Effects of ocean warming on the larval development of coral reef fishes

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Frontispiece: Coral reef fishes in Papua New Guinea.

Statement of contribution of others

This thesis includes collaborative work with my supervisors Professor Geoffrey Jones, Professor Mark McCormick, Professor Philip Munday and Dr Timothy Clark, as well as Dr Jodie Rummer, Dr Amelia Wenger, Professor Rhondda Jones, Miwa Takahashi, and Dr Rohan Brooker. While conducting these collaborative projects, experimental design, data collection, technical analysis, and ecological interpretation were primarily done by me. My co-authors provided intellectual guidance, financial support, and assistance with fieldwork, otolith preparation, experimental design, statistical analysis and editing.

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General Abstract

There is an urgent need to better understand how organisms respond to changing temperatures as evidence for rapid climate warming accumulates. Climate change models predict that tropical ocean temperatures will increase by up to 4^{o}C this century and affect the plankton communities that are the basis of food webs in most marine ecosystems. Most coral reef fishes have a bipartite life cycle with larvae that develop in the pelagic environment. Sensitivity to elevated temperatures may be magnified during the larval stage, which is the key stage for mortality, dispersal and connectivity. Previous field and experimental studies have shown that larval coral reef fishes typically exhibit increased growth rates and shorter pelagic larval durations (PLDs) with increasing temperatures within their natural temperature range. Based on this knowledge, it has been predicted that enhanced growth associated with increasing temperatures will lead to faster metamorphosis and less time in the dangerous pelagic environment. However, previous field studies have generally excluded populations from the warmest latitudes, have infrequently tested the impacts of elevated temperatures experimentally, and have not investigated the potential climate-related interactions between temperature, food availability and digestion, which may have negative effects on larval growth rates and development.

Latitudinal and temporal gradients in ocean temperature may be useful for predicting the likely response of marine species to climate warming. The ranges of coral reef fishes extend into the warmest oceanic waters on the planet, but the comparative life-history traits across their full latitudinal range are unknown. To answer this critical knowledge gap, I examined the potential effects of a temperature gradient on some key early life-history traits of two coral reef fishes, the damselfish *Pomacentrus moluccensis* and the wrasse *Halichoeres melanurus,* among 8 sites spanning the southern tropics from northern Papua New Guinea to the southern Great Barrier Reef (**Chapter 2**). Recently settled juveniles were collected and their otolith microstructure was analysed to estimate PLD, average daily growth and size at settlement. Latitudinal comparisons revealed a non-linear relationship between sea-surface temperature (SST), and these early life history traits. Pelagic larval durations declined with increasing temperature up to 28-29^{o}C, above which they stabilised or increased. Larval growth increased with increasing temperature to 28-29^{o}C before stabilising or decreasing. Size at settlement tended to be highest at mid-latitudes, but

overall declined with increasing temperature above 28.5°C in both species. These results indicate that the thermal optima for growth and development is reached or surpassed at low latitudes such that populations at these latitudes may be particularly vulnerable to global warming.

Likely impacts of long-term changes are often inferred from spatial gradients in temperature or short-term temperature fluctuations. However, the effects of climate change may only be apparent on decadal time scales, and there are few studies that extend over such long periods. In **Chapter 3**, I examined the influence of temperature and other associated environmental variables on key early life history traits of the coral reef damselfish, *Pomacentrus moluccensis* based on ten cohorts of newly settled fish collected over 13 years from around Lizard Island (Great Barrier Reef, Australia). Multiple regression techniques were used to measure the strength of the association between these traits and developmental temperature, rain, wind speed and solar radiation. Pelagic larval durations generally declined and growth rates generally increased with increasing temperatures to ~28°C, above which PLD tended to increase and growth rates tended to decrease. This pattern mirrored the existing latitudinal spatial patterns in these parameters (Chapter 2). This study confirmed that ~28°C is likely to be a thermal optimum, and significant warming above this level will detrimentally impact on this species. In addition, other environmental factors associated with climate change including rainfall, wind speed and solar radiation can have significant effects on larval development and should be considered in predictions of climate change effects on larval fish.

Both temperature and food supply can influence the development, growth, and metabolism of marine fishes, particularly during larval stages. However, little is known about the relative importance and potential interacting effects of ocean warming and changes to food supply on the performance of larval fishes. In **Chapter 4**, I tested this by raising larvae of the coral reef anemonefish, *Amphiprion percula*, in an orthogonal experiment comprising three temperatures (current day, +1.5°C and +3°C) and three feeding schedules. Overall, larvae grew more slowly and took longer to settle at higher temperatures and less frequent feeding, with a highly significant interaction between these factors. Fish from the lower feeding regimes had a lower body condition and decreased survivorship to metamorphosis. Routine oxygen consumption rates ($\dot{M}O_{2routine}$) were a third higher for larvae raised at +3°C than those raised at current temperatures. The elevated $\dot{M}O_{2routine,}$ and therefore greater energy use at higher

temperatures may leave less energy available for growth and development, resulting in the longer time to metamorphosis.

Finally, in **Chapter 5**, I examined food processing, digestion, and growth of larval *A. percula* at current-day and at an increased temperature (+3^{o}C). Larvae exhibited rapid, temperature-independent growth in the 24 h following satiation feeding. $\dot{M}O_{2routine}$ and peak $\dot{M}O_2$ during digestion were 55 ± 16% and 28 ± 11% higher at 31.5^{o}C. Elevated temperature had no significant effect on the energy used to digest and assimilate a meal (0.53 ± 0.05 J), digestion duration (6.3 ± 0.3 h), or the percent of total meal energy used for digestion (11.7 ± 1%). These results suggest that even if fish larvae can secure the food necessary to satisfy higher routine metabolism in a warmer ocean, they may not be able to process food at the necessary speed to maintain current-day growth rates.

Overall, this thesis shows that, contrary to some optimistic suggestions, climate warming is likely to have negative impacts on larval coral reef fishes. Impacts are likely to be most severe for low latitude populations living in the naturally warmest temperatures, which appear to be already living close to their thermal optima. Since routine metabolic rate increases with increasing temperature, individuals must consume more food to maintain the same level of growth at higher temperatures. However, the planktonic communities that are food for many coral reef fish are predicted to become more variable or decline with climate change, and even if food is plentiful larvae may not be able to process it at the necessary speed to maintain current-day growth rates. Elevated temperatures and reduced food supplies are therefore likely to lead to slower larval growth and protracted development in the pelagic environment, with effects on larval survival and dispersal, and population connectivity and persistence.

Table of Contents

List of Tables

List of Figures

Chapter 1. General Introduction

The earth's climate is changing at an unprecedented rate, mainly due to anthropogenic carbon dioxide (CO_2) input into the atmosphere through rapid industrialisation, fossil fuel combustion, cement manufacture, and land use changes such as deforestation (Solomon et al., 2007, IPCC, 2013). Rising carbon dioxide in the atmosphere is causing a greater retention of heat resulting in global warming. Each of the past three decades has been warmer than all the previous decades in the instrumental record, and the decade of the 2000s was the warmest (IPCC, 2013). Depending on the magnitude of future CO_2 emissions, the average surface temperature is projected to rise another 0.3-4.8^{o}C by the end of the century (IPCC, 2013). However, we are currently tracking one of the highest carbon dioxide emissions scenarios (Peters et al., 2012), and as likely as not, will be facing over 4^{o}C of warming, relative to the years 1850-1900, by the end of the century unless the trend of accelerating emissions is changed. Climate change is expected to have serious consequences for ecosystems globally, resulting in an overall loss of diversity, disruptions to ecosystem function, and a reduction in the ecological goods and services provided to human societies (Thomas et al., 2004, Dawson et al., 2011, Diffenbaugh and Field, 2013, Hollowed et al., 2013, Wheeler and Von Braun, 2013, Burrows et al., 2014).

Climate change and marine ecosystems

Marine ecosystems are centrally important to the biology of the planet, yet a comprehensive understanding of how climate change is affecting them is still being developed (Hoegh-Guldberg and Bruno, 2010, Doney et al., 2012). In the ocean, the primary consequences of increasing CO_2 are increasing ocean temperatures (Bindoff, 2007, Bopp et al., 2013), ocean acidification (Doney et al., 2009), and an expansion of oligotrophic waters (Doney et al., 2012). These physical and chemical changes are expected to result in shifts in the timing, structure, and magnitude of phytoplankton production (Richardson, 2008, Barton et al., 2013, Bopp et al., 2013). Changes to plankton communities will vary, but in many locations these communities may become less productive because higher temperatures favour longer, less productive planktonic food chains (McKinnon et al., 2007, Morán et al., 2010), and because greater thermal stratification of the water column will reduce nutrient enrichment of the surface layers that are the most important for planktonic productivity (Brander, 2010, Doney et al., 2012). Bopp, et al (2013) predicted a global reduction in marine primary productivity

of 8.6% by the 2090s compared to the 1990s, primarily driven by greater thermal stratifications reducing enrichment of the euphotic zone. Plankton is the base for almost all marine food chains and changes to the availability of this food source are likely to have important effects on marine organisms across life stages (Richardson, 2008, Munday et al., 2009a).

Globally, sea surface temperature (SST) has already increased by about 0.7^{o}C over the last 100 years (Bindoff, 2007) resulting in shifts in current strength and direction, ocean stratification, and nutrient input into surface waters with wide-ranging biological effects such as decreased ocean productivity, altered food web dynamics, reduced abundance of habitat-forming species, shifting species distributions, and a greater incidence of disease (Hoegh-Guldberg and Bruno, 2010). The latest IPCC (2013) estimates for ocean-wide SST increases are ~0.6-2.0^{o}C by the end of the century, and this warming is likely to amplify these processes.

Tropical marine ecosystems

Temperature increases will not be uniform throughout the oceans and tropical waters are predicted to have some of the strongest warming with SST temperature increases larger than 4^{o}C possible by the end of the century, depending on emission scenario (Bopp et al., 2013). Temperature influences a host of organism physiological rates and cellular processes and has been referred to as 'the ecological master factor' (Brett, 1976). Ectotherms do not physically regulate their internal body temperature and are therefore strongly influenced by external temperatures and are expected to be especially vulnerable to elevated temperatures. Most marine organisms are ectothermic and thus their metabolic pathways are strongly influenced by water temperature. Tropical ectothermic species may be especially vulnerable to rising temperatures because many have a narrower thermal tolerance range than equivalent temperate species and tend to live in temperatures closer to their thermal optima (Pörtner and Farrell, 2008, Sunday et al., 2011). Therefore, increasing environmental temperatures in the tropics are likely to exceed the thermal optimum for many tropical species (Deutsch et al., 2008, Tewksbury et al., 2008, Wright et al., 2009), and modify the timing of key events such as reproduction that utilise temperature cues (Walther et al., 2002, Parmesan, 2006).

Ocean warming and coral reefs

Tropical coral reef ecosystems are especially sensitive to ocean warming, with widespread bleaching and mortality of reef forming corals recorded at temperatures just 1-2 degrees above the long-term average (Glynn, 1991, Hoegh-Guldberg et al., 2007). All major coral reef regions have undergone declines in coral cover (Gardner et al., 2003, Bruno and Selig, 2007, Ateweberhan et al., 2011). A reduction in live coral and structural complexity has been shown to cause losses in fish diversity and abundance (Jones et al., 2004, Graham et al., 2006, Pratchett et al., 2011) and is increasing the extinction risk for coral reef fish species (Pratchett et al., 2008).

Direct physiological consequences of climate change on reef fishes will further compound the effect of coral degradation. Because water temperature controls cell function and metabolic rate in ectotherms, it can influence marine fishes throughout all life stages across a range of attributes and life-stages. Recent laboratory experiments have shown that higher summer temperatures, in the range predicted by the end of this century, (i.e. up to 3^{o}C higher than current summer averages), lead to reductions in aerobic scope (Nilsson et al., 2007), critical swimming speeds (Johansen and Jones, 2011), somatic growth (Munday et al., 2008b), and reproductive output (Donelson et al., 2010) of adult reef fishes. However, not all life stages are equally thermally sensitive and therefore not equally susceptible to environmental change.

Ocean warming and its effects on larval reef fishes

The persistence of population in a changing climate will be constrained by their most vulnerable life stages. Most coral reef organisms have a complex life cycle with a relatively sedentary and site-attached adult phase and a larval phase that undergoes development in the pelagic environment. For many marine fish species it is the small pelagic larval stage that is the most vulnerable to environmental stressors such as temperature changes due to their inability to temperature compensate and to key systems still developing, such as ventilator and circulatory systems (Pankhurst and Munday, 2011, Pörtner and Peck, 2011). The larval stage of coral reef fishes is the most important for dispersal and connectivity among adult populations (Cowen and Sponaugle, 2009, Jones et al., 2009), and because mortality rates are extremely high during this life phase (Bailey and Houde, 1989, Leis, 1991, Peck et al., 2012), small changes in larval growth, development, or survival rates could have large impacts on adult population dynamics (Houde, 1989, D'Alessandro et al., 2013).

To predict future responses to temperature change it is critical to define the thermal reaction norms - the shape of the relationship between a particular trait and temperature. Thermal reaction norms typical exhibit an approximately dome-shaped relationship, where rates increase with temperature up to an optimal level (thermal optimum), then decrease rapidly with further increases in temperature (Pörtner and Farrell, 2008, Tewksbury et al., 2008). However, most research to date has shown that the thermal reaction norms for growth of larval fishes tend to be approximately linear until the lethal upper thermal limit is reached (Sponaugle and Cowen, 1996, Rombough, 1997). Meta-analyses (Houde, 1989, Laurel and Bradbury, 2006), experiments (McCormick and Molony, 1995, Green and Fisher, 2004) and field studies (Meekan et al., 2003, Sponaugle et al., 2006) have indicated that larval growth rates generally increase with increasing temperature. Additionally, within the temperature range currently experienced by reef fishes, warmer years generally appear to favour good recruitment events for a variety of coral reef fishes (Meekan et al., 2001, Wilson and Meekan, 2002, Cheal et al., 2007). Based on this knowledge, it has been predicted that climate warming could have positive outcomes for coral reef fish larvae because enhanced growth will lead to faster metamorphosis and less time in the dangerous pelagic environment (O'Connor et al., 2007). Already studies are using these assumptions to parameterise models predicting the effects of climate change on connectivity, and dispersal patterns among meta-populations (e.g. Kendall et al., 2013). However, previous studies on the effects of ocean warming on larval coral reef fish have some important limitations.

Critical knowledge gaps

Many species of coral reef fishes are distributed across a large latitudinal range from the equator to subtropical waters (Jones and McCormick, 2002). Indeed, many span temperature ranges that are greater than the projected increase in ocean temperature by the end of the century (Munday et al., 2008a). Their responses to the gradient in temperatures across their range can provide clues as to how they will respond to a warmer climate (Deutsch et al., 2008, Dillon et al., 2010, Sunday et al., 2011). However, there is little information on intraspecific latitudinal variation in important early life-history traits of fishes across their distribution range (but see Booth and Parkinson, 2011, Takahashi et al., 2012). In particular, few studies of larval fish development in relation to temperature have included populations at equatorial latitudes, which may be particularly sensitive to global warming (Sunday et al., 2011, Rummer et al., 2013). Recent research has indicated that the warmest waters close to

the equator (Takahashi et al., 2012), and abnormally warm water during El Niño events (Lo-Yat et al., 2011) are associated with slower larval growth and reduced survival. However, no studies have examined the existing thermal responses of larval fishes over their entire latitudinal range, which provides a means to gauge the shape of the thermal reaction norm. This knowledge gap was recently highlighted by Leis et al. (2013) who called for more studies investigating the effects of latitudinal and long-term temporal variation of early life history traits of fishes to improve understanding of connectivity and dispersal patterns to inform fisheries management, conservation, and to predict climate-driven changes to marine systems.

There have been relatively few experimental studies where larvae were raised at elevated temperatures and reviews have typically relied on extrapolating from existing relationships between growth and temperature. Additionally, there has been little research into the capacity for growth and development at elevated temperatures in the context of energy dynamics and food supply. Most larval coral reef fish eat plankton (Leis, 1991, Sampey et al., 2007). Climate change is predicted to modify planktonic communities with an ocean-wide reduction in oceanic productivity (Bopp et al., 2013). However, these changes will not be uniform across the ocean, and will be particularly strong in the tropics, with decreases in primary productivity of up to 30% in the tropical Indian and west tropical Pacific predicted (Bopp et al., 2013). The effects of food supply on larval fishes are well known, with both faster growth and shorter PLDs observed with increasing food supply (McCormick and Molony, 1992, Green and McCormick, 1999, Meekan et al., 2003, Sponaugle et al., 2009). However, in relation to climate change, and the potential change in plankton productivity, it is the interaction between food supply and temperature that may hold the most relevance. While the growth rate of fishes with an unlimited food supply generally increases with increasing temperature, the effects of increasing temperature may be detrimental in food-poor environments.

Higher metabolic rates with increasing temperature may lead to faster growth, but only if the availability of food is sufficient to fuel the higher metabolic demands (Munday et al., 2008a). Food is rarely unlimited in the natural environment, and on a fixed ration, increasing temperature may lead to slower growth, due to increasing energy demands. The existence of potential synergistic effects between the different stressors emphasizes the need to study them together. The paucity of empirical data for larval coral reef fishes in response to elevated temperature is largely due to the inherent difficulties in working on such tiny

vertebrates. This has been identified as a critical knowledge gap hindering the parameterization of quantitative models aimed at forecasting the effects of climate change on fish communities (Jørgensen et al., 2012). Controlled studies at high temporal resolution are required to shed light on the potential effects of climate warming and food availability on the metabolism and food processing efficiency of larval fishes.

Larval fishes exhibit three inter-related developmental traits that have a bearing on successful recruitment to adult populations and that will likely respond to changes in temperature: PLD, larval growth, and size at settlement. Increased growth rates are likely to lead to a reduced PLD because there are strong correlations between growth rates and PLD, with fast-growing larvae often exhibiting shorter larval durations (Houde, 1989, McCormick and Molony, 1995, Sponaugle and Cowen, 1996, Green and Fisher, 2004). The relationship between growth rates and PLD will influence size at settlement (Sponaugle et al., 2006). Metamorphosis from the larval to the juvenile stage is thought to be a critical period characterised by high levels of mortality (Caselle, 1999, Almany and Webster, 2006). Size at settlement is important because a larger size is often associated with increased growth and survival after settlement (Sogard, 1997). Coral reef fishes generally metamorphose from the larval to the juvenile phase at larger sizes at lower temperatures (McCormick and Molony, 1995, Radtke et al., 2001, Green and Fisher, 2004, Sponaugle and Grorud-Colvert, 2006). The relationship between larval life history traits and temperature has mostly been studied in laboratory experiments or using temporal variation in temperature within a single location or region. Few studies have examined relationships among larval life history traits across the entire latitudinal temperature range of a species.

Like all vertebrates, larval fishes must expend energy to process and assimilate the nutrients from each meal. Termed specific dynamic action (SDA), the energy used during digestive processes can account for up to 25% of daily energy expenditure for adult fishes, yet nothing is known of the SDA and digestive efficiency of larval fishes (Secor, 2009). Previous studies of ectotherms have shown that elevated environmental temperatures lead to an increase in the magnitude and a decrease in the duration of the SDA (Secor, 2009). Therefore, ocean warming has the potential to significantly modify food-processing capacity. The advantage of this for a larval fish should be that the larvae could process meals faster and be ready for another one, potentially increasing their overall energy intake. Investigations into the SDA of larval fishes could provide important insights into the impacts of ocean warming on this important life stage.

Research aims and thesis outline

This thesis combines both large-scale observational approaches and controlled laboratory experiments to investigate the potential effects of ocean warming, including its interactions with other environmental stressors, on larval coral reef fishes. The large-scale observational focus was on coral reef species that occur across a broad latitudinal gradient that currently encompasses a larger temperature range than that expected through global warming. The effects of ocean warming will not be uniform across the tropics, and investigation into spatial and temporal patterns in relation to water temperature can provide clues to how fish populations will fare in future conditions. Controlled experimental studies would allow measurements of the direct and interacting effects of predicted temperature increases and of varying food supply, on key early life history traits of coral reef fishes.

Chapter 2 describes how latitudinal variation in larval growth, pelagic larval duration, and size at settlement was investigated by sampling two species of common coral reef fishes, the demersal egg laying damselfish *Pomacentrus moluccensis* and the broadcast spawning wrasse *Halichoeres melanurus*. Recently settled juveniles were collected from eight sites across 21 degrees of latitude, from northern Papua New Guinea (2.3^{o} S) to the southern Great Barrier Reef (23.3^{o} S). Otolith microstructure was analysed to estimate PLD, daily growth, and size at settlement and this was related to individual developmental temperature.

The next chapter **(Chapter 3)** describes 13 years of inter-annual variation in *P. moluccensis* early life traits around Lizard Island, Great Barrier Reef, Australia. Otolith microstructure was again analysed to estimate PLD, daily growth, and size at settlement but this time was also related to further environmental factors (solar radiation, rainfall, and wind strength) along with developmental temperature.

Once an understanding of the natural variation was understood, the focus of the research shifted to experimentally testing the impacts of elevated temperatures and varying food supply on larval development. **Chapter 4** investigated the potentially interacting impacts of elevated temperatures and varying access to food on larval development. Larvae of the coral reef anemone fish, *Amphiprion percula*, were used in an orthogonal experiment comprising three temperatures and three feeding schedules. Temperatures were chosen to represent current-day summer averages (29.2°C) and end-of-century climate change projections of +1.5°C (30.7°C) and +3°C (32.2°C), and feeding schedules were chosen to represent a reduction in access to food (fed daily, every two days, or every three days). PLD,

average larval growth, condition at settlement, survival to settlement and larval metabolic rate were measured.

In **Chapter 5** the focus narrows further to the impact of ocean warming on fine-scale growth and digestive performance. For this chapter, I tested the effect of acutely increased temperature on the growth and digestive performance of a coral reef fish (*A. percula*) during its larval phase. First, I measured the length and weight of 340 fed and unfed larvae raised at a typical current temperature (28.5^{o}C) and a temperature likely to be common by the end of the century (31.5^{o}C), across fine temporal scales. Second, I determined routine metabolic rate and the energetic cost of digestion (SDA) at both temperatures.

Together, the following four chapters advance our knowledge of the impacts of ocean warming on larval coral reef fishes by examining temperature effects from macroecological to ecophysiological scales and in the context of synergistic effects of multiple stressors. Knowledge of these impacts will provide critical information for assisting in the management and conservation of marine species and ecosystems in a warmer future.

Chapter 2. Latitudinal variation in larval development of coral reef fishes: implications of a warming ocean

This chapter has been submitted for publication in the Journal Proceedings of the Royal Society B: Biological Sciences. Authors: IM McLeod, MI McCormick, PL Munday, TD Clark, AS Wenger, RM Brooker, M Takahashi, and GP Jones.

Abstract

Latitudinal gradients in water temperature may be useful for predicting the likely responses of marine species to global warming. The ranges of coral reef fishes extend into the warmest oceanic waters on the planet, but the comparative life-history traits across their full latitudinal range are unknown. Here, we examined differences in early life-history traits of two coral reef fishes, the damselfish *Pomacentrus moluccensis* and the wrasse *Halichoeres melanurus,* among 8 locations across 21^{o} of latitude, from northern Papua New Guinea (2.3^{o}S) to the southern Great Barrier Reef (23.3^{o}S). Recently settled juveniles were collected and otolith microstructure was analysed to estimate pelagic larval duration (PLD), daily growth, and size at settlement. Latitudinal comparisons revealed a non-linear relationship between temperature and each of PLD and larval growth. Water temperature during larval development ranged between 25 and 30^{o}C among sites, with the warmest sites closest to the equator. PLDs declined with increasing temperature around 28-29^{o}C, above which it stabilized in *P. moluccensis* and increased in *H. melanurus*. Larval growth increased with increasing temperature to ~28-29^{o}C before stabilising in *P. moluccensis* or decreasing in *H. melanurus*. Size at settlement tended to be highest at mid-latitudes, but overall declined with increasing temperature above 28.5^{o}C in both species. These results indicate that the thermal optima for growth and development is reached or surpassed at low latitudes such that populations at these latitudes may be particularly vulnerable to global warming.

Introduction

There is an urgent need to better understand how organisms respond to changing temperatures as overwhelming evidence for rapid global warming accumulates (Bopp et al., 2013, IPCC, 2013). The latitudinal ranges of both marine and terrestrial species are shifting pole-ward in response to increasing temperatures (Chen et al., 2011, Hickling et al., 2006, Poloczanska et al., 2013). Tropical species are predicted to be among the most sensitive to global warming as they naturally experience some of the highest temperatures, with relatively little seasonal change (Tewksbury et al., 2008). Yet, many tropical species are distributed over a broad latitudinal range and their responses to the gradient in temperatures across their range can provide clues as to how they will respond to an exceedingly warmer climate (Deutsch et al., 2008, Dillon et al., 2010, Sunday et al., 2011). In particular, establishing the relationships between latitude and critical life history traits will assist in defining the thermal reaction norm (Angilletta, 2009) for these traits and indicate how close populations are to their thermal limits.

The persistence of populations in a changing climate will be constrained by their most vulnerable life stages. For many marine fish species it is the small pelagic larval stage that is the most vulnerable to environmental stressors such as temperature changes (Pankhurst and Munday, 2011, Pörtner and Peck, 2011). The larval stage is critically important for dispersal and connectivity among adult populations (Cowen and Sponaugle, 2009, Jones et al., 2009), and because mortality rates are extremely high during this life phase (Leis, 1991, Peck et al., 2012), small changes in larval growth, developmental, or survival rates could have large impacts on adult population dynamics. For example, Lo-Yat *et al.* (2011) documented a severe reduction in larval supply to the adult reef fish population in French Polynesia associated with abnormally warm waters during an El Niño event. Therefore, identifying temperature effects on fish larvae is essential to predict the impacts of climate warming on marine fish populations and fisheries.

In the tropics, reef-building corals appear to be close to their thermal maxima and episodes of mass coral bleaching in response to extreme temperatures are predicted to become more common as the oceans continue to warm (Hoegh-Guldberg, 1999, Hoegh-Guldberg et al., 2007). In contrast, we only have a preliminary understanding of how coral reef fishes will respond to temperature changes. To predict future responses to temperature change it is critical to define the thermal reaction norms - the shape of the relationship

between a particular trait and temperature. Thermal reaction norms typically exhibit a dome-shaped relationship, where rates increase with temperature up to an optimal level (thermal optimum) then decrease gradually with further increases in temperature (Pörtner and Farrell, 2008, Tewksbury et al., 2008). However, most research to date has shown that the thermal reaction norms for growth of larval fishes tend to be approximately linear until the upper lethal temperature is reached (Rombough, 1997, Sponaugle and Cowen, 1996). Meta-analyses (Houde, 1989, Laurel and Bradbury, 2006), experiments (McCormick and Molony, 1995, Green and Fisher, 2004) and field studies (Meekan et al., 2003, Sponaugle et al., 2006) have indicated that larval growth rates generally increase with increasing temperature. Additionally, within the temperature range currently experienced by reef fishes, warmer years generally appear to favour good recruitment events for a variety of coral reef fishes (Meekan et al., 2001, Wilson and Meekan, 2002, Cheal et al., 2007). Based on this knowledge, it has been predicted that climate warming could have positive outcomes for larval coral reef fishes because enhanced growth will lead to faster metamorphosis and less time in the dangerous pelagic environment (O'Connor et al., 2007).

Many species of coral reef fishes are distributed across a large latitudinal range from the equator to subtropical waters (Jones and McCormick, 2002). Indeed, many span temperature ranges that are actually greater than the projected increase in ocean temperature by the end of the century (Munday et al., 2008a). With a few notable exceptions (Booth and Parkinson, 2011, Takahashi et al., 2012), there is little information on intraspecific latitudinal variation in important early life-history traits of fishes across their distribution range. Few studies of larval fish development in relation to temperature have included populations at equatorial latitudes, which may be particularly sensitive to global warming (Sunday et al., 2011, Rummer et al., 2013). Indeed, recent research has indicated that in the warmest waters close to the equator (Takahashi et al., 2012), abnormally warm water during El Niño events (Lo-Yat et al., 2011) or elevated temperatures in line with ocean warming (Chapter 4) were associated with slower larval growth and reduced survival. However, no studies have examined the existing thermal responses of larval fishes over their entire latitudinal range, which provides a means to gauge the shape of the thermal reaction norms. Leis *et al.* (2013) emphasized the urgent need for more studies investigating latitudinal and temperature effects on larval life history traits to improve understanding of connectivity and dispersal patterns to inform fisheries management, conservation, and to predict climate-driven changes to marine systems.

Larval fishes exhibit three inter-related developmental traits that have a bearing on successful recruitment to the adult populations and will likely respond to changes in temperature: larval growth, pelagic larval duration (PLD) and size at settlement. Increased growth rates are likely to lead to a reduced PLD because there are strong correlations between growth rates and PLD, with fast-growing larvae often exhibiting shorter larval durations (Houde, 1989, McCormick and Molony, 1995, Sponaugle and Cowen, 1996, Green and Fisher, 2004). The relationship between growth rates and PLD will influence size at settlement (Sponaugle et al., 2006), which is important because a larger size at settlement is often associated with increased growth and survival after settlement (Sogard, 1997). The relationship between developmental temperature and size at settlement of coral reef fishes generally follows the 'developmental temperature–size rule' (Atkinson, 1994) with a larger settlement size at lower temperatures (McCormick and Molony, 1995, Radtke et al., 2001, Green and Fisher, 2004, Sponaugle and Grorud-Colvert, 2006). The relationship between larval life history traits and temperature has mostly been studied in laboratory experiments, or using temporal variation in temperature within a single location or region, and few studies have examined relationships among larval life history traits across the entire latitudinal temperature range of a species.

The aim of this study was to examine the effects of a temperature gradient on key early life-history traits of two coral reef fishes across their entire latitudinal range in the southern hemisphere. We sampled recently settled juveniles of the yellow damselfish, *Pomacentrus moluccensis* and the tail-spot wrasse, *Halichoeres melanurus* from 8 locations that spanned 21^{o} of latitude, from the southern Great Barrier Reef (GBR) to within 2^{o} of the equator in northern Papua New Guinea (PNG; Fig. 2.1). PLD, pre-settlement growth rates, and size at settlement were estimated by examining the microstructure of otoliths (ear bones), which exhibit daily rings and settlement marks. The variations in these traits were examined in relation to the average water temperatures experienced by the larvae during development to determine if the reaction norms were linear among latitudes, as suggested by existing experimental and observational studies, or if there is a shift in the shape of the reaction norm at higher temperatures close to the equator. A deflection in growth rates and size at settlement, or increase in PLD, could indicate that equatorial populations are living close to their thermal limits.

Materials and Methods

Study species and collection

The yellow damselfish, *Pomacentrus moluccensis* (Pomacentridae; Bleeker, 1853) and the tail-spot wrasse, *Halichoeres melanurus* (Labridae; Bleeker, 1851) are common in shallow coral reefs in the western Pacific (Randall et al., 1990, Green, 1998). *P. moluccensis* lay demersal eggs during a reproductive season from October to March on the Great Barrier Reef (GBR) (Milicich et al., 1992, Booth et al., 2000), and throughout the year in equatorial regions, such as northern Papua New Guinea (PNG; Srinivasan and Jones, 2006). *H. melanurus* is a broadcast spawner with a reproductive season ranging from late summer at Lizard Island in the northern GBR (Green, 1998), to year round at equatorial regions (Srinivasan and Jones, 2006). Previous estimates suggest that *P. moluccensis* and *H. melanurus* have free-swimming pelagic phases lasting 15-23 days (Wellington and Victor, 1989, Bay et al., 2006a) and 20-24 days (Victor, 1986), respectively.

To investigate variation in larval traits in relation to latitude and water temperature, newly settled individuals (*P. moluccensis* <25mm TL, *H. melanurus* <30 mm TL) were collected from the reef using hand nets and clove oil anaesthetic from eight and seven locations respectively, spanning a latitudinal gradient from near the equator in PNG to the southern GBR (21^0 latitude; 2.1; Table 2.1). Samples were collected from 2009-2013, when young recruits were available and collection was logistically possible. After capture, fish were euthanized on ice and TL was measured to the nearest 0.1 mm using callipers

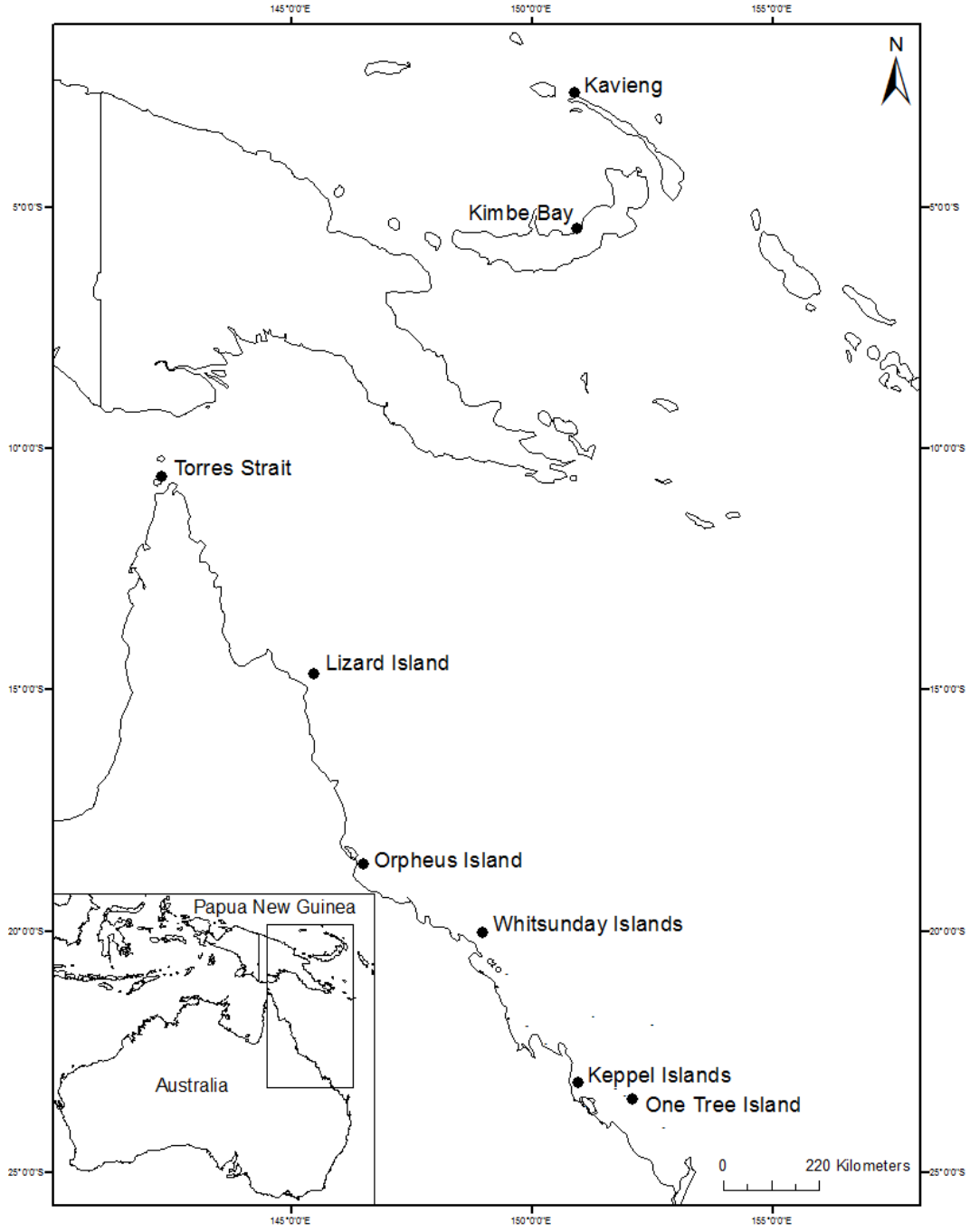

Figure 2.1. Map of eastern Australia and Papua New Guinea indicating study sites for *Pomacentrus moluccensis* and *Halichoeres melanurus*: One Tree Island (23°30'S, 152°04'S), Keppel Islands (23°10'S, 150°57'E), Whitsunday Islands (20.03'S, 148°57'S), Orpheus Island (18°37'S, 146°29'E), Lizard Island (14°40'S, 145°27'E), Torres Strait (10°34'S, 142°18'S), Kimbe Bay (5°25's, 150°56'E) and Kavieng (2°36'S, 150°52'E).

Otolith preparation and analysis

A pair of otoliths (sagittae) were extracted from each fish, cleaned in distilled water and stored dry. A cross-section through the nucleus of one otolith was prepared as described by Wilson & McCormick (1997). Each sectioned otolith was viewed through a compound microscope, using immersion oil at 200-400 x magnification and 1-6 digital images were taken, depending on the size of the otolith. If more than one image was taken, images were merged using Photoshop (v. 6). The otolith radius, the distance from the core to settlement mark, and the hatch ring radius were measured from the merged photograph using image analysis software (ImageJ v.1.45s, National Institutes of Health, USA).

The settlement mark of *P. moluccensis* was characterized by a sudden decline in daily ring increment widths (Type I transition mark; Wilson and McCormick, 1999). The settlement mark of *H. melanurus* was characterized by a wide band (Type II transition mark; Wilson and McCormick, 1999). For *H. melanurus* we interpreted the inner edge of the transition band to indicate settlement, corresponding to the time when settling larvae begin metamorphosis, and the outer edge of the band as corresponding with the completion of metamorphosis as described by Victor (1982).

The number of daily increments from the nucleus to the settlement mark was counted to estimate PLD for *P. moluccensis*. For *H. melanurus* we added 2 days to the count of pre-settlement increments to account for the time between fertilization and deposition of daily increments, as being typical of wrasse species (Victor, 1982, Cowen, 1991). The number of days post settlement was estimated by counting the number of rings after the settlement mark. Blind counts of daily increments were conducted twice. A third blind count was conducted when the error between the first two counts was more than 10%. When the closest two of the three counts differed by more than 10% the slide was rejected (N = 21). When slides were accepted, an average of the two counts was used for further analysis.

Daily rings have previously been validated for *P. moluccensis* (Brunton and Booth, 2003). We assumed otolith growth rings were daily for *H. melanurus* because (1) daily growth rings have been validated for the congenic species *H. miniatus* (Munday et al., 2009b) and *H. bivittatus* (Victor 1982), and (2) in a pilot study we established there was a strong linear correlation ($r^2 = 0.823$) between the number of days post-hatch and the TL of 190 juvenile *H. melanurus* (11.6-27.7 mm TL; I. M. McLeod, unpublished data).

Estimates of size at age based on back-calculation assume that otolith growth rate is linearly correlated with somatic growth in fish (Thorrold and Hare, 2002). A strong linear relationship ($r^2 = 0.80$) has been found in previous studies for *P. moluccensis* (Fowler, 1990). In a pilot study we established that there was a strong ($r^2 = 0.88$) linear relationship between the otolith length and TL of 190 *H. melanurus* juveniles (11.6-27.7 mm TL; I. M. McLeod, unpublished data). TL (mm) at settlement of newly settled juveniles were back-calculated using the biological intercept method developed by Campana and Jones (1992), using the equation:

$$L_S = L_C + (O_a - O_c) \times (L_c - L_o) \times (O_c - O_o)^{-1}$$

Where L_S = length at settlement, L_C = length at capture, L_O = length at age zero = 2.5 mm for *P. moluccensis* (Fisher, 2005) and 1.62 mm for *H. melanurus* (as estimated as the mean larval size at hatching of two congenic species, *H. poecilopterus* and *H. tenuispinnis* (Kimura et al., 1998), O_a = otolith radius at settlement, O_C = otolith radius at capture, O_O = otolith radius at hatch. When hatch rings were not clear enough to measure accurately (N = 53 among species) the mean hatch ring diameter for the same species and location was used. Average pre-settlement growth rates (mm day^{-1}) were calculated by dividing the estimated changes in TL of each fish by their individual PLD. The date of settlement of individual fish was estimated by subtracting post-settlement age (days) from the sample collection date, plus five days of metamorphosis for *H. melanurus.* The estimate of five days was used because (1) this is the mean number of days for metamorphosis to complete for the congeneric species *H. bivittatus*, and (2) it was possible to discern several (usually five) faint increments making up the transition band in some otoliths.

Water temperature data

Historical temperature data (2004-2012) for sites excluding Kaving were obtained from two sub-tidal temperature loggers at 0.5-10 m at each site except Kavieng (accessed from Australian Institute of Marine Science website; http://data.aims.gov.au/seatemp; downloaded 1 February 2013). Historical temperature data (2004-2012) for Kavieng were obtained from the Integrated Global Ocean Services System (IGOSS) satellite sea surface temperatures taken in 1° latitude/longitude grids near Kavieng (2.5°S; 150.5°E). The time period between 2004-2012 was chosen for the long-term averages because that time period had the most complete data available. Mean water temperature during the pelagic duration for each fish was calculated as the mean temperature for the site between the individual estimated hatching

date and settlement date. Because there were no temperature data available for when the fish were collected from Torres Strait were in their pelagic larval stage, the mean temperature for 2004-2012 for the Torres Strait during the relevant dates was used. Because of the differences in the timing of sampling, the average (mean ± s.e.m.) temperature experienced by larvae at locations did not always reflect the latitudinal gradient.

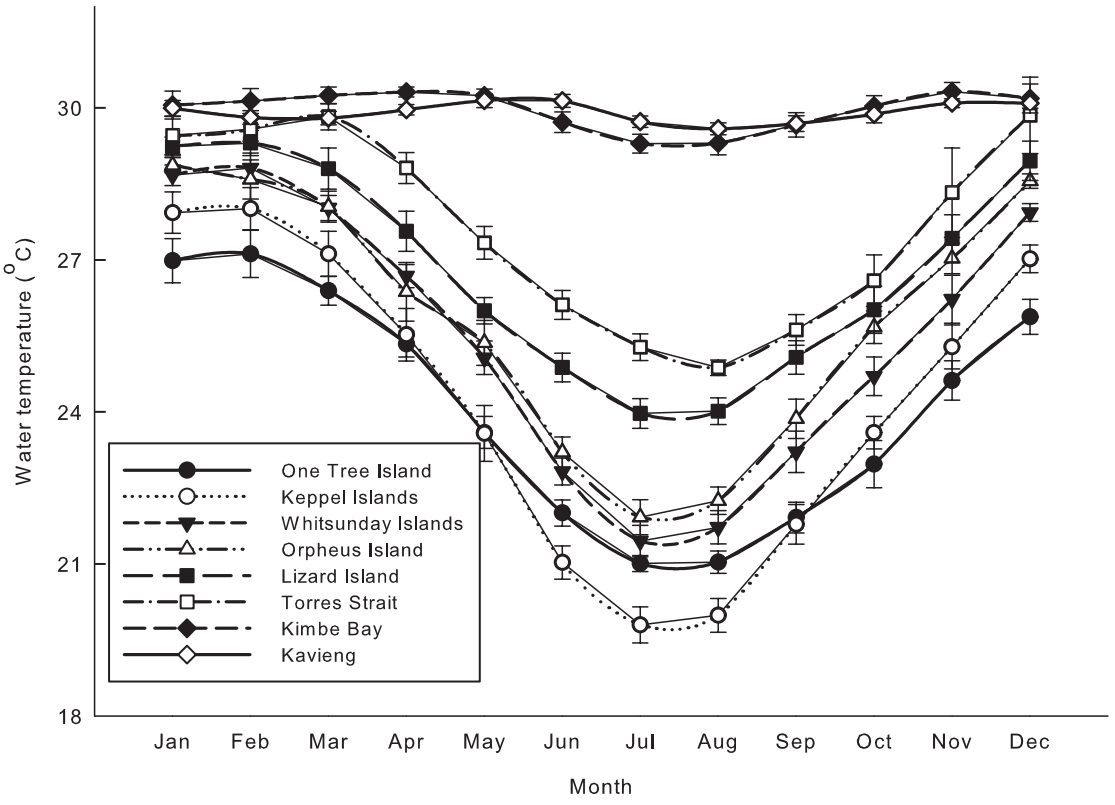

Figure 2.2. Monthly average water temperature (2004-2012) at study locations: One Tree Island, Keppel Island, Whitsunday Island, Orpheus Island, Lizard Island, Torres Strait, Kimbe Bay and Kavieng. Error bars denote s.e.m.

Statistical analysis

Analysis of variance (ANOVA) was used to compare mean PLD, pre-settlement growth rates, and settlement size among locations. Assumptions of homogeneity of variance and normality were examined using residual analysis. Tukey's tests were used to distinguish any

significant difference among means found by ANOVA. Variation in early life history traits in relation to temperature and among latitudes was analysed using quadratic regression analysis of the mean values of each trait at each latitude. Because we were most interested in the overall relationship between water temperature and early life history traits regression analysis, site was not included in the regression model. SPSS v 21 (IBM SPSS inc. 2012) was used for all statistical analysis.

Results

Pelagic larval duration

Pelagic larval duration (PLD) ranged from 16-24 days for *P. moluccensis* and 19-27 days for *H. melanurus*. Temperature explained 44.0% of the variation in *P. moluccensis* PLD and 33.9% of the variation in *H. melanurus* PLD among latitudes. *P. moluccensis* PLDs were longest at the Whitsunday Islands where larvae experienced the coolest developmental environment and shortest at Torres Strait (Figure 2.3a). *H. melanurus* PLDs were longest at Orpheus Island where larvae experienced the coolest developmental environment and shortest at Lizard Island (Figure 2.3b). Latitudinal variation in PLD was best described by a curvilinear relationship with water temperature (Fig. 2.3a-b). PLDs declined with increasing temperatures to 28-28.5^{o}C for both species where they then stabilised with increasing temperature for *P. moluccensis* and tended to increase with increasing temperature for *H. melanurus*. Despite high levels of variability within locations, PLDs were significantly different among latitudes (Tables 2.1-2.3).

Larval growth

Average daily growth ranged from 0.48-0.81 mm d^{-1} for *P. moluccensis* and 0.45-0.77 mm d^{-1} for *H. melanurus*. *P. moluccensis* larval growth was slowest at the Whitsunday Islands where larvae experienced the coolest developmental environment and fastest at Torres Strait (Figure 2.3a). *H. melanurus* larval growth was slowest at Orpheus Island where larvae experienced the coolest developmental environment and fastest at Lizard Island (Figure 2.3b). Temperature explained 28.9% and the variation in *P. moluccensis* growth and 39.6% of the variation in *H. melanurus* growth among latitudes. Latitudinal variation in larval growth was best described by a curvilinear relationship with water temperature (Fig. 2.3c-d). Larval growth increased with increasing temperatures to approximately 28^{o}C then decreased

at higher temperatures for both species. Despite high levels of variability within locations growth rates were significantly different among latitudes (Tables 2.1-2.3).

Settlement size in total length (TL)

Size at settlement ranged from 10.45-15.03 mm for *P. moluccensis* and 8.88-14.00 mm for *H. melanurus*. Temperature explained 15.2% and 24.5% of the variation in size at settlement of *P. moluccensis* and *H. melanurus*, respectively. Despite high levels of variability within locations size at settlement was significantly different among latitudes (Tables 2.1-2.3). Latitudinal variation in size at settlement was best described by a curvilinear relationship with water temperature (Fig. 2.3f-g). Size at settlement was highest at moderate temperatures and latitudes, and declined above 28.5^{o}C. The smallest *P. moluccensis* sizes at settlement were found in Kavieng and Lizard Island and the largest at the Keppel Islands (Table 2.1, Figure 2.3e). The smallest *H. melanurus* sizes at settlement were found in the PNG sites of Kavieng and Kimbe and the largest at the Keppel and Whitsunday Islands (Table 2.1, Figure 2.3).

Table 2.1. Mean (± s.e.m) water temperature that individual larval fish were exposed to, pelagic larval duration (PLD), growth rate and settlement size of sampled populations of *Pomacentrus moluccensis*. *Homologous subgroups are displayed by letters.

Location	Sampling month	*n*	Water temp. (^{o}C)	Latitude (^{o}S)	PLD (days)	Growth rate (mm day^{-1})	Settlement size (TL, mm)
Kavieng	August	39	29.8 ± 0.02	2.36	18.3 ± 0.20 (A)	0.666 ± 0.0091 (A)	12.11 ± 0.12 (A)
Kimbe Bay	October	31	28.4 ± 0.01	5.25	18.6 ± 0.15 (AB)	0.667 ± 0.0070 (A)	12.42 ± 0.12 (AB)
Torres Strait	December	28	28.2 ± 0.09	10.34	18.0 ± 0.20 (A)	0.686 ± 0.0079 (A)	12.34 ± 0.13 (ABC)
Lizard Island	January	37	29.7 ± 0.02	14.40	19.1 ± 0.17 (BC)	0.629 ± 0.0069 (B)	11.98 ± 0.09 (ABCD)
Orpheus Island	December	30	26.2 ± 0.06	18.37	20.4 ± 0.19 (DE)	0.620 ± 0.0079 (B)	12.59 ± 0.13 (CD)
Whitsunday Islands	November	43	25.6 ± 0.05	20.03	21.8 ± 0.19 (F)	0.582 ± 0.0068 (C)	12.67 ± 0.12 (CD)
Keppel Islands	January	29	28.0 ± 0.04	23.10	19.8 ± 0.22 (CD)	0.680 ± 0.0085 (A)	13.42 ± 0.12 (DE)
One Tree Island	February	24	27.0 ± 0.09	23.30	21.4 ± 0.27 (EF)	0.607 ± 0.0118 (BC)	12.95 ± 0.17 (E)

Table 2.2. Mean (± s.e.) water temperature that individual larval fish were exposed to, pelagic larval duration (PLD), growth rate, and settlement size of sampled populations of *Halichoeres melanurus*.

Location	Sampling month	*n*	Water temp. (^{o}C)	Latitude (^{o}S)	PLD (days)	Growth rate (mm day^{-1})	Settlement size (TL, mm)
Kavieng	August	39	29.8 ± 0.01	2.36	23.7 ± 0.18 A	0.546 ± 0.0054 A	10.55 ± 0.08 A
Kimbe Bay	October	26	28.9 ± 0.03	5.25	22.4 ± 0.18 B	0.586 ± 0.0076 B	10.66 ± 0.12 A
Torres Strait	December	32	28.5 ± 0.08	10.34	21.8 ± 0.24 B	0.591 ± 0.0091 B	10.74 ± 0.14 A
Lizard Island	February	21	28.7 ± 0.02	14.40	21.7 ± 0.28 B	0.631 ± 0.0166 C	11.00 ± 0.23 AB
Orpheus Island	December	31	26.1 ± 0.01	18.37	25.3 ± 0.20 C	0.525 ± 0.0072 A	11.49 ± 0.15 BC
Whitsunday Islands	March	19	28.0 ± 0.11	20.03	24.3 ± 0.29 A	0.590 ± 0.0105 B	11.76 ± 0.15 C
Keppel Islands	January	22	28.0 ± 0.02	23.10	23.3 ± 0.23 A	0.610 ± 0.0173 BC	11.78 ± 0.20 C

Table 2.3. The results of ANOVA, comparing the means of early life history traits of Pomacentrus moluccensis and Halichoeres melanurus among populations sampled at eight and seven locations respectively.

Life-history trait	d.f.	*F*	*P*
P. moluccensis			
PLD	7, 263	52.4	< 0.001
Growth rate	7, 263	13.0	< 0.001
TL at settlement	7, 263	22.7	< 0.001
H. melanurus			
PLD	6, 191	35.4	< 0.001
Growth rate	6, 191	15.2	< 0.001
TL at settlement	6, 191	14.6	< 0.001

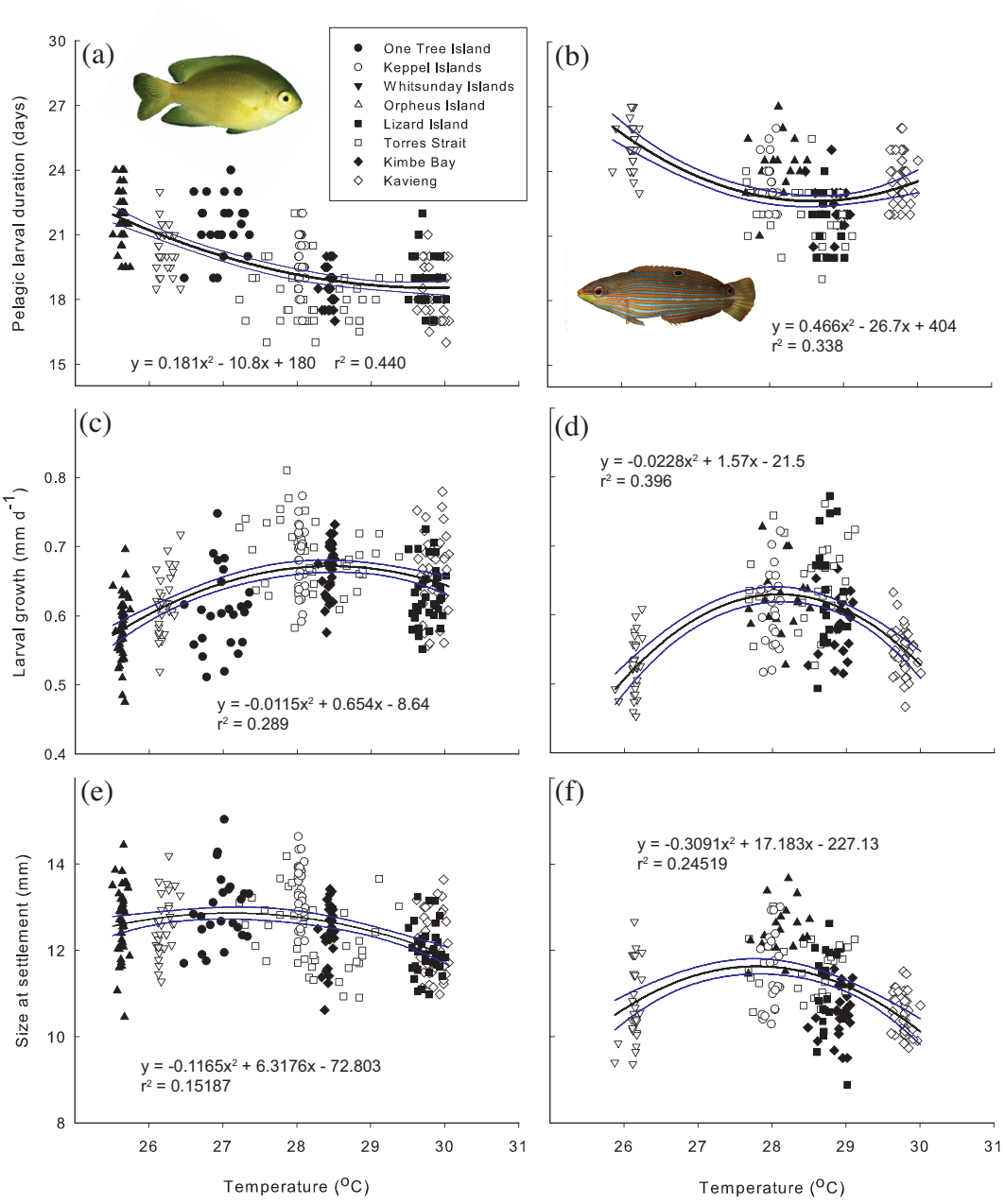

Figure 2.3. Pelagic larval duration (a-b), pre-settlement growth rates (c-d), and total length at settlement (e-f) of *Pomacentrus moluccensis* and *Halichoeres melanurus,* in relation to mean water temperature during larval development at 8 locations: One Tree Island (filled circles), Keppel Island (open circles), Whitsunday Islands (filled triangles), Orpheus Island (open triangles), Lizard Island (filled squared), Torres Strait (open squares), Kimbe Bay (filled diamonds), and Kavieng (open diamonds). The statistical outputs of the regressions, the regression lines and the 95% confidence intervals of the regression model are presented on the figure.

Discussion

The potential impacts of global warming across latitudes remain poorly understood. Here, we showed that the latitudinal patterns of pelagic larval duration (PLD), larval growth rates, and size at settlement of two coral reef fish species were significantly correlated with water temperature. Among latitudes the thermal reaction norms for PLD, larval growth rate, and settlement size were non-linear, with the major shift in the relationships between temperature and all three traits occurring at 28-29°C. The decrease in growth rate at the warm near-equatorial sites contrasts with predictions of previous studies that suggest linear increases in growth rate across the natural thermal range. Curvilinear responses to temperature are a general indication of thermal thresholds and optima for fitness-related traits (Pörtner and Farrell, 2008, Angilletta, 2009). The slower growth and development rates in warmer waters close to the equator suggest that those populations are already living at temperatures beyond the optimum for these traits.

The thermal optima for growth and development of the two coral fishes in this study appear to be around 28-29^{o}C, and that above this temperature, the thermal optima may be exceeded. The observed positive correlations between growth rate and temperature, and negative correlation between PLD and temperature up to 28.5^{o}C, are consistent with previous studies on larval coral reef fishes (McCormick and Molony, 1995, Wilson and Meekan, 2002, Meekan et al., 2003, Green and Fisher, 2004, Takahashi et al., 2012). Sponaugle *et al.* (2006) found a linear relationship between growth and temperature in of the coral reef wrasse, *Thalassoma bifasciatum*, but recruitment declined above 28.5^{o}C, suggesting a thermal threshold had been reached. Takahashi *et al.* (2012) found a linear increase in the larval growth of *P. moluccensis* with increasing temperature (25.4-29.3^{o}C) during the breeding season (November-February) at Lizard Island. However, developmental temperatures were higher for this species and location in the present study (29.7^{o}C), and *P. moluccensis* larval growth rates were lower than the maximum found by Takahashi *et al.* (2012), indicating that optimal temperatures for growth might have been surpassed at this location once temperatures increased above 29.3^{o}C.

The observed dome-shaped patterns between larval traits and temperature may be explained in part by local adaptation of northern populations. Although there is

ample evidence of within-species temperature-dependant physiological responses of early life history traits the actual effects in nature might be minimised through adaptation of key traits. The high levels of gene flow in coral reef fishes, including *P. moluccensis*, between latitudes on the GBR (Doherty et al., 1995, Bay et al., 2006b, Jones et al., 2010) might limit the potential for local adaptation. In contrast, strong genetic structure has been described between populations coral reef fish species in Kimbe Bay (and presumably Kavieng) and the GBR (Messmer et al., 2005, Jones et al., 2010), possibly as a result of landmasses serving as a barrier to larval dispersal. The isolation of these northern populations may have facilitated regional adaptation to the thermal environment, and consequently a different temperature dependency. However, in the present study there was little difference in the relationship between temperature and early life history traits among populations from the GBR and the northern PNG sites. Despite the potential for local adaptation there is evidence for a species-wide optimal temperature for growth and development.

Local environmental factors other than temperature may also have contributed to the observed differences in larval traits among locations. In larval fishes, the process of growth reflects the interaction of an individual's developmental physiology with a range of physical and biological factors (Bergenius et al., 2005) such that location-specific environmental factors apart from developmental temperature can have important effects. Prolonged larval development can be associated with poor environmental conditions, such as reduced food or sub-optimal temperatures, so that it takes longer for the larvae to reach a state where they are developmentally prepared for metamorphosis (McCormick and Molony, 1992, McCormick and Molony, 1995, Green and Fisher, 2004). Other physical factors apart from temperature or food supply can also have important effects on larval development. For example, wind speed and direction determine small-scale turbulence in the water column and may indirectly influence how larvae encounter and capture prey (Bergenius et al., 2005), turbidity levels can have important effects on feeding success (Utne-Palm, 2004, Peck et al., 2012), and excess turbidity can slow larval development (Wenger et al., 2013). Variation in solar radiation and along-shore wind, but not developmental temperature, accounted for the majority of the variability in larval growth of a coral reef surgeonfish, *Acanthurus chirugus,* at San Blas archipelago in Panama (Bergenius et al., 2005). There is temporal variation in environmental conditions at each location,

and temporal variation in larval development would be expected. Because each location was only sampled once or twice, this is a potential weakness of this study. Differences in these environmental factors undoubtedly influenced the rates of growth and development measured in the present study, yet despite this, there were clear temperature-related patterns in growth and development.

Increased water temperature is expected to accelerate physiological processes in larvae, provided temperatures do not exceed the thermal optima for this life stage (Munday et al., 2008a). Larval coral reef fishes can have exceptionally high rates of aerobic metabolism (Nilsson et al., 2007) during this period of rapid growth and ontogenetic development, which has been shown to increase with increasing temperatures for a larval coral reef damselfish (Chapter 4). Elevated routine metabolic rate and therefore energy use at higher temperatures may leave less energy available for growth, especially if food supplies are low or digestive capacity is limited, and this may have contributed to the lower growth rates in the warmest waters in the present study.

Larval body size at settlement was highly variable at all sites and weakly correlated with temperature. Most (75-85%) of the variability in size at settlement was due to site differences apart from developmental temperature. Size at settlement likely depends on complex, site-specific interactions between temperature, food supply, oceanographic processes, predation, and availability of suitable settlement habitat. Despite the weak correlation between temperature and size at settlement in the present study, the patterns generally followed the prediction of the 'developmental temperature - size rule' with smaller sizes being present at elevated temperatures closer to the equator. Size at settlement for both species tended to decline at the warmest temperatures. Sponaugle *et al.* (2006) suggested that the negative correlation between settlement size and water temperature, also found in previous studies (McCormick and Molony, 1995, Radtke et al., 2001, Green and Fisher, 2004), is related to the length of time in the pelagic environment with slow-growing larvae in cooler water settling at larger size because they spend more time in the planktonic stage. However, this hypothesis relies on there being adequate food supply to fuel this growth. Size at settlement may reflect trade-offs between spending longer in the dangerous pelagic environment or settling too small or with poor body condition with negative effects on post-settlement survival. The absence of differences in size at

settlement among the GBR sites as expected by temperature-size rule might reflect differing food levels or other site-specific environmental factors.

Settlement size plays an important role in influencing the mortality rates of recently settled juvenile fish, which is estimated to be as high as 25% on the first day after settlement (McCormick and Hoey, 2004). A larger size at settlement may offer some survival advantages (Sogard, 1997, Perez and Munch, 2010). However, larger size at settlement does not always lead to higher survival because the preferred settlement size to survive through size-selective mortality may be dependent on site-specific biological factors, including the composition and abundance of predatory species, and the types and availabilities of habitat (Levin, 1994, McCormick, 1994). For example, Grorud-Colvert & Sponaugle (2011) showed that the survival rates of a coral reef wrasse, *Thalassoma bifasciatum,* were higher at smaller settlement sizes. As ocean temperatures increase with global warming it could be expected that larval fish will metamorphose and settle at smaller sizes in the future but the consequences of this change are currently unknown.

The thermal reaction norms for important larval traits were broadly similar for the two study species from different families with different breeding modes (benthic egg laying vs broadcast spawner). The fact that they are present over such an extensive latitudinal range implies that both species are thermal generalists, and thus may be more resilient to thermal variation than thermal specialists that have smaller geographic ranges centred on the equator (Calosi et al., 2010). Further research into variation in the relationship between larval traits and temperature among species will be important for predicting the impacts of climate warming on fish communities and their associated fisheries.

Our findings have new implications for predicting the consequences of global change for marine species. They strongly suggest that there will be latitudinal variation in the impacts of ocean warming on larval coral reef fishes, with high latitude populations at particular risk. Previous conclusions about the relationships between key larval traits and temperature will need to be reconsidered. O'Conner et al. (2007) presented a unified model for the temperature dependence of larval development in marine animals based on a meta-analysis of published laboratory studies. Recent studies have used the results of this meta-analysis to predict that PLDs will be reduced and larval survival will increase in a warming ocean, consequently,

influencing connectivity and dispersal patterns (Munday et al., 2009a, e.g. Kendall et al., 2013, Underwood et al., 2013). However, we predict that coral reef fish larvae at high latitudes are likely to grow faster and settle earlier with small increases in ocean temperature (as predicted by existing models), but at lower latitudes the thermal optimum may be exceeded with global warming leading to slower growth and extended larval duration.

Climate change models predict an increase in global sea surface temperatures of 2.2-3.8^{o}C by the end of the 21st century under 'business as usual' scenarios of carbon emissions (Bopp et al., 2013). This may take many higher latitude populations up to their thermal limit, and those currently at their limit may be severely impacted by changes in larval development. Similar latitudinal variation in vulnerability patterns have been described for terrestrial ectotherms, suggesting that low latitude species are already living towards the upper edge of their thermal limits, and will be most vulnerable to a changing climate.

Chapter 1. Interannual variation in the larval development of a coral reef fish in response to temperature and associated environmental factors

This chapter was prepared for publication in the journal Coral Reefs.

Abstract

Climate change is predicted to increase ocean temperatures and influence weather patterns, which are likely to have a major impact on the larval development of marine organisms. Likely impacts of long-term changes are often inferred from spatial gradients in temperature or short-term temperature fluctuations. However, the effects of climate change may only be apparent on decadal time scales, and there are few studies that extend over such long periods. Here, I examine the influence of temperature and other associated environmental variables on key early life history traits of the coral reef damselfish, *Pomacentrus moluccensis*, based on ten cohorts of newly settled fish collected over 13 years from around Lizard Island (Great Barrier Reef, Australia). Pelagic larval duration (PLD), larval growth, and size at settlement were estimated through otolith microstructure analysis. Multiple regression techniques were used to measure the strength of the association between these traits and developmental temperature, rain, wind speed, and solar radiation. Temperature accounted for 19.2%, 20.2%, and 18.7% of the variability in PLD, growth, and settlement size respectively. PLDs generally declined and growth rates generally increased with increasing temperatures to ~28^{o}C, above which PLDs tended to increase and growth rates tended to decrease. This pattern mirrored exactly the existing latitudinal spatial patterns in these parameters for this species (Chapter 2). Size at settlement did not differ between ~25 to ~28^{o}C, but tended to decrease with increasing temperature above ~28^{o}C. Wind speed explained 6% of the remaining variability in PLD, with higher wind speed generally associated with longer PLDs. The highest ranked regression models explained 25% and 41% of the remaining variability in growth and size at settlement respectively, with increasing wind, rain and solar radiation associated with slower growth rates and smaller sizes at settlement. This study confirmed that ~28^{o}C is likely to be a thermal optima, and

significant warming above this level will detrimentally impact on this species. In addition, other environmental factors associated with climate change including rainfall, wind speed, and solar radiation can also have significant effects and should be considered in predictions of climate change effects on larval fish.

Introduction

The earth's climate is changing rapidly through anthropogenic carbon emissions (Solomon et al., 2007, IPCC, 2013). The primary direct consequences on marine systems are increasing ocean temperatures (Bindoff, 2007) and acidity (Doney et al., 2009). In addition, increasing temperatures are predicted to influence patterns of rainfall and sunshine, and the frequency and magnitude of storms (Meehl, 2007, Poloczanska et al., 2007, IPCC, 2013, Lough and Hobday, 2011). The projected impacts of climate change often relate to effects on spatial distributions, such as poleward shifts in distribution or a narrowing of geographic ranges (Burrows et al., 2014, Cheung et al., 2010, Poloczanska et al., 2013). However, such inferences assume that spatial variation in responses to gradients in temperature will be the same as long-term temporal responses to temperature change at any one site. Other inferences about effects of climate change have been based on short-term field observations of seasonal changes (Bergenius et al., 2005, e.g. Sponaugle and Grorud-Colvert, 2006, Takahashi et al., 2012), or experiments conducted over only a few months (e.g. Johansen and Jones, 2011). Clearly, the effects of climate change may only become apparent over decadal time-scales, with a multitude of factors contributing to normal inter-annual variation in environmental demographic processes. To date, there have been few observational studies on the effects of temperature on key demographic processes that have extended over such time periods.

The ability of populations to persist in a changing climate will be governed by how their most vulnerable life stages respond to the environment. For many marine fish species it is the small pelagic larval stage that is the most vulnerable to environmental stressors (Pankhurst and Munday, 2011, Pörtner and Peck, 2011). Mortality rates are extremely high during this life phase (Leis, 1991, Peck et al., 2012), and subtle differences in mortality rates can cause order-of-magnitude differences in recruitment from year to year (Houde, 1989, Peck et al., 2012). Larval

fishes exhibit three inter-related developmental traits that each has a bearing on the successful recruitment to adult populations: pelagic larval duration (PLD), larval growth, and size at settlement. Increased growth rates are likely to lead to a reduced PLD because there are strong correlations between growth rates and PLD, with fast-growing larvae often exhibiting shorter larval durations (Houde, 1989, Sponaugle and Cowen, 1996, Green and Fisher, 2004). Slower growing larvae can be subject to selective mortality (Anderson, 1988, Berkelmans et al., 2004, D'Alessandro et al., 2013). The relationship between growth rates and PLD will influence size at settlement (Sponaugle et al., 2006), which is important because a larger size at settlement is often associated with increased growth and survival after settlement (Sogard, 1997). Variations in these three life history traits can also influence larval mortality and dispersal, with important implications for patterns of recruitment to adult population and connectivity and dispersal among populations (O'Connor et al., 2007, Cowen and Sponaugle, 2009, Munday et al., 2009a).

In larval fishes, the processes of growth and development reflects the interaction of their developmental physiology with a range of physical and biological factors (Bergenius et al., 2005). Temperature is one of the most relevant abiotic factors influencing growth and development in fishes (Munday et al., 2008a). Thermal reactions norms, the shape of the relationship between a particular trait and temperature, typically exhibit a dome-shaped relationship for ectotherms, where rates increase with temperature up to an optimal level, then decrease with further increases in temperature (Pörtner and Peck, 2011). However, whether or not such models explain long-term temporal variation in response to temperature is unknown. A few studies have examined the effects of seasonal temperature variation (over several months to a few years) on the larval development of reef fishes (e.g. McCormick and Molony, 1995, Radtke et al., 2001, Sponaugle and Grorud-Colvert, 2006, Takahashi et al., 2012) and commonly found that increasing water temperature was associated with extended PLDs, slower larval growth, and smaller settlement sizes. These results have been supported by experimental studies (e.g. Green and Fisher, 2004, Gagliano et al., 2007b), and by meta-analysis of field and laboratory studies (Laurel and Bradbury, 2006, O'Connor et al., 2007). Some authors have extrapolated from the linear relationship between current-day temperatures and larval development to predict faster larval growth and shorter PLDs in a warmer future, with important

effects of larval survival and dispersal (Munday et al., 2009a, Kendall et al., 2013). These predications may be valid but have not been tested in the context of decadal observations at one site.

Ocean temperatures also vary with latitude, and a few studies have tested how within-species early life history traits vary with latitude. Bay et al. (2006b) found that PLD was a plastic trait in a number of GBR fish species across latitudes. In one of the broadest latitudinal studies to date, Chapter 2 showed that up to ~28-29^{o}C effects of developmental temperature were similar to those found in above temporal studies (faster growth rates and shorter PLDs with increasing temperature), but this trend slowed or reversed in the warmest waters at low latitudes. Whether the relationship is similar between temporal and spatial gradients is also largely unknown. Comparisons between long (decadal) temporal scales and wide spatial scales (Chapter 2) over a similar temperature range will let us know if 'snap shot' studies over spatial scales with differing temperatures ranges could inform us about temporal changes, with important implications for climate change predictions.

Other climate-related factors, such as wind speed, rainfall and solar radiation are also likely to influence early life-history traits, both directly, and indirectly through influencing larval food intake. Wind speed and rainfall determine small-scale turbulence in coastal waters and may therefore indirectly be an important influence on rates at which larval fish encounter and capture prey (Utne-Palm, 2004, Bergenius et al., 2005), which would likely impact growth rates and survival. For example, wind-induced turbulence explained 25% of the variability in larval supply of two species of tropical lizardfishes in Panama (Lemberget et al., 2009). Theoretically, with increasing turbulence, prey contact rates and prey capture success increase and decrease, respectively, for predators, and this yields a dome-shaped relationship between larval food consumption rates and turbulence (MacKenzie and Kiorboe, 1995). North Sea field studies have revealed that larval growth rates are affected by turbulence, illustrated by the dome-shaped effect of turbulence on the growth rates of larval Atlantic herring (Gallego et al., 1996). Solar radiation may either aid feeding by increasing the visibility of prey, or indirectly through its impact on secondary production. Light intensity may also affect vertical distribution, enabling larvae to feed over a range of depths where prey may be concentrated (Kouwenberg et al., 1999). However, solar radiation may directly reduce larval growth rates and survival

through the damaging effects of ultraviolet radiation on nucleic acids or through epidermal damage (Zagarese and Williamson, 2001) or reduce survival by making larvae more visible to their predators.

The aim of this study was to examine the long-term effects of a temperature gradient and associated environmental factors on key early life-history traits of the damselfish, *Pomacentrus moluccensis*, based on collections of juveniles collected over 13 years at Lizard island (GBR). Otolith microstructure analysis was undertaken to estimate PLD, average larval growth, and size at settlement. Regression analyses were applied to determine the influence of water temperature, and other associated environmental factors (wind speed, rainfall and solar radiation) on these life history traits.

Materials and Methods

Study site and species

This study was undertaken at the Lizard Island Group (14^{o}40' S, 145^{o}27' E), a mid-shelf reef, situated 30 km from the Australian mainland in the northern Great Barrier Reef. The species investigated was the yellow damselfish, *Pomacentrus moluccensis* (Pomacentridae; Bleeker, 1853), a common coral-associated species on shallow coral reefs in the western Pacific (Randall et al., 1990). *P. moluccensis* lay demersal eggs during a reproductive season from October to March on the Great Barrier Reef (Randall et al., 1990) that hatch into free-swimming larvae that develop in the pelagic environment for 15-23 days (Booth et al., 2000, Milicich et al., 1992).

Sample collection

A cross-section through the nucleus of one otolith was prepared as described by Wilson & McCormick (1997). Each sectioned otolith was viewed through a compound microscope, using immersion oil at 200-400x magnification and 1-6 digital images were taken, depending on the size of the otolith. If more than one image was taken, images were merged using Photoshop (v.6). The otolith radius, the distance from the core to settlement mark, and the hatch ring radius were measured from the merged photographs using image analysis software (ImageJ v.1.45s, National Institutes of Health, USA). The settlement mark of *P. moluccensis* was characterized

by a sudden decline in daily ring increment widths (Type I transition mark; Wilson and McCormick, 1999). The number of daily increments from the nucleus to the settlement mark was counted to estimate PLD. The number of days post-settlement was estimated by counting the number of rings after the settlement mark. Blind counts of daily increments were conducted twice. A third blind count was conducted when the error between the first two counts was more than 10%. When the closest two of the three counts differed by more than 10% the slide was rejected (N = 12). When slides were accepted, an average of the two counts was used for further analyses. Daily rings have previously been validated for *P. moluccensis* (Brunton and Booth, 2003). Estimates of size at age based on back-calculation assume that otolith growth rate is linearly correlated with somatic growth in fish (Thorrold and Hare, 2002). A strong linear relationship (r^2 = 0.80) has been found in previous studies for *P. moluccensis* (Fowler, 1990). TL (mm) at settlement of newly settled juveniles were back-calculated using the biological intercept method developed by Campana and Jones (1992), using the equation:

$$L_S = L_C + (O_a - O_c) \times (L_c - L_o) \times (O_c - O_o)^{-1}$$

where L_S = length at settlement, L_C = length at capture, L_O = length at age zero = 2.5 mm for *P. moluccensis* (Fisher, 2005). When hatch rings were not clear enough to measure accurately (N = 36) the mean hatch ring diameter for the same cohort was used. Average pre-settlement growth rate (mm day^{-1}) was calculated by dividing the estimated changes in TL of each fish between hatching and settling, by their individual PLD. The date of settlement of individual fish was estimated by subtracting post-settlement age (days) from the sample collection date.

Environmental data

Historical mean daily water temperature data from two sub-tidal temperature loggers at approximately 2 and 8 m depth were obtained from the Australian Institute for Marine Science (http:/data.aims.gov.au/aimsrtds/datatool.xhtml, downloaded 10 January 2014). Average daily rainfall and total daily solar radiation were obtained from Cape Flattery Weather Station, approximately 35 km from Lizard Island. (www.bom.gov.au/climate/data/stations, downloaded 10 January 2014). Wind speed from 2006-2011 was obtained from Agincourt Reef, 140 km south of Lizard Island (http:/data.aims.gov.au/aimsrtds/datatool.xhtml, downloaded 10 January 2014) and

wind speed from 1998 was obtained from an anemometer on Lizard Island (Lyle Vail, unpublished data).

Comparison of environmental and biological variables

The influence of the developmental temperature, average daily rainfall, average wind speed, and solar radiation on individual PLD, average daily growth and size at settlement was examined using multiple regressions. The assumptions of linearity, independence of errors, homoscedasticity, unusual points and normality were met. As strong correlations among independent variables can mask or exaggerate outcomes of multiple regression models, especially where the data are unbalanced, scatter plots and correlations were examined for all pairs of environmental variables. Developmental temperature was strongly correlated with all other environmental variables: for example, high rainfall levels only occurred when temperatures were also high. Because of strong *a priori* knowledge about the effects of temperature on larval development (Chapters 2 and 4) this variable was dealt with first in isolation. Since the relationship between each of the early life history traits and temperature showed curvature, each was modelled with a quadratic regression, and the residuals from these regressions were calculated. Stepwise regression (backwards and forwards) was then used to determine how much of the variability in the residuals could be explained by the remaining environmental variables rain, wind and solar irradiation, and their interactions. Where scatter plots between any of these variables and the residuals suggested curvature, a quadratic term for that variable was also included as an option for the stepwise regression. The Akaike information criterion was used to rank the possible models. All statistical analyses were run using TIBCO Spotfire S+ 8.2 for Windows.

Results

Intercohort variation in larval traits

Pelagic larval duration (PLD) ranged from 15-24 days. Mean PLD differed significantly among cohorts (Table 3.2), with the highest mean PLD (21.47 ± 0.30) observed in October 2009 and the lowest PLD (17.55 ± 0.34) observed in January 2008 (Table 3.1).

Average daily growth ranged from 0.44-0.79 mm d^{-1}. Growth rates differed significantly among cohorts (Table 3.2), with the highest average growth (0.69 ± 0.01 mm d^{-1}) in February 2010 and the lowest growth (0.50 ± 0.01 mm d^{-1}) in January 2011 (Table 3.1).

Total size at settlement (TL mm) ranged from 11.0 to 16.1 mm. Mean size at settlement differed significantly among cohorts (Table 3.2). The maximum average length at settlement (15.0 ± 0.13 mm) was observed in February 2010 and the lowest mean size at settlement (12.10 ± 0.11 mm) was recorded in January 2011 (Table 3.1). Size at settlement was slightly less variable than PLD and growth rates (CV: size at settlement = 7.6%, PLD = 8.3%, growth = 11.0%).

Relationships between larval traits and temperature

Average temperatures varied among years for the different cohorts, from a low of 25.53^{o}C (± 0.01) in October 2009 to a high of 29.6^{o}C (± 0.01) in January 2011 (Table 3.1). A quadratic regression model showed that temperature accounted for 19.2% of the variability in PLD (Fig. 3.1a, Table 3.3). The longest PLDs were associated with the lowest temperatures, PLDs generally declined with increasing temperatures to ~28^{o}C then tended to increase at higher temperatures. The highest ranked regression model explained 6% of the remaining variability (Table 3.4). Hence, temperature explained most of the variation among cohorts in this trait.

A quadratic regression model showed developmental temperature accounted for 20.2% of the variability in growth rates (Fig. 3.1b, Table 3.3). Growth rates generally increased with increasing temperatures to ~28^{o}C then tended to decline a higher temperatures. The highest ranked regression model showed that temperature explained 25% of the remaining variability (Table 3.4). Hence, temperature explained most of the variation among cohorts in this trait.

A quadratic regression model accounted for 18.7% of the variability in size at settlement (Fig. 3.1c, Table 3.3). Size at settlement was not significantly different at temperatures from ~25 to 28^{o}C, but tended to decrease with increasing temperature above ~28^{o}C. The highest ranked regression model explained 41% of the remaining variability, suggesting other factors were strongly contributing to variation in settlement size (Table 3.4).

Relationships between larval traits and other environmental factors

For PLDs, the highest ranked regression model explained only 6.0% of the remaining variability, with wind speed as the only significant influencing variable (Table 3.4). PLDs displayed a dome-shaped relationship with wind speed, with PLDs tending to increase with wind speed up to 20 km h^{-1} then decreasing at wind speeds higher than 25 km h^{-1}.

Rain, wind speed and solar radiation were identified as having a significant influence on growth, along with significant interactions between rain and solar radiation, and wind and solar radiation (Table 3.4). Increasing wind, rain and solar radiation all tended to decrease growth rates. There was an interaction between wind speed and solar radiation. At low wind speeds (20 km h^{-1}) increasing solar radiation tended to be associated with decreasing growth rates, but at higher wind speeds (>20 km h^{-1}) increasing solar radiation had no effect (Fig. 3.2). Additionally, when rainfall was low, increasing solar radiation was associated with a decrease in growth rate, but at high rainfall the effect of solar radiation was reduced (Fig. 3.3).

Rain, wind speed and solar radiation were identified as having a strong negative influence on size at settlement, along with significant interactions between rain and solar radiation, and wind and solar radiation (Table 3.4). Increasing wind, rain and solar radiation all tended to decrease size at settlement. The interaction between wind and solar radiation was driven by a decreasing effect of solar radiation at wind speeds higher than 20 km h^{-1} (Fig. 3.4a, 3.4b). The interaction between rain and solar radiation was driven by a decreasing effect of solar radiation at high rainfall (Fig. 3.4c, 3.4d).

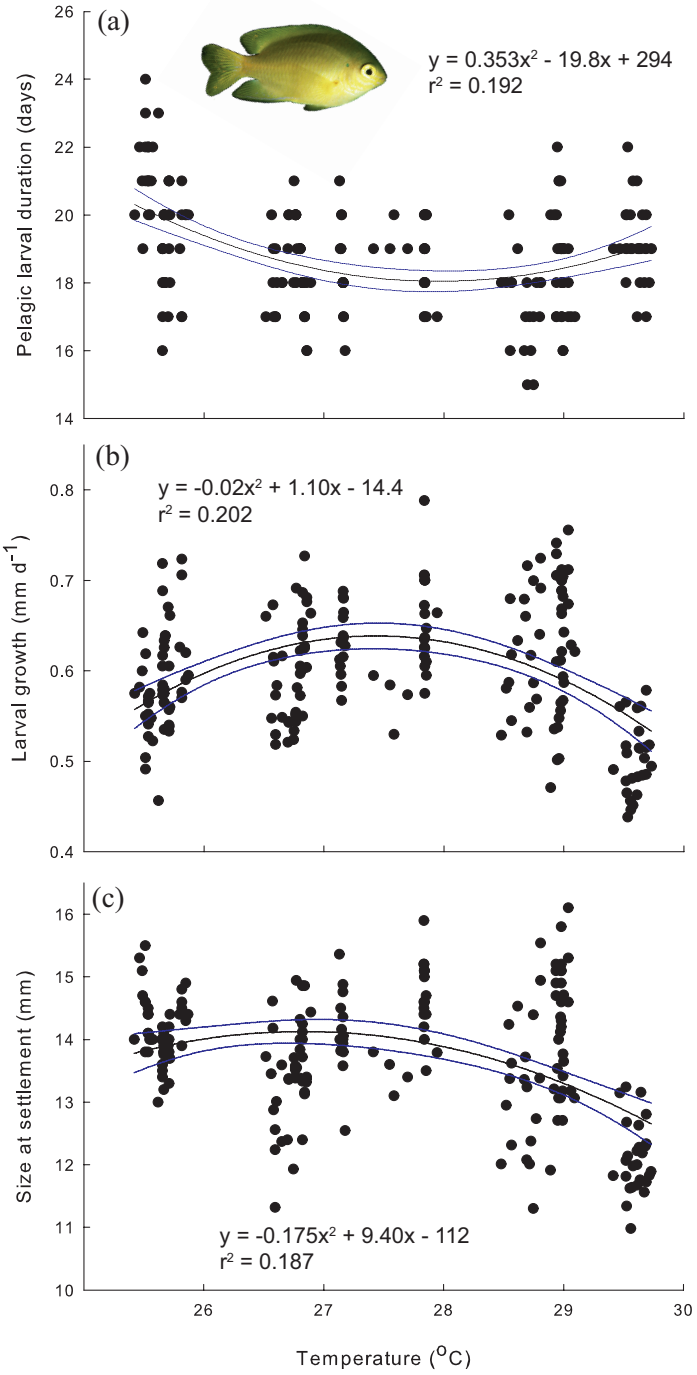

Figure 3.1. Pelagic larval duration (a), pre-settlement growth rates (b), and total length at settlement (c) of *Pomacentrus moluccensis*, in relation to mean water temperature during development at Lizard Island. The statistical outputs of the regressions, the regression lines and 95% confidence intervals of the regression models are presented on the figure.

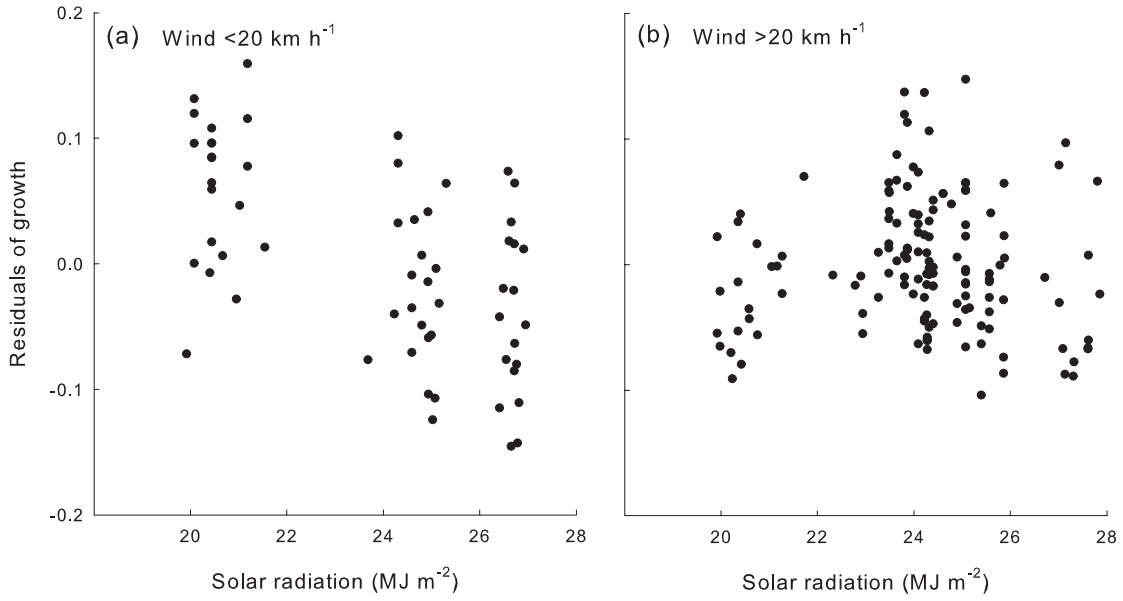

Figure 3.2. Partial regression plot of the residuals of the temperature and growth analysis of *Pomacentrus moluccensis* in relation to solar radiation at (a) low (<20 km h^{-1}) and (b) high wind speeds (>20 km h^{-1}).

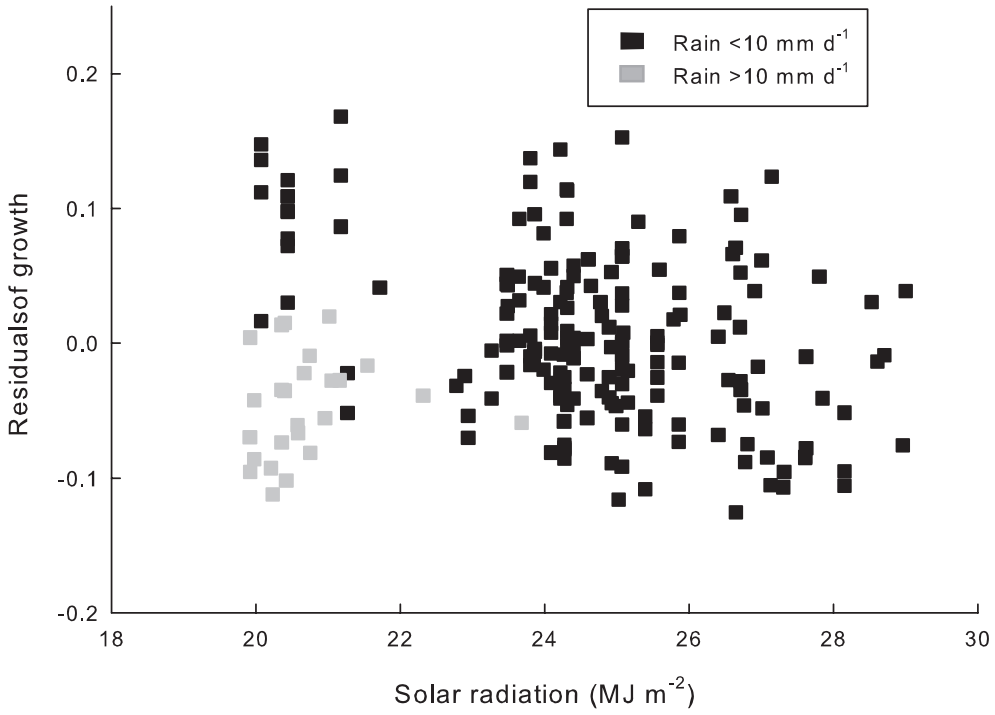

Figure 3.3. Partial regression plot of the residuals of the temperature and growth analysis of *Pomacentrus moluccensis* in relation to solar radiation at low (<10 mm d^{-1}) and high (>10 mm d^{-1}) average daily rainfall.

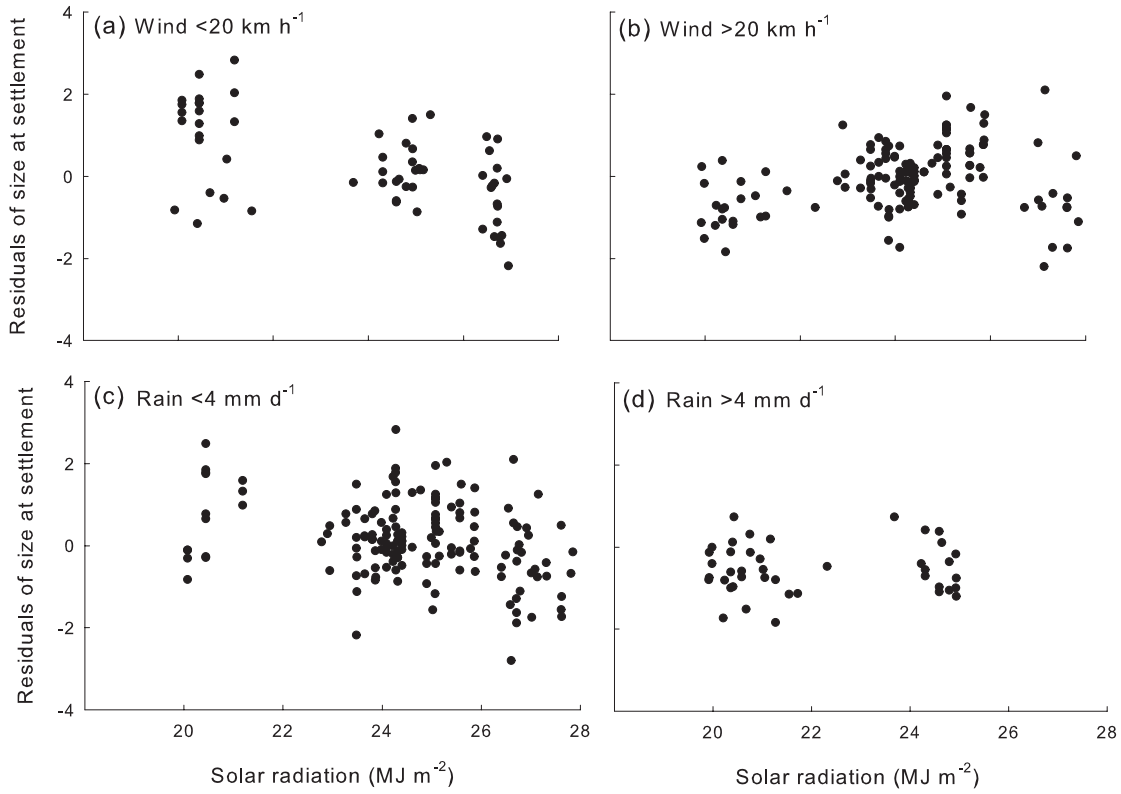

Figure 3.4. Partial regression plots of the residuals of the temperature and size at settlement analysis of *Pomacentrus moluccensis* in relation to solar radiation at (a) low wind speeds (<20 km h^{-1}), (b) high wind speeds (>20 mm d^{-1}), (c) low average daily rainfall (<4 mm d^{-1}) and (d) high average daily rainfall (>4 mm d^{-1}).

Table 3.1. Mean (± s.e.m.) pelagic larval durations, growth rates and settlement sizes of each cohort of *Pomacentrus moluccensis* from Lizard Island, and mean water temperature, solar radiation, wind speed and rainfall during their individual larval development.

Sampling month	N	PLD (days)	Growth rate (mm day^{-1})	Settlement size (mm TL)	Water temp. (^{o}C)	Solar radiation (MJ m^{-2})	Wind (km h^{-1})	Rainfall (mm d^{-1})
Dec 1998	16	18.75 (±0.45)	0.60 (±0.016)	13.59 (±0.17)	28.98 (±0.01)	24.70 (±0.08)	13.52 (±0.09)	4.51 (±0.11)
Dec 2006	21	18.43 (±0.28)	0.58 (±0.011)	13.17 (±0.19)	26.69 (±0.03)	27.58 (±0.24)	24.09 (±0.35)	0.26 (±0.06)
Jan 2008	20	17.55 (±0.34)	0.61 (±0.016)	13.12 (±0.24)	28.63 (±0.03)	26.55 (±0.16)	18.37 (±0.15)	1.24 (±0.60)
Oct 2009	17	21.47 (± 0.30)	0.55 (±0.011)	14.35 (±0.15)	25.53 (±0.01)	25.55 (±0.08)	20.35 (±0.30)	0.01 (±0.0001)
Nov 2009	33	19.09 (±0.24)	0.61 (±0.009)	13.99 (±0.07)	25.71 (±0.01)	24.21 (±0.07)	28.69 (±0.27)	0.92 (±0.03)
Dec 2009	22	18.82 (±0.22)	0.64 (±0.013)	14.50 (±0.15)	27.79 (±0.03)	25.10 (±0.03)	23.60 (±0.08)	1.70 (±0.03)
Feb 2010	15	18.13 (±0.26)	0.69 (±0.010)	15.01 (±0.13)	28.98 (±0.01)	20.49 (±0.10)	18.16 (±0.13)	3.53 (±0.02)
Oct 2010	14	18.57 (±0.31)	0.63 (±0.010)	14.21 (±0.13)	27.15 (±0.003)	23.28 (±0.07)	27.00 (±0.09)	0.78 (±0.06)
Jan 2011	27	19.15 (± 0.22)	0.50 (±0.007)	12.10 (±0.11)	29.60 (±0.02)	20.67 (±0.12)	20.73 (±0.24)	12.41 (±0.33)
Oct 2011	22	18.18 (± 0.26)	0.62 (±0.017)	13.74 (±0.13)	26.87 (±0.05)	24.33 (±0.21)	27.42 (±0.37)	3.37 (±0.13)

Table 3.2. The results of ANOVA, comparing the means of early life-history traits of *Pomacentrus moluccensis* among cohorts from Lizard Island.

Life-history trait	d.f.	*F*	*P*
PLD	9	11.56	<0.001
Growth rate	9	19.60	<0.001
TL at settlement	9	33.90	<0.001

Table 3.3. Summary of results of curvilinear regression analyses that compared water temperature (^{o}C) with pelagic larval duration (days), average larval growth (mm d^{-1}), and total length at settlement (mm) of *Pomacentrus moluccensis*. See text for details of analyses techniques. R^2 = Coefficient of determination, *Adj* (R^2) = adjusted R square.

Dependent variable	*d.f.*	*s.e.*	R^2	*Adj* (R^2)	F	*P*	Independent variables	Regression coefficient	t	*Sig.*
PLD	2, 204	1.41	0.19	0.18	24.42	<0.001	Temperature	-19.8	6.14	<0.001
							Temperature^2	0.35	-5.66	<0.001
Growth	2, 204	0.06	0.20	0.19	25.8	<0.001	Temperature	1.10	7.02	<0.001
							Temperature^2	-0.020	-7.05	<0.001
Size at settlement	2, 204	0.94	0.19	0.18	23.4	<0.001	Temperature	9.40	4.04	<0.001
							Temperature^2	-0.18	-4.15	<0.001

Table 3.4. Summary of results of multiple regression analyses of temperature residuals on rainfall (mm d^{-1}), solar radiation (MJ m^{-2}), wind speed (km h^{-1}) with pelagic larval duration (days), average larval growth (mm d^{-1}), and total length at settlement (mm) of *Pomacentrus moluccensis*. See text for details of analyses techniques. R^2 = Coefficient of determination, *Adj* (R^2) = adjusted R square, DV = Dependent variable, IV = Independent variable.

DV	*d.f.*	*s.e.*	R^2	*Adj* (R^2)	F	*P*	IV	Reg. coef.	t	*Sig.*
PLD	2, 204	1.37	0.06	0.05	6.84	<0.001	Wind	0.43	2.44	0.02
							$Wind^2$	-0.011	-2.74	<0.001
Growth	5, 201	0.06	0.25	0.24	13.9	<0.001	Rain	-0.049	-3.63	<0.001
							Wind	-0.041	-2.79	<0.006
							Solar	-0.054	-4.05	<0.001
							Rain: Solar	0.0018	2.87	<0.005
							Wind: Solar	0.0016	2.69	<0.008
Size at settlement	7, 199	0.73	0.41	0.39	20.0	<0.001	Rain	-2.28	-4.07	<0.001
							Wind	-1.11	-4.31	<0.001
							Solar	-1.37	-5.49	<0.001
							$Rain^2$	0.022	2.76	0.006
							Rain: Solar	0.073	3.97	<0.001
							Wind: Solar	0.042	4.11	<0.001

Discussion

This study strongly indicates a signature of temperature variation and other environmental parameters on variation in important larval development traits over a decadal time scale. Pelagic larval durations, larval growth and size at settlement all varied significantly among the cohorts sampled. Although temperature changes did not indicate a gradual warming impacting on successive cohorts, strong fluctuations in temperature among years supported the model that PLDs reach a minimum, and larval growth a maximum at 28^{o}C. Patterns in the response to temperature mirrored exactly the current large-scale, latitudinal variation in these traits (Chapter 2). Hence, for many places, and for some places in some years, larval fishes are already exceeding the thermal optima for larval development. In addition, other environmental factors that may be associated with changes in water temperature, including wind, rainfall and solar radiation are also potentially increasing the stress on larval development.

Effects of temperature

Temperature accounted for 19.2%, and 20.2% of the variability in PLD and larval growth respectively, exhibited by 10 cohorts of *P. moluccensis* over 13 years at Lizard Island. PLDs generally declined and growth rates generally increased with increasing temperatures to ~28^{o}C, above which PLD tended to increase and growth rates tending to decrease. The observed positive correlations between growth rate and temperature, and negative correlation between PLD and temperature up to ~28^{o}C, are consistent with previous studies on larval coral reef fishes (Green and Fisher, 2004, McCormick and Molony, 1995, Meekan et al., 2003, Takahashi et al., 2012, Wilson and Meekan, 2002) and with the results of Chapter 2 of this thesis. The results of the large-scale spatial study (Chapter 2) are highly consistent with this long-term study, hence single 'snap shots' based on spatial patterns may be able to be used to make valid predictions about temporal change and thus prediction about a warmer future.

The results of this decadal study are also consistent with shorter-term studies, which have looked at developmental responses to seasonal change. Takahashi *et al.* (2012) found a linear increase in the larval growth of *P. moluccensis* with increasing temperature during one breeding season at Lizard Island. However, when the

relationship between temperature and growth was investigated over a longer time series, and greater temperature range, in the present study, a dome-shaped relationship between developmental temperature and growth rates was identified. Dome-shaped relationships are generally an indication of thermal optima. Thus the thermal optimum for growth and development of *P. moluccensis* at Lizard Island appears to be ~28^{o}C, and above this temperature, the thermal optimum may be exceeded.

Size at settlement did not differ between ~25 to ~28^{o}C, but tended to decrease with increasing temperature above ~28^{o}C. This relationship is consistent with that found for the two species investigated in Chapter 2 of this thesis. Size at settlement likely depends on complex, site-specific interactions between temperature, food supply, oceanographic processes, predation, and availability of suitable settlement habitat. Despite the weak correlation between temperature and size at settlement in the present study, the patterns generally followed the prediction of the 'developmental temperature-size rule' with smaller sizes being present at the highest temperatures. Sponaugle *et al*. (2006) suggested that the negative correlation between settlement size and water temperature, also found in previous studies (Green and Fisher, 2004, McCormick and Molony, 1995, Radtke et al., 2001) were related to the length of time in the pelagic environment with slow-growing larvae in cooler water settling at larger size because they spend more time in the planktonic stage. However, this hypothesis relies on there being adequate food supply to fuel this growth. Size at settlement may reflect trade-offs between spending longer in the dangerous pelagic environment or settling too small or with poor body condition with negative effects on post-settlement survival.

Effects of other environmental factors

The overall weak correlation between developmental temperatures and early life history traits emphasises the potential importance of other factors apart from developmental temperature in larval development. Our results suggest that increasing solar radiation may have negative effects on larval growth, and be associated with a smaller size at settlement. However, the effects of increasing solar radiation seemed to be less at high wind speeds or rainfall. These results are roughly consistent with those of Bergenius et al. (2005), who found that larval growth rates of a tropical surgeonfish were negatively associated with increasing solar radiation in the dry season but not in

the wet season in Panama. Traditionally, it was thought that sunlight has a positive, indirect effect on larval growth by warming surface waters and increasing food production (Cushing, 1995, Heath, 1992). However, recent research suggests that the early life-history stages of fish and invertebrates are particularly sensitive to the UV radiation present in natural sunlight (reviewed in Häder et al., 2011). Adult coral reef fishes can adapt to UV stress by incorporating UV-absorbing substances, which they acquire through their diet, into their eyes and epithelial slime (Chatzifotis et al., 2005). However early life history stages (embryos in eggs and early larvae) are known to be highly sensitive to UV damage because they still lack the photo-protective pigments and/or extensions of the integument (Zagarese and Williamson, 2001). Moreover, Boeuf and Le Bail (1999) suggest that beyond an upper intensity, solar radiation may decrease growth and reduced survival because of its impacts on visual development, with in turn would effect feeding activities. Also, sunlight could make larvae more susceptible to predators and this may also force fish deeper in the water column, away for the depth of optimal light intensities for feeding and growth (Fortier and Harris, 1989). It is possible that excessive UV radiation at high levels of solar radiation had negative effects on larval growth in the present study, but these effects were mediated when high rainfall or windy conditions reduced UV penetration of surface waters.

Increasing rainfall was associated with a reduction in larval growth rates and size at settlement. Reduced growth rates with increasing rainfall were also found for larval surgeonfish *Acanthurus chirurgus* by Bergenius et al. (2005), who hypothesised that heavy rains in the wet season may decrease salinity in surface layers and this may be detrimental to young larvae due to their small surface to volume ratio and lack of protective scaling against changes in the osmotic environment. Heavy rains are associated with increased run-off from rivers and nutrients in inshore environments (D'Croz et al., 1999), potentially resulting in better feeding conditions for larvae. However, turbid upper layer of fresh water may also reduce light penetration and thus feeding by young fish (Gallego et al. 1996; Dower et al. 1997). It is unlikely that rain-induced turbidity was a factor in the present study because Lizard Island is located on the mid-shelf and is subject to very little runoff.

Increasing wind-speed was correlated with extended PLD, slower larval growth and a reduced size at settlement. Wind also interacted with the relationship between larval growth and levels of solar radiation. At low average wind speeds (<20km h^{-1})

increasing solar radiation was associated a reduction of growth rates and size at settlement, however at higher wind speeds, there appears to be no influence of levels of solar radiation on growth rates and size at settlement. It has been hypothesised that winds may affect growth in larval fish due to their influence on small-scale turbulence. At optimum levels, turbulence is thought to increase the probability of encounter between larval fish predators and their prey, thus increasing growth rates and survivorship (Bergenius et al., 2005). The results of the present study indicate that higher winds generate turbulence at above optimum levels for *P. moluccensis*.

The majority of the variation in all three larval performance variables was not explained by the environmental factors tested. Thus other biological factors must have an important influence. Gagliano and McCormick (2007) showed that an increased maternal food supply led to an increase in the energy provisioning of the eggs of a coral reef damselfish at Lizard Island. Thus, maternal food supply would likely influence larval growth and development. Food supply is also an important factor influencing larval development and could only be loosely estimated with the available data. Further studies into other biological and environmental influences on larval development would be required for a more complete understanding of the drivers of variation in larval development.

Conclusions

The Great Barrier Reef has been identified as a climate change 'hot spot' (Poloczanska et al., 2007), and there is increasing evidence of long-term habitat change extending over several decades (De'ath et al., 2012) However, while there are many long-term studies underway that have focussed on patterns of coral cover and fish abundance (e.g. Cheal et al., 2008, Pratchett et al., 2008) decadal changes in developmental responses to temperature are only beginning to receive attention. The close match between the current latitudinal responses to temperature (Chapter 2) and the thermal maximum described here for Lizard Island over a 13-year period strengthens the hypothesis that the 28^{o}C point is pivotal for these species. Larval development is already being impaired at locations that now regularly exceed these temperatures.

This study also suggests that along with developmental temperature, other environmental variables including solar radiation, rainfall, and wind may be used to predict the growth of *Pomacentrus moluccensis* larvae. To date, most studies of the

effects of the environment on life history characteristics of marine fishes have examined only one environmental variable. We suggest predictions of climate change impacts on larval coral reef fishes may need to incorporate thermal optima predictions and climate change related environmental variables supplemental to developmental temperature to predict climate change effects of larval fishes.

Chapter 4. Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability

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Abstract

Climate change models predict that tropical ocean temperatures will increase by up to 4°C this century and affect plankton communities that are food for marine fish larvae. Both temperature and food supply can influence development time, growth, and metabolism of marine fishes, particularly during larval stages. However, little is known of the relative importance and potential interacting effects of ocean warming and changes to food supply on the performance of larval fishes. I tested this for larvae of the coral reef anemonefish, *Amphiprion percula*, in an orthogonal experiment comprising three temperatures and three feeding schedules. Temperatures were chosen to represent current-day summer averages (29.2°C) and end-of-century climate change projections of +1.5°C (30.7°C) and +3°C (32.2°C), and feeding schedules were chosen to represent a reduction in access to food (fed daily, every second day, or every third day). Overall, larvae took longer to settle at higher temperatures and less frequent feeding, with a highly significant interaction between these factors. Time to metamorphosis was fastest at low temperature and high food availability (10.5 ± 0.2 days) and slowest at high temperature and low food availability (15.6 ± 0.5 days; i.e. 50% slower). Fish from the lower feeding regimes had a lower body condition and decreased survivorship to metamorphosis. Routine oxygen consumption rates ($\dot{M}O_{2routine}$) were highest for fish raised at 32.2°C and fed every third day (1620 ± 107 mg kg^{-1} h^{-1}) and lowest for fish raised at 29.2°C and fed daily (1220 ± 101 mg kg^{-1} h^{-1}; i.e. 35% lower). Elevated $\dot{M}O_{2routine}$, and therefore greater energy use at higher temperatures may leave less energy available for growth and development, resulting in the longer time to metamorphosis. Overall, these results suggest that larval fishes will

be severely impacted by climate-change scenarios that predict both elevated temperatures and reduced food supply.

Introduction

Climate change models predict that tropical sea surface temperatures will increase by up to 4°C this century (Bopp et al., 2013). Tropical ectothermic species may be especially vulnerable to rising temperatures because many have a narrower thermal tolerance range than equivalent temperate species (Pörtner and Farrell, 2008, Sunday et al., 2011), and tend to live closer to their thermal optimum. Therefore, even relatively small increases in temperature could lead to declines in individual performance (Stillman, 2003, Tewksbury et al., 2008). Furthermore, many biotic effects of warming are mediated through metabolic rate, which is a fundamental measure of physiological activity. In ectotherms metabolic rate increases exponentially rather than linearly with temperature in ectotherms (Gillooly et al., 2001, Dillon et al., 2010). Consequently, an increase in metabolic rate caused by warming would require organisms to increase food intake to gain enough energy to cover basic functions including growth. However, food is rarely unlimited in the natural environment, and therefore, understanding the interaction between warming and food supply is important for predicting climate change impacts on ectotherms in the tropics.

Coral reef ecosystems may be especially sensitive to ocean warming because coral bleaching and subsequent mortality is often linked to high temperatures (Glynn, 1991, Hoegh-Guldberg, 1999). Degradation of coral reef habitat has negative consequences for many coral-associated organisms, such as coral reef fish (Wilson et al., 2006, Munday et al., 2008a, Pratchett et al., 2008). Direct physiological consequences of climate change on reef fishes will further compound these effects. Recent laboratory experiments have shown that higher summer temperatures, in the range predicted by the end of this century, (i.e. up to 3^{o}C higher than current summer averages), lead to reductions in aerobic scope (Nilsson et al., 2007), critical swimming speeds (Johansen and Jones, 2011), somatic growth (Munday et al., 2008b), and reproductive output (Donelson et al., 2010) of adult reef fishes.

Sensitivity to temperature may be magnified during early life history stages (e.g. eggs and larvae), which are also the key stages for mortality, dispersal, and connectivity (Houde, 1989, Wood and McDonald, 1997). Larval coral reef fishes

typically exhibit increased growth rates and shorter pelagic larval durations (PLDs) with increasing temperatures within their natural temperature range (McCormick and Molony, 1995, Wilson and Meekan, 2002, Green and Fisher, 2004, Sponaugle et al., 2006, Takahashi et al., 2012). However, little is known about the effects that climate change associated elevated temperatures, above the normal range, will have on these traits (Pankhurst and Munday, 2011). While some studies have predicted that increased temperatures will lead to shorter PLDs, increased growth rates, and higher survivorship (e.g. O'Connor et al., 2007) others have suggested that more variable survival is likely due to higher starvation risk associated with higher metabolism (Houde, 1989, Pankhurst and Munday, 2011, Munday et al., 2012) and that connectivity patterns may be altered due to shorter PLDs (Munday et al., 2009a).

The impact of climate change on the availability of food for larval coral reef fishes is uncertain. Elevated ocean temperatures are predicted to affect the structure of plankton communities that are a food source for larvae (Hays et al., 2005, Richardson, 2008, Barton et al., 2013). Changes to plankton communities will vary, but in many locations these communities may become less productive because higher temperatures favour longer, less productive planktonic food chains (McKinnon et al., 2007, Morán et al., 2010). Greater thermal stratification of the water column will reduce nutrient enrichment of the surface layers that are the most important for planktonic productivity (Poloczanska et al., 2007, Brander, 2010, Doney et al., 2012). Steinacher, et al. (2010) reported results from four climate change models predicting a 2-20% reduction in global marine primary production by 2100, with declines in mid to low latitudes due to reduced nutrient input into the eutrophic zone. Future changes in plankton communities will be superimposed on a resource that is inherently variable on a broad range of spatial and temporal scales (Sorokin and Sorokin, 2009). As a result of likely effects of increasing ocean temperature on the productivity of plankton communities, and their inherent variability, it is important to understand the consequences for planktivorous organisms.

The effects of food supply on larval fishes are well known, with both faster growth and shorter PLDs observed with increasing food supply (McCormick and Molony, 1992, Green and McCormick, 1999, Meekan et al., 2003, Sponaugle et al., 2009). However, in relation to climate change, and the potential change in plankton productivity, it is the interaction between food supply and temperature that may hold

the most relevance. While the growth rate of fishes with an unlimited food supply generally increases with increasing temperature, the effects of temperature may be detrimental in food-poor environments. Higher metabolic rates with increasing temperature may lead to faster growth, but only if the availability of food is sufficient to fuel the higher metabolic demands Food is rarely unlimited in the natural environment, and fish on a fixed ration may not grow more slowly with increasing temperature due to increasing energetic demands.

In this study, I experimentally investigated the independent and interacting effects of elevated water temperature and varying food availability on the larval development and performance of the coral reef damselfish *Amphiprion percula.* I reared larvae at three temperatures and three food supplies in a full orthogonal design to examine effects on larval duration, larval growth, body condition, mortality, and metabolic rate. I tested the following specific hypotheses: (1) elevated temperatures would decrease PLD and increase average daily growth, length at metamorphosis, body condition at metamorphosis and survivorship to metamorphosis, (2) decreased food supply would increase PLD and decrease daily growth, length at metamorphosis, body condition at metamorphosis, and survivorship to metamorphosis and (3) increased temperature would increase metabolic rate, leading to a lower performance on the same level of food availability.

Materials and methods

Study species and brood stock maintenance

Five breeding pairs of *Amphiprion percula* were captured from reefs in the Cairns region of northern Great Barrier Reef (GBR) and transported to the Marine and Aquaculture Research Facilities Unit at James Cook University, Townsville, Australia. Pairs were maintained at 29 ± 0.5^{o}C in 60 l outdoor aquaria and fed 0.075 g of Aquaculture Nutrition 12/20 pellets (Proaqua Pty Ltd, Queensland Australia) twice per day. Pairs were provided with half a terracotta pot for shelter and to serve as a structure for egg deposition. The pots were inspected each morning for the presence of eggs.

Larval rearing conditions

On the afternoon when hatching was predicted (6-8 days after eggs were laid), pots containing the eggs were transferred to another 60 l aquarium (28.5^{o}C) inside an experimental laboratory, where hatching occurred within a few hours after darkness. Larvae were reared in the 60 l aquarium for two days in a semi-closed system; kept isolated during the day to facilitate feeding, then slowly flushed with filtered seawater each morning prior to light. Green *Nannochloropsis* spp. paste (Reed Mariculture, California, USA) was added to the water each morning after flushing until the bottom of the aquarium could not be seen, equating to approximately 4 million cells ml^{-1} (Moorhead and Zeng, 2011). This was done to both dissipate light and maintain the nutritional value of the rotifers (*Brachionus* sp.) that were fed to the larvae at a density of 10 rotifers ml^{-1} each morning for the first two days. The number of larvae surviving until the third day ranged from ~50-400 per clutch.

Experimental design

Larval *A. percula* were subjected to three feeding regimes and three temperatures in a full orthogonal design (i.e. nine treatments). Food availability was manipulated by increasing the time lag between feeds, with larvae provided with constant food either daily, every second day, or every third day. Temperatures were chosen to represent current-day summer averages in the Cairns region of the GBR where the brood stock were collected (29.2°C) and relevant end-of-century climate change projections for this location; +1.5°C (30.7°C) and +3°C (32.2°C).

On the third morning post-hatch (before feeding), larvae that were visually in good condition (i.e., displaying normal swimming behaviour and balance), were gently collected in a glass beaker and arbitrarily distributed among 9-18 (1-2 vessels per treatment, depending on the number of larvae in the clutch) 3 l culture vessels (5-10 larvae per vessel) made of 150 mm polyvinyl chloride pipe as described in Moorhead and Zeng (2011). Three culture vessels were placed in each of 3-6 temperature-controlled water baths (1-2 for each temperature treatment). The temperature of the water baths and the location of the vessels within the water baths were randomly modified for each run of the experiment to negate any influence of vessels or location within the laboratory on results.

Table 4.1: Number of culture vessels (replicate tanks), number of larval *Amphiprion percula* stocked in each treatment level at the start of the experiment, number of vessels containing live larvae at the end of the experiment, and number of surviving larvae among vessels at the conclusion of the experiment. Abbreviations: L = fed every third day, M = fed every second day, H = fed every day.

Temp. (^{o}C)	Food availability	Number of vessels stocked	Number of larvae stocked at start	Number of vessels with surviving larvae	Number of surviving larvae among vessels
29.2	L	11	104	7	20
29.2	M	11	105	6	23
29.2	H	11	105	8	51
30.7	L	11	105	8	21
30.7	M	11	105	7	37
30.7	H	11	105	9	46
32.2	L	11	105	8	28
32.2	M	11	105	8	38
32.2	H	11	105	8	36

All larval fish were fed immediately after transfer to the experimental setup. In addition to the rotifers, their diet was enriched with newly hatched *Artemia sp.* nauplii (INVE technologies, Thailand LTD) fed at a rate of 1 ml^{-1}. The tanks were 'greened' with *Nannochloropsis* spp. as described above. Temperatures were adjusted slowly (0.5-1^{o}C every 8 hours, depending on treatment) over 24 hours. Feeding manipulations began the day after transfer. Water exchange of vessels was carried out each morning using filtered seawater before the lights came on to flush out uneaten prey and faeces. The photoperiod was maintained at 14 hours light: 10 hours dark during the trials.

Larvae were carefully checked for metamorphosis by torchlight each morning before they had an opportunity to feed. Larvae were considered metamorphosed when their post-orbital stripe became fully pigmented, which always occurred between 8-19 days post hatch. This pigmentation coincides with a shift in habitat use and a change to benthic colouration (I. McLeod, pers. obs.), which is typical of most damselfishes (McCormick et al., 2002) and has been used as a diagnostic tool for metamorphosis and settlement for a congeneric species *A. melanopus* (Green and McCormick, 1999). The entire protocol was run seven times using progeny from four pairs of adult fish to achieve the necessary sample sizes (11 vessels per treatment among trials).

Standard length and body condition

Metamorphosed larvae were removed from culture vessels and euthanized using an overdose of clove oil. Larvae were then immediately transferred to a 4% phosphate buffered formaldehyde solution and the following measurements were taken within 48 hours. Larvae were removed from the preservative, blotted dry, weighed (nearest 0.1 mg) and photographed in a lateral position on a 0.5 mm plastic grid. Standard length (SL) to the nearest 0.01 mm was estimated from each fish from the digital photograph as described above. Body condition (hereafter: condition factor) was calculated as blotted weight at metamorphosis after controlling for standard length at metamorphosis using analysis of covariance (ANCOVA).

Pelagic larval duration and growth

Individual PLD was calculated as the number of days between hatching and metamorphosis. Larval growth was assumed as linear during the larval stage as it is for the congeneric species *A. melanopus* (Green and McCormick, 1999). Individual growth rates were estimated according to the formula: $R_g = (L_m - L_h) / T_m$, where R_g is the rate of growth in mm day^{-1}, L_m is the standard length (mm) at metamorphosis, L_h is the standard length at hatching (= 3.79 mm), and T_m is the time (days) from hatching to metamorphosis. Standard length at hatching was determined by sampling ten newly hatched *A. percula* each from three clutches. These larvae were euthanizing using an overdose of clove oil then photographed in a lateral position on a 0.5 mm plastic grid. Standard length (SL) to the nearest 0.01 mm was estimated from each fish from the digital photograph using image analysis software (ImageJ version 1.45s, National

Institute of Health, USA). The mean standard length among clutches was used for the hatch length in the above calculations.

Survivorship

Survivorship was calculated as the number of larvae surviving until metamorphosis divided by the number of larvae placed into each vessel.

Larval respirometry

Intermittent-flow respirometry was used to determine routine O_2 consumption rates ($\dot{M}O_{2routine}$), a standard measure of metabolic rate (Willmer et al., 2009). Oxygen consumption rates were measured at eight days post hatch for larvae raised at the lowest (29.2^{o}C) and highest (32.2^{o}C) temperatures and the lowest (food available every third day) and highest (food available daily) food availability. A total of 43 larvae were used (7-16 per treatment, Table 5.2). All larvae used in these trials were starved for 24-26 hours before trials began.

Larvae were placed individually into 4.8 ml glass vials that served as respirometry chambers. Chambers were submerged in an aquarium maintained at the same temperature at which the larvae were reared. To reduce light levels and external disturbance a lid was placed over the opaque aquarium during the habituation period and left in place during measurements of $\dot{M}O_{2routine}$. Water was continuously recirculated within each respirometer using a closed circuit connected to a peristaltic pump, which ensured homogeneous oxygen tension throughout the apparatus. The total volume of the respirometer chamber and tubing circuit was 10 ± 1 ml. A submersible pump connected to a timer was used to flush the respirometer chambers with aerated water intermittently at 5 ml min^{-1}, and excess water flushed from each respirometer overflowed from a small standpipe that extended just above the water surface in the aquarium.

Preliminary observations indicated that larvae took ~15 min in the respirometer to exhibit normal behaviour, an observation that was supported by $\dot{M}O_{2routine}$ measurements plateauing after this time (I. McLeod, unpublished data). To avoid the early period of stress, $\dot{M}O_{2routine}$ measurements commenced 15 min after the larvae entered the respirometer and continued for a total of four 15 min measurements separated by 5 min flush cycles.

Temperature-compensated O_2 concentration of the water within each chamber was continuously recorded (1 Hz) using oxygen-sensitive REDFLASH dye on contactless spots (2 mm) adhered to the inside of each chamber and linked to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany) via fibre-optic cables. To reduce background bacterial oxygen consumption, seawater used for the respirometers was UV-sterilized, and the system was cleaned with 70% ethanol each day, or more often if background respiration exceeded 10%. In addition, background respiration was measured before and after each trial and used to correct fish $\dot{M}O_{2routine}$ measurements assuming a linear change in background respiration (i.e., subtracted from the values calculated for the whole animal). At the end of the respirometry trials larvae were euthanized with an overdose of clove oil, blotted dry with a paper towel and weighed with scales accurate to 0.1 mg.

Data analysis

Two-factor ANOVA followed by Tukey's post-hoc comparisons of means were used to test the effect of temperature and food availability on PLD, average daily growth, standard length at metamorphosis, individual metabolic rate, and weight adjusted metabolic rate. The assumptions of normality and homogeneous variance for each performance variable were tested using Levene's test and graphically analysed using residual and qq plots. A natural log transformation was required for the PLD data and a square root transformation for individual metabolic rate data to conform to the homogeneity of variance condition.

A two-factor ANCOVA was used to test the effect of temperature and food availability on weight at metamorphosis at a standard length at metamorphosis (this adjusted weight is our measure of condition factor). There was a linear relationship between weight and SL for each treatment, as assessed by visual assessment of a scatterplot. Standardized residuals for the treatments and for the overall model were normally distributed, as assessed by Shapiro-Wilks test. There was homoscedasticity and homogeneity of variances, as assessed by visual inspection of a scatterplot, and Levene's Test of Homogeneity of Variance respectively. Post-hoc analysis for the ANCOVA was performed with a Bonferroni adjustment.

Individual fish within tanks were pooled across tanks for the analysis. A nested ANOVA design was not appropriate because some tanks only had one fish surviving

to settlement. Using the mean value for each tank produced near-identical results in performance variables (revealed through ANOVA and ANCOVA). The exception was average daily growth, where there was no significant interaction between the food supply and temperatures using the mean tank values, but there was a significant interaction after pooling individuals across tanks. This was because of low statistical power due to the limited number of tanks per treatment (6-9 among trials).

There were significant differences in performance variables between clutches, but consistent trends in effects of temperature and food availability (revealed through careful analysis of stem and leaf plots and histograms). Because clutch effects were not the focus of the current study, individuals were pooled across clutches for the analysis. Logistic regression on the individual fish was used to ascertain the effects of temperature and food availability on the likelihood that individual larvae survived to metamorphosis. Individual larvae were also pooled across tanks for this analysis.

Larval respirometry data for a total of 43 larvae were analysed using LabChart version 6.1.3, (AD Instruments, Colorado Springs, CO, USA). $\dot{M}O_{2routine}$ (mg kg^{-1} h^{-1}) was calculated from the average of the final three slopes of O_2 concentration versus time, minus the background O_2 consumption. The Q_{10} temperature coefficient for average daily growth and PLD was calculated using the formula: $Q_{10} = (R2 / R1)^{10 / (T2 - T1)}$, where T1 and T2 are the temperatures over which the change was recorded, R1 is the value of the measured variable at T1, and R2 is the value of the variable at T2. All statistical analyses were conducted using the statistical package SPSS Statistics version 20 (IBM™ SPSS™ Inc. 2011).

Results

Pelagic larval duration and growth

The average pelagic larval duration (PLD) of *A. percula* ranged from 10.5 ± 0.2 (mean ± s.e.) days in the 30.7°C and high food treatment to 15.6 ± 0.5 days in the 32.2°C and low food availability (i.e., a 50% difference; Fig. 4.1a). There was a strong and significant ($F_{4,291}$ = 6.4, P = <0.0001) interaction between temperature and food availability on PLD (Table 4.1). The significant interaction was due to a much greater effect of food availability on PLD at high temperatures, compared to the baseline temperature (29.2°C). Post-hoc tests showed that at 29.2°C individual PLDs were

significantly longer in the medium (fed every second day) than in the high (fed every day) food availability treatment. At 30.7^{o}C, PLDs were significantly longer at the medium and low food availability treatments compared to the high food availability treatments, and PLDs were significantly longer at the low compared to the medium food availability treatment.

There was also a strong and significant ($F_{4,291}$ = 4.6, P = 0.01) interaction between temperature and food availability on the average growth rate between hatching and metamorphosis (Table 4.1, Fig. 4.1b). The significant interaction was due to a much greater effect of the food availability on average daily growth at high temperatures, compared to the baseline (29.2^{o}C) temperature. Specifically, temperature had no effect on growth at the high or medium food availability treatments, but at the low food availability treatment, growth was significantly lower at 32.2^{o}C than 29.2^{o}C. Growth ranged from 0.27 ± 0.0052 mm d^{-1} in the 30.7^{o}C and high food supply to 0.018 ± 0.01 mm d^{-1} days in the 32.2^{o}C and low food availability treatment (i.e., a 31% decrease, Fig. 4.1b).

Standard length

A. percula metamorphosed at 6.5 ± 0.5 mm standard length (SL) across all treatments (Fig. 4.1c). There was no significant effect of temperature ($F_{2,291}$ = 1.8, P = 0.17) or access to food ($F_{2,291}$ = 0.09, P = 0.92) on SL at metamorphosis, and no interaction between temperature or food supply on SL at metamorphosis ($F_{4,291}$ = 0.43, P = 0.79; Table 4.1).

Body condition at metamorphosis

After adjusting for standard length at metamorphosis (through an ANCOVA), there was a significant ($F_{4,290}$ = 4.41, P = 0.02) interaction between temperature and food availability on the average blotted weight at metamorphosis (condition factor; Table 4.1, Fig. 4.1d). The significant interaction was due to a greater effect of food availability at the 30.7^{o}C treatments than at 29.2^{o}C. Condition factor ranged from 11.2 at 30.7^{o}C and high food availability to 9.47 at the low food availability at that temperature. Condition factor was always lower at the low food availability treatments that in the high and medium food treatments.

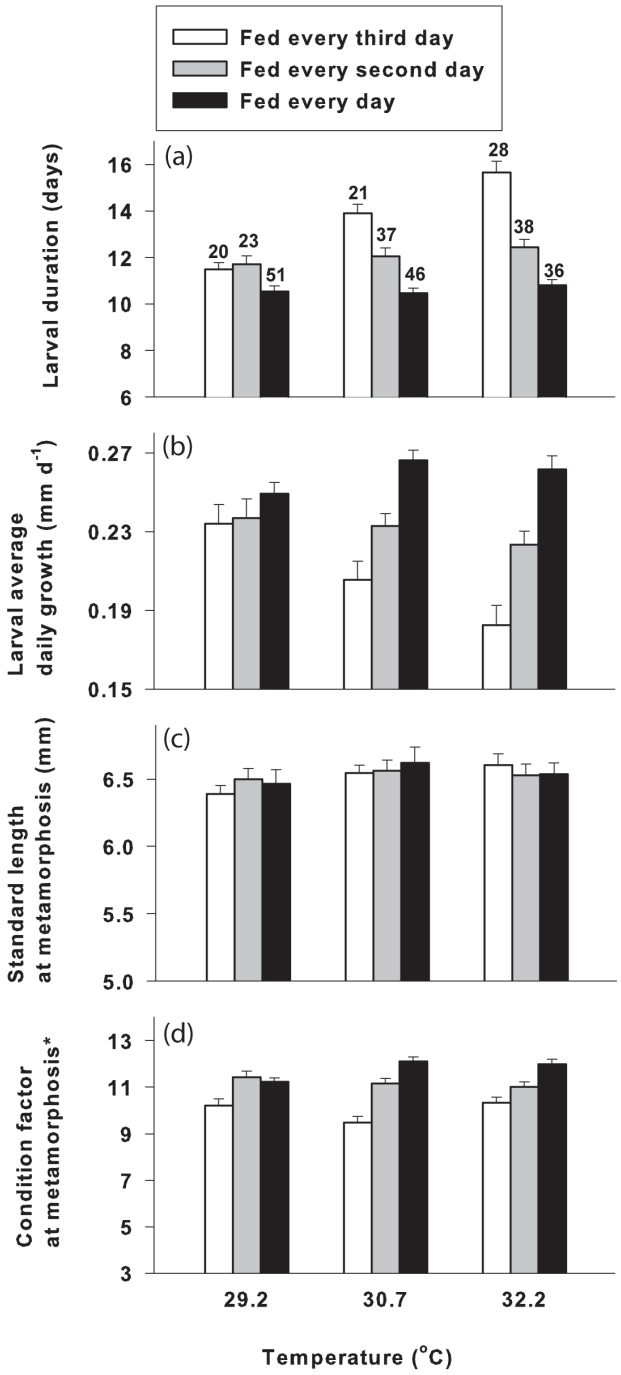

Figure 4.1: Mean (a) pelagic larval duration, (b) larval average daily growth, (c) standard length at metamorphosis, and (d) Body condition at metamorphosis (*weight in mg adjusted for standard length) for *Amphiprion percula* raised at 29.2°C, 30.7°C and 32.2°C on a low (white bar), medium (light grey bar) or high (dark grey bar) food availability. Error bars denote ± standard error.

Table 4.2: ANOVA and ANCOVA table for pelagic larval duration, average daily growth, standard length at metamorphosis, condition at metamorphosis (ANCOVA), and routine metabolism for larval *A. percula* reared in nine combinations of water temperature and food availability.

Source	df	MS	F	*P*
Pelagic larval duration				
Temperature	2	0.366	15	<0.0001*
Food availability	2	1.42	58.3	<0.0001*
Temperature x food availability	4	0.156	6.39	<0.0001*
Error	291	0.024		
Average daily growth				
Temperature	2	0.0000748	4.198	0.016*
Food availability	2	0.001	35.132	<0.0001*
Temperature x food availability	4	0.0000821	4.609	0.001*
Error	291	0.0000178		
Standard length at metamorphosis				
Temperature	2	0.381	1.782	0.17
Food availability	2	0.018	0.084	0.919
Temperature x food availability	4	0.092	0.431	0.786
Error	291	0.214		
Condition factor at metamorphosis (ANCOVA)				
Temperature	2	1.15	0.687	0.504
Food availability	2	69.8	41.944	<0.0001*
Temperature x food availability	4	7.346	4.41	0.002*
Error	290	1.67		
$\dot{M}O_{2routine}$ (*Individual)*				
Temperature	1	0.000	19.95	<0.0001*
Food availability	1	0.000	10.7	0.002*
Temperature x food availability	1	0.0000255	2.56	0.117
Error	39	0.00001		
$\dot{M}O_{2routine}$ *(weight adjusted)*				
Temperature	1	306	6.48	0.015*
Food availability	1	1.82	0.039	0.845
Temperature x food availability	1	1.94	0.041	0.84
Error	39	47.2		

Survival

Among treatments 31.8% of larvae survived to metamorphosis, ranging from 19.0% in the 29.2°C low food availability treatment to 49.0% in the 29.2°C, high food treatment. The logistic regression model ($x^2(2) = 16$, $P < 0.001$) explained 49.7% of the variance in survival to metamorphosis and correctly classified 62.7% of cases. Food availability but not temperature was a statistically significant predictor of survival to metamorphosis. Larvae in the high food availability (42.4% survival) treatments were 1.4 times more likely to survive than those in the medium food availability (31.1% survival) treatments and 1.9 times more likely to survive to metamorphosis than those at the low food availability treatments (21.9% survival).

Larval respirometry

Both temperature ($F_{1,39} = 20$, $P < 0.0001$) and food availability ($F_{1,39} = 10.7$, $P = 0.002$) significantly affected individual larval O_2 consumption. However, there was no interaction between temperature and food supply ($F_{1,39} = 2.56$, $P = 0.117$). Mean O_2 consumption ranged from 0.0059 ± 0.00082 mg O_2 h^{-1} at 29.2°C and fed every third day to 0.013 ± 0.0059 mg O_2 h^{-1} for the 32.2°C and fed every day (i.e. a 120% increase; Fig. 4.2a). However, there was a significant effect of food ration on fish size, so when $\dot{M}O_{2routine}$ was corrected for the individual weights of the larval fish, only temperature significantly affected $\dot{M}O_{2routine}$ ($F_{1,39} = 6.5$, $P = 0.015$; Fig. 4.2b). Mean $\dot{M}O_{2routine}$ was 1220 ± 101 mg O_2 kg h^{-1} at 29.2°C and 1620 ± 107 mg O_2 kg h^{-1} at 32.2°C (i.e. a 33% increase). The Q_{10} coefficient for $\dot{M}O_{2routine}$ calculated over this 3°C increase in temperature was 2.59.

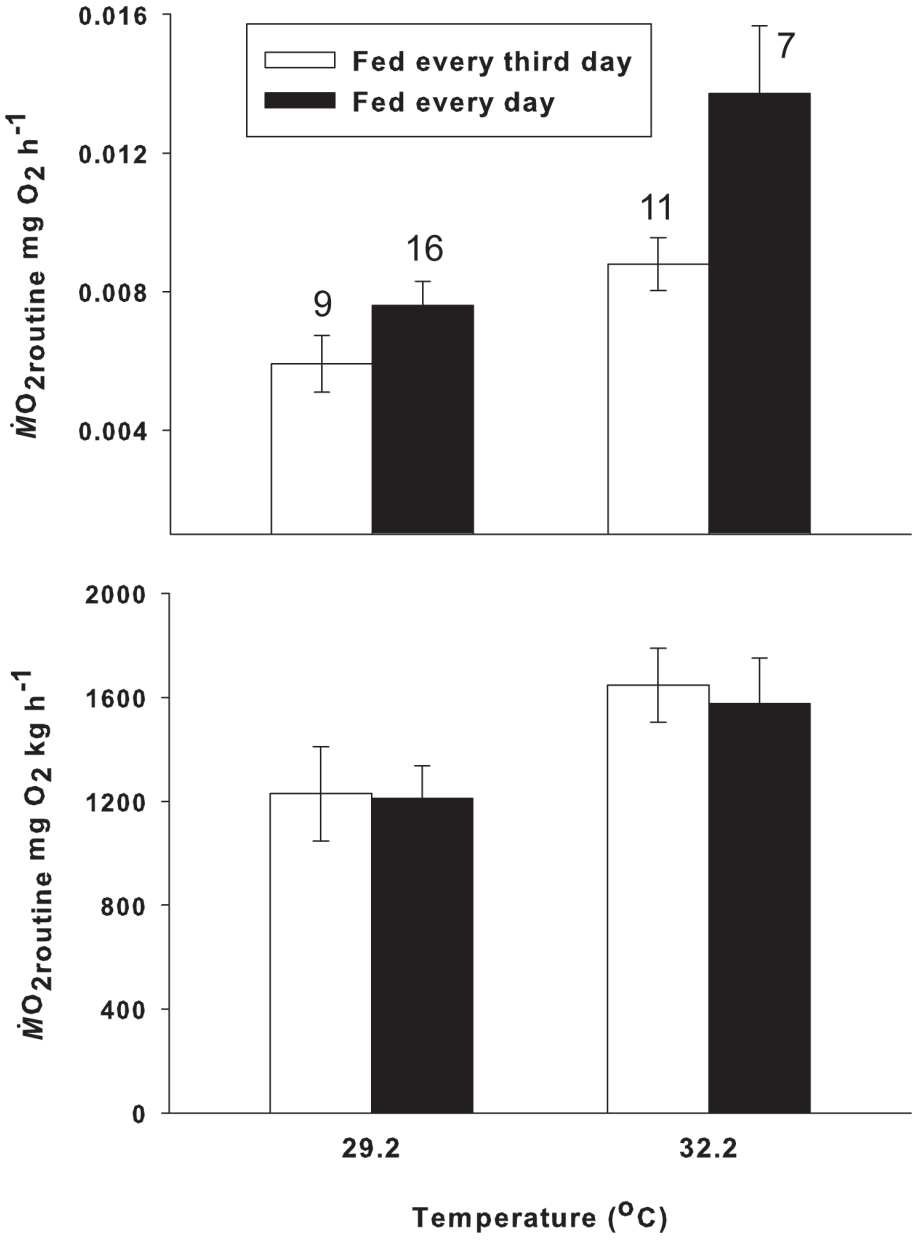

Figure 4.2: Mean (a) $\dot{M}O_{2routine}$ mg O_2 h^{-1}, (b) $\dot{M}O_{2routine}$ mg O_2 kg h^{-1} for 8 days old *Amphiprion percula* raised at 29.2^{o}C and 32.2^{o}C on a low (white bar) or high (black bar) food supply. Error bars denote ± standard error.

Discussion

Our results suggest that climate-induced increases in ocean temperature and variation in planktonic food supply may impact the development and metabolism of larval reef fishes. Overall, larval *A. percula* grew more slowly and took longer to metamorphose and settle at higher temperatures and with reduced access to food, with a highly significant interaction between these factors. However, neither temperature nor access to food affected the length at metamorphosis. Fish from the lower access to food treatments had a lower condition factor and decreased survivorship. Temperature affected routine oxygen consumption rates ($\dot{M}O_{2routine}$), which were significantly higher at 32.2°C than at 29.2°C. The difference in individual $\dot{M}O_{2routine}$ between the high and low food availability treatments were explained by the lower weight of the larvae fed every third day. When the metabolic rate was corrected for individual weight, there was no influence of food availability on $\dot{M}O_{2routine}$.

The direct effects of temperature included an increase in PLD, a lower growth rate, and an increase in metabolic rates at higher temperatures. Previous research investigating the effects of temperature on larval coral reef fish growth and PLD showed that higher temperatures lead to increased growth and shorter PLDs (e.g. McCormick and Molony, 1995, Green and Fisher, 2004, Sponaugle et al., 2006, Takahashi et al., 2012). Furthermore, in a recent review, Munday et al. (2009a) postulated that the limited evidence available suggests that a 3°C increase in sea surface temperatures would reduce the PLD of larval reef fishes by 12-25%. However, this estimate was made by extrapolating results from experiments and field studies looking at temperatures within their natural range, because there were few data available regarding the effects of elevated temperatures on these traits. Importantly, our results show that developmental rates and PLDs may not continue a linear relationship with temperature beyond the range of temperatures typically experienced by the population. Consequently, extrapolations based on present-day variation may be inappropriate for projecting the consequences of future temperature increases on the early life history traits of tropical fishes.

Food availability affected survivorship to metamorphosis, with reduced survivorship in the lower food availability treatments. *Amphiprion percula* are capable of feeding immediately upon hatching, and feeding treatments were not commenced

until the fourth day post-hatch. Hjort (1914) first emphasized the importance of food limitation at the time of first feeding for fish larvae. If food was reduced over the first three days, overall survivorship may have been reduced further, suggesting that our findings, although substantial and significant, may still be conservative. In nature, mortality is extreme in the larval phase, and so small changes in mortality rates could have important ramifications for recruitment to adult populations (Jones, 1991, Leis, 1991).

Access to food had a major impact on growth rates and PLD, with longer PLDs and slower average growth rates in the lower food availability treatments. Food availability also had a profound effect on condition factor at metamorphosis, with lower food availability treatments resulting in lower weight for a given body length. These results are consistent with previous experiments that tested the effects of reduced food for larval coral reef fish (e.g. McCormick and Molony, 1992, Green and McCormick, 1999). Lowered condition factor and growth associated with suboptimal feeding in the larval environment is likely to affect the likelihood of survival well after the fish have metamorphosed and settled (Hoey and McCormick, 2004, Gagliano et al., 2007a, McCormick and Gagliano, 2009). Poor feeding history has a lasting effect on metabolism, and this may lead to behavioural trade-offs associated with the balance between feeding and predator vigilance (Metcalfe and Monaghan, 2001). Furthermore, fishes that have had poor growth histories sometimes compensate by over-performing in growth once feeding conditions improve, and this over-performance in growth can be at the cost of behaviours that directly affect survival (Pechenik, 1990, Gagliano and McCormick, 2007).

The interacting effects of elevated temperature and reduced food availability resulted in much longer PLDs and slower growth rates, suggesting that the harmful effects of ocean warming are likely to be most severe if accompanied by a declining planktonic food supply. The increase in PLD would be likely to lead to important ecological consequences, because the larval life stage has the highest risk of mortality through predation (Bailey and Houde, 1989, Houde, 1989). A longer PLD increases the length of time that larval fish are exposed to the high-risk pelagic environment, thus indirectly reducing the probability of survival. This contrasts with predictions of increased larval survival by some analyses that have primarily synthesized PLD data for many species within their present-day temperature range (e.g. O'Connor et al.,

2007). Predictions of increased larval survival may be inaccurate, especially for tropical species, if they do not account for the shape of the thermal reaction norm in PLD above the range of natural variation and incorporate the possible effects of reduced food supply (Steinacher et al., 2010). For those larvae that survive longer PLDs, this might have some implications for the spatial scale of larval dispersal, and thus, the scale of connectivity for some populations, which could have flow-on effects for population dynamics and sustainability (Munday et al., 2009a),

It is difficult to estimate the relative importance of temperature and food supply for larval performance in the wild using the results of this study, because very little is known about the temporal variability in food supply and larval feeding in the wild. Owing to this lack of information and logistical constraints, the food treatments used in the present study were chosen to represent high, medium, and low access to food for comparative purposes, and may not be indicative of natural food supply. Nevertheless, they enabled us to examine experimentally the relationship between metabolism and food availability, and how performance variables are affected. In our experiments, the difference in effect sizes show that food supply was more important than temperature for PLD, larval growth, and condition factor. These results contrast with those of Meekan *et al*. (2003) who showed that over two consecutive summers, water temperature explained 30% and zooplankton abundance only 3.5–4.1% of the variance in growth for a tropical damsel fish, *Pomacentrus coelestis*.

In our study, metabolic rate increased with increased temperature, a result consistent with findings from previous studies (reviewed by Houde, 1989, Rombough, 1997, Peck et al., 2012). In a recent review, Peck *et al*. (2012) found that a 10°C increase in temperature was accompanied by a 1.2- to 4.3-fold increase in larval $\dot{M}O_{2routine}$ (Q_{10} = 1.2-4.3) across 15 families of marine fishes. In the present study, the Q_{10} was ~2.6, similar to the average Q_{10} (2.31) found by Peck *et al.* (2012). Elevated $\dot{M}O_{2routine}$ and therefore energy use at higher temperatures may leave less energy available for growth, resulting in the longer time to metamorphosis, especially when food supplies are low. In our laboratory experiment, the tanks were static, with no flow during the day, and with high concentrations of nutritious prey, so overall energy use is likely to be lower than in nature. Consequently, the effects of reduced access to food may be more severe in the natural environment than the results of this experiment

indicate, because larvae in nature must swim against ocean currents and are likely to consume prey of lesser nutritional value.

Larvae settled at a remarkably consistent length across treatments, indicating that reaching a precise size was important for metamorphosis in *A. percula*. Fish in the lower access to food treatments also had a lower condition factor, indicating that it is taking longer for the fish in the lower food availability treatments to become competent to settle, rather than delaying metamorphosis as some other coral reef will do when denied access to the preferred settlement structure (McCormick, 1999). There is more variation in age than size at metamorphosis among marine fish species (Chambers and Leggett, 1987). However, our results contrast with results for a congener, *A. melanopus*, in which length at settlement was significantly longer at lower temperatures (Green and Fisher, 2004) and shorter in lower food availability treatments (Green and McCormick, 1999). A larger size at settlement may offer some survival advantages (Sogard, 1997, Perez and Munch, 2010). Perhaps the disadvantage of settling at a smaller size outweighs the potential reduction in survivorship that would result from an extended PLD.

Conclusions

This study highlights the potential interacting effects that higher ocean temperatures and reductions in food supply will have on larval coral reef fishes. The $\dot{M}O_{2routine}$ was higher at higher temperatures, indicating a potential mechanism for the differences in growth and development in relationship to food supply. Indeed, the greater effect of temperature in the lowest food availability conditions could be because there is insufficient food to meet the increased metabolic costs. Overall, our results indicate that despite previous predictions of a positive influence of ocean warming on larval development and survival, variable food supply alters how temperature affects growth and development. These observations demonstrate that increased temperature and reduced food supply will affect the life history and demography of larval fish, possibly affecting recruitment processes and population dynamics. Further work is required to determine whether these results are general for a range of other fish species. The potential for coral reef fish populations to adapt to climate-change-associated elevated temperatures and variable access to food during the larval phase is unknown, but is a critical area of future research (Munday et al., 2013). Future studies could use

quantitative genetic breeding designs to test the heritability of individual variation in the response of larval fish to elevated temperature and reduced food supply. Such experiments are logistically challenging, but ultimately essential for predicting the potential for populations to adapt to rapid climate change. This work is urgent, given the potential susceptibility of fish larvae to almost every predicted change facing the oceanic environment.

Chapter 5. The digestive system of larval coral reef fishes may not keep pace with climate warming

This chapter is in review with the Journal Oecologia. Authors: IM McLeod and TD Clark.

Abstract

Climate change is predicted to increase ocean temperatures and alter plankton communities that are food for many marine fish larvae, yet little is known of the effects of temperature on the digestive efficiency and fine-scale growth responses of these tiny vertebrates. Here, we tested the effect of acutely increased temperature (3-6 day acclimation) on the growth and digestive performance of a coral reef fish (*Amphiprion percula*) during its larval phase. First, we measured the length and weight of 340 larvae across fine temporal scales at a typical current temperature (28.5^{o}C) and a temperature likely to be common by the end of the century (31.5^{o}C). Second, we determined routine metabolic rate and specific dynamic action (SDA) at both temperatures. Larvae fed voraciously and exhibited rapid, temperature-independent growth in the 24 h following satiation feeding; body length and weight increased by 9.4 ± 2% and 22 ± 5%, respectively. Routine metabolic rate ($\dot{M}O_{2routine}$) and peak $\dot{M}O_2$ during digestion were 55 ± 16% and 28 ± 11% higher at 31.5^{o}C. Elevated temperature had no significant effect on SDA (0.53 ± 0.05 J), digestion duration (6.3 ± 0.3 h), or the percent of total meal energy used for digestion (11.7 ± 1%). While based on relatively acute thermal challenges, these results suggest that even if fish larvae can secure the food necessary to satisfy higher routine metabolism in a warmer ocean, they may not be able to process food at the necessary speed to maintain current-day growth rates. The techniques and approaches used here should promote further research on the metabolic and growth responses of larval fishes under more chronic thermal exposures.

Introduction

Fish larvae are the smallest vertebrates and can maintain impressive growth rates of up to 50% of body mass per day (McCollum et al., 2006), dependent on temperature, metabolism, food supply/quality and food processing efficiency (Peck et al., 2012). Since climate change models predict an increase in sea surface temperatures of up to 4°C this century and an alteration in the plankton communities that are food for many marine fish larvae (Bopp et al., 2013), the consequences for larval growth and survival are likely to be significant but remain poorly understood.

Mortality is almost absolute (i.e., >99%) during the larval stage of many fish species, thus even small changes in mortality rates can have significant impacts on adult populations (Leis, 1991, Peck et al., 2012, Pittman et al., 2013). Larval fishes typically exhibit increased growth rates and shorter pelagic larval durations (PLDs) with increasing temperatures within their natural temperature ranges, with tropical fish larvae reported to show the most rapid growth and shortest PLDs (Leis et al., 2013). Based on this knowledge, it has been predicted that climate warming could have positive outcomes for coral reef fish larvae because enhanced growth will lead to faster metamorphosis and less time in the dangerous pelagic environment (O'Connor et al., 2007, Kendall et al., 2013). However, current knowledge is generally based on correlation rather than experimentation, and recent research has cast doubt over current theories once the potential climate-related interactions between temperature and food availability are considered (Chapter 4).

Like all vertebrates, larval fishes must expend energy to process and assimilate the nutrients from each meal. Termed specific dynamic action (SDA), the energy used during digestive processes can contribute significantly to daily energy budgets of adult fishes, yet little is known of SDA and digestive efficiency of larval fishes (Secor, 2009). Since the SDA response is dependent on environmental temperature in ectothermic vertebrates (Secor, 2009), climate warming has the potential to significantly modify food-processing capacity but this has not been examined in larval fishes.

The paucity of empirical data for larval coral reef fishes in response to elevated temperature is largely due to the inherent difficulties in working on such tiny vertebrates. This has been identified as a critical knowledge gap hindering the

parameterization of quantitative models aimed at forecasting the effects of climate change on fish communities (Jørgensen et al., 2012). Controlled studies at high temporal resolution are required to shed light on the potential effects of climate warming and food availability on the metabolism and food processing efficiency of larval fishes.

Here, I use an integrated approach to better understand how fish larvae are likely to respond to warmer oceans with different prey availability. Using controlled experimental approaches, I examined food processing, digestion and growth of larval *Amphiprion percula*, a coral reef damselfish. First, I quantified growth at fine temporal scales by measuring the length and weight of fed and unfed larvae at a current-day temperature (28.5^{o}C) and a temperature likely to be more common by the end of the century (31.5^{o}C) every 3 h for 24 h. Second, to gain an understanding of the underlying physiological mechanisms associated with the growth data, we determined the routine metabolic rate ($\dot{M}O_{2routine}$) and the postprandial metabolic response (SDA) of larvae at 28.5^{o}C and 31.5^{o}C. By obtaining postprandial metabolic measurements for one of the smallest vertebrates investigated to date, we provide insight into the interactive effects of temperature and prey availability on the growth and physiological performance of fish larvae.

Materials and methods

Study species and brood stock maintenance

Pairs of the damselfish, *Amphiprion percula*, were captured from the Palm Island region of the central Great Barrier Reef, Australia and transported to James Cook University, Townsville, Australia where they were maintained at the mean temperatures of the source location (22.5^{o}C-28.5^{o}C annually). Pairs were fed 0.075 g of Aquaculture Nutrition 12/20 pellets (Proaqua Pty Ltd, Queensland Australia) twice per day and were provided with half a terracotta pot for shelter and to serve as a structure for egg deposition. The pots were inspected each morning during the breeding season for the presence of eggs.

Larval rearing conditions

On the afternoon when hatching was predicted (6-8 days after egg deposition), pots containing the eggs were transferred to another 60 l aquarium (28.5^{o}C) inside an experimental laboratory, where hatching occurred within a few hours after darkness. Larvae were reared in the 60 l aquarium for two days in a semi-closed system; the system was kept isolated during the day to facilitate feeding, and slowly flushed with filtered seawater each morning prior to light. Green *Nannochloropsis* spp. paste (Reed Mariculture, California, USA) was added to the water each morning after flushing until the bottom of the aquarium could not be seen, equating to approximately 4 million cells ml^{-1} (Moorhead and Zeng, 2011). This was done both to dissipate light and maintain the nutritional value of the rotifers (*Brachionus* sp.) that were fed to the larvae at a density of 10 rotifers ml^{-1} each morning for the first two days. From the third day, larvae were fed newly hatched *Artemia sp.* at a rate of 2 ml^{-1}.

On the morning of the third day post-hatch (DPH), larvae that were visually in good condition (i.e. displaying normal swimming behaviour and balance) were gently collected in a glass beaker and arbitrarily distributed among twelve 3 l culture vessels made of 150 mm polyvinyl chloride pipe as described by Moorhead and Zeng (2011). On the fourth DPH temperatures were increased slowly (1^{o}C every 8 hours) over 24 hours in half the tanks until the temperature reached 31.5^{o}C. The two temperatures were chosen to represent the current-day summer average in the Palm Island region of the GBR where the brood stock were collected (28.5°C), and relevant end-of-century climate change projections for this location equating to the current summer average + 3°C (31.5°C).

Growth trials

To establish the rates of growth over 24 h, 8-DPH larval *A. percula* were subjected to two feeding regimes (fed and unfed) at the two holding temperatures (28.5^{o}C and 31.5^{o}C) in an orthogonal design. The experimental setup consisted of three 3 l culture vessels per treatment (12 tanks in total). Between 20 and 30 larvae were placed in each culture vessel. The fish in the fed treatments were fed newly hatched Artemia sp. (2 ml^{-1}), while the unfed group remained fasted.

At the start of the experiment (09:00 hours) and every 3 h after, 3-10 larvae were killed, blotted dry, weighed, and measured (SL). Larvae weighing less than 2.5 mg

(i.e., >1.5 SD below the mean mass of all larvae) were judged to be non-feeding and were discarded from the analyses (N = 24). The entire protocol was run three times using progeny from three pairs of *A. percula* to achieve high sample sizes (340 larvae among treatments).

Respirometry

Best practices in intermittent-flow respirometry (Clark et al., 2013) were used to determine postprandial oxygen consumption rates of a subset of 6-9 DPH larval *A. percula*. Larvae were gently transferred using a plastic pipette into individual 4.8 ml glass vials that served as respirometry chambers. Background respiration was quantified in all chambers before each trial, and in all cases was negligible. Four respirometers were used in each trial and they were submerged in an aquarium maintained at the same temperature at which the larvae were reared. Water was continuously recirculated within each respirometer using a closed circuit of tubing individually connected to a peristaltic pump, which ensured homogeneous oxygen tension throughout the apparatus. The total volume of the respirometer chamber and tubing circuit was 10 ± 1 ml. A submersible pump connected to a timer was used to flush the respirometer chambers with aerated water (5 ml min^{-1}) for 15 min in every 30 min period, and excess water flushed from each respirometer overflowed from a small standpipe that extended just above the water surface in the aquarium. Temperature-compensated oxygen concentration of the water within each chamber was continuously recorded (0.5 Hz) using oxygen-sensitive REDFLASH dye on contactless spots (2 mm) adhered to the inside of each chamber and linked to a Firesting Optical Oxygen Meter via fibre-optic cables (Pyro Science. K., Aachen, Germany). Once all four respirometers contained one individual larvae, the lights in the experimental room were turned off and the trials commenced and ran for 10.5 hours. Oxygen consumption rates were calculated from the decline in oxygen concentration over time during the 15 min period between flush cycles. At the end of the respirometry trials larvae were killed, blotted dry with a paper towel and weighed with scales accurate to 0.1 mg. After the larvae were removed from the chambers, three 15 min blanks were run with a 5 min flush cycle between each to quantify any changes in background microbial respiration rates.

To reduce background microbial respiration, seawater used for the respirometers was UV-sterilized, and the system was cleaned with 70% ethanol before each trial. Given the large surface area to volume ratio of the respirometers, it was imperative to account for background $\dot{M}O_2$ throughout the trial. To quantify the build-up of background $\dot{M}O_2$, 12 blank trials without fish were run at each temperature for 10.5 hours. Analysis of these blanks revealed that the build-up of background oxygen consumption rate fitted a sigmoidal function. The average $\dot{M}O_2$ of the three blank trials at the end of each experiment was used as the end point for further back-calculation. The function of the mean for each temperature at each half-hour time period was used to fit background $\dot{M}O_2$ for each fish. Background $\dot{M}O_2$ was subtracted from fish $\dot{M}O_2$ dynamically across the 10.5 h duration of the trials.

To account for the difference in weight while the digesting fish grew overnight in the respirometers, we assumed a linear increase in weight between the group sampled at time 0 h (representing the unfed control group) and the fed fish sampled at time 24 h (when the gut was empty and digestion complete), and mass-specific $\dot{M}O_2$ was calculated dynamically throughout the trials.

The energy content of Artemia sp. in the gut of each larvae (after 12 h of access to food) was estimated by assuming a linear increase in weight of the fish (not including food in the gut) during the 24 hours of the experiment, then using the mean weights from the growth experiment to: (1) back calculate the fish weight at 12 hours, (2) back calculate the weight of the fish plus the Artemia in its gut at 12 hours, (3) subtract 1 from 2 to estimate the weight of Artemia in the gut when the individual fish were placed into the respirometry chambers, and (4) calculate meal energy intake using the energy content of newly hatched Artemia sp. shown in Table 5.1.

Table 5.1. Nutritional content of the newly hatched *Artemia sp*. fed to the larval *Amphiprion percula*.

Constituent	(%)
Lipid	2.364*
Protein	7.009*
Ash	0.860*
Moisture	87.07*
Nitrogen free extract (digestible carbohydrate)	2.690*
Energy (J g^{-1})	2511**

*Nutrient content of newly hatched *Artemia sp.* was obtained from David Francis, Australian Institute of Marine Science, (unpublished data).

**Energy content calculated assuming that lipid, protein and carbohydrates have energy equivalents of 37.70KJ g^{-1}, 16.77KJ g^{-1}, and 16.77KJ g^{-1} respectively (Beamish and Trippel, 1990).

Respirometry data for 23 digesting larvae, 20 unfed larvae, and 24 blank trials without fish were analysed using LabChart version 6.1.3 (ADInstruments, Sydney, Australia). The duration of the postprandial increment in metabolic rate ($\dot{M}O_{2dur}$) was calculated as the time between when the larvae was removed from access to food and placed in the respirometer, and the return of $\dot{M}O_2$ to stable baseline levels. $\dot{M}O_2$ was considered to be at baseline levels and digestion complete when the 1.5-h running mean $\dot{M}O_2$ (i.e., three successive $\dot{M}O_2$ measurements) was within two standard deviations of the minimum 1.5-h mean $\dot{M}O_2$ (German, 2011). Post-digestion $\dot{M}O_2$ was calculated for each fish using the lowest mean $\dot{M}O_2$ during any 1.5-h period (i.e., three successive $\dot{M}O_2$ measurements).

Using the baseline $\dot{M}O_2$ levels at the end of the digestion period, specific dynamic action (SDA) was calculated for each individual as the excess energy expended above baseline throughout the digestion period. Peak metabolic rate during

digestion ($\dot{M}O_{2peak}$) was calculated as the highest mean $\dot{M}O_2$ over any 1.5 h period (i.e., three successive $\dot{M}O_2$ measurements). Factorial scope was calculated for each individual as $\dot{M}O_{2peak}$ / $\dot{M}O_{2routine}$. The SDA coefficient was calculated as the percentage of meal energy that was used during the SDA process. Conversion of $\dot{M}O_2$ to its energy equivalent was performed assuming 14.32 J of energy was expended per 1 mg of O_2 consumed (Clark et al., 2010). The Q_{10} temperature coefficient for the measured variables was calculated using the formula: $Q_{10} = (R2 / R1)^{10 / (T2 - T1)}$ where T1 and T2 are the temperatures over which the change was recorded, R1 is the rate of a process at T1, and R2 is the rate of the same process at T2.

5.1.1. Data analysis

Condition factor was calculated as the mean residual values after regressing the lengths and weights of all larvae in the growth trials. Independent sample T-tests revealed no significant effect of temperature on the measured variables within feeding levels at the end of the growth experiment, thus individual larvae were pooled between temperatures for all further analyses. Differences in mean weight, length, or condition factor between fed and unfed larvae at each time of sampling were revealed using independent sample T-tests, as were differences in metabolic variables between temperatures. Differences in length, weight or condition factor for fed and unfed larvae over time were revealed through ANOVA with a post-hoc two-sided Dunnet's Test using time 0 as the control category.

Results

Growth

Larvae in the fed treatments were 41.6 ± 4% heavier, 7.2 ± 2% longer, and had a higher condition factor after 12 h of access to food, reflecting the full gut and initial stages of the growth phase of the fed fish (Figure 5.1. a-c). After 24 h (12 h access to food then 12 h of fasting in darkness) fed larvae had grown 22.0 ± 5% heavier and 9.4 ± 2% longer than the control fish that were sampled at the start of the experiment. A return of the condition factor to pre-feeding levels (Figure 5.1c) and visual verification that the gut was empty confirmed that these changes were due to growth. In contrast, unfed fish had no significant change in weight, were 4.8 ± 2% longer, and had a lower condition factor after 24 h (Figure 5.1 a-c).

Table 5.2: ANOVA table of larval *Amphiprion percula* weight, standard length and condition factor over time.

Effect	*d.f*	*Mean Square*	*F*	*P*
Weight (fed)	8, 205	7.440	11.723	<0.001*
Weight (unfed)	8, 154	0.622	1.876	0.067
Length (fed)	8, 205	0.980	9.212	<0.001*
Length (unfed)	8, 154	0.208	2.605	0.011
Condition factor (fed)	8, 205	3.764	10.455	<0.001*
Condition factor (unfed)	8, 154	2.210	8.467	<0.001*

Metabolism

$\dot{M}O_{2routine}$ after digestion and peak $\dot{M}O_2$ during digestion were 55.0 ± 16% and 27.8 ± 11% higher at 31.5^{o}C, respectively. However, the elevated temperature had no significant effect on SDA (0.52 ± 0.05 J), digestion duration (6.3 ± 0.3 h), or the percent of total meal energy used for digestion (SDA coefficient; 11.7 ± 1%; Table

5.2). Post-SDA $\dot{M}O_2$ did not significantly differ from unfed $\dot{M}O_{2routine}$ at either temperature (Figure 5.1d), confirming that digestion was complete.

Table 5.3: Summary of SDA $\dot{M}O_2$ results (means ± s.e.m.) for larval *Amphiprion percula* at 28.5°C and 31.5°C. P value refers to comparisons between temperatures.

Variable	28.5 (°C)	31.5 (°C)	*P*	Q_{10}
Unfed $\dot{M}O_{2routine}$ (mg kg^{-1} h^{-1})	1235 ± 89.8	1873 ± 247	0.029*	4.03
Post SDA $\dot{M}O_{2routine}$ (mg kg^{-1} h^{-1})	1111 ± 126	1722 ± 175	0.010*	4.24
$\dot{M}O_{2peak}$ (mg kg^{-1} h^{-1})	2208 ± 178	2820 ± 211	0.033*	2.26
$\dot{M}O_{2dur}$ (h)	5.95 ± 0.61	6.54 ± 0.46	0.218	
Meal energy (J)	5.32 ± 0.29	4.14 ± 0.21	0.003*	
SDA (J)	0.51 ± 0.057	0.53 ± 0.07	0.612	
SDA coefficient (%)	10.14 ± 1.34	12.96 ± 1.74	0.106	

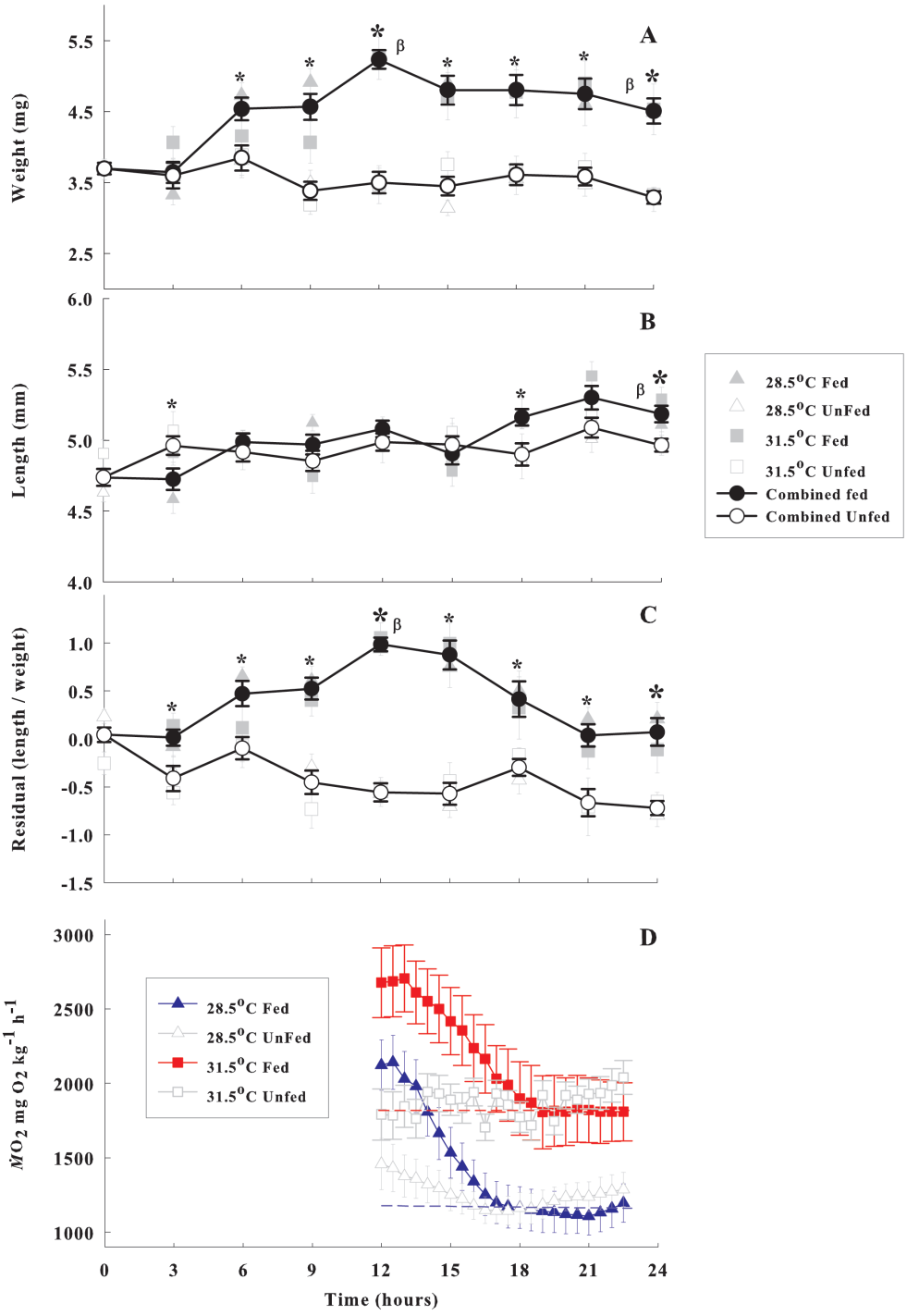

Figure 5.1: Mean weight (a), standard length (b), condition factor (c), of fed and unfed larval *Amphiprion percula* at 28.5°C and 31.5°C over 24 h, and metabolic rate (d) over 12 h. The red and blue dashed lines show $MO_{2routine}$ at 28.5°C and 31.5°C respectively. (*) Denotes significant differences between fed and unfed treatments. (β) Denotes significant differences compared to time 0.

Discussion

This study is the first to quantify fine-scale feeding and growth patterns in a larval coral reef fish. I found that larval *A. percula* were voracious predators, showing extremely distended abdomens and a 41.6 ± 4% increase in body mass during the 12-h feeding period. When access to food was removed and the fed larvae were given 12 h to complete digestion, body length and mass plateaued at values that were, respectively, 9.4 ± 2% and 22.0 ± 5% higher than the pre-feeding control values.

In an attempt to understand some of the underlying mechanisms associated with such exceptional growth rates, this study became the first to measure the postprandial metabolic response of any larval coral reef fish. While it has been previously documented that larval coral reef fish maintain exceptional rates of aerobic metabolism (Nilsson et al., 2007), no study has investigated the metabolic consequences and efficiency of digestion. This represents a significant knowledge gap, since meal digestion and assimilation are likely to be fundamental components of the daily energy budget of pelagic larval fishes as they strive to attain the critical body size necessary for settlement and metamorphosis.

Amphiprion percula larvae had an SDA coefficient of 11.7 ± 1% when feeding on Artemia, indicating that around 12% of meal energy was expended during meal processing and assimilation. This represents a greater efficiency than the >25% reported for most other adult fishes when feeding on natural foods (Secor, 2009) but is similar to that of larval whitefish, *Coregonus lavaretus*, fed Artemia (Huuskonen et al., 1998; 13%). Interestingly, we found no evidence for a reduced SDA duration at 31.5^{o}C in comparison with fish at 28.5^{o}C, which contrasts with the general observation for ectothermic vertebrates that SDA duration decreases with temperature (Secor, 2009). This may have implications for food processing capacity in a warming world, as a greater food intake will be required to satisfy higher baseline energy expenditure prior to partitioning excess energy into growth. Indeed, the finding of similar SDA duration between the two temperatures suggests that SDA cycles may necessarily overlap in a warmer world if the larvae are to obtain sufficient energy to ensure growth rates are not compromised.

The dominant prey items for low latitude marine fish larvae are copepods from the Calanoidae and Cyclopidae families and other crustacean larvae (nauplii) (Sampey

et al. 2007, Llopiz 2013). Copepods have a higher nutritional value than Artemia (Conceicao et al. 2010). However, the relative digestive effort required to process these two food items by larval damselfish are unknown. Repeating these experiments using copepods as the food source would provide useful and potentially more ecologically relevant results. The role of prey switching and feeding optimisation in the wild in regard to growth and digestion offers a logistically challenging but intriguing avenue for future research.

The peak of the SDA response is thought to be associated with large allocations of energy into protein synthesis (McCarthy and Fuiman, 2011). Due to the generally limited metabolic scope in fish larvae (Lucas and Priede, 1992), any short-term allocation of energy into protein synthesis may limit the energy that can be allocated to other functions such as foraging or predator evasion. Indeed, reduced activity after feeding has been observed in a number of taxa including adult fishes (Owen et al., 1998), and has been suggested for larval fishes based on measurements of aerobic metabolism (Wieser et al., 1988, Cunha et al., 2007). If climate warming leads to more regular and overlapping SDA events as proposed in the present study, larval fishes may be compromised in their capacity to simultaneously perform other important aerobic processes such as sustained swimming and predator avoidance.

Little is known about the capacity for adaptation or acclimation of larval fishes to increased temperature and this is an important area for future research (Munday et al., 2013). While based on relatively acute thermal challenges, the larvae tested at elevated temperatures in our study were acclimated to the elevated temperatures for most of their post-hatch life so they had a reasonable amount of time to acclimate. However, recent research has shown that some tropical reef fish species have the capacity for transgenerational acclimation to elevated temperatures (Donelson et al., 2012b). We hope the techniques and approaches used here will promote further research on the metabolic and growth responses of larval fishes under more chronic thermal exposures to increase the accuracy of projections of climate warming impacts.

The novel, controlled experiments conducted in the present study give rise to a deeper understanding of how climate warming may influence larval coral reef fishes. A 3^{o}C increase in temperature caused a 55.0 ± 8% increase in $\dot{M}O_{2routine}$, which would necessitate a significant increase in food consumption just to maintain energy balance unless metabolic acclimation/adaptation can completely compensate. This is likely to

leave less energy available for growth, especially when food supplies are low. In Chapter 2 I showed that elevated temperatures coupled with restricted food supply dramatically reduced growth rates in larval *A. percula*, consequently leading to a significantly longer PLD, which would likely translate to reduced survival. Combined with predictions of plankton communities becoming more ephemeral as the climate warms (Bopp et al., 2013), our results suggest that the success of coral reef fish larvae may become even more variable and patchy into the future, with important implications for population dispersal, connectivity and persistence.

Chapter 6. General Discussion

Climate change models predict that tropical ocean sea surface temperatures will increase by up to 4°C this century (Bopp et al., 2013). This will dramatically alter both the physical environment for marine animals and the plankton communities that are the basis of food webs in most marine ecosystems (Hoegh-Guldberg and Bruno, 2010, Doney et al., 2012, Poloczanska et al., 2013). The four data chapters of this thesis employed a variety of observational, analytical and experimental approaches to investigate the interacting effects of varying temperature, food supply and associated environmental variables on larval growth, development, metabolic rate and digestion in coral reef fishes. The results of this thesis provide significant insights into the effects of ocean warming on the larval phase of coral reef fishes, and the consequences of these changes for coral reef ecosystems.

Chapter 2 described variation in key early life history traits of two coral reef fish species across most of their latitudinal range in the southern tropics. Curvilinear relationships were identified between developmental temperatures and pelagic larval duration (PLD), larval growth and size at settlement. Growth and developmental rates generally increased with increasing developmental temperature, however the thermal optima (~28-29°C) for growth and development appears to be reached or surpassed at low latitudes such that populations at these latitudes may be particularly vulnerable to ocean warming. Chapter 3 used long-term sampling at Lizard Island, Great Barrier Reef, Australia to reveal that the relationships between developmental temperature and PLD, larval growth, and size at settlement are similar to those found among latitudes in Chapter 2, with a thermal optima at ~28°C. Additionally, environmental variables that are likely to be modified by climate-change such as rainfall, wind speed and solar radiation were shown to influence larval growth and development. Chapter 4 experimentally explored the impacts of elevated developmental temperatures and varying access to food on the growth, development and metabolic processes of larval coral reef fishes. Results from this chapter showed that the interaction between elevated temperature and limited food availability slowed larval growth and led to a longer PLD. Finally, Chapter 5 provided the first experimental quantification of the effects of elevated temperatures on fine-scale feeding and growth patterns for a larval coral reef fish species. While it has been previously documented that larval coral reef fish maintain exceptional rates of aerobic metabolism (Nilsson et al., 2007), no study

has investigated the metabolic consequences and efficiency of digestion. Elevated temperature had no significant effect on the energy used to digest and assimilate a meal, digestion duration, or the percent of total meal energy used for digestion. The implications of these findings for understanding the effects of ocean warming on the larval development of coral reef fish are discussed below.

Effects of current-day temperature variation on larval development

One of the key aims of this thesis was to define the species-wide thermal reaction norms for the growth and development of larval coral reef fishes because this information is vital for predicting the impacts of future warming. Chapter 2 showed that the latitudinal patterns of PLD, larval growth, and size at settlement of two coral reef fish species were significantly correlated with water temperature. Among latitudes, the thermal reaction norms for PLD, larval growth rate, and settlement size were non-linear, with the major shift in the relationships between temperature and all three traits occurring at 28-29°C. A similar curvilinear relationship between developmental temperature and the above early life history traits was found in Chapter 3, where samples were taken over 13 years at Lizard Island spanning a similar temperature range to the latitudinal gradient. At Lizard Island, the major shift in the relationships between temperature and all three traits occurred at ~28^{o}C, with slower growth, longer PLD and smaller size at settlement above this temperature. Additionally, when the effects of elevated temperature on the development of larval coral reef fish were experimentally tested (Chapters 4 and 5), there appeared to be little capacity for increased growth or developmental rates at elevated temperatures, even when high quality food was abundant.

The observed positive correlations between growth rate and temperature, and negative correlation between PLD and temperature up to ~28-29^{o}C, are consistent with previous studies on larval coral reef fishes (McCormick and Molony, 1995, Radtke et al., 2001, Wilson and Meekan, 2002, Meekan et al., 2003, Green and Fisher, 2004, Takahashi et al., 2012). The decrease in growth rate in the naturally warmest waters close to the equator and during the warmest months at Lizard Island contrasts with predictions of previous studies that suggest linear increases in growth rate across natural thermal ranges. However, previous studies into latitudinal variation have typically excluded equatorial populations (but see Takahashi et al., 2012), and

temporal studies have typically been short-term and may have missed cohorts that developed during particularly warm periods (but see Lo-Yat et al., 2011). The results of Chapters 2 and 3 of this thesis indicate that when the entire natural thermal range is investigated the thermal reaction norm is dome-shaped rather than linear.

Curvilinear responses to temperature are a general indication of thermal thresholds and optima for fitness-related traits (Pörtner and Farrell, 2008, Angilletta, 2009). The slower growth rates in the warmest waters close to the equator suggest that those populations are already living at temperatures close to or beyond the optimum for these traits. Populations at these low latitudes experience very little natural variation in temperature and often breed year-round (Srinivasan and Jones, 2006), and thus may be particularly vulnerable to ocean warming. Similar latitudinal variation patterns in vulnerability to ocean warming were revealed in a recent study of the effects of elevated temperature on adult coral reef fishes where equatorial populations were found to be living close to or beyond optional temperatures for aerobic scope (Rummer et al., 2013). These recent findings add evidence to support the hypothesis that marine species have similar latitudinal variation in vulnerability patterns to those that have been postulated for terrestrial ectotherms, with low latitude species already living towards the upper edge of their thermal limits, and being most vulnerable to a changing climate (Deutsch et al., 2008, Tewksbury et al., 2008, Nguyen et al., 2011).

The results of Chapter 3 show that environmental variables that are likely to differ among locations, such as rainfall, wind speed and solar radiation are likely to influence the early life history traits of coral reef fishes. Differences in environmental factors undoubtedly influenced the rates of growth and development measured in the present study, yet despite this, there were clear, consistent temperature-related patterns in growth and development.

Larval development in a warmer future

Resolving the effects of ocean warming on the larval stage of coral reef fishes is challenging because temperature affects a multitude of environmental factors that may in turn affect various processes at different levels of biological organisation. The effects of future warming will differ depending on where the population sits on the thermal reaction norm, larval food supply, other site-specific environmental factors, and the capacity for adaptation (Munday et al., 2008a, Munday et al., 2009a). The

effects of ocean warming on larval development are unlikely to be uniform among latitudes. Because low latitude populations appear to be living close to their thermal optimum for larval development, future increases in temperature are likely to lead to detrimental effects. Previous conclusions about the relationship between key larval traits and temperature may need to be reconsidered for low latitude areas.

Increased water temperature is expected to accelerate physiological processes in larval fishes, provided temperatures do not exceed the thermal optima for this life stage (Munday et al., 2008a). Larval coral reef fishes can have exceptionally high rates of aerobic metabolism (Nilsson et al., 2007) during this period of rapid growth and ontogenetic development. Resting metabolic rate was shown to increase as expected with increasing temperatures (Chapters 4 and 5). However, relatively large amounts of food will be needed to fuel this increased metabolic rate and rapid growth (Houde, 1987). Climate change decreases in primary productivity have been predicted to be severe in some tropical waters (Bopp et al., 2013). This reduction in primary productivity is likely to lead to a reduction in zooplankton communities that are food for larval fishes (Leis, 1991, Sampey et al., 2007). Reduced food supplies are associated with slower growth and protracted PLD (McCormick and Molony, 1992, Green and McCormick, 1999, Meekan et al., 2003, Sponaugle et al., 2009). The negative effects of restricted food supply are likely to be most profound when coupled with high temperatures and thus higher routine metabolic rates and energy use, leaving less energy available for growth. Furthermore, the results of Chapter 5 suggest that even if larvae can procure more food in a warmer ocean they may not be able to process it fast enough to maintain growth rates.

The El Niño-Southern Oscillation cycle offers a glimpse into potential future conditions because it involves warming of the oceans at large spatial (thousands of km) and temporal (annual) scales. Lo-Yat et al. (2011) showed that an El Niño event associated with temperatures up to 3.5^{o}C above mean values and low productivity (and presumed food supply) coincided with a 51% reduction in larval survival compared to long-term mean values. The authors concluded that increasing SST and declining productivity might lead to increased mortality of larvae in the plankton due to insufficient food, resulting in lower rates of larval supply to reefs.

The effects of warming will not be limited to the larval stages of reef fishes. Reproduction in marine fishes typically occurs within a narrow temperature range, and

an increase of 2-3°C can be sufficient to reduce reproductive activity or increase egg mortality (Munday et al., 2009a, Donelson et al., 2010, Pankhurst and Munday, 2011). Fishes on equatorial reefs often breed throughout much of the year (Srinivasan and Jones, 2006, Abesamis and Russ, 2010) and reproductive success in these populations could be severely compromised if higher temperatures in the future mean that the thermal optimum for reproduction is exceeded for large parts of the year (Munday et al., 2008a). Breeding tends to be more seasonal at higher latitudes and is often associated with increasing water temperature in spring (Munday et al., 2008a). Increased water temperatures could cause an earlier start to the breeding season for species at higher latitudes, when temperature thresholds for reproduction are not exceeded. Alternatively, a split breeding season might develop, with reproduction occurring either side of the summer maximum (Munday et al., 2009a). Thus, variation in the timing of reproduction could mitigate the potential negative effects of elevated temperatures at higher latitudes but the potential for this is limited at low latitudes.

Early life history traits influence reef fish recruitment dynamics (Bergenius et al., 2002, Wilson and Meekan, 2002), which can in turn influence population survival and have important implications for management of fish stocks (Rothschild, 1986, Booth, 1995). Slower larval growth is often coupled with an increase in PLD. An increase in PLD would likely have important ecological consequences, because the larval life stage has the highest risk of mortality through predation (Bailey and Houde, 1989, Houde, 1989). This contrasts with hopeful predictions of increased larval survival by some analyses that have primarily synthesized PLD data for many species within their present-day temperature range (e.g. O'Connor et al., 2007). Recent studies have used the results of this meta-analysis to predict that PLDs will be reduced and larval survival will increase in a warming ocean, consequently, influencing connectivity and dispersal patterns (Munday et al., 2009a, e.g. Kendall et al., 2013, Underwood et al., 2013). Predictions of increased larval survival and shorter PLDs may be inaccurate, especially for tropical species, if they do not account for the shape of the thermal reaction norm in PLD above the range of natural variation and incorporate the possible effects of reduced food supply (Steinacher et al., 2010). For those larvae that survive longer PLDs, this might have some implications for the spatial scale of larval dispersal, and thus, the scale of connectivity for some populations, which could have flow-on effects for population dynamics and sustainability (Munday et al., 2009a).

Opportunities for future research

Temperature increases will not happen in isolation and will be associated with increasing ocean acidification, and other anthropogenic stressors such as coastal development, declining water quality, and increases in turbidity in many coastal areas (Brodie et al., 2012, Doney et al., 2012, Ban et al., 2014). Many of these stressors are likely to have synergistic effects, exacerbating the response to elevated temperatures. Research into the site-specific synergistic impacts of stressors will be required to understand the full impacts of climate change on local fish populations. This will also be important in determining the most ecologically and economically susceptible geographic locations and countries, and ultimately, tailoring mitigation and conservation strategies to effectively conserve the biodiversity of tropical ecosystems and the services they provide to millions of people.

The present study showed that the tropics-wide thermal reaction norms for important early life history traits were similar between two species of coral reef fishes from different families with different breeding modes (benthic egg laying versus broadcast spawner). However, the fact that these species are common over such an extensive latitudinal range implies that both species are thermal generalists and may be more robust to thermal variation than thermal specialist species. Indeed, *P. moluccensis* performed relatively well in comparison with other reef fish in experiments testing the effects of elevated temperature on aerobic scope (Gardiner et al., 2010, Rummer et al., 2013), and Nilsson et al. (2009) found that two species of cardinalfish (Apogonidae) were more sensitive to elevated temperatures than damselfish. Furthermore, large-bodied fish are critical for sustaining coral reef fisheries, and recent evidence has shown that larger coral reef fish may be more severely influenced by elevated temperatures (Johansen et al., 2014). These results demonstrate that some coral reef fish species are more thermally sensitive than others and further research into inter-specific variation, with a focus on commercially important species, in the relationship between larval traits and temperature would be important for predicting impacts of ocean warming on fish populations.

A major research effort over the past decade has demonstrated that many marine organisms living under-current day conditions are sensitive to future environmental conditions (Munday et al., 2013), and this thesis adds to this body of knowledge. However, little is known about the capacity for acclimation (phenotypic changes) or

adaptation (genetic change through selection) of larval fishes to increased temperature, and this is an important area for future research (Munday et al., 2013). The results from Chapters 2 and 3 show that despite the potential for acclimation and adaptation, larvae consistently performed sub-optimally at the warmest temperatures. However, in these studies developmental temperature co-varies with many other environmental variables, and potentially with location-specific demographic factors.

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). Studies to date have found little evidence for thermal acclimation by adults of coral reef fish species (Nilsson et al., 2009, Nilsson et al., 2010). However, much of the developmental plasticity of coral reef fishes occurs in the early life stages (Warner, 1997, Munday et al., 2006), and recent studies have shown that some coral reef fishes possess the capability to modify their physiology during development to cope with future ocean temperatures (Donelson et al., 2011, Donelson et al., 2012a, Grenchik et al., 2013). There is emerging evidence that transgenerational effects can have positive benefits on the performance of offspring that experience the same conditons as their parents (Donelson et al., 2012b, Miller et al., 2012, Munday et al., 2013). For example, Donelson et al. (2012b) showed that juvenile damselfish were able to compensate completely for the negative effects of elevated water temperature on metabolic rate and aerobic scope when their parents were also reared at elevated temperatures. However, this acclimation may come at a cost or involve trade-offs with other traits (Angilletta Jr et al., 2003). For example, the damselfish possessing thermal acclimation in the above study were smaller and in poorer condition than fish reared at current-day temperatures (Donelson et al., 2012b). These recent findings have challenged the assumption that tropical reef fish have limited capacity to cope with rising ocean temperature. However, much of this research has used the coral reef damselfish species, *Acanthochromis polyacanthus*, which is unusual because it does not have a dispersing larval phase. The potential for developmental or transgenerational acclimation to elevated temperatures during the larval phase of coral reef fish is largely unknown but is an important area for future research.

Concluding remarks

Overall, this thesis has shown that, contrary to some optimistic suggestions, climate warming is likely to have negative impacts on larval coral reef fishes. Impacts are likely to be most severe for low latitude populations with the naturally warmest SST, which appear to be already living close to their thermal optima. Since routine metabolic rate increases with increasing temperature, individuals must consume more food to maintain the same level of growth at higher temperatures. However, the planktonic communities that are food for many coral reef fish are predicted to become more variable or decline with climate change, and even if food is plentiful larvae may not be able to process food at the necessary speed to maintain current-day growth rates. Elevated temperatures and reduced food supplies are therefore likely to lead to slower larval growth and protracted development in the pelagic environment, which are likely to have direct implications on larval survival, population connectivity and persistence.

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Appendix A: Publications arising from thesis

Publications derived from thesis

- **McLeod IM**, Rummer JL, Clark TD, Jones GP, McCormick MI, Wenger AS & Munday PL (2013) Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability**.** *Conservation Physiology* **1** doi: 10.1093/consphys/cot24.
- **McLeod IM** & Clark TD (In review). The digestive system of coral reef fishes may not keep pace with global warming. *Oecologia.*
- **McLeod IM**, McCormick MI, Munday PL, Clark TD, Takahashi M, Brooker RM, Wenger AS & Jones GP (Submitted). Latitudinal variation in larval development of coral reef fishes: implications for a warming ocean. *Proceedings of the Royal Society B: Biological Sciences.*
- **McLeod IM**, Jones GP & McCormick MI (In Preparation). Inter-annual variation in the larval development of a coral reef damselfish at Lizard Island, Australia. *Coral Reefs.*

Additional publications during PhD candidature

- **McLeod IM**, Parsons DM, Morrison MA, Van Dijken, SG & Taylor RB (2014) Mussel reefs on soft sediments: a severely reduced but important habitat for macroinvertebrates and fishes in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **48** 48-59.
- Wenger AS, McCormick MI, Endo GGK, **McLeod IM**, Kroon F & Jones GP (2013) Suspended sediment prolongs larval development in a coral reef fish. *Journal of Experimental Biolog*y. In press.
- Brooker RM, Munday PL, **McLeod IM** & Jones GP (2013). Habitat preferences of a corallivorous reef fish: predation risk versus prey availability. *Coral Reefs* **32** 613-622.

- Wenger AS, McCormick MI., **McLeod IM** & Jones GP (2013). Suspended sediment alters predator-prey interactions between two coral reef fishes. *Coral Reefs* **32** 369-374.
- **McLeod IM**, Parsons DM, Morrison MA, Le Port A &Taylor RB (2012). Factors affecting the recovery of soft-sediment mussel reefs in the Firth of Thames, New Zealand. *Marine and Freshwater Research* **63** 78-83.