Warren, Donald Thomas James (2017) ***Influence of elevated temperature on juvenile damselfish performance and their scope for adjustment through developmental plasticity*****. PhD thesis, James Cook University.**

Influence of elevated temperature on juvenile damselfish performance and their scope for adjustment through developmental plasticity

Thesis submitted by:

Donald Thomas James Warren

BSc (Hons) in Biology

The University of Iowa, Iowa, USA

In March 2017

For the degree of **Doctor of Philosophy**

in the College of Science and Engineering

and Centre for Excellence for Coral Reef Studies

James Cook University

Statement of contribution of others

This thesis included collaborative work with my supervisors, Professor Mark I. McCormick, Professor Philip L. Munday, and Dr Jennifer M. Donelson as well with Dr Maud Ferrari. 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, financial support, or statistical support.

Financial support was provided by the Australian Research Council (McCormick, MI), the ARC Centre of Excellence for Coral Reef Studies (McCormick MI & Munday PL), Ian Potter Foundation (Donelson JM), University Technology, Sydney (Donelson JM) and James Cook University Graduate Research School Funding Scheme (Warren DT).

Declaration of ethics

The research presented in this thesis was conducted in accordance with the JCU Animal Ethics Committee (A2079, A2080). Collecting permits for study species were acquired by the Great Barrier Reef Marine Park Authority (G10/33239.1, G12/35117.1) and Queensland fisheries (170251).

Acknowledgements

My first thanks must be given to my supervisory panel, **Prof Mark I. McCormick, Prof Philip L. Munday,** and **Dr Jennifer M. Donelson** for graciously imparting their wisdom, support, direction, and encouragement. Mark, thank you for all your help supporting my field studies and providing your input and lab to achieve this success. Phil, your feedback on writing has been both the most extensive and constructive advice I have received. Your ability to articulate my thoughts before I do is greatly appreciated. Jenni, I would have never accomplished the amount and quality of data collected without your guidance at MARFU. This thesis would not have been made possible without any of your contributions.

Next, I must thank the support of my family. I would not have been able to leave my US home to begin this endeavour if I had not first received permission from my mother, Janet. Her selfless devotion to ensuring I achieve all that I may dare attempt gives me motivation to strive for more. I thank my father, Don, for being the role model I yearn to be and regret he was not able to live to also share in this accomplishment. I know he would be proud. I thank my siblings, Louise, Jack, Charlie, and Olivia for looking up to me with leaving to take on this challenge. I wish to remain the positive role they describe to me.

I thank my officemate, Blake Spady, and close friend, Tory Chase, for making this degree more enjoyable. Blake, our time in the office has created some of my fondest and funniest memories throughout this degree. I am grateful for your input in experimental building ideas and feedback on research questions and writing. Tory, you have been my help both off campus on hiking and weekend trips to recalibrate myself and on campus of your time as postgraduate representative to untangle the complex

inner workings of JCU. I thank my housemates, Ryan Ramasamy and Lex Landry, to have shared such a wonderful living experience with each of you. Thanks to my lab mates, Maria Palacios and Bridie Allan, for making the field season an unforgettable time enriched with laughter.

I thank the technicians at MARFU: Simon Wever, Ben Lawes, and Andrew Thompson who collectively keep this facility a thriving hub for supporting research students, such as myself. I thank Matt Glue who was a fantastic volunteer during my field studies. I also thank my fellow research students of the McCormick and Munday lab for their help in field studies at Lizard Island Research Station as well as at MARFU.

I finally thank Emily Shaw for her support in my romantic life. I am happy to have found someone as great as you to share such an amazing romantic experience with. I hope to continue to share more wonderful times together and look forward to the adventures we will take on in the future.

General Abstract

Understanding how individuals and populations respond to higher temperatures is crucial for making predictions about the vulnerability of communities to global climate change. Studies within a climate-change context commonly expose adult animals to acutely elevated temperatures, but seldom account for the inherent capacity of individual and populations to change on longer time scales. Tropical ectotherms, like those inhabiting coral reefs, are expected to be particularly sensitive to thermal change as they have evolved in relatively stable thermal environments. However, recent evidence suggests that coral reef fishes are physiologically plastic if exposure to thermal change occurs during early life stages. Yet, it is unknown whether this thermal plasticity extends to individual performance in ecological processes and whether the link between physiological and ecological performance is temperature sensitive. This study aimed to determine the effects of projected future temperatures from climate change on the competitive performance, antipredator escape performance, and physiology of juvenile coral reef damselfishes, and to explore the extent of thermal plasticity individuals have for these processes.

To determine the ability of species to cope with long-term elevations in average temperature, a baseline of how individuals respond to elevated temperature in the short-term is required. This measure can be thought of as the thermal sensitivity of current-day populations for rising temperature. To address this aim, newly settling juveniles of *Pomacentrus nagasakiensis* and *P. chrysurus* were collected using light traps at Lizard Island, northern Great Barrier Reef. Juveniles were then randomly allocated to one of four temperature treatments for seven days (**Chapter 2**). Size-matched intraspecific pairs were then placed into competitive arenas where aggressive interactions for a coral shelter were video recorded. There was little effect of temperature on aggressive

interactions for *P. chrysurus*, whereas *P. nagasakiensis* displayed substantial increases in aggression levels with temperature. Furthermore, temperature changed the dynamics of contests of *P. nagasakiensis*, leading to fiercer contests. The differences in thermal sensitivity between species suggests reef assemblages may be restructured under warmer conditions.

The next aim was to determine the extent of thermal plasticity for competitive interactions through longer-term exposure to rising temperatures. This chapter used juveniles of *Pomacentrus amboinensis* and *P. moluccensis* collected from the reefs near the Cairns region, Australia. Individuals were divided into ambient temperature for the collection season (29 °C) and two elevated temperature treatments (30 and 31 °C) for either 4d or 90d exposure periods. **Chapter 3** followed methods similar to chapter 2 to record competitive performance, however with the added comparison of performance between the 4d and 90d exposure periods to test for thermal plasticity. Both intra- and inter-specific competitive contests were used to link differences in species thermal performance with interspecific competitive dominance. When comparing the control with 4d exposure to elevated temperatures, there were opposing responses by species, with *P. moluccensis* significantly increasing aggressive interactions whereas *P. amboinensis* exhibited a decreasing trend in aggression at higher temperatures. After 90d, *P. moluccensis* showed signs of beneficial plasticity as aggression trended back to control levels while *P. amboinensis* showed a further decrease in aggression. Interspecific contests showed a trend for *P. moluccensis* to win more contests in elevated temperatures. These results indicate that differences in species thermal performance may impact dominance hierarchies in fish assemblages.

Chapter 4 used a similar experimental design of elevated temperature and extended exposure to explore the extent of thermal plasticity in antipredator escape performance for *P. amboinensis* and *P. moluccensis*. Many fish undertake a C-start escape response when faced with the threat of predation. Recordings from a high-speed camera show temperature effected behavioural decision-making more than physical ability to escape. Higher temperature altered the directionality of escape responses with both species exhibiting more reactions towards the stimulus. Only *P. moluccensis* showed a beneficial response after 90d, with directionality of response returning to control levels. There was a slight increase in responsiveness to the stimulus at elevated temperatures, suggesting a decrease in the stimulus threshold to trigger a C-start. However, this was reversed after 90d as both species showed significant decreases in responsiveness and even overcompensated as levels fell below control.

Finally, I tested the plasticity of physiology through varying exposure durations to elevated temperature and explored the link between physiological and competitive performance (**Chapter 5**). Previous work has attempted to correlate metabolic rates with aggression and competitive dominance to better understand the ecological relevance of physiological performance; however, there is uncertainty about how these traits relate to one another. Individual oxygen consumption was recorded via closed chamber respirometry as a measure of metabolic rate and then paired with competitive data from chapter 3. Maximum metabolic rate ($MO_{2\ \mathrm{Max}}$), routine metabolic rate ($MO_{2\ \mathrm{Routine}}$), and aerobic scope ($MO_{2\ \mathrm{Max}} - MO_{2\ \mathrm{Routine}}$; AS) significantly increased with short-term exposure to elevated temperature. After 90d, all three metabolic traits for *P. moluccensis* had virtually returned to control levels, indicating plasticity fully compensated the effects of temperature while *P. amboinensis* only showed partial plasticity. Metabolic performance was then matched with competitive data from chapter 3 and individuals were categorised as dominant or subordinate. There was no association of individuals with greater metabolic rates becoming dominant necessarily.

However, individuals more thermally sensitive with greater changes in metabolism tended to become subordinate in contests. With extended exposure, individuals that displayed greater signs of plasticity by returning to control metabolic rates were more likely to become dominant.

This research showed projected elevated temperature will alter competitive ability, antipredator escape responses, and metabolic rates for several species of damselfish. After prolonged exposure to temperature, there were signs of beneficial plasticity of some traits for certain species. The extent of the effects and plasticity observed varied species to species. Such species-specific responses will have implications at the community level, potentially restructuring dominance hierarchies within and between species, and altering species assemblages.

Table of Contents

List of tables

Table 2.1 Experimental design matrix. Control (grey) fish were compared to individuals with 40 exposure (solid) to elevated temperatures. Fish with 4d (solid) exposure were compared to 90d (open) exposure at each elevated temperature.

Table 5.1 Planned comparisons of metabolic rates for temperature and exposure durations. There were six comparisons for each metabolic trait (MO2$_{\text{Routine}}$, MO2$_{\text{Max}}$, and AS) and replicated for each species, *Pomacentrus amboinensis* (*a*) and *P. moluccensis* (*b*). The first four comparisons measured control treatments against both temperatures at both exposure durations and the last two compared 4d and 90d exposure durations for each elevated temperature separately.

List of Figures

Fig 2.1. Seasonal and diurnal temperature fluctuation at Lizard Island, northern Great Barrier Reef. Seasonal fluctuation (23-30.6° C) from daily means recorded by long-term temperature loggers Dec 2013-2014. Inset shows the daily fluctuation (27.1-30.4° C) for the collection season, recorded every 20 min.

Fig 2.2. Diagram of competition arena. Size-matched pairs were placed into habituation chambers at the beginning of a trial for 5 min. Next, revolving doors were opened simultaneously, allowing fish to exit and explore their portion of the arena. Once both fish had emerged, video recording commenced from an above camera and the clear removable partition was lifted, exposing fish to the coral shelter and each other. A 5-min recording was taken starting from the first interaction. Videos were later analysed and 3 interactions were quantified: (i) displays, defined as a lateral fin flare towards the opponent; (ii) attacks, defined as a chase or biting of the opponent; and (iii) avoidances, swimming away from an opposing attack or display.

Fig 2.3. Influence of temperature on aggression scores between dominant and subordinate fish. Mean aggression score difference (± SE), taken as aggression score of dominant competitor minus the subordinate score, for intraspecific contests at each of four temperatures. Only *P. nagasakiensis* (solid) showed an effect of temperature with aggression lowest at ambient and increasing in either direction. Italicised letters above error bars represent Tukey's HSD means comparisons. *P. chrysurus* (open) was unaffected by temperature and aggression scores significantly differed from *P. nagasakiensis* at ambient and 30° C (shown by *). n = 17-20 pairs.

Fig 2.4. Aggression score and proportion of interaction behaviour by each competitor. (*a*) Mean aggression scores (± SE) of dominant (open) and subordinate (solid) competitors for intraspecific contests involving *P. chrysurus* (left) or *P. nagasakiensis* (right). *P. nagasakiensis* showed a significant effect of temperature on aggression score with subordinate competitors more affected compared to dominants. (*b*) Mean proportion (± SE) of attacks (shaded), displays (solid), and avoids (open) elicited by the dominant competitor. (*c*) Mean proportions of behavioural interactions elicited by subordinate competitors. As temperature increased dominant *P. nagasakiensis* decreased displaying behaviour and increased proportions of attacks while subordinates decreased displays but increased number of avoids.

Figure 3.1 Experimental setup for competition trials. Fish began in habituation chambers (circles) for 10 min. Revolving doors were opened and fish were allowed to emerge. Once emerged, a second partition was raised (dotted line), exposing fish to each other and a coral skeleton for shelter. A 10-min video recording was taken of their interactions.

Figure 3.2 Mean aggression score difference ± SE, calculated as winner fish score minus loser score between competitors by temperature of 4d (grey) and 90 exposure treatments (open). Species composition listed by row: intraspecific *Pomacentrus amboinensis* (*a*), intraspecific *Pomacentrus moluccensis* (*b*), and interspecific (c). Statistical significance ($p < 0.05$) between 4d and 90d treatments represented with (*). All treatments n = 10.

Figure 3.3 Proportion of wins by species for interspecific contests. *P. moluccensis* (grey) and *P. amboinensis* (open) tested in “control” (29 °C) and “elevated” (30 + 31 °C combined) temperature treatment. Elevated temperature treatments are split by 4d and 90d exposure.

Figure 4.1 C-start responsiveness of fish to a drop stimulus for juvenile *Pomacentrus amboinensis* (*a*) and *Pomacentrus moluccensis* (*b*) at 4d (left) or 90d (right) exposure durations to elevated temperatures. Types of reaction are: no reaction (black), avoidance (shaded), and C-start (open).

Figure 4.2 Directionality of C-start for juvenile *P. amboinensis* (*a*) and *P. moluccensis* (*b*) at 4d (left) or 90d (right) exposure durations to elevated temperatures. Direction was determined whether individuals that made initial movement towards (shaded) or away from (open) the stimulus.

Figure 4.3 Kinematic performance of fast starts by juvenile *P. amboinensis* (left) and *P. moluccensis* (right) at 4d (open) or 90d (grey) exposure durations to elevated temperatures. Variables measured were response latency (*a)*, max speed (*b*), and escape distance (*c*).

Figure 5.1. Metabolic rates across temperature and exposure durations. $MO_{2\ Routine}$ (*a*,*d*), $MO_{2\ Max}$ (*b*,*e*), and net aerobic scope (*c*,*f*) (mg O_2 kg^{-1} h^{-1} ± SE) are given for two damselfish species, *Pomacentrus amboinensis* (left) and *P. moluccensis* (right). Thermal sensitivity was determined by comparing 4d exposure (solid) to temperature with control, and capacity for plasticity was measured through comparisons of 4d with 90d exposure (open). Treatments with (*) represent significant difference from control and (Δ) represent significant difference **Figure 5.2.** Metabolic rates for dominant and subordinate competitors**.** Metabolic performance of dominant (solid) and subordinate (open) competitors of *Pomacentrus amboinensis* and *P. moluccensis* are given. $MO_{2\ Routine}$ (*a*,*d*), $MO_{2\ Max}$ (*b*,*e*) and net aerobic scope (AS; *c*,*f*) (mg O_2 kg^{-1} h^{-1} ± SE). Temperature exposure durations were 4d (left subdivide) and 90d (right subdivide). Treatments with (*) represent significant difference from control while

(Δ) represent significant difference between exposure durations (Planned comparisons, $p < 0.05$).

Figure 5.3. Difference in metabolic rates between competitors shown as relative metabolism. $MO_{2\ Routine}$ (*a*), $MO_{2\ Max}$ (*b*), and AS (*c*) are given relative to the subordinate individual in a trial (mg O_2 kg^{-1} h^{-1} ± SE) for two damselfish species *Pomacentrus amboinensis* (left) and *P. moluccensis* (right). Effect of short-term temperature exposure was determined by comparing 4d (solid) with control treatments (*, planned comparison $p < 0.05$). Thermal plasticity was examined by comparing 4d with 90d exposure (open).

Chapter 1: General introduction

Global climate change

Climate change is a threat to the natural world requiring accurate predictions of how it will impact species, communities, and ecosystems. Since the industrial revolution, anthropogenic carbon dioxide (CO_2) emissions have increased atmospheric CO_2 from 280 ppm to 400 ppm, the highest level in the past 800,000 years (Lüthi et al. 2008). This addition of CO_2 has created an enhanced greenhouse effect within the Earth's atmosphere, raising the average surface temperature by nearly 0.85 °C (Collins et al. 2013). According to the Intergovernmental Panel on Climate Change (IPCC), atmospheric CO_2 is projected to reach nearly 1000 ppm by 2100 if the current trajectory of CO_2 emissions is maintained, which would cause global temperature to rise by as much as 4.5 °C (IPCC 2013). Global climate change has already caused changes to the distribution, behaviour, and physiology of many species of plants and animals (Parmesan 2006; Walther et al. 2002). As global temperatures continue to rise, further disruption of ecological processes is predicted, potentially leading to higher extinction rates and declining ecosystem function (Parmesan 2006; Scheffers et al. 2016).

Rising temperatures and marine ecosystems

Due to the high thermal capacity of water, the world's oceans absorb over 90 % of the additional heat trapped by the enhanced greenhouse effect (Hoegh-Guldberg et al. 2013)). The IPCC predicts that sea surface temperatures will increase by as much as 3 °C by 2100 (Collins et al. 2013). This increase will likely exceed the thermal optimum for many tropical species (Tewksbury et al. 2008), disrupt the use of temperature as a cue in the timing of key events like reproduction and migration (Walther et al. 2002), and alter the distribution and abundance of many other species (Parmesan 2006). Many marine birds and mammals coincide seasonal temperature change with migration patterns and increasing temperatures can result in delayed onset of these movement patterns (Doney et al. 2012). Temperatures rise will also alter species distributions across latitudes as populations shift towards cooler locations, increasing the number of invasive species and creating new interspecific interactions (Milazzo et al. 2013). Due to unequal thermal tolerances among species, rising temperatures can affect community assemblages leading to restructuring of dominance hierarchies and impacting predator-prey interactions (Hoegh-Guldberg & Bruno 2010). These examples stress the need for accurate predictions for the effect of climate change on marine species as well as their responses.

Ectotherms and tropical species

Ectothermic species may be especially sensitive to global warming as they rely on ambient environmental temperature for body heat. This means that performance is tightly linked to surrounding temperature. Since most marine organisms (e.g. fish, molluscs, crustaceans) are ectothermic, ambient temperature is therefore a major determinant of many biological processes including metabolism (Farrell 2002), growth (Pauly 1980) and locomotion (Wakeling & Johnston 1998). Changes in these processes can be due to various mechanisms either at the whole-animal level of balancing energy demand with consumption or at the cellular level due to the temperature dependency of chemical reactions and enzyme efficiencies (Cossins & Bowler 1987). For example, metabolic demands in ectotherms rise nearly exponentially with temperature (Clarke & Johnston 1999). This increase in energy requirement can alter behavioural habits such as increased foraging time to meet food intake rather than pursuing mates or finding shelter.

The effect of temperature change on the performance a given trait for a species can be visualised using a thermal performance curve (TPC; Schulte et al. 2011). TPCs are a useful tool that helps show a species' thermal tolerance range and illustrate performance in temperature-dependent traits. Performance curves begin at a species' minimum critical temperature, generally increase with temperature, peak at a thermal optimum, then rapidly fall off as the maximum critical temperature is approached (Angilletta et al. 2002; Schulte et al. 2011). Although the thermal tolerance range for a species can vary among populations and life stages, tolerance ranges often reflect the temperature range experienced in their habitats (Sunday et al. 2011). TPCs can be generated for traits at the whole-organism level to study fecundity, growth, metabolic rate, and locomotor speed or at the molecular level to determine the rate of enzyme activity, oxygen consumption, and nerve connectivity. Recently, TPCs have received a rise in interest as they can provide insight on how species will perform with future temperature increase due to climate change (Deutsch et al. 2008).

While studies have now begun to address the effects of global climate change, much of the effort has been devoted to temperate species, particularly in the northern hemisphere (Chambers et al. 2013). Yet, tropical species may be especially vulnerable to rising temperatures because they occupy more thermally stable habitats and are therefore predicted to have a relatively narrow range of thermal tolerance (Tewksbury et al. 2008).. When exposed to temperatures 3 °C above current summer maximums, several species of reef fish reduced aerobic scope by nearly half and show little capacity for additional oxygen consumption for temperatures above 3 °C indicating coral reef fishes may be living close to their thermal optimum for aerobic capacity (Munday et al. 2012). Aerobic scope is a measure of aerobic capacity and is an important attribute as it is believed to represent the energy budget of a species (Portner & Farrell 2008).

Rising temperatures can accelerate development for juvenile reef fish as elevated temperatures increase larval growth rate, decrease the age of metamorphosis, and increase swimming ability (McCormick & Molony 1995; Meekan et al. 2003). However, higher temperatures will require higher food intake and when food is limited, individuals suffer loss of body condition, growth rate, and larval duration below current-day control temperatures (McLeod et al. 2013). Increased temperature also impact predator-prey interactions with higher predation rates and prey item switching (Ferrari et al. 2015). Collectively, these examples demonstrate the sensitivity reef ecosystems will be to future ocean warming.

Potential for plasticity

While there is much concern over the threat and negative impacts of rapid climate change, there are also ways that organisms can cope with changing environments. Species might maintain their performance under elevated temperature through phenotypic plasticity or adaptive evolution. Adaptation occurs through selection on genetic variation which is inherited from one generation to the next. Over a number of generations, selection shifts the average phenotype of the population towards the fitness peak (Angilletta 2009). By contrast, phenotypic plasticity enables individuals to change morphology, physiology, or behaviour without genetic change (Schulte et al. 2011). While both plasticity and adaptation will likely be driving responses to climate change over the coming decades, plasticity may be particularly useful as it can operate over more rapid timescales similar to global climate change.

Phenotypic plasticity can generally be divided into 3 categories; reversible, irreversible, and transgenerational (Angilletta 2009). Reversible plasticity is observed in adults and occurs frequently in nature with seasonal variation (Angilletta 2009).

Reversible plasticity happens within an individual's lifetime, is not considered permanent, and can serve as a method to cope with natural environmental variation (Angilletta 2009). Reversible plasticity is particularly useful for species living in variable habitats (Gabriel et al. 2005). Irreversible plasticity is usually a result of exposure to environmental conditions during earlier life stages, namely development, and aptly referred to as developmental plasticity. For example, adult zebrafish show enhanced swimming performance at elevated temperature if they had been first exposed to those same temperatures as embryos (Scott & Johnston 2012). Finally, plasticity can occur on a transgenerational scale when the performance of the offspring is influenced by non-genetic parental contributions. Non-genetic mechanisms that lead to transgenerational plasticity can be parents varying energy provisions, such as yolk reserves (Uller 2008), or through epigenetic processes, such as DNA methylation, influencing transcription of genes (Bonduriansky 2012; Sunday et al. 2014).

The extent of plasticity can depend on the thermal environment a species occupies. Temperate species that experience wide fluctuations in temperature annually, have been shown to possess remarkable capacity for physiological plasticity to a range of temperatures (Johnston & Dunn 1987). Meanwhile, tropical species are expected to be more thermally intolerant (Wright et al. 2009) due to their relatively stable environments and this has been shown to be true for some coral reef fishes (Nilsson et al. 2010). Yet, studies of plasticity in tropical fish have focused on exposure of adults for times scales from a couple of days to a few weeks (Nilsson et al. 2010; Gardiner et al. 2010; Rummer et al. 2014). Recently, studies exposing reef fish during embryonic stages and even transgenerational exposure periods have produced promising results for plasticity (Donelson et al. 2012, 2011). Targeting younger individuals is important because this can unlock greater plasticity not observed in later stages of life (Angilletta 2009).

Adaptive plasticity is generally considered to induce changes that are beneficial to performance, allowing individuals to better cope with new environmental conditions. However, detection and response to varying environments can be energetically costly (DeWitt et al. 1998). Resources spent on physiological or morphological modifications may be at the expense of growth rate and other functions (Hoffmann 1995). If the cost outweighs the benefit in performance, then plasticity may not be observed (Angilletta et al. 2003). Furthermore, phenotypic plasticity is limited and if the effect of environmental change is too great, plasticity may only be partial.

Knowledge gaps

The effects of environmental temperature on individual performance will be particularly important at life history bottlenecks (Fuiman et al. 2010). Animals migrating between habitats are often faced with the challenges of new competitors and predators. For reef fish transitioning from larval to juvenile stage, predation pressure can be highest. At 3-4 weeks of age, juveniles reef fish make their final metamorphosis and must transition from a pelagic larval stage back to the reef to join the adult population. Mortality due to predation in tropical marine fishes at settlement can average more than 50 % in the first 2 days (Almany & Webster 2006). Fish that can successfully fight for and hold adequate shelter may have a higher chance at survival to later life stages. While aggressive interactions among reef fishes has been closely examined (McCormick & Weaver 2012; Poulos & McCormick 2014) the effect that increased temperature will have on these interactions is still widely unknown.

The effect that rising temperature will have on competitive interactions will be, in part, influenced by the capacity for thermal plasticity in reef fish. At settlement, thermal plasticity in juveniles is useful as individuals may recruit to reefs with thermal regimes different to what they experienced during their larval phase. Individuals with a greater thermal plasticity may be able to maintain competitive performance better than others. This can put a selective pressure on juvenile populations favouring more thermally tolerant competitors. Yet, the capacity for thermal plasticity in competitive contests is not known. Studying thermal plasticity and competitive interactions will be help define intraspecific and interspecific interactions in reef communities under climate change projections.

If a competitive contest is lost and an individual finds themselves directly faced with a predatory attack without shelter, a C-start escape response is used in attempt to avoid capture. Many amphibians and fishes undertake C-start escape responses to a startling stimulus (Bullock 1984) involving a sudden acceleration away from the direction of perceived threat (Domenici 2010). C-start responses are triggered when 1 of the 2 large Mauthner cells, running laterally along both sides, is stimulated, usually in the laboratory by perturbance of the water's surface. C-starts that result in successful evasion usually consist of highly tuned responsiveness and locomotor performance (Domenici 2010). Yet, studies have shown that C-starts are sensitive to environmental temperature with impacts differing between species and ontogenetic stages (Johnston & Temple 2002; Wilson et al. 2010). To date, few studies have explored the capacity for plasticity in escape responses. Exploring the extent of plasticity in escape responses to climate change temperatures will demonstrate the outlook of predator-prey interactions in the future.

Along with investigating the effects of increased temperature on performance in ecological processes, e.g. competitive behaviour and escape performance, there is also a need to determine the direct influence of temperature through measuring physiological traits. This is because physiological performance is generally linked to performance in many ecological traits (Biro & Stamps 2010; Killen et al. 2013). Previous study on freshwater fish species have shown a relationship between physiological performance and competitive dominance (Cutts et al. 2001, 1998; Metcalfe et al. 1995) and this has also been shown to be true for a coral reef damselfish (Killen et al. 2014). However, determining how thermal plasticity of physiological performance can contribute to competitive dominance has not yet been tested.

Aims and objectives

The aim of this thesis is to elucidate the effects of elevated temperature on individual performance and the capacity for plasticity in coral reef fishes. Measures of competitive performance, metabolic rates, and antipredator escape performance as well as the linkages among them were investigated by exposing juvenile reef fish to elevated temperature for varying durations.

The first data chapter **(Chapter 2)** measured the effects of elevated temperature on intraspecific competitive interactions in two species of damselfish, *Pomacentrus chrysurus* and *Pomacentrus nagasakiensis*. Following short-term exposure to higher temperature (+4 C above ambient) size-matched pairs of fish were placed into arenas with a fragment of coral to compete for shelter. Aggressive interactions (e.g. displays, bites, and chases) were recorded and an aggression index was calculated to compare across temperatures and species.

The next three chapters explored the capacity for plasticity to increased temperature. Juvenile *Pomacentrus amboinensis* and *Pomacentrus moluccensis* were exposed to elevated temperatures (+1 and +2 °C above summer average) for either short-term (4d) or long-term (90d) durations. The effect of temperature and exposure duration was recorded for aggressive interactions during competition (**Chapter 3**), C-start escape responses (**Chapter 4**), and metabolic rates (**Chapter 5).** Comparing results from the short-term treatments with current-day controls showed the level of thermal sensitivity to climate change temperatures in reef fish and the difference between short-term and long-term exposures at elevated temperature, if any, would suggest evidence of plasticity.

Chapter 3 used the above described experimental design to determine the effect on aggressive interactions during competitive contests. Competitive interactions were recorded similar to chapter 2, however this design allowed for comparisons between exposure durations to elevated temperature to indicate capacity for plasticity. Matched pairs also consisted of within and between species combinations to examine differences in thermal tolerances across species.

The next chapter **(Chapter 4)** tested the effect of climate change temperatures and thermal plasticity on antipredator escape response performance. This was measured by analysing slow motion video recording of individuals subjected to a startling stimulus.

Once the effects of elevated temperature and duration of exposure for competition contests were understood, the next step was to address individual physiology. **Chapter 5** used static respirometry to measure maximum metabolic rate, minimum metabolic rate, and aerobic scope. Metabolic measures were then matched to competitive performance to determine any links between physiology and competitive dominance and whether physiological plasticity can contribute to competition.

Together, these four data chapters advance our knowledge of the impacts of ocean warming on coral reef fishes and the long-term effects of climate change on reef communities. Understanding these effects, the ability to cope with this temperature increase, and the link between physiological and ecological performance will be key to creating a more comprehensive picture of how communities may respond to a changing environment.

Chapter 2: Intrageneric differences in the effects of acute temperature exposure on competitive behaviour between damselfishes

Authors: Donald T. Warren & Mark I. McCormick

2.1 Abstract

Projected increases in global temperatures brought on by climate change threaten to disrupt many biological and ecological processes. Tropical ectotherms, like many fishes, can be particularly susceptible to temperature change as they occupy environments with narrow thermal fluctuations. While climate change models predict temperatures to increase over decades, thermal fluctuation is already experienced on a seasonal scale, which may affect the ability to capture and defend resources across a thermal gradient. For coral reef fish, losers of competitive interactions are often more vulnerable to predation, and this pressure is strongest just after settlement. Competitive interactions may determine future success for coral reef fishes, and understanding how temperature experienced during settlement can influence such interactions will give insight to community dynamics in a future warmer world. We tested the effect of increased temperatures on intraspecific competitive interactions of two sympatric species of reef damselfish, the Nagasaki damselfish *Pomacentrus nagasakiensis*, and the whitetail damselfish *Pomacentrus chrysurus*. Juvenile fishes were exposed to one of four temperature treatments, ranging from 26-32° C, for seven days then placed into competitive arenas where aggressive interactions were recorded between sized matched individuals within each species. While there was no apparent effect of temperature treatment on aggressive behaviour for *P. chrysurus,* we observed up to a four-fold increase in aggression scores for *P. nagasakiensis* with increasing temperature. Results

suggest that temperature experienced as juveniles can impact aggressive behaviour, however species-specific thermal tolerances lead to behavioural affects that differ among closely related species. Differential thermal tolerance among species may lead to restructuring of the interaction network that underlies the structure of reef assemblages.

2.2 Introduction

Temperature is an important abiotic factor that can have profound effects on behaviour, physiology, and life-history traits (Schulte et al. 2011). For many species, temperature dictates basic daily activities such as locomotion (Rome 2007), aggression (Pruitt et al. 2011), and boldness (Biro et al. 2010). Currently, climate change models predict sea surface temperature will rise as much as +3 °C by 2100 (Collins et al. 2013), impacting the ecology of many species (Walther et al. 2002). Yet, changes in temperature already occur temporally on a daily and seasonal scale or spatially such as across latitude or with water depth. Even within tropical ecosystems, which are considered relatively thermally stable, coral reef lagoons can experience seasonal thermal variation of up to 8 °C (McCabe et al. 2010). This effect of temperature change can be more pronounced in ectotherms as their metabolism tends to increase nearly exponentially with rising temperature (Clarke & Johnston 1999). Studying how reef species respond to temperature change can be helpful for determining the impact future climate change will have.

While climate change models predict temperature change to occur over several decades, the effect of warmer temperatures on reef fish can be observed today on a seasonal scale. Many benthic marine species have a planktonic larval phase that coincides with the lunar cycle, where juveniles recruit back to the reef to join the adult population (Leis & Mccormick 2002). This oscillation with moon patterns results in waves of newly settling juveniles over the reproduction season. Cohorts of juveniles that settle at the beginning of the season will experience cooler temperatures, have less competition for shelter sites, and have a head start on growth. Comparatively, cohorts that settle later in the season will experience warmer temperatures, must compete for shelter sites, and be behind on growth. As temperatures continue to rise, temperatures normally experienced during mid-late summer may occur earlier in the season. Determining the effects of temperature experienced by newly recruiting fish on individual performance will give insight to how increases in future averages may affect coral reefs.

The effects of environmental temperature on individual performance will be particularly important at life-history bottlenecks (Fuiman et al. 2010). During reef fish recruitment, over half of juveniles are consumed by predation in the first two days (Almany & Webster 2006). Juveniles that acquire and defend a shelter have a greater chance of survival. In general, relatively dominant individuals can secure shelter sites that provide better refuge from predators and access to food sources compared to their subordinate counterparts (McCormick 2009; 2012). This creates a selective bottleneck on juveniles where aggression and competitive dominance can generate an advantage. Yet, how temperature experienced during this phase may influence aggressive interactions is poorly understood. Determining how temperature may affect these aggressive interactions in fish will add to the understanding of how reef community dynamics may be affected over seasonal temperature fluctuations and will be informative in a climate change context.

The present study explores how temperature experienced during recruitment can impact intraspecific competitive interactions in two commonly found damselfish across the Great Barrier Reef, the whitetail damselfish *Pomacentrus chrysurus* and the

Nagasaki damselfish *Pomacentrus nagasakiensis*. Newly settled recruits were collected and reared in four temperatures for 7 days. Size-matched pairs were placed into competition arenas and their aggressive interactions over a coral shelter resource were quantified. We predicted that aggressive interactions would be lowest at ambient temperatures and increase as treatment temperature moved further from their ambient condition. However, it is likely that both species have different thermal tolerances, therefore, we predicted the magnitude of change will be difference for each species.

2.3 Methods

Study species, collection, and holding facilities

The study species were the whitetail damselfish, *P. chrysurus*, and the Nagasaki damselfish, *P. nagasakiensis.* These species are found throughout the Great Barrier Reef (Allen 1991) and have been the focus of multiple ecological and behavioural experiments (e.g. Ferrari et al. 2011; Chivers et al. 2014). As adults, *P. chrysurus* is generally found on dead and algal-covered corals while *P. nagasakiensis* is more associated with degraded corals (Feary et al. 2007). However, many recruiting juveniles settling to the reef can compete for similar habitats (Feary et al. 2007; Jones et al. 2004) before moving to their adult associated sites. Newly metamorphosed juveniles (3-4 weeks old; standard length, $\bar{x} \pm$ SD; *P. chrysurus* 13.26 ± 0.78 mm; *P. nagasakiensis* 15.46 ± 0.97 mm) were collected using light traps (M. G. Meekan et al. 2001) at Lizard Island on the northern Great Barrier Reef (-14°67′S, 145°44′E), Australia during November 2015 and held in replicate 32 L tanks on a flow-through system. Individuals were randomly allocated to 1 of 4 temperature treatments: 26, 28 (ambient), 30, and 32 °C. Temperatures revolved around ambient (28 °C) and ranged according to temperature fluctuation for the reproduction season (Oct-Jan). Temperature data was based on temperature loggers deployed around Lizard Island in common settlement locations (Fig 2.1) and verified as standard for the season against long-term loggers (AIMS 2014). The 32 °C treatment represented potential maximum summer temperatures for 2100 (Collins et al. 2013). To reduce thermal stress, tank temperatures were changed 1.5 °C/day from ambient and held at treatment temperature for 7 days prior to testing (as per Gardiner et al. 2010). Holding temperatures and photoperiods fluctuated ± 0.5 °C around the mean and 12:12 hr, respectively. Fish were fed *Artemia* nauplii to satiation twice during daylight hours.

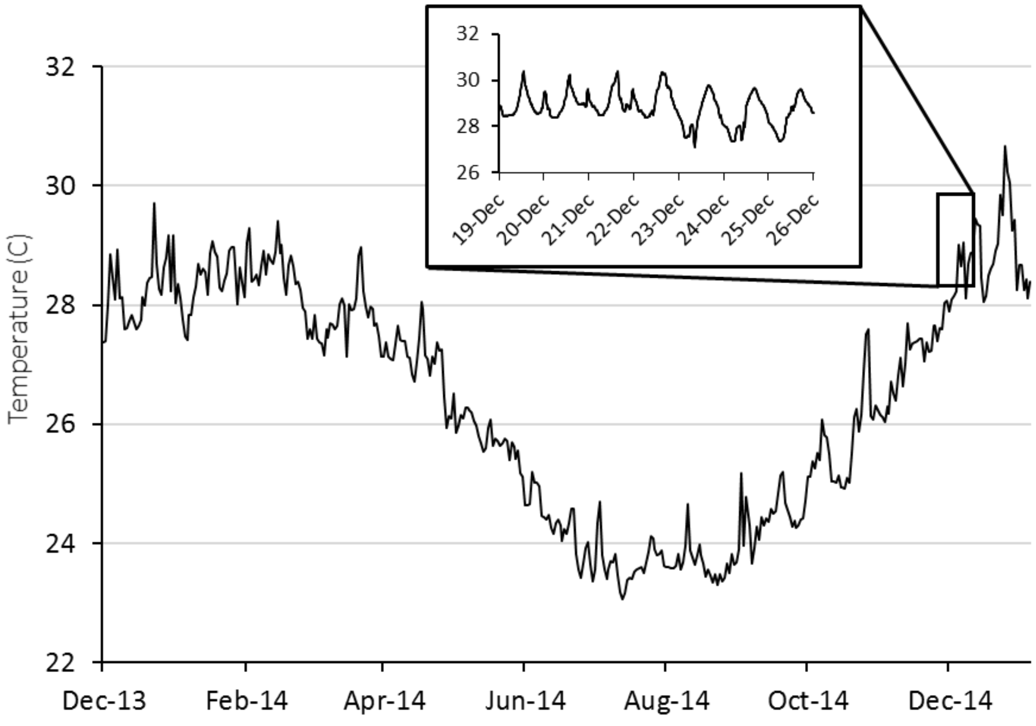

Fig 2.1. Seasonal and diurnal temperature fluctuation at Lizard Island, northern Great Barrier Reef. Seasonal fluctuation (23.0-30.6° C) from daily means recorded by long-term temperature loggers Dec 2013-2014. Inset shows the daily fluctuation (27.1-30.4° C), recorded every 20 min, for the collection season.

Experimental design

The effect of temperature on each species was measured by conducting intraspecific contests within temperature treatments. No trials occurred between fish from the same holding tank to control for the possibility of a pre-established hierarchy, and fish were used only once in the study to maintain novelty of the design and prevent any winner effect (Poulos & McCormick 2014). In total, there were 8 treatment combinations: 4 temperatures for each of 2 species. Replicates ranged from 17-20 pairs per treatment, due to mortality.

Competitive interaction trials

Size differences between competitors can influence the outcome of competitive interactions (McCormick 2009; McCormick & Weaver 2012). To control for this size effect, fish were measured and pairs were matched within 10% of their standard length to reduce the effect of size on outcome (Warren et al. 2016). The fish pairs were tested the day following measurement to decrease the effect of handling stress on the outcome. Fish were fasted for 12-24 h prior to competitive trials. Competitive arenas and procedures were modified from Warren et al. (2016). Arenas consisted of an oval tank (37 x 30 cm) filled with seawater to a depth of 10 cm with a fragment of live coral (*Pocillopora damicornis* ~5 cm^3) in one half and two habituation chambers in the other half (Fig. 2.2). The coral provided shelter and served as a resource for competition. At the start of each trial, a size-matched pair of fish was transferred via water-filled jars to separate habituation chambers for 5 min. After the habituation period, revolving doors on both chambers were slowly opened simultaneously, allowing the two fish to emerge and explore their portion of the arena. Habituation chambers were separated by a solid fixed divider, preventing fish from seeing each other, thus making emergence an independent decision. A clear partition allowed fish to view the coral on the other side but stopped either fish from reaching the shelter before the other. This partition prevented a priority effect of previous residency, a result known to influence outcomes of competitive interactions (Almany 2003; Geange et al. 2016). Once both fish exited their chambers, the clear partition was raised, exposing both fish to one another as well as allowing direct access to the coral fragment. Competitive interactions were video-recorded from the first encounter for 5 min and later analysed for three aggressive behaviours: (i) displays, defined as a lateral fin flare towards the opponent; (ii) attacks, defined as a chase or biting of the opponent; and (iii) avoidances, swimming away from an opposing attack or display. These variables were used to calculate an aggression score = attacks + displays – avoids (McCormick 2009; Warren et al. 2016). The individual with the higher aggression score was deemed dominant and the contest winner for that pair. Aggression was used as a measure for competitive performance as aggression provides a good indicator of contest outcomes (McCormick 2009; McCormick & Weaver 2012).

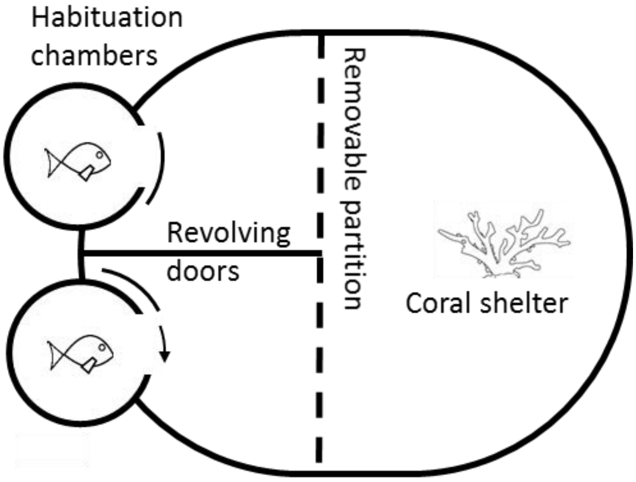

Fig 2.2. Diagram of competition arena. Size-matched pairs were placed into habituation chambers at the beginning of a trial for 5 min. Next, revolving doors were

opened simultaneously, allowing fish to exit and explore their portion of the arena. Once both fish had emerged, video recording commenced from an above camera and the clear removable partition was lifted, exposing fish to the coral shelter and each other. A 5-min recording was taken starting from the first interaction. Videos were later analysed and 3 interactions were quantified: (i) displays, defined as a lateral fin flare towards the opponent; (ii) attacks, defined as a chase or biting of the opponent; and (iii) avoidances, swimming away from an opposing attack or display.

Statistical analysis

Differences in size for each pair, calculated as winner fish size minus loser size, were normally distributed and did not differ from 0 (one-sample t-test, $t = 1.72$, $df = 157$, $p = 0.08$). This analysis showed that size matching was successful and the remaining difference in sizes did not decide contest outcomes. Aggression scores for each competitor were calculated, and the difference, taken as the winner score minus the loser score, was computed. Aggression score differences were analysed with a two-way ANOVA using species and temperature as fixed factors. To ensure aggression score differences were not being driven by one competitor alone, separate two-way ANOVAs, one for each species, were generated with individual aggression score as dependent variable and competitor status (categorised as dominant or subordinate) and temperature as fixed factors.

To examine how temperature affected the dynamics of a contest, the proportions of attacks, displays, and avoids elicited by each competitor were analysed using a two-way MANOVA with temperature and competitor as fixed factors and the proportion of interactions for each of the three behaviours as the dependant variables, again separate models were generated for each species. Any significance found was further investigated with Tukey's HSD post-hoc analyses. Bonferroni correction was used for hypothesis tests on individual variables for each species (Adjusted $\alpha = 0.017$). Preliminary analyses tested the presence of a tank effect by including holding tank as a random factor. However, this did not change the results, so holding tank was excluded from subsequent analyses. Data were found to conform to the assumptions of normality and homogeneity using residual analyses.

2.4 Results

There was a significant difference in species' response of aggression score differences to temperature. For *P. nagasakiensis*, aggression scores were lowest at ambient and increased as temperature moved from ambient in either direction ($F_{1,145} = 10.24$, $p = 0.002$; Fig 2.3). Conversely, aggression score differences for *P. chrysurus* were unaffected by temperature and were significantly greater than *P. nagasakiensis* at ambient and 30 °C (signified with *; Fig 2.3). When aggression score differences were separated by competitor, (i.e. dominant and subordinate scores), we found the change in aggression with temperature was more apparent in subordinate competitors of *P. nagasakiensis* ($F_{1,146} = 12.45$, $p = <0.001$; Fig 2.4d). Temperature also affected the proportion of interactions elicited by each competitor during contest of *P. nagasakiensis* ($F_{6,290} = 6.91$, Wilk's $\Lambda = 0.76$, $p < 0.001$). At elevated temperature, dominant *P. nagasakiensis* decreased displays ($F_{1,73} = 11.98$, $p < 0.001$) and increased attacking behaviour ($F_{1,73} = 8.69$, $p < 0.001$; Fig 2.4e). Concurrently, subordinate *P. nagasakiensis* decreased displays ($F_{1,73} = 11.64$, $p < 0.001$) and increased avoidance behaviour ($F_{1,73} = 10.92$, $p < 0.001$; Fig 2.4f). For *P. chrysurus,* neither dominant nor subordinates showed a change in interaction proportions across temperatures (Fig 2.4b and 2.4c, respectively).

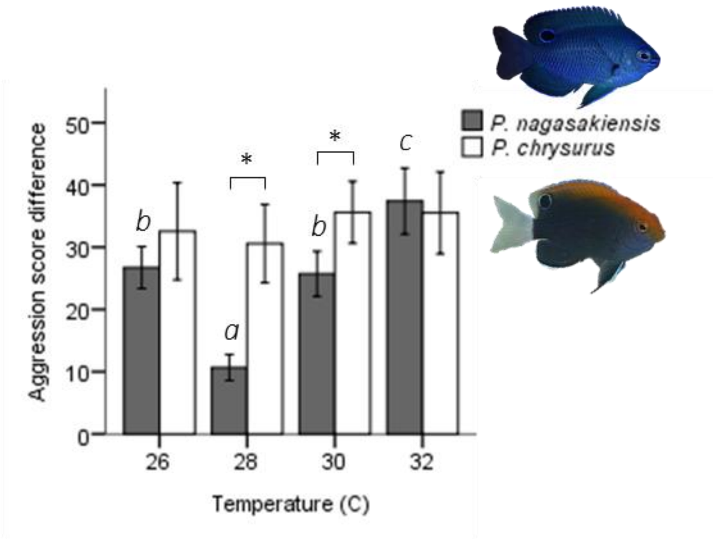

Fig 2.3. Influence of temperature on aggression scores between dominant and subordinate fish. Mean aggression score difference (± SE), taken as aggression score of dominant competitor minus the subordinate score, for intraspecific contests at each of four temperatures. Only *P. nagasakiensis* (solid) showed an effect of temperature with aggression lowest at ambient and increasing in either direction. Italicised letters above error bars represent Tukey's HSD means comparisons. *P. chrysurus* (open) was unaffected by temperature and aggression scores significantly differed from *P. nagasakiensis* at ambient and 30° C (shown by *). n = 17-20 pairs.

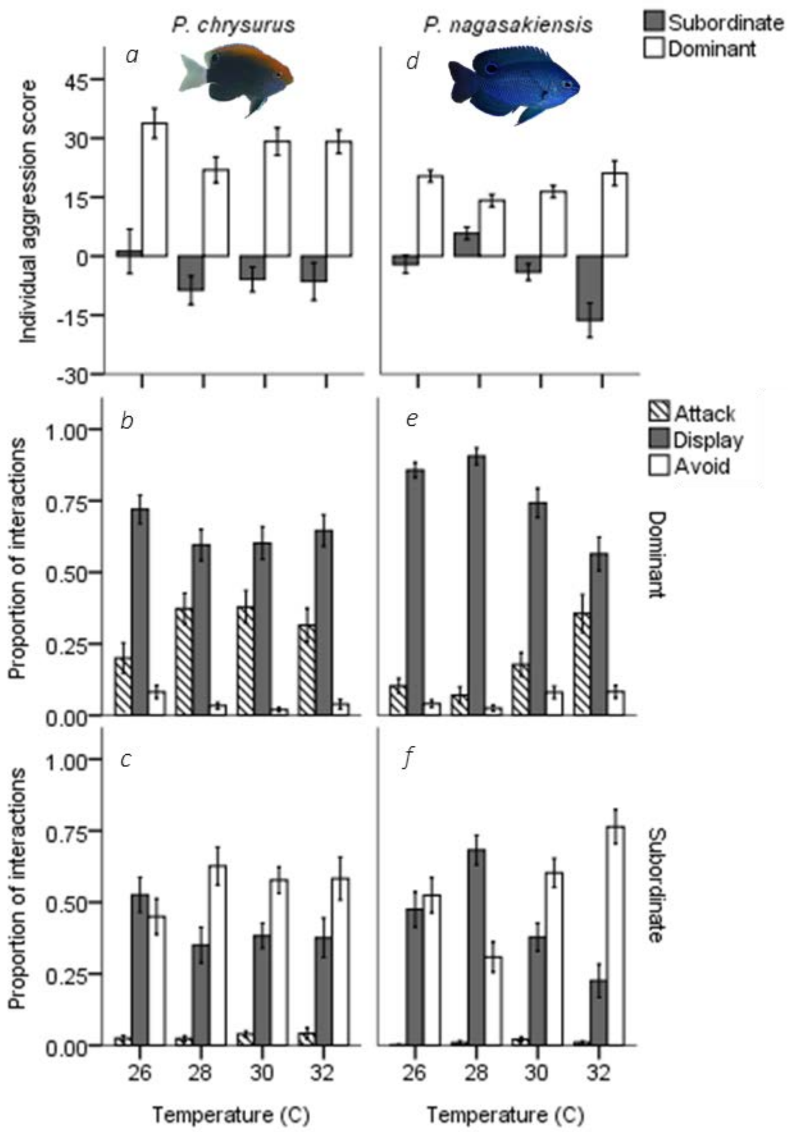

Fig 2.4. Aggression score and proportion of interaction behaviour by each competitor. (*a*) Mean aggression scores (± SE) of dominant (open) and subordinate (solid) competitors for intraspecific contests involving *P. chrysurus* (left) or *P. nagasakiensis* (right). *P. nagasakiensis* showed a significant effect of temperature on aggression score with subordinate competitors more affected compared to dominants. (*b*) Mean proportion (± SE) of attacks (striped), displays (solid), and avoids (open) elicited by the dominant competitor. (*c*) Mean proportions of behavioural interactions elicited by subordinate competitors. As temperature increased dominant *P.*

nagasakiensis decreased displaying behaviour and increased proportions of attacks while subordinates decreased displays but increased number of avoids.

2.5 Discussion

Determining the effect of temperature on ecological processes will be crucial for making realistic predictions about how communities may change in response to a warming ocean (Walther et al. 2002). While previous studies have looked at the effects of temperature on aggressive interactions in freshwater systems (De Staso & Rahel 1994; Reese & Harvey 2002; Taniguchi et al. 1998), few have focused on marine species. The current study found that aggression levels during competitive contests can increase with temperature experienced during settlement. Furthermore, the effect that temperature will have can be specific to each species. The effect of temperature on competition suggests that aggression may increase as summer averages increase with climate change. However, the species-specific response illustrates that differential thermal tolerance may lead to restructuring of reef-assemblages and dominance hierarchies within reef communities.

We found aggression scores differences for *P. nagasakiensis* were lowest when recruits were exposed to ambient temperature and increased when temperature changed in either direction. Increasing aggression with temperature is expected as activity level, boldness, and aggression have previously been shown to increase with temperature in a closely related damselfish (Biro et al. 2010). This may be due to the increased metabolism that coincides with rising temperatures for ectotherms (Clarke & Johnston 1999). When aggression score differences were separated into individual aggression score components (i.e. dominant and subordinate), we found that temperatures above ambient had a greater effect on subordinates. This may be due to an increasing stress of losing at elevated temperatures. Losing a competitive contest can be stressful for an individual as subordinates will spend the duration avoiding aggressive displays and denied access to shelter (Senar et al. 2000). This stress can manifest as elevated cortisol levels and metabolic rates (McCormick 2016), which may make them less resilient to other stressors such as temperature change. However, aggression showed a similar increase at 2 °C below ambient (26 °C) as 2 °C above (30 °C). This change in aggressive performance contradicts trends predicted for physiological performance potentially indicating reducing temperature was more important to the behavioural response.

Temperature also impacted the dynamics of contests through altering the proportions of competitive behaviours elicited by each competitor. At ambient temperature, dominant competitors won an encounter by displaying most often, followed by attacking, and avoiding least often. Conversely, subordinates showed avoidance behaviour most often, then displays, and attacked least of all. This is different to previous research on competitive interactions in other species of damselfish showing dominants win contests by attacking most, then displaying (Killen et al. 2014; Warren et al. 2016). As water temperature increased, there was a shift in the behaviour of dominant *P. nagasakiensis* to exhibit less displays and more attacks, while subordinates increased avoids and displayed less. This suggests that higher temperatures can both increase overall aggression during contests as well as lead to fiercer competition. Aggressive interactions can already be costly in energy expenditure and increase risk of injury (Briffa & Sneddon 2007). This transition to fiercer competition means individuals may be investing even more into contests, leaving less resources for other activities.

When comparing species, temperature had little effect on aggression score differences for *P. chrysurus* and was overall greater than *P. nagasakiensis* at 28 (ambient) and 30 °C. This shows a species-specific response to elevated temperature within congeneric species. Specificity by species to temperature has been shown in other work on competition in the damselfish *Pomacentrus amboinensis* and *Pomacentrus moluccensis* (Warren et al. 2016). Here, there was opposing trends in aggression after short-term exposure to elevated temperature. Differences in aggression help demonstrate species' thermal performances across temperatures and may give insight to how species may fare with future warming. The increase of aggression with temperature for *P. nagasakiensis* suggests the thermal optimum for this species may be above current summer average with future warming potentially yielding better performance. Comparatively, the lack of change in aggression suggests *P. chrysurus* may have a broader thermal optimum with changes in temperature showing little effect on performance. Interestingly, the selected temperature range can also overlap with diurnal fluctuation questioning whether there could be a similar fluctuation of aggression in a day.

Increasing temperature impacted aggressive contests and resulted in fiercer competition, however, the effect of temperature was specific to each species. Differences in thermal performance between species is most likely ubiquitous for many fishes within trophic groups. Species that are be dominant in current-day temperatures may be outcompeted at warmer temperatures, causing a shift in hierarchy assemblages and potentially species distributions. From here, there is a need to investigate what is driving these changes in competition and how our observed changes in aggressive behaviour will cascade into the reef community. Future studies should include competing species in interspecific trials, direct assessment of temperature on physiology, and longer-term exposure periods to temperature to give a more comprehensive picture of how climate change might be expected to influence coral reef fishes.

Chapter 3: Duration of exposure to elevated temperature affects competitive interaction in juvenile reef fishes

This chapter was published in PLoS ONE (2016), doi:10.1371/journal.pone.0164505

Authors: Donald T. Warren, Jennifer M. Donelson, Mark I. McCormick, Maud C. O. Ferrari, and Philip L. Munday

3.1 Abstract

Climate change will affect key ecological processes that structure natural communities, but the outcome of interactions between individuals and species will depend on their thermal plasticity. We tested how short- and long-term exposure to projected future temperatures affects intraspecific and interspecific competitive interactions in two species of coral reef damselfishes. In conspecific contests, juvenile Ambon damselfish, *Pomacentrus amboinensis*, exhibited no change in aggressive interactions after 4d exposure to higher temperatures. However, after 90d of exposure, fish showed a nonadaptive reduction in aggression at elevated temperatures. Conversely, 4d exposure to higher temperature increased aggression towards conspecifics in the lemon damselfish, *Pomacentrus moluccensis*. 90d exposure began to reduce this pattern, but overall there was little effect of temperature. Aggression in interspecific contests increased with short-term exposure, but was significantly lower after long-term exposure indicative of acclimation. Our results show how the length of exposure to elevated temperature can affect the outcome of competitive interactions. Furthermore, we illustrate that results from intraspecific contests may not accurately predict interspecific interactions, which will challenge our ability to generalise the effects of warming on competitive interactions.

3.2 Introduction

Climate change will alter the physiology, behaviour, and geographical distribution of many species (Botkin 2007; Huey et al. 2012; Parmesan 2006). However, the impact of these changes on population and community structure will depend on the outcome of biological interactions with other species (Harley et al. 2006; Hughes 2000). Due to differences in thermal tolerances, some species will be more capable of maintaining performance at higher temperatures than others (Milazzo et al. 2013; Pörtner 2001). Consequently, differences in thermal tolerance could dramatically alter the outcome of ecological interactions, such as competition and predation (Portner & Farrell 2008; Walther et al. 2002). Yet, thermal tolerances can change if exposure to new thermal conditions occurs for a sufficient amount of time at critical periods (Huey & Kingsolver 1989; Schulte et al. 2011). Furthermore, the extent of phenotypic change can depend on the length of exposure, with longer-term periods believed to unlock greater plasticity (Angilletta 2009; Munday et al. 2013). While many studies are now investigating the effects of higher temperatures on physiological processes (Johansen & Jones 2011; Mora & Ospína 2001; Nilsson et al. 2009), incorporating longer exposure lengths during early life has received much less attention. In climate change studies, relevant exposure will be critical for predicting the outcome of ecological processes in a future warmer world (Huey et al. 2012; Munday et al. 2013; Sentis et al. 2015).

Much of our understanding of how species might respond to future warming comprises of relatively short-term experiments using adults (Frank Seebacher et al. 2012; Johnson & Bennett 1995). Yet, exposure of juveniles to higher temperatures during early development can potentially induce greater plasticity in phenotypic traits and greater thermal tolerance in later life stages than just exposure of adults (Donelson et al. 2011; Schaefer & Ryan 2006; Scott & Johnston 2012; Tracy & Walsberg 2001). These early life influences can then produce long-lasting alterations in phenotype, affecting individual success later in life (West-Eberhard 2003). Changes in phenotype that prove beneficial to an individual are known as acclimation and could be a method to maintain performance in a new environment (Nettle & Bateson 2015). However, acclimation may not always fully compensate for the negative effects of environmental stress and can result in partial acclimation or even overcompensation (Huey & Berrigan 1996; Precht 1958). Furthermore, not all induced changes will be beneficial to future performance (Woods & Harrison 2002, 2001). Instead, prolonged exposure to elevated temperature may result in a further degrade in performance compared to present-day levels (Huey et al. 1999; Leroi et al. 1994; Wilson et al. 2002). Understanding the influence of early environmental experience is important, because early life stages are critical in population regulation (Caley et al. 1996). Many juveniles are vulnerable to resource restriction and even small changes in resource acquisition can affect attributes like growth and survival (Moksnes 2004; Newman 1998). At this age, competitive interactions greatly influence which individuals will survive to the next life stage (Andersen et al. 2011; Nilsson & Gårdmark 2001).

Performance in tropical ectotherms is believed to be particularly sensitive to higher temperature as they occupy relatively stable thermal environments and often live close to their thermal maximums (Hoffmann et al. 2013; Tewksbury et al. 2008). Small increases in temperature may have large effects on competitive performance in these environments (Gilman et al. 2010). However, the effect of longer-term exposure to elevated temperatures on competitive ability has not been tested. The objective of the present study was to determine how the length of exposure to elevated thermal conditions in post-settlement recruits affects the outcome of competitive interactions within and between two species of coral reef damselfish, *Pomacentrus moluccensis* and *P. amboinensis*. These species are known competitors for shelter over a broad geographic range (McCormick & Weaver 2012), making them ideal candidates for this study. We raised juveniles of both species at three temperatures and two exposure lengths to test: (1) the effects of elevated temperature on aggressive interactions and (2) compare performance from short-term to long-term exposure to higher temperature. If these species are currently living close to their thermal optimum at average summer temperatures (29 °C), then we expected that short-term exposure would have a negative impact on competitive interactions by reducing aggression in contests. After long-term exposure, we predicted either acclimation to occur and aggression would be restored towards control levels, or that extended exposure would accumulate stress and lead to a further decline in aggression performance.

3.3 Methods

Study species, collection, and holding facilities

The study species were the lemon damselfish, *P. moluccensis*, and the Ambon damselfish, *P. amboinensis.* These species are commonly used in a wide range of ecological, behavioural and physiological experiments (Allan et al. 2013; Grenchik et al. 2013; McCormick & Weaver 2012). They co-occur in the same habitat for the majority of their geographic range, spanning from the Coral Sea to Southeast Asia (Allen 1991). Much research undertaken on their interactions has been done at Lizard Island on the northern Great Barrier Reef, Australia (-14°67′S, 145°44′E). Both species prefer to settle to live coral (McCormick et al. 2010; McCormick 2012) and in the wild *P. moluccensis* is exclusively found on live coral, while *P. amboinensis* is found on a broader range of habitats including dead coral and rubble (McCormick 2012;

McCormick & Weaver 2012). These species feed on similar food items as juveniles (McCormick & Weaver 2012) and *P. moluccensis* is normally outcompeted for preferred habitat and is forced to occupy a position near the top of a coral head, while *P. amboinensis* occupies the safer bottom part of the habitat patch (Feary et al. 2007). Body size is strongly related to dominance within (McCormick 2012; McCormick & Meekan 2007) and between the two species (McCormick & Weaver 2012).

Like many reef fishes, these species have a pelagic larval phase that prevents rearing individuals from their embryonic phase. After 3-4 weeks, larvae make their final metamorphosis and recruit back as juveniles to join the reef community. This transition period is an important life history bottleneck as there are more juveniles than available shelter, forcing individuals to compete for space (Almany & Webster 2006). Collection efforts targeted the smallest (~20 mm standard length), and therefore youngest of these recruits. Fish were collected from reefs in the Cairns region (-16°78′S, 146°26′E) of the Great Barrier Reef, Australia during January 2014 and transferred to experimental facilities at James Cook University. Individuals were randomly allocated to replicate 40 litre tanks in three temperature treatments: 29 °C (current-day summer average for the collection region; control; AIMS 2014), 30 °C, and 31 °C (projected future temperatures by 2100; Collins et al. 2013). Elevated temperature treatments were split into two exposure lengths: 4d or 90d. We used 4d for our short-term exposure to explore the impacts of elevated temperature on competitive behaviour without causing a thermal stress response (Gardiner et al. 2010). Previous work using *P. moluccensis* has shown no acclimation to similar elevated temperatures to occur for up to 22 days, supporting that our 4d treatment would not be confounded by reversible acclimation (Nilsson et al. 2010). We chose a 90d exposure for our long-term treatment based on previous developmental studies using this species (Grenchik et al. 2013) and another closely related species of damselfish (Donelson et al. 2011). Daily temperature variation and photoperiods followed a natural cycle for the collection region, ± 0.6 °C around the mean and 12:12h, respectively (AIMS 2014).

Experimental design

To determine the effects of short-term exposure to elevated temperature on competitive interactions we conducted intraspecific and interspecific trials with fish exposed to30 °C or 31 °C for 4d. Holding tank temperatures were raised 1 °C/day (to reduce the effect of heat shock; (Gardiner et al. 2010; Nilsson et al. 2010) and held at target temperature for 4d before testing. To determine the effects of long-term thermal exposure on competitive interactions, we conducted intraspecific and interspecific trials with fish maintained at 30 °C or 31 °C for 90d.

Our control treatments maintained fish at 29 °C for entirety of the experiment. To control for any effect of time spent in captivity, the 4d treatments were first held at control temperature, then exposed to their randomly assigned temperature 4 days before the end of the long-term duration. This allowed all fish to finish temperature treatments at the same time. No trials occurred between fish from the same holding tank to control for the possibility of a pre-established hierarchy and no two competitors were matched more than once to prevent a winner effect. In total, there were 40 holding tanks for the five treatment combinations: control, two combinations for short-term exposure (30 and 31 °C), and two combinations for long-term exposure (30 and 31 °C; S1 Table). Ten replicates were conducted for each treatment combination.

Competitive interaction trials

Size difference between competitors is a known factor in determining the outcome of competitive contests (McCormick & Weaver 2012). Consequently, all fish were measured just before the experimental period (standard length mm, $\bar{x}$ = 29.1 ± 4.1 SD) and pairs were created by matching fish within 10 % of their standard length (Poulos & McCormick 2014). Competitive arenas and procedures followed Killen *et al.* (2014). At the start of the trial, fish were placed individually in habituation chambers for 10 min (Fig 3.1), consisting of PVC cylinders with a revolving door. After a 10-min habituation period, the doors on both chambers were carefully opened simultaneously and fish were allowed to emerge. Once both fish exited their habituation chambers, a central partition was raised, exposing both fish to one another as well as to a fragment of coral skeleton (~5 cm^3). The coral provided shelter and served as a resource for competition. Competitive interactions were video recorded for 10 min and later analysed for three behavioural traits: (i) displays, defined as a lateral flare of its fin towards the opponent; (ii) attacks, defined as a chase or biting of the opponent; and (iii) avoidances, swimming away from an opposing attack or display. These variables were used to calculate an aggression score = attacks + displays – avoids (McCormick 2009). The fish with the higher aggression score was deemed dominant and the winner for that pair. Aggression was used as a measure for competitive performance as it provides a good indicator of contest outcomes (Killen et al. 2014).

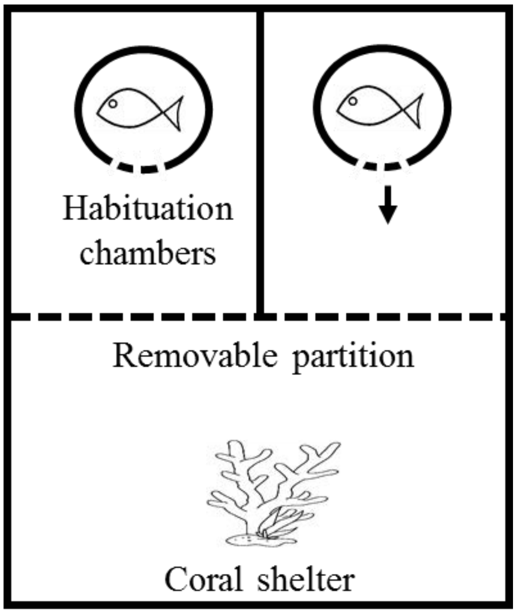

Fig 3.1. Experimental setup for competition trials. Fish began in habituation chambers (circles) for 10 min. Revolving doors were opened and fish were allowed to emerge. Once emerged, a second partition was raised (dotted line) exposing fish to each other and a coral skeleton for shelter. A 10-min video recording was taken of their interactions.

Data analysis

Size differences for each pair, calculated as the size of the winner fish minus the size of the loser fish, were normally distributed and did not differ from zero (one-sample t-test, $t_{150} = 0.213$, $p = 0.832$). This indicated size matching was successful and the remaining size differences had no effect on the outcome of contests. Subsequent analyses were performed without correcting for any size differences. For each trial, individual aggression scores were calculated and the difference of the winner minus the loser score was computed. Aggression score differences were analysed with separate ANOVAs to measure the effect of short-term exposure to elevated temperature and to compare short-term with long-term exposure. This was repeated for each species in the intraspecific treatments and the interspecific treatment for a total of six models. Preliminary analysis included holding tank in the model to test for tank effect, but no effect was found. Consequently, results reported do not include this variable in the design.

While increased temperature could affect the difference in aggression score between competitors, it could also affect the absolute level of aggression as well as the total number of aggressive interactions during a trial. To ensure aggression score difference was an accurate reflection of overall aggressive behaviour, aggression score of winner fish only and total number of aggressive interactions were analysed similar to aggression score differences. For interspecific trials, proportion of wins by species were compared across temperature and exposure length with a chi-square test of independence.

3.4 Results

Intraspecific competition

For *P. amboinensis,* there was no change in aggression score difference following 4d exposure to higher temperatures (Fig. 3.2*a*). After 90d, fish had a significantly smaller difference in aggression scores at 30 °C and 31 °C compared to the 4d treatment ($F_{1,38} = 10.7$, $P = 0.002$; Fig. 3.2*a*). For *P. moluccensis,* there was a trend to increase aggression score differences following 4d exposure (Fig. 3.2*b*) and if we only considered the aggression score of the winner, this effect was significant ($F_{2,27} = 3.46$, $P = 0.046$). There was no difference in aggression score differences between 4d and 90d (Fig. 3.2*b*). The aggression score of the winner only, and the total number of aggressive interactions by the two individuals, mirrored aggression score differences in both the 4d and 90d treatments confirming aggression score differences were an appropriate measure of aggressive behaviour during a trial.

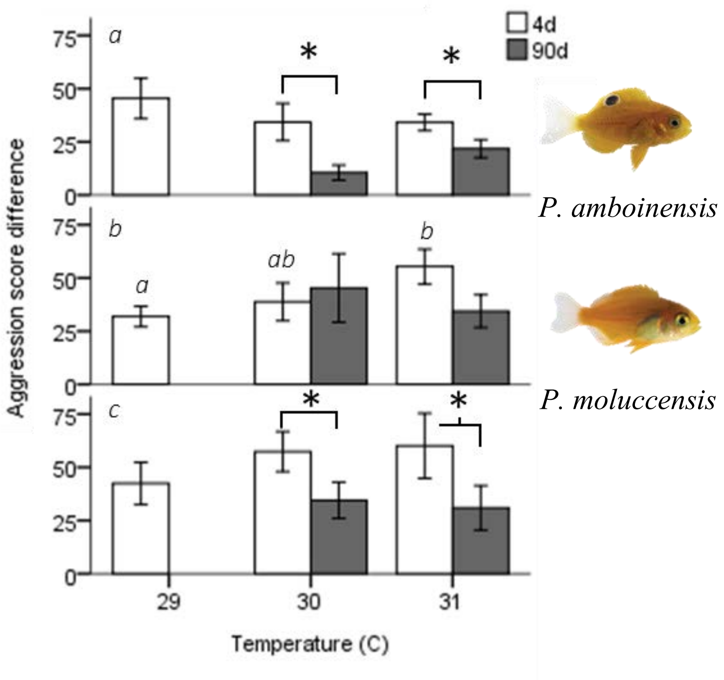

Fig 3.2. Mean aggression score difference ± SE, calculated as winner fish score minus loser score, between competitors by temperature of 4d (solid) and 90d exposure treatments (open). Species composition listed by row: intraspecific *Pomacentrus amboinensis* (*a*), intraspecific *P. moluccensis* (*b*), and interspecific (*c*). Statistical significance ($p < 0.05$) across control and 4d at elevated temperature represented by letter and comparison of 4d and 90d treatments represented with (*). All treatments n = 10.

Interspecific competition

There was no change in aggression score differences among temperature treatments for contests with 4d exposure, although there was a tendency for the aggression scores to increase with temperature (Fig. 3.2*c*). Fish with 90d of exposure had lower aggression score differences at elevated temperatures compared with 4d ($F_{1,38} = 5.14$, P = 0.029;

Fig. 3.2*c*). The aggression score of the winner only and total number of aggressive interactions by the two individuals, mirrored aggression score differences in both the 4d and 90d treatments.

There was no significant difference in the proportion of contests won by species across the three temperatures after 4d exposure. However, when the 30 and 31 °C treatment groups were combined into an "elevated temperature" group, there was a trend from favouring *P. amboinensis* at control temperatures to favouring *P. moluccensis* in elevated temperatures ($\chi^2 = 3.5$, $df = 1$, $p = 0.07$; Fig. 3.3). After 90d exposure, this trend was reduced, though proportions did not fully return to controls.

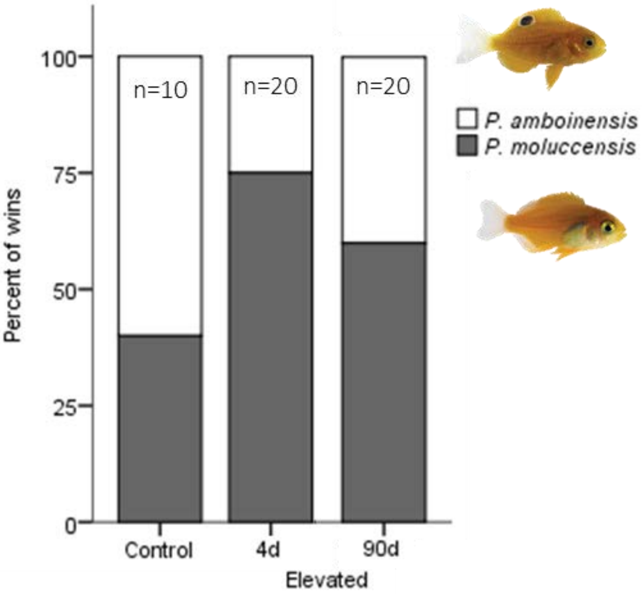

Fig 3.3. Proportion of wins by species for interspecific contests. *Pomacentrus moluccensis* (solid) and *P. amboinensis* (open) tested in "control" (29 °C) and "elevated" (30 + 31 °C combined) temperature treatment. Elevated temperature treatments are split by 4d and 90d exposure.

3.5 Discussion

Climate change is predicted to affect the outcome of ecological interactions within and between species, with potentially far-reaching consequences for population dynamics and community structure (Williams et al. 2008). Elevated temperatures can lead to negative effects on individual performance (Parmesan 2006), but individual performance may acclimate and return to control levels after long-term exposure to these temperatures (Angilletta 2009; Nettle & Bateson 2015). Alternatively, longer exposure could result in a further decline in performance (Wilson et al. 2002; Woods & Harrison 2002). We found aggression after short-term exposure was maintained in intraspecific contests with *P. amboinensis*, whereas there was a trend towards increasing aggression in *P. moluccensis*. Long-term exposure reduced aggression scores in *P. amboinensis,* but *P. moluccensis* was unchanged. Interestingly, the aggressive response of species in interspecific contests differed from what would have been predicted from each species' performance in intraspecific contests. This suggests that predicting the outcomes of species interactions in the future may not be possible from measuring the behavioural performance of species independently.

Elevated temperatures influenced the outcome of competitive interactions for interspecific contests. There was a tendency for increased aggression scores differences with short-term exposure to elevated temperature. After long-term exposure, acclimation appears to have occurred as aggression was significantly lower compared to fish with short-term exposure. In fact, acclimation may have overcompensated as aggression was lower than control in the long-term treatment. Exposure length also appeared to influence the relative proportions of wins by each species. While *P. amboinensis* was favoured in control conditions, *P. moluccensis* tended to win more contests after short-term exposure to elevated temperatures. This trend was less apparent with long-term exposure, though proportions did not fully return to controls. Changes in species dominance at elevated temperature can occur in competitive hierarchies of both marine (Kordas et al. 2011) and freshwater species (Taniguchi et al. 1998), but whether these changes are diminished or reversed with longer-term exposure during early life has not been tested.

Elevated temperature had different effects on each species for intraspecific competition, possibly due to differential thermal sensitivity and performance optima. Temperature had only small effects on competitive interactions in *P. moluccensis.* Short-term exposure had an increasing trend with temperature, while long-term exposure showed a relatively flat response with the biggest difference between exposure lengths at 31 °C. The lowered aggression with longer exposure at 31°C matches previous research showing evidence of acclimation in aerobic capacity for this species, from a nearby reef region, after early development in similar temperatures (Grenchik et al. 2013). These results suggest this species may fare well within the +2 °C future temperature increases. In contrast, *P. amboinensis* maintained aggression levels with short-term exposure, but long-term exposure resulted in a reduction of aggressive performance. This indicates there was no acclimation to elevated temperature. Instead, this shows how prolonged exposure will not always induce phenotypic changes that are beneficial to an individual's performance. Other studies have also reported non-beneficial, or non-adaptive, responses after long-term exposure periods to temperature (Hoffmann 1995; Huey & Stevenson 1979; Huey et al. 1999). These non-adaptive responses have been attributed to insufficient duration of exposure for acclimation to occur (Levins 1969), conditions too extreme for any acclimation to occur (Woods & Harrison 2002), or costs of acclimation outweighing its benefits

(DeWitt et al. 1998). Alternatively, the longer exposure period could have resulted in accumulated physiological stress that caused performance to decline. In our study, one or more of these may have acted to create a non-beneficial response.

As global temperatures continue to rise, individuals will be exposed to increased temperatures throughout their early life stages. Thermal plasticity will likely be a key process in determining performance in ecological interactions in future warmer environments. This is the first study to show that extended exposure to elevated temperature affects competitive interactions in coral reef fishes. Our intraspecific results suggest that *P. amboinensis* should perform poorly relative to *P. moluccensis* in interspecific contests at elevated temperature. While this was true for short-term treatments, we found this trend was less apparent after long-term exposure. This highlights that interspecific competitive interactions can be complex and extrapolation of results from within species to predicting between species contests may not be accurate. Future studies should also consider exposure periods similar to, if not longer than, our study and be combined with physiological measurements on stress responses when attempting to project the outcome of competitive interactions between species in a future warmer world.

Chapter 4: Extended exposure to elevated temperature affects escape response behaviour in coral reef fishes

This chapter is published at Peer J (2017), doi:10.7717/peerj.3652

Authors: Donald T Warren, Jennifer M Donelson, and Mark I McCormick

4.1 Abstract

The threat of predation, and the prey's response, are important drivers of community dynamics. Yet, global climate change poses a threat to predation avoidance techniques such as fast-start performance observed in marine fishes. While it is known that projected future temperature increases can influence performance and behaviour in the short-term, little is known about how species respond to extended exposure during development. Using a repeated drop stimulus, we show that length of thermal exposure of juveniles to elevated temperature significantly affects escape responses in two species of damselfish, the lemon damsel *Pomacentrus moluccensis,* and the Ambon damselfish *Pomacentrus amboinensis.* Short-term (4d) exposure to warmer temperature effected directionality and responsiveness for both species. After long-term (90d) exposure, only *P. moluccensis* showed beneficial plasticity, with directionality returning to control levels. Responsiveness also decreased in both species, possibly to compensate for higher temperatures. There was no effect of temperature or length of exposure on latency to react, maximum swimming speed, or escape distance suggesting that the physical ability to escape was maintained. Evidence suggests that elevated temperature may impact some fish species through its effect on the behavioural decisions they make while under threat rather than having a direct influence on their physical ability to perform an effective escape response.

4.2 Introduction

Predator-prey interactions are one of the key drivers structuring communities (Lima & Dill 1990). Predation pressure can alter prey morphology, regulate population size, and control biodiversity (Sih et al. 1985). Similarly, antipredator responses of prey reduce the efficacy of predators and enhance survival. Factors that influence predator attack success or prey escape performance will alter the outcome of these interactions, potentially causing a cascade of trophic effects. Increased temperature has already been shown to affect antipredator responses in terrestrial ectotherms (Cury de Barros et al. 2010), freshwater fishes (Weetman et al. 1998), and marine species (Allan et al. 2015). Understanding how changes in environmental temperature can affect key ecological processes is crucial to predicting the composition of future communities.

Rising environmental temperatures can substantially impact the behaviour and locomotor performance components of escape responses in fish (Domenici 2010). Previous studies have shown that temperature can influence locomotor performance through physiological mechanisms like muscle performance (Johnston et al. 1990) and enzyme activity (Johnson & Bennett 1995). Additionally, temperature can affect behavioural components of escape responses, including responsiveness (Preuss & Faber 2003) and directionality (Szabo et al. 2008). The effect of environmental temperature will depend on both the level of thermal change compared to the normal thermal conditions, and the length of time thermal change is experienced (Munday et al. 2012). With sufficient exposure time, plasticity can occur resulting in changes in phenotype, which potentially reduce the negative impacts of rising temperature (Angilletta 2009). Exposure during the early stages of life is believed to unlock greater plasticity in traits (West-Eberhard 2003), and this has been found to be true for coral reef fishes (Donelson et al. 2011). While many studies have investigated the effects of short-term exposure to climate change temperatures in adult fishes, to date few studies have examined how juvenile fishes respond to long-term exposure to near-future temperature increases.

The interaction between a predator and its prey is an intricate sequence that, at its end, leads to a predator striking at a prey and the prey being captured or escaping (Lima & Dill 1990). Many amphibians and fishes undertake a C-start escape response to a startling stimulus (Bullock 1984) involving a sudden acceleration away from the direction of the perceived threat (Domenici & Blake 1997). C-start responses are controlled by two large neurons called Mauthner cells, one for each lateral half of the body (Eaton et al. 1977). When a stimulus is detected, the corresponding Mauthner cell triggers the escape response resulting in a rapid contraction of white muscle fibres. At first, the fish bends into a C-shape (stage 1), then contralateral contraction releases this tension which propels the fish to flee the stimulus in the opposite direction (stage 2) (Eaton et al. 1977). The initiation of C-starts are neurally controlled with maximum performance believed to be limited by muscle power output (Wakeling & Johnston 1998). However, fish rarely perform at their maximal limits (Webb 1986) and modulation of performance during an escape response has been shown to occur in different behavioural contexts, showing there is potential for optimisation (Domenici 2010; Korn & Faber 2005; Ramasamy et al. 2015). This supports the economic model that individuals balance the energetic cost of escaping with the threat of predation (Ydenberc & Dill 1986). How long-term exposure to elevated temperature can alter behavioural components of an escape response is still unknown.

The present study examines whether short-term or long-term exposure to elevated temperature regimes affect the fast-start escape response in two tropical damselfishes. Using a repeatable drop stimulus, we compared escape responses of juveniles from current-day controls (29 °C) to individuals exposed to 50 and 100 year projections (30 and 31 °C; Collins et al. 2013) for either 4d or 90d. Specifically, we tested: (1) the effects of short-term exposure to elevated temperature as an indication of thermal sensitivity and (2) how short-term exposure differed to long-term exposure at elevated temperature as an indication of plasticity. We predicted these species are currently living close to their thermal optimum for summer temperature and that short-term exposure to elevated temperature would have a negative impact on escape responses. If beneficial plasticity was possible with long-term exposure, individuals exposed for 90d would exhibit improvement in performance that either approached or returned to present-day control levels.

4.3 Methods

Study species, collection, and holding facilities

To examine how short-term and long-term exposure to elevated temperatures influences escape performance, two model reef fish species were used (the Lemon damselfish, *Pomacentrus moluccensis*, and the Ambon damselfish, *Pomacentrus amboinensis*). These species have been used in past studies on escape performance (Allan et al. 2013) and behaviour (McCormick & Weaver 2012) making them familiar study species. These species undergo embryonic development during a pelagic larval phase. After 3-4 weeks, larvae make their final metamorphosis and recruit back to the reef to join the adult population. At this stage, thermal plasticity is ecologically important as individuals often settle to reefs with thermal regimes different to what they experienced as larvae. These species generally live up 5-7 years and reach maturity after 1 year of age (Allen 1991; Frédérich & Parmentier 2016) Recently settled recruits were collected from the Cairns region (-16°78′S, 146°26′E) of the Great Barrier Reef, Australia in January 2014.

Fish were transported back to the closed system holding facilities at James Cook University, Townsville and housed in 40 litre replicate tanks randomly allocated into one of three temperatures: 29 °C (current-day summer average for the collection region; control; AIMS 2014), 30 °C, or 31 °C. To avoid thermal stress, the temperature was raised 1 °C per day (Gardiner et al. 2010; Nilsson et al. 2010). Elevated temperatures were split into two exposure lengths: 4d or 90d. A 90d period was chosen for our long-term exposure treatment based on previous developmental studies on this species (Grenchik et al. 2013) and a closely related species of damselfish (Donelson et al. 2011). A 4d period was chosen for our short-term exposure treatment to observe the effects of increased temperature without causing a thermal stress response (Gardiner et al. 2010). No thermal benefit in plasticity has been shown to occur in *P. moluccensis* for up to 22 days of exposure to similar elevated temperatures supporting our 4d treatment would not be confounded by reversible plasticity (Nilsson et al. 2010). Temperature variation and photoperiod followed natural diurnal cycle for the collection region, ± 0.6 °C around the mean and 12:12h respectively (AIMS 2014).

Experimental design

The escape performance of fishes was quantified using a repeatable drop stimulus. In total, there were 40 holding tanks split into 5 treatments: control, 2 short-term exposure treatments (30 and 31 °C), and 2 long-term exposure treatments (30 and 31 °C). The control treatment held fish maintained at 29 °C for the duration of the study (90d). The 2 long-term treatments contained fish held at 30 or 31 °C for 90d. The short-term treatments maintained fish at 29 °C then were moved to either 30 or 31 °C by raising the temperature by 1 °C/day then held at target temperature for 4d.

Experimental procedure

After the exposure period, individuals were measured (standard length, $\bar{x}$ ± SD; *P. amboinensis* n = 58, 30.57 ± 5.79 mm, *P. moluccensis* n = 83, 28.08 ± 3.59 mm) and tested for escape performance the following day. Escape performance was tested in a circular arena (30 cm diameter) filled to a depth of 10 cm to reduce movement in the vertical plane and illuminated with fluorescent lighting strips. The walls and top had opaque covers to prevent outside disturbance. To elicit an escape response, a startling stimulus consisting of a weight with a tapered end was released through a tube suspended above the centre of the arena. The suspended tube stopped ~1 cm above the water's surface concealing the stimulus weight as it fell thus creating a more sudden disturbance. A monofilament line was used to raise the weight and was long enough that the end touched just the water's surface when released, preventing collision with the individual. To start a trial, fish were placed into a central habituation ring (10 cm diameter) via a water filled sample jar and given a one-minute habituation period. Following the habituation period, the central ring was gently raised and the stimulus weight was released, striking the water in attempt to elicit an escape response. The escape response was recorded using a high-speed camera (Casio Exilim Ex-F1; 600fps) directed at a 45° angled mirror placed beneath the testing tank. If a C-start was performed, the individual was returned to its holding tank and trials began for the next fish. If no response was elicited, individuals were replaced into the habituation ring for another minute and the trial was run again. Individuals were given three attempts to perform a C-start before being returned to their holding tanks and labelled "no reaction".

Response variables

Response variables were measured using the fish's centre of mass (~35% SL from the snout; Webb 1976) and tracked using ImageJ software (v1.48) with the manual tracking plugin. We chose our variables as they have been used in previous study as a good indicator of escape ability (Walker et al. 2005). Only stages 1 and 2 of the C-start, described as directional changes in the anterior part of the body (Domenici & Blake 1997) were quantified.

Non-locomotor

a) Responsiveness: proportions of fish that responded with a “C-start”, an “avoid” to the stimulus by swimming away but not performing a desired C-start, or “no reaction” to the stimulus.
b) Directionality: categorised fish that undertook a C-start by whether the head turned “away” or “toward” the stimulus during stage 1 of the response.
c) Response latency (ms): time between the stimulus onset and first movement of the individual.

Locomotor

d) Escape distance (mm): distance travelled during the response to the end of stage 2.
e) Maximum swim speed (body lengths s^{-1}): maximum velocity achieved at any time during the escape response.

Data analysis

Statistical analyses were performed using IBM SPSS statistics (v23.0.0.2). Proximity of fish to the stimulus at the onset and body size can affect escape responses (Webb 1976). The central habituation ring helped reduce differences in starting proximity to stimulus and the remaining distances of fish to the stimulus did not differ among treatments (one-way ANOVA $F_{4,110} = 0.959$, $P = 0.433$). Maximum swim speed (m s^{-1}) was converted to body length s^{-1} to control for any effect of size. Control temperature did not consist of two exposure combinations like the elevated temperatures, therefore a full-factorial 3×2 design was not possible. Instead, the five treatments (control, 2 short-term, and 2 long-term) were categorised into one "treatment combination" factor. A binomial logistic regression was used to ascertain the effects of treatment combination on the likelihood of an individual to elicit a C-start after the stimulus and the likelihood of that individual to turn away from the stimulus. Response latency, maximum swim speed, and escape distance were used as dependent variables analysed separately with one-way ANOVAs ($\alpha = 0.05$) and treatment combination as the fixed factor. These analyses were repeated for both species (replicates ranged from 7-12 for *P. amboinensis* and 9-19 for *P. moluccensis*). Preliminary analysis of response latency, maximum swim speed, and response distance used a linear mixed effects model with holding tank as a random factor to test for a tank effect. However, AIC values were lower for models not including random tank factor with 1AIC <2 to models that did include random tank factor. Consequently, this term was dropped from the final model.

4.4 Results

There was no significance in the logistic regression model for responsiveness in *P. amboinensis* ($\chi^2(4) = 4{:}18$, $p = 0{:}38$; Fig. 4.1A). At control, 84.62% of individuals responded with a C-start, 7.69% showed no reaction, and 7.69% displayed avoidance behaviour. Exposure of 4 days to either 30 or 31 °C had very little effect on responsiveness. However, for individuals that did not display a C-start, all showed no reaction to the stimulus and none displayed avoidance behaviour. After 90 days, fish were 2.19 times less likely (71.43%) to perform a C-start at 30 °C and 3.14 times less likely (63.64%) at 31 °C. Additionally, avoidance behaviour was returned (14.29%) and divided non C-start individuals in half with those showing no reaction (14.29%). There was significance in the regression model for directionality in *P. amboinensis* ($\chi^2(4) = 11{:}23$, $p = 0.024$; Fig. 4.1B). The model explained 28.70% (Nagelkerke R^2) of the variance in directionality of C-starts and correctly classified 67.60% of cases. Compared to controls, fish exposed to 30 °C for 4 days were 5 times more likely (33.33%) to turn towards the stimulus and 6.67 times more likely (40%) after 90 days. At 31 °C, fish were 25 times more likely (71.43%) to turn towards the stimulus, which was the same for both exposure durations. Temperature nor exposure duration had any significant effect on response latency ($F_{4,42} = 1:32$, $p = 0.27$, partial $\eta^2 = 0.053$; Fig. 4.2A), maximum swim speed ($F_{4,42} = 0.6$, $p = 0:66$ partial $\eta^2 = 0.056$; Fig. 4.2B), or response distance ($F_{4,42} = 0:65$, $p = 0.62$, partial $\eta^2 = 0.058$; Fig. 4.2C) for *P. amboinensis*.

For *P. moluccensis*, logistic regression model for responsiveness was statistically significant ($\chi^2(4) = 16.58$, $p = 0.002$; Fig. 4.1A). The model explained 28% (Nagelkerke R^2) of the variance in proportion of individuals that elicited a C-start and correctly classified 80% of cases. At control, 82.61% of fish displayed a C-start. Of the fish that did not C-start, most showed avoidance behaviour (13.04%) and no reaction (4.35%) least of all. Responsiveness rose to absolute (i.e., 100% of individuals performed a C-start) after 4 days of exposure to either 30 or 31 °C. When 90 day exposure was compared to controls, individuals were 1.45 times less likely (76.47%) to perform a C-start at 30 °C and 3.55 times less likely (57.14%) at 31 °C. For individuals that did not display a C-start, there was an increase in no reaction up to 38% at 31 °C (from 4.35% at control). There was no significance in the regression model for directionality ($\chi^2(4) = 5.98$, $p = 0.20$; Fig. 4.1B). Fish with 4 days of exposure to 30 °C were 2.8 times more likely (50%) to turn towards the stimulus compared to controls and 4.2 times more likely (60%) at 31 °C. However, this was reduced after prolonged exposure where directionality was very similar to control (23.08% and 25% for 30 and 31 °C, respectively). There was no significant effect of temperature and exposure duration on response latency ($F_{4,60} = 1.23$, $p = 0{:}30$, partial $\eta^2 = 0.052$; Fig. 4.2A), maximum swim speed ($F_{4,60} = 0.19$, $p = 0.94$, partial $\eta^2 = 0.013$; Fig. 4.2B), nor response distance ($F_{4,60} = 0.85$, $p = 0.49$, partial $\eta^2 = 0.054$; Fig. 4.2C) for *P. moluccensis*.

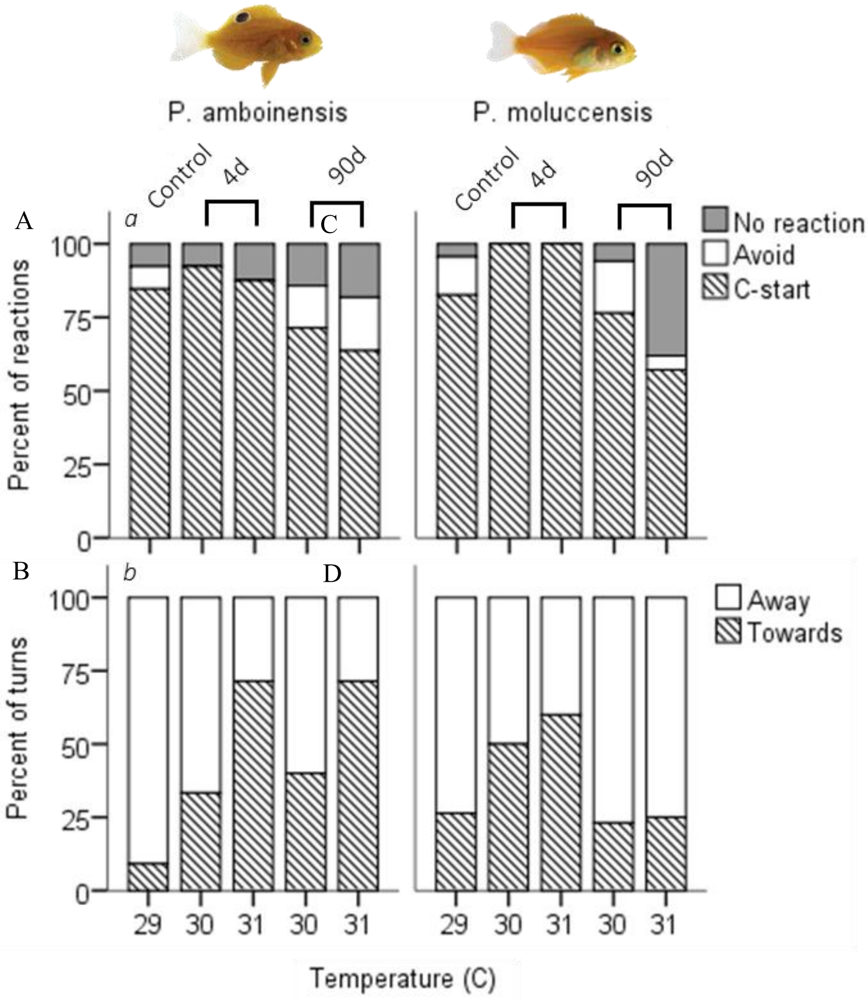

Fig 4.1. **Responsiveness and directionality to the startling stimulus**. Percent of reactions represents C-start responsiveness of juvenile *Pomacentrus amboinensis* (A) and *Pomacentrus moluccensis* to a drop stimulus at control, 4d or 90d exposure durations to elevated temperatures. Types of reaction are: no reaction (grey), avoidance (open), and C-start (striped). Directionality categorises only C-start individuals by the percent of turns made by *P. amboinensis* (B) and *P. moluccensis* (D) where the first movement of head was either away (open) or towards (striped) the stimulus.

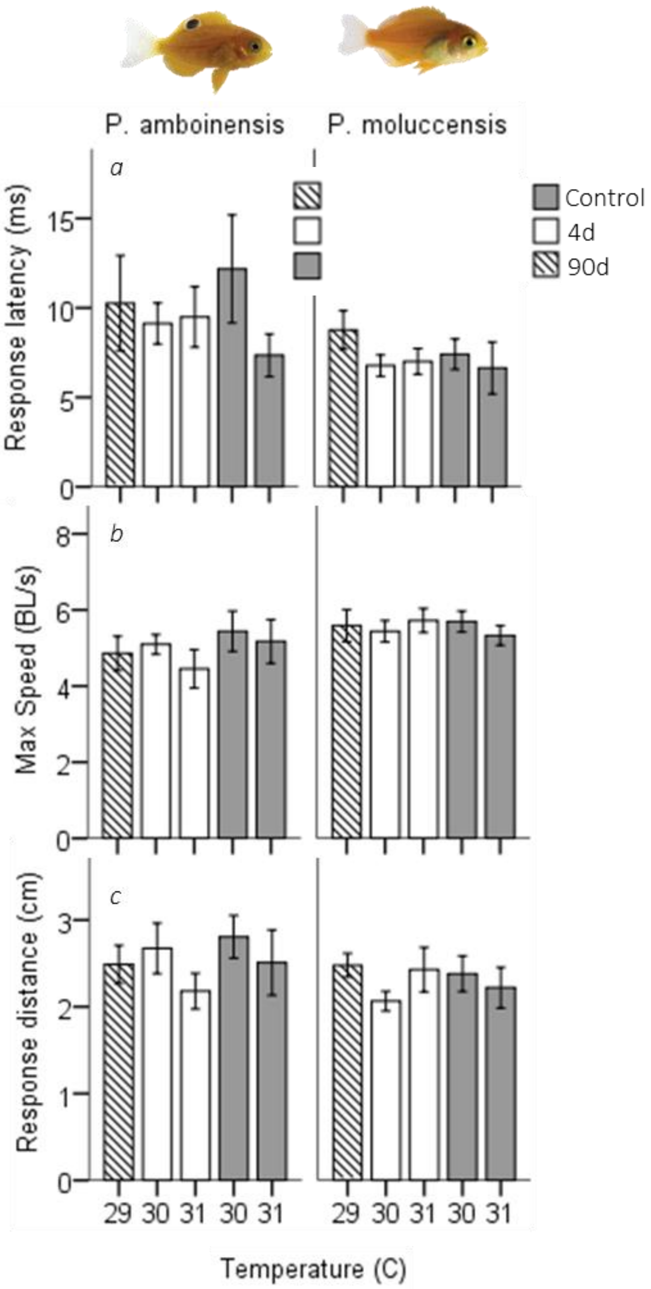

Fig 4.2. **Measures of kinematic variables to the drop stimulus**. Kinematic performance of fast starts by juvenile *P. amboinensis* and *P. moluccensis* at control (striped), 4d (open) or 90d (grey) exposure durations to elevated temperatures. Variables measured were response latency, max speed (body lengths s^{-1}), and escape distance (A, B, and C respectively for *P. amboinensis* and D, E, and F respectively for *P. moluccensis*).

4.5 Discussion

Studying how escape responses are affected by elevated temperature will be crucial when making predictions on the ability of prey to escape predators under climate change conditions (Gilman et al. 2010). Supporting our predictions, we found short-term exposure to elevated temperature negatively impacted directionality for both species. However, there was little effect on responsiveness, response latency, maximum, swim speed, and escape distance. After long-term exposure, directionality in *P. moluccensis* followed our predictions by reducing the number of turns towards the stimulus and returning to control levels. For *P. moluccensis*, long-term exposure also altered responsiveness with fish responding less often with a C-start response at elevated temperatures. Such alterations in the C-start responsiveness to predators is likely to change prey survival, while the differential effects of temperature on the two closely related species suggests that future temperature elevation may affect community composition through differential survival.

The ability for prey to perceive and avoid a predatory threat is essential to survival. Directionality in escape responses is important as turning towards a stimulus would increase exposure time to a predator (Domenici 2010). Short-term exposure to elevated water temperature negatively impacted directionality by increasing number of turns towards the stimulus. Previous study on goldfish also found directionality was impaired at increased temperatures compared to controls (Szabo et al. 2008). Altered directionality may have been due to an impairment of sensorimotor control (Foreman & Eaton 1993). While not significant, short-term exposure to elevated temperatures increased C-start responsiveness in *P. moluccensis* from 80% to 100%, and reduced avoidance reactions to 0% for both species. Generally, proportion of C-starts has been shown to increase with exposure to temperature both above (Szabo et al. 2008) and below control treatments (Preuss & Faber 2003), suggesting responsiveness may increase with thermal stress, at either extreme. Hyperactivity under stress can be beneficial to survival, as a fish that does not react during a predator attack will most likely get eaten. However, this strategy can risk expending additional energy on a false or non-threatening stimulus.

Extended exposure to new environmental conditions can alter the physiological and behavioural response of individuals (Angilletta 2009). We found directionality in *P. moluccensis* showed signs of beneficial plasticity after long-term exposure by returning to control levels. Long-term exposure may have reduced the negative impact of sensorimotor control brought on by thermal stress. Physiological plasticity to similar temperatures (+2 °C) in this species has also been shown to occur for aerobic metabolism (Grenchik et al. 2013) potentially demonstrating that *P. moluccensis* could exhibit beneficial plasticity to future projected temperatures in a range of traits. The proportion of C-starts reduced while avoidance behaviour increased with long-term exposure in both species. Decreasing the proportion of C-starts deviates further from control levels, therefore cannot be considered beneficial. However, future sea temperature increases are expected to exceed the thermal optimum for many reef fish (Tewksbury et al. 2008) forcing species to adopt energy saving strategies (Ydenberc & Dill 1986). Reducing the proportion of C-starts and replacing with avoidance behaviour may help compensate for the costs of increasing thermal conditions.

While other studies have shown that elevated temperature affects the locomotor performance in fast-starts (Beddow et al. 1995; Webb & Zhang 1994), we found projected increased sea temperatures had no effect on maximum swim speed or escape distances in our species, regardless of exposure duration. Similarly, we found no effect on response latency with temperature and exposure duration. The lack of change in performance at these temperatures indicates once a C-start was initiated, the physical ability to escape is maintained. This suggests future warming may have little effect on mortality rates. Although promising, this may be problematic because metabolic demands for fish increase with rising ambient temperature (Grenchik et al. 2013; Nilsson et al. 2010) and C-starts are high energy manoeuvres (Jayne & Lauder 1993). Without adjusting physiological costs, individuals would fall into an energy debt. Thus, the behavioural modulation of reducing C-start responsiveness after long-term exposure observed, may act as a means of balancing the increasing cost of C-starts at higher temperature.

To conclude, both species showed negative effects of increased temperature after short-term, though the impact was greater for *P. amboinensis*. After long-term exposure, *P. moluccensis* adjusted to temperature with beneficial plasticity and energy saving strategies. These results show the C-start escape response possesses thermal plasticity and extended exposure can induce beneficial changes. The difference in response by species matches a previous study showing *P. moluccensis* possessed a higher capacity for thermal plasticity in aggressive interactions (Warren et al. 2016). Differences in thermal plasticity for even closely related species suggest other reef fishes may also possess unequal capacities for plasticity. Changes in escape behaviour will ultimately affect mortality rates in prey and the species-specific response suggests some species will better cope with future temperatures than others leading to changes in populations sizes. Precise measurement of escape success, though, will require future study with only the prey, only the predator, and both exposed to elevated temperature as predators will also likely have specific responses to thermal conditions. Studies investigating the factors that influence predator-prey interactions, like this one, are crucial because these interactions determine population sizes and replenishment, leading to changes in community structure.

Chapter 5: Thermal plasticity of metabolic performance through extended exposure shows some impact on competitive dominance in two juvenile damselfish species

Authors: Donald T. Warren, Mark I. McCormick, & Jennifer M. Donelson

5.1 Abstract

Dominance within a social group can provide better access to food sources, higher quality shelter sites and preferred mates. Due to the potentially high energetic cost of competition, individuals with greater metabolic performance may be expected to have a competitive advantage. Yet, the link between dominance and physiology is still unclear with previous works attributing dominance to various metabolic traits. Understanding this link is important as future climates will be warmer and are predicted to have substantial impacts on metabolism. In this study, I measured metabolic rates in two species of juvenile damselfish, *Pomacentrus amboinensis* and *Pomacentrus moluccensis,* after short-term or long-term exposure to elevated temperatures. Metabolic rates were then linked to aggressive behaviour for each individual using previously collected data of size-matched intraspecific competitive contests. I found short-term exposure to elevated temperature resulted in substantial increases in maximum metabolic rate ($MO_{2\ Max}$), routine metabolic rate ($MO_{2\ Routine}$), and net aerobic scope (AS) in both species. There were significant reductions in metabolic variables after extended exposure, suggesting plasticity. In particular, *P. moluccensis* showed greater plasticity with virtually complete return in all three metabolic traits to control levels. When temperature was elevated to 31 °C (+2 °C above control) for 4d, individuals of *P. amboinensis* that displayed marked increases in metabolic rates tended to become subordinate in competitive contests. This suggests thermally sensitive

individuals may struggle in competitive interactions at elevated temperatures. However, oxygen consumption for dominants and subordinates was similar after 90d suggesting plasticity may have acted to equalise metabolic rates of both competitors. For *P. moluccensis*, there was little difference in metabolic rates between dominant and subordinate competitors after 4d. Yet, individuals that displayed greater decreases in metabolic rate after 90d exposure tended to become dominant suggesting thermal plasticity may invoke a competitive advantage. Contrary to previous research, individuals with greater plasticity, not metabolic rate, may have a competitive advantage.

5.2 Introduction

Social status can have major long-term and short-term consequences for the life-history characteristics and fitness of animals. High status individuals can obtain better access to resources, preferential mates, and have an enhanced survival rate compared to their lesser ranked counterparts in a variety of organisms (Fournier & Festa-Bianchet 1995; Metcalfe et al. 1990; Thorpe et al. 1992). In general, factors such as body size, aggression, and prior residence can be used to predict dominance (Huntingford & Turner 1987; Poulos & McCormick 2014). However, the contribution that individual physiological performance plays in the outcome of competitive interactions is less understood. Previous work on linking these traits has focused on resting metabolic rate with studies showing a general positive correlation with dominance (Biro & Stamps 2010; Metcalfe et al. 2016). This may be because higher metabolic rates correspond with a greater need to acquire food and therefore individuals must be more aggressive to compete for resources (Metcalfe et al. 1995). In contrast, increasing routine metabolic rate may reduce aggression if energy output is finite. This would leave individuals possessing greater metabolic rates with less resources to allocate to competitive activity (Careau et al. 2008). In addition, aerobic capacity (the difference between minimal and maximal metabolic rate) has also been proposed as an important trait influencing aggressive behaviour as this reflects the extent of oxygen-consuming processes an individual may undertake (Killen et al. 2007). If metabolism plays a key role in the determination of competitive dominance, then it may make it a good indicator of ecological success. Yet, currently the relationship between the two are poorly understood.

Physiological performance of ectotherms is reduced as environmental temperatures move away from the thermal optimum for an individual (Angilletta 2009; Portner & Farrell 2008; Schulte et al. 2011). This effect can be exacerbated in tropical species as they inhabit relatively stable thermal environments making them especially sensitive to thermal change (Deutsch et al. 2008; Huey et al. 2012; Tewksbury et al. 2008). At temperatures above optimum, the minimum energy required to maintain cell function increases with temperature, thus individuals require more energy for cell function and basic life processes (Clarke & Johnston 1999). Concurrently, limitation of the circulatory and ventilatory systems can mean individuals are unable to meet the greater maximum oxygen demand, leading to declines in aerobic function (Pörtner & Knust 2007). For coral reef fish, even slight increases of 2-3 °C can have substantial effects on oxygen consumption (Nilsson et al. 2009; Gardiner et al. 2010; Johansen & Jones 2011; Rummer et al. 2014). Yet, phenotypic plasticity can occur after exposure to new environmental conditions and alter thermal physiological responses (Angilletta 2009). While plasticity can be beneficial, allowing individuals to improve performance in an altered environment, its energetic cost can result in no plasticity occurring or even negative effects due to internal trade-offs (DeWitt et al. 1998). Previously, capacity for thermal plasticity has been observed in traits including aggressive interactions (Warren et al. 2016) and aerobic metabolism (Donelson et al. 2011; Grenchik et al. 2013) in fishes after extended exposure during early life to elevated temperature. How thermal plasticity of aerobic physiology to elevated temperatures may influence the results of competitive outcomes is still unknown.

The thermal regime experienced by marine fishes can alter over a range of temporal scales. On a seasonal scale, temperature fluctuation can be largely driven by yearly changes in solar irradiance (Leichter et al. 2006). On the Great Barrier Reef, Australia temperatures in tropical latitude locations can drop to 22 °C in the winter and up to 29 °C in summer, though the yearly fluctuation for higher-latitudes can be more than this (Beggs et al. 2011). Within a day, tropical coral reefs can experience 2-3 °C change and potentially more for shallow lagoons (McCabe et al. 2010). Currently, extreme climate change scenarios project coral reefs may reach temperatures as much as +4 °C above current averages by the end of the century, however more modest models project +1-2 °C by 2100 (Collins et al. 2013). This is frightening as several coral reef fish species have been shown to be currently living close to their thermal optimum for aerobic performance (Rummer et al. 2014). Consequently, variation in temperature over both short and long-term scales may significantly impact physiological and ecological performance of some fish species within the communities they inhabit.

Coral reef damselfish present a model system for examining the link between physiological performance and aggressive behaviour because competition for resources can be very intense during early life stages, i.e., immediately after juvenile fish migrate from their pelagic larval stage to settle to the reef (McCormick 2012). At this stage, over half of juveniles succumb to predation in the first two days (Almany & Webster 2006). Juveniles fiercely compete within and among species for optimal shelter sites offering refuge from predators and better access to food (McCormick & Weaver 2012). Many damselfish also exist in social groups where dominance status determines rank in the queue for later breeding (McCormick 2016). Therefore, the drive of competition for shelter during the settlement stage presents a selective bottleneck where aggressive dominance can be advantageous (Meekan et al. 2010). Yet, the extent of thermal plasticity of metabolic rates to elevated temperature and its contribution to competitive dominance has not been studied.

The present study examined the capacity of individuals to metabolically respond to acute and chronically exposed elevated temperatures. This response was then related to their success in size matched intra-specific contests. Two closely related species with differing responses to elevated temperature were compared (Warren et al. 2016), the Ambon damselfish *Pomacentrus amboinensis* and the lemon damselfish *Pomacentrus moluccensis*. The thermal sensitivity to elevated temperature was first examined by measuring maximum metabolic rate ($MO_{2\ Max}$), routine metabolic rate ($MO_{2\ Routine}$) and net aerobic scope (AS), after short-term exposure (4d) to elevated temperatures (+1 and +2 °C above summer average of 29 °C; AIMS 2014). Short-term temperature treatments were then compared to treatments with longer-term exposure (90d) to elevated temperature to investigate the capacity for thermal physiological plasticity. Finally, metabolic data was paired with previously collected competitive data of size matched intraspecific contests for a shelter resource to investigate the relationships between metabolic performance and competitive dominance for each individual.

5.3 Methods

Study species, collection, and holding facilities

The study species used were *P. moluccensis*, and *P. amboinensis*. These species co-occur and compete for the same habitat across their geographic distribution, spanning the Coral Sea up to Southeast Asia (Allen 1991). These species have been used in past physiology studies (Grenchik et al. 2013; Killen et al. 2014; Rummer et al. 2014) and competitive studies (McCormick & Weaver 2012; McCormick et al. 2013), making them familiar species. Like many reef fishes, these species undergo a pelagic larval phase of 2-3 weeks (Brothers et al. 1983), after which will make their final metamorphosis and settle to join the reef-associated juvenile population. Much of their later success can depend on events that happen early in life that influence their growth and behavioural decisions (McCormick 2016). This open life-cycle hampers rearing in the laboratory, Therefore, recently settled fish (1-2 months) were collected from reefs in the Cairns region (-16°78´S, 146°26´E) of the Great Barrier Reef, Australia during January 2014. Collection efforts targeted the smallest, and therefore the youngest individuals (standard length and mass, $\bar{x}$ ± SD; *P. moluccensis* 28.14 ± 3.69 mm, 1.09 ± 0.44 g; *P. amboinensis* 30.30 ± 5.86 mm, 1.45 ± 0.89 g).

Experimental design

Fishes were maintained at the experimental facilities at James Cook University, Townsville, in 40 L replicate tanks with flowing seawater. Juvenile fish were randomly divided into 3 temperature treatments: 29 °C (current-day summer average for the collection region; control; AIMS 2014), 30 °C, or 31 °C (based on projected temperatures for 2050 and 2100 respectively; Collins et al. 2013). Elevated temperature treatments were divided into 2 exposure durations: 4d or 90d. A 4d exposure duration was chosen to explore the effects of thermal sensitivity without causing a thermal stress response (Nilsson et al. 2010). The 90d duration was based on previous developmental studies using this species (Grenchik et al. 2013) and a closely related species of damselfish (Donelson & Munday 2012). In total, there were 10 treatments: 2 short-term exposures, 2 long-term, 1 control, and repeated for each species. Replicates ranged 18-20 individuals per treatment allowing for 9-10 competitive contests. Thermal sensitivity to elevated temperature was measured by comparing fish at control (29 °C) temperatures to individuals exposed to either 30 or 31 °C for a 4d period. To determine the extent of thermal plasticity, 4d exposure treatments to elevated temperatures were compared with 90d exposure treatments.

Holding tank temperatures were raised 1 °C/day to reduce the effect of heat shock (Gardiner et al. 2010). To control for any effect of time spent in captivity, the 4d treatments were first held at control temperature, then exposed to the randomly assigned temperature 4 days before the end of the long-term duration. This allowed all fish to finish temperature treatments at the same time. The control treatment remained at 29 °C for the entirety of the experiment. Holding temperatures and photoperiods fluctuated on a natural diurnal scale for the collection region (0.6 °C around the mean and 12:12h, respectively). Food was not limited and fish were fed a high performance commercial fish pellet (NRD 0.5-0.8 mm; Primo aquaculture) once or twice daily to satiation. Excess food was removed and tanks were cleaned via vacuum siphon weekly or as needed. After the relevant exposure period, wet weight and standard length were recorded and fish were tagged with coloured elastomer for individual recognition. This tagging procedure is known not to affect growth or mortality (Hoey & McCormick 2006). Fish were given a minimum of 24 h after tagging before respiration trials followed by an addition 24 h before competitive trials to help reduce handling stress.

Metabolic trials

The effects of water temperature on aerobic performance were determined by measuring routine oxygen consumption ($MO_{2\ Routine}$), maximum oxygen consumption ($MO_{2\ Max}$), and calculating net aerobic scope ($MO_{2\ Max} - MO_{2\ Routine}$). Fish were fasted for 24 h prior to testing to reduce influence of digestion on oxygen consumption. $MO_{2\ Routine}$ measurements were completed through static respirometry without a mixing mechanism (Seebacher et al. 2014; Sinclair et al. 2006). Previous work has shown that natural activity in coral reef damselfish provide sufficient mixing of the respiration chamber even in the absence of a mixing device for reliable results (Rodgers et al. 2016). Fish were placed in to 590 ml chambers, with continuous supply of temperature-controlled and aerated seawater. Chambers were darkened on all sides to reduce outside disturbance and fish were given a 2 h habituation period before testing commenced. This habituation time has been shown to be adequate for $MO_{2\ Routine}$ to stabilize after introduction to a respirometry chamber (Rummer et al. 2014). Next, habituation chambers were sealed and the fall of oxygen (O_2 %) was recorded for 30 mins using an oxygen sensor (Witrox oxygen meter, Loligo Systems) as an indication of routine oxygen consumption. $MO_{2\ Max}$ was measured immediately after $MO_{2\ Routine}$ based on methods by Nilsson et al. (2009; 2007) and Seebacher et al. (2013). Fish were placed into a cylindrical swim chamber (830 ml, 90 mm inner diameter) submerged in a temperature controlled reservoir (see Fig. 1 in Nilsson et al. 2007). A magnetic stir plate beneath the tank powered a stir bar (60 mm) to create a circular current for the fish to swim against. The speed of the current was controlled by the magnetic plate and slowly increased until the fish could sustain a maximal swimming speed while maintaining its position in the water column and without making (anaerobic) burst and glide movements. Once maximum speed was achieved, the fall of oxygen was recorded every second for 5 min. Oxygen levels within the chambers remained between 70-100% for both $MO_{2\ \mathrm{Routine}}$ and $MO_{2\ \mathrm{Max}}$ measurements.

Competitive interaction trials

This study paired metabolic data with aggression score data from competitive contests conducted by Warren et al. (2016). Fish were given at least one day of rest following respiration trials and then placed in competitive trials with individuals size-matched within 10 % of mean standard length. Pairs were placed into competitive arenas (12 × 48 × 32 cm) with a coral fragment (~5 cm^3) for shelter and as a resource for competition (see Fig 1 in Warren et al. 2016). Aggressive interactions were video recorded from above via a GoPro camera Hero3 for 10 mins for subsequent analysis of 3 behaviours: (i) displays, lateral fin flare towards opponent; (ii) attacks, chasing or biting of the opponent; and (iii) avoids, swimming away from an opposing attacks or display. Aggression scores for each individual were calculated as aggression = attacks + displays – avoids (McCormick & Weaver 2012; Warren et al. 2016).

Statistical analysis

Statistical analysis was performed using SPSS statistics (v24). $MO_{2\ \mathrm{Max}}$ and $MO_{2\ \mathrm{Routine}}$ ($mg\ O_2\ kg^{-1}\ h^{-1}$) was calculated for each fish from the recorded drop in oxygen concentration in the respiration chambers. Net aerobic scope was calculated as $MO_{2\ \mathrm{Max}} - MO_{2\ \mathrm{Routine}}$. One-factor analyses of variance tested the equality of each metabolic trait ($MO_{2\ \mathrm{Routine}}$, $MO_{2\ \mathrm{Max}}$, and AS) among the 5 treatment combinations (a fixed factor) for each species separately. Planned comparisons were then used to determine the nature of differences between treatments. To determine the effect of an individual's metabolic response to temperature on dominance within a contest, metabolic rates were categorised by dominance status (dominant or subordinate) and analysed with a repeated measures ANOVA. Here, each dominant-subordinate contest pair was the repeated measure (within-subject variable) and treatment combination was the between-subject factor. The magnitude of difference in metabolic rates between competitors (taken as the dominant rate minus subordinate and referred to as relative metabolism) was analysed with a one-way ANOVA using $MO_{2\ \mathrm{Routine}}$, $MO_{2\ \mathrm{Max}}$, and AS relative to the subordinate as separate dependent variables and treatment combination as the fixed factor. All models were repeated for each species separately. Residual analysis verified assumptions of normality and homogeneity.

5.4 Results

Metabolic rate

There was no effect of temperature nor exposure treatment on $MO_{2\ \mathrm{Routine}}$ in *P. amboinensis* (Fig 1*a*). However, there were significant effects on $MO_{2\ \mathrm{Max}}$ ($F_{4,88} = 4.17$, $p = 0.004$; Fig 5.1*b*) and AS ($F_{4,88} = 7.7$, $p < 0.001$; Fig 5.1*c*). Compared to control, short-term 4d exposure to elevated temperature increased $MO_{2\ \mathrm{Max}}$ as much as 67% and AS by 119%. After 90d, $MO_{2\ \mathrm{Max}}$ and AS showed a significant reduction compared to 4d, but this was only at 30 °C (Table 5.1; Fig 5.1*b,c*). For *P. moluccensis*, there were significant effects of treatment combination on $MO_{2\ \mathrm{Routine}}$, ($F_{4,94} = 3.99$, $p = 0.005$; Fig 5.1*d*), $MO_{2\ \mathrm{Max}}$ ($F_{4,94} = 17.53$, $p < 0.001$; Fig 5.1*e*) and AS ($F_{4,94} = 18.14$, $p < 0.001$; Fig 5.1*f*). Short-term exposure to elevated temperatures increased $MO_{2\ \mathrm{Routine}}$ up to 32%, $MO_{2\ \mathrm{Max}}$ by 66%, and AS by as much as 92%. The 90d exposure lead to significant reductions in all metabolic traits at both elevated temperatures (Table 5.1; Fig. 5.1*d,e,f*). In fact, all metabolic traits after 90d exposure were not different to control (planned comparisons, $p > 0.05$).

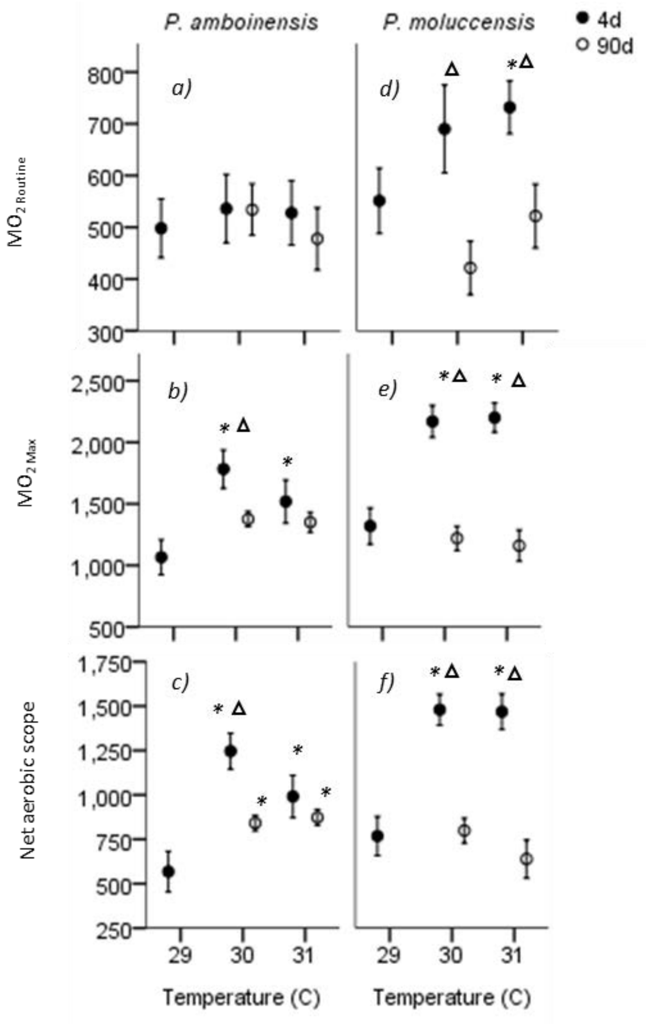

Fig 5.1. Metabolic rates across temperature and exposure durations. $MO_{2\ Routine}$ (*a*,*d*), $MO_{2\ Max}$ (*b*,*e*), and net aerobic scope (*c*,*f*) (mg O_2 kg^{-1} h^{-1} ± SE) are given for two damselfish species, *Pomacentrus amboinensis* (left) and *P. moluccensis* (right). Thermal sensitivity was determined by comparing 4d exposure (solid) to temperature with control, and capacity for plasticity was measured through comparisons of 4d with 90d exposure (open). Significant difference from control represented with (*) and (Δ) represents significant difference between exposure durations ($p < 0.05$).

Table 5.1. Planned comparisons of metabolic rates for temperature and exposure durations. There were six comparisons for each metabolic trait ($MO_{2\ \mathrm{Routine}}$, $MO_{2\ \mathrm{Max}}$, and AS) and replicated for each species, *Pomacentrus amboinensis* (*a*) and *P. moluccensis* (*b*). The first four comparisons measured control treatments against both temperatures at both exposure durations and the last two compared 4d and 90d exposure durations for each elevated temperature separately.

Treatment	**Compared to**	$\mathbf{MO_{2\ Routine}}$	$\mathbf{MO_{2\ Max}}$	**Net AS**
(*a*) *P. amboinensis*				
Control	30 °C, 4d	ns	$t_{88} = -3.96, p < 0.001$	$t_{88} = -5.4, p < 0.001$
	30 °C, 90d	ns	ns	$t_{88} = -2.23, p = 0.028$
	31 °C, 4d	ns	$t_{88} = -2.46, p = 0.016$	$t_{88} = -3.32, p = 0.001$
	31 °C, 90d	ns	ns	$t_{88} = -2.49, p = 0.014$
30 °C, 4d	30 °C, 90d	ns	$t_{88} = 2.3, p = 0.023$	$t_{88} = 3.31, p = 0.001$
31 °C, 4d	31 °C, 90d	ns	ns	ns
(*b*) *P. moluccensis*				
Control	30 °C, 4d	ns	$t_{94} = -4.82, p < 0.001$	$t_{94} = -5.26, p < 0.001$
	30 °C, 90d	ns	ns	ns
	31 °C, 4d	$t_{88} = -2.01, p = 0.047$	$t_{94} = -4.99, p < 0.001$	$t_{94} = -5.18, p < 0.001$
	31 °C, 90d	ns	ns	ns
30 °C, 4d	30 °C, 90d	$t_{94} = 2.99, p = 0.004$	$t_{94} = 5.38, p < 0.001$	$t_{94} = 5.04, p < 0.001$
31 °C, 4d	31 °C, 90d	$t_{94} = 2.31, p = 0.023$	$t_{94} = 5.82, p < 0.001$	$t_{94} = 6.06, p < 0.001$

Metabolism and competitive performance

There was a significant effect of dominance status on $MO_{2\ \mathrm{Routine}}$ for *P. amboinensis* (Wilk's $\Lambda = 0.91$, $F_{1,39} = 4.26$, $p = 0.046$; Fig 5.2*a*). However, there was no effect on $MO_{2\ \mathrm{Max}}$ (Wilk's $\Lambda = 0.97$, $F_{1,39} = 1.16$, $p = 0.28$; Fig 5.2*b*) nor AS (Wilk's $\Lambda = 0.99$, $F_{1,39} = 0.1$, $p = 0.75$; Fig 5.2*c*). There was no interaction of dominance status with treatment combination for any trait. However, there was a trend for differences in all metabolic traits between competitors to increase with short-term temperature exposure when compared to control. In fact, subordinates had significantly greater $MO_{2\ \mathrm{Routine}}$ (planned comparisons, $F_{1,83} = 4.52$, $p = 0.036$; Fig 5.2*a*) and $MO_{2\ \mathrm{Max}}$ ($F_{1,83} = 5.08$, $p = 0.027$; Fig 5.2*b*) at 31 °C. After 90d, this difference was close to zero, like controls. There was a non-significant trend for all relative metabolic rates to become more negative than controls with short-term temperature exposure, suggesting metabolic rates may be greater in subordinates at higher temperatures. In fact, all relative metabolic rates were significantly lower at 31 °C compared to controls ($MO_{2\ Routine}$, $t_{39} = 2.15$, $p = 0.037$; Fig 5.3*a*; $MO_{2\ Max}$, $t_{39} = 2.64$, $p = 0.012$; Fig 5.3*b*; AS, $t_{39} = 2.26$, $p = 0.029$; Fig 5.3*c*). After 90d, relative metabolism for all traits trended back towards control levels and both dominant and subordinate individuals had very similar metabolic rates (Fig. 5.3).

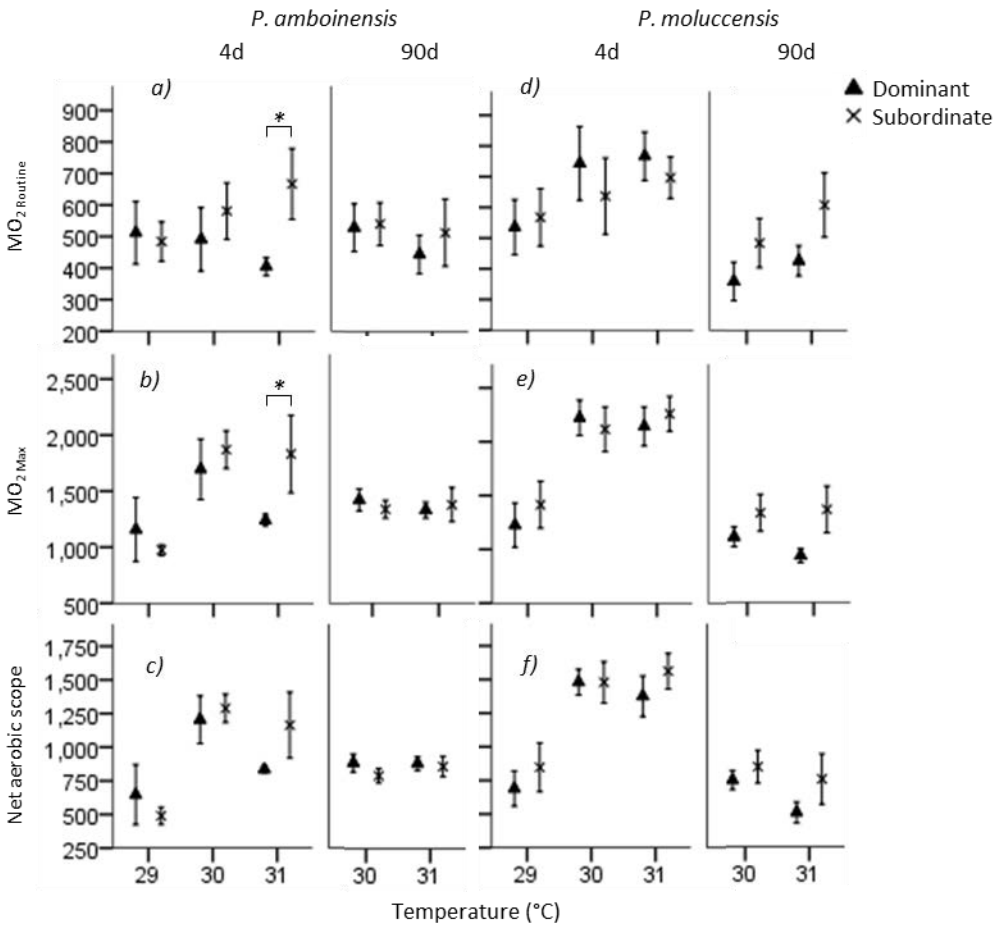

Fig 5.2. Metabolic rates for dominant and subordinate competitors. Metabolic performance of dominant (triangle) and subordinate ("x") competitors of *Pomacentrus amboinensis* and *P. moluccensis* are given. $MO_{2\ \mathrm{Routine}}$ (*a*,*d*), $MO_{2\ \mathrm{Max}}$ (*b*,*e*) and net aerobic scope (AS; *c*,*f*) (mg O_2 kg^{-1} h^{-1} ± SE). Temperature exposure durations were 4d (left subdivide) and 90d (right subdivide). Treatments with (*) represent significant difference between dominant and subordinate competitors (Planned comparisons, $p < 0.05$).

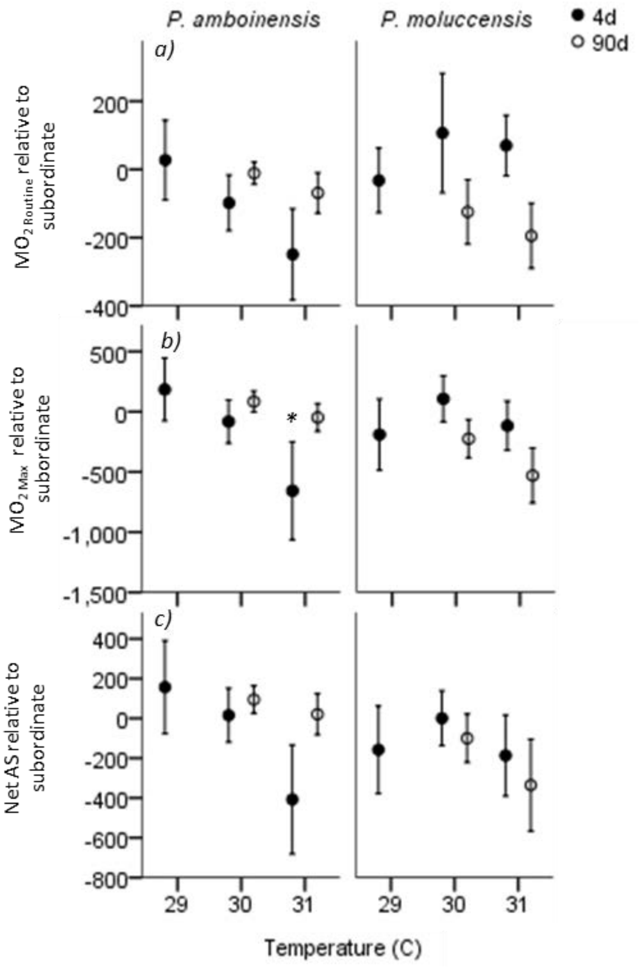

Fig 5.3. Difference in metabolic rates between competitors shown as relative metabolism. Relative MO_2 Routine (*a*), MO_2 Max (*b*), and AS (*c*) were calculated as dominant competitor minus subordinate (mg O_2 kg^{-1} h^{-1} ± SE) for competitive contests of two damselfish species, *Pomacentrus amboinensis* (left) and *P. moluccensis* (right). Effect of short-term exposure to elevated temperature was determined by comparing fish with 4d exposure (solid) to 30 or 31 °C with control treatments (29 °C). Significant difference from control represented with (*, planned comparison $p < 0.05$). Thermal plasticity was examined by comparing 4d with 90d (open) exposure at each elevated temperature, though no significance was found.

For *P. moluccensis,* there was no effect of dominance status on MO_2 Routine (Wilk's $\Lambda = 0.99$, $F_{1,44} = 0.45$, $p = 0.5$; Fig 5.2*d*), MO_2 Max (Wilk's $\Lambda = 0.92$, $F_{1,44} =$

3.74, $p = 0.06$; Fig 5.2*e*) nor AS (Wilk's $\Lambda = 0.92$, $F_{1,44} = 3.48$, $p = 0.068$; Fig 5.2*f*). There was also no interaction of dominance status on treatment combination, meaning temperature exposure impacted metabolic rates of both competitors similarly (Fig 5.2*d,e,f*). Yet, there was a trend for dominant competitors to have lower of all metabolic traits in the 90d treatment, suggesting that individuals who displayed more thermally plastic responses tended to become dominant. *P. moluccensis* also showed no effect of treatment combination on any relative metabolic rates. There were only trends for relative metabolic rate to be more negative after 90d exposure to elevated temperature compared 4d (Fig 5.3d*,e,f*), but these were not significant.

5.5 Discussion

There was evidence of thermal plasticity in aerobic performance after extended exposure to elevated temperature, though not equal across species. Short-term exposure to elevated temperature substantially increased $MO_{2\ Max}$ and AS for *P. amboinensis*, and $MO_{2\ Routine}$, $MO_{2\ Max}$, and AS for *P. moluccensis*. After extended exposure, both species showed some reductions back to control, but this was greater for *P. moluccensis* as all metabolic traits had completely returned to control levels. This suggests that while both species possess the capacity for thermal plasticity, capacity may be greater for *P. moluccensis*. When metabolic rates for each individual were paired with aggression scores in Warren et al. (2016), no single metabolic trait was strongly associated with a competitive advantage. More thermally sensitive individuals of *P. amboinensis* tended to become subordinate, but plasticity may have acted to equalise metabolic traits for competitors after extended exposure. For *P. moluccensis*, short-term temperature influenced both competitors similarly. However, individuals that displayed greater plasticity after extended exposure tended to become dominant. Evidence within this study support other studies showing that damselfish can demonstrate thermal plasticity, which was aided by exposure time (Donelson et al. 2011; Grenchik et al. 2013; Warren et al. 2016), and may produce a competitive advantage at elevated temperatures.

There were distinct differences in thermal sensitivities and the plasticity of metabolic responses between the two damselfish tested. For *P. amboinensis*, short-term temperature had virtually no effect on $MO_{2\ \mathrm{Routine}}$ but increased $MO_{2\ \mathrm{Max}}$ and AS, with the biggest difference from control at 30 °C. With extended exposure to elevated temperatures, fish lowered their $MO_{2\ \mathrm{Max}}$ to being indistinguishable from control levels. This may indicate this species is close to its physiological optimum and that its capacity for physiological plasticity may only partially restore metabolic performance. In contrast, short-term exposure to elevated temperature had substantial effects on all three metabolic traits in *P. moluccensis*, with a complete return to control levels after 90 days. Previous studies of this species have reported increases in $MO_{2\ \mathrm{Routine}}$, $MO_{2\ \mathrm{Max}}$, and AS after short-term exposure to similar temperatures (Nilsson et al. 2009; Gardiner et al. 2010; Rummer et al. 2014). Yet, these studies have not incorporated extended exposure duration that may induce plasticity. Our results indicate both species showed some capacity for thermal plasticity, though the greater response in *P. moluccensis* suggests this species may be better able to metabolically adapt to a future warmer ocean. Decreasing $MO_{2\ \mathrm{Routine}}$ after extended exposure would be beneficial as it represents a lower cost of basic living processes. However, reducing aerobic scope and $MO_{2\ \mathrm{Max}}$ may restrict the maximal and total range for aerobic activity, which could be detrimental. This suggests there may be long-term costs to sustaining increases in $MO_{2\ \mathrm{Max}}$ and AS at elevated temperatures.

Differences in thermal sensitivity and plasticity for competing individuals may influence the outcome of intraspecific competitive interactions. While there was no evidence for a single metabolic trait that predicted dominance in competitive contests, there was a trend for all relative metabolic traits to become more negative than control with increasing temperature for *P. amboinensis*. This suggests that subordinate individuals are more likely to be those with higher metabolic rates. This contradicts previous study showing a positive relationship between dominance and $MO_{2\ \mathrm{Routine}}$ (Cutts et al. 1998; Metcalfe et al. 1995; Yamamoto et al. 1998) or AS (Killen et al. 2014). However, these studies did not include the potential stress of elevated temperature. Individuals more thermally sensitive should display greater increases in metabolic rates. Additionally, the stress of losing a competitive interaction can also increase metabolic rate (Senar et al. 2000). In our study, competition may have acted as a secondary stressor to elevated temperature resulting in individuals already thermally sensitive to be at a competitive disadvantage. For *P. moluccensis*, short-term temperature had similar impacts on physiological performance for dominant and subordinates alike. This suggests there may be little variation in thermal sensitivity within the population. There was also little variation between competitors in plasticity response after extended exposure, however those displaying greater reductions tended to become dominant in contests. This suggests within species, individuals with greater potential for thermal plasticity may have a competitive advantage.

Variation of thermal plasticity within populations will likely be a key factor to coping with temperature change. Across populations, adaptation to local environments can result in differing responses to temperature change for the same species (Eliason et al. 2011; Gardiner et al. 2010). Yet, not all individuals within a population will possess similar tolerance to temperature change either. Even within the more thermally tolerant species, *P. moluccensis*, we found some individuals showed a greater plasticity response to new thermal environments than their conspecifics. These more plastic fish then tended to become dominant when matched in competitive contests. Previously, within population variation of metabolic rates has been acknowledged (Metcalfe et al. 2016) though strong correlations with hereditary or experienced environment have not been found. While studies have recently begun to show tropical reef fish can possess capacity for thermal plasticity, this study shows how within population variation in plasticity may benefit competition. This may act as a selective mechanism that favours individuals with greater capacity for plasticity. An interesting field of study will be the investigation of how this individual variation in thermal plasticity can affect other population processes. Directions for future research may include determining the extent of this within population variation and its influence on other behaviours and performance in different ecological processes.

Chapter 6: General discussion

Understanding the impacts of future climate change on coral reef fishes is crucial as tropical reefs are particularly vulnerable to temperature change (Tewksbury et al. 2008). The four chapters of this thesis employ various laboratory and field studies to measure the effects of increased temperature and exposure duration on competition, antipredator performance, and physiology of reef damselfishes. Exposing individuals to elevated temperature provided information on the thermal sensitivity of reef fishes. By exposing fishes to elevated temperature for different time periods, this thesis indicates the level of thermal plasticity reef damselfish currently possess to cope with future climate change. Results suggest that elevated temperature may indeed have negative impacts, however there is plasticity after longer periods of exposure to elevated temperature. Furthermore, there is a distinct species-specific response to increased temperature and level of plasticity. This specificity may lead to restructuring of reef assemblages and community hierarchies.

Effects of increased temperature on reef fishes

The key aims of this thesis were to describe how projected future temperatures will impact several traits in damselfish to gain a more comprehensive understanding of the vulnerability of reef fishes to climate change. My focus was on the influence of temperature on intra- and interspecific interactions, as these are the building blocks of social organisation, assemblage composition and community dynamics. **Chapters 2 & 3** quantified aggressive interactions in four species of damselfish at current-day controls and elevated temperatures based on climate change projections. Results showed that aggression levels during a contest were influenced by water temperature and the magnitude of change was species-specific. While previous work has studied aggressive interactions in damselfish (Killen et al. 2014; McCormick & Weaver 2012; Poulos & McCormick 2014) this thesis is one of the first studies to examine how water temperature may alter those interactions.

Measuring individual aggression level during competitive bouts is important because competitively dominant individuals may have an advantage. Increasing aggression during competitive bouts can be advantageous as dominant individuals can acquire higher quality shelter locations offering access to food sources and better protection against predators, which can consume over half of recruiting larval fish in the first two days (Almany & Webster 2006). Two of the species, *P. nagasakiensis* and *P. moluccensis,* displayed a trend for an increase in aggression with temperature. This aligns with previous study showing greater levels of activity, boldness, and aggression at elevated temperatures within species (Biro et al. 2010) and may be due to the increased metabolic rate at rising temperatures in fish (**Chapter 5**; Gardiner et al. 2010; Nilsson et al. 2010; Rummer et al. 2014). This suggests these species may be living below their thermal optimum for competitive performance with future projections of warming leading to a competitive benefit.

For *P. amboinensis,* aggressive scores were reduced with temperature. If the capacity to be aggressive is advantageous, this suggests that this species may already be living at or even above its thermal optimum at current-day temperatures. Further increases in temperature would approach their thermal maximum and competitive performance would begin to decline. Here, individuals must balance the energetic cost of aggressive behaviour with the benefit of acquiring a shelter resource. On one side, more aggressive individuals may gain a shelter, however, this must compensate for the energetic cost of aggressive encounters and potential risk of injury. Finally, the level of aggressive behaviour of *P. chrysurus* had overall greater averages and was unaffected by temperature change. The lack of change in aggression suggests this species may have a broader performance curve with changes in temperature resulting in only small changes in behavioural performance. Broader performance curves are more associated with thermal generalist to accommodate a wider range of temperatures they may experience. While these species have specific site preferences on the reef, e.g. live coral, coral rubble, or algal-covered patches (Feary 2007), many damselfishes can be found at similar depths and exposed to similar temperature regimes (Allen 1991). Alternatively, the higher overall average in aggression scores may be the result of a greater pressure for competition regardless of surrounding temperature. This could be due to the experimental design if the competition arena did not appropriately reflect home range size.

The contrasting trends in aggression with temperature for *P. amboinensis* and *P. moluccensis* may explain how species dominance changes with temperature during interspecific contests. At ambient temperatures, the outcome of the contest was close between species, though favoured *P. amboinensis*. Previous work has shown that when body size and priority of access to resources is accounted for, *P. amboinensis* is dominant over *P. moluccensis* (McCormick & Weaver 2012). The current study shows that at elevated temperatures, this outcome reversed as *P. moluccensis* trended to win more contests. Interestingly, reversal in competitive dominance between these species has also shown to occur under elevated CO_2 levels (McCormick et al. 2013), suggesting *P. moluccensis* is robust to various environmental stressors.

Though the relationship between aggression and temperature can have implications for future ocean warming, the effect of temperature can also be observed currently with respect to natural thermal fluctuation. Fish species with a planktonic larval stage, such as fishes of the genus *Pomacentrus*, settle in pulses with the lunar cycle over the summer season (Milicich et al. 1992). Individuals that settle earlier in the season will experience cooler temperatures while the later arriving individuals will be exposed to warmer temperatures during settlement. Early arriving cohorts may have more selections for shelter sites, less competition for food sources, and have a head start on growth giving this cohort an advantage. Conversely, later recruiting cohorts may have an advantage if they settle closer to their thermal optimum, increasing individual performance. Depending on the thermal performance of the species, the timing of recruitment could play a substantial role in survival rate of juveniles (Poulos 2016). Future studies could address whether optimal performance temperature for species correlates with recruitment abundance over seasonal temperatures.

Rising temperature was also shown to have an effect on antipredator escape responses of juvenile fishes. **Chapter 4** explored how elevated water temperature could impact the performance in C-starts that fish use when faced by the threat of a predatory strike. Contrary to other work, temperature had less effect on the physical ability to react and instead appeared to alter the decision-making process. For *P. amboinensis* and *P. moluccensis*, elevated temperatures decreased the level of avoidance behaviour to a startling stimulus and impaired the directionality during an escape response leading to more reactions turning towards the simulated predator. This supports the notion that C-start reactions have remarkable adaptability to continuously varying environments (Korn & Faber 2005) and performance is context-dependent with a large input of behavioural control (Domenici 2010).

The proportion of individuals that reacted to a threat, i.e. responsiveness, is important because individuals that do not react during a predatory attack have a higher probability of being eaten. For *P. moluccensis*, the proportion of C-start reactors increased to 100% at elevated temperatures. This suggests that temperature reduced the stimulus threshold for a burst start, potentially by reducing the input resistance in neurons (Faber 1989). However, temperature impaired directionality as both species turned towards the stimulus more often at elevated temperature. Turning towards a potential threat can increase exposure time and chance of capture. Previous work on C-starts has shown elevated temperature can increase responsiveness and impair directionality (Szabo et al. 2008). However, similar responses in these traits has been observed after exposure to colder temperatures as well (Preuss & Faber 2003) suggesting these traits may be sensitive to any temperature change. The mechanism of directionality during an escape response results in signalling from sensory cells to stimulate of one of the two large Mauthner cells (Domenici 2010). Temperature may have impacted this signalling mechanism leading to individuals reacting towards the threat.

Finally, **Chapter 5** measured how water temperature can influence physiology and link changes in metabolic rates to competitive dominance. Results showed that maximum oxygen consumption, routine oxygen consumption, and aerobic scope all increased with water temperature for both *P. moluccensis* and *P. amboinensis*. Individual metabolic performance was then paired with competitive performance from **Chapter 3** to determine how competitive dominance relates to physiology. There was no association of individuals with greater metabolic rates than their opponents to become dominant in competitive contests. However, there was a trend for subordinate *P. amboinensis* to have slightly greater $MO_{2\ Routine}$, $MO_{2\ Max}$, and AS after short-term exposure to elevated temperature. This difference was diminished with extended exposure. Comparatively, short-term temperature exposure had similar effects on both competitors of *P. moluccensis*. With extended exposure, there was a trend for dominant competitors to have lower of all three metabolic traits.

An increase in metabolism for temperatures 2 °C above ambient is expected as ectotherms increase energy demand in warmer environments (Clarke & Johnston 1999) and this matches with previous study on several reef fish (Gardiner et al. 2010; Rummer at al. 2014) with short-term exposure to similar temperatures. In theory, individuals with greater metabolic rates should outperform their competitors and therefore have an advantage during aggressive encounters (Metcalfe et al. 2016). Yet, the stress of losing competitive contests can also increase metabolic rates (Senar et al. 2000), which could be amplified under additional thermal stress. Studies have found a variable relationship between metabolic performance and dominance, with some finding dominance linked to routine metabolic rate (Metcalfe et al. 1995) while others report dominance is linked with aerobic scope (Killen et al. 2014), and one showing no link with metabolism at all (Seppänen et al. 2009). Furthermore, measuring traits like $MO_{2\,Max}$ would be irrelevant if competitors do not reach these extremes during contests, which has been shown to occur when competitors are placed into separate but neighbouring respirometry chambers (Killen et al. 2014). Regardless of species, the present study shows no single trait was related to competitive dominance.

Potential for plasticity

Along with quantifying the effects of increased temperature, there is also the need to investigate the level of phenotypic plasticity that fishes possess, which will allow for adjustment to new thermal environments. Until recently, it was predicted that tropical species had a limited capacity for plasticity to increasing temperatures (Deutsch et al. 2008; Wright et al. 2011), and this was shown to be true for metabolic rates in some coral reef fishes (Nilsson et al. 2009). My study joins more recent works that demonstrate there is capacity in juvenile fishes for developmental plasticity of physiology to projected temperatures (Donelson et al. 2011; Grenchik et al. 2013;

Chapter 5). Furthermore, the present study is the first to show that exposing juveniles to elevated temperature for prolonged durations results in plasticity for performance in ecological processes like competition (**Chapter 3**) and antipredator responses (**Chapter 4**).

P. amboinensis and *P. moluccensis* exhibited different trends in their plasticity for aggressive interactions after extended exposure to elevated temperature (**Chapter 3**). For *P. amboinensis*, there was a further decrease in aggression in 90d treatments compared to 4d exposure at the highest temperature. In contrast, aggressive behaviour in *P. moluccensis* trended back to control levels after 90d, with the biggest difference from 4d treatments at 31 °C. The reduction in aggression after 90d shows a loss of competitive performance, however may be a sign of trade-offs. Competitive behaviour can be energetically expensive and increase the risk of injury (Briffa & Sneddon 2007), and warmer temperatures are already costly environments for ectotherms. The increasing energy costs to compete at elevated temperature may not have outweighed the benefits of dominance resulting in little pressure to compete and mild aggressive behaviour between competitors.

There was also evidence of thermal plasticity in C-start performance after extended exposure to elevated temperature (**Chapter 4**). The negative effect of short-term exposure to elevated temperature on directionality was mitigated for *P. moluccensis* by long-term exposure, with values returning to control levels. However, *P. amboinensis* showed no signs of plasticity as there was no change in directionality of the response between exposure durations. The proportion of individuals that reacted with a C-start to the startle stimulus after 90d was significantly lower than 4d for both species, but avoidance behaviour was restored. The reduction in responsiveness suggests the stimulus threshold for eliciting a C-start increased and this may be a mechanism to avoid energy debt at higher temperatures. Previous work has shown decreased responsiveness may occur through repeated stimulation over short intervals leading to habituation (Eaton et al. 1977) or varying stimulus strengths affecting the perception of threat (Domenici 2010). However, an increased stimulus threshold may mean individuals are not responding to a potential danger. This may explain the increase in avoidance behaviour, as this behaviour offers a less costly method of escape. Plasticity likely reflected a trade-off between energy expenditure of performing a C-start with the perception of a threatening stimulus.

Plasticity was also found in metabolism for *P. amboinensis* and *P. moluccensis* after 90d exposure (**Chapter 5**). For *P. amboinensis,* $MO_{2\ Max}$ and AS after 90d were significantly reduced at 30 °C while $MO_{2\ Max}$, $MO_{2\ Routine}$, and AS for *P. moluccensis* were all significantly lower after 90d for both temperatures. Previously, developmental plasticity has been observed to occur in freshwater systems as a means to restore swimming performance at various acclimation temperatures (Johnson & Bennett 1995; Scott & Johnston 2012). For coral reef fish, developmental plasticity can reduce the negative effects of elevated temperature in routine metabolic rate (Donelson et al. 2011; Grenchik et al. 2013). Interestingly, the reduction in metabolism back to control levels after 90d in *P. moluccensis* occurred concurrently with a reduction in overall contest aggression levels. Coincidently, *P. amboinensis* showed little plasticity in metabolic rates with extended exposure and contest aggression levels deviated further from control. This suggests that although there were only minor differences in metabolic rate between competitors, the overall level of aggression was reflective of metabolism.

This study adds to the growing number of examples of plasticity in coral reef fishes (Donelson et al. 2011; Grenchik et al. 2013). This study shows the capacity for thermal plasticity does not only occur in physiology but also ecological processes and responses, like competitive behaviour and antipredator escape responses. The accumulation of research demonstrating plasticity from earlier life stages illustrates that, while sensitive to temperature change, coral reef fishes may be capable of coping with future temperatures better than previously expected. Yet, there is a cost of maintaining plasticity (Hofmann & Todgham 2010; Reylea 2002) that may involve trade-offs (e.g. loss of growth rate or body condition; Angilletta 2006, 2009), and plasticity may not occur if the benefits do not outweigh costs. Consequently, to fully understand the mechanisms of plasticity, a range of traits need to be studied as a benefit in performance for one trait may not be seen for others.

Species-specific thermal tolerances

Results from these four chapters show that thermal sensitivity and the potential for adjusting to rising temperature will vary from species to species (**Chapters 2-5**). Due to this, some species will be more vulnerable to temperature change and possess a lower capacity for thermal plasticity. One species, *P. moluccensis,* showed exceptional ability to cope with increased temperature for multiple traits and displayed a tremendous capacity for plasticity. In fact, *P. moluccensis* showed signs of becoming the dominant species at elevated temperatures when in contests with the less plastic *P. amboinensis*. Changes in species dominance hierarchy with temperature have been observed for other systems (Taniguchi et al. 1998), showing differential thermal tolerance is most likely ubiquitous across systems. These differences will not only affect interactions among competing species within trophic groups but also across trophic levels between predators and prey altering capture rates. This shows how temperature may affect future reef assemblages and dominance hierarchies.

Concluding remarks

This thesis suggests that short-term exposure to future climate change temperatures is likely to have negative impacts on a range of traits in reef fishes. However, there is a capacity for adjusting performance through plasticity after long-term exposure to temperature. Tropical species are already predicted to be more vulnerable to rising temperatures and this research shows within tropical ecosystems, there will be species particularly sensitive to thermal changes. However, this study involved testing the performance of two populations from the same latitude, while these species can be found from the southern Great Barrier Reef and as far north as Indonesia (Allen 1991). This geographic range encompasses a thermal range greater than the projected increase in ocean temperatures suggesting populations are likely adjusted to their local thermal environment. Previous work on physiological performance in several damselfish species across a latitudinal gradient show strikingly similar performance optima, around 31 °C, even for higher latitude sites that rarely experience those temperatures (Rummer et al. 2014). This would give the basis for distribution change as summer averages for equatorial locations may soon exceed performance optima for metabolism (Perry et al. 2005). Although many damselfish are site attached after settling, their demersal larval stage allows for redistribution of populations. Future research could involve similar cross latitude studies to determine if performance curves for other traits (like competition) are consistent across locations. Measures of multiple traits should be included to gain a comprehensive understanding of how performance in various processes will respond to future warming.

References

AIMS. (2014). *Water temperature for Janurary 2014, Arlington and Moores reefs*. Retrieved from http://data.aims.gov.au/aimsrtds/datatool.xhtml?from=2014-01-01&thru=2014-06-03&period=DAY&aggregations=MIN,MAX&channels=1751,100203,1896

Allan B J M, Domenici P, McCormick M I, Watson S A, & Munday P L. (2013). Elevated CO2 affects predator-prey interactions through altered performance. *PLoS ONE*, *8*(3), e58520. doi:10.1371/journal.pone.0058520

Allan B J M, Domenici P, Munday P L, & Mccormick M I. (2015). Feeling the heat: the effect of acute temperature changes on predator – prey interactions in coral reef fish. *Conservation Physiology*, *3*, 1–8. doi:10.1093/conphys/cov011.Introduction

Allen G. (1991). *Damselfishes of the World*. Mergus Publishers, Melle, Germany.

Almany G R. (2003). Priority effects in coral reef fish communities. *Ecology*, *84*(7), 1920–1935. doi:10.1890/0012-9658(2003)084[1920:PEICRF]2.0.CO;2

Almany G R, & Webster M S. (2006). The predation gauntlet: Early post-settlement mortality in reef fishes. *Coral Reefs*, *25*(1), 19–22. doi:10.1007/s00338-005-0044-y

Andersen I L, Nævdal E, & Bøe K E. (2011). Maternal investment, sibling competition, and offspring survival with increasing litter size and parity in pigs (Sus scrofa). *Behavioral Ecology and Sociobiology*, *65*(6), 1159–1167. doi:10.1007/s00265-010-1128-4

Angilletta M J. (2006). Estimating and comparing thermal performance curves.

Journal of Thermal Biology, *31*, 541–545. doi:10.1016/j.jtherbio.2006.06.002

Angilletta M J. (2009). *Thermal adaptation: a theoretical and emprirical synthesis*. Oxford University Press.

Angilletta M J, Niewiarowski P H, & Navas C A. (2002). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, *27*(4), 249–268. doi:10.1016/S0306-4565(01)00094-8

Angilletta M J, Wilson R S, Navas C A, & James R S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology and Evolution*, *18*(5), 234–240. doi:10.1016/S0169-5347(03)00087-9

Beddow T, Leeuwen J, & Johnston I. (1995). Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish Myoxocephalus scorpius. *The Journal of Experimental Biology*, *198*(Pt 1), 203–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9317640

Beggs H, Zhong A, Warren G, Alves O, Brassington G, & Pugh T. (2011). RAMSSA - An operational, high-resolution, regional Australian multi-sensor sea surface temperature analysis over the Australian region. *Australian Meteorological and Oceanographic Journal*, *61*(1), 1–22. doi:10.22499/2.6101.001

Biro P A, Beckmann C, & Stamps J A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1678), 71–77. doi:10.1098/rspb.2009.1346

Biro P A, & Stamps J A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology* *and Evolution*, *25*(11), 653–659. doi:10.1016/j.tree.2010.08.003

Bonduriansky R. (2012). Rethinking heredity, again. *Trends in Ecology and Evolution*, *27*(6), 330–336. doi:10.1016/j.tree.2012.02.003

Botkin D, Saxe H, Araujo M, & Betts R. (2007). Forecasting the effects of global warming on biodiversity, *57*(3), 227–236. doi:10.1641/B570306

Briffa M, & Sneddon L U. (2007). Physiological constraints on contest behaviour. *Functional Ecology*, *21*(4), 627–637. doi:10.1111/j.1365-2435.2006.01188.x

Brothers E B, Williams D M, & Sale P F. (1983). Length of larval life in twelve families of fishes at “One Tree Lagoon”, Great Barrier Reef, Australia. *Marine Biology*, *76*(3), 319–324.

Bullock T H. (1984). Comparative neuroethology of startle, rapid escape, and giant fiber-mediated responses. In R. C. Eaton (Ed.), *Neural Mechanisms of Startle Behavior* (pp. 1–13). Boston, MA: Springer US. doi:10.1007/978-1-4899-2286-1_1

Caley M J, Carr M H, Hixon M A, Hughes T P, Jones G P, & Menge B A. (1996). Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics*, *27*(1), 477–500. doi:10.1146/annurev.ecolsys.27.1.477

Careau V, Thomas D, Humphries M M, & Réale D. (2008). Energy metabolism and animal personality. *Oikos*, *117*, 641–653. doi:10.1111/j.0030-1299.2008.16513.x

Chambers L E, Altwegg R, Barbraud C, Barnard P, Beaumont L J, Crawford R J M, … Wolfaardt A C. (2013). Phenological Changes in the Southern Hemisphere. *PLoS ONE*, *8*(10), e75514. doi:10.1371/journal.pone.0075514

Chivers D P, McCormick M I, Mitchell M D, Ramasamy R A, & Ferrari M C O. (2014). Background level of risk determines how prey categorize predators and non-predators. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1787), 20140355–20140355. doi:10.1098/rspb.2014.0355

Clarke A, & Johnston N M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, *68*(5), 893–905. doi:10.1046/j.1365-2656.1999.00337.x

Collins M, Knutti R, Arblaster J, Dufresne J, Fichefet T, & Friedlingstein P. (2013). Long-term Climate Change: Projections, Commitments and Irreversibility. In T. Stocker, D. Qin, G. Plattner, M. Tognor, J. Boschung, A. Nauels, … P. Midgley (Eds.), *Climate Change 2013: The Physical Science Basis. Contributing work to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Cossins A R, & Bowler K. (1987). The direct effects of temperature changes. In *Temperature Biology of Animals* (pp. 23–60). Dordrecht: Springer Netherlands. doi:10.1007/978-94-009-3127-5_2

Cury de Barros F, Eduardo de Carvalho J, Abe A S, & Kohlsdorf T. (2010). Fight versus flight: the interaction of temperature and body size determines antipredator behaviour in tegu lizards. *Animal Behaviour*, *79*(1), 83–88. doi:10.1016/j.anbehav.2009.10.006

Cutts C J, Adams C, & Campbell A. (2001). Stability of physiological and behavioural determinants of performance in Arctic char (Salvelinus alpinus). *Canadian Journal of Fisheries and Aquatic Sciences*, *58*(5), 961–968.

doi:10.1139/cjfas-58-5-961

Cutts C J, Metcalfe N B, & Taylor a C. (1998). Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *Journal of Fish Biology*, *52*(5), 1026–1037. doi:10.1111/j.1095-8649.1998.tb00601.x

De Staso J, & Rahel F J. (1994). Influence of water temperature on interactions between juvenile colorado river cutthroat trout and brook trout in a laboratory stream. *Transactions of the American Fisheries Society*, *123*(April), 289–297. doi:10.1577/1548-8659(1994)123<0289:IOWTOI>2.3.CO;2

Deutsch C A, Tewksbury J J, Huey R B, Sheldon K S, Ghalambor C K, Haak D C, & Martin P R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(18), 6668–6672. doi:10.1073/pnas.0709472105

DeWitt T J, Sih A, & Wilson D S. (1998). Cost and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, *13*(97), 77–81. doi:10.1111/j.1558-5646.2009.00647.x

Domenici P. (2010). Context-dependent variability in the components of fish escape response: Integrating locomotor performance and behavior. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *313 A*(2), 59–79. doi:10.1002/jez.580

Domenici P, & Blake R W. (1997). The kinematics and performance of fish fast-start swimming. *The Journal of Experimental Biology*, *200*, 1165–1178.

Donelson J M, & Munday P L. (2012). Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. *Journal of Animal Ecology*, *81*(5), 1126–1131. doi:10.1111/j.1365-2656.2012.01982.x

Donelson J M, Munday P L, Mccormick M I, & Nilsson G E. (2011). Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Global Change Biology*, *17*(4), 1712–1719. doi:10.1111/j.1365-2486.2010.02339.x

Donelson J M, Munday P L, McCormick M I, & Pitcher C R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, *2*(1), 30–32. doi:10.1038/nclimate1323

Doney S C, Ruckelshaus M, Duffy J E, Barry J P, Chan F, English C A, … Talley L D. (2012). Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, *4*(1), 11–37. doi:10.1146/annurev-marine-041911-111611

Eaton R C, Bombardieri R A, & Meyer D L. (1977). The Mauthner-initiated startle response in teleost fish. *The Journal of Experimental Biology*, *66*, 65–81.

Eliason E J, Clark T D, Hague M J, Hanson L M, Gallagher Z S, Jeffries K M, … Farrell A P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science*, *332*(6025), 109–112. doi:10.1126/science.1199158

Faber D S, Fetcho J R, & Korn H. (1989). Neuronal networks underlying the escape response in goldfish. *Annals of the New York Academy of Sciences*, *563*, 11–33. doi:10.1111/j.1749-6632.1989.tb42187.x

Farrell A P. (2002). Cardiorespiratory performance in salmonids during exercise at high temperature: Insights into cardiovascular design limitations in fishes. *Comparative Biochemistry and Physiology - A Molecular and Integrative*

Physiology, *132*(4), 797–810. doi:10.1016/S1095-6433(02)00049-1

Feary D A, Almany G R, McCormick M I, & Jones G P. (2007). Habitat choice, recruitment and the response of coral reef fishes to coral degradation. *Oecologia*, *153*(3), 727–737. doi:10.1007/s00442-007-0773-4

Ferrari M C O, Dixson D L, Munday P L, Mccormick M I, Meekan M G, Sih A, & Chivers D P. (2011). Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: Implications for climate change projections on marine communities. *Global Change Biology*, *17*(9), 2980–2986. doi:10.1111/j.1365-2486.2011.02439.x

Ferrari M C O, Munday P L, Rummer J L, Mccormick M I, Corkill K, Watson S-A, … Chivers D P. (2015). Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities. *Global Change Biology*, *21*, 1848–1855. doi:10.1111/gcb.12818

Foreman M B, & Eaton R C. (1993). The direction change concept for reticulospinal control of goldfish escape. *The Journal of Neuroscience*, *13*(10), 4101–4113.

Fournier F, & Festa-Bianchet M. (1995). Social-dominance in adult female mountain goats. *Animal Behavior*, *49*(6), 1449–1459. doi:10.1016/0003-3472(95)90066-7

Frédérich B, & Parmentier E (Eds.). (2016). *Biology of Damselfishes*. Boca Raton, FL 33487-2742, USA: CRC Press, Taylor & Francis Group, Boca Raton, FL 33487-2742, USA.

Fuiman L A, Meekan M G, & McCormick M I. (2010). Maladaptive behavior reinforces a recruitment bottleneck in newly settled fishes. *Oecologia*, *164*(1), 99–108. doi:10.1007/s00442-010-1712-3

Gabriel W, Luttbeg B, Sih A, & Tollrian R. (2005). Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. *The American Naturalist*, *166*(3), 339–353. doi:10.1086/432558

Gardiner N M, Munday P L, & Nilsson G E. (2010). Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE*, *5*(10), e13299. doi:10.1371/journal.pone.0013299

Geange S W, Poulos D E, Stier A C, & McCormick M I. (2017). The relative influence of abundance and priority effects on colonization success in a coral-reef fish. *Coral Reefs*, *36*(1), 151–155. doi:10.1007/s00338-016-1503-3

Gilman S E, Urban M C, Tewksbury J, Gilchrist G W, & Holt R D. (2010). A framework for community interactions under climate change. *Trends in Ecology and Evolution*, *25*(6), 325–331. doi:10.1016/j.tree.2010.03.002

Grenchik M K, Donelson J M, & Munday P L. (2013). Evidence for developmental thermal acclimation in the damselfish, Pomacentrus moluccensis. *Coral Reefs*, *32*(1), 85–90. doi:10.1007/s00338-012-0949-1

Harley C D G, Hughes A R, Hultgren K M, Miner B G, Sorte C J B, Thornber C S, … Williams S L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228–241. doi:10.1111/j.1461-0248.2005.00871.x

Hoegh-Guldberg O, & Bruno J F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*(2), 1523–1528. doi:10.1126/science.1189930

Hoegh-Guldberg O, Poloczanska E S, & Richardson A J. (2013). Australia's marine resources in a warm, acid ocean. In P. Christoff (Ed.), *Four Degrees of Global*

Warming: Australia in a Hot World (p. 84). Routledge, 270 Madison Avenue, New York, NY 10016.

Hoey A S, & McCormick M I. (2006). Effects of subcutaneous fluorescent tags on the growth and survival of a newly settled coral reef fish, Pomacentrus amboinensis (Pomacentridae). *Proceedinas of 10th International Coral Reef Symposium*, *2006*, 420–424. doi:10.1007/s00442-004-1489-3

Hoffmann A A. (1995). Acclimation: Increasing survival at a cost. *Trends in Ecology and Evolution*, *10*(1), 1–2. doi:10.1016/S0169-5347(00)88949-1

Hoffmann A A, Chown S L, & Clusella-Trullas S. (2013). Upper thermal limits in terrestrial ectotherms: How constrained are they? *Functional Ecology*, *27*(4), 934–949. doi:10.1111/j.1365-2435.2012.02036.x

Huey R B, & Berrigan D. (1996). Testing evolutionary hypotheses of acclimation. *Animals and Temperature: Phenotypic and Evolutionary Adaptation*, *59*, 205–237.

Huey R B, Berrigan D, Gilchrist G W, & Herron J C. (1999). Testing the Adaptive Significance of Acclimation: A Strong Inference Approach. *American Zoologist*, *39*, 323–336. doi:10.1093/icb/39.2.323

Huey R B, Kearney M R, Krockenberger A, Holtum J a M, Jess M, & Williams S E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *367*(1596), 1665–79. doi:10.1098/rstb.2012.0005

Huey R B, & Kingsolver J G. (1989). Evolution of thermal sensitivty of ectotherm performance. *Tree*, *4*(5), 131–135.

Huey R B, & Stevenson R D. (1979). Integrating Thermal Physiology and Ecology of Ectotherms - Discussion of Approaches. *American Zoologist*, *19*(1), 357–366.

Hughes L. (2000). Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution*, *15*(2), 56–61. doi:10.1016/S0169-5347(99)01764-4

Huntingford F A, & Turner A K. (1987). The consequences of animal conflict. In F. A. Huntingford & A. K. Turner (Eds.), *Animal Conflict* (pp. 227–250). Dordrecht: Springer Netherlands. doi:10.1007/978-94-009-3145-9_9

Jayne B C, & Lauder G V. (1993). Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. *Journal of Comparative Physiology A*, *173*(4), 495–508. doi:10.1007/BF00193522

Johansen J L, & Jones G P. (2011). Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. *Global Change Biology*, *17*(9), 2971–2979. doi:10.1111/j.1365-2486.2011.02436.x

Johnson T, & Bennett A. (1995). The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *The Journal of Experimental Biology*, *198*, 2165–75.

Johnston I A, & Dunn J. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Symposia of the Society for Experimental Biology*, *41*, 67–93.

Johnston I A, Fleming J D, & Crockford T. (1990). Thermal acclimation and muscle contractile properties in cyprinid fish. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *259*(2 28-2), 231–236.

Johnston I A, & Temple G K. (2002). Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. *J Exp Biol*, *205*(Pt 15), 2305–2322. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12110664

Jones G P, McCormick M I, Srinivasan M, & Eagle J V. (2004). Coral decline threatens fish biodiversity in marine reserves. *Proceedings of the National Academy of Sciences*, *101*(21), 8251–8253. doi:10.1073/pnas.0401277101

Killen S S, Costa I, Brown J a, & Gamperl A K. (2007). Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings. Biological Sciences / The Royal Society*, *274*(1608), 431–438. doi:10.1098/rspb.2006.3741

Killen S S, Marras S, Metcalfe N B, McKenzie D J, & Domenici P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology and Evolution*, *28*(11), 651–658. doi:10.1016/j.tree.2013.05.005

Killen S S, Mitchell M D, Rummer J L, Chivers D P, Ferrari M C O, Meekan M G, & Mccormick M I. (2014). Aerobic scope predicts dominance during early life in a tropical damselfish. *Functional Ecology*, *28*(6), 1367–1376. doi:10.1111/1365-2435.12296

Kordas R L, Harley C D G, & O'Connor M I. (2011). Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. *Journal of Experimental Marine Biology and Ecology*, *400*(1–

2), 218–226. doi:10.1016/j.jembe.2011.02.029

Korn H, & Faber D S. (2005). The Mauthner cell half a century later: A neurobiological model for decision-making? *Neuron*, *47*(1), 13–28. doi:10.1016/j.neuron.2005.05.019

Leichter J J, Helmuth B, & Fischer A M. (2006). Variation beneath the surface: Quantifying complex thermal environments on coral reefs in the Caribbean, Bahamas and Florida. *Journal of Marine Research*, *64*(4), 563–588. doi:10.1357/002224006778715711

Leis J M, & Mccormick M I. (2002). The biology, behavior, and ecology of the pelagic, larval stage of coral reef fishes. In P. F. Sale (Ed.), *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem* (pp. 171–199). Academic Press, Elsevier Science, New York, NY.

Leroi A M, Bennett A F, & Lenski R E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(5), 1917–1921. doi:10.1073/pnas.91.5.1917

Levins R. (1969). Thermal acclimation and heat resistance in Drosophila species. *The American Naturalist*, *103*(933), 483–499.

Lima S L, & Dill L M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*(4), 619–640. doi:10.1139/z90-092

Lüthi D, Le Floch M, Bereiter B, Blunier T, Barnola J-M, Siegenthaler U, … Stocker T F. (2008). High-resolution carbon dioxide concentration record 650,000-

800,000 years before present. *Nature*, *453*(7193), 379–382. doi:10.1038/nature06949

McCabe R M, Estrade P, Middleton J H, Melville W K, Roughan M, & Lenain L. (2010). Temperature variability in a shallow, tidally isolated coral reef lagoon, *115*, 1–17. doi:10.1029/2009JC006023

McCormick M I. (2009). Behaviourally mediated phenotypic selection in a disturbed coral reef environment. *PLoS ONE*, *4*(9), e7096. doi:10.1371/journal.pone.0007096

McCormick M I. (2012). Lethal effects of habitat degradation on fishes through changing competitive advantage. *Proc Roy Soc Lond B*, *279*(1744), 3899–3904. doi:10.1098/rspb.2012.0854

McCormick M I. (2016). Protogyny in a tropical damselfish : females queue for future benefit. *PeerJ*, 4:e2198. doi:10.7717/peerj.2198

McCormick M I, & Meekan M G. (2007). Social facilitation of selective mortality. *Ecology*, *88*(6), 1562–1570. doi:10.1890/06-0830

McCormick M I, & Molony B W. (1995). Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. *Marine Ecology Progress Series*, *118*, 59–68. doi:10.3354/meps118059

McCormick M I, Moore J A Y, & Munday P L. (2010). Influence of habitat degradation on fish replenishment. *Coral Reefs*, *29*(3), 537–546. doi:10.1007/s00338-010-0620-7

McCormick M I, Watson S-A, & Munday P L. (2013). Ocean acidification reverses competition for space as habitats degrade. *Sci. Rep.*, *3*, 3280.

doi:10.1038/srep03280

McCormick M I, & Weaver C J. (2012). It pays to be pushy: Intracohort interference competition between two reef fishes. *PLoS ONE*, *7*(8), e42590. doi:10.1371/journal.pone.0042590

Mcleod I M, Rummer J L, Clark T D, Jones G P, McCormick M I, Wenger A S, & Munday P L. (2013). Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability, *1*(1), cot024. doi:10.1093/conphys/cot024

Meekan M G, Carleton J H, McKinnon A D, Flynn K, & Furnas M. (2003). What determines the growth of tropical reef fish larvae in the plankton: Food or temperature? *Marine Ecology Progress Series*, *256*, 193–204. doi:10.3354/meps256193

Meekan M G, von Kuerthy C, McCormick M I, & Radford B. (2010). Behavioural mediation of the costs and benefits of fast growth in a marine fish. *Animal Behaviour*, *79*(4), 803–809. doi:10.1016/j.anbehav.2009.12.002

Meekan M G, Wilson S G, Halford A, & Retzel A. (2001). A comparison of catches of fishes and invertebrates by two light trap designs, in tropical NW Australia. *Marine Biology*, *139*(2), 373–381. doi:10.1007/s002270100577

Metcalfe N B, Huntingford F A, Thorpe J E, & Adams C E. (1990). The effects of social-status on life-history variation in juvenile salmon. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, *68*(12), 2630–2636.

Metcalfe N B, Taylor A C, & Thorpe J E. (1995). Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour*, *49*, 431–436.

Metcalfe N B, Van Leeuwen T E, & Killen S S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology*, *88*(1), 298–321. doi:10.1111/jfb.12699

Milazzo M, Mirto S, Domenici P, & Gristina M. (2013). Climate change exacerbates interspecific interactions in sympatric coastal fishes. *Journal of Animal Ecology*, *82*(2), 468–477. doi:10.1111/j.1365-2656.2012.02034.x

Milicich M J, Meekan M G, & Doherty P J. (1992). Larval supply: a good predictor of recruitment of three species of reef fish (Pomacentridae). *Marine Ecology Progress Series*, *86*(2), 153–166. doi:10.3354/meps087153

Moksnes P O. (2004). Interference competition for space in nursery habitats: Density-dependent effects on growth and dispersal in juvenile shore crabs Carcinus maenas. *Marine Ecology Progress Series*, *281*, 181–191. doi:10.3354/meps281181

Mora C, & Ospína A F. (2001). Tolerance to high temperatures and potential impact of sea warming on reef fishes of Gorgona Island (tropical eastern Pacific). *Marine Biology*, *139*(4), 765–769. doi:10.1007/s002270100626

Munday P L, McCormick M I, & Nilsson G E. (2012). Impact of global warming and rising CO2 levels on coral reef fishes: what hope for the future? *Journal of Experimental Biology*, *215*(22), 3865–3873. doi:10.1242/jeb.074765

Munday P L, Warner R R, Monro K, Pandolfi J M, & Marshall D J. (2013). Predicting evolutionary responses to climate change in the sea. *Ecology Letters*, *16*(12), 1488–1500. doi:10.1111/ele.12185

Nettle D, & Bateson M. (2015). Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? *Proc R Soc B*, *282*, 20151005. doi:10.1098/rspb.2015.1005

Newman R A. (1998). Ecological constraints on amphibian metamorphosis: Interactions of temperature and larval density with responses to changing food level. *Oecologia*, *115*(1–2), 9–16. doi:10.1007/s004420050485

Nilsson G E, Crawley N, Lunde I G, & Munday P L. (2009). Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology*, *15*(6), 1405–1412. doi:10.1111/j.1365-2486.2008.01767.x

Nilsson G E, Östlund-Nilsson S, & Munday P L. (2010). Effects of elevated temperature on coral reef fishes: Loss of hypoxia tolerance and inability to acclimate. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *156*(4), 389–393. doi:10.1016/j.cbpa.2010.03.009

Nilsson G E, Ostlund-Nilsson S, Penfold R, & Grutter A S. (2007). From record performance to hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef. *Proceedings of The Royal Society B.*, *274*(January), 79–85. doi:10.1098/rspb.2006.3706

Nilsson J, & Gårdmark a. (2001). Sibling competition affects individual growth strategies in marsh tit, Parus palustris, nestlings. *Animal Behaviour*, *426*(June), 182–196. doi:10.1006/anbe.2000.1602

Parmesan C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(2006), 637–669. doi:10.1146/annurev.ecolsys.37.091305.110100

Pauly D. (1980). On the interrelationships between natural mortality, growth parameters, and mean environmental temparature in 175 fish stocks. *Journal Du Conseil*, *39*(2), 175–192.

Perry A L, Low P J, Ellis J R, & Reynolds J D. (2005). Climate change and distribution shifts in marine fishes. *Science*, *308*(5730), 1912–1915. doi:10.1126/science.11111322

Pörtner H. (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, *88*(4), 137–146. doi:10.1007/s001140100216

Portner H O, & Farrell A P. (2008). Physiology and climate change. *Science*, *322*(5902), 690–692. doi:10.1126/science.1163156

Pörtner H O, & Knust R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, *315*(5808), 95–98. doi:10.1126/science.1135471

Poulos D E. (2016). *Priority effects and the dynamics of coral reef fish assemblages*. James Cook University.

Poulos D E, & McCormick M I. (2014). Who wins in the battle for space? The importance of priority, behavioural history and size. *Animal Behaviour*, *90*, 305–314. doi:10.1016/j.anbehav.2014.02.003

Precht H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blodded animals. In *Physiological Adaptation* (pp. 50–78). Amercan Physiological Society, Washington, D.C.

Preuss T, & Faber D S. (2003). Central cellular mechanisms underlying temperature-dependent changes in the goldfish startle-escape behavior. *The Journal of*

Neuroscience : The Official Journal of the Society for Neuroscience, *23*(13), 5617–5626. doi:23/13/5617 [pii]

Pruitt J N, Demes K W, & Dittrich-Reed D R. (2011). Temperature mediates shifts in individual aggressiveness, activity level, and social behavior in a spider. *Ethology*, *117*(4), 318–325. doi:10.1111/j.1439-0310.2011.01877.x

Ramasamy R A, Allan B J M, & McCormick M I. (2015). Plasticity of escape responses: Prior predator experience enhances escape performance in a coral reef fish. *PLoS ONE*, *10*(8), e0132790. doi:10.1371/journal.pone.0132790

Reese C D, & Harvey B C. (2002). Temperature-dependent interactions between juvenile steelhead and Sacramento pikeminnow in laboratory streams. *Transactions of the American Fisheries Society*, *131*, 599–606. doi:10.1577/1548-8659(2002)131<0599

Rodgers G G, Tenzing P, & Clark T D. (2016). Experimental methods in aquatic respirometry: The importance of mixing devices and accounting for background respiration. *Journal of Fish Biology*, *88*(1), 65–80. doi:10.1111/jfb.12848

Rome L C. (2007). The effect of temperature and thermal acclimation on the sustainable performance of swimming scup. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *362*(1487), 1995–2016. doi:10.1098/rstb.2007.2083

Rummer J L, Couturier C S, Stecyk J A W, Gardiner N M, Kinch J P, Nilsson G E, & Munday P L. (2014). Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology*, *20*(4), 1055–1066. doi:10.1111/gcb.12455

Schaefer J, & Ryan A. (2006). Developmental plasticity in the thermal tolerance of zebrafish Danio rerio. *Journal of Fish Biology*, *69*(3), 722–734. doi:10.1111/j.1095-8649.2006.01145.x

Scheffers B R, De Meester L, Bridge T C L, Hoffmann A A, Pandolfi J M, Corlett R T, … Watson J E M. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, *354*(6313), 719. doi:10.1126/science.aaf7671

Schulte P M, Healy T M, & Fangue N A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, *51*(5), 1–12. doi:10.1093/icb/icr097

Scott G R, & Johnston I A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proceedings of the National Academy of Sciences*, *109*(35), 14247–14252. doi:10.1073/pnas.1205012109

Seebacher F, Beaman J, & Little A G. (2014). Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Functional Ecology*, *28*(1), 137–148. doi:10.1111/1365-2435.12156

Seebacher F, Holmes S, Roosen N J, Nouvian M, Wilson R S, & Ward A J W. (2012). Capacity for thermal acclimation differs between populations and phylogenetic lineages within a species. *Functional Ecology*, *26*(6), 1418–1428. doi:10.1111/j.1365-2435.2012.02052.x

Seebacher F, Ward A J, & Wilson R S. (2013). Increased aggression during pregnancy comes at a higher metabolic cost. *J Exp Biol*, *216*(Pt 5), 771–776. doi:10.1242/jeb.079756

Senar J C, Polo V, Uribe F, & Camerino M. (2000). Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. *Animal Behaviour*, *59*, 103–110. doi:10.1006/anbe.1999.1281

Sentis A, Morisson J, & Boukal D S. (2015). Thermal acclimation modulates the impacts of temperature and enrichment on trophic interaction strengths and population dynamics. *Global Change Biology*, *21*(9), 3290–3298. doi:10.1111/gcb.12931

Seppänen E, Tiira K, Huuskonen H, & Piironen J. (2009). Metabolic rate, growth and aggressiveness in three Atlantic salmon Salmo salar populations. *Journal of Fish Biology*, *74*(3), 562–575. doi:10.1111/j.1095-8649.2008.02142.x

Sih A, Crowley P, Mcpeek M, Petranka J, & Strohmeier K. (1985). Predation, competition, and prey communities : A review of field experiments author. *Annual Review of Ecology and Systematics*, *16*, 269–311.

Sinclair E L E, Thompson M B, & Seebacher F. (2006). Phenotypic flexibility in the metabolic response of the limpet Cellana tramoserica to thermally different microhabitats. *Journal of Experimental Marine Biology and Ecology*, *335*(1), 131–141. doi:10.1016/j.jembe.2006.03.010

Sunday J M, Bates A E, & Dulvy N K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceeding of the Royal Society of London B: Biological Science*, *278*(1713), 1823–1830. doi:10.1098/rspb.2010.1295

Sunday J M, Calosi P, Dupont S, Munday P L, Stillman J H, & Reusch T B H. (2014). Evolution in an acidifying ocean. *Trends in Ecology & Evolution*, *29*(2), 117–125. doi:10.1016/j.tree.2013.11.001

Szabo T M, Brookings T, Preuss T, & Faber D S. (2008). Effects of temperature acclimation on a central neural circuit and its behavioral output. *J Neurophysiol*, *100*, 2997–3008. doi:10.1152/jn.91033.2008

Taniguchi Y, Rahel F J, Novinger D C, & Gerow K G. (1998). Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*(8), 1894–1901. doi:10.1139/f98-072

Tewksbury J J, Huey R B, & Deutsch C A. (2008). Putting the heat on tropical animals. *Science*, *320*(5881), 1296–1297. doi:10.1126/science.1159328

Thorpe J E, Metcalfe N B, & Huntingford F A. (1992). Behavioural influences on life-history variation in juvenile Atlantic salmon, Salmo salar. *Environmental Biology of Fishes*, *33*(4), 331–340. doi:10.1007/BF00010944

Tracy R L, & Walsberg G E. (2001). Developmental and acclimatory contributions to water loss in a desert rodent: Investigating the time course of adaptive change. *Journal of Comparative Physiology - B Biochemical, Systemic, and Environmental Physiology*, *171*(8), 669–679. doi:10.1007/s003600100218

Uller T. (2008). Developmental plasticity and the evolution of parental effects. *Trends in Ecology and Evolution*, *23*(8), 432–438. doi:10.1016/j.tree.2008.04.005

Wakeling J M, & Johnston I A. (1998). Muscle power output limits fast-start performance in fish. *The Journal of Experimental Biology*, *201*, 1505–1526.

Walker J A, Ghalambor C K, Griset O L, McKenney D, & Reznick D N. (2005). Do faster starts increase the probability of evading predators? *Functional Ecology*, *19*(5), 808–815. doi:10.1111/j.1365-2435.2005.01033.x

Walther G R, Post E, Convey P, Menzel A, Parmesan C, Beebee T J C, … Bairlein F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389–395. doi:10.1038/416389a

Warren D T, Donelson J M, McCormick M I, Ferrari M C O, & Munday P L. (2016). Duration of exposure to elevated temperature affects competitive interactions in juvenile reef fishes. *Plos One*, *11*(10), e0164505. doi:10.1371/journal.pone.0164505

Webb P W. (1976). The effect of size on the fast-start performance of rainbow trout Salmo cairdneri, and a consideration of piscivorous predator-prey interactions. *Journal of Experimental Biology*, *65*, 157–177. doi:10.1016/S1546-5098(05)23009-1

Webb P W. (1986). Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (Micropterus salmoides). *Canadian Journal of Fisheries and Aquatic Sciences*, *43*(4), 763–771. doi:10.1139/f86-094

Webb P W, & Zhang H B. (1994). The relationship between responsiveness and elusiveness of heat-shocked goldfish (Carrassius auratus) to attacks by rainbow-trout (Oncorhynchus mykiss). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, *72*(3), 423–426.

Weetman D, Atkinson D, & Chubb J. (1998). Effects of temperature on anti-predator behaviour in the guppy, Poecilia reticulata. *Animal Behaviour*, *55*, 1361–1372. doi:10.1006/anbe.1997.0666

West-Eberhard M. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Williams S E, Moritz C, Shoo L P, Isaac J L, Hoffmann A a, & Langham G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol*, *6*(12), e325. doi:10.1371/journal.pbio.0060325

Wilson R S, Franklin C E, & Franklin C E. (2002). Testing the beneficial acclimation hypothesis, *17*(2), 66–70. doi:10.1016/S0169-5347(01)02384-9

Wilson R S, Lefrançois C, Domenici P, & Johnston I A. (2010). *The effect of environmental factors on the unsteady swimming performance of fish*. (P. Domenici & B. G. Kapoor, Eds.), *Fish Locomotion: an Eco-Ethological Perspective*. Enfield, NH, USA: Science Publishers.

Woods H A, & Harrison J F. (2001). The beneficial acclimation hypothesis versus acclimation of specific traits: physiological change in water - stressed Manduca sexta caterpillars. *Physiological and Biochemical Zoology : Ecological and Evolutionary Approaches*, *74*(1), 32–44.

Woods H A, & Harrison J F. (2002). Interpreting rejections of the beneficial acclimation hypothesis: When is physiological plasticity adaptative? *Evolution*, *56*(9), 1863–1866.

Wright S J, Muller-landau H C, & Schipper J A N. (2009). The future of tropical species on a warmer planet. *Conservation Biology*, *23*(6), 1418–1426. doi:10.1111/j.1523-1739.2009.01337.x

Yamamoto T, Ueda H, & Higashi S. (1998). Correlation among dominance status, metabolic rate and otolith size in masu salmon. *Journal of Fish Biology*, *52*(2), 281–290. doi:10.1111/j.1095-8649.1998.tb00799.x

Ydenberc R C, & Dill L M. (1986). The Economics of Fleeing from Predators.

Advances in the Study of Behavior, *16*(1986), 229–249. doi:10.1016/S0065-3454(08)60192-8

Appendix 1: Publications not arising from this thesis

Chivers D, McCormick MI, Warren DT, Allan BJ, Ramasamy RA, Arvizu B, Glue M, Ferrari M *in review*. Competitive superiority versus predation savvy: The two sides of behavioural lateralization. *Functional Ecology*.

Krist AC, Kay AD, Scherber E, Larkin K, Brown BJ, Lu Diana, Warren DT, Riedl, R, Neiman M 2016. Evidence for extensive but variable nutrient limitation in New Zealand lakes. *Evolutionary Ecology* doi:10.1007/s10682-016-9855-2.

Palacios MM, Warren DT, McCormick MI 2015. Sensory cues of a top-predator indirectly control a reef fish mesopredator. *Oikos* 000:001-009, doi:10.1111/oik.02116.