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Climate change and the future for coral reef fishes: the potential for acclimation

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Jennifer Marie DONELSON BSc (Hons) Qld

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Statement on the Contributions of Others

This thesis includes collaborative work with my supervisors Prof. Philip Munday, Prof. Mark McCormick and Dr. C. Roland Pitcher, as well as Prof. Ned Pankhurst, Dr. Patricia Pankhurst and Dr. Göran Nilsson. While undertaking these collaborations, I was responsible for research concept and design, data collection, analysis and interpretation of results. My co-authors provided intellectual guidance, editorial assistance, financial support and technical assistance.

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General Abstract

Knowledge of the likely responses species to rapid climate change is important for effective future conservation and management. Critical to this is an understanding of the ability of species to cope with environmental alterations through acclimation (phenotypic changes) and/or adaptation (genetic changes through selection). The response of species to environmental change has generally been predicted by observing their current-day thermal sensitivity, yet this may not be sufficient to determine responses to climate change over the next 50-100 years. Tropical ectotherms are expected to be one of the most sensitive groups to climate change since they have evolved in relatively stable thermal conditions and do not physiologically regulate their internal body temperature. The lack of thermal variability experienced had led to the belief that tropical ectotherms will have limited ability to acclimate, however this hypothesis has not been rigorously tested. This study utilized a common coral reef fish *Acanthochromis polyacanthus* to investigate the potential for acclimation to future ocean conditions over multiple years and multiple generations.

To begin understanding the long-term impacts of climate change, knowledge of how current-day populations respond to the predicted environmental changes is required. This can serve as a baseline for testing the ability to acclimate over multiple years and multiple generations. The effects that elevations in sea water temperature and modifications to food availability have on coral reef fish were established by maintaining breeding pairs of *A. polyacanthus* in 3 water temperatures and 2 food levels crossed in a fully orthogonal design (***Chapter 2***). Water temperatures were the current-day average for the collection location (summer mean = 28.5°C) and temperatures predicted to become the average for this region over the next 50 to 100 years (+1.5°C = 30.0°C and +3.0°C = 31.5°C). Pairs were provided with either a high or low quantity diet based on average and minimum feeding rates in the wild. Both water temperature and food supply affected growth rate and reproductive ability including the quality and quantity of gametes produced. Reduced breeding at warmer temperatures may mean significant declines in *A. polyacanthus* populations as the ocean warms.

Variations in offspring quality due to parental effects are likely to affect their performance during early life, however, the importance of parentally sourced differences can depend on the environmental conditions juveniles experience. In highly competitive or stressful environments beneficial parental effects may be more important than when conditions are favourable. Offspring produced under future ocean conditions in Chapter 2 were reared under 3 levels of food availability to investigate differences in performance during early life (***Chapter 3***). The growth and survival of offspring depended on the combination of parental effects and juvenile environment quality. In poor environments offspring produced by elevated temperature parents had reduced survival. In contrast, in all food availabilities compensatory growth mediated the initial parental effects to the size, suggesting that parental effects induced by climate change may not be persistent. The benefits of compensatory growth can be short lived, however, this accelerated growth may trade-off with other important life history attributes.

Since a range of fish attributes were found to be influenced by sea water temperature, the potential for developmental acclimation to mediate the underlying driver of effects (metabolic rate differences) was investigated. Offspring produced by current-day adults were reared for their entire life-cycle at current-day and elevated (+1.5°C and +3.0°C) water temperatures (***Chapter 4***). Some evidence for metabolic acclimation was identified in fish reared at 3.0°C greater than the current-day average during summer, however no acclimation was exhibited by fish reared at 1.5°C above current-day temperatures. Fish possessing developmental acclimation at +3.0°C were smaller and in poorer condition than fish reared at current-day temperatures, suggesting that even with acclimation there may be significant consequences for future populations of tropical fishes caused by global warming.

Generally it is believed that the potential for thermal acclimation is influenced by the thermal variation experienced by a population. By comparing two geographically separated populations of *A. polyacanthus* (approximately 950km apart), which vary in average temperatures but not the seasonal or daily variation experienced, I found that acclimation ability differed regardless of thermal variability (***Chapter 5***). Specifically, fish from the higher-latitude location were able to fully acclimate resting metabolic rate (RMR) and aerobic scope, while the lower-latitude location could only partially compensate RMR at the warmest temperature. This indicates that acclimation capacity of populations may vary on relatively small scales (<800kms), and that understanding such variation will be critical for predicting the impacts of climate change.

Finally, this study investigated the potential for developmental acclimation to mediate the negative effects to reproduction and gametes produced at elevated water temperatures, as well as the potential for parents to produce offspring with enhanced performance in future ocean conditions (transgenerational acclimation; ***Chapter 6***). With developmental acclimation reproductive ability was enhanced at +1.5°C, but limitations still existed for breeding pairs at +3.0°C. Evidence was found for transgenerational acclimation in both elevated temperature treatments, with offspring (F2) produced by +1.5 and +3.0°C breeding pairs (F1) exhibiting metabolic attributes equivalent to control fish at present-day average temperatures. Improvement in metabolic attributes is expected to lead to improvements in growth, condition and reproductive ability in this generation.

The present research showed that, contrary to expectations, the coral reef fish *A. polyacanthus* has a substantial potential to cope with alterations to the ocean environment predicted with climate change. Additionally, it provides the first evidence for rapid transgenerational thermal acclimation in reef fish. Such acclimation could reduce the impact of warming temperatures and allow populations to persist across their current range.

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Chapter 1: General Introduction

Climate change has been occurring rapidly over the last 40 years and in the next 50-100 years it is expected to significantly alter environmental conditions across the globe. Due to industrialisation, rising carbon dioxide in the earth's atmosphere is causing a greater retention of heat with warming of up to 4.5°C possible by 2100 (IPCC 2007; Hobday and Lough 2011). Warming that has already occurred globally due to industrialisation has caused range shifts and alterations in the timing of key events for a diverse range of plants and animals (Walther *et al*. 2002; Perry *et al.* 2005; Parmesan 2006; Hickling *et al*. 2006). As global temperatures continue to increase more ranges shifts are predicted and extinction rates are expected to rise for species that are unable to shift their distributions (Thomas *et al.* 2004). The majority of our current understanding of the biological effects of climate change is concentrated on temperate regions of the world and in the northern hemisphere, thus there is a necessity to understand the implications of climate change to a broader range of ecosystems and regions.

Generally, tropical regions are not expected to warm as much as temperate and polar regions, and the ocean will warm more slowly than the atmosphere. Increases of up to 4°C are projected in terrestrial temperature (IPCC 2007) and up to 3°C in sea surface temperature by 2100 depending on emission scenarios (Lough 2007; Munday *et al*. 2009). Increasing environmental temperature is likely to exceed the thermal optimum for many tropical species (Deutsch *et al.* 2008; Tewksbury *et al*. 2008; Wright *et al*. 2009), as well as modify the timing of key events such as reproduction which utilize temperature cues (Walther *et al*. 2002; Parmesan 2006). Changes in ocean temperature are expected to modify circulation patterns and stratification leading to alterations in upwelling of nutrient enhanced deep water (Sarmiento *et al.* 2004; Harley *et al.* 2006; Rost *et al.* 2008). This will have significant impacts on phytoplankton production and community composition, and consequently reductions in plankton abundance with warming (McKinnon *et al.* 2007; Poloczanska *et al.* 2007; Brander 2009). Plankton is the base for almost all marine food chains and changes in quality and quantity of this food source is likely to have substantial effects to marine organisms across life stages (Richardson 2008; Munday *et al.* 2009).

Implications for tropical ectotherms

Ectotherms do not physiologically regulate their internal body temperature and are therefore strongly influenced by the surrounding thermal environment. Consequently, they are expected to be especially vulnerable to rising temperatures from climate change. Most marine organisms are ectothermic and thus their metabolic pathways are strongly influenced by water temperature (Bret 1971; Hazel and Prosser 1974; Houde 1989; Clarke and Johnston 1999). This means thermal influences to physiology will affect individual performance and influence ecological interactions (Pörtner and Knust 2007; Hofmann and Todgham 2010).

There is increasing evidence that terrestrial ectotherms from tropical regions will be affected more strongly by the relatively small increases in temperature than species from temperate regions (Deutsch *et al.* 2008; Tewksbury *et al*. 2008; Wright *et al.* 2009). This is due to tropical species possessing a narrower thermal niche and a smaller range between critical minimum and maximum temperatures (Kleypas *et al.* 1999; Tewksbury *et al*. 2008). These thermal characteristics are believed to be due to tropical species evolving in a relatively thermally stable environment, and maintaining plasticity to a range of temperatures being costly (Hofmann and Todgham 2010; Relyea 2002). Furthermore, tropical species not only appear to possess restricted thermal ranges, but future predicted environmental conditions are expected to exceed the maximum critical temperate for many (Deutsch *et al.* 2008; Tewksbury *et al.* 2008, Hughes *et al.* 2003). Due to these thermal attributes, some capacity to modify critical thermal characteristics will be required for tropical species to persist at present locations.

For aquatic species the ability to perform aerobically has been identified as a key determinate of species persistence and performance (Pörtner and Knust 2007; Pörtner and Farrell 2008; Hofmann and Todgham 2010). The ability to maintain aerobic function is reduced as environmental temperature shifts away from the thermal optimum (Pörtner 2002; Pörtner and Knust 2007). Declines in aerobic function can indicate the onset of thermal limitation due to the circulatory and ventilatory systems unable to supply oxygen demands (Pörtner 2002; Pörtner and Knust 2007; Farrell 2009). Additionally, at temperatures greater than the optimum, the minimum energy required to maintain cell function (resting metabolic rate: RMR) increases with temperature (Bret 1971; Houde 1989; Clarke and Johnston 1999), and thus individuals require more energy for basic life processes. In addition to the direct impact of temperature on the oxygen consumption of organisms, as water temperature increases the solubility of oxygen is reduced and consequently the amount available. Understanding of key physiological attributes (resting metabolic rate and aerobic scope) for tropical aquatic species will be important to predicting their likely response to climate change.

Potential effects to marine fishes

Since water temperature controls cell function and metabolic rate in ectotherms, it can influence marine fishes throughout all life stages in a range of attributes (e.g. growth and development rate). However, not all life stages are equally thermally sensitive and therefore not equally susceptible to climate change. Reproductive individuals and the early life stages of fishes are expected to be the most sensitive to environmental variations (Rombough 1997; Pörtner and Farrell 2008). During reproduction, fish are believed to be inhibited by elevations in temperature due to gonads requiring a substantial amount of oxygen on top of the already large general oxygen demands with warmer water temperatures (Pörtner and Farrell 2008), as well as temperature directly affecting the production of gonadal steroids (Manning and Kime 1985; Van der Kraak and Pankhurst 1997; Pankhurst and Thomas 1998). During the early stages of development, the high thermal sensitivity of marine fishes is believed to be due to their inability to temperature compensate (Rombough 1997) and to key systems still developing, such as the ventilatory and circulatory systems (Pörtner and Farrell 2008).

Variations in water temperature during early life can modify the rate of development of embryos, larvae and juveniles for many species (McCormick and Molony 1995; Rombough 1997; Benoit and Pepin 1999; Wilson and Meekan 2002; Meekan *et al.* 2003; Green and Fisher 2004; Sponaugle *et al.* 2006). During embryogenesis an increase in water temperature can result in a reduction in time till hatching (Blaxter and Hempel 1963; Rombough 1997; Benoit and Pepin, 1999) and a decline in survival (Gagliano *et al.* 2007). In contrast, increased growth rate caused by warmer water temperature during pelagic development has been correlated to a greater survival (Meekan and Fortier 1996; Bergenius *et al.* 2002; Wilson and Meekan 2002), most likely due to a reduced susceptibility to size selective predation (Bailey and Houde 1989; Sogard 1997). However, if food is in low abundance elevations in temperature may increase larval mortality due to increased metabolic rate and thus reduced survival on endogenous reserves (Blaxter and Hempel 1963; Pepin 1991). Once settled, the rate of juvenile development (Ellis *et al.* 1997; Barber and Jenkins 2001), gender (Conover and Kynard 1981) and successful maturation (Davies *et al.* 1986) may also be influenced by water temperature.

Reproduction and production of viable gametes is only possibly within a narrow portion of the total temperature range an organism lives in (Van der Kraak and Pankhurst 1997; Hofmann and Todgham 2010). For many marine fishes reproductive activity is known to be highly sensitive to elevations or reductions in temperature (Manning and Kime 1985; Pankhurst and Thomas 1998; Van der Kraak and Pankhurst 1997; Hilder and Pankhurst 2003; Pankhurst and Porter 2003; Burt *et al.* 2011). Effects to breeding adults can have further flow on effects to the gametes and subsequent offspring produced (Buckley *et al.* 1990; Brown *et al.* 2006; Burt *et al.* 2011). For example, female Atlantic halibut maintained in warmer conditions produced fewer eggs with poorer viability and fertilization success (Brown *et al.* 2006). Due to the thermal sensitivity of reproduction, changes to timing of breeding or the length of the breeding season would be expected with warming (Munday *et al.* 2008a). However, not all organisms will adjust the timing of life-history events in a similar way, which presents a significant risk for a mismatch in various trophic levels (Edwards and Richardson 2004). The majority of research into the effects of elevated temperature on reproduction and subsequent effects to progeny of marine fishes has been completed on temperate species (exception for *Acathochromis polyacanthus,* Hilder and Pankhurst 2003). There is an urgent need to understand whether effects on tropical fishes will be similar or of a greater magnitude due to their evolution in a thermally stable environment.

Coping with a changing environment

There are multiple ways organisms can cope with climate change that might allow them to maintain their performance and persist at their current location despite rising temperatures. One possibility is acclimation through phenotypic plasticity, which can occur in physiological, morphological or behavioural attributes (Angiletta 2009). There are two main types of acclimation; developmental acclimation which are permanent responses to the environment during early ontogeny, and reversible acclimation which includes controlled responses in relation to daily or seasonal environmental fluctuations (Angiletta 2009). Reversible acclimation is generally found when thermal variation occurs within an organisms’ lifetime and the ability to acclimate is generally related to the variability experienced. Species or populations that experience larger thermal fluctuations generally show an increased ability to modify attributes in relation to altered environmental temperatures (Bradshaw 1965; de Jong 1995). This has lead to the concern that tropical species will not possess a large capacity to acclimate since they have evolved in a thermally stable environment (Stilman 2003; Deutsch *et al.* 2008; Tewksbury *et al*. 2008; Wright *et al*. 2009). However, the potential for developmental acclimation, which often occurs when the environment varies between generations, has not been considered in these studies.

Acclimation is most commonly thought of when it is beneficial in nature and allows individuals to better cope with an altered environment. However, acclimation will not always be beneficial because both detection and response to a varying environment can energetically cost (DeWitt *et al.* 1998; Angiletta *et al.* 2003; Angiletta 2009). Trade-offs can exist when modifications of one attribute interacts with another. For example, an increase in refuge-seeking behaviour in order to reduce the time spent in adverse environmental conditions may result in a reduction in the time spent foraging and the amount of food consumed (Krause *et al.* 2000). Additionally, energy spent on physiological or morphological modifications may have otherwise been used on other functions (Hoffmann 1995; DeWitt *et al.* 1998; Angiletta 2009). Since acclimation is a plastic change (i.e. phenotypic), not genetic, limits to responses may also exist (DeWitt *et al.* 1998). If the amount of plasticity possible is less than the effect of the environmental change, acclimation will not be able to fully compensate for the impacts of an altered environment. Therefore, selection of successful phenotypes which allow adaptation or transmission of beneficial phenotypes by parents to offspring is likely to be needed for long-term coping with climate change.

Phenotypic contributions to offspring by parents (parental effects) can strongly influence the early development and survival of marine fishes. In most cases the maternal contribution is studied due to the greater contribution in gamete size (Kerrigan 1997, McCormick 2003, 2006; Gagliano and McCormick 2007; see Green 2008 for review), however, paternal contributions are also possible (Hunt and Simmons 2000; Moller and Cuervo 2000). The maternal phenotype can influence gamete size, number of offspring produced, the amount of yolk provisioning, as well as the size of offspring produced (see Green 2008 for review). Transgenerational plasticity, where parents positively alter the phenotype of their offspring in relation to environmental conditions, has been seen in insects and plants in relation to predatory defences (Agrawal *et al.* 1999; Mondor *et al.* 2005). For example, insects that develop in environments with high levels of predators produce offspring with morphological changes to reduced predation, when compared to parents reared in low predation environments (Agrawal *et al.* 1999). No research has yet investigated the potential for parental effects to mediate impacts of climate change, however, it represent a potential pathway of coping with adverse environmental conditions.

Acclimation in tropical fishes

Evidence for thermal acclimation in tropical fishes is limited to studies on physiology, metabolic rate and swimming ability. Research to date has show little to no ability for thermal acclimation of juvenile or adult tropical reef fishes (Tullis and Baillie 2005; Nilsson *et al.* 2009, 2010; Gardiner *et al.* 2010; Johansen and Jones 2011). This finding of limited acclimation ability is consistent with other tropical marine species (Stillman 2003), but contrasts with temperate marine fishes, which show a substantial capacity to alter physiology in relation to a modified thermal environment (e.g. Johnston and Dunn 1987; Johnston *et al.* 1998; Johnston and Temple 2002; Sylvestre *et al.* 2007). To date, all studies investigating thermal effects in tropical marine fishes have been completed over short times scales of only days to a few weeks, because this length of time has been shown to be sufficient for thermal acclimation in temperate marine fishes (Barrionuevo and Fernandes 1998). This increased ability for temperate species to modify physiology in relation to environmental temperature is probably due to their evolution and persistence in a more variable thermal environment.

While present research suggests a limited capacity for acclimation in tropical marine fishes the full range of pathways through which acclimation can occur has yet to be investigated. Present studies may have been completed over too short a time to allow acclimation to occur, due to physiological modifications for tropical species possibly taking longer. Additionally, most studies have been conducted on adults or older juveniles and thus may not be allowing for developmental acclimation to occur. Phenotypic plasticity is commonly found to be more prevalent during early life in a range of taxa (Wright and McConnaughay 2002; Bateson *et al.* 2004), including larval and newly settled marine fish where developmental plasticity has been identified in a range of attributes (Warner 1997; West-Eberhard 2003; Munday *et al.* 2006). Therefore, thermal acclimation during early life could be possible for tropical reef fish and the potential for developmental acclimation warrants further investigation. No research has yet been conducted over the longer term to explore the potential for acclimation to occur across generations (transgenerational), which also represents a significant pathway for coping with climate change.

Study species

The present research examined the potential effects of predicted global warming on coral reef fishes as well as the potential for thermal acclimation. This study utilised the damselfish *Acanthochromis polyacanthus* (Fig 1.1) as a model species since it is a common member of reef's across the Great Barrier Reef and Indo-Pacific region and is easily reared in captivity. *A. polyacanthus* form breeding pairs, with most reproduction occurring over a 4 month period during the summer (October to February; Robertson 1973). Eggs are laid on substrate within caves (Thresher 1983) and care is provided to the benthic eggs by both parents (Pankhurst *et al.*1999). This species does not possess a planktonic larval stage and after hatching, the young remain with the parents for up to 45 days (Kavanagh 2000). Unlike many other coral reef fishes with an extended pelagic larval phase, the offspring of *A. polyacanthus* can be reared with high success. Additionally, maturation in *A. polyacanthus* is attained within 2 years which makes them suitable for multi-generational experiments.

Figure 1.1: *Acanthochromis polyacanthus* adult with juvenile brood in the wild.

The present research utilises two populations of *A. polyacanthus* from the Great Barrier Reef (GBR); the Palm Island region (23°27’S, 151°57’E) and Heron Island (23°27’S, 151°57’E). These two populations represent the lower extent (Heron Island) and middle (Palm Island) of the species’ range on the GBR. The geographic range of *A. polyacanthus* is from 15°N–26°S and 116°E–169°E, which encompasses a total temperature span (inclusive of seasons) of approximately 20°C to 31°C across this range. Similar temperature ranges are experienced by many other broadly distributed coral reef fishes (Munday *et al.* 2008b). The majority of this research utilises fish from the Palm Island population to test the long-term impacts of climate change.

Aims and thesis outline

This thesis investigates the potential effects of a modified future environment on coral reef fish and the ability to acclimate and adjust to new environmental conditions. While there is some understanding that both changes in temperature and food availability (which is expected to be reduced for many locations) are likely to affect coral reef fishes, there are few studies that incorporate temperature changes relevant to climate change and even fewer that incorporate both the environmental factors (exception see Munday *et al.* 2008a). Specifically, this study will explore the affect that elevations in sea water temperature predicted by 2100 and potential changes to food availability will have on reproductive ability and early development of a coral reef fish. The impact of temperature and food availability on reproductive attributes are investigated in ***Chapter 2***, with a fully orthogonal design combining 3 temperature treatments and 2 food levels. Chapter 3 explores the survival, growth and morphological characteristics of offspring produced by adult breeding pairs in Chapter 2, as well as their performance in environments that differ in temperature and food availability.

Once an understanding of the potential impacts of temperature increase on coral reef fish has been established, the focus of the thesis shifts to testing the potential for fish to cope with elevated water temperature over longer time scales. First, in ***Chapter 4***, the potential for developmental thermal acclimation during early ontogeny is established. In ***Chapter 5*** the focus broadens to compare the developmental acclimation ability of the two populations (Palm Island and Heron Island) to determine if acclimation ability is consistent with the thermal environment experienced by the two populations. These two populations experience different average sea surface temperatures, but similar daily and seasonal fluctuations. Finally, in ***Chapter 6*** the potential for parents to pass on favourable traits to their offspring that may assist in coping with future climate change (transgenerational acclimation) is tested. Fish were maintained for 3 generations (wild F0, F1 and F2) under present-day and future predicted temperatures (+1.5°C and +3.0°C) to investigate transgenerational acclimation in coral reef fish for the first time. By examining the ability of species to modify key traits, both within and across generations, this thesis advances our understanding of the potential long-term impacts of climate change on fish populations. Knowledge on this potential to cope with climate change over multiple generations will be key to managing and conserving organisms and ecosystems in the future.

Chapter 2: Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish

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Authors: J. M. Donelson, P. L. Munday, M. I. McCormick, N. W. Pankhurst and P. M. Pankhurst

2.1 Summary

Climate change is predicted to increase ocean temperatures and alter plankton communities that are food for many marine fishes. To examine the effects of increased sea surface temperature and fluctuating food levels on reef-fish reproduction, breeding pairs of the coral reef damselfish *Acanthochromis polyacanthus* were maintained for a full summer breeding season in an orthogonal experiment comprising 3 temperature and 2 food levels. Water temperatures were the current-day average summer temperature for the collection location (28.5°C) and temperatures predicted to become close to the average for this region over the next 50 to 100 yr (30.0 and 31.5°C). Pairs were fed either a high or low quantity diet based on average and minimum feeding rates in the wild. Both water temperature and food supply affected reproductive output. Fewer pairs bred at elevated water temperatures and no pairs reproduced at either of the higher temperatures on the lower quantity diet. Furthermore, eggs produced were smaller at 30.0 and 31.5°C compared to those at 28.5°C. Histological analysis of the gonads and steroid hormone measurement did not reveal any apparent differences in patterns of oogenesis among treatments. However, spermatogenesis was reduced at high temperatures despite some increases in plasma androgen levels. Reduced breeding rate at warmer

temperatures combined with reduced sperm production indicates the potential for significant declines in *A. polyacanthus* populations as the ocean warms.

2.2 Introduction

Climate change is predicted to cause major changes to the ocean environment over the next 50 to 100 yr, including significant increases in average sea-surface temperatures and fundamental changes to plankton communities that are the basis of food webs in most marine ecosystems (Guinotte *et al.* 2003; Hays *et al.* 2005; Harley *et al.* 2006; IPCC 2007). While ocean temperature is projected to increase less in tropical locations compared to temperate locations, tropical species might be more sensitive to small temperature increases because they have evolved in a more thermally stable environment (Tewksbury *et al.* 2008). Most tropical marine species are ectotherms, and small increases in temperature can affect a wide range of traits including physiological condition, growth rate, swimming ability, reproduction and behavior (Houde 1989; Przeslawski *et al.* 2008; Munday *et al.* 2009). Several recent studies have shown that increases in ocean temperature consistent with climate change predictions can affect growth rates and aerobic capacity of tropical reef fishes (e.g. Munday *et al.* 2008b; Nilsson *et al.* 2009). However, whether temperature increases will affect the reproductive performance of these species is almost completely unknown (Munday *et al.* 2008a). Reproduction in fishes is only possible within a narrow range of the temperatures that can be tolerated by adults (Van Der Kraak and Pankhurst 1997). Consequently, elevated temperatures could potentially depress reproductive success of tropical reef fishes if populations are already living close to their thermal optima, with potentially serious consequences for population sustainability.

A range of reproductive attributes can be influenced by temperature including the frequency of reproduction (Hilder and Pankhurst 2003), clutch size and egg size (Brown *et* *al.* 2006), embryo development and duration (Taranger and Hansen 1993; Rombough 1997; Hilder and Pankhurst 2003), gonad maturation (Davies *et al.* 1986), and the secretion and action of reproductive hormones (Van Der Kraak and Pankhurst 1997). To date, most research into the effects of temperature on these reproductive traits has been conducted on temperate and freshwater fishes (exception Hilder and Pankhurst 2003). Little is known about how any of these traits will be influenced by rising ocean temperatures for tropical marine species (Munday *et al.* 2008a). It might be predicted, however, that reproduction of tropical marine fishes will be highly sensitive to increasing ocean temperatures because these species have evolved in a relatively stable thermal environment and they experience less seasonal fluctuation in water temperature than do most temperate marine fishes.

For ectotherms such as fish, increasing temperature causes an increase in metabolic rate (Burel *et al.* 1996; Pörtner and Knust 2007; Nilsson *et al.* 2009). Consequently, daily energetic requirements are expected to rise with increasing temperature and individuals will either need to procure more food to maintain current-day growth, maintenance and reproductive functions, or they will need to reduce energy allocation to some activities and functions. If additional food is able the increase in rate of digestion will lead to an increase in energy available to tissues. The increase in energetic requirements at elevated temperatures creates the potential for trade-offs to occur between various activities (Angilletta *et al.* 2003). For example, if energy allocation to reproduction is maintained at higher temperature, there might be less energy available for growth. Therefore, predicting the consequences of climate change for most tropical marine fishes depends on understanding the interacting effects of temperature and food supply on life history characteristics and whole-organism function. In addition to the direct physiological effects of temperature, increased water temperature is predicted to affect the structure of plankton communities that are a food source for many marine fishes (Hays *et* *al.* 2005). Changes to plankton communities will not be uniform, but in many locations these communities may become less productive because higher temperatures favour longer, less productive planktonic food-chains (McKinnon *et al.* 2007) and greater thermal stratification of the water column will reduce nutrient enrichment of the surface layers that is important for planktonic productivity (Poloczanska *et al.* 2007; Brander 2009). Fish can increase the time spent foraging to enhance their energy intake, however food is usually not in unlimited supply (Buckley *et al.* 2004). Furthermore, the likelihood of predation also increases with increased foraging time (Biro *et al.* 2007), which might cause individuals to forage at sub-optimal levels. As a result of likely changes to the productivity of plankton communities, it is important to understand how changes to sea surface temperature will affect individuals depending on different levels of food availability, not just when food is unlimited and easy to access.

This study investigated the independent and interacting effects of water temperature and food availability on reproductive output and body condition of the coral reef fish *Acanthochromis polyacanthus*. Adult breeding pairs collected from the central Great Barrier Reef, Australia, were maintained at 3 temperature and 2 food levels in a fully orthogonal design.

2.3 Materials and methods

Study species and collection

The tropical damselfish *Acanthochromis polyacanthus* was used in the present study because it is amenable to laboratory breeding. *A. polyacanthus* form breeding pairs, with most reproduction on the Great Barrier Reef occurring over a 4 mo period from October to February (Robertson 1973). Eggs are laid on substrate within caves (Thresher 1983) and care is provided to the benthic eggs by both parents (Pankhurst *et al.* 1999). After hatching, the young remain with the parents for up to 45 d (Kavanagh 2000).

Adult pairs of *Acanthochromis polyacanthus* were collected in June and July 2007 (austral winter) from a number of locations in the Palm Island region of the central Great Barrier Reef. Fish were collected from Orpheus Island (18° 37’ S, 146° 30’ E), Fantome Island (18° 41’ S, 146° 31’ E), Pelorus Island (18° 33’ S, 146° 29’ E), and Bramble Reef (18° 24’ S, 146° 42’ E). Care was taken to collect only established breeding pairs, which were identified by the behavioral interaction of individuals towards each other and defense of a common territory. Pairs were collected using a barrier net and hand nets. The standard length of each fish was recorded on collection. These fish were supplemented with breeding pairs collected the previous year from Orpheus Island, making a total of 42 adult pairs. Supplementary pairs represented only 5 of the 42 pairs employed in the experiment. However, to verify that differences did not exist between these 2 groups, the values obtained for supplemented individuals were compared to the means ±1 SE of all non-supplemented pairs in their treatment group. In all cases the values for the 5 pairs fell within the specified range. Pairs were established in separate 60 l aquariums inside an environmentally-controlled aquarium room at James Cook University (Townsville, Australia). Each aquarium was supplied with a continuous flow of filtered, aerated seawater. A large shelter composed of half a terracotta pot and 2 bricks underneath to elevate it was placed in each aquarium. The age of adult fish was determined at the conclusion of the experiment by counting annual rings from the sagittal otolith (Fowler 1990). Individuals were 2 to 8 yr old (mean ± SE = 4.5 ± 0.14 yr) and there was no significant difference in average age between treatments (factorial ANOVA temperature: $F_{2,\,65} = 0.126$, $p = 0.882$; food: $F_{1,\,65} = 0.671$, $p = 0.416$; temp × food: $F_{2,\,65} = 0.044$, $p = 0.957$). Age was tested against all reproductive characteristics and was found to not relate significantly. Trends in these characteristics were best described by their relationship to temperature and food.

Experimental design

Adult pairs were kept at the average winter ocean temperature for the Palm Island region until 6 August 2007. Predictions for increases in water temperature on the Great Barrier Reef range from 1 to 3°C in the next century (Lough 2007). On 6 August 2007 water temperature was slowly raised over a 2 wk period in 2/3 of the aquariums to split the pairs into 3 treatment groups: (1) average sea water temperature for Palm Island region, (2) 1.5°C higher than average, and (3) 3.0°C higher than the average for the Palm Island region. The water temperature in each treatment group was subsequently raised every week until the average summer water temperature for the region (28.5°C) was attained in the lowest temperature treatment (Figure 2.1). This resulted in temperature treatments of 28.5°C (±0.05 SE) for current day, 30.0°C (±0.05 SE) for an increase of 1.5 and 31.5°C (±0.06 SE) for an increase of 3.0°C. Weekly increases in water temperature were matched to the 10 yr average increase in water temperatures during spring in the Palm Island region. Fish were kept in their treatment groups until 28 March 2008 when the experiment was concluded. The geographic range of *A. polyacanthus* is from 15°N–26° S and 116° E–169°E, which encompasses an average summer temperature range of approximately 27 to 31°C. Similar temperature ranges will be experienced by many other broadly distributed coral reef fish (Munday *et al.* 2008a). Day length was controlled with artificial lighting and adjusted weekly to match the natural cycle for the central Great Barrier Reef.

Breeding pairs at each temperature were randomly assigned to 2 feeding treatments (high or low ration) to produce a fully orthogonal design with 6 to 8 breeding pairs at each combination of temperature and food ration. The high food treatment consisted of 0.376 g Aquaculture Nutrition NRD 12/20 pellets ind. d–1 (1.0% body weight, protein 55%, fat 9%, fiber 1.9%), and the low treatment of 0.188 g ind. d–1 (0.5% body weight, i.e. half the high ration). The above feeding levels were validated in a natural setting in the Palm Island region by observing the activity level of breeding pairs throughout the day, and subsequent collection and measurement of gut fullness in the late afternoon. It was found that fish consumed from 0.7 to 2.6% (average: 1.47%) of their body weight per day. This average amount of plankton would convert to approximately 4.37 calories and the lower range would be 2.15 calories (Clarke and Bishop 1948). In addition, it was determined that fish spent 30% of their time on average on vigorous activities other than feeding, such as territorial defense. Therefore, the experimental feeding levels were adjusted to be 0.5 and 1.0% to account for the lack of time fish in the experiment would spend on these activities in an aquarium situation, as well as the enhanced energy content of formulated diet.

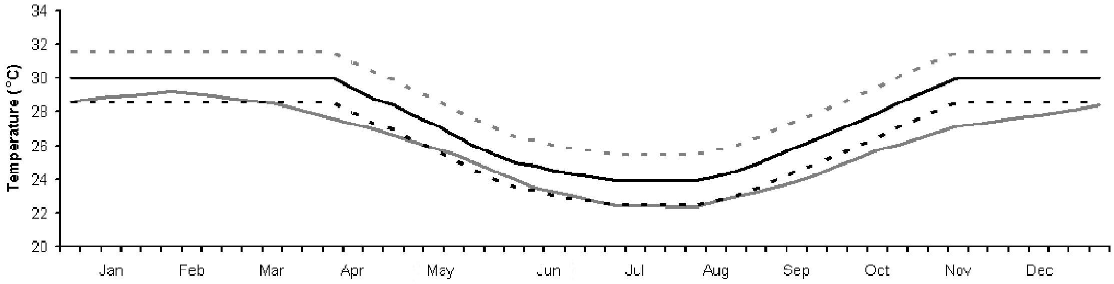

Figure 2.1. Seasonal water temperature at the Palm Island Region and in experimental treatments. The solid grey line shows average weekly water temperature at Orpheus-Palm Island based on 14 years of daily water temperature records from the JCU/AIMS weather station. Experimental treatment temperatures were: present day (black dashed), +1.5°C (solid black) and +3.0°C (grey dashed).

Reproductive output

Nesting sites were checked daily at 09:00 h for the presence of eggs. An underwater photograph was taken of each first clutch for estimation of the number of eggs laid. To measure egg size, a sample of 15 eggs (representing 2 to 6% of a clutch) was collected from random locations within each clutch using tweezers. Eggs were photographed under a stereomicroscope directly after removal from the clutch. Cross sectional egg area was estimated from the photographs using Optimas 6.5 software (Media Cybernetics). The number of eggs in each clutch at laying was estimated by viewing the digital photograph on a computer screen using Image Tool (UTHSCSA). To approximate the reproductive output of each spawning, the number of eggs in each clutch was multiplied by the average egg area of the 15 sample eggs for that clutch. Nest sites were checked daily to determine the period (d) from laying to hatching (embryonic duration).

Growth and body condition

Temperature, food supply and reproductive output can all influence energy available for growth and maintenance activities; we therefore compared growth rates and fat deposits between temperature and food treatments. Estimating growth and fat reserves among treatments also enabled us to determine whether reduced reproduction at elevated temperatures and low food rations could be due to energy shortages in these treatments.

To determine growth, standard length (to the nearest 0.5 mm) and wet weight (to the nearest 0.01 g) was measured for each fish following euthanasia. Fat within the stomach region (visceral fat) was quantified to investigate differences in the amount of stored energy in fish from different temperature and food treatments. The gut was dissected from the body cavity and immediately frozen. Subsequently, all visible fat deposits were carefully removed from the external surfaces of the stomach with tweezers and weighed to the nearest 1 mg.

Gamete production and hormones

To investigate if water temperature and food supply affected gonadal steroidogenesis, blood samples were taken from each fish at the end of the experiment to estimate levels of the steroid hormones 17β-estradiol (E2), testosterone (T) in females, and T, 11-ketotestosterone (11KT) in males based on their demonstrated roles in female and male reproduction respectively (Pankhurst *et al.* 1999). Each fish was caught with a large hand net and a blood sample taken by caudal puncture using pre-heparinized syringes within 2 min of capture. Blood was immediately stored on ice and then placed in a refrigerator and centrifuged within 5 h. Blood plasma was then frozen at –80°C until steroid measurement. Plasma samples were extracted with ethyl acetate and resuspended in assay buffer; plasma levels of E2, T and 11KT were determined by radioimmunoassay as described in Pankhurst et al. 1999. Extraction efficiency was determined by recovery of 3H-labelled steroid from replicates of a plasma pool and was 71.6, 65.2, and 83.6% for T, E2 and 11KT respectively. Assay values were corrected accordingly. All samples were measured in a single assay for each steroid. Finally, the gonads of euthanazed fish were fixed in 4% formaldehyde, 5% acetic acid, and 1.3% calcium chloride (FAACC) for 2 mo. Gonads were then embedded in paraffin wax, sectioned longitudinally at 5 µm, and stained with Mayer's alum haematoxylin and Young's eosin-erythrosine. Proportions of mature, vitellogenic oocytes (in ovaries) and spermatozoa (in testis) were estimated by scoring the presence of vitellogenic follicles or spermatozoa at intervals along a transect (40 and 21 µm intervals for ovary and testis respectively); transects ran along the longest dimension of a representative longitudinal section from the gonad of each fish. A longitudinal section was used to account for a variation in gamete production between the posterior and anterior parts of the gonad.

Data analysis

Reproductive characteristics: Due to a lack of breeding in 2 temperature and food combinations (30.0 and 31.5°C with low food rations), 2 separate analyses were used to compare each of the other reproductive characteristics (egg area, clutch size and reproductive output) among temperatures and between food ration. Clutch size was compared among temperatures with a Kruskal-Wallis test due to data not satisfying ANOVA assumptions, and was then compared between high and low food rations at 28.5°C using a t-test. Egg area and reproductive output was compared among temperatures with ANOVA and then with a *t*-test between food levels. Reproductive output data was log transformed to improve normality of distribution prior to analysis. Significant main effects in ANOVA were examined using Fisher's least significant difference (LSD) post hoc analysis. A Kruskal-Wallis test was used to compare embryo duration between treatments. All analyses were completed with Statistica 8.0 (StatSoft).

Growth and body condition: Growth is often correlated with initial length in fishes. Therefore, to account for variation in growth that could be attributed to variation in initial length of fish within treatments, ANCOVA was used to compare growth during the experiment depending on the initial length of each fish. Temperature and food levels were regarded as fixed factors, growth (mm) was the dependent variable and initial standard length (mm) was the covariate. Fisher's LSD post hoc analysis was used to compare means where significant treatment effects were identified. Differences between fat deposits depending on temperature and food treatments were also tested with ANCOVA. Temperature and food levels were regarded as fixed factors, fat (mg) was the dependent variable and final standard length (mm) was the covariate.

Gonadal condition and plasma steroid levels: The proportion of spermatozoa in male gonads and the proportion of vitellogenic oocytes in female gonads were arc sin transformed prior to statistical analysis to meet the assumptions of ANOVA. Their relationship (depending on treatment) was tested with a factorial ANOVA (temperature and food fixed factors) followed by Fisher's LSD post hoc analysis. Plasma levels of T and E2 in females were square root transformed, and 11KT levels in males were log transformed, prior to analysis. Hormone levels were compared among temperature and food treatments using factorial ANOVA. Fisher's LSD post hoc analysis was used to compare means where significant treatment effects were identified.

2.4 Results

Reproductive characteristics

Temperature and food ration both affected the number of pairs that reproduced, with no pairs reproducing at elevated temperatures and low ration (Table 2.1). Seven of 8 pairs at 28.5°C on the high-ration diet produced egg clutches. In contrast, on the high ration diet, only 3 of 8 pairs reproduced at 30.0°C and 3 of 7 pairs at 31.5°C; no pairs reproduced at these temperatures on the low ration diet. Despite the obvious reductions in the proportion of pairs that reproduced at higher temperatures, there was no difference in the timing of breeding, with fish at all temperatures laying egg clutches within 3 wk of each other.

Egg size declined at higher temperatures (Figure 2.2a; ANOVA: $F_{2,10} = 6.27$, $p = 0.017$). In contrast, food ration did not significantly affect egg size at 28.5°C (Figure 2.2a; $t = 0.514$, $df = 8$, $n = 10$, $p = 0.621$). Although clutch size exhibited a declining trend with increasing temperature and a reduction in food availability (Figure 2.2b), neither were found to be statistically significant (Kruskal-Wallis temperature: $\chi2 = 4.569$, $df = 2$, $p = 0.102$; food: $t = 1.908$, $df = 8$, $n = 10$, $p = 0.332$). Reproductive output, which combines both egg size and the number of eggs produced, exhibited a decrease with increasing temperature that was not statistically significant (Figure 2.2c; $F_{2,10} = 4.01$, $p = 0.055$). There was no significant difference in reproductive output between food treatments at 28.5°C (Figure 2.2c; $t = 1.29$, $df = 8$, $n = 10$, $p = 0.232$).

Embryonic duration also decreased with increasing temperature. Specifically a 3.0°C rise in water temperature caused a reduction of approximately 1.5 d in embryonic duration (mean at 28.5°C: 10.33 ± 0.17 d; at 31.5°C: 9 ± 0.00 d; Kruskal-Wallis: $\chi 2 = 3.343$, $df = 2$, $n = 13$, $p = 0.03$). Food availability did not significantly affect embryo duration at 28.5°C (mean for high ration: 10.43 ± 0.20 d; for low ration: 10 ± 0.00 d).

Table 2.1 *Acanthochromis polyacanthus*. Effect of temperature and food availability on the number of pairs that spawned.

Food level	Temperature (°C)	No. of pairs that spawned	Total number of pairs
High	28.5	7	8
	30.0	3	8
	31.5	3	7
Low	28.5	3	7
	30.0	0	7
	31.5	0	6

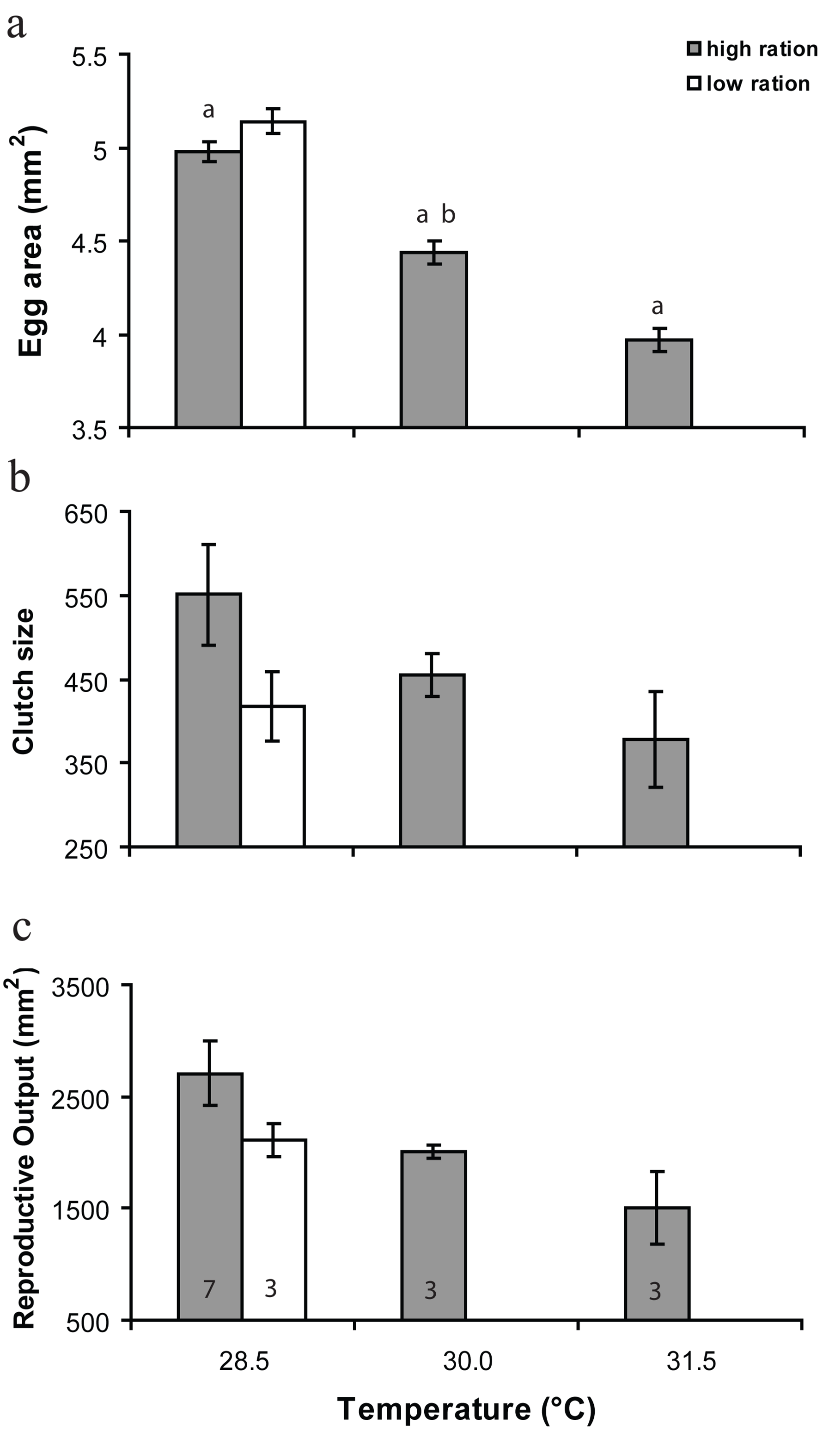

Figure 2.2 *Acanthochromis polyacanthus.* Mean (a) egg area, (b) clutch size and (c) reproductive output (mean egg area × clutch size) in relation to temperature and ration level. Values are mean ± SE. Numbers within bars are replicates and letters represent differences between temperatures within the high food level ($p < 0.05$).

Growth and body condition

There was no difference in the standard length (mm) of fish among treatments at the start of the experiment (ANOVA temperature: $F_{2,\,78}$ = 1.854, p = 0.163; food: $F_{1,\,78}$ = 1.403, p = 0.240; temperature × food: $F_{2,\,78}$ = 1.374, p = 0.259). Growth rate declined with increasing temperature (Figure 2.3; ANCOVA: $F_{2,\,58}$ = 4.10, p = 0.010) and with reduced food availability (ANCOVA: $F_{1,\,58}$ = 14.38, p < 0.001). Growth was greatest for fish in the current day temperature on the high food ration (Figure 2.3), but declined rapidly with increasing water temperature and decreased food supply. Post-hoc tests showed that differences in growth between food levels were significant at 28.5 and 30.0°C, but not at 31.5°C. Growth rate also depended on the initial length of fish (ANCOVA: $F_{1,\,58}$ = 27.29, p < 0.001).

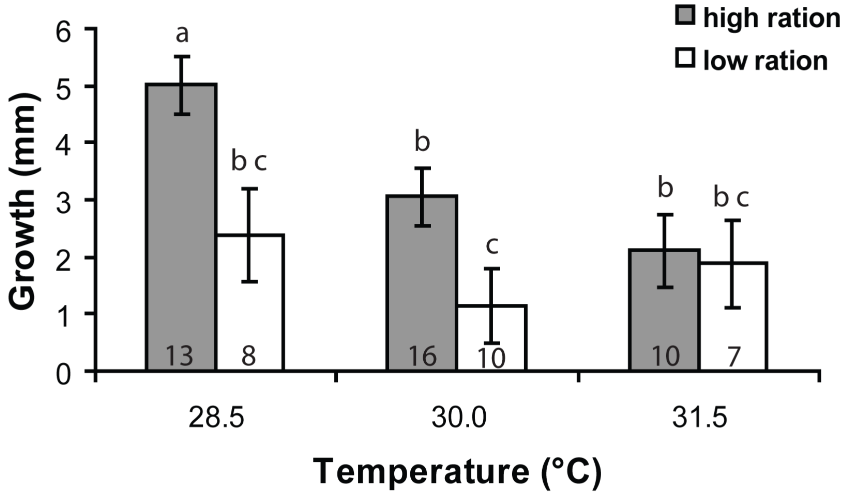

Figure 2.3 *Acanthochromis polyacanthus.* Mean growth (±SE) at different temperature and ration levels during the experimental period after correction for the relationship between growth and initial length. Numbers within bars are replicates and letters represent differences between all treatments (p < 0.05).

The amount of visceral fat in the body cavity was substantially greater in fish given the high ration diet, regardless of temperature (Figure 2.4; ANCOVA: $F_{1,\,67}$ = 38.99, p <

0.001). While not as distinct, visceral fat also increased significantly with temperature (ANCOVA: $F_{2,\ 67} = 3.96$, $p = 0.024$), indicating that reduced reproduction at the high temperatures was not primarily due to poor nutritional condition. The amount of visceral fat also depended on initial length (ANCOVA: $F_{1,\ 67} = 6.55$, $p = 0.013$).

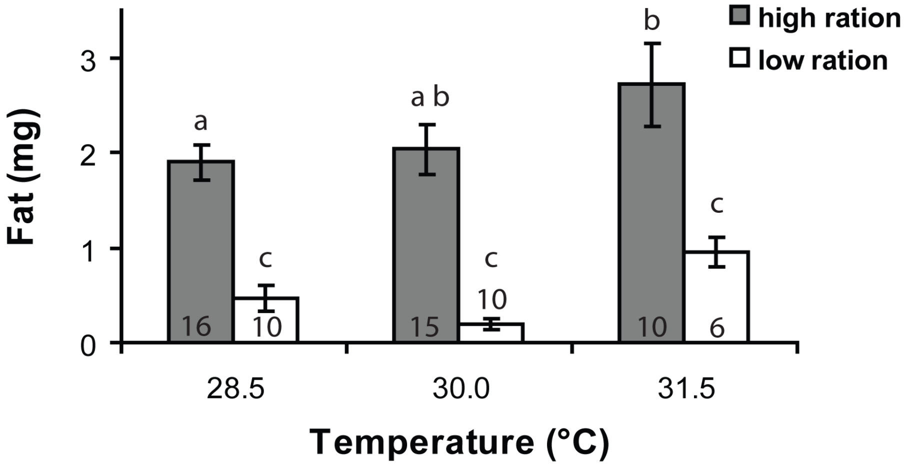

Figure 2.4 *Acanthochromis polyacanthus.* Mean weight of visceral fat (± SE) at different temperature and ration levels during the experimental period after correction for the relationship between growth and initial length. Numbers within bars are replicates and letters represent differences between all treatments ($p < 0.05$).

Gonadal condition and plasma steroid levels

The proportion of spermatozoa in testes differed among temperatures and food rations (Figure 2.5; factorial ANOVA temperature: $F_{2,\ 28} = 12.001$, $p < 0.001$; temperature × food: $F_{2,\ 28} = 4.118$, $p = 0.027$). The proportion of spermatozoa decreased with increasing water temperature, but the response was greater on the low food ration compared to the high food ration. At 31.5°C, males on the low food ration had fewer spermatozoa than males on the high ration; however, in both other temperatures low ration fish always had more spermatozoa.

The proportion of vitellogenic stage oocytes in ovaries was also significantly related to temperature (factorial ANOVA: $F_{2, 28} = 6.453$, $p = 0.005$). However, this effect was due to the ovaries of 3 fish having very few vitellogenic oocytes in the low ration group at 30.0°C. The mean proportion of vitellogenic oocytes in ovaries for this group (35%) was significantly different from all other groups (mean > 64%) ($p < 0.02$ for all combinations).

In females, plasma levels of T and E2 did not differ between temperatures or food ration (Table 2; factorial ANOVA T temperature: $F_{2, 26} = 2.606$, $p = 0.930$; food: $F_{2, 26} = 0.097$, $p = 0.758$; E2 temperature: $F_{2, 26} = 0.487$, $p = 0.620$; food: $F_{2, 26} = 0.954$, $p = 0.338$). In contrast, plasma levels of 11KT in males were affected by both temperature and food availability (Fig. 6; factorial ANOVA temperature: $F_{2, 25} = 5.412$, $p = 0.011$; food: $F_{1, 25} = 8.372$, $p = 0.008$), and levels of testosterone were affected by an interaction between temperature and food ration (Table 2.2; temperature × food: $F_{2, 27} = 3.425$, $p = 0.047$). Increasing plasma levels of 11KT were seen with increasing temperature (Figure 2.6), but only in the high food treatments. Post-hoc tests showed that there was no significant differences in 11KT levels between high and low ration fish at the lowest temperature, but that there were significant differences at both 30.0 and 31.5°C. Post-hoc tests showed that the significant interaction of temperature and food on testosterone level was due to (1) 28.5°C fish on high food ration having lower testosterone levels than 28.5°C fish on low ration, and (2) 28.5°C fish on high food ration having lower testosterone levels than 30.0 and 31.5°C fish on high ration (Table 2.2, $p < 0.05$).

Table 2.2 *Acanthochromis polyacanthus*. Effect of temperature and ration on plasma levels of testosterone (T) and 17ßestradiol (E2). Values are mean ±SE Figure 2.6. *Acanthochromis polyacanthus*. Effect of temperature and ration level on plasma levels of 11-ketotestosterone (11KT) in males (±SE). Numbers within bars are replicates and letters represent differences between all treatments (p < 0.05)

Food level	Temperature (°C)	Male	Female	
		T	T	E_2
High	28.5	1.74 ± 0.16	2.40 ± 0.28	1.73 ± 0.32
	30.0	2.93 ± 0.52	2.58 ± 0.58	1.81 ± 0.91
	31.5	3.00 ± 0.51	3.74 ± 0.88	1.82 ± 0.43
Low	28.5	2.74 ± 0.47	2.54 ± 0.25	1.61 ± 0.58
	30.0	2.00 ± 0.40	2.13 ± 0.18	3.27 ± 1.21
	31.5	3.16 ± 0.92	3.96 ± 1.65	1.9 ± 1.45

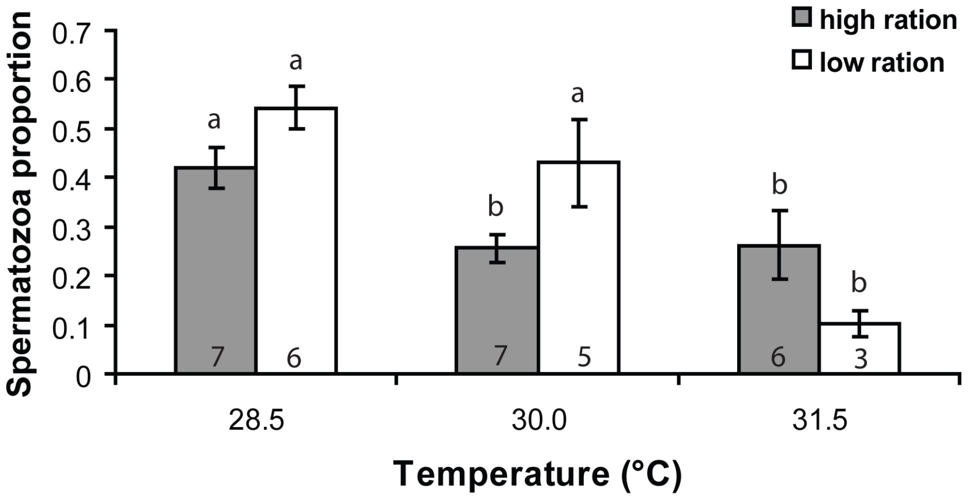

Figure 2.5 *Acanthochromis polyacanthus*. Mean proportion of spermatozoa in male gonads (±SE) between temperature and ration levels. Numbers within bars are replicates and letters represent differences between all treatments (p < 0.05).

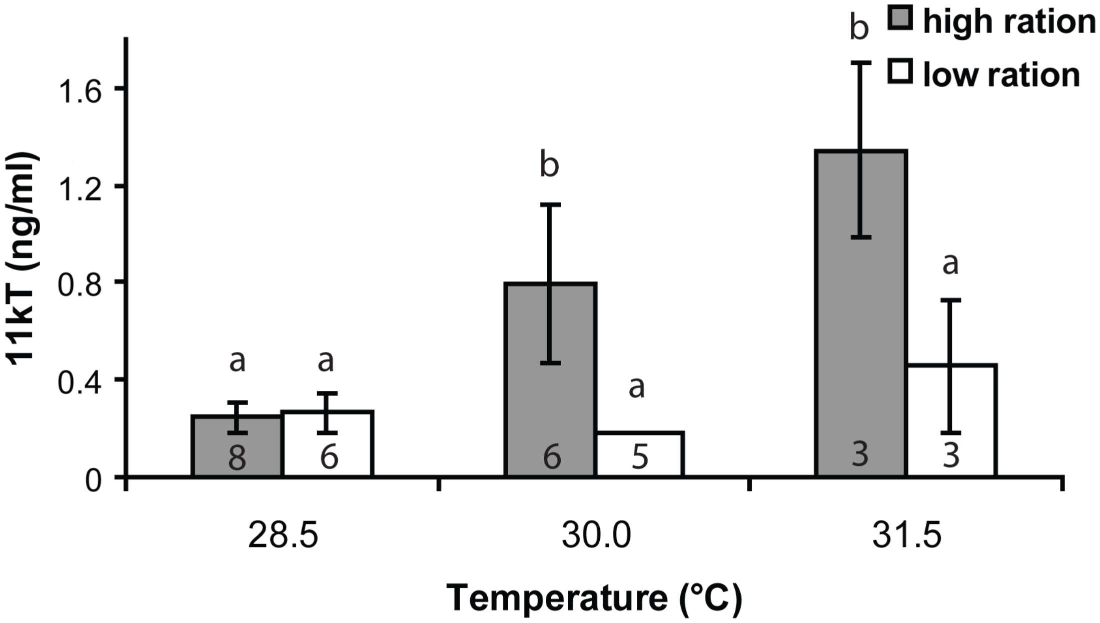

Figure 2.6 *Acanthochromis polyacanthus*. Effect of temperature and ration level on plasma levels of 11-ketotestosterone (11KT) in males (±SE). Numbers within bars are replicates and letters represent differences between all treatments ($p < 0.05$)

2.5 Discussion

Climate change is predicted to increase average water temperature and affect food supply for marine fishes, but how these changes will influence reproductive performance is still poorly understood. We found that both temperature and food level had substantial effects on some reproductive attributes and physical condition of a common reef fish, *Acanthochromis polyacanthus*. Most noticeably, increases in water temperature caused a substantial reduction in reproduction, with complete reproductive failure at elevated temperatures and low food supply. Fish that reproduced at higher temperatures produced smaller eggs, which has important implications for juvenile success (Donelson *et al.* 2008). There was no indication of plasticity in the timing of reproduction relative to water temperature, with individuals at all temperatures commencing breeding within a week of each other. Although the lack of breeding in some pairs may represent a delay in reproduction, and thus some plasticity in this trait, the loss of a season's breeding in a population with average ages of 2 to 8 yr would represent a significant loss of individual fitness. Given the apparent lack of plasticity in the timing of reproduction, the present results suggest that reproductive failures and declines in *A. polyacanthus* populations are likely to occur as the ocean warms.

Populations might adapt to increasing temperature over many generations or acclimate within a generation over multiple years, but the potential for this to occur in species such as *Acanthochromis polyacanthus* is currently unknown (Munday *et al.* 2008a). A few investigations of acclimation or adaptation potential on a time scale necessary for climate change have been completed (see Angilletta 2009), however none involve tropical marine fishes. In most cases observed adaptation took from 21 to 432 generations allowing for epigenetic pathways to assist in the process, but in one instance adaptation was observed within only 2 generations (zooplankter; Van Doorslaer *et al.* 2007). Understanding the acclimation and adaptation potential for reef species is vital for predicting the long term effects of climate change.

Elevated water temperature and low ration resulted in cessation of reproduction in the present study. Even when a greater amount of food was available reproductive capacity was still diminished, with fewer pairs breeding than at lower temperatures. The presence of similar amounts of visceral fat in fish on the high ration diet at all temperatures suggests that daily energetic requirements might still have being met at higher temperatures. Consequently, it appears that elevated water temperature alone can affect reproductive success and could result in reduced reproductive capacity of tropical reef fish regardless of food availability. There is limited information on the combined effects of temperature and food availability on reproduction in ectothermic vertebrates, but studies on invertebrates show similar trends to those observed here, with egg production suppressed at high temperatures even when ample food was provided (Woodward and White 1981; Snell 1986; Lee *et al.* 2003). Exposure of *Acanthochromis polyacanthus* in the present study to elevated temperatures produced significant reductions in egg size. Egg size is a useful indicator of current reproductive condition, and a predictor of subsequent larval success. Smaller eggs are often produced by fish in poorer physical condition (Marteinsdottir and Steinarsson 1998; Donelson *et al.* 2008), and smaller eggs usually produce smaller or poorer conditioned offspring (Green 2008). Furthermore, the quality of larvae and juveniles are known to affect the risk of mortality, with smaller and poorer conditioned individuals often being selected against (Hoey and McCormick 2004; Grorud-Colvert and Sponaugle 2007; McCormick and Meekan 2007). Consequently, it is possible that a smaller proportion of progeny produced by fish at warmer temperatures would ultimately survive.

The reduced growth rate of adults at warmer temperatures also has important implications for reproductive performance. Increased size of reproductively active individuals is known to beneficially affect many components of reproduction including fecundity (Morita and Takashima 1998; Lambert *et al.* 2000), egg size (Vallin and Nissling 2000; Johnston and Legget 2002) and offspring size (Tamada and Iwata 2005). In consequence, a reduction in growth rate resulting from ocean warming could result in fish producing fewer offspring and progeny of reduced fitness. It was expected in the present study that adults might exhibit a trade-off between reproduction and growth; however, both attributes diminished with increasing temperature suggesting that such a trade-off did not occur. Instead, a trade-off may have existed between reproduction and growth versus energy stores, which may have been conserved for future reproduction. Additionally, visceral fat is not the only location of stored energy within a fish (Cowey and Sargent 1972); therefore investigation of muscle protein and liver lipids may have emphasized or altered the current picture.

Elevated water temperature had effects on both plasma steroid levels and gamete production in male fish in the present study. The reduction in spermatozoa observed in this experiment could indicate a possibility for reduced fertilization success as water temperatures increase. However, no direct measurements of fertilization capacity were made in the present study, so the possible effects on fertility are not known. Levels of the gonad steroid 11KT increased with temperature, but only in the presence of high food availability. Levels of 11KT in *Acanthochromis polyacanthus* increase with reproductive maturity and in relation to recent or imminent spawning events (Pankhurst *et al.* 1999). The failure to maintain elevated 11KT at high temperature and low ration in the present study is consistent with the hypothesis that elevated 11KT is required to maintain reproductive function in males. More significant inhibitory effects have been seen in other species, where increasing temperatures were reported to reduce both plasma T and 11KT levels *in vivo* in male rainbow trout (Manning and Kime 1985). This was consistent with studies in other species showing increased levels of steroid inactivation by conjugation at high temperature (Kime 1979). A similar effect to that seen in the present study was described for male carp *Cyprinus carpio*; increased levels of 11KT were produced at high temperatures, but increased levels of the conjugate T-glucuronide were also produced (Kime and Manning 1986). However, not all effects of elevated temperature appear to be generated by increasing rates of conjugation. Males of the red sea bream *Pagrus major* showed decreased mRNA levels of the enzyme 11β-hydroxylase (which mediates the conversion of T to 11KT) in association with reduced spermatogenesis after 8 wk exposure to temperatures of 20°C and above (Lim *et al.* 2003).

The proportion of mature oocytes in the gonad and the level of reproductive hormones of females did not exhibit a strong relationship with temperature or food treatments. However, the observed reduction in egg size suggests that exposure to elevated temperatures did have an effect on vitellogenesis in the present study.

Reductions in final egg size in thermally stressed fish have been reported in rainbow trout (Pankhurst and Thomas 1998) and Atlantic salmon *Salmo salar* (King *et al.* 2003). This was associated with reductions in plasma E2 levels, and levels of the yolk precursor vitellogenin (in turn synthesized by the liver in response to stimulation by E2) in Atlantic salmon, but no change in plasma E2 in rainbow trout (Pankhurst *et al.* 1996; Pankhurst and Thomas 1998). Later studies on Atlantic salmon showed that depression of E2 levels for only part of the period of vitellogenesis could generate reductions in plasma vitellogenin, egg size and subsequent fertility and survival (King *et al.* 2007). It is possible that the same effect has occurred here but has not been detected using only single time point sampling. Effects to eggs may also have occurred through modification of biochemical components, which can affect a range of egg and progeny attributes (Brooks *et al.* 1997), however this was not explicitly tested in the present study.

There was evidence in the present study of a partial protective effect of nutritional condition on reproductive processes at higher temperature, as some pairs were still reproductively active. The basis for this effect is not known, but data from field studies suggest that the effect is exercised through the endocrine system. Assessment of reproductive condition of *Acanthochromis polyacanthus* from reef sites, covering a range of habitats and fish densities, showed that lowest reproductive output was associated with sites of high overall fish density and consequently the highest competition for access to plankton crossing the reef (Pankhurst *et al.* 2008). *In vitro* incubation of ovarian follicles from these sites showed impaired steroidogenesis with, in particular, reduced production of E2. This could be partially restored by treatment with an analogue of luteinizing hormone (LH), suggesting that fish from high density sites had suppressed pituitary release of LH. The same effect may be operating in the present study among fish on the low ration. The results of the present study are generally consistent with the trend of decreasing reproduction with increasing temperature in a range of species (Van Der Kraak and Pankhurst 1997). However, an additional factor here is the relatively small temperature increase required to produce negative effects in tropical fish.

The difference between lower and upper thresholds for reproduction in salmonids is typically 12 to 14°C (Taranger and Hansen 1993, Pankhurst *et al.* 1996, King and Pankhurst 2000), whereas in the present study there was a reduction in the number of reproductively active pairs with an increase of as little as 1.5°C. A lower temperature threshold for reproductive activity in *Acanthochromis polyacanthus* has been established at 24 to 25°C (Hilder and Pankhurst 2003), and on the basis of the present study an upper limit of 30 to 31°C under optimal feeding conditions. In combination it suggests a maximum range of 7°C for this species. If this trend is consistent, the implication for tropical systems in comparison to temperate is that smaller directional shifts in ocean temperature may be required to produce inhibitory effects on stages of reproduction.

We found that temperatures 1.5 to 3.0°C higher than the current summer average significantly influenced reproduction and growth of adult *A. polyacanthus*. When temperatures were elevated, and only a low level of food was available, reproduction ceased. Even when a greater amount of food was supplied fewer pairs reproduced, and there was a marked reduction in egg size at higher temperature. This might result in reduced spawning at elevated temperatures, with associated declines in population size. Alternatively, ocean warming may shift the seasonality of reproduction in *A. polyacanthus* and other species. The capacity of populations to adjust to warmer conditions will depend partially on the level of seasonal change in the system, and how close current seasonal minima and maxima are to temperature thresholds for inhibitory effects. Studies on the potential for long-term acclimation or adaptation of reproductive traits to increased temperature are logistically difficult, but should be a priority for future research given the significant effects detected here.

Chapter 3: Interacting effects of parental and juvenile environment on a coral reef fish

This chapter was prepared for Coral Reefs.

Authors: J. M. Donelson, P. L. Munday and M. I. McCormick.

3.1 Summary

Changes to tropical sea surface temperature and plankton communities are expected to occur over the next 100 years due to climate change. There is a limited understanding of how these environmental changes are likely to impact coral reef fishes, especially in terms of population replenishment through the quality of progeny produced. The present study investigated the effect that elevated sea water temperature and changes to plankton availability may have on the production of offspring by the reef fish *Acanthochromis polyacanthus*, as well as the performance of progeny in environments of varying food availability. An orthogonal design of 3 water temperatures and 2 food availabilities (high and low ration) was used, with water temperatures being the current-day average for the collection location (28.5°C), +1.5°C (30.0°C) and +3.0°C (31.5°C), representing a range of likely temperatures by 2100. Generally, an increase in the water temperature adults were maintained in resulted in a reduction in the size, weight and amount of yolk possessed by newly hatched offspring. Offspring whose parents were maintained under elevated temperature (30.0°C, high ration) had lower survival than offspring produced by parents at the current day temperature (28.5°C, high ration) at 15 days post-hatching, but only when juveniles were reared under conditions of low or no food availability. In contrast, by 30 days post-hatching the growth and condition of these offspring produced by elevated temperature parents (30.0°C high ration) was the best of all treatment groups in all juvenile

environments. This result illustrates the potential for initial parental effects to be meditated by compensatory growth early in life (within 1 month) and that parental effects are not everlasting. These findings suggest that the performance of juvenile reef fish in future ocean conditions will not only depend on initial parental effects, but the interaction between their parentally mediated phenotype and their present environmental conditions.

3.2 Introduction

Substantial changes to tropical oceans are expected to occur within the century due to climate change. This includes increases in tropical sea surface temperature of up to 3°C by 2100 (Lough 2007; Munday *et al.* 2009) and alterations to ocean currents and surface mixing (Sarmiento *et al.* 2004; Harley *et al.* 2006; Rost *et al.* 2008). Changes to ocean mixing will influence primary productivity leading to flow-on effects to plankton production, which is the major food source for many marine organisms (Hays *et al.* 2005; Richardson 2008; Rost *et al.* 2008; Brown *et al.* 2010). In general, the expectation is for less productive tropical oceans due to greater thermal stratification of the water column reducing nutrient enrichment of the surface layers that is important for planktonic productivity (Poloczanska *et al.* 2007; Brander 2009). Also, warmer waters tend to support longer and less productive food chains (McKinnon *et al.* 2007).

The majority of coral reef organisms, including fishes, are ectotherms. This means their basic metabolic processes are influenced by environmental temperature (Bret 1971; Hazel and Prosser 1974; Houde 1989; Clarke and Johnston 1999). Research on terrestrial ectotherms indicates that tropical species are especially sensitive to increases in environmental temperature because they tend to live close to their thermal optimum and they possess a narrow range of temperatures over which they can performance successfully (Deutsch *et al.* 2008; Tewksbury *et al.* 2008; Wright *et al.* 2009). For aquatic ectotherms, metabolic performance declines as water temperature increases above the thermal optimum. Specifically, the energy required to function at rest (resting metabolic rate) increases, while the ability to perform aerobically decreases (Pörtner and Knust 2007, Pörtner and Farrell 2008; Farrell 2009). These relationships between metabolic attributes and increasing water temperatures have been seen across a range of coral reef fish species (Nilsson *et al.* 2009; Gardiner *et al.* 2010; Nilsson *et al.* 2010; Johansen and Jones 2011). Since the energy required at rest is increasing with warming it might be expected that organisms will consume more food to compensate. However, in food restricted situations trade-offs between non-essential activities, such as reproduction and growth, would be expected (Angiletta *et al.* 2003).

Both temperature and food availability can directly affect reproductive attributes in marine fishes. Elevated temperatures can influence the secretion and action of reproductive hormones (Van Der Kraak and Pankhurst 1997), egg size and number (Brown *et al.* 2006; **Chapter 2**), the frequency of reproduction (Hilder and Pankhurst 2003), and offspring size and quality (Brown *et al.* 2006; **Chapter 2**). Variation in food available to parents can affect the energy available for reproduction, and consequently the number, size, energy content or biochemical composition of the eggs produced (see Green 2008 for review). These variations can have flow on effects to the size and conditions of offspring produced (Kerrigan 1997; McCormick 2003; Gagliano and McCormick 2007; Green 2008) as well as how they perform in various environmental conditions (Donelson *et al.* 2009). Studies on the combined effects of temperature and food availability to reproduction and early larval development indicate that in most cases variation in temperature has a greater effect than food (Meekan *et al.* 2003; **Chapter 2**), however when both environmental attributes are unfavourable, the effects are more pronounced than either single variable (**Chapter 2**).

Differences in offspring characteristics in early ontogeny, whether from parental effects or previous environmental conditions, may be compensated for when their present environment is favourable. For example, compensatory growth is often observed, where smaller individuals can rapidly increase their growth catching up to larger individuals in the cohort (Arendt *et al.* 2001; Metcalfe and Monaghan 2001; Morgan and Metcalfe 2001; Johnsson and Bohlin 2006; Álvarez and Metcalfe 2007; Gagliano and McCormick 2007). However, this rapid growth can come at a cost to other life history traits and physical attributes (Arendt *et al.* 2001; Metcalfe and Monaghan 2001; Morgan and Metcalfe 2001; Álvarez and Metcalfe 2007), potentially leading to poorer survival (Johnsson and Bohlin 2006). Additionally, differences between offspring caused by parental effects may only be important under stressful juvenile environments (Marshall *et al.* 2006; Donelson *et al.* 2009). Consequently, predicting the effects that changes in ocean temperature and food supply will have on marine populations is challenging, because it depends both on how the parental environment affects offspring quality and condition, and how these offspring respond to changed environmental conditions (Munday *et al.* 2009). To better understand this problem we need to investigate how offspring produced by parents maintained under future climate predictions perform in varying quality juvenile environments.

In this study we tested the effects that future predicted ocean temperatures and food availability have on the life-history attributes of offspring produced by pairs of the coral reef fish *Acanthochromis polyacanthus*. We then examined how the offspring performed in different quality juvenile environments (three levels of food availability). Specifically, we tested the ability of offspring to survive on endogenous reserves, a low, a medium and a high food level, as well as their growth in these environmental conditions. By comparing key life history attributes including growth, condition and survival in different juvenile environments, the importance of interactions between the parental and juvenile environment in shaping the early life history of reef fish could be established.

3.3 Materials and methods

Study species and adult rearing conditions

Forty-two adult pairs of *A. polyacanthus* were collected from the central region of the Great Barrier Reef (Australia) during the Austral winter and housed in 60 l aquariums at the James Cook University Marine and Aquaculture Research Facility. Breeding pairs were split into three temperature treatments: current-day average temperature (summer mean = 28.5°C), 30.0°C (current +1.5°C) and 31.5°C (current +3.0°C). The current-day summer means were calculated from November to February over 14 years of daily water temperature records from the JCU/AIMS weather station. Breeding pairs at each temperature were randomly assigned to two feeding treatments (high or low ration) to produce a fully orthogonal design with 6 to 8 breeding pairs at each combination of temperature and food ration. The high food treatments consisted of 0.376g Aquaculture Nutrition NRD 12/20 pellets (1.0% body weight) per fish per day, which is approximately the average amount of food consumed per day at the collection location (**Chapter 2**). The low treatment was half of the high at 0.188g per fish per day (0.5% body weight) and represented the minimum amount of food fish were consuming per day (**Chapter 2**).

Hatching characteristics

Nesting sites were checked daily for the presence of clutches. On the day of hatching, twenty haphazardly selected juveniles were removed from each clutch for measurement of physical attributes. The sampled fish were euthanased with an overdose of clove oil and then preserved in 4% phosphate buffered formaldehyde solution. Weight, standard length (SL), and yolk area were measured after 2 days of preservation. Standard length was estimated by photographing fish under a stereomicroscope and subsequently using image analysis (Optimus 6.5, Media Cybernetics). The length of each fish and the area of each yolk sac were measured to the nearest 0.01 mm three times and the average recorded. Fish were then blotted dry and weighed to the nearest mg.

Breeding pairs did not reproduce evenly in all combinations of temperature and food levels, with pairs in both the +1.5°C and +3.0°C on low food ration failing to reproduce at all (Donelson *et al.* 2010). In the 28.5°C (current-day) high ration treatment combination 7 clutches were produced, 28.5°C low ration a total of 3 clutches, 30.0°C (+1.5°C) high ration produced 3 clutches and 31.5°C (+3.0°C) high ration produced 3 clutches. In addition, limited numbers of offspring in the 31.5°C treatment survived through to hatching, thus post-hatching experiments were unable to be completed on this treatment.

Survival on endogenous reserves

Survival on endogenous reserved provides an indication of the quantity and quality of parental provisioning to offspring. To determine if the length of time offspring can survive on their endogenous resources varied between treatments, a random sample of 20 newly hatched individuals from each clutch were reared without food. Juveniles were haphazardly selected from each clutch within 2 hours of hatching and transferred to individual 2 l plastic aquariums (260×120×95mm) in an environmentally controlled room (completed as per Donelson *et al.* 2008). Fish were maintained in the temperature of their parents throughout the experiment. For the current-day treatment the mean temperature over the experimental period was 28.49°C (±0.03 SE) and for the +1.5°C treatment the mean temperature was 30.11°C (±0.05 SE). The light regime was kept at the summer average for the parental collection location; 12.75 h light: 12.25 h dark. Each aquarium containing a juvenile was checked daily at approximately 09:00 and 17:00 h to determine if that individual was still alive.

Juvenile rearing conditions

Sixty juveniles per breeding pair were haphazardly sampled at hatching and were transferred into individual 2 l plastic aquariums (160×160×9mm) in their corresponding parental temperature and supplied with a constant flow of seawater at temperatures stated above. Twenty fish were randomly assigned to each three feeding treatments. On the first day of the experiment all fish were fed *Artemia* nauplii at a concentration of 2 individual ml^{-1} (approximately 4000 individuals per aquaria). Subsequently, they were fed *Artemia* nauplii at a concentration of 2 individual ml^{-1} every day (high food treatment), two out of every three days (medium food treatment), or every third day (low food treatment). From day 7 onwards juveniles were fed *Artemia* at 1 individual ml^{-1} and approximately 2mg of INVE Aquaculture Nutrition 2/4 NRD pellets at each feed. From day 15 onwards juveniles were fed approximately 5mg of INVE Aquaculture Nutrition 2/4 NRD pellets at each feed. Any juveniles that died within in the first 24 hours after relocation were replaced, as this mortality was attributed to the stress of movement. Subsequently, containers were checked daily at 09:00 and deaths within the last 24 hour period were recorded.

Half of the individuals still alive were sampled at day 15 of the experiment and all the remaining individuals were sampled at day 30 post-hatching. Fish were euthanised and measured on the day of sampling prior to preservation. SL was estimated by photographing fish under a stereomicroscope and subsequently using image analysis to the nearest 0.01 mm. Fish were then blotted dry with a paper towel and weighed (to nearest 0.001 g).

Data analysis

To determine whether parental temperature and food level affected offspring characteristics at hatching a one-factor MANOVA was run on SL, weight, yolk area and Fulton's K condition. In this analysis, parental temperature and food availability were combined as one variable due to the absence of data from the elevated temperatures and low food levels (i.e. only 4 groups were tested 28.5°C high ration, 28.5°C low ration, 30.0°C high ration and 31.5°C high ration). Where significant differences were identified univariate ANOVAs and Fisher's LSD post-hoc tests were undertaken. To determine whether parental temperature and food treatment affected survival on endogenous reserves a multiple sample survival analysis was used. This analysis is an extension of the Gehan's generalized Wilcoxon test. Where significant differences were found two sample Cox's F-test survival analysis were run on each combination of parental treatments with an adjusted significance level of 0.0167. For survival in the three juvenile food levels separate multiple sample survival analyses were completed. If significant differences were identified two-sample Gehan's Wilcoxon tests were run with adjusted significance levels of 0.0167. To investigate whether parental treatment and juvenile food level affected growth of juveniles a factorial MANOVA was undertaken on SL, weight and Fulton's K condition separately at 15 d and 30 d post-hatching. At 15 d post-hatching SL and weight were logarithmic transformed to meet homogeneity assumptions. ANOVAs and Fisher's LSD post-hoc tests were used to explore the nature of significant MANOVAs. All statistical analysis was completed with Statistica 9.0.

3.4 Results

Hatching characteristics

The physical characteristics of individuals at hatching depended on their parents' temperature and food treatment (Figure 3.1; MANOVA: $F_{12,828}$=15.7, p<0.001; Univariate: SL: $F_{3,277}$=25.11, p<0.001, weight: $F_{3,277}$=18.68, p<0.001, yolk area: $F_{3,277}$=22.78 p<0.001, condition: $F_{3,277}$=22.78, p<0.001). Both SL and weight declined with increasing parental temperature (Figure 1a & b; p<0.004), while offspring from 28.5°C low ration parents were longer than offspring from any of the high ration groups (p<0.001). Yolk area was smallest in fish produced by 30.0°C parents (Figure 3.1c; $p<0.001$), but still significantly less in 31.5°C high ration and 28.5°C low ration offspring compared to 28.5°C high ($p<0.03$). Contrastingly, the condition of offspring was similar between the three high ration parents ($p>0.05$) and only significantly reduced in the 28.5°C low ration offspring (Figure 3.1d; $p<0.002$).

Survival on endogenous reserves

The combination of parental temperature and food level significantly influenced the rate of offspring mortality on endogenous reserves (Fig. 2; $X^2=84.73$, df=2, $p<0.001$). Offspring produced by parents on the high food ration in 28.5°C survived up to 4 days longer (up to 13 days post-hatching) than offspring from low ration pairs in 28.5°C (Cox's F-test: $F_{278,120}=3.806$, $p<0.001$) and high ration pairs in 30.0°C (Cox's F-test: $F_{278,120}=2.790$, $p<0.001$). Both 30.0°C high and 28.5°C low ration treatment offspring survived a maximum of 10 days post-hatching and were not significantly different (Cox's F-test: $F_{120,120}=1.457$, $p>0.0167$).

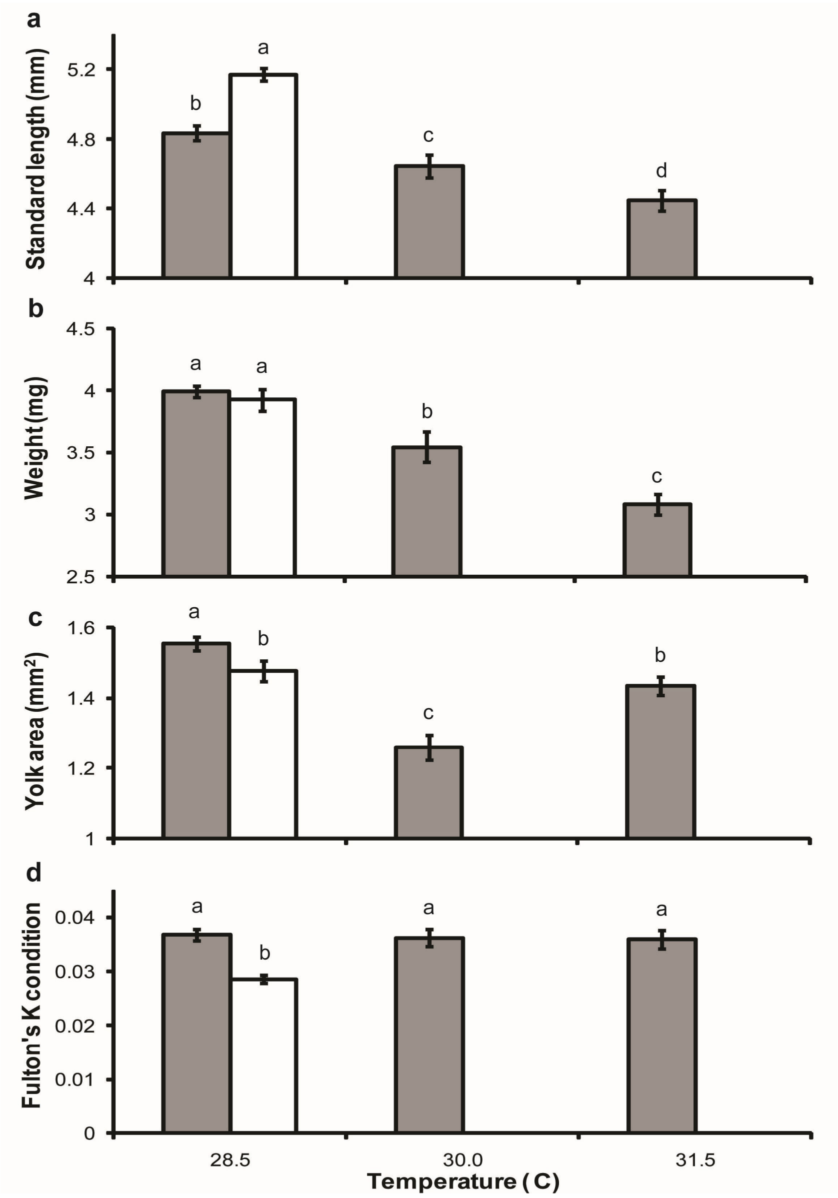

Figure 3.1. Mean standard length (a), weight (b), yolk area (c) and Fulton's K condition index (±SE) of newly hatched *Acanthochromis polyacanthus* that resulted from breeding pairs kept under a combination of three water temperatures and two food levels. Shaded bars, high food levels; white bars low food levels. Letters over bars represent the groupings from Fisher's LSD post-hoc tests ($p<0.05$).

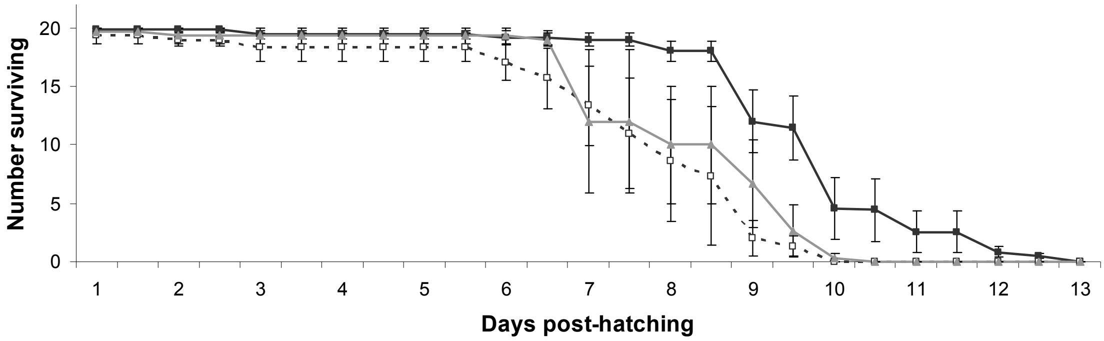

Figure 3.2. Mean survival (± SE) of juvenile *Acanthochromis polyacanthus* on endogenous reserves in relation to parental maintenance temperature and ration level. Black squares, parents kept under 28°C and high ration; open squares, parents under 28°C and low ration; grey triangles, parents under 30°C and high ration.

Juvenile survival under different food availability

Greater than 85% survival occurred in the high and medium juvenile food environments regardless of parental treatment over the first 15d post-hatching (Figure 3.3a & b). In contrast, up to 70% mortality occurred in the low quantity juvenile food (Figure 3.3c). Furthermore, in the low ration juvenile environment differences in offspring survival depending on parental temperature and food ration (X^2=7.93, df=2, p=0.019). Offspring from parents fed high ration at 28.5°C had on average 69% survivorship at day 15, and survived significantly better than offspring from 28.5°C low ration parents (z=3.839, n=200, p<0.001) and high ration parents at 30.0°C (z=5.218, n=200, p<0.001). However, no differences were seen between the survival of offspring from 28.5°C low ration parents (mean 42% on day 15) and high ration parents at 30.0°C (mean 32% on day 15; z=1.717, n=120, p=0.086). In the medium ration environment no differences were seen in survival of any parental treatment (X^2=0.790, df=2, p=0.674). In contrast, in the high ration juvenile environment fish produced by parents fed low food levels at 28.5°C had significantly greater mortality levels than the fish produced by high ration parents at 30.0°C (full model: X^2=7.93, df=2, p=0.019; 30.0°C high: z=2.451, n=120, p=0.014).

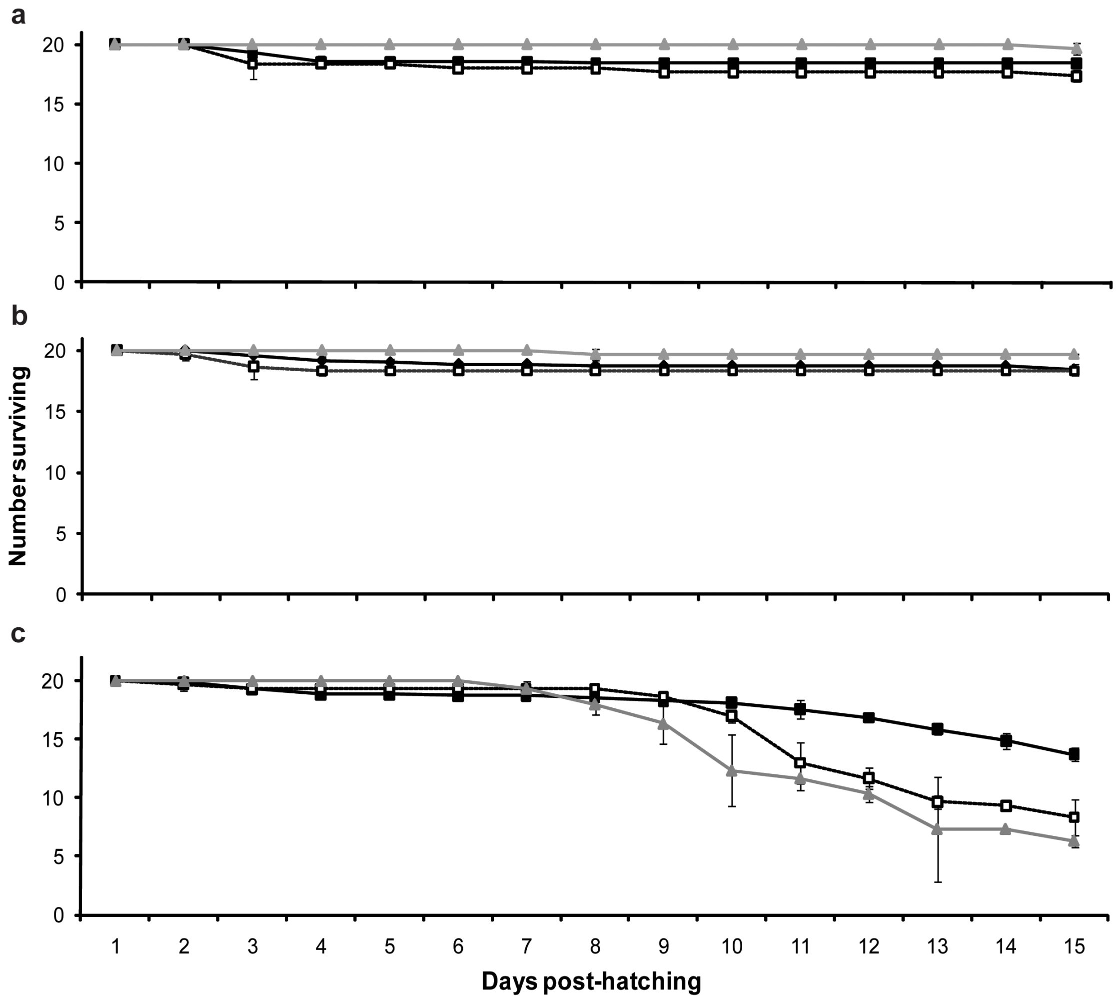

Figure 3.3 Mean survival (± SE) of juvenile *Acanthochromis polyacanthus* reared under three conditions: a) juvenile high-food, b) juvenile medium-food and c) juvenile low-food environments. Survival is plotted in relation to parental maintenance temperature and ration level. Black squares, parents kept under 28°C and high ration; open squares, parents under 28°C and low ration; grey triangles, parents under 30°C and high ration.

Growth in various food quality environments

Juvenile growth and condition was strongly affected by the food level available in their environment at both 15 days (Table 3.1; MANOVA: $F_{6,584}$=51.0, p<0.001; Univariate: SL: $F_{2,301}$=236.0, p<0.001; Weight: $F_{2,231}$=276.6, p<0.001; Fulton's K condition: $F_{2,301}$=21.17, p<0.001) and 30 days post-hatching (Table 3.2; MANOVA: $F_{6,444}$=44.64, p<0.001 ; Univariate: SL: $F_{2,231}$=135.15, p<0.001; Weight: $F_{2,231}$=143.70, p<0.001; Fulton's K condition: $F_{2,231}$=5.65, p=0.004). The importance of the parental environment (combined temperature and food level) differed depending on the attribute measured and the time post-hatching. At 15 days, parental environment did not significantly influence SL ($F_{2,301}$=2.30, p=0.101), but did affect weight ($F_{2,301}$=3.89, p=0.022) and interacted with juvenile food level to affect condition ($F_{2,301}$=3.98, p=0.004; Table 1). Specifically, the weight of fish from 28.5°C low ration parents was less than both other parental treatments (p<0.007). Body condition did not differ between any parental group in the low food level juvenile environment, but in moderate food availability offspring from 28.5°C high ration parents performed significantly better (p<0.03) and in high availability 28.5°C low ration offspring were in significantly worse condition (p<0.001). At 30 days post-hatching the parental treatment significantly affected SL ($F_{2,231}$=4.11, p=0.018), weight ($F_{2,231}$=3.31, p=0.038) and body condition (Table 3.2; $F_{2,231}$=6.23, p=0.002). However, offspring from various parental treatments performed differently depending on the attribute measured. Offspring produced by 28.5°C high ration parents were in the poorest condition at 30 days (p<0.009), while offspring from 28.5°C low ration parents were significantly shorter (p<0.016) and 30.0°C parental high ration offspring were significantly heavier than both other groups (p=0.004).

Table 3.1. Effect of parental treatment (breeding temperature and food ration) and juvenile food ration on standard length, weight and Fulton's K condition at 15 days post-hatching *Acanthochromis polyacanthus*. Values are mean ±SE.

	Parental treatment		
	28.5°C high ration	28.5°C low ration	30.0°C high ration
Juvenile high ration	SL = 8.48 ± 0.08	SL = 8.42 ± 0.12	SL = 8.44 ± 0.14
	Weight = 18.62 ± 0.49	Weight = 16.67 ± 0.68	Weight = 17.90 ± 0.82
	Condition = 0.030 ± 0.0005	Condition = 0.027 ± 0.0005	Condition = 0.029 ± 0.0006
Juvenile medium ration	SL = 7.53 ± 0.05	SL = 7.48 ± 0.09	SL = 7.47 ± 0.12
	Weight =12.61 ± 0.33	Weight = 11.56 ± 0.49	Weight = 11.50 ± 0.59
	Condition = 0.029 ± 0.0004	Condition = 0.027 ± 0.0006	Condition = 0.027 ± 0.0006
Juvenile low ration	SL = 6.27 ± 0.06	SL = 5.94 ± 0.15	SL = 6.37 ± 0.17
	Weight = 5.91 ± 0.22	Weight = 5.42 ± 0.56	Weight = 6.86 ± 0.70
	Condition = 0.024 ± 0.0005	Condition = 0.025 ± 0.0012	Condition = 0.026 ± 0.0014

Table 3.2. Effect of parental treatment (breeding temperature and food ration) and juvenile food ration on standard length, weight and Fulton's K condition at 30 days post-hatching *Acanthochromis polyacanthus*. Values are mean ±SE.

	Parental treatment		
	28.5°C high ration	28.5°C low ration	30.0°C high ration
Juvenile high ration	SL = 12.67 ± 0.20	SL = 12.16 ± 0.21	SL = 12.84 ± 0.32
	Weight = 61.17 ± 2.71	Weight = 57.21 ± 3.19	Weight = 68.17 ± 5.14
	Condition = 0.030 ± 0.0004	Condition = 0.028 ± 0.0005	Condition = 0.031 ± 0.0007
Juvenile medium ration	SL = 11.26 ± 0.16	SL = 10.60 ± 0.19	SL = 11.01 ± 0.26
	Weight = 41.68 ±1.78	Weight = 36.46 ± 1.85	Weight = 44.8 ± 3.14
	Condition = 0.029 ± 0.0003	Condition = 0.030 ± 0.0006	Condition = 0.033 ± 0.0006
Juvenile low ration	SL = 8.24 ± 0.33	SL = 7.66 ± 0.30	SL = 8.33 ± 0.38
	Weight = 15.45 ± 2.22	Weight = 12.75 ± 1.97	Weight = 16.9 ± 2.64
	Condition = 0.027 ± 0.001	Condition = 0.027 ± 0.0022	Condition = 0.028 ± 0.0014

3.5 Discussion

Evidence of parental effects to offspring characteristics at hatching were found when parents were maintained in future predicted temperature and food conditions. As the temperature experienced by parents increased, the size of offspring became smaller and yolk reserves declined. The performance of these juveniles in various environmental conditions depended on their parental treatment, however performance was not always consistent with offspring characteristics at hatching. Offspring from parents kept under 30.0°C and high ration conditions were smaller at hatching, but by 30 days post-hatching were generally longer, heavier and in better condition. In contrast, offspring from 28.5°C low ration parents were largest at hatching, but smallest by 30 days. When poor juvenile food environmental conditions were present the survival of 30.0°C high ration and 28.5°C low ration offspring were significantly worse than 28.5°C high ration. This suggests that progeny performance in future ocean conditions will not only depend on their environment, but the interaction between their parentally mediated phenotype and their present environmental conditions.

For coral reef fish the first few weeks of life are known to be critical for success and small differences in body size and growth rate may substantially influence survival (Sale and Ferrell 1988; Almany and Webster 2006). In our experiments, parents maintained in future predicted temperature conditions produced smaller and lighter offspring with less yolk provisioning. Mortality is size selective in juvenile reef fish (Meekan and Fortier 1996; Holmes and McCormick 2009) and consequently offspring produced by parents' in future warmer oceans may suffer greater mortality than current day individuals if it takes longer for mortality size thresholds to be reached. Alternatively, if the same proportion of mortality remains it would be expected that individuals in the future will be smaller for the same age. Body condition has also been identified as a potential determinant of survival during the early life of reef fish (Hoey and McCormick 2004; Holmes and McCormick 2009) and the condition of offspring from all high-ration parents was similar regardless of increasing temperature. Thus, the early juvenile survival in future populations will depends on the nature of selectivity agents and what offspring traits are most influential, which may not be the same as current day.

Generally, elevations in parental environmental temperature had a greater effect on the attributes of offspring at hatching than parental food availability. Specifically, an increase of only 1.5°C caused a greater reduction in size and yolk reserves of offspring than half the food provisioning to parents. It may also indicate that temperature directly affects reproductive pathways that influence gamete quality (final oocyte maturation and vitellogen production; Pankhurst *et al.* 1996; King *et al.* 2007) and consequently offspring attributes. There is substantial support for elevations in temperature negatively effecting reproductive pathways and production of gametes of marine fish (Manning and Kime 1985; Pankhurst and Thomas 1998; Van der Kraak and Pankhurst 1997; Hilder and Pankhurst 2003; Pankhurst and Porter 2003), and this occurred in breeding adults utilised for the present study (Donelson *et al.* 2009).

The importance of parental effects on offspring survival varied depending on the food available in the juvenile environment. Similar juvenile survival was found if there was a low level or no food provided in the juvenile environment. In these environments offspring from 28.5°C high ration parents surviving significantly better than offspring from parents that experienced an increase of +1.5°C or a reduction in food availability. Offspring from parents maintained under 28.5°C low ration or 30.0°C high ration survived similarly in these poor juvenile environments suggesting that a 1.5°C increase or half the food available to parents may produce comparable future survival results. In contrast, in moderate and high food juvenile environments this trend was lost, supporting the hypothesis that parental effects are most important in stressful (i.e. food stressed) environmental conditions (Marshall *et al.* 2006; Donelson *et al.* 2009). However, a large amount of research shows that parental effects are more influential at the beginning of life and with time the current environment conditions of offspring become the determining factor (Donelson *et al.* 2009; van der Sman *et al.* 2009). Interestingly, in high juvenile food conditions the two parental treatments that survived similarly in poor environments (28.5°C low and 30.0°C high) were significantly different from each other, with fish produced by 30.0°C parents surviving significantly better. This suggests that there may not be a simple recipe to survival and characteristic that determine survival vary with juvenile environmental quality.

It is well established that the amount of food available to a juvenile will substantially influence their growth and condition (Jones 1986; Forrester 1990; Booth and Alquezar 2002). Our data fitted the expected trends with juveniles being longer, heavier and in better body condition with increasing food availability. However, offspring performance within these environments with different available food depended on their parent's temperature and feeding history. At 15 days post-hatching in a low food environment, juveniles from all parental treatments grew similarly, whereas in moderate and high food environments larval growth was affected by parental treatment. This observation is in contrast to previous findings that beneficial parental effects are only important in poor quality environments (Donelson *et al.* 2009). However, our ability to detect differences between parental groups in the low-food juvenile environment may be limited by high juvenile mortality.

By 30 days post-hatching offspring from 30.0°C parents were generally performing the best for all physical attributes measured. Initially at hatching these offspring were shorter and lighter than offspring produced by either 28.5°C parental group. This indicates that while fish started out with potential physical limitations they were able to compensate for differences across all juvenile environments (Arendt et al. 2001; Morgan and Metcalfe 2001; Johnsson and Bohlin 2006; Álvarez and Metcalfe 2007; Gagliano and McCormick

2007). Even in poor environmental conditions, where mortality was significantly greater for offspring from 30.0°C high ration parents, individuals that persisted from this treatment were in better condition. In contrast, offspring produced by 28.5°C low ration parents were the longest at hatching, but by 30 days were significantly shorter than the two other parental treatments, which in part could be due to selective mortality. These findings suggest that initial measures of size and condition may be good indicators of growth and survival when fish first arrive on the reef (Meekan and Fortier 1996; Hoey and McCormick 2004), but other phenotypic parental contributions not captured by size and condition estimated may subsequently determine performance (e.g. yolk quality or hormones). Additionally, a portion of the difference observed between 28.5°C and 30.0°C may be directly due to the temperature differences of the juvenile environment, since offspring were carried through on their parent’s temperature. Small increases in temperature are known to enhance growth and development rate (Rombough 1997; Meekan and Fortier 1996; Bergenius *et al.* 2002; Wilson and Meekan 2002; Sponagule *et al.* 2006). However, for this to be the only cause of differences between 28.5°C and 30.0°C high ration offspring, 30.0°C juveniles would have been expected to be unable to maintain a high rate of growth and condition under low ration conditions in the juvenile environment.

This study highlights the potential impacts that elevated sea temperature and reductions in food availability to adults will have on their progeny. Furthermore, some of these parental effects to progeny will determine juvenile survival in poor environmental conditions. The study also illustrates that observed differences at hatching due to moderate ocean warming (+1.5°C and no reduction in food) are able to be compensated for over relatively short time frames (15-30 days). To determine the long-term effects of hatching differences and compensatory growth longer running grow-out experiments are required. The potential for long-term thermal acclimation has not been tested in this study and the importance of parental effects to offspring may be reduced with complete development in future climate conditions.

Chapter 4: Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish

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Authors: Jennifer M. Donelson, Philip L. Munday, Mark I. McCormick and Göran E. Nilsson

4.1 Summary

Determining the capacity of organisms to acclimate and adapt to increased temperatures is key to understanding how populations and communities will respond to global warming. While there is evidence that elevated water temperature affects metabolism, growth and condition of tropical marine fish, it is unknown if they have the potential to acclimate, given adequate time. We reared the tropical reef fish *Acanthochromis polyacanthus* through its entire life-cycle at present day and elevated (+1.5°C and +3.0°C) water temperatures to test its ability to thermally acclimate to ocean temperatures predicted to occur over the next 50-100 years. Fish reared at 3.0°C greater than the present day average reduced their resting oxygen consumption (RMR) during summer compared with fish reared at current day temperatures and tested at the elevated temperature. The reduction in RMR of up to 69 mg O_2 kg^{-1} h^{-1} in acclimated fish could represent a significant benefit to daily energy expenditure. In contrast, there was no acclimation to summer temperatures exhibited by fish reared at 1.5°C above current day temperatures. Acclimated fish at +3.0°C were smaller and in poorer condition than fish reared at present day temperatures, suggesting that even with acclimation there will be significant consequences for future populations of tropical fishes caused by global warming.

4.2 Introduction

Average sea surface temperatures are expected to increase up to 3.0°C by the end of this century due to global warming (Meehl *et al*. 2007; Poloczanska *et al*. 2007; Munday *et al.* 2009). Whether marine organisms have the capacity to cope with such a rapid increase in temperature is critical to understanding the likelihood of species persistence and population sustainability (Donner *et al*. 2005; Brierley and Kingsford 2009; Hofmann and Todgham 2010). There is increasing evidence that species located in tropical regions might be sensitive to temperature increases of just a few degrees, due to them having evolved in a relatively thermally stable environment (Tewksbury *et al.* 2008; Wright *et al.* 2009; Nilsson *et al*. 2009). Specifically, some tropical species exhibit a narrower thermal reaction norm than related temperate species and this is expected to reduce their ability to cope with temperatures above their thermal optimum (Stillman 2003; Tewksbury *et al.* 2007; Pörtner and Farrell 2008). Consequently, understanding the potential for species to acclimate or adapt to rapid temperature change will be especially important for predicting the consequences of global warming for populations and communities of tropical species.

Poikilotherms, like fish, are strongly influenced by increases in environmental temperature (Fry 1967; Hazel and Prosser 1974; Houde 1989; Clarke and Johnston 1999). A wide range of attributes may be affected including resting metabolic rate, maximum oxygen uptake, growth rate, lipid composition and enzyme activity (Hazel and Prosser 1974; Clarke and Johnston 1999). Resting metabolic rate (RMR), generally measured as resting oxygen consumption, and maximal oxygen uptake have been suggested to be critical factors in determining persistence at locations during climate change (Pörtner and Knust 2007). Specifically, at higher temperatures, the maximum capacity for oxygen uptake can no longer keep pace with the rise in resting metabolism, causing a reduction in the scope for processes like feeding and reproduction (Brett 1971; Portner and Knust 2007; Farrell *et al.* 2008; Pörtner and Farrell 2008). Furthermore, the temperature for optimal metabolic scope is believed to coincide with optimal growth (Del Toro-Silva *et al.* 2008), and optimal growth rate does not readily shift with acclimation temperature (Brett, 1971; Guderley *et al.* 2001). For some tropical reef fishes recent research has shown that aerobic scope (the difference between maximum and resting metabolism) decreases when water temperature is raised above normal summer temperature (Nilsson *et al.* 2009), and that increased seawater temperature can limit the growth of juvenile and adult reef fishes (Munday *et al.* 2008b), as well as their reproductive capacity (Hilder and Pankhurst 2003; Donelson *et al.* 2010). Therefore, most of the available evidence suggests that populations of tropical reef fish will be significantly affected by global warming.

Thermal acclimation is one means of coping with increased temperature. Acclimation involves the phenotypic altering of physiological, behavioral or morphological characteristics to better suit an environment (Fry 1967; Hazel and Prosser 1974; Randall *et al.* 2000, Woods and Harrison 2002). Two general classes of acclimation are recognized: reversible and developmental acclimation. Developmental acclimation consists of permanent responses to the environment during early ontogeny while reversible acclimation includes controlled responses in relation to daily or seasonal environmental fluctuations (Angilletta 2009). While either form of acclimation has the potential to produce some benefits to the individual, neither is likely to be cost free. There are possible energetic costs associated with modifications for which the energy used could have been spent on other functions (Hoffmann 1995; Angilletta *et al.* 2003). For example, physiological acclimation to increased temperature may reduce the energy available for growth or reproduction. Furthermore, acclimation to elevated maximum temperatures might have consequences for performance at minimum temperatures (Dulai *et al.* 1999; Seebacher *et al.* 2005; Glanville and Seebacher, 2006) if there is a trade-off between performance at maximum and minimum temperatures.

Previous research on temperature acclimation in marine fishes has primarily involved temperate species, which are likely to have a greater scope for acclimation than tropical species since they naturally experience a greater range of temperature fluctuations (Stillman 2003). Moreover, the main focus has been on cold acclimation and its effects to mitochondrial densities, metabolic rate, swimming performance and muscular development (Johnston and Dunn 1987; Johnston *et al.* 1998; Johnston and Temple 2002; Sylvestre *et al.* 2007). Research on polar species, which have evolved in a thermally stable environment, primarily indicate a very low capacity for acclimation (Steffensen 2002), although some evidence for thermal acclimation has been found (Franklin *et al.* 2007; Robinson and Davison 2008).

So far, only three studies have investigated thermal acclimation in tropical coral reef species, and no metabolic acclimation was observed (Tullis and Baillie 2005; Nilsson *et al.* 2009; Nilsson *et al.* 2010). However, since these studies only investigated the potential for acclimation in juveniles and adults over a short period (up to three weeks), the question of whether coral reef fishes are capable of developmental thermal acclimation remains unanswered.

We tested the ability of a common reef fish, *Acanthochromis polyacanthus*, to undergo developmental metabolic acclimation as a means of coping with tropical ocean temperatures that are predicted to occur over the next 50-100 years due to global warming. Specifically, fish were reared from shortly after hatching to maturity in present day and elevated temperatures (+1.5°C and +3.0°C) to determine if continuous exposure to elevated temperatures induced an acclimation response in their RMR at maximum (summer) and minimum (winter) temperatures. The experimental temperatures were chosen to represent tropical ocean warming that could occur around 2050 (+1.5°C) or by 2100 (+3.0°C) (Preston and Jones 2006; Lough 2007). In addition, the physical characteristics of the fish were measured to determine if acclimation had a significant physiological cost.

4.3 Materials and methods

Experimental design

Established pairs of *Acanthochromis polyacanthus* were collected from the Palm Island region (18° 37’ S, 146° 30’ E) of the central Great Barrier Reef in July to August 2007 and maintained in 60 l aquariums inside an environmentally-controlled facility at James Cook University, Townsville, Australia. Pairs were maintained at the mean present day ocean temperature for the collection location (Figure 2.1) and provided with the average food consumed by wild pairs (0.376g per fish Aquaculture Nutrition NRD 12/20 pellets: 1.0% body weight, see Donelson *et al.* 2010 for more details). In *A. polyacanthus* care is provided to the benthic eggs by both parents and after hatching the young remain with the parents for 30 to 45 days (Kavanagh 2000). The geographic range of *A. polyacanthus* is from 15°N–26°S and 116°E–169°E, which encompasses a total temperature span (inclusive of seasons) of approximately 20°C to 31°C across this range. Similar temperature ranges are experienced by many other broadly distributed coral reef fishes (Munday *et al*. 2008a).

During the austral summer 2007-2008 breeding bouts from 8 pairs were used for the current study. Offspring from these pairs were kept with their parents for 30 days post-hatching. At this time individuals from each clutch were divided into 3 groups of 18 and gradually adjusted (>5 hrs) to one of the three treatment temperatures: either the present day mean temperature at the collection location, +1.5°C or +3.0°C treatment (Figure 4.1). Temperature was kept within ±0.3°C of the desired treatment mean. Temperatures at the collection location have fluctuated between 0.2-2.5°C in a single day over the last 14 years, but on average vary only 0.45°C on a daily basis (JCU/AIMS weather station).

Splitting clutches in this way ensured that each experimental treatment contained similar genetic diversity and controlled for possible genetic effects on acclimation ability. Sibling fish were kept in groups of 6 in 40 l aquaria until 6 months old when the smallest 2 fish were removed to reduce density in aquaria. No differences in mortality were observed among treatments, with close to 100 percent survival attained at each temperature. At 8 months individuals were sorted into pairs consisting of one male and female non-sibling fish and maintained in 40 l aquaria for the duration of the experiment to limit aggressive interactions between same sex individuals. Fish were fed to satiation a mixture of newly hatched *Artemia* nauplii and Aquaculture Nutrition NRD with increasing size.

Resting metabolism

RMR was estimated at the end of summer during March and April 2009 (fish were approximately 1 year old and 13.5g ± 0.3 SE) and during winter in July and August 2009 (fish were approximately 1.5 years old and 16.2g ± 0.4 SE). Fish were starved for 24 hours prior to testing to remove any effects of digestion on oxygen consumption. The test temperatures used during these periods were 28.5°C, 30.0°C and 31.5°C in summer and 22.5°C, 24.0°C and 25.5°C in winter. These temperatures are the summer and winter averages for the three treatments: present day, +1.5°C and +3.0°C respectively. Fish were initially tested at their temperature treatment and then given at least 3 days of rest. All tested fish were then divided equally in two groups and gradually adjusted to one of the other two treatment temperatures over a 2 hour period. Fish were maintained at the new temperature for 7 days before RMR was tested again at the new temperatures. All experiments were conducted within a temperature controlled room and during respirometry temperatures did not vary more than 0.3°C from the intended temperature. For measurements undertaken from March to April a total of 170 trials were completed with 28 fish from present day, 33 from +1.5°C and 30 from +3.0°C treatments. During the July to

August measurement period, 111 trials were completed with 19 fish from current day, 20 fish from +1.5°C and 19 fish from +3.0°C. Wet weight (nearest mg) and standard length (mm) of all fish were measured during March-April 2009, when acclimation was found to occur. These measurements were taken directly following RMR measurements, when fish has not fed for 24 hours, thereby ensuring that feeding activity did not affect wet weight measures.

Respirometry methods were modified from Nilsson and Östlund-Nilsson (2004) and Nilsson *et al.* (2009). Each fish was allowed to acclimatize in the respirometer (a 1000 ml Perspex cylinder with 68 mm inner diameter) for 1 h with a constant water flow. A longer acclimatization time of 3 h did not alter respiration values obtained (ANOVA: $F_{3,16}=0.701$, $p=0.565$). Following acclimatization the chamber was sealed and oxygen concentrations were monitored with an oxygen electrode (WTW OXI 340i or OXI 3310, Germany) for 30 min or until oxygen concentrations had fallen to 60% of air saturation. The respirometer was submerged in a temperature-controlled aquarium to maintain a stable temperature. Subsequently, the wet weight of each fish was measured to the nearest mg.

Statistics

RMR (mg of O_2 consumed kg^{-1} h^{-1}) for each fish was calculated from the recorded fall in oxygen concentration in the respirometer (measured as % of air saturation), taking into account the O_2 solubility in sea water at each test temperature. One-way and factorial ANOVA, with rearing temperature and/or testing temperature as fixed factors, was used to compare RMR in summer and winter test periods. Summer data were square root transformed prior to analysis to improve the homogeneity of variances. Fisher's LSD post-hoc tests were completed where necessary.

To determine if acclimation had a cost to growth of body condition, regression analysis was used to examine the relationship between standard length and weight of fish in each temperature treatment during summer, as this was when acclimation was found to occur. A power function was fitted as this best described the relationship between standard length and weight. Additionally, the length-weight relationship converted to Fulton's K condition factor (equation: (10000*weight)/L^3) was used as an indicator of condition. An increase in weight proportional to length has been found to relate significantly to muscle and liver energy content (Lambert and Dutil 1997), survival time in stressful conditions (Robinson *et al.* 2008) and fecundity (Lambert and Dutil 2000), and is thus considered to be a good general indicator of physiological condition. ANOVA was used to test for differences in both Fulton's K and standard length between the temperature treatments. Fisher's LSD post-hoc tests were executed where necessary. All statistics were completed with Statistica 8.0 - (StatSoft Inc).

4.4 Results

Resting metabolism at rearing temperatures

When resting oxygen consumption (RMR) for each treatment group was measured at their rearing temperatures (present day, +1.5 °C and +3.0 °C), it increased with temperature at both the summer maxima ($F_{2,82}$= 16.77, p<0.001; Fig. 2a) and winter minima ($F_{2,53}$= 22.89, p<0.001; Figure 4.1b). During winter all three treatments were significantly different from each other and each increase of 1.5°C caused an increase of approximately 30 mg O_2 kg^{-1} h^{-1} in oxygen consumption. However, during summer differences were only found between fish at the present day temperature and the two elevated temperatures, but not between the elevated temperature groups. Thus the +1.5°C and +3.0°C treatment groups showed similar RMR values of approximately 370 mg O_2 kg^{-1} h^{-1} (Figure 4.1a).

Resting metabolism with acute temperature change

Fish were tested for potential RMR differences between the three treatments throughout the range of summer and winter average temperatures (Figure 4.2). When exposed to acute temperature changes RMR values of the groups were similar at all temperatures in the winter series, regardless of acclimation temperature, revealing no effect of long-term acclimation (Figure 4.2b). This was also the case in the summer series at both 28.5°C and 30.0°C (Figure 4.2a). However, differences in RMR were seen at 31.5°C, with both the present day and +1.5°C fish showing significantly higher RMR (up to 69 mg O_2 kg^{-1} h^{-1} higher) than the +3.0°C fish (Figure 4.2a). Thus, the data provides evidence of thermal acclimation in the +3.0°C treatment group to the elevated temperature experienced during summer, without affecting RMR at winter minimum temperatures.

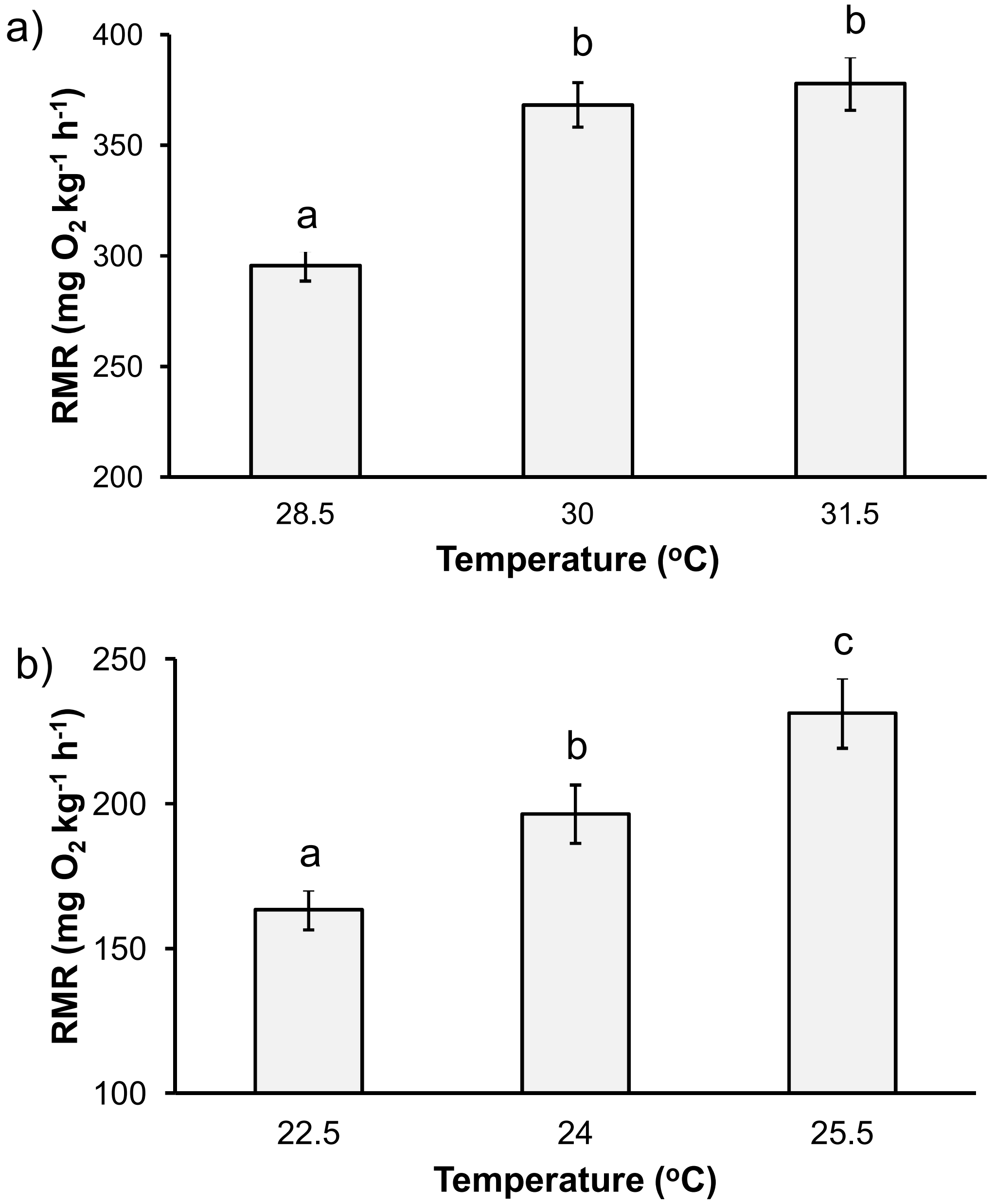

Figure 4.1. Resting metabolic rate (±SE) of fish reared and tested at their average summer (a) and winter (b) temperatures. Letters above bars represent significant differences at $p<0.05$ determined by Fisher's LSD.

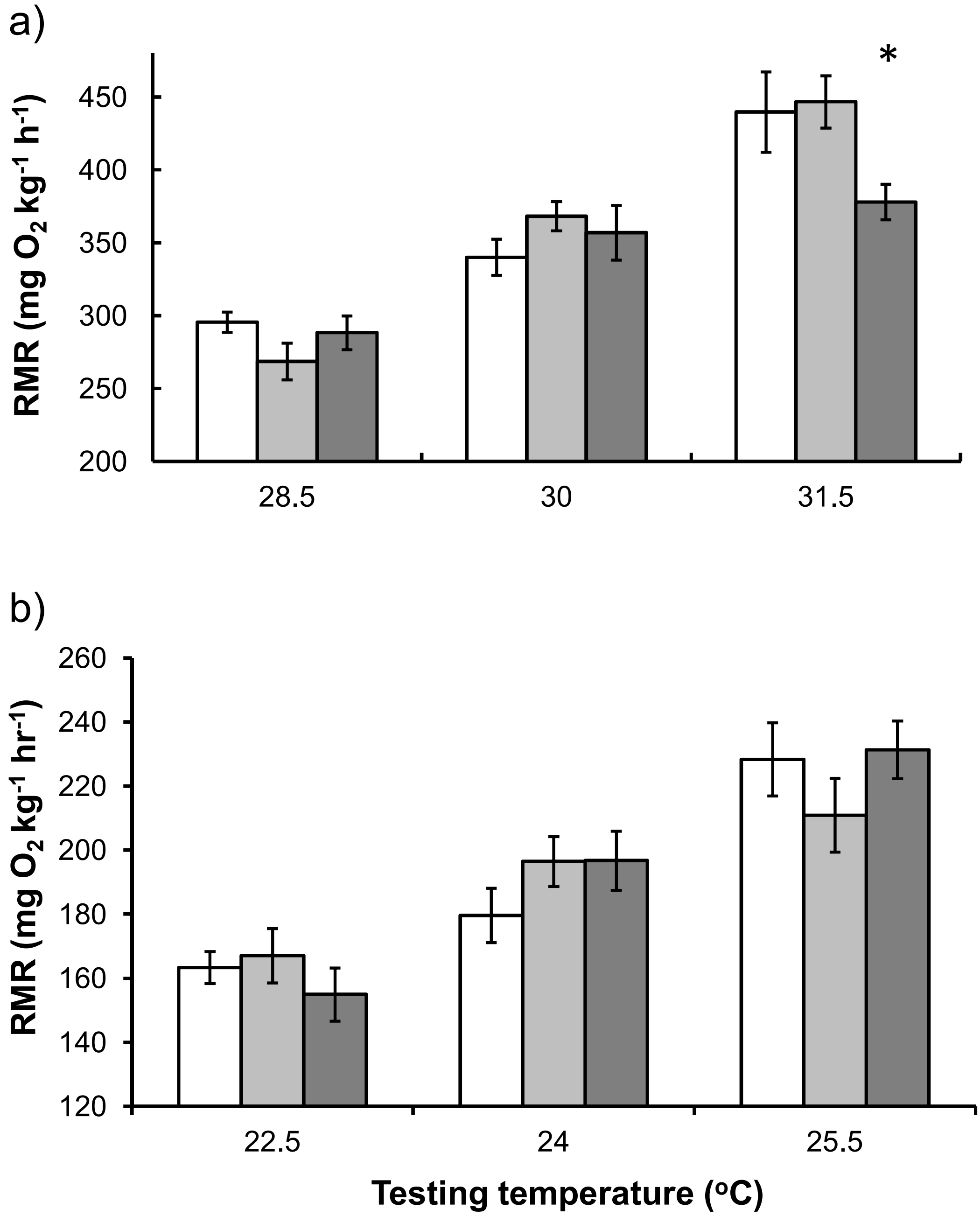

Figure 4.2. Resting metabolic rate (±SE) of fish reared under present day (white), +1.5°C (light grey) and +3.0°C (dark grey) and tested at the summer (a) and winter (b) averages of temperatures. Asterisk represents significant differences within a testing temperature at $p<0.05$ determined by Fisher's LSD.

Body condition and growth

There was a close relationship between body mass and standard length for fish in each temperature treatment (Figure 4.3; $r^2 > 0.92$ for all relationships). The physical condition (i.e. body mass at a given length) of fish depended on the temperature treatment they were reared at for 1 year ($F_{2,92} = 29.27$, $p < 0.001$; Figure 4.3). Fish kept under present day conditions (mean Fulton's K: 0.468 ± 0.0051 SE) were heavier for a given length than fish from either the +1.5 (mean Fulton's K: 0.431 ± 0.0040 SE) or +3.0°C (mean Fulton's K: 0.422 ± 0.0043 SE) treatment ($p < 0.001$). In contrast, there was no difference in the length-weight relationship between +1.5 or +3.0°C treatment fish ($p = 0.149$). The differences in condition between fish at present day temperatures compared with the +1.5 or +3.0°C treatments were more pronounced at lengths greater than 65-70mm (Figure 4.3).

The average length of fish also varied depending on treatment ($F_{2,92} = 28.5$, $p < 0.001$), with fish reared at +3.0°C (mean: 66.1mm ± 0.96 SE) being on average shorter than fish from either present day (mean: 67.9mm ± 1.06 SE) or +1.5°C treatments (mean: 70.4mm ± 0.81 SE; $p < 0.001$).

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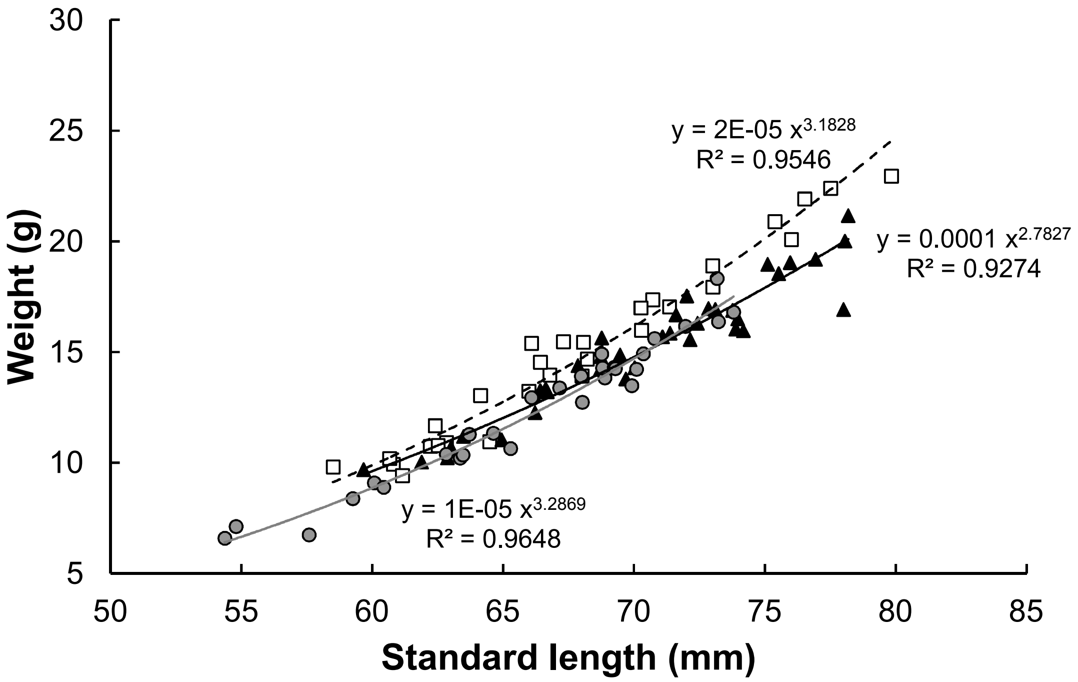

Figure 4.3. Weight of fish after one year in present day (squares), +1.5°C (triangles) and +3.0°C (circles) temperature treatments. Lines of best fit are power functions.

4.5 Discussion

Acclimation is an important mechanism for coping with a changing or fluctuating environment. Previous studies have found no evidence for thermal acclimation of metabolic rates in tropical reef fishes (Tullis and Baillie 2005; Nilsson *et al.* 2009, 2010). In contrast, we detected thermal acclimation through a reduction in resting oxygen consumption (RMR) in fish that had been reared from shortly after hatching to 1.5 years old at 3°C above average temperatures (but not in fish reared at 1.5°C above average temperatures). This indicates that at least some tropical reef fishes can undertake developmental plasticity to acclimate their resting metabolic rates as water temperatures increase due to global warming. The reduction in daily metabolic demands is likely to be beneficial by allowing redistribution of saved energy to other activities. However, fish acclimated to a higher than normal temperature were on average smaller than fish kept at present day temperatures, suggesting that increased temperature, or acclimation *per se*, incurred some physiological cost. Furthermore, fish at both elevated temperatures were in poorer condition than fish reared at present day temperatures. This demonstrates that even with acclimation, warmer sea temperatures are likely to have significant impacts on reef fish populations.

The low RMR exhibited by +3.0°C fish at the summer temperature (31.5°C) compared with present day and +1.5°C fish tested at this temperature indicates that these fish spent less energy on daily maintenance than fish that exhibited acute temperature responses. This acclimation would potentially provide individuals with extra energy for a range of activities, including feeding and reproduction. The RMR of +3.0°C fish tested at 31.5°C was not different from the RMR of +1.5°C fish tested at 30.0°C and these two groups had similar body condition (body mass at a given length). This suggests that the additional energy made available through acclimation at 31.5°C was possibly used to maintain body condition. Further support for this notion is seen in the comparison of RMR between fish at elevated temperature treatments (+1.5°C and +3.0°C) and the present day temperature treatment. The RMR of +1.5°C and +3.0°C fish in their respective temperatures was higher than that of fish reared at present day temperatures, and both these groups had lower body condition than fish reared in the present day temperature treatment. In combination, these comparisons indicate that elevated temperature has an effect on body condition, but this effect was reduced at the highest temperatures through acclimation of resting metabolic rate.

Despite acclimation of RMR to the summer average in fish reared at 3.0°C above present day averages, there was no evidence for acclimation in fish reared at 1.5°C above. The absence of acclimation to 30.0°C in the +1.5°C fish is likely to be related to the costs and benefits of acclimation. Previous studies have shown that for this population of *Acanthochromis polyacanthus* a reduction in growth, body condition, and reproduction occurs at ≥31.0°C, regardless of food availability (Munday *et al*. 2008b; Donelson *et al.* 2010). Although reductions in growth and reproduction have also been observed at 30.0°C (Donelson *et al.* 2010) they are less severe than at 31.5°C. The smaller loss of performance caused by exposure to 30.0°C compared with 31.5°C may explain why physiological acclimation only occurred in the higher temperature treatment.

The reduced average length of +3.0°C fish compared to fish reared at +1.5°C and present day temperatures suggests that metabolic acclimation during development may have incurred a physiological cost. Energy used for acclimation and maintenance of body condition may have otherwise been used for somatic growth. It is also likely that physiological processes involved in growth, like protein synthesis and cell proliferation, influence resting metabolic rate. Such processes may have to be suppressed during developmental acclimation to allow for a maintained (i.e. reduced) resting metabolic rate at an elevated water temperature, thereby retarding growth. Alternatively, reduced growth may have occurred because +3.0°C fish could only partially compensate for temperature effects through RMR (Fry 1967). This is suggested by the fact that RMR was still significantly higher in the +3.0°C acclimated group compared to those reared at present day temperatures. RMR is expected to be mirrored by metabolic scope, which coincides with optimum growth (Del Toro-Silva *et al.* 2008). Therefore, the reduced growth rate might have occurred because only partial compensation was possible. Regardless of the specific cause, our results suggest that future populations living at +3.0°C conditions will consist of smaller individuals that are in poorer condition than present day populations at the same location.

Reduced physical condition at elevated temperatures is likely to have important ramifications for reef fish populations in a natural setting. It is well documented that survival rates are positively associated with body condition in juvenile reef fishes (Hoey and McCormick 2004; Grorud-Colvert and Sponaugle 2007; Holmes and McCormick 2009). Therefore, fewer fish will potentially survive to maturity at elevated temperatures, because they will be in poorer condition. Reduced condition will also affect reproductive output, with fewer and smaller offspring being produced compared to good condition counterparts (Marteinsdottir and Steinarsson 1998; Donelson *et al.* 2008). In addition, fish from the +3.0°C treatment group were shorter on average, which also has ramifications for individual fitness, including reduced size at maturity which in turn may reduce reproductive output (Morita and Takashima 1998; Vallin and Nissling 2000; Johnston and Legget 2002). Alternatively, maturity may be delayed until a size threshold is reached, which could increase the likelihood of mortality prior to maturity and reduce the size of the breeding population (Morita *et al.* 2005).

Although acclimation to maximum temperatures might be expected to affect performance at minimum temperatures, this is not always the case. Acclimation to one temperature extreme often does not coincide with poor performance at the opposite temperature extreme (Gvoždík *et al.* 2007; Angilletta 2009; exceptions Seebacher *et al.* 2005; Glanville and Seebacher 2006). Consistent with these observations, thermal acclimation observed in the 31.5°C treatment group did not affect RMR at any of the predicted winter temperatures. This suggests that acclimation may be an important process whereby tropical reef fish can cope with increased average summer temperatures in the future without a loss of performance at winter temperatures. The reduction in RMR of ~ 70 mg O_2 kg^{-1} h^{-1} in acclimated fish represented a Q_{10} (the rate of change depending on temperature) reduction of 1.31, which is similar to that observed when acclimation in polar fishes occurs (Q_{10} change: 0.62-1.29; Franklin *et al.* 2007; Robinson and Davison 2008), but is less than that recorded for temperate species (Q_{10} change: 1.55-3.97; Morris, 1965; Walsh *et al.* 1983; Sébert *et al.* 1995). This demonstrates that acclimation potential of some tropical fishes may not be as large as species that experience greater seasonal variation in maximum and minimum temperatures.

While the present study only investigates the potential for acclimation in one species it is expected that developmental acclimation will be common among coral reef fishes. In many ectotherms developmental acclimation, or developmental plasticity, is known to be critical to producing phenotypes suited to specific environments (Beck 1983; Seebacher 2005). This plasticity is common in the dispersive larval stage of reef fish, when they are likely to experience environmental unpredictability (Warner 1997; West-Eberhard 2003). Here we have shown that *A. polyacanthus*, a species without a dispersive larval phase, can exhibit thermal acclimation during its lifetime. Thus, it is likely that other species of reef fish will exhibit thermal acclimation because developmental plasticity in other traits is well documented (Munday *et al.* 2006), although this remains to be tested.

One hour acclimatization to the metabolism chamber is relatively short time compared to other studies and a longer time can be required to obtain accurate RMR values of some species (Sloman *et al.* 2000; Nilsson and Östlund-Nilsson 2004). However it is unlikely that our RMR values are inflated by the relatively short acclimatization since the values obtained are similar to those reported in Nilsson *et al.* (2009) for the same species. Additionally, it is possible that a greater acclimation time is needed at warmer temperatures or at temperatures different to rearing conditions. However, it would then be expected for the +3.0°C group at 31.5°C (where acclimation was found to occur) to have a greater RMR than when it was tested at 30.0°C or for groups tested at their rearing temperature to obtain lower RMR than the other treatment groups. We suggest that the reduced acclimatization time required is due to the fish being habituated to their aquarium conditions since birth. Furthermore, this habituation may also influence RMR values, but again this is not likely since present metabolism estimates are similar to wild estimates (Nilsson *et al.* 2009).

Our study is one of the first to demonstrate that acclimation to high temperature may come at the cost of reduced somatic growth. Other research to identify costs associated with thermal acclimation (quantified as the production of heat shock proteins) has been conducted on *Drosophila* with differences found in cell growth (Feder *et al.* 1992), fecundity (Krebs and Loeschcke 1994) and survival (Krebs and Feder, 1997). To our knowledge, ours is the first study on an organism outside the Instecta to suggest that energy employed to facilitate acclimation could have been utilized on another function. This emphasizes the need to explore the costs as well as the benefits associated with thermal acclimation across a range of key life history and performance traits.

Thermal acclimation provides a mechanism that could assist tropical fish populations to cope with warming ocean temperatures. It seems likely that developmental acclimation will play a role in reducing the impacts of global warming, improving the chances that populations will persist across their present geographic ranges. However, acclimation has limitations and may not be sufficient for populations already living close to their thermal maximum, thus selection of thermally tolerant genotypes over multiple generations will also be vital. Even with acclimation, our results demonstrate that individuals in future populations will have reduced fitness, which will have significant ramifications for population sustainability.

Chapter 5: Thermal Sensitivity Does Not Determine Acclimation Capacity for a Tropical Reef Fish

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Authors: J. M. Donelson and P. L. Munday

5.1 Summary

Thermal reaction norms are expected to indicate the sensitivity of species to global warming. However, given time, species may be able to acclimate and adapt to increasing temperature. Consequently, for accurate predictions of long-term effects to be made from short-term experiments, thermal reaction norms must be a good indicator of acclimation potential. We found this was not the case for the tropical reef fish *Acanthochromis polyacanthus*. Specifically, two geographically separated populations exhibited similar metabolic performance across a range of temperatures at each location (present-day, +1.5°C and +3.0°C), but large differences in acclimation potential. Fish from the higher-latitude location were able to fully acclimate RMR and aerobic scope, while the lower-latitude location could only partially compensate RMR at the warmest temperature. This indicates that acclimation capacity of populations may vary on relatively small scales, and that understanding such variation will be critical for predicting the impacts of climate change.

5.2 Introduction

Global warming has already caused range shifts in many species (Walther *et al*. 2002; Perry *et al.* 2005; Parmesan 2006; Hickling *et al*. 2006) and is predicted to increase extinction rates as temperatures rise above the thermal limits of species that have limited capacity to shift their distributions (Thomas *et al.* 2004). There is growing evidence that tropical species may be especially sensitive to global warming because they have narrower thermal niches and reduced ability to cope with temperature increases compared with species from higher latitude (Stillman 2003; Deutsch *et al.* 2008; Tewksbury *et al*. 2008; Wright *et al*. 2009), possibly because they have evolved in a relatively stable thermal environment. One pathway organisms can cope with adverse environmental conditions is through acclimation, a form of plasticity, which involves the modification of behavioural, physical or morphological characteristics in relation to changes in their environment (Angiletta 2009). Since tropical regions often experience relatively small temperature fluctuations, and maintaining plasticity can be costly (Relyea 2002; Hofmann and Todgham 2010), it seems unlikely that plasticity or acclimation capacity for a large range of temperatures would be preserved in most tropical species. The low variability in temperature may also cause populations to be adapted to their local thermal regime. If temperatures are stable and predictable there would be fitness benefits in fine tuning physiological performance to the thermal environment (Bradley 1982). This may limit plasticity to temperature change (Stillman 2003) and cause populations to exhibit very different capacities to cope with global warming (Eliason *et al.* 2011).

Physiological performance of ectotherms, including fish, is strongly influenced by environmental temperature (Fry 1967; Hazel and Prosser 1974; Houde 1989). Recent research on aquatic ectotherms has identified metabolic attributes as the key factors in determining persistence in a warming environment (Pörtner and Knust 2007; Pörtner and Farrell 2008). As water temperature increases so does the minimum energy needed to maintain physiological cell functions (RMR: resting metabolic rate) as well as the maximum rate of oxygen uptake (MMR: maximum metabolic rate) (Brett 1971). However at temperatures greater than an optimum, the maximum rate of oxygen uptake (MMR) cannot increase at the same rate as RMR, reducing the scope for aerobic activity (Steinhausen *et al.* 2008; Farrell 2009). This reduction in aerobic function is linked to a decline in the capacity of critical activities such as reproduction and growth (Pörtner and Knust 2007; Pörtner and Farrell 2008). The thermal sensitivity and plasticity of these metabolic traits is poorly understood in tropical marine species compared to temperate species, however new research indicates that RMR, MMR and aerobic scope are strongly affected by temperature in coral reef fishes (Nilsson *et al.* 2009; Gardiner *et al.* 2010; Johansen and Jones 2011; **Chapter 4**). Evidence for thermal acclimation in coral reef fishes is limited to one study on developmental acclimation (**Chapter 4**), while all others have found limited capacity for acclimation in adult and juvenile reef fish over days to weeks (Nilsson *et al.* 2009; Nilsson *et al.* 2010). These preliminary results are consistent with the hypothesis that tropical regions may be especially susceptible to global warming because tropical species have limited thermal plasticity (Stillman 2003). However, many tropical marine organisms also inhabit a large geographic range and consequently experience a wide temperate range throughout their distributions (Munday *et al.* 2008a), indicating that they must also have some capacity for acclimation and local adaptation over longer time scales.

The temperature variation experienced by a population can influence the width of the thermal norm and the amount of thermal plasticity that is expressed (Via 1993; Angiletta *et al.* 2006; Angiletta 2009). If optimal performance is expected at temperatures most frequently experienced by populations, populations from a warmer environment are predicted to perform better in warm conditions than populations from a cooler environment (Lynch and Gabriel 1987; Huey and Kingsolver 1989; Gilchrist 1995). Similarly, populations that experience a more variable thermal environment perform well at a greater range of temperatures than populations which only experiences a narrow range of temperature variation. This leads to the expectation that populations which experience similar season variation will possess similar thermal reaction norms and similar ability to acclimate through time. However, this is not always the case, with countergradient variation in thermal reaction norms frequently observed, where the cooler population outperforms the warmer across all temperatures (Conover and Present 1990; Schultz *et al.* 1996; Álvarez *et al.* 2006; Gardiner *et al.* 2010; Baumann and Conover 2011). Countergradient variation occurs when genetic effects to performance and the thermal environmental variation are negatively associated (Conover *et al.* 2009). Thus, the thermal reaction norm a population exhibits will depend not only on its thermal history, but also on the biological attributes that are under selective pressures and any trade-offs among traits that occur as a result of thermal specialization.

This study aimed to compare thermal acclimation capacity of a coral reef fish (*Acanthochromis polyacanthus*) from two geographically separated populations (~800km), which differ in maximum and minimum temperatures, but not the range of seasonal or daily variation. The two populations are also well established as being genetically distinct (Planes *et al.* 2001) and no mixing is possible as they lack a pelagic larval phase (Robertson 1973). Juveniles from each location were reared until maturity at seasonally cycling temperatures matching averages of the sampling locations, and at average temperatures predicted to occur over the next 50-100 years (+1.5°C and +3.0°C). Thermal reaction norms of each population were estimated by testing the metabolic performance of fish reared at present-day average temperatures in all three temperatures (present-day, +1.5°C and +3.0°C). The potential for thermal acclimation in each population was then estimated by comparing the metabolic performance of fish that have developed in elevated temperature regimes (+1.5°C and +3.0°C) to fish that have been reared in present-day temperatures and tested at elevated temperatures.

5.3 Materials and methods

Study species and experimental treatments

The tropical coral reef damselfish *Acanthochromis polyacanthus* is widespread in the Indo Pacific (15°N–26°S and 116°E–169°E). Across its range populations experience a total temperature span (inclusive of seasons) of approximately 20°C to 31°C. Fish were collected from two locations on the Great Barrier Reef (GBR), Heron Island (23°27'S, 151°57'E) and the Palm Islands (18°37'S, 146°30'E). These two populations represent the lower extent (Heron Island) and middle (Palm Island) of the species' range on the GBR. The Palm Island region fish were offspring produced by wild pairs collected in July-August 2007 and spawned in December 2007-February 2008. The Heron Island fish were collected as recently hatched juveniles in February-March 2009 (mean weight: 2.5g). Fish were reared until maturity for up to 28 months in seasonally cycling temperature treatments matching present-day averages for the collection locations and +1.5°C and +3.0°C above these averages. The elevated temperature treatments match sea surface temperatures projected to occur on the GBR by 2050-2100 (Lough 2007; Munday *et al.* 2009). Fish were divided into the three temperature treatments within 3 months of hatching.

Experimental temperatures were modelled on averages for the 10 year period, 1999-2008, from each location. Temperatures were modified weekly to mimic seasonal temperature changes. While fish at Heron Island experience cooler temperatures than Palm Island fish (Heron Island mean: summer = 27.0°C and winter = 21.8°C; Palm Island mean: summer = 28.5°C and winter = 23.2°C), the two locations experience similar seasonal variation in temperature (difference between winter and summer means: Heron Island = 5.2°C, Palm Island = 5.3°C; mean daily variation: Heron Island = 1.29°C, Palm Island = 1.12°C).

Metabolic attributes

Respirometry was conducted as per Nilsson *et al.* (2009) and **Chapter 4**. Both resting metabolic rate (RMR) and maximum metabolic rate (MMR) were measured directly, which allowed the calculation of factorial aerobic scope (MMR/RMR) and net aerobic scope (MMR-RMR). For RMR measurements, each fish was allowed to acclimatize in the respirometer (a 4 l Perspex cylinder with 144 mm inner diameter) for 1 h with a constant water flow. Following acclimatization the chamber was sealed and oxygen concentrations were monitored with an oxygen electrode (WTW OXI 340i or OXI 3310, Germany) for 30 min. Oxygen concentrations remained above 70% of air saturation during trials. Fish were given at least 3 hours rest before measuring MMR. For measurement of MMR the chamber was placed upright creating a circular swimming area (see Nilsson *et al.* 2007 for details). Water current was created by a 60 mm magnetic stirring bar inside the sealed chamber and the speed was set to the maximum aerobic swimming speed of the fish. Maximum swimming speed was determined to be when the fish was swimming maximally without losing ground. Oxygen level in the water was measured for 5 to 10 min. For both trials the respirometer was submerged in a temperature-controlled aquarium to maintain a stable temperature. Subsequently, the wet weight of each fish was measured to the nearest mg.

All fish were tested at the average summer temperatures of their treatment; Heron Island present-day = 27.0°C, +1.5°C = 28.5°C and +3.0°C = 30.0°C, and for the Palm Island region present-day = 28.5°C, +1.5°C = 30.0°C and +3.0°C = 31.5°C. Fish that were maintained at the present-day summer average, and thus had no opportunity for long-term acclimation, were acclimatized at the two higher temperatures of their location for 7 days and tested to investigate the acute effects of temperature.

Statistics

Difference between metabolic attributes of no acclimation and acclimation fish from Heron and Palm Island were tested with a factorial ANOVA, with location, acclimation treatment and temperature (present-day, +1.5°C and +3.0°C) all fixed factors. When significant effects of treatment were found, Fisher's LSD post-hoc tests were used to establish where significant differences existed ($p<0.05$). RMR data was log transformed to satisfy homogeneity of variance assumptions.

5.4 Results

Fish with no potential for acclimation exhibited an increase in RMR with increasing water temperature (Figure 5.1a; Table 5.1). MMR did not vary with increasing temperature, but was generally greater in fish from Heron Island across all temperatures, though not significant at +1.5°C (Figure 5.1b; Table 5.1; present-day: $P<0.001$, +1.5°C: $P=0.137$, +3.0°C: $P<0.01$). Additionally, non-acclimated Heron Island fish achieved a significantly greater factorial ($P<0.05$) and net aerobic scope ($P<0.01$) than Palm Island fish at their summer average temperatures, however this did not remain with an increase of either 1.5°C or 3.0°C (Figure 5.1c & d). When comparing performance differences between non-acclimated fish at shared absolute temperatures (i.e. 28.5°C and 30.0°C), both RMR and factorial aerobic scope were superior in Palm Island fish at 28.5°C (Figure 5.2a & c; $P<0.05$), as well as RMR at 30.0°C ($P<0.001$). Heron Island fish still exhibited a greater MMR at 28.5°C and 30.0°C, but was only significant at 30.0°C (Figure 5.2b; $P<0.01$). This elevated MMR consequently caused no significant differences between populations in net aerobic scope at either temperature (Figure 5.2d).

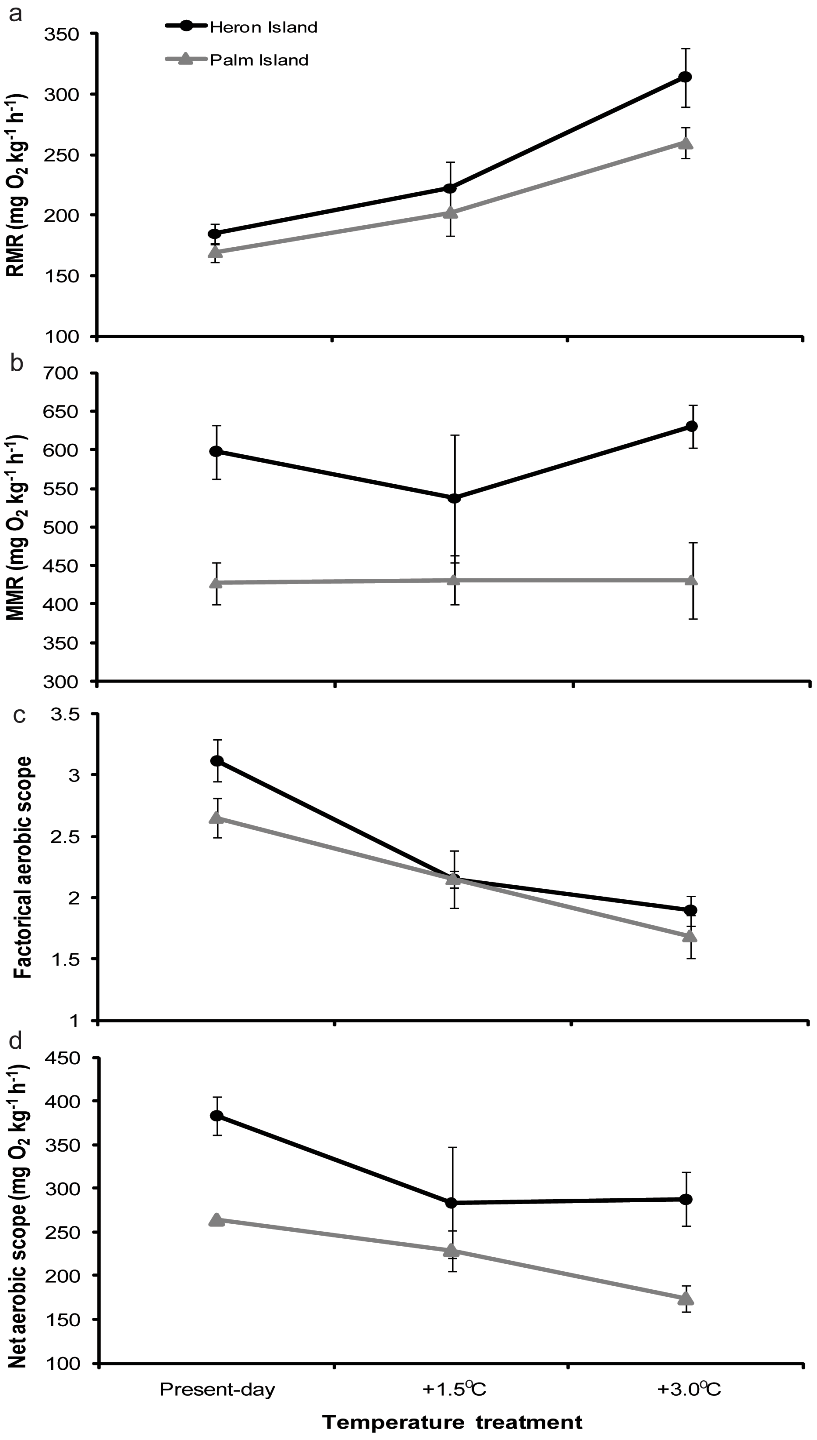

Figure 5.1. Mean resting metabolic rate (a), maximum metabolic rate (b), factorial aerobic scope (c) and net aerobic scope (d) of Heron and Palm Island fish at present-day, +1.5°C, +3.0°C temperatures (±SE).

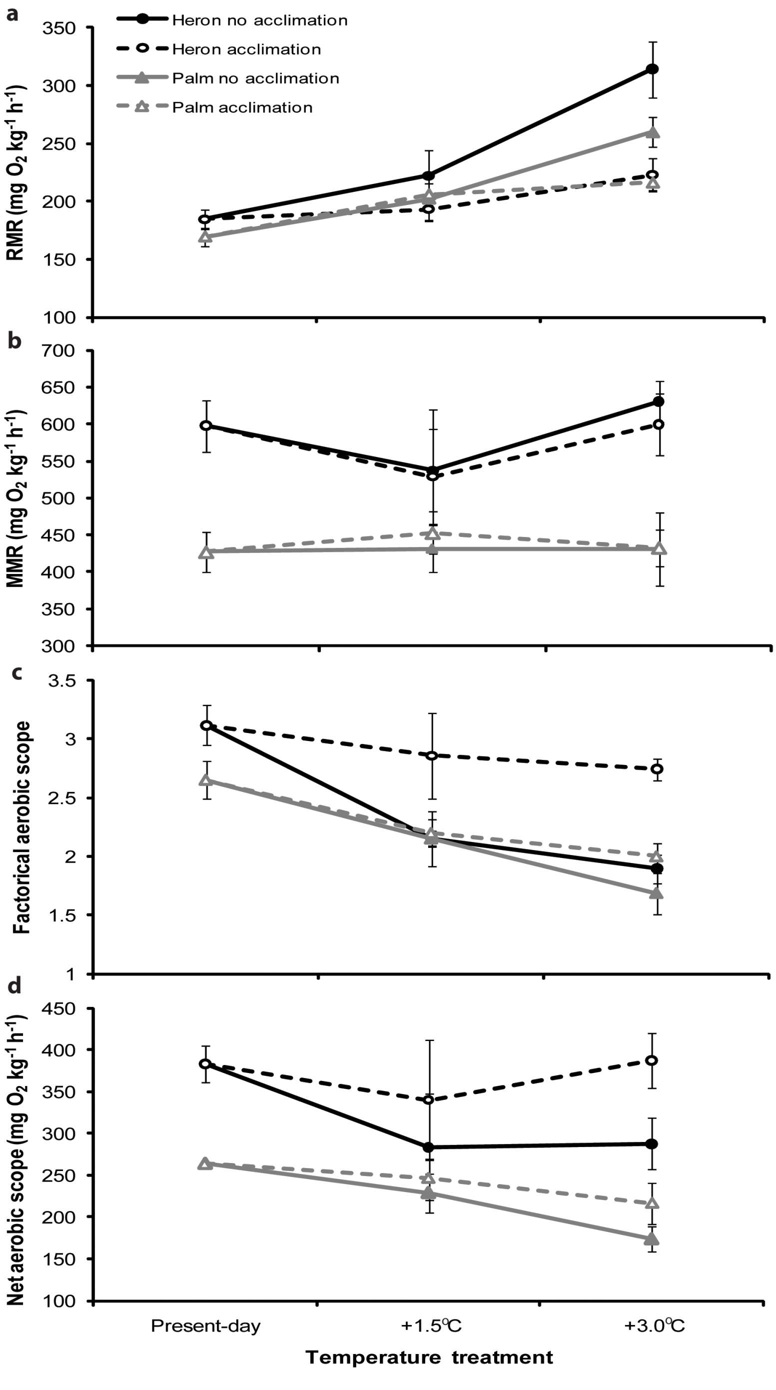

Figure 5.2. Mean resting metabolic rate (a), maximum metabolic rate (b), factorial aerobic scope (c) and net aerobic scope (d) of Heron and Palm Island fish with acclimation or no acclimation across testing temperatures (±SE).

Evidence of thermal acclimation was identified in both populations, but to differing extents. The Heron Island population exhibited a greater capacity for acclimation of RMR, factorial and net aerobic scope (Figure 5.2; Table 5.1; all except net scope 28.5°C significant $P<0.05$). Palm Island fish were only capable of acclimation at 31.5°C for RMR (Figure 5.2a; $P<0.05$). In contrast, acclimated Heron Island fish performed better than acclimated Palm Island fish at +1.5°C and +3.0°C as well as 28.5°C and 30.0°C (Figure 5.2). Specifically, both aerobic scope estimates at 30.0°C were significant (factorial scope: $P<0.01$, net scope: $P<0.001$) and all aerobic scope values at +1.5°C and +3.0°C (Figure 5.2c & d; factorial scope +1.5°C: $P<0.001$, +3.0°C: $P<0.01$; net scope +1.5°C: $P<0.01$, +3.0°C: $P<0.001$). Additionally, all attributes that were originally significant between populations in non-acclimated fish, were no longer significant with acclimation (RMR at 28.5 & 30.0°C and factorial aerobic scope at 28.5°C all $P>0.05$). The only attribute that exhibited no change with acclimation was MMR which was not strongly influenced by temperature originally (Figure 5.2b).

Table 5.1. Factorial ANOVA comparison of RMR ($\log_{10}$ transformed), MMR, factorial and net aerobic scope of fish depending on location (Heron vs Palm Island), acclimation and temperature treatment (present-day, +1.5°C, +3.0°C). Significant P values are indicated in bold.

	RMR	**MMR**	**Factorial scope**	**Net scope**
Location	**$F_{1,84}=4.08$** **$P<0.05$**	**$F_{1,82}=37.97$** **$P<0.001$**	**$F_{1,71}=18.26$** **$P<0.001$**	**$F_{1,71}=33.97$** **$P<0.001$**
Acclimation	**$F_{1,84}=11.97$** **$P<0.001$**	$F_{1,82}=0.01$ $P=0.911$	**$F_{1,71}=10.46$** **$P<0.01$**	$F_{1,71}=3.54$ $P=0.063$
Temperature	**$F_{2,84}=31.63$** **$P<0.001$**	$F_{2,82}=0.68$ $P=0.509$	**$F_{2,71}=25.74$** **$P<0.001$**	**$F_{2,71}=4.02$** **$P<0.05$**
Location * Acclimation	$F_{1,84}=2.90$ $P=0.092$	$F_{1,82}=0.192$ $P=0.662$	$F_{1,71}=3.94$ $P=0.051$	$F_{1,71}=0.68$ $P=0.412$
Location * Temperature	$F_{1,84}=0.06$ $P=0.946$	$F_{1,82}=1.29$ $P=0.281$	$F_{2,71}=0.22$ $P=0.801$	$F_{2,71}=0.98$ $P=0.382$
Acclimation * Temperature	**$F_{2,84}=5.02$** **$P<0.01$**	$F_{2,82}=0.06$ $P=0.940$	**$F_{2,71}=3.37$** **$P<0.05$**	$F_{2,71}=1.26$ $P=0.287$
Location * Acclimation * Temperature	$F_{2,84}=3.94$ $P=0.051$	$F_{2,82}=0.06$ $P=0.943$	$F_{2,71}=1.18$ $P=0.313$	$F_{2,71}=0.21$ $P=0.807$

5.5 Discussion

Acclimation is a vital means for organisms to cope with a changing environment. We found differences in acclimation ability to future predicted water temperatures between populations, with full compensation possible in Heron Island fish compared to only partial compensation of RMR at +3.0°C for Palm Island fish. This distinction in acclimation ability suggests that the high-latitude Heron Island population will be able to persist at the current location without loss of performance, but the mid-latitude Palm Island population is likely to suffer a reduction in metabolic capacity for critical activities as ocean temperatures rise. In contrast, the two populations exhibited almost identical metabolic response to acute temperature increases (i.e. their thermal reaction norms). Consequently, the differences in acclimation ability were not evident when comparing the thermal sensitivity of the two populations using standard methods (7 days acclimatization to the new temperature) (Nilsson *et al.* 2009, Gardiner *et al.* 2010). When a longer acclimation time of up to 22 days has been employed to test for reversible acclimation the finding of no acclimation ability remains (Nilsson *et al.* 2010). This highlights the limitation of short-term experiments that do not allow the possibility for developmental acclimation in predicting responses to climate change.

Improvement in crucial metabolic attributes through developmental acclimation will reduce the impact of elevated water temperature to coral reef fish. Reductions in resting metabolic rate should equate to lower daily energy expenditure, and is likely to provide fish with additional energy for non-critical functions such as reproduction or growth. In addition, the increase in aerobic scope exhibited by the Heron Island population will provide improvements to fish at elevated temperatures across a range of characteristics. Specifically, aerobic scope is known to relate to growth rate, swimming capacity, foraging ability (Pörtner and Knust 2007; Johansen and Jones 2011) and a reduction in aerobic scope relates to declines in abundance of fish (Pörtner and Knust 2007). Furthermore, a reduced aerobic capacity can cause a decreased ability for individuals to deal with normal environmental fluctuations or variations in habitat conditions (Johansen and Jones 2011).

Evidence for local thermal adaptation was found at the two locations, with both achieving similar RMR when comparing summer averages, +1.5°C and +3.0°C. Additionally, Palm Island fish obtained lower RMR than Heron Island fish at absolute temperatures of 28.5°C and 30.0°C as well as a greater factorial aerobic scope at 28.5°C. For *A. polyacanthus* local adaptation might be expected due to the lack of pelagic larvae, dispersal being limited (Robertson 1973), and populations being genetically distinct from the early Pliocene (Planes *et al.* 2001). Local adaptation of RMR would be beneficial since it defines the amount of energy required at rest and under limited food situations will influence the amount of spare energy for activities such as growth and reproduction (Bret 1971). Furthermore, since RMR influences attributes that determine fitness it seems probable that it would be under selection (Lauder *et al.* 1994).

In addition to the evidence for local thermal adaptation, there was also evidence for performance differences between populations not related to their thermal environment (i.e. countergradient variation; Angiletta 2009; Conover *et al.* 2009). Firstly, Heron Island fish achieved significantly higher MMR across all testing temperatures, as seen by Gardiner and colleagues et al (2010) at this location. In addition, fish from Heron Island had greater factorial and net aerobic scope at their summer average than Palm Island fish at their summer average, due to higher MMR but not a lower RMR. There are multiple possible physiological explanations for the ability of Heron Island fish to achieve greater MMR including a greater cardiac output and consequently blood flow across the gills, larger gill surface area, increased oxygen carrying capacity of blood through haemoglobin or red blood cells, or an increased capacity to remove oxygen from the blood by the tissues (Gardiner *et al.* 2010; Perry and Gilmour 2010). Regardless of the mechanism it seems that the underlying cause of differences would be genetic since there is not substantial difference in variation of thermal environment, but significant differences in genetics (Planes *et al.* 2001). Furthermore, the differences in thermal environmental oppose the direction of physiological capacity.

In general the ability to cope with variation in environmental temperature is thought to be linked to the thermal variation a population experiences (Angiletta *et al.* 2006; Angiletta 2009). However, the present study found countergradient variation in acclimation capacity of *A. polyacanthus* populations. With developmental acclimation, Heron Island fish were able to reduce RMR down to levels equivalent to Palm Island across the thermal range investigated and to outperform Palm Island fish in numerous aerobic scopes measures. As well as being genetically distinct, the two populations investigated are believed to have been colonised by separate invasion events in the past 5 million years (Planes *et al*. 2001). This, as well as both populations experiencing similar thermal variation, suggests that differences in acclimation capacity may be linked to historical differences rather than recent thermal environment, especially since a genetic basis for variation in thermal reaction norms has been established in a range of taxa (Knies *et al.* 2006; Driessen *et al.* 2007; Yamahira *et al.* 2007). Furthermore, it is possible that acclimation capacity has not yet undergone selection as present temperatures have not exceeded the thermal performance breadth of the species.

Knowledge of acclimation capacity is critical to fully understand the capacity for species to cope with predicted temperature increase. Simply testing how individuals cope with short-term temperature changes are unlikely to give a true indication of their response to climate change. It is also apparent that not all populations will respond similarly to temperature increases, even with similar thermal histories. In addition, research should be expanded to include a range of reef fish species from a range of families and with varying life styles (e.g. mobile vs sedentary species). Contrary to predictions, the present findings suggest that some tropical reef fish populations possess a large capacity to modify key metabolic attributes through developmental acclimation to warmer conditions.

Chapter 6: Rapid Transgenerational Acclimation of a Tropical Reef Fish to Climate Change

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Authors: J. M. Donelson, P. L. Munday, M. I. McCormick and C. R. Pitcher

6.1 Summary

Understanding the capacity of species to acclimate and adapt to expected temperature increases is critical for making predictions about the biological impacts of global warming, yet it is one of the least certain aspects of climate change science. While it is expected that some species may have capacity to acclimate or adapt to environmental changes, evidence is lacking. Tropical species are considered to be especially sensitive to climate change because they live close to their thermal maximum and exhibit limited capacity for acclimation. Here, we demonstrate that a tropical reef fish is highly sensitive to small increases in water temperature, but can rapidly acclimate over multiple generations. Acute exposure to elevated temperatures (+1.5°C & +3.0°C) predicted to occur this century caused a 15% & 30% loss of aerobic scope, respectively. However, complete compensation in metabolic attributes occurred when both parents and offspring were reared their entire life at elevated temperature. Such acclimation could reduce the impact of warming temperatures and allow populations to persist across their current range. This study reveals the importance of transgenerational acclimation as a mechanism for coping with rapid climate change and highlights that single generation studies risk underestimating the potential of species to cope.

6.2 Introduction

Ocean temperatures are expected to become adverse for many marine species within the next 50-100 years due to global warming (Hoegh-Guldberg *et al*. 2007; Poloczanska *et al*. 2007; Munday *et al.* 2009), however acclimation and adaptation could allow future populations to adjust to increased temperatures (Stillmann 2003; Hofmann and Todgham 2010). Acclimation of physiological processes, such as metabolic function, usually occurs within a generation (Angiletta 2009). Although, parental effects (influences on offspring phenotype that are not solely due to offspring genotype) can facilitate some acclimatory processes between generations (termed transgenerational effects; Rossiter 1996; Mousseau and Fox 1998). Tropical species are expected to have less capacity for thermal acclimation than temperate species because they have evolved in a more stable thermal environment (Stillman 2003; Tewksbury *et al.* 2008; Wright *et al.* 2009). There is also some evidence that plasticity and capacity for genetic adaptation may be linked (Lucassen *et al.* 2006), suggesting that limited capacity for thermal acclimation may relate to restricted potential for long-term adaptation to global warming.

For water-breathing ectotherms, such as fish, the capacity to meet increased oxygen demands at elevated temperature will determine their persistence across locations with ocean warming (Pörtner and Knust 2007). Specifically, as ambient temperature increases, a greater rise in resting metabolic rate (RMR) occurs relative to maximum metabolic rate (MMR) reducing scope for aerobic activity (Pörtner and Knust 2007). This decline in aerobic scope affects critical biological functions, including behaviour, growth and reproduction due to limited capacity of circulatory and ventilatory systems to match oxygen demands (Pörtner and Knust 2007; Pörtner and Farrell 2008). At present we have a limited understanding of how aquatic species might be able to alter their physiology over multiple generations to enable persistence in a warmer environment (Skelly *et al.* 2007; Hofmann and Todgham 2010).

6.3 Materials and methods

Study species and climate change predictions

The coral reef damselfish *Acanthochromis polyacanthus* is a widespread Indo Pacific species (15°N–26°S and 116°E–169°E). Fish were collected from the Palm Island region (18° 37' S, 146° 30' E) of the central Great Barrier Reef, which experiences a mean temperature range of 23.2°C to 28.5°C. Average sea surface temperatures in the Great Barrier Reef, Australia, are predicted to increase up to 3°C by 2100 due to global warming (Lough 2007; Meehl *et al.* 2007). Temperature increases of this magnitude during summer are known to have negative effects to metabolic rate, reproduction, growth and physical condition of *A.* polyacanthus (Hilder and Pankhurst 2003; Munday *et al.* 2008b; Donelson *et al.* 2010). The longevity of *A. polyacanthus* in the collection region is approximately 9 years (Kingsford and Hughes 2005).

Multigenerational rearing and experimental design

Nine established pairs of *A. polyacanthus* were collected in July to August 2007 and maintained in 60 l aquariums inside an environmentally-controlled facility at James Cook University, Townsville, Australia. Pairs were maintained at the mean present-day ocean temperature for the collection location and provided with the average food consumed by wild pairs (Chellappa *et al.* 1995). During the austral summer 2007-2008, breeding bouts from 8 F0 pairs were used for the current study. Offspring from these pairs were kept with their parents for 30 days post-hatching. At this time F1 individuals from each clutch were divided into 3 groups for rearing in 3 seasonally cycling temperatures regimes; splitting clutches in this way ensured that each experimental treatment contained similar genetic diversity and allowed investigation of possible genetic effects on acclimation ability. One treatment group was kept at the present-day average temperature cycle at the collection location (+0.0°C), while the other two groups were gradually adjusted to, and reared at, two higher temperature treatments: either +1.5°C or +3.0°C (Figure 6.1). Temperature was kept within ±0.2°C of the desired treatment mean. Temperatures at the collection location naturally fluctuating between 0.2-2.5°C in a single day, but on average vary only 0.45°C on a daily basis (JCU/AIMS weather station). Sibling fish were kept in groups of 6 in 40 l aquaria for 1 year after hatching, at which time density was reduced to pairs (See **Chapter 4** for more details).

During the austral summer 2009-2010 nesting sites of F1 pairs were checked daily at 9:00 for the presence of eggs. Following the observation of a clutch, tanks were checked again daily at 11:00 for the presence of hatched offspring. Directly after hatching groups of 30-40 F2 individuals were removed from the parents and reared in 25 l aquaria under temperature treatments (Figure 6.1) until 3 months post-hatching when metabolic testing occurred. Offspring from +0.0°C fish (4 clutches) were separated into 3 groups, of which one group was kept at the natal temperature and two groups were gradually adjusted to the two higher temperature treatments (+1.5°C and +3.0°C). Offspring from +1.5°C (5 clutches) and +3.0°C (3 clutches) F1 pairs remained at their natal temperature until testing. F2 offspring raised at +0.0°C had "no acclimation" and thus demonstrate the acute effects of temperature, while offspring raised at +1.5°C and +3.0°C could demonstrate "developmental acclimation". The F2 offspring produced by +1.5°C and +3.0°C treatment F1 adults comprised the "transgenerational acclimation" treatment.

At the conclusion of the breeding season (April 2010) all mature F1 fish were euthanised and measurements of standard length (to nearest 0.01mm), body weight (to 0.01g) and liver weight (to 0.001g) were taken. Hepato-somatic index was calculated as liver weight as a percentage of the total body weight.

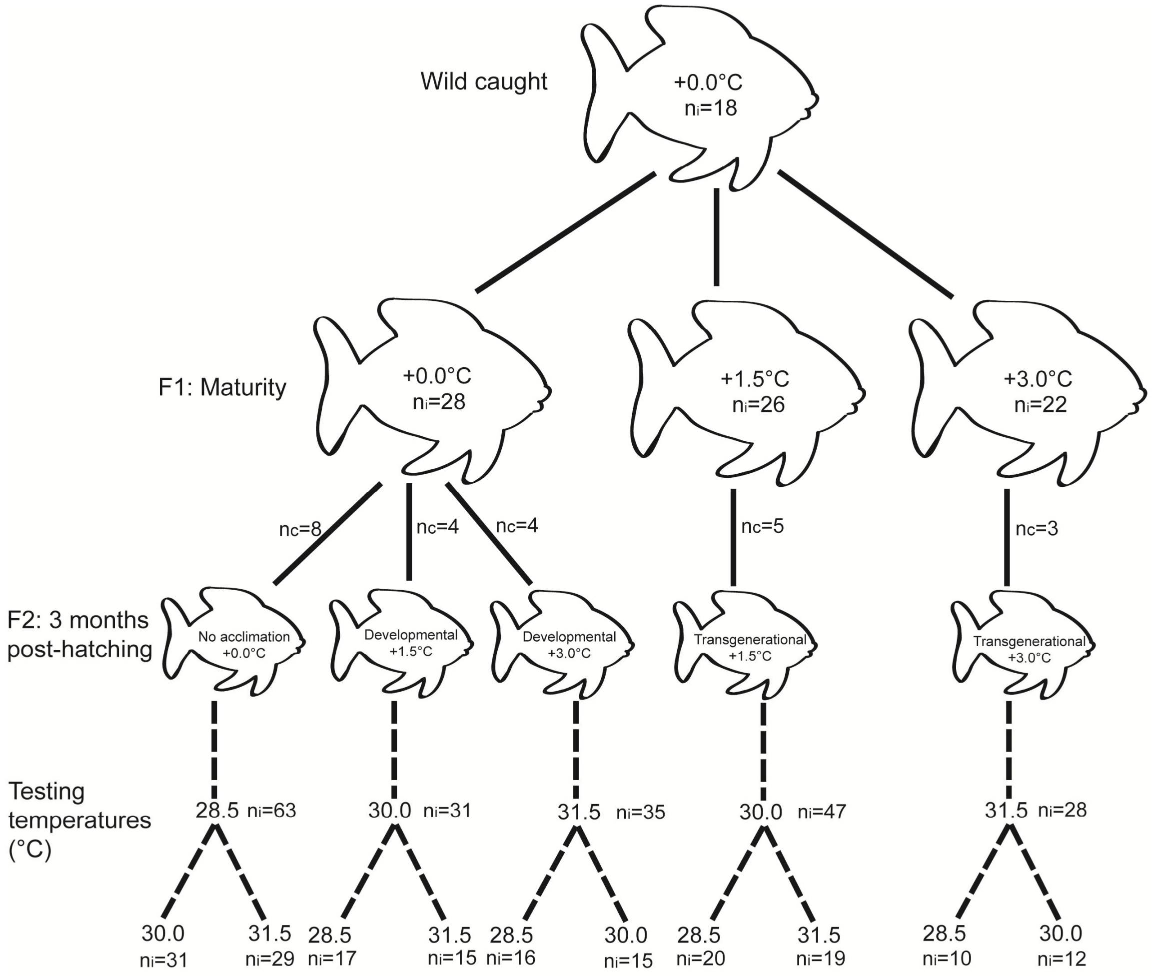

Figure 6.1 Experimental design tree. Experimental design where fish icons represent acclimation treatments and temperature regimes that subjects were raised in from hatching until maturation (2 years F1) or 3 months (F2). F2 offspring were first tested in their acclimation treatment summer average temperature before being tested in one of the other two treatment temperatures. Numbers inside fish represent total numbers of individuals in a treatment (N) and numbers outside represent numbers of clutches (n_c) or individuals (n_i) tested.

Metabolism methods

Resting metabolic rate (RMR) and maximum metabolic rate (MMR) were measured directly in mg O_2 kg^{-1} h^{-1}, using a closed respirometer (Nilsson *et al.* 2009; Donelson *et al.* 2011), which allowed the calculation of aerobic scope (MMR/RMR). For RMR measurements, each fish was allowed to acclimatize in the respirometer (a 350 ml Perspex cylinder with 68 mm inner diameter) for 1 h with a constant water flow. Following acclimatization the chamber was sealed and oxygen concentrations were monitored with an oxygen electrode (WTW OXI 340i or OXI 3310, Germany) for 30 min. Oxygen concentrations remained above 70% of air saturation during all respirometry. Fish were given at least 2 hours before measuring MMR. For measurement of MMR the chamber was placed upright so the chamber could be opened at the bottom for addition of the fish and created a circular swimming area (Nilsson *et al.* 2007). Water current was created by a 60mm magnetic stirring bar inside the sealed chamber and the current speed was set to the maximum aerobic swimming speed of the fish. Oxygen level in the water was measured for 5 to 10min during which oxygen decline was stable (see Nilsson *et al.* 2007 for further details). For both trials the respirometer was submerged in a temperature-controlled aquarium to maintain a stable temperature. Subsequently, the wet weight of each fish was measured to the nearest mg.

Fish were from 182 mg to 538 mg in all treatments and were starved for 24 hours prior to testing to remove any effects of digestion on oxygen consumption. Testing temperatures were 28.5°C, 30.0°C and 31.5°C, corresponding to the summer averages of each of the rearing treatment groups (+0.0°C +1.5°C and +3.0°C respectively). Fish were initially tested at their rearing temperature treatment and then given 1 to 2 days of rest. All tested fish were then divided equally in two groups and gradually adjusted to one of the other two treatment temperatures over a 2 to 3 hour period. Fish were maintained at the new temperature for 5 to 6 days before metabolic attributes were tested again at the new temperatures. All experiments were conducted within a temperature controlled room and during respirometry temperatures did not vary more than 0.2°C from the intended temperature.

Statistical analysis

RMR, MMR and aerobic scope were analysed by factorial ANOVA with both testing temperature and rearing treatment as fixed factors. A $\log_{10}$ transformation was used to satisfy ANOVA assumptions. Fisher's LSD post-hoc tests were used to compare treatment means following ANOVA. The same analysis was used to evaluate the response in RMR, MMR and aerobic scope of offspring from grandparent #41 (which was found to be selected for at +3.0°C) compared to all other grandparents, however transgenerational +3.0°C group was not included as all groups tested originally were from grandparent #41. The effect of rearing treatment on the hepato-somatic index, weight and standard length of F1 offspring was tested with two separate one-factor ANOVAs. Mann-Whitney U tests were used to compare hepato-somatic index of breeding and non-breeding fish within each rearing treatment.

6.4 Results and Discussion

We reared the tropical damselfish *Acanthochromis polyacanthus* for multiple generations in present-day (+0.0°C) and predicted future increased water temperatures (+1.5 and +3.0°C) to test their capacity for metabolic acclimation to ocean warming. The metabolic performance of F2 fish at their summer average rearing temperature was influenced by whether individuals had the opportunity for no acclimation, developmental acclimation or transgenerational acclimation (Figure 6.2) For fish with no opportunity to acclimate, an increase in temperature caused an increase in resting metabolic rate (RMR), a decline in maximum metabolic rate (MMR) at the warmest temperature, and a progressive decline in aerobic scope (MMR/RMR), which are common trends for tropical coral reef fish (Nilsson *et al.* 2009; Donelson *et al.* 2011). Developmental acclimation significantly reduced RMR, compared to no acclimation, but only for +3.0°C fish at 31.5°C (Figure 6.2a). A lack of increase in MMR for developmental acclimation fish resulted in no significant increase in aerobic scope (Figure 6.2b & c). In contrast, transgenerational acclimation enabled fish to completely restore their aerobic scope (Figure 6.2c). Transgenerational acclimation individuals achieved increased scope, when compared to fish not acclimated, at 30.0°C by reducing RMR and, at 31.5°C by both reducing RMR and increasing MMR (Figure 6.2). The magnitude of the reduction of RMR in developmental and transgenerational acclimation fish at 31.5°C was similar; indicating that physiological mechanisms to reduce RMR may be limited and further acclimation or adaptation of this trait may not be possible. Increased MMR was exhibited only by transgenerational acclimation groups at 31.5°C (Figure 6.2b). Changes in tissue mitochondrial densities or key oxygen transport characteristics are likely explanations of differences in MMR (Perry and Gilmour 2010).

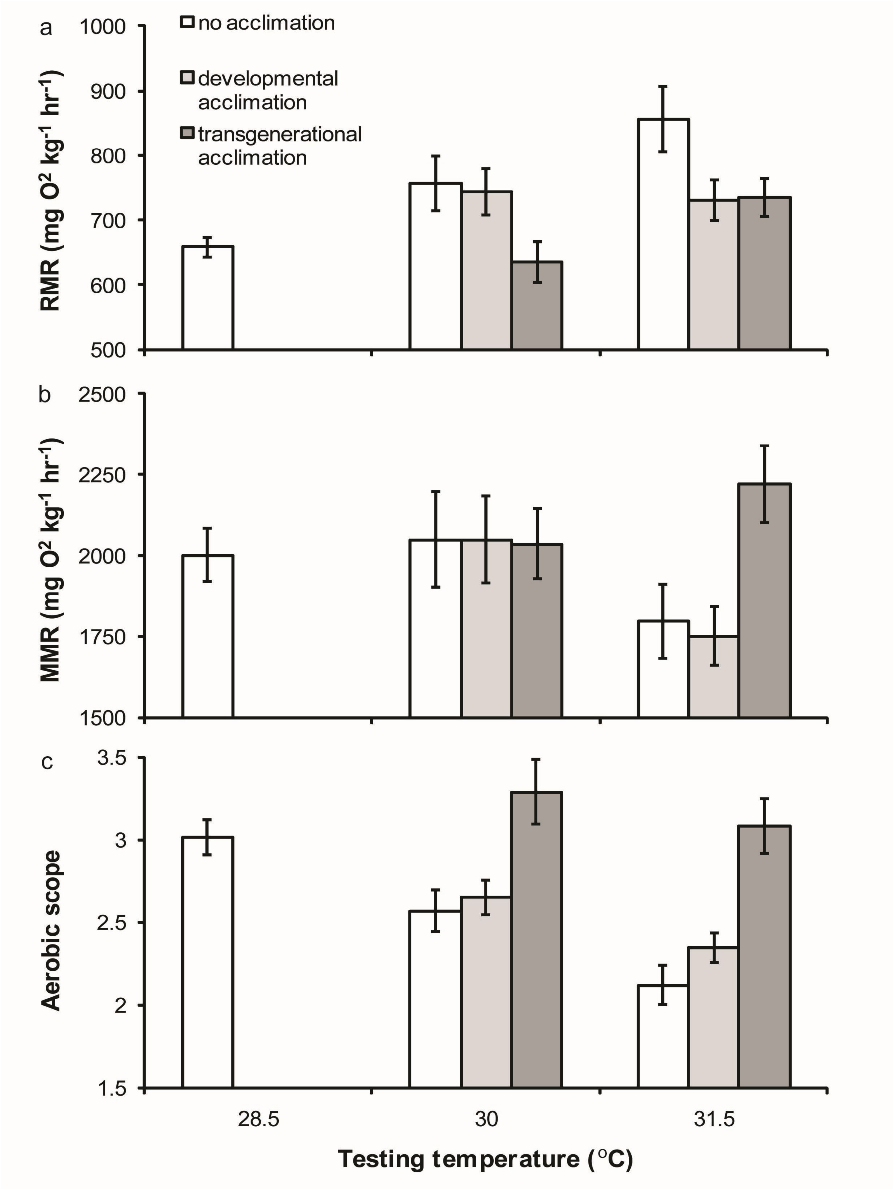

Figure 6.2 Metabolic performance of acclimation groups at their summer average treatment temperature. Resting metabolic rate (a), maximum metabolic rate (b), and aerobic scope (c) of fish (*Acanthochromis polyacanthus*) tested at their average summer temperature and +0.0°C fish tested across all temperatures (mean ± s.e.m.). Significant differences between means are identified when error bars do not overlap.

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Not only did the performance of fish at their average summer temperature differ between acclimation groups, but so did their performance across the three testing temperatures (Figure 6.3; Table 6.1). The most striking difference was that the transgenerational acclimation group, at both +1.5 and +3.0°C, maintained scope across all temperatures, whereas no other treatment groups did (Figure 6.3c). The mechanism for maintaining aerobic scope is likely to be through a widening of the thermal performance breadth (the range of temperatures over which an organism performs well; Huey and Stevenson 1979) since it is rare for thermal optima to shift readily with temperature (Guderley *et al.* 2001), and no reduction in performance at cooler temperatures was observed. This increase in aerobic scope was achieved by a reduction in RMR at all temperatures (Figure 6.3a), relative to fish with no acclimation, and an increase in MMR at 31.5°C (Figure 6.3b). Fish with developmental acclimation at either +1.5°C or +3.0°C did not differ in their response across test temperatures compared to non-acclimated fish for most metabolic measures (except RMR of +3.0°C at 31.5°C). The difference in performance between developmental and transgenerational acclimation fish demonstrates that parental influences may be critical in coping with future temperatures, by allowing MMR and subsequently aerobic scope to be maintained. Furthermore, increases of only +1.5°C are enough for F1 parents to produce offspring with superior performance at all summer temperatures.

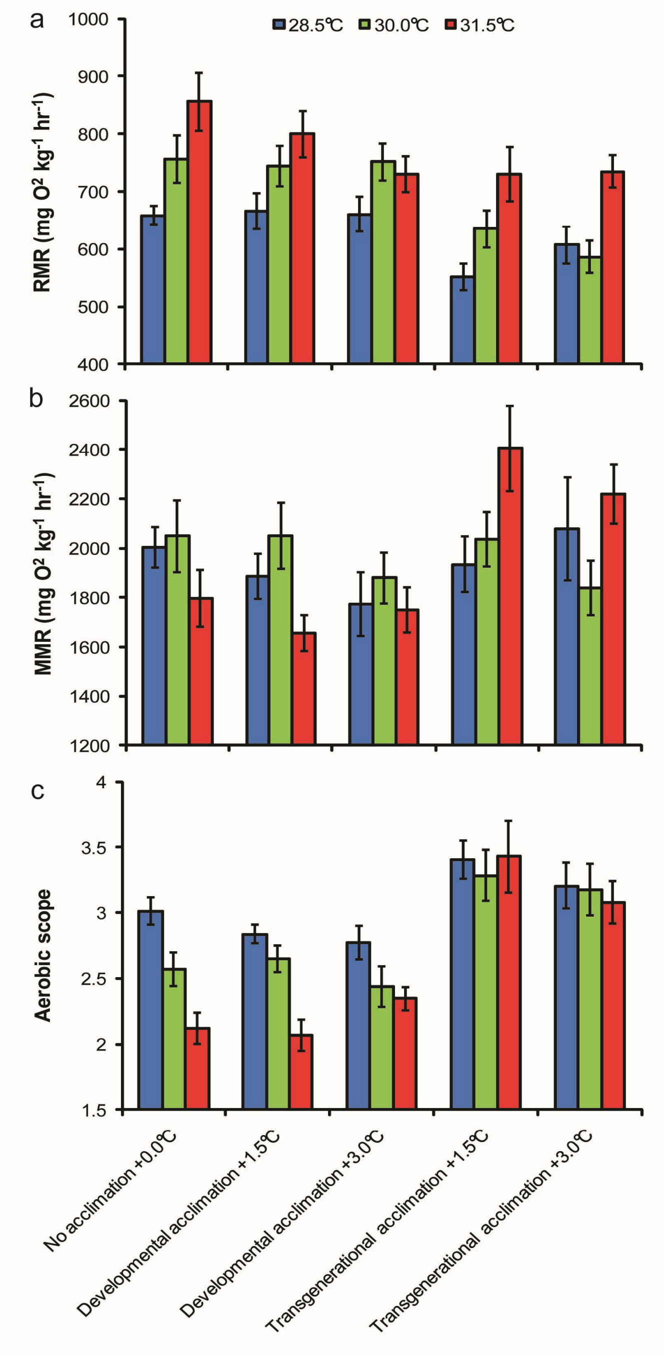

Figure 6.3 Metabolic performance of acclimation groups across all summer treatment temperatures. Resting metabolic rate (a), maximum metabolic rate (b), and aerobic scope (c) of fish across the three summer average temperatures depending on acclimation treatments (mean ± s.e.m.). Significant differences between means are identified when error bars do not overlap.

Table 6.1 Statistical results of differences in metabolic attributes of F2 acclimation treatment groups at 3 testing temperatures. Factorial ANOVA comparison of resting metabolic rate (RMR), maximum metabolic rate (MMR) and aerobic scope ($\log_{10}$ transformed) of F2 fish across testing temperatures for all acclimation treatments. Significant p values are indicated in bold.

	RMR		MMR		Scope	
Testing temperature	$F_{2,344}$=15.3	**p<0.001**	$F_{2,316}$=0.00	p=0.97	$F_{2,290}$=12.05	**p<0.001**
Acclimation treatment	$F_{2,344}$=7.2	**p<0.001**	$F_{2,316}$=2.4	p=0.05	$F_{2,290}$=17.25	**p<0.001**
Testing temperature* Acclimation treatment	$F_{2,344}$=1.1	p=0.392	$F_{2,316}$=2.1	**p=0.036**	$F_{2,290}$=2.11	**p=0.035**

From the F1 generation, 64% of fish reproduced in +0.0°C, 54% in 1.5°C and 36% in +3.0°C. Successful reproduction in fish is linked to energy storage (Tyler and Sumpter 1996; Brooks 1997). An indicator of the amount of stored energy, and physical condition of fish generally, is the liver to body weight ratio (hepatosomatic index = HSI; Chellapa *et al.* 1995). The mean HSI of all F1 fish was significantly higher in elevated temperature treatments (Table 6.2), suggesting that enhanced liver development and energy storage may be critical for reproduction in a warmer environment. Further, while the HSI of breeding and non-breeding F1 fish in either the +0.0°C or +1.5°C treatments did not differ, those F1 fish that reproduced at +3.0°C had significantly greater HSI than non-breeders at the same temperature (Table 6.2). This further indicates that a larger amount of stored energy may be required for reproduction at elevated temperatures. By comparing +1.5°C and +3.0°C F1 fish we see a trade-off in energy partitioning between liver development and linear growth. These two groups possess similar RMR (Donelson *et al.* 2010), however +1.5°C fish are significantly larger in length and +3.0°C fish have a significantly greater HSI (Table 6.2).

Table 6.2 Average size and condition of F1 adults in temperature treatments. Mean (± s.e.m.) standard length (SL), weight and HSI of F1 adults at the conclusion of the experiment are shown. Bold indicates significant differences between temperature treatments (ANOVA) and between breeding and non-breeding fish within a temperature treatment (Mann-Whitney U).

Temperature treatment	SL	Weight	HSI	HSI Breeders	HSI Non-breeders	Mann-Whitney U HI Breeder vs Non-breeders
+0.0°C	78.82 ± 1.23	21.55 ± 1.14	0.93 ± 0.05	0.93 ± 0.08	0.93 ± 0.05	Z=0.33 n=24 p=0.75
+1.5°C	79.13 ± 1.09	21.10 ± 0.91	1.09 ± 0.05	1.07 ± 0.08	1.09 ± 0.07	Z=0.46 n=25 p=0.64
+3.0°C	74.79 ± 1.18	17.73 ± 1.00	1.32 ± 0.05	1.51 ± 0.08	1.21 ± 0.05	**Z=12.05 n=22 p=0.007**
ANOVA difference b/w treatments	**$F_{2,69}$=4.73 p<0.01**	**$F_{2,69}$=4.22 p=0.019**	**$F_{2,69}$=11.99 p<0.001**			

Eight genetic lineages from the F0 wild stock were evenly represented in the F1 population, allowing the investigation of the potential for genetic selection. Evidence of rapid selection for particular genetic lineages was observed in the F1 +3.0°C temperature, with 75% of all pairs that reproduced at this temperature comprising an offspring of wild pair #41, whereas this genetic lineage was less prominent among reproducing pairs at +1.5°C and +0.0°C treatments (57% and 44% respectively). The selection of a genetic lineage at +3.0°C indicates that in addition to transgenerational acclimation, rapid selection of genotypes tolerant to thermal regimes could also occur in tropical marine fishes. Importantly, the increased contribution of wild pair #41 to the F2 generation did not affect our interpretation of complete acclimation in aerobic scope by transgenerational acclimation groups, because improved performance seen in F2 transgenerational acclimation was observed across all lineages not just offspring from #41 grandparents (Table 6.3).

Table 6.3 Statistical results of differences in metabolic attributes between fish from wild grandparent #41 and all other grandparents. Factorial ANOVA comparison of resting metabolic rate, maximum metabolic rate and aerobic scope ($\log_{10}$ transformed) between offspring of F0 wild pair #41 and all F0 pairs (Parental ID). The full-factorial ANOVA design was tested, but only the combinations involving parental ID are shown below.

	RMR		MMR		Scope	
Parental ID	$F_{1,286}$=3.2	p=0.075	$F_{1,262}$=0.1	p=0.767	$F_{1,238}$=1.538	p=0.216
Testing temperature * Parental ID	$F_{2,286}$=0.3	p=0.758	$F_{2,262}$=0.4	p=0.671	$F_{2,238}$=0.935	p=0.415
Acclimation treatment* Parental ID	F3$_{,286}$=0.06	p=0.598	$F_{4,262}$=0.8	p=0.503	$F_{4,238}$=0.953	p=0.696
Testing temperature * Acclimation treatment* Parental ID	$F_{6,286}$=0.08	p=0.597	$F_{8,262}$=1.1	p=0.371	$F_{8,238}$=1.546	p=0.163

The rapid transgenerational acclimation observed in our experiments to temperatures expected by 2050-2100, including complete restoration of aerobic capacity, could allow species to persist across their present locations. The ability to acclimate and maintain aerobic capacity would also be expected to maintain performance in characteristics such as growth and swimming ability at elevated temperatures (Pörtner and Knust 2007; Pörtner and Farrell 2008). Non-genetic parental effects or epigenetic inheritance (phenotypic differences not originating from variations in DNA base sequences; Jablonka and Raz 2009) most likely explains the transgenerational acclimation to increased temperature. Since the magnitude of response in the transgenerational +1.5°C and +3.0°C F2 offspring were equivalent, it is likely that the same mechanism operated in both treatments. Additionally, it suggests that an increase of only +1.5°C in the parental generation is sufficient to “prepare” offspring and enhance performance at +3.0°C. One likely mechanism by which parents influenced the performance of offspring in the present study is through variation in their epigenetic state, which affects gene expression, potentially producing offspring of phenotypes more suited to their environment (Bonduriansky and Day 2009). Another possible mechanism is by selection within the F1 generation for particular genotypes, which produce progeny with enhanced metabolic performance due to genetic differences. While, detailed physiological and genetic investigations would be needed to identify the means of transmission, these results clearly show that the attributes of progeny have been altered compared with their parents in response to elevated temperatures.

This study provides evidence that, contrary to some expectations, a tropical marine species has the capacity for acclimation and adaptation to temperature increases over time scales much shorter than the rate of anthropogenic climate change. Genetic diversity was also reduced at the warmest temperature due to differential breeding success among familial lineages, which has a range of implications for future populations, including disease resistance and plasticity to further environmental changes (Reed and Frankham 2003). Furthermore, physiological modifications involved in acclimation and adaptation may come at a cost to others attributes such as growth (Angiletta 2009; Donelson *et al.* 2011), Nevertheless, the discovery that advantageous offspring phenotypes are produced within two generations could indicate that tropical marine species are more capable of coping with global warming than has been suggested and illustrates the limitation of short-term experiments for predicting the long-term impacts of climate change (Leuzinger *et al.* 2011).

Chapter 7: General Discussion

Understanding the potential for acclimation to reduce the impacts of climate change is often discussed, but rarely investigated (Hughes *et al.* 2003; Bradshaw and Holzapfel 2006; Munday *et al.* 2008a; Munday *et al.* 2009; Hofmann and Todgham 2010). Knowledge of the ability of species to acclimate is critical to predicting future species diversity, abundance, range shifts and the likely persistence of existing populations. By exploring the potential for coral reef fish to cope with climate change over years and generations, the present study found that *A. polyacanthus* is capable of both developmental and transgenerational thermal acclimation. These findings challenge the commonly held assumption that tropical reef fish have limited capacity to cope with rising ocean temperatures.

Thermal effects on coral reef fishes

Most coral reef fish species studied to date appear to be sensitive to elevated temperature, with increases of only a few degrees Celsius having substantial effects to adult metabolism (Nilsson *et al.* 2009, 2010; Gardiner *et al.* 2010; ***Chapter 4***), reproduction (***Chapter 2***), growth and body condition (Munday *et al.* 2008b; ***Chapter 2***). The implication of this research is that the performance of future populations are likely to be significantly impacted and that many species may not be able to persist through out their current ranges. Reductions in key metabolic attributes represent a significant threat as changes are known to determine individual performance and set the limits for the distribution of wild populations (Pörtner and Knust 2007; Farrell *et al.* 2008; Pörtner and Farrell 2008). These effects to metabolism (Nilsson *et al.* 2009, 2010; Gardiner *et al.* 2010), as well as the direct influence of water temperature to reproduction (Van der Kraak and Pankhurst 1997; ***Chapter 2***), pose significant risks to future populations of reef fishes, with reductions in the quantity and quality of gametes likely and complete cessation of reproduction possible if adults also experience poor food availability (***Chapter 2***).

Alterations to gametes have subsequent effects to offspring quality, specifically offspring are smaller, lighter and have less endogenous provisioning (***Chapter 3***). This is likely to lead to increased mortality in a natural setting when mortality is size selective (Bailey and Houde 1989; Sogard 1997; Meekan and Fortier 1996; Holmes and McCormick 2009). However, the performance of these progeny will also depend on the quality of juvenile environment they experience (Marshal *et al.* 2006; Donelson *et al.* 2009; ***Chapter 3***) and the selectivity of mortality agents they encounter (Hoey and McCormick 2004; Holmes and McCormick 2009). Despite the influence of the parental environment, juveniles are also capable of compensating for size limitations at hatching through accelerated growth in the first weeks of life (***Chapter 3***). This suggests that parental effects to offspring are not everlasting and the phenotype of progeny may be more strongly determined by their present juvenile environment than parental effects.

Thermal acclimation

Theory predicts that tropical organisms will have limited capacity to deal with rising temperatures because they have evolved in a thermally stable environment (Deutsch *et al.* 2008; Tewksbury *et al*. 2008; Wright *et al*. 2009). This is supported by research to date finding little or no evidence for thermal acclimation by adults of tropical marine species, including coral reef fishes (Stillman 2003; Nilsson *et al.* 2009, 2010). In contrast, the present study provides exciting new evidence that tropical marine fish possess the capability to modify their physiology during development to cope with future ocean temperatures (***Chapter 4-6***). The distinction between the current and previous studies is the type of acclimation being investigated. Previous research has tested late-stage juveniles and adult reef fish, possibly only allowing for reversible acclimation (Nilsson *et al.* 2009, 2010), while the present study reared fish from shortly after hatching permitting developmental acclimation to occur.

The developmental thermal acclimation found to occur in *A. polyacanthus* resting metabolic rate is likely to provide significant benefits to fish by reducing the energy required for daily cell function (Bret 1971; Hazel and Prosser 1974; Houde 1989; Clarke and Johnston 1999). Reductions to the energy necessary for basic processes could provide extra energy for other activities such as growth and reproduction. Additionally, improvement in aerobic capacity (observed in the Heron Island population, ***Chapter 5***) will lead to further improvements in growth and enhanced swimming ability (Portner and Knust 2007; Farrell *et al.* 2008; Pörtner and Farrell 2008; Johanssen and Jones 2011). The capacity to maintain key metabolic attributes through developmental acclimation is likely to reduce the impacts of climate change and potentially allow persistence of species throughout their current range. Although this study only tested one species, it seems likely that other tropical reef fishes will also exhibit some capacity to cope with rising ocean temperature through this developmental pathway. Studies with other species are now needed to test the ubiquity of developmental thermal acclimation in reef fishes.

A number of theories have been proposed to explain relationships observed between acclimation temperature and performance. Original theory suggested that acclimation to a particular temperature would allow individuals to outperform others not acclimated (Beneficial acclimation hypothesis; Leroi *et al*. 1994) and that all changes that occur in response to a modified thermal environment would enhance performance (Angiletta 2009). However, these prediction are rarely supported by data (Huey *et al.* 1999; Angiletta 2009), including the present study since acclimation was not found to occur at all temperatures and all attributes were not beneficially influenced by temperature (***Chapter 4***). The explanation for the contrast between theoretical predictions and experimental evidence is that acclimation is likely to involve energetic costs, but such costs are usually unknown and rarely considered when making predictions from theory. Both detection and response to a varying environment has energetic expenses and the benefits will depend on the predictability of the variation in the thermal environment (Kingsolver and Huey 1998; Angiletta 2009). The present study found evidence for energy trade-offs between metabolic acclimation and growth rate, suggesting that energy spent on physiological changes to reduce resting metabolic rate could have otherwise been spent on growth (***Chapter 4***). Consequently, to fully understand the potential for reef fish to cope with climate change a range of fundamental traits need to be investigated to predict future performance and persistence.

At the population level both “colder is better” (larger body size) and “warmer is better” (smaller body size) hypotheses have been proposed to explain differences in performance when they are linked to differences in body size attained in either environment (Huey *et al.* 1999). When the cooler population outperforms the warmer across all temperatures irrespective of body size, it is termed countergradient variation (Conover and Schultz 1995). Evidence for contergradient variation was found between Heron and Palm Island populations in the present investigation, with the Heron Island population able to fully acclimate metabolic attributes (***Chapter 5***). There is increasing support for countergradient thermal relationships in performance across a range of fish species (Conover and Present 1990; Álvarez *et al.* 2006; Baumann and Conover 2011; Gardiner *et al.* 2010), again suggesting that persistence in a warmer environment may not equate to beneficial performance in that environment. In addition, difference in thermal performance between populations may be genetic in nature and therefore not determined by environmental variability (Knies *et al.* 2006; Driessen *et al.* 2007; Yamahira *et al.* 2007). Differences in acclimation ability between Heron and Palm Island populations (***Chapter* 5**) could be due to them being colonised by separate invasion events (Planes *et al.*, 2001). Thus, acclimation capacity may be linked to a population’s history, just as much as it is to the thermal environment currently experienced. This suggests that considering phylogenetic history in comparisons of thermal tolerance and acclimation history may further help reconcile theoretical predictions and empirical evidence.

A close relationship between the thermal variability experienced by a population and the potential for acclimation is expected (Angiletta *et al.* 2006; Angiletta 2009), since maintaining the ability for plasticity can be costly (Hofmann and Todgham 2010; Relyea 2002). However, the timing and nature of environmental variability will also determine acclimation capacity. Three main predictions arise from this logic: 1) stable environments both within and between generations will favour thermal specialists with limited acclimation ability, 2) environments which are stable within a generation but vary between generations will favour developmental acclimation and 3) environments that vary within a generation will favour reversible acclimation (Angiletta 2009). Current evidence on thermal acclimation in coral reef fish shows support for the second relationship. Most reef fish, once settled, do not move between reefs and are unlikely to experience large amounts of thermal variability over their lifetime. However, there is ample opportunity for variation in thermal environment between generations since most reef fishes possess a planktonic larval stage and may not settle in a similar thermal environment to their parents (Pineda *et al.* 2007). Therefore, the ability to modify key attributes in relation to a juveniles' new thermal environment would be beneficial. This concept is further supported by the prevalence of developmental plasticity in the early life stages of coral reef fishes for a range of traits (Warner 1997; Munday *et al*. 2006). Interestingly, the species used in the current study has evolved to not longer possess a planktonic larval phase and thus the parental and offspring environment are the same. This may provide further evidence that the potential for plasticity is more ancestrally derived than controlled by current environmental variation. While the populations tested in this study exhibit the ability to developmentally acclimate, populations of reef fishes living close to the equator may not be capable of acclimation due to the environment being very stable both within and between generations.

Transgenerational acclimation

In addition to developmental acclimation, my research demonstrated the potential for parental effects to fully restore critical metabolic attributes, within just two generations at elevated temperatures (***Chapter 6***). Beneficial transgenerational plasticity has been previously identified in both plants and insects in relation to predation risk in the parental environment (Agrawal *et al.* 1999; Mondor *et al.* 2005), however this is the first study to identify transgenerational acclimation as a potential pathway to mediate the impacts of climate change. The enhancement of key metabolic attributes, improved with developmental acclimation, are expected to equate to improved growth, swimming and reproductive ability compared to the performance of present-day populations at elevated temperatures (Pörtner and Knust 2007; Pörtner and Farrell 2008). The prevalence of transgenerational acclimation in coral reef fishes is currently unknown and may not be expected to be common since there is generally not a link between parental and juvenile environment. However, there is increasing evidence that a large proportion of reef fish recruit to their natal reefs (Jones *et al.* 1999; Swearer *et al.* 2002; Jones *et al.* 2005; Almany *et al.* 2007) and other forms of parental effects are common (Kerrigan 1997, McCormick 2003; Gagliano and McCormick 2007; Green 2008), thus it just as likely that transgenerational acclimation will be detected in other species. Uncovering the potential for parental effects to mediate impacts of climate change is a highly significant development in our understanding of the pathways marine species use to cope with adverse environmental conditions and the prevalence in a range of taxa warrants further investigation.

Concluding remarks

This thesis demonstrates that understanding the potential for species to acclimate and adapt to rapid environmental change is critical for predicting the nature and extent of biological impacts that will occur this century due to human-induced climate change. By rearing multiple generations of *A. polyacanthus* at future predicted ocean temperatures this study showed that both developmental and transgenerational acclimation represent potential means for coral reef fishes, and possibly a greater range of ectotherms, to cope with climate change. Simply testing how present-day populations and individuals cope with short-term temperature changes are unlikely to give a true indication of their response to a changing environment and may overestimate the likely impacts. It is also evident that population genetic history is important and that not all populations will respond similarly to temperature increases, even if they currently experience similar thermal environments. Nevertheless, acclimation likely comes at some cost, and understanding these costs will be a key part to the development of any theory that attempts to marry environmental change and changes in the reaction norms of performance attributes. Only with further investigation of acclimation ability in a range of species will a sufficiently complete understanding be possessed to make predictions on the likely future biological impacts of climate change.

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Appendix A: Publications not arising from the thesis

Donelson JM, Munday PL, McCormick MI (2009) Parental effects on offspring life histories: when are they important? *Biology Letters* 5:262-265.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Døving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc Natl Acad Sci USA* 106:1848-1852.

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