat gradient than in systems with a lower diversity of predators. Future studies should compare the relative importance of intrinsic versus extrinsic factors in influencing information retention across systems that have evolved under different levels of predator stability.
Chapter 5: Thermal environment and nutritional condition affect the efficacy of chemical alarm cues produced by prey fish

Govinda D.H. Lienart, Maud C.O. Ferrari, Mark I. McCormick
This chapter has been submitted to Environmental Biology of Fishes

5.1 Summary

A wide variety of prey animals possess damage-released chemical alarm cues that evoke antipredator responses in both conspecifics and heterospecifics. A few studies have indicated that food availability may influence body condition and in doing so, affect the production of alarm cues. We examined whether food availability (high and low) and temperature (27, 30 and 32 °C) interacted to affect the production of chemical alarm cues by juveniles of a marine tropical prey fish, Pomacentrus moluccensis. Results indicate that poorly-fed fish had depleted energy reserves with rising temperatures compared to well-fed fish. Fish with a poor feeding history also showed depressed growth rate across all temperature treatments. The alarm cues produced by experimental fish triggered a stronger antipredator responses as temperature increased up to 30 °C, regardless of the feeding history or body condition of the donor. However, at 32 °C, alarm cues from poorly-fed fish did not elicit as effectively an antipredator response in conspecifics compared to those produced by well-fed fish. The results highlight that warming oceans, in isolation but also in conjunction with changes in food supply, may have a drastic impact on chemically-mediated predator-prey interactions.

5.2 Introduction

In aquatic environments, many prey animals possess chemical alarm cues, which are involuntarily released upon laceration of the epidermis
during a predation event (McCormick and Larson 2007; Ferrari et al. 2010b). These cues have been found to trigger dramatic, short-term, increases in antipredator behaviour in both conspecifics and some sympatric heterospecifics (Brown et al. 1995; Mirza and Chivers 2003). Exposure of prey to alarm cues can also elicit a suite of long term responses, including facilitation of learned recognition of a predator (Larson and McCormick, 2005; Smith et al 2008) and induction of morphological and life history changes (Chivers et al. 2008; Lönnstedt et al. 2013a). While there is empirical evidence that the signal receiver increases its fitness and likelihood of survival through sensory integration of alarm cues (Mirza and Chivers 2000; Lönnstedt et al. 2013b), the benefits of the sender to invest in the production of alarm cues are less obvious. Some studies suggest that the release of alarm cues by an injured prey may function as a distress call (Chivers et al. 1996; Lönnstedt and McCormick 2015), though whether this was the initial driver of their evolution is doubtful (Chivers et al. 2007a). A great deal of research has addressed the role and function of alarm cues in mediating behavioural and life history defenses in prey. However, there is a paucity of research investigating factors that may influence the chemistry of alarm cues and its associated effectiveness in eliciting antipredator responses in conspecifics (Brown et al. 2004a; Chivers et al. 2013a). In particular, factors influencing the physical condition of the sender can be expected to affect the qualitative (e.g. chemical structure) and/or quantitative (i.e. concentration) properties of the alarm cues produced (Brown et al. 2004a; Roh et al. 2004; Manek et al. 2013).

A few studies have indicated that body condition and size of the sender can have a marked influence on the production of alarm cues (e.g. McCormick and Larson 2008; Lönnstedt and McCormick 2011a). Fish with low body condition due to restricted food availability have been found to produce alarm cues that did not elicit responses as pronounced as those produced from fish of high body condition. Low levels of energy reserves due to a poor feeding history may not allow prey to invest in alarm cues as much as it would have in a high food environment (Brown et al. 2004a; Roh et al. 2004; McCormick and Larson 2008). In addition to body
condition, the body size and morphology has also been shown to influence the properties of alarm cues (Mirza & Chivers, 2002; Chivers et al. 2007b). For instance, studies have shown that juvenile coral reef fish displayed a stronger response to alarm cues from similar sized individuals than to alarm cues of larger (and older) individuals (Lönnstedt and McCormick 2011a; Mitchell and McCormick 2013). Whether such body size dependent changes in alarm cue properties is due to alteration in the alarm cue formation or an inclusion of additional chemicals indicating size is uncertain. In general, these studies highlight the important role that food availability and diet may play in determining the amount and/or quality of the alarm cue produced.

Interestingly, no studies have yet considered how factors other than food availability and diet may affect the energy balance and growth of the sender and its associated production of alarm cues. Temperature may be important as it is regarded as an ‘ecological master factor’ affecting every aspect of the physiology and performance of organisms (Brett 1971; Clark and Fraser 2004; Roessig et al. 2004; Englund et al. 2011). Water-breathing ectothermic species, such as fish, are especially sensitive to environmental changes in water temperature because their body temperature directly depends on the thermal environment in which they live (Clark and Fraser 2004; Green and Fisher 2004). The relationship between body temperature and measures of performance in ectothermic species is often characterized by a dome shaped thermal performance curve (Clark and Fraser 2004; Handeland et al. 2008). As such, a fish living with an unlimited supply of food generally increases its growth rate with rising temperature, up to an optimal temperature, after which growth declines dramatically (Jobling 1997; Courtney Jones 2015). However, food is seldom unlimited in the wild and fish on a limited food ration may not grow as fast with rising temperatures, owing to the increasing metabolic demands (Jobling 1997; Okamoto et al. 2012; McLeod et al. 2013). Thus, food availability and temperature can be considered as two major environmental drivers which are expected to influence the production of alarm cue in a potentially interactive way, via changes in the animal’s energy budget and growth performance.
In the present study, we used juveniles of the lemon damselfish, *Pomacentrus moluccensis*, to assess the effects of temperature and food availability on donor condition and how it may influence the production of chemical alarm cues. Like most tropical ectothermic organisms, *P. moluccensis* evolved under relatively stable temperature conditions and is therefore very vulnerable to any change in its thermal environment (McLeod et al. 2013; 2015; Rummer et al. 2014). Studies on closely related congeneric species on the northern Great Barrier Reef have indicated that their thermal optimum is only ~1 °C above the regional average maximum summer temperature (30 °C; Rummer et al. 2014). Consequently, the expected warming of tropical oceans projected to occur by the end of this century of 2-4 °C may be detrimental for the species and many other tropical marine species (Lough 2007; IPCC 2013). We generally expect that with increasing temperature and food-mediated physiological stress, fish would decrease their body condition and consequently have limited energy available to invest in the production of alarm cues. In addition, it may also be expected that a food- and temperature-mediated growth rate may lead to a body size effect on the properties of produced alarm cue, whereby the receiver may respond stronger to cues obtained from fish of similar size. To test these predictions, fish were collected at Lizard Island (Great Barrier Reef, Australia) and were reared on low and high food rations at temperatures below, close to, and above regional maximum summer temperature (27, 30 and 32 °C, respectively) in a fully orthogonal design for 9 days. At the end of the treatment period, we measured body condition and dimension of the fish and tested the effectiveness of produced alarm cues in eliciting an antipredator response in conspecifics.

5.3 Methods

5.3.1 Study species, collection and maintenance

This study was conducted at Lizard Island (145°27’E, 14 °41’S), northern Great Barrier Reef, Australia between December 2013 and January 2014.
Juvenile lemon damselfish, *P. moluccensis*, were used as the study species. This species is common on shallow Indo-Pacific coral reefs, and is typically associated with branching hard corals (Booth and Beretta, 2002). Fish were collected as newly metamorphosed juveniles from the fringing reef using hand nets and a solution of anaesthetic clove oil. After collection, fish were transported back to the research station, where they were transferred into 18-L maintenance tanks (40 x 30 cm and 15 cm deep) in groups of 15-20 individuals (14-17 mm total length range; mean = 15.677; SD = 0.821, n = 123) for 72 h. Fish were fed newly hatched *Artemia* twice per day (2000 *Artemia*/L) to allow for recovery from the stress of capture and standardize feeding history prior to the start of the experiment. All fish were maintained in a 12:12 h light: dark regime. Flow-through aquaria systems were fed directly from the surrounding lagoon so that the water temperatures in holding aquaria mirrored that found in the natural environment (min: 26 °C; max: 30 °C). Immediately after the maintenance period, fish were either put under treatment and subsequently used as donor fish for the production of alarm cues, or directly transferred into observation tanks and used as receiver to test the effectiveness of the alarm cues produced from fish that had been under treatment.

**5.3.2 Experimental treatments**

Fish used as alarm cue donors were randomly allocated to one of the 12 thermally insulated 18-L flow-through seawater aquaria representing each combination of the two feeding levels and the three temperature treatments. Each experimental aquarium contained 12 fish. Fish were either poorly-fed (300 *Artemia*/l twice daily) or well-fed (1000 *Artemia*/l twice daily). These feeding levels were established based on treatments used in similar feeding experiments on congeneric species (Lönnstedt and McCormick 2011b; Lienart et al. 2014). Tanks of fish from each feeding treatment were exposed to one of the following temperature treatments: 27, 30 or 32 °C. To establish these temperature regimes, we used monthly mean temperature data obtained from loggers deployed at 0.6 m depth.
around Lizard Island between September 2010 and August 2014 (Australian Institute of Marine Science http://data.aims.gov.au/; Fig. 5.1). The 27 °C treatment represent the approximate mean temperature regime at the initiation of the recruitment season, 30 °C represent the mean maximum summer temperature and 32 °C represent the projected temperature under IPCC predictions in the next 50-100 years (Lough 2007; IPCC 2013).

**Figure 5.1** Seasonal water temperature at Lizard Island, northern Great Barrier Reef, Australia (temperature logger deployed at 0.6 m). Solid black line: mean monthly temperature; grey shaded area: minimum and maximum temperature range; horizontal black dotted lines represent the selected experimental temperature regimes (27 °C, 30 °C and 32 °C)

Chillers or electric batten heaters (300 W) were used to control the temperature of the seawater. Fish were acclimated to the different temperature treatment by slowly manipulating the temperature over a 48 h period (from ~28.5 °C). Fish were kept in each temperature and food treatment combination for 9 days. An airstone within each tank kept *Artemia* in suspension and distributed throughout the tank, so all fish had similar access to food during the treatment period.
5.3.3 Body measurements of donor fish

Following the treatment period, juveniles were photographed in a lateral position on a 1x1-cm grid. Standard length and body depth to the nearest 0.01 mm was estimated from each fish from the digital photograph using image analysis software (ImageJ version 1.45s, National Institute of Health, U.S.A., http://rsbweb.nih.gov/ij/). We also measured body mass of fish to the nearest 0.0001 g. The morphometrics of 11-16 fish were measured from each of the 6 treatment groups.

Energy reserves of *P. moluccensis* were estimated by quantifying hepatocyte density in liver. Studies have indicated that the hepatocyte density is sensitive to variation in the nutritional condition of an individual. With decreasing body condition, glycogen and lipid stores in the liver decline, leading vacuoles to shrink (e.g., Green and McCormick 1999). This leads to lower levels of vacuolation and consequently, a higher number of hepatocytes per liver area (e.g., Storch and Juario 1983; Hoey et al. 2007). In the present study, all larval samples were individually preserved in FAAC (4% formaldehyde, 5% acetic acid, 1.3% calcium chloride). The complete body of the fish was embedded in paraffin and sectioned serially along the sagittal plane (5 micron) with a microtome. Histological sections were prepared with Schiff’s reagent (periodic acid) and then counterstained with haematoxylin (PAS-H). *Photographs* of the slides were taken through the eyepiece of a dissecting *microscope* and subsequently analyzed using ImageJ. Counts of hepatocyte within three quadrats (47.657 x 47.657 micrometer at 40x magnification) were undertaken for a randomly chosen liver section of each replicate fish. We measured hepatocyte density of 4-7 fish in each of the 6 treatment groups (2 food levels x 3 temperatures).

5.3.4 Stimulus preparation

Alarm cue solutions were prepared from *P. moluccensis* individuals reared under the different temperature and food treatments. The donor fish were individually euthanized via cold shock (an ice-slurry in seawater; in
accordance with James Cook University Animal Ethics; permit no. A2005 and A2080). Death in juveniles is commonly identified as a sudden lack of opercula movement, which generally occurred within 10 sec. However, fish were immersed in an ice-slurry for the full 2 min to ensure complete brain death. Thermal shock was chosen above other killing methods because of the speed of death and it also prevents the release of potentially confounding body odours (e.g., blow to the head) or the introduction of foreign odours (e.g., anaesthesia overdose). Chemical alarm cues were collected by making five vertical cuts (each 0.5 cm long) and 1 cut along the lateral line (1 cm long) of the left flank of each donor fish with a clean scalpel. The length of the incision among all fishes was kept constant for standardization of the cue solution. Fish were then rinsed with 20 mL of seawater, and the solution was filtered to remove any solid material. Alarm cue solutions were prepared within 5 min of injection into the observation tank to avoid any time-related decrease in potency. For each behavioural trial, we injected the alarm cues obtained from one donor fish.

5.3.5 Behavioural trials

We used receiver fish as bioassay to test the effectiveness of the chemical alarm cues produced by donor fish in eliciting an antipredator response in conspecifics. Observations of fish behaviour were conducted in 13-L flow-through aquaria. The observation tanks were fed directly from the surrounding lagoon and had a temperature of ~28.5 °C. Each tank had a 3-cm layer of sand, a small PVC tube (50 mm diameter) for shelter at one end and an air stone at the opposite end. An injection tube was attached to the air stone tube to allow food and cues to be introduced with minimal disturbance to the fish and be dispersed rapidly within the tank. Each tank was surrounded on three sides with black plastic to visually isolate the fish, and a black plastic curtain was hung in front of the tanks to minimize disturbance to the fish during observation.

Prior to the start of the observational trials, the flow-through system was turned off to avoid flushing out the injected cues and food. Trials were
conducted between 0800 and 1600 hr each day. The foraging rate of the fish was observed for 4 min before and then 4 min after the introduction of chemical alarm cues from the temperature-by-food reared fish or the saltwater control. A decreased foraging rate to predation threat is a well-known antipredator response in a number of prey species, including coral reef fishes (e.g., Lienart et al. 2014; Mitchell et al. 2012). The foraging rate included the total number of feeding strikes displayed by the fish, irrespective of whether they were successful at capturing food items (*Artemia*).

Prior to the start of the behavioural trial, fish were fed to remove the possibility of a ‘feeding frenzy’ effect at the start of the bioassay. This feeding period consisted of injecting 2.5 mL of food (at 250 *Artemia*/mL) in the tank, followed by 20 mL of saltwater to flush completely the food into the tank. The pre-stimulus observation period initiated 4-min later when an additional 2.5 mL of food (250 *Artemia*/mL) was introduced and flushed with 20 mL of seawater. At the end of the pre-stimulus observation period, we injected 2.5 mL of food (250 *Artemia*/mL), followed by 20 mL stimulus and flushed with 20 mL of seawater. The post-stimulus observation period began 1 min later and lasted for 4 min. The stimulus consisted of either one of the six chemical alarm cue solutions (2 food by 3 temperature combinations) or a saltwater control. We tested 16-22 fish in each of the 6 treatment groups. Individuals were tested only once.

5.3.6 Statistical analysis

*Morphometric measurements and hepatocyte density*

Since standard length, body depth and body mass of fish are not independent of one another, we used a two-factor multivariate ANOVA (MANOVA) to investigate the effect of food and temperature on the overall morphology of fish. We subsequently ran ANOVAs to investigate the nature of the significant difference found by MANOVA. A two-factor ANOVA was used to explore whether there was an effect of food and temperature on the density of hepatocytes. Tukey’s HSD post-hoc tests
were used to explore the differences found by ANOVA. Prior to analyses assumptions of normality and homoscedasticity were explored with residual analysis. Density of hepatocytes required log\textsubscript{10} transformation to meet these assumptions.

**Behavioural measurements**

We initially ran a two-factor ANOVA on pre-stimulus feeding strikes using temperature and food level as factors to test for differences in baseline values among treatment groups prior to exposure to the experimental stimulus. We quantified the proportional change in the behavioural response of fish to the experimental stimulus by computing the percent change in number of feeding strikes from the pre-stimulus baseline ([post-pre]/pre). This metric was used as our response variable in all subsequent analysis. To investigate the effect of food level and temperature on the production of alarm cues by donor fish, we performed a two-factor ANOVA on the behavioural response (i.e., feeding strikes) by the receiver to the stimuli. Given the high number of groups and associated risks of type I error, we ran separate Tukey HSD post-hoc tests across temperature for each of the feeding levels (the water control was included in each test). Assumptions of normality and homoscedasticity were examined with residual analysis and found to be met.

**5.4 Results**

*Morphometric measurements and hepatocyte density*

Multivariate analysis indicated that temperature (Pillai’s = 0.017, F\textsubscript{6,138} = 0.201, \(P = 0.976\)) and temperature x food level (Pillai’s = 0.038, F\textsubscript{6,138} = 0.441, \(P = 0.085\)) were not found to affect morphometric measurements of the fish. However, food level had a significant effect on morphometric measurements (Pillai’s = 0.456, F\textsubscript{3,68} = 19.024, \(P < 0.001\); S 5.1 for overview table). Univariate analysis confirmed that food had a significant effect on standard length (F\textsubscript{1,83} = 36.211, \(P < 0.001\), body depth (F\textsubscript{1,83} =
41.646, $P<0.001$) and body mass ($F_{1,79} = 41.388, P < 0.001$) (see overview table in supplementary S5.1 and S5.2). Tukey’s post-hoc analyses revealed that at each temperature level, well-fed fish had a significantly larger standard length (all $P < 0.020$), deeper body depth (all $P < 0.012$), and heavier body mass (all $P < 0.036$) compared with poorly-fed fish (Fig. 5.2).

A two-factor ANOVA showed that there was a significant interaction between temperature and food level on hepatocyte density, and therefore body condition (ANOVA: $F_{2,31} = 16.994, P < 0.001$). Tukey’s post-hoc tests revealed that poorly- and well-fed fish reared at 27 °C did not differ in their hepatocyte density ($P = 0.860$). However, at higher temperatures, poorly-fed fish appeared to show higher hepatocyte densities compared with well-fed fish (at 30 °C, $P = 0.011$; at 32 °C, $P < 0.001$). Post-hoc tests further revealed that well-fed fish showed similar levels of hepatocyte densities across all temperature regimes (all $P > 0.6$). In contrast, poorly-fed fish showed a significantly lower hepatocyte density at 27 °C compared with poorly fish kept at higher temperatures (at 30 °C, $P = 0.002$; at 32 °C, $P < 0.001$). However, poorly-fed fish at 30 °C and 32 °C showed similar hepatocyte densities ($P = 0.579$) (Fig. 5.3).
Figure 5.2 Mean (±SE) (a) standard length (cm), (b) body depth (cm) and (c) body mass (g) for poorly-fed and well-fed juvenile *Pomacentrus moluccensis* maintained at either 27 °C, 30 °C or 32 °C for 9 days. Numbers within bars are number of replicates, and letters represents Tukey’s HSD groupings of means among all treatments (p < 0.05)
Figure 5.3 Mean (±SE) number of hepatocytes (per micrometer$^2$) for poorly-fed or well-fed juvenile Pomacentrus moluccensis maintained at either 27 °C, 30 °C or for 9 days. Numbers within bars are the number of replicates, and letters represent Tukey’s HSD groupings of means among all treatments (p < 0.05)

**Behavioural measurements**

We found no difference in the pre-stimulus number of feeding strikes among treatment groups (food level: $F_{1,118} = 0.752$, $P = 0.388$; temperature: $F_{2,118} = 2.008$, $P = 0.139$; food x temperature: $F_{2,118} = 0.161$, $P = 0.851$). We found an interaction between food level and temperature on the proportional change in number of feeding strikes ($F_{2,97} = 5.383$, $P = 0.006$) (See S 5.3 for overview table of the results). An increase in temperature from 27 to 30 °C in the donors appeared to significantly
increase the proportional change in feeding strikes in conspecifics exposed to their alarm cues, regardless of feeding levels (poorly-fed: $P < 0.011$; well-fed: $P < 0.012$). A further increase in temperature from 30 to 32 °C led only alarm cues obtained from well-fed fish ($P < 0.991$), but not poorly-fed fish ($P < 0.027$), to trigger equivalent high values in the proportional change in feeding strikes in conspecifics. Multiple comparisons further indicated that all fish exposed to alarm cue differed significantly in the proportional change in feeding strikes with fish exposed to saltwater control (poorly-fed: all $P < 0.026$; well-fed: all $P < 0.007$) (Fig. 5.4).

**Figure 5.4** Mean (±SE) proportional change in feeding strikes for juvenile *Pomacentrus moluccensis* exposed to a saltwater control or to a chemical alarm solution obtained from poorly or well-fed juveniles maintained at either 27 °C, 30 °C or 32 °C for 9 days. Numbers above bars are the number of replicates. Minuscule and majuscule letters below bars represent Tukey’s HSD significant differences between groups among...
well-fed and poorly-fed fish, respectively. The asterisk (*) above bar of saltwater control indicate Tukey’s HSD a significant difference with all other groups.

5.5 Discussion

Previous studies have found that alarm cues from donors with low body condition due to limited food availability do not elicit an alarm reaction in conspecifics as pronounced as alarm cues obtained from well-fed donors in good condition (Brown et al. 2004a; Roh et al. 2004; McCormick and Larson 2008). Our results further underscore that the feeding history of prey influences the effectiveness of the alarm cues produced in eliciting an antipredator response in conspecifics. Moreover, the study is the first to show that the thermal environment in which an organism lives can, in isolation and in conjunction with food supply, further influences the effectiveness of the produced alarm cues in mediating antipredator responses in conspecifics.

An increasing number of studies demonstrate that temperature and food availability can interact to profoundly effect physiological condition, performance and behaviour of ectothermic organisms (Arendt and Hoang 2005; Donelson et al. 2010; McLeod et al. 2013, Lienart et al. 2014). A recent study using comparable levels of feeding and temperature treatments as the current study, indicated that the antipredator responses of juvenile reef fishes maintained at 27 °C were not affected by feeding history (Lienart et al. 2014). However, the study also found that a relatively small increase in temperature of 3 °C led poorly-fed fish, but not well-fed fish, to take greater risk under predation risk. The findings of the present study provide further support that fish with a poor feeding history may take more risk in warmer water due to their higher energy demands. While poorly-fed fish showed similar levels of depressed growth rate regardless of temperature, they showed depleted energy reserves with rising temperatures. Such poor body condition is very likely driven by a temperature-mediated increase in metabolic rate and a lack of food to fuel
associated higher energy demands (Clarke and Johnston 1999; Clarke and Fraser 2004; Hayes et al. 2014).

Studies investigating the effect of low body condition on the production of alarm cues by donor prey have often highlighted that the quality and/or quantity of these cues may be compromised by a lack of available energy (e.g. Wisenden and Smith 1997; Roh et al. 2004). Surprisingly, we found that poorly-fed fish, reared at 27 °C and 30 °C, do not appear to show a constrained production of alarm cues compared with well-fed fish. The high production of alarm cues despite a low energy budget suggests that alarm cues may not be energetically expensive to produce. There is a good possibility that alarm cues are a mere by-product or eventually end-product of a biochemical pathway involved in the production of an active agent with another primary function. In particular, it has been hypothesized that alarm cues may have originally evolved as part of an antipathogenic response in the skin and assumed secondarily the role of an alarm cue (Chivers et al. 2007a). Some authors have further suggested that club cells, which are thought to play a critical role in the immunological system of the animal, may also function as production and storage unit of alarm cues (Chivers et al. 2007a; Halbgewachs et al. 2009; Manek et al. 2014). These cells have no duct to the surface, and can only be released following mechanical damage, which do not require energetically costly cellular transport compared with a voluntary releasing mechanism (Chivers et al. 2007a). There may potentially be energetic costs associated with the sequestration process to specific compartments of the body (e.g. club cells), storage, maintenance, and breakdown (Pennings et al., 1996; Brown et al., 2001; Cronin, 2001) of alarm cues. However, animals have a whole set of biochemical machinery available to reduce some of the energetic expenditures involved in these cellular processes (Schäfer & Penefsky, 2008; Wilmer et al., 2009). For instances, many biochemicals are lipid soluble and can often with limited energetic requirements be transported via simple or facilitated diffusion through the lipophilic membranes of cells and tissues (Parsons, 2004; Willmer et al., 2009). Overall, these predictions may explain why a rising temperature up to the maximum summer temperature in the study area (30 °C) still
allowed prey to increase the qualitative and/or quantitative nature of the chemical alarm produced, despite its low energy budget. If sufficient metabolites are available, rising temperature within the thermal tolerance of an organism may increase rates that biochemical reactions occur (Clarke and Fraser 2004). This may have potentially led to a higher accumulation of the biochemicals constituting the alarm cues, thereby increasing its effectiveness in eliciting an antipredator in conspecifics.

Well-fed fish managed to maintain the production of chemical alarm cues at temperatures as high as 32 °C, which is the predicted temperature on a business-as-usual climate change scenario within the next 50 to 100 years (IPCC 2013). However, the present study found that at 32 °C poorly-fed fish showed not only particularly low energy storage but also a drop in the effectiveness of the produced alarm cues to trigger an antipredator response in conspecifics. A threshold level may have been reached, after which they could no longer afford to produce the chemicals that constitute the alarm cues. Alternatively, we speculate that the intensity of the imposed stressors was such that the production of alarm cues was affected through alteration of the immunological system of the fish, including the club cells. Several studies have shown a reduction in club cell densities for fish that were food stressed (McCormick and Larson 2008; Stabell and Vegusdal 2010). Interestingly, stress hormones such as cortisol have also been shown to depress club cell densities (Blom et al. 2000; Halbgewachs et al. 2009; Caruso et al. 2010). However, whether there is a causal link between food and thermal stressors, cortisol, immunological system, club cells, and the chemistry of alarm cues remains unclear. Unfortunately, in the present study, we have not been able to investigate the effect of food and temperature on club cell investment in coral fish juveniles because no obvious sign of club cells were found within the epidermis. Nevertheless, skin extract obtained from juvenile fish still elicited antipredator behaviour in conspecifics. Our juvenile fish may not have been sufficiently developed to allow the identification of club cells using standard haematoxylin and eosin staining techniques. Interestingly, Carreau-Green et al. (2008) reported that adult minnows displayed an antipredator response to skin extracts from larval
minnows that have not yet developed club cells. This suggests that alarm cues may not just be produced in the club cells but elsewhere in the skin or the body. A better knowledge of the role of the immunological system and in particular club cell in the production of alarm cues may provide a better mechanistic understanding of how environmental stressors may influence the chemistry of alarm cues.

The lack of a comprehensive understanding of the biochemical mechanisms behind the processes involved in the production of chemical alarm cues makes interpretation of patterns observed during this study challenging. Some authors have highlighted for several species, including coral reef fishes, that prey have a stronger intensity of response to alarm cues from individuals of similar size (Mirza & Chivers, 2002; Lönnstedt and McCormick 2011a; Mitchell and McCormick 2013). However, not only the size of the donor, but also the physiological condition of the prey can influence the quality and/or quantity of the alarm cues produced (Brown et al. 2004a; Roh et al. 2004; McCormick and Larson 2008). Since all the signal-receivers in the present study had near-identical body sizes and feeding histories, it is reasonable to suggest that the observed variability in the response to the alarm cues between experimental treatments were predominantly mediated by differences in physical condition among donor fish. Nevertheless, because both body size and condition of donor were affected by temperature and food availability, the present study cannot determine the relative importance of previously mentioned factors on cue production. Furthermore, alteration of the quality and/or quantity of the produced alarm cues may have happened at different parts of the production sequence: during the biosynthesis of alarm cues, during the sequestering of the alarm cues into the epidermis, or possibly during storage of the alarm cues in the epidermis. Regardless of the mechanism by which temperature and food availability impact the amount and/or quality of alarm cues sequestered by a fish, the ecologically-relevant temperature and food scenarios used in the present experiment clearly influence the production of alarm cues released upon physical damage. Similar environmentally driven changes in alarm cue production by donor prey in the wild can be expected to dramatically alter trophic dynamics and
ecosystem functioning due to the fundamental role these alarm cues play in predator-prey interactions amongst aquatic organisms. This study highlights the need for a better understanding of the chemical nature of alarm cues and associated biochemical pathways, and how environmental factors may influence their production, quality and storage.
Chapter 6: General discussion

Variability in thermal regimes and food supply has the potential to dramatically shape the strength of predator-prey interactions within aquatic ecosystems. This prediction mainly relies on the fact that the vast majority of species living in aquatic realms are ectotherms (Willmer et al., 2000; Sokolova & Lannig, 2008). Since an ectotherm’s metabolic rate is greatly affected by its thermal environment, any change in temperature will influence its energy acquisition and expenditure, thus modifying nutritional needs (Clarke & Johnston, 1999). As a consequence, temperature and food availability can be expected to have profound consequences for trophic dynamics between predator and prey animals. While the physiological effects of temperature and food availability have been extensively investigated (e.g. Johnston & Dunn, 1987; McLeod et al., 2013; Clarke & Fraser, 2004; Courtney Jones et al., 2015), few studies have looked at how these key drivers act together to affect population and community processes, such as the dynamics between predators and their prey. In the present dissertation, I addressed specific aspects of this knowledge gap by characterizing, in a series of laboratory experiments, the effects of food availability and temperature on the propensity of juvenile coral reef fishes to take risk when facing predation threat. The outcome of this research highlight that there is a variety of ways by which temperature and historic food availability can affect the trade-off between avoiding the threat of predation and other fitness-enhancing activities such as foraging. These environmentally-induced shifts in behavioural patterns can often be explained by the animal’s physical state and the individual’s balancing their current and future energetic needs.

6.1 Food supply, temperature and starvation avoidance

Theoretical and empirical studies have commonly stressed the importance of a prey’s feeding history as a driving component that should affect risk-taking behaviour, with hungry animals decreasing their antipredator
behaviour to avoid starvation (Lima & Dill, 1990; Houston et al., 1993; McCormick & Larson, 2008; Krause et al., 2011). Surprisingly, while increasing temperature can also impose a dramatic energetic cost for ectotherms (Gillooly et al., 2011), no attempt has been made to test whether this variable can, in conjunction with food availability, further shape behavioural decision of prey when facing predation threat. I addressed this research question in Chapter 2. Interestingly, my data indicates that temperature in isolation does not appear to influence cost-benefit trade-offs between predator avoidance and foraging in well-fed fish. Fish with a good feeding history reared at 27 °C showed similar high intensities in their antipredator response as those reared under regional average maximum summer temperature (30 °C). Comparable results were found for a higher latitude species, whereby fish reared at either 3 or 8 °C responded with similar magnitude to predation threat, regardless of temperature (Killen et al., 2011). Nevertheless, these findings contrast with a very recent study, which reported for a temperate fish species maintained at either 15 or 23 °C that a temperature increase within the thermal tolerance of the animal reduced the impact of a predation risk on foraging behaviour (Pink & Abrahams, 2015). While many factors may have led to such differences among studies, it is very likely that the nutritional condition of the experimental animal may have played a major role in determining the extent to which temperature affected risk-taking behaviour.

As such, to the best of my knowledge, the study described in Chapter 2, is one of the first to experimentally demonstrate that prey animals with a poor nutritional condition due to limited food availability take more risks under a predation threat with increasing temperature. Although I did not find differences in body size among treatments, there were clear differences in behavioural decisions, which may indicate that observed patterns were mainly driven by intrinsic starvation-avoidance mechanisms. Interestingly, the results shows that poorly fed fish at 27 °C still display a detectable antipredator response to predation threat. However, fish kept in 3 °C warmer water, with low food rations, fed at a high rate even under the threat of predation. This indicates that the requirement for food is greater
at higher temperatures, so poorly fed fish should be willing to incur greater costs to obtain it. Interestingly, in Chapter 5, I found (using similar levels of food and temperature to Chapter 2) that poorly fed fish, not well fed fish, displayed decreased energy reserves with rising temperatures. These findings support the conclusion that the risk-prone behaviour observed in poorly-fed fish treatment maintained at 30 °C is very likely to be caused by a temperature-mediated increase in metabolic rate and a lack of food to fuel the higher energy demands (Clarke and Johnston, 1999; Clarke & Fraser, 2004; Hayes et al. 2014). These findings may have important implications in our understanding of other biological processes. It would be of particular ecological relevance to further investigate whether intra- and interspecific competition for food resources can further affect an individual’s thermal sensitivity and associated risk-taking behaviour.

6.2 Food supply and complementarity of predator-related information

High physiological demands can lead prey to prioritize foraging behaviour at the expense of increased exposure to predation threat (Smith, 1981; Lönnstedt & McCormick, 2011b) (Chapter 2). However, it can be expected that the extent to which such risk-taking behaviour may happen will be counteracted by the likelihood of being preyed upon (Helfman, 1989; Vavrek, 2009). As such, following the complementarity hypothesis, prey that receive information concerning a predation threat from more than one sensory mode will have more certainty on the level of immediate risk posed by the threat, and will be able to make a more informed decision (Smith & Belk, 2001; Mikheeve et al., 2006). My experiment in Chapter 3 was the first study to directly test whether the complementarity of sensory information to inform risk was affected by an animal’s energetic requirement. Here, prey were reared under different levels of food and then exposed to either odour, sight or combined cues of a known predator. Surprisingly, poorly fed fish, despite low nutritional condition, significantly decreased their foraging response when exposed to a single predator cue and further enhanced the intensity of the antipredator response when
exposed to additional sensory information. I found very similar behavioural results for moderately fed fish. However, these behavioural patterns strongly contrasted with well-fed fish, which had a well-developed antipredator response to any predation threat, regardless of the sensory source of information or number of senses that informed the threat. Although these behavioural patterns are not completely in line with the initial prediction, these results strongly suggest that the extent to which additional sensory information on predation can lead to a further enhancement of the response will be dependent of the prey’s feeding history.

Studies on related topics have often interpreted such state-dependent risk-taking behaviour from a starvation-avoidance perspective, whereby individuals with low energy reserves are expected to take more risks to avoid starving to death (e.g. Giaquinto & Volpato, 2001; Smith et al., 2001; Fraker, 2008). However, results in Chapter 3 highlight that despite the higher level of vigilance in well-fed fish, there was no difference in liver cell densities, suggesting energy reserves were similar. Body size did differ however, and well-fed fish were significantly larger than fish from other treatments; well-fed fish have obviously allocated much of their excess energy to growth. This implies that subtle differences in body size may also play a role in influencing risk-taking behaviours. Individuals with a larger body size generally have a lower basal metabolism relative to their body size and higher storage capacity, which may partly explain cautious behaviour in well-fed fish (Kleiber, 1932). In addition, larger individuals may have accumulated more fitness-associated assets, progressively leading to a reduction of the gains obtained from a unit of food relative to the chance of being preyed upon (Clark, 1994; Reinhardt & Healey, 1999; Reinhardt & Healey, 1999). While body size and level of energy reserves can play key role in determining risk-taking behaviour, other studies have indicated that prey also have the ability to integrate a whole range of body and life-history traits, including metabolic rate, reproductive stage, immune condition, age, morphology and habitat shifts (Grand, 1999, Kortet et al., 2010; Sih et al., 2015; Katwaroo-Anderson, in press). However, the existing literature reflects, and also
underscores, a lack of comprehensive understanding of the relative importance of different intrinsic body traits in mediating antipredator behaviour of prey to risk of predation. This highlights the need for more in-depth studies on the dynamics of foraging rewards and their relationship to fitness in the light of predation risk.

**6.3 Size and context-specific adjustment of antipredator response**

While many studies are based on the prediction that high-asset individuals are more cautious (Chapter 2, 3), other authors have stressed that higher assets in the form of higher condition can increase the propensity to take risk (Johnsson, 1993; Luttbeg & Sih, 2010; Arendt & Hoang, 2005). In particular, it may be expected that as prey grow, it will reduce its level of vigilance to gape-limited predators (Nilsson et al., 1995; Urban, 2007). Such type of state-dependent safety behaviour may allow animals to maintain high foraging rates and thus continue to garner energy to maintain their high condition (Luttbeg & Sih, 2010; Sih et al., 2015). While several studies have indeed shown that an increase in prey body size can lower mortality to gape-size limited predators (Schmitt & Holbrook, 1984; Holmes & McCormick, 2010), very few studies have investigated how prey may adjust their response in accordance to such a context-specific change in vulnerability (but see Ferrari et al., 2012a; Brown et al., 2011). Ferrari et al. (2010c) predicted in their theoretical model, that acquired information on predation threat should be outdated and lose its relevance more quickly in prey under high growth rate because they outgrow their predators sooner than slow growing individuals (Ferrari et al., 2010c). This prediction implies that environmental variability affecting growth may affect temporal patterns in behavioural responses to predation risk (Ferrari et al., 2011a; Ferrari et al., 2012a). In particular, it may be predicted that a temperature-mediated increase in growth rate could be correlated with an increased devaluation rate of the informational value of a learned predator cue.

In Chapter 4, I tested these predications by rearing predator-conditioned prey fish under different temperature-mediated growth trajectories (27 °C vs. 30 °C) for 14 days and assessed weekly whether
prey still responded to the initially acquired predator information. The results from Chapter 4 provide evidence that with time, prey gradually decrease the intensity of their response to a learned predator cue. These findings, along with a few other studies, suggest the existence of an adaptive mechanism causing a decline of the informational value of a learned predator cue over time (e.g. Mirza & Chivers, 2000; Gonzalo et al., 2009). This mechanism should give prey the ability to not persevere with maladaptive behaviour towards outdated or irrelevant information (Kraemer & Golding, 1997; Ferrari & Chivers, 2013). However, while I found that all fish appeared to show a decline in antipredator response over a 14 days period, there were no significant differences in the decline of the response among the different growth trajectories. This contrast with a similar study on tadpoles (Ferrari et al., 2011a; Ferrari et al., 2012a), for which a temperature-mediated increases in growth did lead to higher devaluation rates in the intensity of the antipredator response. It may be that the temperature-mediated growth trajectories in the current study were too subtle to find detectable differences in behavioural patterns. Future studies may want to use a larger temperature range or manipulate the feeding history of the individuals in order to obtain more dramatic changes in growth.

6.4 Temperature, food supply and efficacy of donor alarm cues

In chapter 2, 3 and 4, I mainly emphasised the different pathways to which temperature and food availability can influence a prey’s body state and associated changes in the perception of predation risk. However, temperature and food availability can also be expected to influence a prey’s behavioural decisions through alteration of the quality and/or quantity of the available predation-related stimuli to which it will be exposed. Studies have indicated that low food availability can compromise the energy budget of prey, thereby restricting the efficacy of the produced alarm cue in eliciting antipredator responses in conspecifics (Brown et al., 2004a; Roh et al., 2005; McCormick & Larson, 2008). However, to date, no studies have tested how temperature can further influence the
properties of the produced alarm cue. Because temperature, in conjunction with nutritional variability, can have profound effects on the energy budget and growth pattern in fish (McLeod et al., 2013; Courtney Jones et al., 2015), it can be expected to find concurrent alteration in the properties of the produced alarm cue. I tested these predictions as part of my Chapter 5 by rearing fish under different food-by-temperature treatments, and then assessed the fish physical state and effectiveness of the produced alarm cues in eliciting an antipredator response in conspecifics.

Surprisingly, the results presented in Chapter 5 indicate that an increase in temperature from 27 °C up to 30 °C correlated with a higher efficacy of the produced alarm cues by donor fish to elicit antipredator responses in conspecifics, regardless of the feeding history or body condition of the donor. Such high efficacy of the produced alarm cue despite a low energy budget suggests that alarm cues may not be energetically expensive to produce. There is a good possibility that alarm cues comprise an integral part of a biochemical pathway involved in the production of an active agent with another primary function. Several authors have argued that the role of alarm cues is likely secondary to their role in the immune system (Chivers et al., 2007a). Interestingly, a further rise of the temperature up to 32 °C led poorly-fed fish, not well-fed fish, to show not only particularly low energy storage but also a drop in the effectiveness of the alarm cues produced to trigger an antipredator response in conspecifics. While the chemicals that constitute the alarm cues may have been directly affected, it may also be speculated that the endured physiological stress reached a threshold whereby the production was affected through alteration of the immunological system of the fish. The difficulty in interpreting the obtained patterns highlights the urgent need for a better understanding of the biochemical mechanisms behind the production of alarm cues. Nevertheless, the results clearly show that temperature and food availability can impact the amount and/or quality of alarm cues sequestered by a fish, which further shapes the trade-offs between predator avoidance and foraging behaviour in conspecifics exposed to these cues.
6.5 Concluding remarks

This thesis demonstrates that a temperature shift across a narrow thermal gradient near the maximum regional summer temperature can dramatically affect body state and behavioural responses of juvenile coral reef fishes. Such high vulnerability to small changes in temperature regimes further support the prediction that juvenile coral reef fishes, like many other tropical ectotherms, live close to their upper thermal limits (Tewksbury et al., 2008; Huey et al., 2009; Rummer et al., 2014). However, the lack of thermal performance reaction-norms for any juvenile coral reef fishes, and most other ectotherms, complicates making general predictions on how particular temperature changes may affect an animal’s performance. Recent studies addressing certain aspects of this knowledge gap have reported a remarkable level of intraspecific variability in the shape of thermal performance norms (e.g. Gardiner et al., 2010; Dowd et al., 2015). As such, there is increasing evidence that the thermal optimum of an individual will not only be shaped by genotypic traits or by the environmental inputs during an individual lifetime, but will also be phenotypically mediated through the thermal regime endured by the parents (e.g. Donelson et al., 2012; Shama et al., 2014; Veilleux et al., 2015). In addition, temperature is likely to have synergetic effects with other stressors, exacerbating the effects of temperature (e.g. Sokolova & Lannig, 2008). Indeed, the results of this thesis indicate that the feeding history of an individual can dramatically affect the shape of its thermal performance curve. Interestingly, although such profound interaction between temperature and food availability may seem obvious, only a relatively small body of experimental research have address their co-occurring effects on functional responses. Another important point that awaits future study is that not all fitness measures may respond in a similar way to a specific stressor. For instance, throughout this dissertation, I found that number of foraging strikes was highly sensitive to the manipulated environmental conditions, but overall activity was not significantly affected.

Examining plasticity in performance of animals across a range of
environmental conditions is not only critical for a better understanding of function at the organismal level, but also of more complex biological processes, such as trophic interactions (Englund et al., 2011; Gilbert et al., 2014; Grigaltchik et al., 2012; Terborgh & Estes, 2010). The current body of work provides empirical evidence that temperature, food availability and risk of predation can in isolation but also concurrently lead to dramatic shifts in trade-off between obtaining energy and nutrients for individual production and avoiding being a resource for other consumers. Interestingly, there were a variety of pathways to which our environmental parameters affected these trade-off, which appeared to be driven by various underlying state-dependent and threat-sensitive mechanisms. While the current study quantified the isolated effects of these pathways, it is obvious that in the wild, such pathways will occur simultaneously to further lead to counteracting or enhancing effects on a particular behavioural pattern. The resulting behavioural response of prey can be expected to have profound top-down effects, mediated by changes in the quantity of ingested food; but also bottom-up effects through changes in exposure to predators while foraging (Abrams, 1984; Matassa & Trussel, 2015). Ultimately, it can be predicted that this process can have cascading impacts on trophic dynamics, food-web stability and energy flow with far-reaching effects up to the ecosystem level (Abrahams et al., 2007; Duffy et al., 2007; Schmitz et al, 2008). Obviously, understanding how biological communities and entire ecosystems will respond, in today’s context and in the light of projected global change, will necessitate considerably more studies. Nevertheless, the current body of work demonstrates, through a series of laboratory experiments, that it is not unreasonable to assume that small ecologically relevant changes in food supply and temperature can independently but also interactively affect the strength of predator-prey interaction within tropical aquatic ecosystems.


336.


morphological and behavioural defences: Prey fish detect the morphology of conspecifics in the odour signature of their predators. *Ethology, 113*(8), 733-739.


293, 2248-2251.


of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture, 283*(1-4), 36-42.


Lönnstedt, O. M., Munday, P. L., McCormick, M. I., Ferrari, M. C. O., &


McCormick, M. I., & Weaver, C. J. (2012). It pays to be pushy: Intracohort interference competition between two reef fishes. PLoS One, 7(8), e42590


Smith, M. E., & Belk, M. C. (2001). Risk assessment in western mosquitofish (Gambusia affinis): do multiple cues have additive


Stabell, O. B., & Vegusdal, A. (2010). Socializing makes thick-skinned individuals: on the density of epidermal alarm substance cells in cyprinid fish, the crucian carp (Carassius carassius). Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology, 196(9), 639-647.


### S 3.1 MANOVA results (Pillai’s Trace) with dependent variables feeding strikes and line crosses of *Pomacentrus moluccensis* and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Value</th>
<th>F</th>
<th>df</th>
<th>Error df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>0.06</td>
<td>3.209</td>
<td>4</td>
<td>414</td>
<td>0.013</td>
</tr>
<tr>
<td>Visual cue</td>
<td>0.377</td>
<td>62.306b</td>
<td>2</td>
<td>206</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Chemical cue</td>
<td>0.177</td>
<td>22.091b</td>
<td>2</td>
<td>206</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Food * Visual cue</td>
<td>0.012</td>
<td>0.617</td>
<td>4</td>
<td>414</td>
<td>0.651</td>
</tr>
<tr>
<td>Food * Chemical cue</td>
<td>0.01</td>
<td>0.507</td>
<td>4</td>
<td>414</td>
<td>0.73</td>
</tr>
<tr>
<td>Visual cue * Chemical cue</td>
<td>0.027</td>
<td>2.856b</td>
<td>2</td>
<td>206</td>
<td>0.06</td>
</tr>
<tr>
<td>Food * Visual cue * Chemical cue</td>
<td>0.041</td>
<td>2.187</td>
<td>4</td>
<td>414</td>
<td>0.07</td>
</tr>
</tbody>
</table>

### S 3.2. ANOVA results across all food levels for dependent variables feeding strikes and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in feeding</td>
<td>Visual Cue</td>
<td>1</td>
<td>4.283</td>
<td>119.573</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>feeding strikes</td>
<td>Chemical Cue</td>
<td>1</td>
<td>1.496</td>
<td>41.765</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Food</td>
<td>2</td>
<td>0.089</td>
<td>2.494</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>Visual Cue * Chemical Cue</td>
<td>1</td>
<td>0.2</td>
<td>5.589</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Visual Cue * Food</td>
<td>2</td>
<td>0.031</td>
<td>0.852</td>
<td>0.428</td>
</tr>
<tr>
<td></td>
<td>Chemical Cue * Food</td>
<td>2</td>
<td>0.036</td>
<td>1.009</td>
<td>0.366</td>
</tr>
<tr>
<td></td>
<td>Visual Cue * Chemical Cue * Food</td>
<td>2</td>
<td>0.145</td>
<td>4.056</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>207</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### S 3.3 ANOVA results at each food level for the dependent variable feeding strikes of *Pomacentrus moluccensis* and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food)

<table>
<thead>
<tr>
<th>Food level</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly fed</td>
<td>Visual cue</td>
<td>1</td>
<td>1.888</td>
<td>64.935</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Chemical cue</td>
<td>1</td>
<td>0.903</td>
<td>31.067</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Visual cue * Chemical cue</td>
<td>1</td>
<td>0.004</td>
<td>0.131</td>
<td>0.719</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>75</td>
<td>0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately fed</td>
<td>Visual cue</td>
<td>1</td>
<td>0.905</td>
<td>78.628</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Chemical cue</td>
<td>1</td>
<td>0.399</td>
<td>34.709</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Visual cue * Chemical cue</td>
<td>1</td>
<td>0.024</td>
<td>2.088</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>63</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-fed</td>
<td>Visual cue</td>
<td>1</td>
<td>1.637</td>
<td>25.045</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Chemical cue</td>
<td>1</td>
<td>0.303</td>
<td>4.635</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>Visual cue * Chemical cue</td>
<td>1</td>
<td>0.454</td>
<td>6.951</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>69</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
S 3.4 ANOVA results across all food levels for dependent variables line crosses and factors chemical predator cues, visual predator cues and food (low versus moderate versus high food)

<table>
<thead>
<tr>
<th>Trait in line crosses</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in line crosses</td>
<td>Visual Cue</td>
<td>1</td>
<td>1.639</td>
<td>16.801</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Chemical Cue</td>
<td>1</td>
<td>0.49</td>
<td>5.024</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Food</td>
<td>2</td>
<td>0.286</td>
<td>2.93</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>Visual Cue * Chemical Cue</td>
<td>1</td>
<td>0.261</td>
<td>2.677</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>Visual Cue * Food</td>
<td>2</td>
<td>0.034</td>
<td>0.353</td>
<td>0.703</td>
</tr>
<tr>
<td></td>
<td>Chemical Cue * Food</td>
<td>2</td>
<td>0.04</td>
<td>0.408</td>
<td>0.665</td>
</tr>
<tr>
<td></td>
<td>Visual Cue * Chemical Cue * Food</td>
<td>2</td>
<td>0.072</td>
<td>0.734</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>207</td>
<td>0.098</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S 4.1 Overview table of number of replicates used during behavioural assessment of *Pomacentrus coelestis*

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Tank</th>
<th>1 day post-conditioning</th>
<th>7 days post-conditioning</th>
<th>14 days post-conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Testing date</td>
<td>SWa</td>
<td>POa</td>
</tr>
<tr>
<td>27°C</td>
<td>A</td>
<td>16/01/14</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>17/01/14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>18/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>19/01/14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sum (n)</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>30°C</td>
<td>E</td>
<td>16/01/14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>17/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>18/01/14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>19/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sum (n)</td>
<td>36</td>
<td>36</td>
</tr>
</tbody>
</table>

*Saltwater; *Predator odour

S 4.2 Overview table of number of replicates used during morphometric assessment of *Pomacentrus coelestis*.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Tank</th>
<th>1 day post-conditioning</th>
<th>7 days post-conditioning</th>
<th>14 days post-conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Testing date</td>
<td>SLa</td>
<td>BDa</td>
</tr>
<tr>
<td>27°C</td>
<td>A</td>
<td>16/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>17/01/14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>18/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>19/01/14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sum (n)</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>30°C</td>
<td>E</td>
<td>16/01/14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>17/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>18/01/14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>19/01/14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sum (n)</td>
<td>36</td>
<td>36</td>
</tr>
</tbody>
</table>

*Standard length; *Body depth

S 5.1 Overall MANOVA results (Pillai’s Trace) with dependent variables standard length, body depth and body mass of *Pomacentrus moluccensis* and as independent factors Food (low versus high) and Temperature (27°C, 30°C or 32°C)
### S 5.2 Univariate results for dependent variables standard length, body depth and body mass of *Pomacentrus moluccensis* with as independent factors food (low versus high) and temperature (27 °C, 30 °C or 32 °C)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Value</th>
<th>$F$</th>
<th>df</th>
<th>Error df</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>0.456</td>
<td>19.024</td>
<td>3</td>
<td>68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.017</td>
<td>0.201</td>
<td>6</td>
<td>138</td>
<td>0.976</td>
</tr>
<tr>
<td>Food * Temperature</td>
<td>0.038</td>
<td>0.441</td>
<td>6</td>
<td>138</td>
<td>0.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard length</td>
<td>Food</td>
<td>1</td>
<td>0.361</td>
<td>36.211</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>2</td>
<td>0.001</td>
<td>0.086</td>
<td>0.917</td>
</tr>
<tr>
<td></td>
<td>Food * Temperature</td>
<td>2</td>
<td>0.001</td>
<td>0.137</td>
<td>0.872</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>83</td>
<td>0.010</td>
<td>0.000</td>
<td>0.976</td>
</tr>
<tr>
<td>Body depth</td>
<td>Food</td>
<td>1</td>
<td>0.118</td>
<td>41.646</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>2</td>
<td>0.000</td>
<td>0.161</td>
<td>0.852</td>
</tr>
<tr>
<td></td>
<td>Food * Temperature</td>
<td>2</td>
<td>0.000</td>
<td>0.008</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>83</td>
<td>0.003</td>
<td>0.000</td>
<td>0.946</td>
</tr>
<tr>
<td>Body mass</td>
<td>Food</td>
<td>1</td>
<td>0.037</td>
<td>41.388</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>2</td>
<td>0.000</td>
<td>0.055</td>
<td>0.946</td>
</tr>
<tr>
<td></td>
<td>Food * Temperature</td>
<td>2</td>
<td>0.000</td>
<td>0.178</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>79</td>
<td>0.001</td>
<td>0.000</td>
<td>0.992</td>
</tr>
</tbody>
</table>

### S 5.3 Univariate results for dependent variables pre-stimulus feeding strikes and proportional change in feeding strikes of *Pomacentrus moluccensis* with as independent factors Food (low versus high) and Temperature (27 °C, 30 °C or 32 °C)

<table>
<thead>
<tr>
<th>Feeding strikes</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-stimulus</td>
<td>Food</td>
<td>1</td>
<td>4025.627</td>
<td>0.752</td>
<td>0.388</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>2</td>
<td>10742.232</td>
<td>2.008</td>
<td>0.139</td>
</tr>
<tr>
<td></td>
<td>Food * Temperature</td>
<td>2</td>
<td>861.661</td>
<td>0.161</td>
<td>0.851</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>118</td>
<td>5350.884</td>
<td>0.014</td>
<td>0.992</td>
</tr>
<tr>
<td>Proportional change</td>
<td>Food</td>
<td>1</td>
<td>0.109</td>
<td>7.708</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>2</td>
<td>0.117</td>
<td>8.307</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Food * Temperature</td>
<td>2</td>
<td>0.076</td>
<td>5.383</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>97</td>
<td>0.014</td>
<td>0.000</td>
<td>0.992</td>
</tr>
</tbody>
</table>