THERMAL PERFORMANCE OF SCLERACTINIAN CORALS

Thesis submitted by

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Dedication

Voor James, omdat er niks leuker is dan zwemmen en niks mooier dan een koraal rif.
Ik hoop dat je het allebei je leven lang mag meemaken.

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To finish a thesis or an ironman, the process is the same. The work is put in when no one is looking, at a lone road, a lone desk, in the dark. When motivation wanes and you want to stop, it is in these moments that you create a force that only continues to grow. During the past four years I learned about science, academia and myself. Now, at the finish line, I want to thank everyone that was involved in this process.

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This thesis was conducted under the supervision of Mia Hoogenboom and each chapter was in collaboration with her. For **Chapter 5**, Christine Ferrier Pages provided additional experimental and editorial assistance. For **Chapter 6**, Allison Paley and Tess Hill provided additional editorial support.

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Table I Statement of contributions to individual chapters

Chapter	Statement of Contribution
Chapter 1 General introduction	SJ wrote the chapter with feedback from MH
Chapter 2 Trajectory of thermal acclimation to heat and cold exposure of a scleractinian coral	SJ and MH conceived the research, SJ performed the experiments and analyses, SJ wrote the first draft of the manuscript and MH contributed to the revisions
Chapter 3 Seasonal acclimation of thermal performance in two species of reef-building corals	SJ and MH conceived the research, SJ performed the experiments and analyses, SJ wrote the first draft of the manuscript and MH contributed to the revisions
Chapter 4 Thermal acclimation strategies of scleractinian corals along a latitudinal gradient on the Great Barrier Reef	SJ and MH conceived the research, SJ performed the experiments and analyses, SJ wrote the first draft of the manuscript and MH contributed to the revisions
Chapter 5 Thermal performance of two temperate and tropical corals	SJ and MH conceived the research, SJ performed the experiments with assistance of CFP, SJ analysed the data and wrote the first draft of the manuscript and MH and CFP contributed to the revisions
Chapter 6 General discussion	SJ wrote the chapter with feedback from MH and editorial support from Allison Paley and Tess Hill

* Initials are SJ = Saskia Jurriaans, MH = Mia Hoogenboom, CFP = Christine Ferrier Pages

ABSTRACT

Temperature has a fundamental influence on the physiology, biology and ecology of all organisms, and varies over time and space. Organisms evolved different strategies to cope with this spatial and temporal thermal heterogeneity. For instance, organisms that inhabit thermally variable environments will function over a wider range of temperatures than organisms that live in relatively constant thermal environments. Reef-building corals including their algal symbionts generally live in warm, tropical environments close to their upper thermal maxima, however their performance at varying environmental temperatures remains poorly documented. The overarching aim of my thesis is to determine how temporal and spatial heterogeneity of the thermal environment influences coral and symbiont performance. Through a series of controlled thermal experiments in this thesis I quantify the rate of photosynthesis of reef-building corals and their algal symbionts (termed the holobiont) at various temperatures using coral colonies from different thermal environments and geographic regions. This study is the first to quantify and compare the thermal optima and performance breadth for holobiont and symbiont performance from different thermal environments using thermal performance curves and thereby providing new insights into the mechanisms underlying thermal acclimation

Acclimation to environmental change takes time and does not necessarily result in full compensation of an organism's performance. In **Chapter 2** I identified the acclimation trajectory of massive *Porites* spp. for a set of host and symbiont physiological traits during exposure to heat (31 °C) and cold (21 °C) for 30 days. Cold acclimation took approximately two weeks and resulted in 'no' or 'inverse' compensation of the performance. In contrast, I found no evidence of heat acclimation holobiont and symbiont performance declined continuously instead of reaching a steady state. These results show that there is no rapid compensatory acclimation response when massive *Porites* spp. are exposed to a change in the

thermal environment, and that compensation of the performance is unlikely to occur in response to short-term variations in temperature. I then investigated the between-season variation in performance of two coral species with contrasting life-history strategies (**Chapter 3**). Acclimation to seasonal variation was species-specific, with an increase of the thermal optimum in summer for a fast-growing and thermally sensitive species (*A. valenciennesi*) and a change of the thermal breadth for a slow-growing and thermally tolerant species (*Porites cylindrica*). Additionally, the symbiont performance was less plastic than the holobiont performance indicating that the reversible acclimation mostly occurs through the coral host.

Comparisons of thermal performance of coral species living in different thermal environments along a latitudinal gradient in the Great Barrier Reef (**Chapter 4**) demonstrated significant geographic variation in the thermal performance among populations. Acclimation of the thermal optimum to the local environment was more accurate for the symbiont performance than for the holobiont. In general, the thermal optimum for holobiont performance was ~4 – 6 °C below the environmental temperature, which may result from an inherent time lag in the mechanisms of acclimation, or from constraints imposed during early ontogeny (i.e., developmental acclimation).

In **Chapter 5** I assessed whether the thermal performance of temperate corals is less sensitive to changes in temperature than that of tropical corals due to their history of exposure to more variable thermal environments. To do this I compared the thermal performance of corals sampled along the GBR latitudinal gradient, with the thermal performance of corals from the Mediterranean Sea. Interestingly, despite clear differences in thermal optima, no observable differences occurred between the performance breadths of temperate versus tropical corals at either the holobiont or symbiont level. This result is likely because all of the

sampled coral species had a wide thermal tolerance, which fully encompassed the total local annual variation in temperature in each location.

Overall, the results of this thesis demonstrate that reef-building corals may be more generalist than previously thought. However, a high degree of inter-colony variability in thermal performance was consistently observed for all of the sampled coral species, even between colonies from the same local population. These findings indicate that despite the mean thermal optima being consistently below the average environmental temperatures for all populations, some individual colonies maintain the capacity to perform well at very high and very low temperatures, which suggest that corals may cope with environmental variability through genetic variation rather than reversible plasticity. Hopefully, such high among-colony variation can contribute to the capacity of coral populations to persist in the face of rapid climate change.

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CHAPTER 1

General Introduction

Temperature has a fundamental influence on the physiology, biology and ecology of all organisms. In general, critical life-sustaining processes like metabolism, growth, fecundity and locomotion occur optimally within a specific temperature range (Angilletta 2009). For instance, metabolic rate is controlled by enzymatic reactions that increase their activity with increasing temperature, yet above an optimal temperature, the activity decreases rapidly and, at even higher temperatures, enzymes denature (Reece et al. 2011). The optimal temperature range for these life-sustaining processes is different among species, and among individuals within a species, and depends on the environmental conditions in which the species evolved, or the individual developed (for example, see Xiang et al. 1996, Mitchell & Lampert 2000, Karlsson & Van Dyck 2005). Consequently, most organisms live in areas with distinct climate conditions that enable them to thrive.

1.1 Thermal heterogeneity of the environment

The environmental temperature varies over space and time, and this variation exerts pressure on the behaviour, physiology and life history of organisms. For instance, following latitudinal gradients over which temperature changes substantially, species diversity is greatest in the tropics and lowest at the poles (Gaston 2000). In contrast, animal body sizes show the opposite pattern, with species that developed at lower temperatures generally being larger than related species that developed at higher temperatures (Blackburn et al. 1999,

Kingsolver & Huey 2008). In addition, the environmental temperature generally becomes more variable and unpredictable (both seasonally and diurnally) at higher latitudes (Janzen 1967). An obvious example is the stronger seasonality of temperature in temperate compared with tropical regions, which can influence the timing of reproduction and development, and affect the behaviour and overall fitness of organisms.

Variability in temperature does not affect all organisms equally, nor does a change in temperature affect an individual the same way at different times during its life cycle. This is because organisms have evolved different strategies to cope with the heterogeneity of their thermal environment. These strategies can be defined by two dimensions: the *thermal sensitivity* and the *thermal regulation* of an organism (Angilletta 2009). Thermal sensitivity is the degree to which the performance of an organism depends on temperature. Organisms with low thermal sensitivity are **thermal generalists** that function over a wide range of temperatures and often inhabit thermally variable environments (Levins 1968), such as marine species that live in the intertidal zone (Somero 2002). Organisms with high thermal sensitivity are **thermal specialists** that function only within a narrow range of temperatures and live in relatively constant environments (Levins 1968), such as many tropical birds and mammals (Janzen 1967). Thermal regulation is the degree to which an organism regulates its own temperature (Angilletta 2009). This ranges from organisms that do not regulate their own body temperature but conform to the environmental temperature, known as **thermal conformers** and including most aquatic invertebrates and amphibians, to organisms that strongly maintain their own body temperatures regardless of the fluctuations in the environment, known as **thermal regulators**[1] such as mammals. Together, the thermal

[1] Thermoregulation can be accomplished by two different ways: through metabolic heat production or external heat gain, which relates to the terms '**endotherm**' and '**ectotherm**'. Endotherm animals generate heat through endogenous metabolic processes, whereas ectotherm animals absorb heat from external sources. However, an endotherm animal is not necessarily a thermal regulator, nor is an ectotherm animal per definition a thermal conformer. In general, endotherms thermoregulate and thermoconformers are ectothermic, but many ectotherms thermoregulate (Withers, 1992).

sensitivity of an organism and its capacity to thermoregulate govern its response to fluctuations in temperature. Indirectly, these responses involve trade-offs in energy allocation and resource acquisition that influence the organism's performance and fitness, and ultimately shape its life history (Angilletta et al. 2003, Angilletta 2009).

1.2 Thermal performance curves

The effect of temperature on the performance of an organism can be quantified with a thermal performance curve (TPC). In this context, performance refers to any measure of an organism's capacity to function such as locomotion, growth or development (Angilletta 2009). Thermal performance traits generally involve physiological processes that respond rapidly to changes in temperature, and are often traits that are measured as rates (e.g. oxygen consumption over time or distance travelled over time; Schulte et al. 2011) which implicitly reflects that performance is governed by the rates of enzyme-driven biochemical reactions that are sensitive to temperature. In general, TPCs are hump shaped (**Figure 1.1**), as performance initially increases with temperature until it reaches a maximum (Pf_{max}).

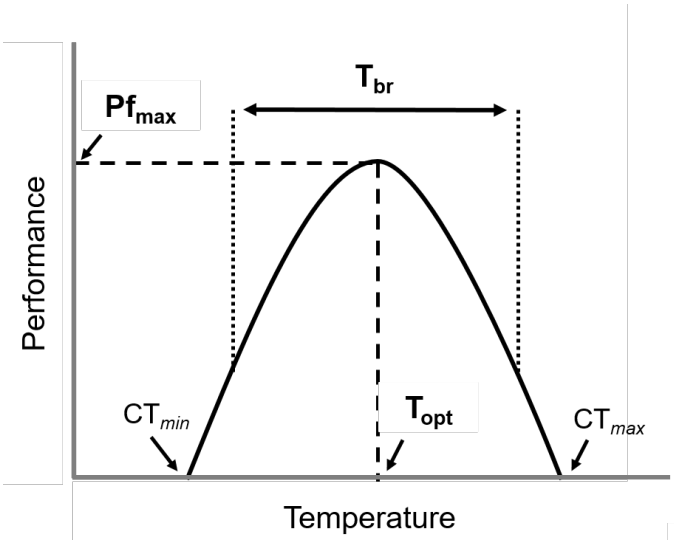


Figure 1.1 Hypothetical thermal performance curve (TPC) with the maximum performance (Pfmax), thermal optimum (T_{opt}), thermal breadth (T_{br}) and critical minimum and maximum thermal thresholds (CT_{min} and CT_{max}).

Logically, this peak is at the optimal temperature for performance (T_{opt}). With further increase of the temperature, performance will decrease (Huey & Stevenson 1979). The intercepts of the curve with the x-axis define the thermal thresholds, with lower and upper critical thermal thresholds, or pejus temperatures *sensu* Pörtner (2001). These thresholds can be used to calculate the thermal tolerance range, that is the temperatures over which performance is positive, although usually the thermal breadth (T_{br}) is estimated, which is the range of temperatures at which the organism performs "well" (usually around 80% of maximum performance; Huey & Stevenson 1979). Therefore, the performance breadth is generally smaller than the absolute thermal tolerance range of an organism.

TPCs are often asymmetrically skewed to the left (i.e., with a steeper decrease in performance at high temperatures compared with the increase in performance at lower temperatures; Martin & Huey 2008). Indeed, there is ongoing debate in the literature as to whether enzyme kinetics should generate asymmetrical or symmetrical TPCs (Gilchrist 1995, Martin & Huey 2008, Asbury & Angilletta 2010). Nevertheless, to estimate the shape of a TPC, and quantify T_{opt} and T_{br}, a function must be chosen that best captures the overall thermal response but not the variation in the data due to random measurement error (Angilletta 2006). By convention, model selection techniques such as Akaike's Information Criterion, seek to identify the simplest function, with the fewest parameters, that captures the majority of the variation in the data (Burnham & Anderson 2003). In the context of fitting TPCs to data, asymmetrical functions, such as Weibull and exponentially modified Gaussian functions, generally contain more parameters and are therefore more complex than symmetrical functions, such as Gaussian and quadratic functions. Consequently, when estimating the shape of the performance curve, Gaussian functions often provide the best fit to the data (Angilletta 2006). The Akaike Information Criterion (AIC) is a commonly used technique to select the model that best describes the data without overfitting (Burnham &

Anderson 2003). Throughout this thesis, I used this model selection technique to compare the fit of different functions to coral thermal performance data.

1.3 Thermal acclimation

Through adaptation and phenotypic plasticity of performance traits, the shape and position of TPCs can vary among species and populations, as well as within the same individual over time. Adaptation involves a genotypic adjustment of the phenotype (e.g., behaviour, physiology or morphology) and requires natural selection on genetic variation to create a population with increased fitness under the environmental conditions of its habitat (Reece et al. 2011). Therefore, adaptation is a process that occurs over large time scales from one generation to the next, and also required time for the new genotypes to disperse throughout the population. In contrast, phenotypic plasticity is a phenotypic adjustment (i.e. acclimatization) of a trait, such as a change in lipid content or growth rate, which allows an individual to optimise its performance in the local environment while maintaining the ability to re-adjust to new environmental conditions if they arise (Reece et al. 2011). Consequently, acclimatization is a process that occurs over smaller time scales than adaptation, such as between seasons, and can be observed on one individual more readily. Individual plasticity falls within two broad categories (Beaman et al. 2016): **reversible acclimation** that occurs constantly throughout an organism's life (Whitman & Agrawal 2009), or **developmental acclimation** that only occurs during early life when a specific trait responds to an environmental cue and becomes fixed during the adult life of the organism (Kinne 1962). Depending on the spatial and temporal heterogeneity of the thermal environment, and the longevity of the organism, a species can be expected to adopt a thermal strategy that maximises fitness through either developmental or reversible acclimation of traits (Berrigan & Scheiner 2004). For instance, in response to seasonal thermal variation, reversible

acclimation is important for maintaining high performance during each season (Gabriel 2005). However, in a homogenous thermal environment, or when within-generation variability in temperature is small, developmental acclimation is favoured as this maximizes performance according to the specific thermal conditions (Angilletta 2009). This leads to a general prediction that developmental acclimation might be more frequently observed in tropical regions where conditions during development are similar to the conditions individuals will experience at maturity. In contrast, reversible acclimation should be more frequently observed at temperate latitudes where conditions change repeatedly within a generation.

Furthermore, acclimation is generally not instantaneous but, rather, occurs via a continuous adjustment of physiological processes over time until a new steady state is reached. Consequently, it is not uncommon to observe a mismatch of the optimal performance with the actual thermal environment due to time lags associated with acclimation (Pfab et al. 2016). Surprisingly, little is known about the actual time course of thermal acclimation, possibly because it may vary between populations, individuals and physiological traits (Schulte et al. 2011, Forsman 2015). A study that investigated the time course of biochemical modifications of rainbow trout during warm (15°C) and cold (5°C) acclimation showed that mitochondrial properties first increased and then decreased during warm acclimation, whereas the pattern was inverse and the response slower during cold acclimation (Bouchard & Guderley 2003). Another study that investigated cold acclimation in fish measured oxidative stress after two days of exposure to 8 °C (Kammer et al. 2011). Nonetheless, when the rate of environmental change is greater than the rate of thermal acclimation, mismatches between T_{opt} and the environment can become increasingly costly and harmful for the organisms (DeWitt et al. 1998, Murren et al. 2015). Clearly, filling this knowledge gap about

the time course of acclimation to temperature change is becoming more urgent because of the potential effects of global warming on aquatic and terrestrial ecosystems.

Phenotypic plasticity can modify the shape or position of a TPC in three ways: by shifting the curve vertically through a change of Pf_{max} (**Figure 1.2 a**), by shifting the curve horizontally through a change of T_{opt} (**Figure 1.2 b**), or by changing the breadth of the curve (**Figure 1.2 c**), which is often accompanied with a change in Pf_{max} (Knies et al. 2006, Angilletta 2009). Due to the large variation in thermal environments, species display a rich diversity of thermal performance curves that vary in thermal optimum, thermal breadth and maximum performance. For instance, the T_{opt} for growth of certain temperate rainforest trees was at lower temperatures than that of tropical species (Cunningham & Read 2003); the thermal breadth for jumping performance of certain frog species was wider in frogs that lived in cooler environments (John-Alder et al. 1988); and tropical *Drosophila melanogaster* populations had a higher critical thermal threshold for heat-induced male sterility than temperate populations (Rohmer et al. 2004). Thermal performance curves facilitate investigation of the variation in thermal performance within and among species from

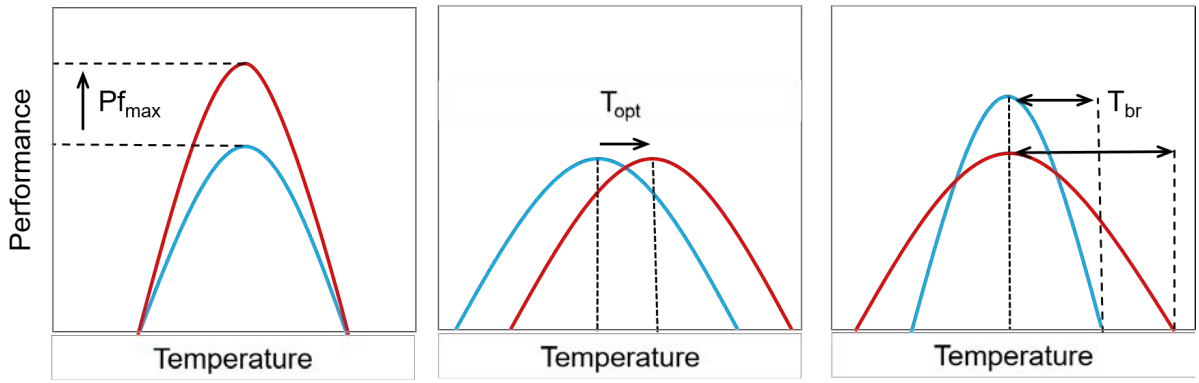


Figure 1.2 Thermal acclimation of the performance can occur through a vertical shift of the performance curve by changing the maximal performance (**a**), a horizontal shift of the performance curve by changing the thermal optimum (**b**), or changing the thermal breadth which is often accompanied by a change in the maximum performance (**c**).

different geographic regions. Such studies also provide an understanding of how environmental conditions shaped thermal physiology and, by quantifying the capacity for local acclimation and adaptation of species, provide insight into the potential of existing populations to persist during periods of climate change.

Changing global temperatures requires species to acclimatize and adapt to new environmental conditions and, therefore, climate changes poses a major threat to the world's ecosystems (IPCC 2014). However, much of our current knowledge of thermal acclimation and adaptation comes from comparative and experimental studies on ectotherms, including lizards (e.g., Huey & Bennett 1987, van Berkum 1988, Xiang et al. 1996, Angilletta et al. 2002), fish (e.g., Pörtner & Knust 2007, Fangue et al. 2008, Donelson et al. 2011) and various insect species (e.g., Sinclair et al. 2012). We can gain new insights into the thermal strategies of ectotherms by investigating species with different life histories, such as corals that have long-life spans, are both hetero- and autotrophic, and cannot use behaviour to thermoregulate. Therefore, to advance our capacity to predict the impacts of climate change on ecosystems worldwide, there is an urgent need for improved knowledge of the mechanisms that underlie thermal acclimation and adaptation for organisms in general.

1.4 Thermal acclimation on coral reefs

Coral reefs harbor the highest concentration of marine biodiversity (Carpenter et al. 2008), and provide spawning, nursery, breeding and feeding grounds for numerous marine organisms (Moberg & Folke 1999). Additionally, coral reefs directly support many millions of people through provision of ecosystem goods and services, such as fisheries, coastal protection, building materials and tourism (Moberg & Folke 1999), valued at hundreds of billions of dollars annually (Costanza et al. 2014). Coral reefs are identified as particularly vulnerable to rising ocean temperatures associated with climate change (Pörtner et al. 2014).

This is alarming because a decline of reef-building corals has extensive ecological, social and economic consequences, including a decrease in biodiversity, reduced coastal protection and loss of income through diminished tourism and coral reef fisheries (Hoegh-Guldberg 1999, Hoegh-Guldberg et al. 2007, Hughes et al. 2017a). However, our understanding of the mechanistic basis of thermal acclimation in corals is limited, and is largely inferred by analogy with other taxa (Gates & Edmunds 1999). Unfortunately, the thermal acclimation strategies used by ectotherms in general cannot be directly extrapolated to corals because, for instance, they are sessile and cannot use behavioral strategies to escape or generate heat. In addition, corals are symbiotic organisms which means that thermal acclimation must be understood at both the level of the coral host (or so-called coral 'holobiont', which includes the coral animal and associated microorganisms, including the symbionts), and of its photosynthetic symbionts.

The foundation for the high biodiversity of coral reefs is the complex three-dimensional reef matrix formed by the calcareous skeletons secreted by reef-building (scleractinian) corals (Graham & Nash 2013), which thrive due to the symbiosis between the scleractinian coral host and the photosynthetic algal symbionts (Symbiodiniaceae). Symbionts are beneficial to the coral host as they convert light energy into organic carbon that the host uses to support metabolic processes. Meanwhile, metabolic waste products produced by the coral host are a source of nutrients for the symbionts (Muller-Parker et al. 2015). Whilst this symbiosis is fundamental for coral reefs to flourish, small changes in the physical parameters of the environment (such as temperature, light or salinity) can lead to the expulsion of symbionts which often results in visibly bleached corals (Jokiel & Coles 1990, Gates et al. 1992, Muller-Parker et al. 2015). Over the past three decades, increased sea surface temperatures related to global warming caused local, regional and global bleaching events, many of which resulted in significant coral mortality (Hoegh-Guldberg 1999, Heron

et al. 2016, Hughes et al. 2017b), and contributed to a decline in the global coral cover (Bruno & Selig 2007, De'ath et al. 2012). Numerous studies estimated the fate of reef corals under future climate scenarios (e.g. Hoegh-Guldberg 1999, Hughes et al. 2003, Carpenter et al. 2008, Teneva et al. 2012), and predicted that the frequency and severity of bleaching events will increase with climate change by mid-century or earlier. Probably for this reason, there is a strong focus in coral research on the bleaching susceptibility due to thermal stress in corals (e.g., Gates et al. 1992, Fitt et al. 2001, Berkelmans & Van Oppen 2006), with an emphasis on quantifying the maximum thermal thresholds for coral bleaching and survival (Coles et al. 1976, Brown et al. 2000, Fitt et al. 2001, Maynard et al. 2008, Berkelmans 2009).

Research into the capacity of corals to cope with increased temperature progressed in four directions (Logan et al. 2014): i) identification of the different Symbiodiniaceae genera hosted by the coral and symbiont shuffling towards more thermal tolerant genera (e.g. Berkelmans & Van Oppen 2006, Silverstein et al. 2015), ii) physiological acclimation of the coral host or symbiont to make the coral more thermally tolerant (e.g. Coles & Brown 2003, Oliver & Palumbi 2011), iii) adaptation of the coral host or symbiont to temperature increase and natural selection on more heat tolerant genotypes (e.g. Barshis et al. 2013), and iv) community shifts towards more heat tolerant coral species (e.g. Van Woesik et al. 2011, Edmunds et al. 2014). While all these studies provide useful insights about coral persistence under to global warming, they focused mainly on the maximum thermal thresholds for coral bleaching and survival. However, climate change is causing a gradual increase in average temperatures together with episodes of abnormally high temperatures (IPCC 2014) and, therefore, the thermal optimum and thermal breadth will determine coral fitness in addition to the maximum thermal thresholds. Thus, in addition to research focused on thermal

thresholds, quantification of thermal performance across the entire temperature range is necessary.

1.5 Thermal performance of corals

Since temperature affects performance at different levels of biological organization, performance traits that measure the whole-organism response are generally different to those traits that directly reflect specific physiological responses and biochemical reactions (Schulte 2015). Coral performance traits that reflect the whole-organism metabolism include oxygen production and consumption (i.e., photosynthesis and respiration rate) and oxidative stress, along with growth rate or calcification rate. These traits likely reflect a composite response of multiple underlying mechanisms but provide an overview of the effects of temperature on the organism. Symbiont specific traits that affect coral performance are related to photosynthesis, but are measured at the level of the photosystems within symbionts, such as maximum photosynthetic quantum yield (F_v/F_m) and electron transport rate. These traits are discussed in more detail in Chapters 2 – 5 of this thesis.

To date, studies that specifically measured thermal performance curves of coral species are virtually non-existent, with only one study documenting the thermal performance of the Mediterranean coral *Oculina patagonica* (Rodolfo-Metalpa et al. 2014). This study showed that the T_{opt} and T_{br} for photosynthesis and some other symbiont-related traits were similar between colonies sourced from four regions with very different thermal regimes. So far, geographic variation in the T_{opt} for coral growth was demonstrated for only one species, *Pocillopora damicornis* (Clausen & Roth 1975), but these results were challenged by another study that showed no difference between optimal temperatures for net productivity of *Montastrea annularis* among sites, despite differences in ambient temperature regimes (Castillo & Helmuth 2005). Lastly, the TPC for calcification rates of the tropical coral

Galaxea fascicularis was similar to that of the azooxanthellate *Dendrophyllia sp.* with an optimal temperature around 25 °C which corresponded approximately to the mean environmental temperatures in summer (Marshall & Clode 2004). The ambiguity about the species-specific and environmental controls on coral thermal tolerance shows that there are gaps in our knowledge about the extent of plasticity of coral thermal performance curves. In addition, despite evidence that both the coral host and symbiont can acclimate to changes in the thermal environment (Coles & Brown 2003, Oliver & Palumbi 2011), knowledge of the mechanisms that underlies these acclimation responses is lacking (Edmunds & Gates 2008).

1.6 Thesis overview

In this thesis, I generate new knowledge in relation to four key knowledge gaps about how thermal performance of corals varies in response to environmental heterogeneity.

Acclimation involves changes at molecular, cellular and physiological levels and, consequently, takes time. Knowledge about the duration of acclimation is important, because it provides insight into the capacity of corals to match their performance to short-term (i.e. daily) and/or long-term (i.e. seasonal) fluctuations in their thermal environment. To date, the research focus on maximal thermal thresholds of coral species resulted in abundant experimental data, but there is inconsistency in the duration of exposure to altered temperatures used in these studies, ranging from a few days (Berkelmans & Willis 1999) to a month (Jokiel & Coles 1977b). Such variation in experimental duration shows that the rate at which corals acclimate to temperature change is unknown. Consequently, the aim of **Chapter 2** was to quantify the duration of the physiological adjustments that occur in the coral host and symbiont following a change in temperature. Therefore, I exposed coral fragments to heat and cold during 30 days, and measured a set of host and symbiont physiological traits daily and weekly to resolve the coral acclimation trajectory and duration.

Reversible acclimation potentially allows corals to continuously adjust their physiology so that the ambient environmental conditions are close to the thermal optimum (Gabriel 2005). However, in tropical environments where there is a relatively small difference in temperature between seasons, it may not be beneficial to continuously adjust the physiology due to the costs and duration of acclimation. Previous studies focussed on the capacity of corals to acclimate their upper thermal threshold, but overlooked whether such changes affect performance at other temperatures experienced within the ambient environment. For instance, reversible acclimation of the bleaching threshold between seasons was demonstrated for certain coral species (Berkelmans & Willis 1999), but there is little understanding of the mechanisms that cause this response. In fact, two scenarios of acclimation are possible through which the upper thermal threshold changes, the thermal optimum could shift (**Figure 1.2 b**) or the thermal breadth could increase (**Figure 1.2 c**). In the former case, increasing T_{opt} to promote survival at high temperature may compromise performance at low temperatures and, in the latter, an increase in T_{br} is often accompanied by a decrease in Pf_{max} such that performance is compromised under the conditions that the coral experiences for most of the year. Therefore, the aim of **Chapter 3** was to understand whether and how the thermal physiology of corals changes between seasons. To achieve this aim, I quantified the performance curves of two corals species in summer and winter, and compared how the maximum performance, thermal optimum and thermal breadth (Pf_{max}, T_{opt}, and T_{br}) of each species differed between seasons.

Species with broad geographic distributions often encounter a wide range of temperatures. However, an individual experiences a much smaller range of these temperatures throughout its life, especially for sessile organisms like corals that live in tropical regions where the thermal environment during development is likely to be similar to that at maturity. Therefore, developmental acclimation and/or reversible acclimation may

allow coral populations to specialize their performance to match their local thermal environments. Consequently, the performance of corals from populations inhabiting reefs with different mean environmental temperatures should vary predictably, with T_{opt} (the optimal temperature for performance) of populations from warm environments occurring at a higher temperature than that of populations from cooler thermal environments. Latitudinal variation in the T_{opt} was observed for insects (Sinclair et al. 2012) and lizards (Huey & Kingsolver 1993) among others, but never among coral populations. The Great Barrier Reef (GBR) offers an ideal environment to investigate geographic variation in thermal performance as the thermal environment on northern reefs is distinctly warmer and less variable than that on southern reefs. Consequently, the aim of **Chapter 4** was to quantify local thermal acclimation of coral populations along a latitudinal gradient in the GBR. For this, I measured the thermal performance of two coral species in the northern, central and southern GBR. A better understanding of the plasticity of the thermal performance of coral species, and how this varies between geographic locations, will provide insight into how global warming might impact the dynamics of coral metapopulations which are naturally distributed across thermally heterogeneous environments.

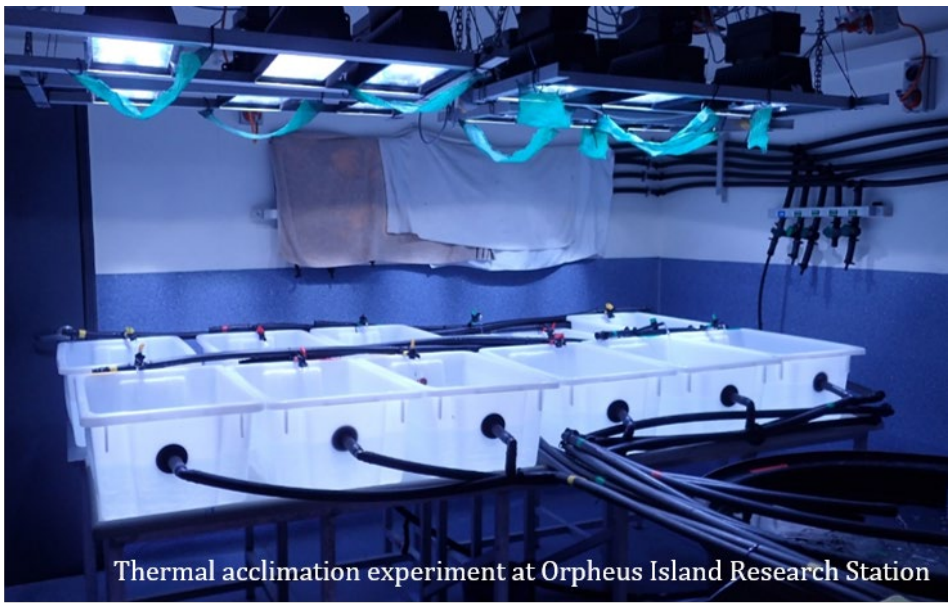
Lastly, temperate and tropical regions generally have distinct climates with, on average, cooler and more variable temperature regimes at temperate latitudes compared with tropical latitudes. It has long been argued in the ecological literature that temperate organisms that experience a broad range of environmental conditions have a broader tolerance range than tropical organisms that experience only a small range of environmental conditions (e.g. Janzen 1967, Stevens 1989). By comparing the thermal performance breadth of temperate organisms with that of tropical organisms, this theory was supported by some studies (e.g. Feder & Lynch 1982, van Berkum 1988) but refuted by other studies (see review by Angilletta 2009). For corals, the number of studies on temperate corals is limited compared to

that of tropical species, making it difficult to compare the thermal sensitivity between these groups. Therefore, in **Chapter 5**, I compared the thermal performance of tropical corals with that of temperate corals to assess whether the performance of temperate corals is indeed less sensitive to changes in temperature and whether one or both groups live at or above the optimal temperature for performance. The comparison of the thermal performance between tropical and temperate organisms became more urgent recently, as it is predicted that the impact of global warming may be significantly greater in the tropics due to the greater sensitivity of these organisms to thermal change (Tewksbury et al. 2008).

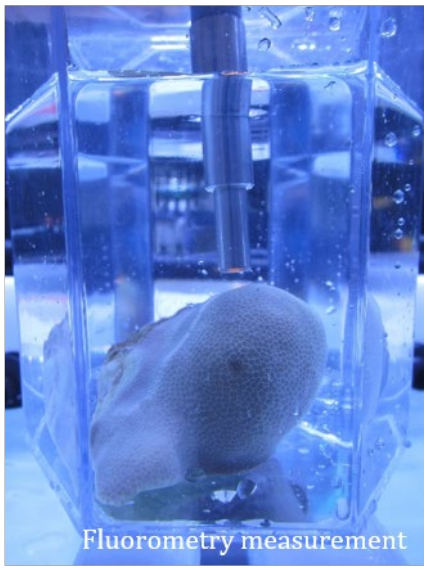
In the final chapter of this thesis (**Chapter 6**), I summarise the key results of my research and evaluate whether and how corals and their symbionts acclimatize to temporal and spatial heterogeneity of the thermal environment. I then identify factors that could promote or constrain coral and symbiont thermal acclimation. I conclude with some suggestions about future research directions that can advance our understanding about the thermal biology of coral reefs, and the implications of my findings in the context climate change.

CHAPTER 2

Trajectory of thermal acclimation to cold and heat exposure of a scleractinian coral[2]


Thermal acclimation experiment at Orpheus Island Research Station


massive *Porites* spp.


Fluorometry measurement

[2] This chapter is prepared for submission to the Journal of Experimental Biology

2.1 Abstract

Beneficial acclimation improves organism performance under environmental change. However, species differ in their capacity to acclimate and in their rate of acclimation, and there are several possible levels of compensation of performance. This study investigated the time course of thermal acclimation, and the level of compensation of performance upon acclimation, for various physiological traits of massive *Porites* spp. when exposed to heat (31 °C) and cold (21 °C) for 30 days. Results showed that heat acclimation did not occur, because traits continuously declined over time since the onset of the new temperature regime rather than converging to a steady state. In contrast, cold acclimation took approximately two weeks and resulted in no or inverse compensation of performance. These results show that there is no rapid compensatory acclimation response when massive *Porites* spp. are exposed to an immediate change in the thermal environment, and that compensation of the performance is unlikely to occur in response to short-term variations in temperature. Instead, massive *Porites* spp. appear to cope with variation in their thermal environment using a thermal generalist strategy.

2.2 Introduction

Species and populations live in variable thermal environments. For instance, temperatures can soar during the day and plummet at night, and these fluctuations can differ from one season to another. Similarly, the mean environmental temperature generally decreases with increasing latitude and elevation (Clarke & Gaston 2006). However, the extent of heterogeneity of the thermal environment that an organism experiences differs between species depending on life-span (Angilletta 2009). For instance, if the temperature variability primarily occurs between seasons, a species with a lifespan of several years will experience

this environment as being heterogeneous, while individuals of a species with a life-span of one month will experience the same environment as being homogeneous, but heterogeneous between generations. Likewise, species with broad spatial distributions covering a range of latitudes will encounter a larger range of temperatures than species with restricted distributions.

Species differ in the degree to which the performance of an individual depends on temperature (i.e. thermal sensitivity; Angilletta 2009). The performance of a species with low thermal sensitivity (often referred to as 'thermal generalists') is not strongly influenced by temperature and these species can maintain physiological functioning over a broad thermal range. In contrast, the performance of a species with high thermal sensitivity ('thermal specialists') depends strongly on temperature, and individuals suffer poor performance when the environmental temperature changes. Therefore, thermal specialists need to adjust their physiology and/or behaviour to minimize the loss of performance when exposed to a new temperature, in a process known as thermal acclimation (Prosser 1991). Ideally, a species would respond instantly to a change in temperature and acclimate perfectly to the new temperature so that its performance is always optimal. In reality, however, organisms differ in both the time it takes to acclimate, ranging from several days to several weeks (Withers 1992), and in the level of performance that is achieved after acclimation. Hence, among-species variation in capacity for thermal acclimation can be scaled according to both the duration and the outcome of acclimation (Loeschcke & Sørensen 2005).

The mechanisms through which temperature is thought to affect performance relate to the acute effects of temperature on biochemical reactions, but this acute response can be altered by exposure to temperature over longer time periods (i.e., by acclimation; Healy & Schulte 2012). Thermal acclimation takes time because it involves a cascade of processes: the change in temperature needs to be detected and then converted into a cellular response that

activates molecules to induce a change in the physiology (Angilletta et al. 2006). Although this cascade generally applies to all individuals, the reaction time of each step, and the number and type of physiological traits involved in acclimation, differs between species (Schulte et al. 2011). Moreover, thermal acclimation of whole-organism performances (e.g., traits like locomotion or growth) can take longer than thermal acclimation of each of the various cellular and physiological traits that interact to influence whole-organism performance (Sidell et al. 1973, Somero 2012). Additionally, the duration of acclimation depends on the magnitude and direction of the temperature change. For instance, given that enzyme reactions are temperature dependent and their rates typically increase with temperature (Somero 1969), acclimation to heat requires different physiological changes than acclimation to cold (Das & Prosser 1967).

Thermal acclimation can lead to different outcomes for the organism (Precht 1958, Hazel & Prosser 1974). During the transition from one temperature state to another (i.e., acute exposure to a new temperature), the performance initially decreases rapidly because cellular functioning is poor at the new temperature (t_0 to t_1, **Figure 2.1**). Subsequently, when exposure is chronic, acclimation can lead to changes in various physiological processes that partially or completely compensate for the lower performance. The first possible outcome of these physiological changes is that acclimation could return performance to the previous steady state, or to a slightly higher or lower level (respectively, 'complete compensation', 'overcompensation', or 'partial compensation' at t_2, **Figure 2.1**). Such partial compensation may represent the presence of physiological constraints (e.g., nutrient limitation, or body size dependence of metabolic rates) that prevent complete compensation, or it may be an adaptive compromise due to trade-offs between multiple traits (Huey & Berrigan 1996). Acclimation could also stabilise performance at the lower level reached at the end of the transition period ('no compensation'), which may an adaptive strategy of organisms living in constant

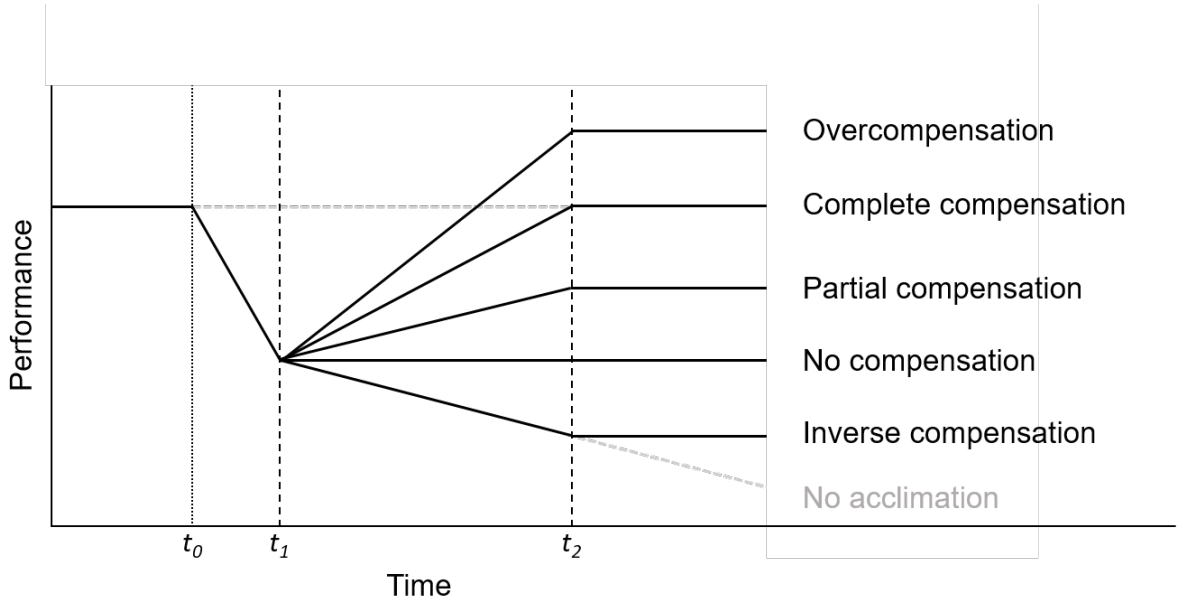


Figure 2.1 Thermal acclimation trajectories and outcomes after exposure to a new temperature. Exposure to a new temperature (at t0) results in a loss of performance, that after some time (t1) will slow down or reverse until it reaches a new steady state (at t2). The performance rate at this new steady state compared to the original rate may be higher (overcompensation), similar (complete compensation), or lower (partial, no or inverse compensation). No acclimation might be when performance did not change in response to a change in temperature, or when performance does not reach a new steady state. (Graph modified from Huey & Berrigan 1996)

environments or when the costs of the physiological changes required for compensation outweigh the benefits (Huey & Berrigan 1996). Finally, acclimation might be constrained such that performance continues to decline over time to reach a steady state at a lower level ('inverse compensation'). In contrast, processes of acclimation do not occur when the performance does not change at the onset of a new temperature (thermal sensitivity of the measured trait is zero), or when performance continues to decline after a change in temperature and does not reach a new steady state is reached (**Figure 2.1**). Understanding the acclimation capacity of a species requires continuous measurement of performance over time following an acute or chronic change in temperature. Moreover, as different traits can

acclimate at different rates, multiple traits that are relevant to physiological functioning should be measured.

Changing global temperatures require species to acclimatize and/or adapt to new environmental conditions to maintain performance. Adaptation depends on the rate of climate change and generation time of the species. If the change in climate is slow and the direction of change is constant, short-lived organisms may adapt successfully through directional selection (Lande, 2009). However, climate change generally occurs across few generations or within generations and often lacks a clear signal to drive directional selection (Seebacher et al. 2015). Consequently, the capacity for thermal acclimation is essential for a species to survive. However, studies documenting the time course of acclimation are sparse in the literature (Somero 2015), and acclimation outcomes often misinterpreted (Huey & Berrigan 1996). For instance, when the performance of an organism is lowered after exposure to a thermal stress, it is often concluded that acclimation did not occur. Yet, as mentioned above, lower performance after a change in temperature can be evidence of 'partial' or 'no' compensation rather than evidence that acclimation did not occur (Edmunds & Gates 2008). Thus, robust interpretations about acclimation require changes in performance to be monitored continuously over time after a change in the temperature regime. Furthermore, the time course of acclimation differs among traits and direction of exposure (i.e. heat-to-cold or cold-to-heat; Withers 1992), emphasizing the need to monitor how performance changes over time for multiple traits that are relevant to the thermal acclimation of the whole organism.

Coral reefs are identified as particularly vulnerable to rising ocean temperatures associated with climate change (Pörtner et al. 2014), and assessing whether corals can acclimate to global climate change has been central in coral research over the past two decades (e.g., Gates & Edmunds 1999, Hoegh-Guldberg 1999, Coles & Brown 2003, Oliver & Palumbi 2011, Howells et al. 2013). Corals live in symbioses with a photosynthetic

symbiont (family Symbiodiniaceae) but changes in temperature can disrupt this symbiosis leading to the expulsion of symbionts from the coral tissues which often results in visibly pale or white 'bleached' corals (Jokiel & Coles 1990, Gates et al. 1992, Muller-Parker et al. 2015). In general, the physiology of both the coral host and the symbiont is compromised by thermal stress, as temperature affects various enzymes and reactions involved in photosynthesis and respiration that can lead to photoinhibition, oxidative stress and cellular damage (Lesser 1997, Warner et al. 1999, Fitt et al. 2009). However, a general lack of knowledge about the trajectory of thermal acclimation in corals means that the duration of thermal acclimation experiments differs between studies, with acclimation times ranging from 24 h (Oliver & Palumbi 2011), to several days (Coles & Jokiel 1977, Berkelmans & Willis 1999, Fitt et al. 2009, Leggat et al. 2011), to 2 - 3 weeks (Howells et al. 2012; Roth et al. 2012) or one month (Jokiel & Coles 1977a). Roth et al. (2012) monitored growth rates and photosynthetic performance of the coral *Acropora yongei* for 20 days after exposure to cold (21 ºC) and heat (31 ºC). In that study, cold exposure initially reduced growth rates and photosynthetic performance, but these traits stabilized after ~2 weeks at a reduced level ('no compensation', **Figure 2.1**). In contrast, effects of heat exposure on growth and photosynthesis were delayed by ~ 5 days, after which the performance of the coral and symbiont declined rapidly and irreversibly ('no acclimation', **Figure 2.1**). Additional studies on different coral species are required to determine whether such dynamics are consistent among coral species.

This study aimed to define the acclimation trajectory and duration of thermal acclimation of massive *Porites* species after exposure to cold and heat. I measured the performance of multiple coral traits influenced by the holobiont physiology (i.e. the coral animal and associated microorganisms, including the symbionts) and symbiont physiology, to assess the acclimation trajectories of processes that determine whole-organism thermal

acclimation in corals. I hypothesized that there would be a rapid initial decrease of the performance followed by the onset of acclimation that would slow down or reverse the decline in performance, with trajectories potentially ranging from no compensation to complete compensation. Specifically, I expected that acclimation at symbiont level would occur quicker than at holobiont level, and thus that the onset of acclimation would be first detected at symbiont level. I also expected that acclimation of the symbiont would result in complete performance, whereas that at holobiont level would be too slow recover completely within the timeframe of the experiment. Therefore, I monitored the set of physiological traits twice daily during the first week after temperature exposure, and daily during the remaining 3 weeks. One month was chosen as we expected steady states to occur within this time, or at least be able to identify the acclimation trajectory. Additionally, the concentration of chlorophyll pigments, proteins and antioxidants was quantified at several time points during acclimation to detect oxidative stress and structural changes in the symbiont photosynthetic apparatus. Harmful reactive oxygen species (ROS) can be generated by the coral host at increasing levels during thermal stress. High ROS concentrations may lead to interference with normal cell functioning, inducing oxidative stress (Lesser 1997). Additionally, ROS synthesis requires energy, which may compromise other energy demanding processes, such as cellular respiration (see review by Sørensen et al. 2003). As a response to thermal and oxidative stress, the concentration of antioxidants, proteins, and chlorophyll and fluorescent pigments may change (Weis 2008, Palmer et al. 2009, Roth & Deheyn 2013). Therefore, I quantified changes in these proteins and pigments over time, in addition to measuring changes in symbiont photophysiology, and whole-coral photosynthesis and respiration. Together these results assess the physiological adjustments, outcome and trajectory of thermal acclimation at various levels of biological organization of massive *Porites* species.

2.3 Materials and Methods

2.3.1 Experimental design

To quantify the dynamics of thermal acclimation I measured several thermally sensitive physiological traits of massive *Porites* spp. exposed to heated, chilled or ambient seawater during one month. Massive *Porites* spp. were used because of their high abundance in the Indo-Pacific region (Done 1982, Veron 2013) and because they are known to be thermally tolerant (Loya et al. 2001). Fragments of different colonies (N = 50 colonies; size approximately 5 by 5 cm; coral identification based on morphological characteristics) were collected by hand using a hammer and chisel while SCUBA diving at 3 – 5 m depth at reefs around Orpheus Island (18° 37′ 06″ S 146° 29′ 37″ E), Great Barrier Reef, Australia. Fragments were immediately transported to Orpheus Island Research Station and randomly distributed among twelve experimental tanks (50 l), five per tank (**Figure 2.2**), set-up in an air-conditioned temperature-controlled room. The tanks were supplied with filtered seawater (15 µm) through a semi-closed flow-through system, where seawater from the reef flat was maintained in three large sumps (500 l) equipped with submerged pumps (Aquapro AP1050, Aquatec, Perth, Australia) that distributed the water to the tanks at a flowrate of approximately 45 l h^{-1}. Overflow from each tank was returned to the corresponding sump and overflow from the sumps returned the water to the reef flat. A small air stone in each sump provided a constant stream of microbubbles for aeration. The ambient water temperature and salinity ranged between 25.5-27 °C and 34-36 PSU respectively. Temperature in the tanks was measured three to four times daily using a hi-accuracy dual thermometer (Traceable 4338, Control Company, Friendswood, USA). Temperature in the sumps was recorded by data loggers every 15 minutes (Hobo model UA-002-08, Onset, Massachusetts, USA). Irradiance was supplied by 12 metal halide lamps (150 W, Oracle, Sylvania, Australia) with

shading in place to provide 185-210 µmol photons m^{-2} s^{-1} (corresponding to the average daily irradiance at ~3 m depth) with a 12 h light/dark cycle. Irradiance was measured with a LI-1400 light logger (LI-COR, Lincoln, USA) with a spherical underwater quantum sensor (LI-193). Corals were fed every three days with freshly hatched *Artemia salina* nauplii. Fragments were given two weeks to recover and acclimate to tank settings.

Following the recovery period, the water temperature in one sump (hereafter 'heated') was increased by 5 ºC (to 31.7 ± 0.2 °C) on the morning of day zero using a diesel generated heater-chiller unit external to the aquarium room and two additional bar heaters (Visi-Therm, 300 W) inside the sump. At the same time, the water temperature in another sump (hereafter 'chilled') was decreased by 5 ºC (to 20.8 ± 0.4 °C) using the same external heater-chiller unit and an additional water chiller (Teco SeaChill TR20, Ravenna, Italy) inside the temperature-controlled room that was connected to the sump. These temperatures (21 °C and 31 °C) approximate the lowest (winter) and highest (summer) temperatures that the corals may experience in the field annually (see also Chapter 2). However, the change in my experiment was more abrupt, as daily fluctuations of the seawater temperature on the reef are on average 0.5 °C (seawater temperatures obtained from the Australian Institute of Marine Science data-

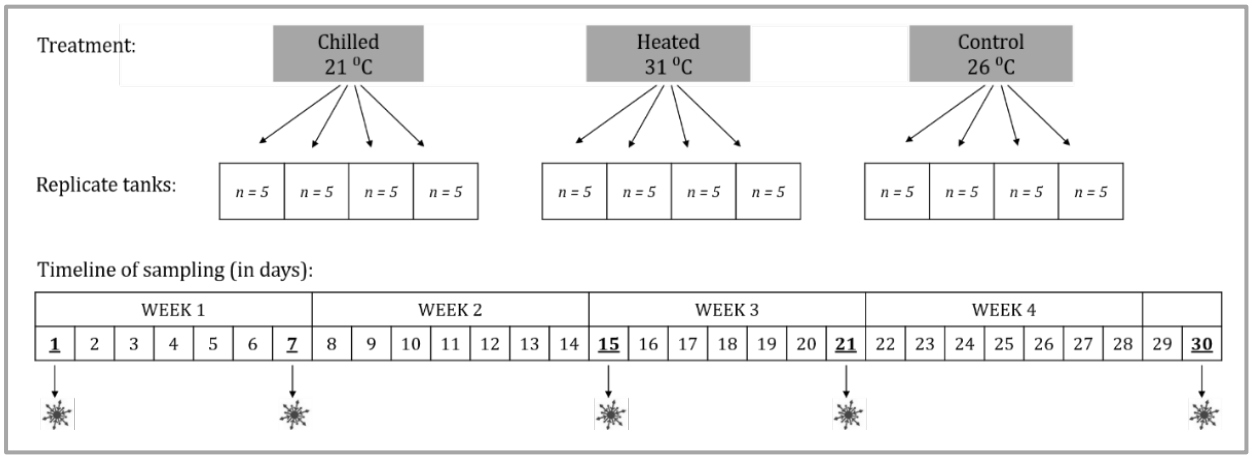


Figure 2.2 Experimental design to test thermal acclimation of massive *Porites* spp. (N = 50). The snowflakes indicate the days at which fragments were collected for tissue analyses.

portal; AIMS 2017). The water temperature in the third sump remained at ambient temperature (26.4 ± 0.6 °C) and this was stabilized from day/night fluctuations by air-conditioning in the room. At the onset of the temperature treatment, the water temperature in the heated and chilled tanks was increased or decreased at a rate of 0.2 °C per hour until it was stabilized at 30.6 ± 0.3 °C in the heated tanks and 21.3 ± 0.2 °C in the chilled tanks (**Figure 2.2**). The water temperature in the ambient tanks was at 25.9 ± 0.4 °C. The first set of measurements were taken the morning following the onset of the temperature change and this continued for 30 days.

I expected that performance would decrease rapidly during the first week of exposure to the new temperature. Therefore, during the first week, every morning and evening, the maximum quantum yield (F_v/F_m) was measured using Pulse Amplitude Modulated (PAM) fluorometry and the oxygen production and consumption was measured using respirometry (details about fluorometry and respirometry are expended later). After seven days, I expected that the changes in performance would occur at a slower pace because thermal acclimation slowed down or reversed the initial decline in performance and respirometry measurements were only performed in the evening. The measuring interval of F_v/F_m did not change, since this is a rapid and non-invasive method to assess the corals' health (Fitt et al. 2001). In addition, daily from day 9 onwards, rapid light curves (RLCs) were performed around noon using PAM fluorometry to assess if the carbon fixation component of photosynthesis was more sensitive than F_v/F_m. For tissue analyses, one randomly chosen fragment from each tank was frozen on day 1, 7, 13, 21 and 30 (**Figure 2.2**).

Observations of the tentacle expansion (categorized as fully expanded, partially expanded, retracted but visible, or not visible, and scored as 4, 3, 2, 1 respectively) were made every evening prior to the measuring the maximum quantum yield, as an indication of polyp activity. Additionally, observations of coral colour were made daily at noon using a

standardized colour chart (Coral Health Chart, CoralWatch, University of Queensland, Australia) to detect tissue paling and/or bleaching (scored on a scale of 1 to 6 from pale to dark). Loss of coloration (paling) of the coral is due to a reduction in the symbiont population and/or a reduction of the photosynthetic pigment per symbiont (Brown 1997), and occurs gradually to eventuate in corals that are 'bleached' white with very low pigment and symbiont concentrations.

2.3.2 Respirometry

Rates of net photosynthesis (Pnet) and respiration (R) were measured in transparent experimental cells made of Plexiglas (five cells, approximately 10 cm in diameter and 10 cm high, containing 1166 ml ± 9 ml) during one hour. Each cell was filled with filtered seawater (15 µm) and four cells contained a coral fragment (randomly chosen from each tank) placed on a PVC stand, while one control cell remained empty (to correct for non-coral oxygen production and consumption). The cells were placed in a water bath that received water from the heated, chilled or ambient sump to control the temperature inside the respirometry cells. A submersible magnetic stirrer plate (MIXdrive 6, 2mag, Muenchen, Germany) and magnetic stirrer bar inside each cell provided continuous mixing of the water. The dissolved oxygen concentration in the water was measured using oxygen probes (LDO101, Hach, Loveland, USA) connected to a logging device (HQ40D, Hach) at one minute interval. Oxygen probes measure the oxygen saturation of the water and therefore respiration rates are likely an underestimation of the absolute respiration rates as measured with higher resolution equipment such as micro sensors (Kühl et al. 1995). However, oxygen probes provide enough accuracy for comparison of differences in respiration at different temperatures. Pnet rates were measured at a light intensity of 300 µmol photons m^{-2} s^{-1} provided by two wide beam lamps (Oracle, Sylvania, Australia) with 150W metal halide light. R rates were measured in

the dark after the photosynthesis measurements. At the end of the respirometry measurements, fragments were returned to their experimental tank. Pnet and R rates of each fragment were corrected by subtracting the differential oxygen concentration of the empty control cell and multiplying by the water volume of the cell. To allow for comparison between fragments and species, Pnet and R rates were normalized to coral skeletal surface area (see "Tissue Analyses" below for details of surface area measurement).

2.3.3 Fluorometry

Symbiont specific traits were measured using a DIVING-PAM fluorometer (Walz, Germany). The maximum quantum yield (F_v/F_m) describes the maximum yield of photosystem II (PSII) when all reaction centres are open. Thermal stress can lower F_v/F_m through inhibition of PS II reaction centres and increased heat dissipation. F_v/F_m was measured with a fibre optic probe at a fixed distance (~ 5 mm) from the coral surface. A red actinic measuring light was applied to determine the minimal chlorophyll fluorescence yield (F_o) after which a suturing light pulse was given which closed all reaction centres and induced the maximal fluorescence yield (F_m). The photochemical yield was then calculated as $(F_m-F_o)/F_m$. Per coral fragment, an average of three F_v/F_m measurements at random points on the coral surface were taken. Measurements were taken on dark-adapted fragments in the morning before the lights would turn on, and in the evening at least two hours after lights turned off.

In addition, rapid light curves (RLCs) were measured on light-adapted fragments (two per tank) by exposing a fragment to a series of nine saturating light pulses, each light pulse followed by a 10 s interval of exposure to a low actinic light intensity that increased in intensity after each step. The saturating light pulses allowed for calculation of the effective quantum yield ($\Delta F/F_m$') which describes the amount of energy used in photochemistry by

PSII under steady-state light conditions. By increasing the light intensity in between the light pulses, fewer reaction centres were open to process the light energy and more light was reemitted as fluorescence, therefore $\Delta F/F_m$' decreased. At each light pulse, rETR was then calculated as

$$rETR = \Delta F/F_m' * PAR * 0.84 * 0.5 \quad \text{(Eq. 2.1)}$$

where PAR was the actinic light intensity, 0.84 was a factor for the assumed light absorbance of the sample and 0.5 was a factor for the ratio of PSII and PSI reaction centres.
Hence, each RLC provided nine measurements of $\Delta F/F_m$' and rETR per fragment, of which the maximum $\Delta F/F_m$' and maximum rETR ($rETR_m$) were recorded. Finally, the excitation pressure (Q_m) over PSII was calculated as

$$Q_m = 1 - (\Delta F/F_m') / (F_v/F_m) \quad \text{(Eq. 2.2)}$$

where $\Delta F/F_m$' is the effective quantum yield of the light-adapted sample and F_v/F_m is maximal quantum yield of the dark-adapted sample. Q_m describes the extent to which the photosynthetic capacity of the fragment is reduced in the light compared with its maximal capacity in the dark. Values close to zero indicate that the reaction centres remain open even when exposed to a high light intensity, suggesting that photosynthetic rates are light-limited, whereas values close to one indicate that the reaction centres are closed, suggestion photoinhibition (Iglesias-Prieto et al. 2004). Q_m was calculated for each day and each tank by dividing the tank average of $\Delta F/F_m$' by the tank average of F_v/F_m.

2.3.4 Tissue analyses

Fragments were transported on dry ice to the laboratory at James Cook University and stored at -80 ºC for tissue analyses. Surface areas were determined by wrapping the upper living tissue of the fragments with aluminium foil (Marsh 1970), flattening the foil on a hard

surface, taking a planar photograph of the foil with a scale bar, and quantifying the surface area by digital image analysis software (ImageJ, version 1.51n, National Institutes of Health, USA). A subsample (~ 4 cm diameter) of each fragment was taken using hammer and chisel, after which fragment surface areas were determined as described above. Tissue was removed from the skeleton of the subsamples by air blasting into 10 ml of phosphate buffer solution (PBS) within a plastic bag. The coral slurry was homogenized for 30 s using a homogenizer (IKA T25, Ultra-Turrax, Germany) and centrifuged for 10 min at 5,000 k. Subsamples of the supernatant were collected for total protein, fluorescent protein and antioxidant extraction and stored at -80 ºC, while the pellet was processed immediately for chlorophyll extraction.

To determine the chlorophyll concentration, 5 ml of 90% acetone was added to the pellet, left at 4 ºC in darkness overnight, centrifuged at 5,000k for 15 min, and added in triplicates (200 µl) to a multiplate well. Absorbance was measured at 630, 663 and 750 nm using a spectrophotometer (Spectramax M2 Reader, Molecular Devices, USA) and chlorophyll (chl) *a* and *c2* concentrations were calculated according to Jeffrey and Humphrey (1975b). The total amount of chl *a* and chl *c2* was standardized to the surface area of the subsample (chl $a + c2$ cm^{-2}).

Total protein content was determined following the method of Palmer et al. (2009). A standard curve was prepared using bovine serum albumen (BSA) with concentrations ranging up to 2 mg ml^{-1}. The stored supernatant (hereafter referred to as samples) and standard curve concentrations were sonicated on ice for 60s, left on ice for 5 min, vortexed for 20 s, left on ice for another 5 min and centrifuged at 2900 k for 5 min. Standard and sample (20 µl) were transferred in triplicate to multiplate microwells and 180 µl of RED 660 Protein Assay Reagent was added into each well and mixed by pipet. The optical density was immediately measured at 660 nm using a spectrophotometer (Spectramax M2 Reader, Molecular Devices, USA). Total protein concentration of each sample was calculated relative to the standard

curve and standardized to the surface area of the subsample as described above (mg protein cm^{-2}).

Fluorescent protein content was measured immediately following the protein determination. For this, each well was excited at 280 nm and the emission spectra measured from 360 nm to 750 nm in steps of 5 nm using a spectrophotometer (Spectramax M2 Reader, Molecular Devices, USA). The relative fluorescence (RFU) was plotted against wavelength and area under the curve was calculated as a measure of total fluorescent. RFU was standardized to total protein content and surface area of the subsample (RFU $protein^{-1}$ cm^{-2}).

Antioxidant concentration was determined using the Oxygen Radical Antioxidant Capacity (ORAC) assay (OxiSelect, Cell Biolabs Inc., USA). Briefly, this assay works by the quenching of a fluorescein probe that is added to the sample. When a radical initiator is added to the sample with the fluorescein probe, peroxyl radicals are produced that quench the fluorescein probe over time. However, in the presence of antioxidants in the sample, peroxyl radical formation is impeded, thereby preventing the quenching of the fluorescence until the antioxidant activity is depleted. By measuring the time of fluorescence decay, the total peroxyl radical antioxidant activity of the sample can be quantified and compared to an antioxidant standard curve. To do this, a standard curve was prepared using 5mM Antioxidant Standard Stock (Trolox) with concentrations ranging up to 200 µl. Standard curve and sample (25 µl) were transferred in triplicate to a microtiter plate and 150 µl of fluorescein probe was added into each well and mixed using a pipette. The plate was incubated for 30 min at 37 ºC, after which 25 µl of radical initiator was added using a multichannel pipette and mixed thoroughly. The fluorescence decay was measured immediately using a spectrophotometer (Spectramax M2 Reader, Molecular Devices, USA) at an excitation wavelength of 480 nm and emission wavelength at 520 nm during one hour at one min intervals. The relative fluorescence of a blank was plotted over time and subtracted

from the fluorescence decay of the samples and standard curve to obtain the net area under the curve for each sample, which was then graphed against the Trolox concentration. The Trolox equivalent (TE) of the samples was compared to the standard curve and standardized to surface area of the subsample (µmol TE cm^{-2}).

2.3.5 Data analyses

Data were analysed using the statistical software R version 3.0.3 (The R Foundation for Statistical Computing) and graphed with Prism GraphPad Software version 7.03. All variables were checked for normality using the Shapiro-Wilk test and log transformed when significant ($p < .05$). Homogeneity of variance was tested using the Levene's test or visually inspected by graphing the data using 'ggplot2 package' when data were unbalanced (note that there are more data points in the heated and chilled treatments than the ambient treatment).

To assess the acclimation trajectory, generalized least squares (GLS) and piecewise regressions fitted using maximum likelihood were fitted to the continuously-measured holobiont and symbiont response variables (i.e., Pnet, R, $\Delta F/Fm'$, F_v/F_m, rETRm) for each temperature treatment (chilled, heated and ambient). For the piecewise regression, breakpoints were set *a-priori* every 10 days during the 30 day period. This allowed me to assess the initial decline in the response rate during the first 10 days, and to determine whether, and at what level, a steady state of the performance occurred by assessing if the slope was significantly different from zero during day 11 – 20 and/or during day 21-30. Piecewise regressions with breakpoints every 10 days provided a significantly better fit than simple regression for both response rates and temperature treatments, except for the Pnet rates in the heated treatment (Appendix **Table A.1** for model comparisons). Simple regressions were fitted to the photosynthesis and respiration rates of the corals at ambient (control) temperature, because these responses were measured less frequently, and were not

expected to change over time. Since fragments were randomly chosen daily for respirometry measurements, several fragments were measured repeatedly. To verify that the repeated measured individuals did not influence the regression, I also regressed the respirometry data with these individuals excluded (Appendix **Figure A. 1**). For $\Delta F/F_m'$, Q_m and $rETR_m$, measurements started after 9 days of thermal exposure, and therefore piecewise regressions were fitted to two time intervals: days 9-20 and days 21-30. Post hoc comparisons to detect differences in response rate (slope) between the three time intervals were made by calculating differences of least squares means. For the response variables F_v/F_m, $\Delta F/F_m'$ and $rETR_m$, multiple coral fragments were measured within a tank each day. In this case, 'tank' was included as random factor to the model and a linear mixed effects model (LME) fit by maximum likelihood was used, and a likelihood ratio test was used to verify whether the factor 'tank' improved the model fit. If the model including 'tank effect' did not explain significantly more variance in the data than the model without this random effect, GLS regressions were fitted instead. Lastly, the tissue parameters (chlorophyll, protein, antioxidant and fluorescent protein content) were analysed using a two-way Analysis of Variance (ANOVA) with time and treatment as a categorical variables (intermediary analyses only done at day 1, 7, 15, 21 and 30). A Tukey posthoc test was used to detect which treatments and days differed.

2.4 Results

2.4.1 Observations

Heat exposure reduced tentacle extension from fully expanded, to partial or not expanded after 17 days (2.6 ± 1.4 on day 9 to 1.4 ± 0.9 on day 17). In contrast, tentacle activity increased slightly after two weeks of cold exposure, with more fragments showing

partially and fully expanded tentacles (2.0 ± 1.3 on day 9 to 2.8 ± 1.3 on day 16). At ambient temperature, the tentacles of most fragments were fully extended (3.7 ± 0.5 on average) and there was no change over time. Additionally, fragments in the heated treatment paled gradually (from 4.5 ± 1.1 on day 9 to 2.8 ± 0.5 on day 30), although bleaching was only observed in two fragments that were sampled from the tanks on day 19 for tissue analyses. In the chilled treatment, there were no visual signs of paling (from 3.6 ± 1.1 on day 9 to 3.8 ± 1.2 on day 30), except that after 9 days, two fragments started to show a slight fluorescent blue colouration, which can be an inflammation (Palmer et al. 2008) or thermal stress response (Palmer et al. 2009). The fragments exposed to ambient temperature were darker compared to the heat and cold exposed fragments (on average 4.7 ± 0.1 for ambient fragments and 4.2 ± 0.6 and 3.9 ± 0.4 for heated and chilled fragments), and did not pale over time.

2.4.2 Respirometry

The experimental set-up did not affect the net photosynthesis or respiration rate, as there was no significant change in performance over time for corals maintained at ambient temperature (**Figure 2.3** and **Table 2.2**). Acclimation of the holobiont physiology to cold and heat resulted in different acclimation trajectories for the net photosynthesis rate and respiration rate **(Figure 2.3**), but I found no evidence of complete or partial compensation for these performance variables. Instead, holobiont performance generally declined over the first 20 days in both treatments and then reached a steady state between days 21 – 30 when exposed to heat (slope = -0.006, $p = 0.32$ for Pnet and -0.002, $p = 0.66$ for R, **Figure 2.3**, **Table 2.1**). However, on average the Pnet rate during this steady state was -0.02 ± 0.07 µmol O_2 h^{-1} cm^{-2}, meaning that oxygen consumption was greater than oxygen production, which is insufficient to sustain coral health in the long term. Collectively these results indicate that

heat acclimation did not occur. In contrast, photosynthesis rates stabilized between days 11 – 20 of cold exposure (slope = 0.004, p = 0.33 for Pnet and slope = 0.002, p = 0.76 for R, **Table 2.1**), suggesting a 'no compensation' trajectory, although rates declined further between days 21 - 30.

2.4.3 Fluorometry

Similar to the holobiont thermal responses, the experimental set-up did not affect the symbiont responses, as there was also no change in $\Delta F/F_m$' or Q_m within the ambient treatment (**Table 2.2**). Likewise, F_v/F_m did not change significantly over time (p value for slope estimates are > 0.05; **Table 2.2**), although the slope across day 0-10 can be considered negative (p = 0.056, **Table 2.2**), suggesting that the start of the experiment may have been stressful which may have influenced the fragments in the chilled and heated treatment as well.

Within the heated and chilled treatments, the physiological responses at symbiont level, F_v/F_m, $\Delta F/F_m$', Q_m (**Figure 2.4**) and $rETR_m$ (**Figure 2.5**) followed different acclimation trajectories but showed no sign of 'complete' or 'partial' acclimation. F_v/F_m declined during the first 10 days of exposure to cold (**Figure 2.4 a**) and heat (**Figure 2.4 c**), with this response being three times stronger in the heated treatment (**Table 2.1**). Following this decline, F_v/F_m reached a steady state in the cold treatment over the remaining days (slope ranged between 0.001-0.002, p > 0.13,**Table 2.1**), whereas the F_v/F_m of heat exposed corals continued to decline further during day 21 to 30 (slope = -0.007, p = 0.002, **Table 2.1**). This suggests that acclimation occurred during cold exposure following the 'no compensation' trajectory, but acclimation did not occur during heat exposure.

Table 2-1 Results of the generalized linear models (for net photosynthesis, respiration and excitation pressure), and linear mixed effects models (for maximum photosynthetic quantum yield, effective photosynthetic quantum yield and maximum electron transport rate) to detect steady states of the physiological responses over 10 day intervals during exposure to cold (21 °C) and heat (31 °C). *P*-values > 0.05 (bold) indicate steady states.

		Cold				Heat			
Response	Days	Slope estimate	S.E.	t-value	*p*-value	Slope estimate	S.E.	t-value	*p*-value
Pnet	0 – 10	-0.011	0.004	-3.006	0.003	-0.025	0.006	-4.231	0.000
	11 – 20	0.004	0.004	0.984	**0.327**	-0.021	0.005	-3.641	0.000
	21 – 30	-0.013	0.004	-2.963	0.004	-0.006	0.006	-1.005	**0.317**
Resp	0 – 10	-0.006	0.005	-1.269	**0.207**	-0.009	0.006	-1.375	**0.172**
	11 – 20	0.002	0.006	0.305	**0.761**	-0.021	0.005	-3.951	0.000
	21 – 30	-0.019	0.006	-3.265	0.001	-0.002	0.005	-0.440	**0.661**
F_v/F_m	0 – 10	-0.003	0.001	-3.070	0.002	-0.009	0.001	-7.741	0.000
	11 – 20	0.001	0.001	0.445	**0.655**	0.001	0.002	0.776	**0.438**
	21 – 30	0.002	0.001	1.502	**0.134**	-0.007	0.002	-3.179	0.002
$\Delta F/F_m'$	9 – 20	-0.001	0.001	-0.624	**0.535**	-0.006	0.002	-2.791	0.007
	21 – 30	0.004	0.002	0.284	**0.777**	-0.007	0.003	-2.594	0.011
Q_m	9 – 20	0.000	0.002	0.147	**0.883**	0.002	0.003	0.682	**0.497**
	21 – 30	0.003	0.002	1.101	**0.274**	0.004	0.004	1.040	**0.301**
$rETR_m$	9 – 30	0.081	0.059	1.365	**0.174**	-0.890	0.097	-9.176	0.000

Table 2-2 Results of the generalized linear models for net photosynthesis, respiration and excitation pressure, and linear mixed effects models for maximum photosynthetic quantum yield, effective photosynthetic quantum yield and maximum electron transport rate to detect changes in the physiological responses of the 'control' group exposed to **ambient** temperature (26 °C). *P*-values > 0.05 (bold) indicate steady states.

Response	Days	Slope estimate	S.E.	t-value	*p*-value
Pnet	0 – 30	-0.003	0.002	-1.397	**0.164**
Resp	0 – 30	0.003	0.002	1.438	**0.152**
F_v/F_m	0 – 10	-0.002	0.001	-1.918	**0.056**
	11 – 20	-0.001	0.002	-0.442	**0.660**
	21 – 30	0.001	0.002	0.698	**0.486**
$\Delta F/F_m'$	9 – 20	-0.005	0.003	-1.616	**0.114**
	21 – 30	-0.000	0.003	-0.074	**0.941**
Q_m	9 – 20	0.000	0.004	0.100	**0.921**
	21 – 30	0.003	0.005	0.690	**0.494**
$rETR_m$	9 – 30	0.073	0.135	0.541	**0.592**

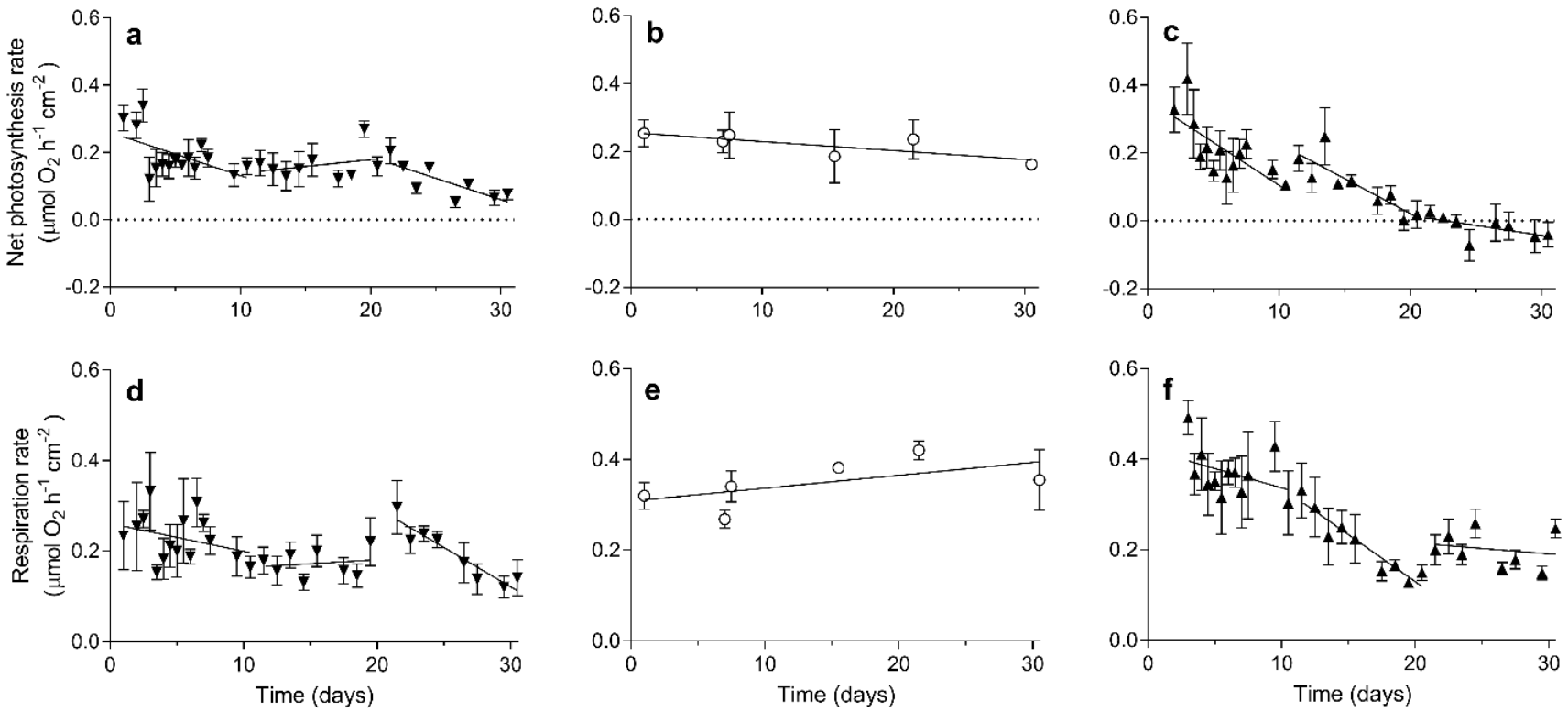


Figure 2.3 The mean net photosynthesis rates (top panels) and absolute respiration rates (bottom panels) of massive *Porites* spp. during 30 days exposed to chilled (21 ^{0}C; **a,d**), ambient (26 ^{0}C; **b,e**) or heated (31 ^{0}C; **c,f**) seawater. During the first 7 days, measurements were taken every morning and evening, the remaining days measurements were taken in evening. Fragments at ambient seawater were measured after 1, 7, 15, 21 and 30 days of exposure. Data points represent averages (n = 4), error bars are standard error of the mean and line shows the linear regression that was fitted to data.

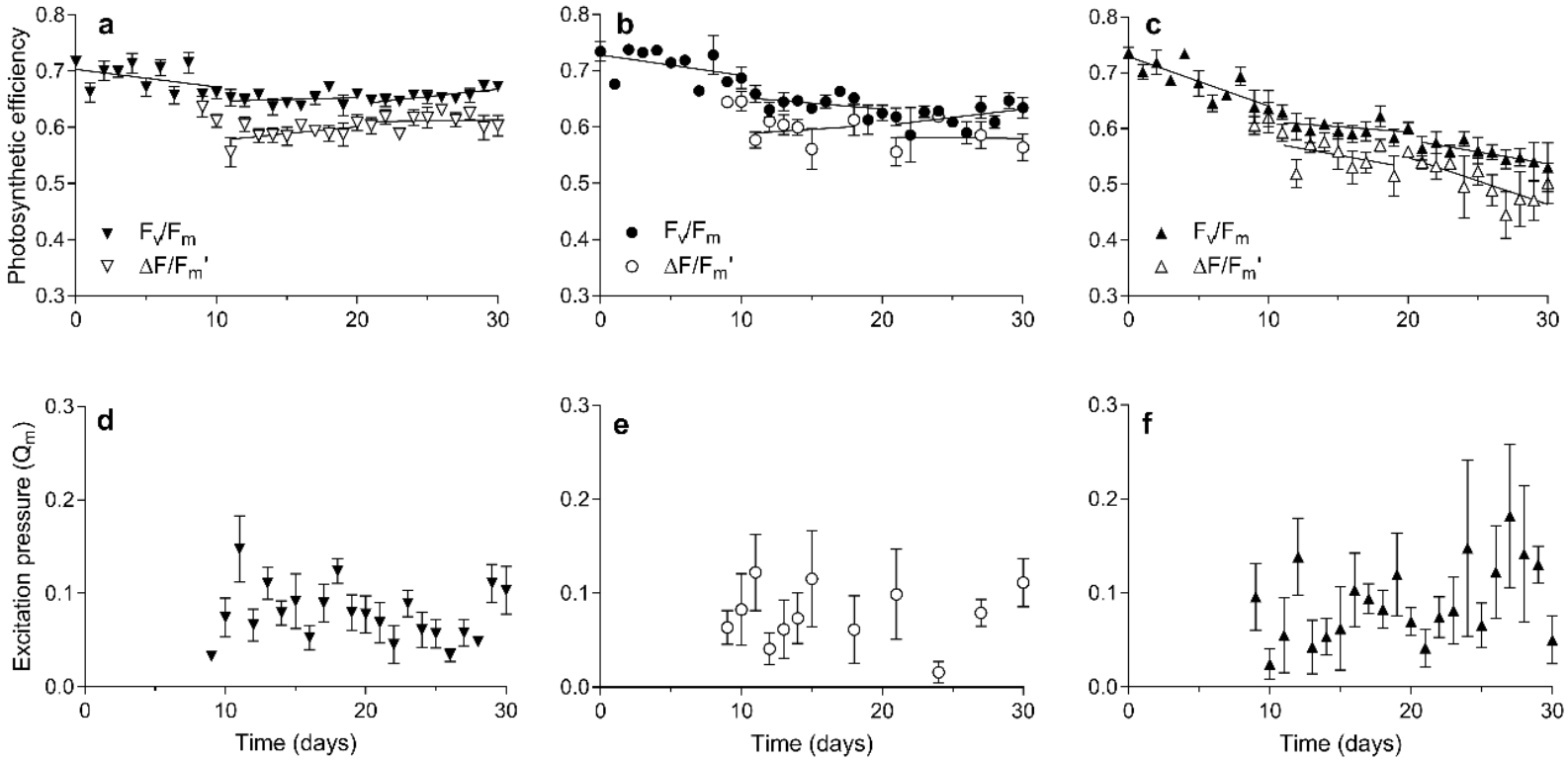


Figure 2.4 Mean maximum quantum yield (F_v/F_m; closed symbols in top panels) and effective quantum yield ($\Delta F/F_m'$; open symbols in top panels) in massive *Porites* spp. during 30 days exposure to chilled (**a**), ambient (**b**) and heated (**c**) seawater, with excitation pressure (Q_m) on photosystem II in bottom panels to chilled (**d**), ambient (**e**) and heated (**f**) seawater. Piecewise regressions were fitted through the responses by 10 days interval, but not displayed for Q_m as the slopes were equal to zero. Datapoints are averages: F_v/F_m was measured on all (remaining) fragments in each tank, $\Delta F/F_m'$ was measured on 8 fragments (2 per tank) and Q_m was calculated by tank averages (n = 4). Errorbars are s.e.m of corresponding sample size.

Simple regressions provided an equally good fit to the data of the effective quantum yield ($\Delta F/F_m$'; **Figure 2.4 a-c**) and excitation pressure (Q_m; **Figure 2.4 d-f**) as piecewise regression (Appendix **Table A.1**), but for consistency with the F_v/F_m analyses, data were analysed using piecewise regressions. During cold exposure, $\Delta F/F_m$' remained constant (**Table 2.1**), which is consistent with the steady state observed for F_v/F_m from day 10 onwards, whereas heat exposure resulted in a continuous decline from day 9 onwards (**Table 2.1**). Because the $\Delta F/F_m$' rates (measured on light-adapted corals) were consistent with the F_v/F_m rates (measured on dark adapted fragments), the photosynthetic capacity during illumination was not affected by the temperature treatment. Consistent with this interpretation, results showed low levels of Q_m in each treatment with no significant change over time (**Table 2.1**), suggesting that photoinhibition did not occur at any time during exposure to heat or cold.

Simple regressions provided a better fit to the $rETR_m$ data in each temperature treatment compared with piecewise regressions, indicating that the trajectory was generally consistent over time (Appendix **Table A.1,** but see also Appendix **Table A.2** for a comparison of the slopes among the time intervals). Indeed, the $rETR_m$ rates in corals

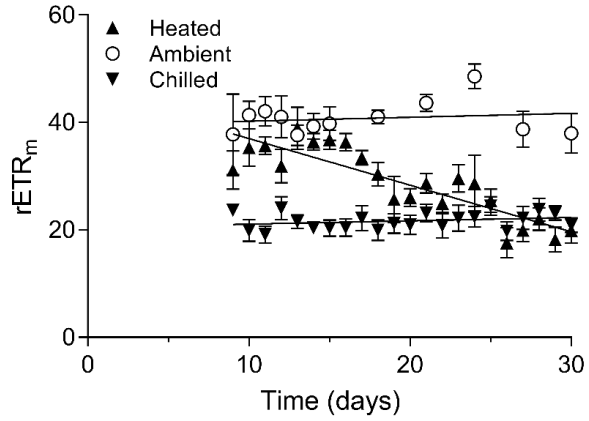


Figure 2.5 Mean maximum electron transport rate of massive *Porites* spp. during 30 day exposure to heated (31 ^{0}C), ambient (26 ^{0}C) or chilled (21 ^{0}C) seawater. Fragments at ambient temperature were measured daily from day 9 to 15 and then at 3 day interval. Data points are averages (n = 8) with error bars for s.e.m.

exposed to cold and ambient remained constant between day 9 to day 30 (**Figure 2.5**), although the average $rETR_m$ was two-fold higher in the ambient treatment compared with the heated treatment (respectively, 40.73 ± 6.69 versus 21.63 ± 5.03). Heat exposure resulted in a continuous decline of $rETR_m$ (**Table 2.1**), starting (on day 9) at rates similar to those of corals exposed to ambient and decreasing to $rETR_m$ rates similar to corals exposed to cold (**Figure 2.5**).

2.4.4 Tissue composition

The observed changes in the tissue composition did not suggest thermal acclimation (**Figure 2.6**). Cold exposure resulted in a strong decrease of the chlorophyll concentration (**Figure 2.6 a-c** and **Table 2-3**) with 50% less total chlorophyll present in coral tissues on day 30 compared with the beginning of the temperature treatments (posthoc, $p = 0.02$). During heat exposure, there was an initial increase of the chlorophyll concentration in the first week

Table 2-3 Results of the two-way analysis of variance to detect variation in the tissue composition between days (day 1, 7, 15, 21 and 30), treatment (chilled, ambient and heated) and the interaction of time and treatment.

Response variable	Factor	df, residuals	F value	*p* – value
Chlorophyll concentration	Time	4, 49	5.199	0.001
	Treatment	2, 49	9.938	0.000
	Interaction	8, 49	1.297	0.267
Protein concentration	Time	4, 49	1.705	0.164
	Treatment	2, 49	1.960	0.152
	Interaction	8, 49	1.101	0.379
Antioxidant content	Time	4, 49	5.440	0.001
	Treatment	2, 49	3.725	0.032
	Interaction	8, 49	2.414	0.029
Fluorescent protein content	Time	4, 45	3.314	0.018
	Treatment	2, 45	1.098	0.342
	Interaction	8, 45	1.311	0.260

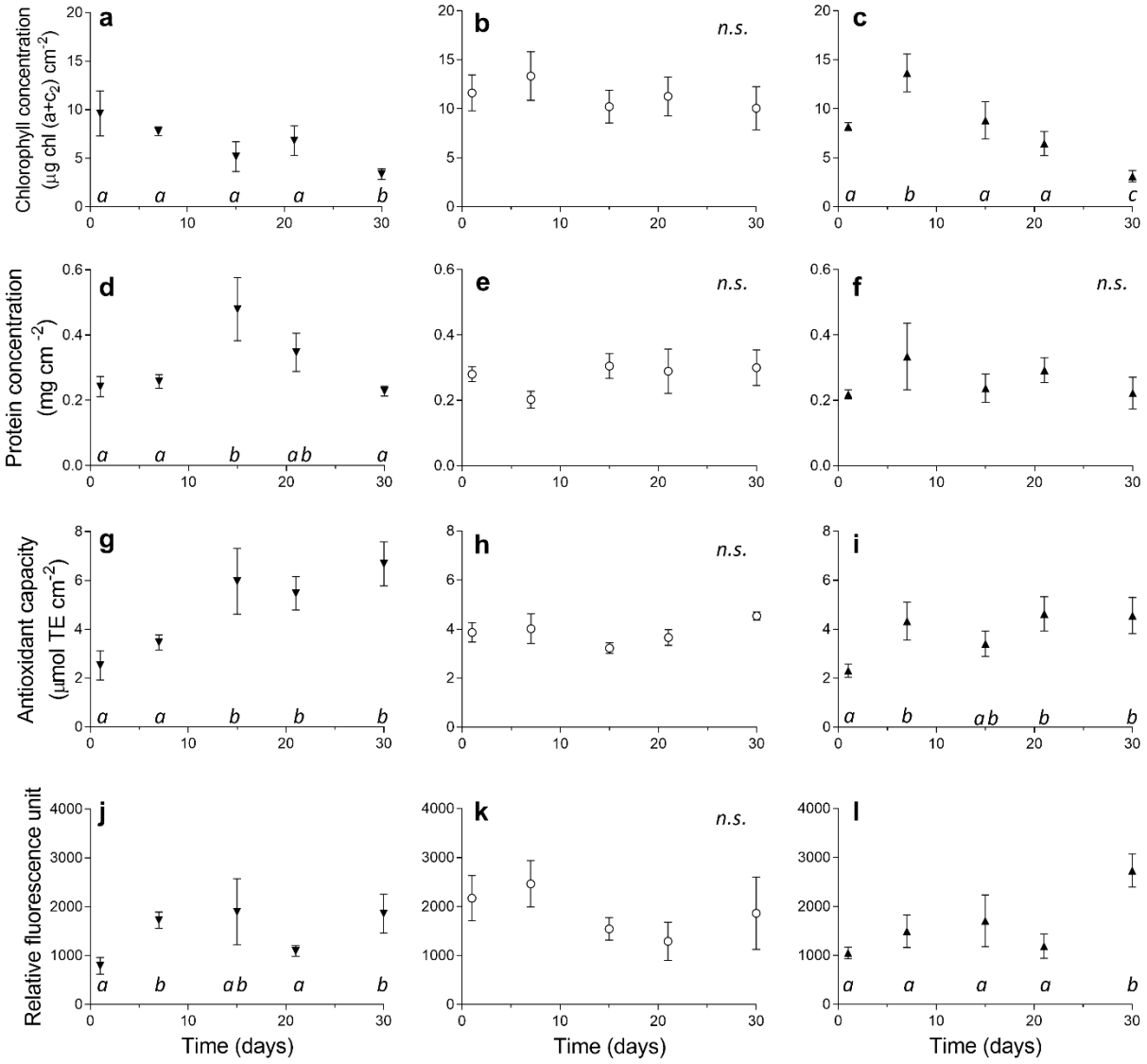


Figure 2.6 Average chlorophyll concentration (**a-c**), protein concentration (**d-f**), relative antioxidant capacity (**g-i**) and relative fluorescence content (**j-l**) for massive *Porites* spp. after 1, 7, 15, 21 and 30 days of exposure to chilled (21 ^{0}C; left column), ambient (26 ^{0}C; middle column), or heated (31 ^{0}C; right column) seawater. Displayed are averages (n = 4) and standard error of the mean. Letters indicate significant differences between days following Tukey's posthoc test, $p < 0.05$, n.s. is noted when no significant differences were detected.

Heat exposure also led to a gradual increase in antioxidant content of coral tissues over the course of three weeks (**Figure 2.6 i**), with two-fold higher antioxidant content on day 30 compared with the start, which could be an acclimation response that did not reach a steady state. Lastly, there was a significant effect of time on the relative fluorescence of coral tissues during exposure to changed temperature (**Table 2-3**), probably driven by the significant increase of FPs during the last week of exposure (**Figure 2.6 j** and **l**). There were no significant changes in the tissue composition over time of the fragments that remained at ambient temperature.

2.5 Discussion

This study aimed to define the acclimation trajectories of several holobiont and symbiont related physiological traits of a coral species when exposed to cold and heat. As expected, the performance of all traits initially declined after an abrupt change in water temperature (i.e., 5 °C in one day). Subsequently, performance either stabilized at a new, but lower, steady state or continued to decline further. Therefore, the acclimation trajectories observed during this study varied between 'no compensation' and 'inverse compensation'. Several symbiont related traits ($\Delta F_v/F_m$', Q_m and $rETR_m$) were relatively insensitive to temperature, although I did not capture changes in these traits immediately after the temperature change. Under both heat and cold exposure, the concentration of fluorescent proteins and antioxidants generally increased in coral tissues over time, while the chlorophyll concentration decreased, suggesting an accumulation of stress over time, and the ongoing upregulation of protective mechanisms, rather than establishment of a new steady-state . However, I also observed differences in the timing of responses to cold versus heat, supporting other evidence in the literature that heat and cold exposure triggers different changes in the physiology of organisms.

2.5.1 Are massive *Porites* spp. thermal generalists?

Massive *Porites* spp. are generally considered to be a thermally tolerant species because they are less susceptible to bleaching (Marshall & Baird 2000) and bleaching-induced mortality (Loya et al. 2001) than many other coral species. This resistance to high temperatures has been attributed to several physiological and morphological characteristics, including (i) a thicker tissue that provides the coral with resources when bleached, and protects the symbionts from high irradiance (Loya et al. 2001), (ii) the ability for heterotrophy so that during bleaching the coral does not solely rely on metabolites from the symbionts (Grottoli et al. 2006), (iii) a high protein turnover that makes the coral less vulnerable to thermal stress (Gates & Edmunds 1999), and (iv) a morphology that facilitates mass transfer between the coral and surrounding seawater which reduces the accumulation of oxygen radicals within tissues (Nakamura & Van Woesik 2001). These properties potentially provide the coral with a capacity to survive prolonged thermal stress and suggest that this species is a thermal generalist. In this study, however, I observed some large and immediate effects of altered temperature on the physiology of massive *Porites* spp., including lower photosynthesis and respiration rates, reduced maximum quantum yield and lower chlorophyll concentrations. Despite these changes, there was no mortality for the duration of the study with only two out of twenty fragments showing substantial bleaching near the end of the exposure to high temperature. This demonstrates that, despite limited thermal acclimation, massive *Porites* spp. showed a high capacity to resist mortality from thermal stress.

An individual with a limited capacity for thermal acclimation will require a generalist strategy in an environment where temperature varies over time (Gabriel 2005). Although there are constraints to such a strategy, such as a lower maximal performance (Huey & Hertz 1984), in highly variable thermal environments there are benefits to an individual to maintain consistent performance across a broad temperature range. To confirm if massive *Porites* spp.

are thermal generalists, the entire temperature range at which performance is positive must be measured (see subsequent Chapters). However, if massive *Porites* spp. are indeed thermal generalists, it can be expected that performance would recover rapidly after the temperature returned to ambient conditions. Although recovery from temperature stress was not measured here, other studies have reported rapid recovery of massive *Porites* spp. after severe stress. For instance, physiological traits including symbiont density and chlorophyll concentration recovered within 30 days following bleaching (D'Croz et al. 2001), and levels of gene expression returned to baseline within days following thermal stress (Kenkel et al. 2011). Together with the results of this study, these findings suggest that massive *Porites* spp. are both resistant and resilient to thermal stress.

2.5.2 No beneficial acclimation

One of the underlying assumptions about thermal acclimation is that acclimation should enhance the performance, or fitness, of an organism, also known as the Beneficial Acclimation Hypothesis (hereafter BAH, Leroi et al. 1994). However, numerous empirical studies on diverse taxa including plants and algae provide examples of cases where no beneficial acclimation was observed (see review by Angilletta 2009). For some studies, this was because the experimental design was more suitable for detecting developmental plasticity rather than beneficial acclimation (Wilson & Franklin 2002). In other cases, beneficial acclimation may not have been observed because, contrary to the BAH which assumes that acclimation is cost-free, there are costs and trade-offs involved with acclimation such that no acclimation may be more beneficial for the organism under certain conditions (Angilletta 2009). For instance, acclimation to high temperatures generally involves synthesis of heat shock proteins (Sørensen et al. 2003), which are energetically costly to produce and consume energy during functioning (Macario & Conway de Macario 2007).

Although my study did not directly test the BAH, none of the performance traits showed compensation after 30 days exposure to heat or cold. Consistent with my results, a well-designed study by Edmunds (2014) using massive *Porites* spp. did not find evidence supporting the BAH with respect to growth rates (holobiont response) or photosynthetic efficiency (symbiont response). In my study, photosynthesis and respiration rates and chlorophyll concentrations declined in response to both low and high temperature treatments, and symbiont related traits, such as F_v/F_m, Q_m and $rETR_m$, remained relatively stable in the cold exposed fragments but declined in the heat exposed fragments. The decreased chlorophyll concentration is most likely due to expulsion of symbionts and loss of pigmentation as a result of high thermal stress (e.g. Brown 1997). However, in this study, the fluorescent and antioxidant levels increased, and protein levels and Q_m were relatively constant, which is inconsistent with high levels of tissue damage due to thermal stress. Fluorescent proteins play a role in the photoprotection by absorbing harmful light (Salih et al. 2000) and as an antioxidant (Palmer et al. 2009). During heat stress, fluorescent proteins can enhance the resistance of corals to bleaching (Salih et al. 2000). The strong increase in fluorescent proteins at the end of my experiment suggests that thermal stress did increase and may have reached a tipping point at the time when the Pnet rates became negative.

Several mechanisms potentially explain the lack of compensation of the holobiont performance following temperature change. First, changes in single traits, such as the upregulation of the antioxidants and fluorescent proteins may have not collectively resulted in a strong signal at the holobiont level. Consequently, there was no compensation of the holobiont performance. Second, compensation of photosynthetic performance requires synthesis of chlorophyll pigments, which is energetically costly and demands relatively high input of nitrogen which can be limiting in natural seawater (Falkowski et al. 1993, Dubinsky & Jokiel 1994). Hence, the carbon and nitrogen costs for chlorophyll synthesis may have

been too high compared to other competing metabolic processes, such as protein synthesis. This scenario is plausible because coral net photosynthesis rates declined over the course of the experiment, therefore restricting the energy supply for host metabolic processes. However, corals were fed regularly with *Artemia*, providing additional carbon, as well as nitrogen, phosphorus and other nutrients that cannot be supplied from photosynthesis (Houlbreque & Ferrier-Pagès 2009). This may have been sufficient to sustain the lack of compensation of the performance. Further research is required to untangle the influence of nutrient availability on the capacity of massive *Porites* spp. to acclimate to temperature change.

Lastly, it is important to note that the outcome of acclimation observed during this study may differ from that in the field, as 'acclimation' in the laboratory under controlled conditions is not functionally the same as 'acclimatization' in the natural environment where temperature changes are more stochastic and other variables co-vary with temperature (Prosser 1991). Under such conditions, thermal specialists that constantly adjust their physiology to meet short-term fluctuations in the thermal environment, are likely to be disadvantaged unless they are able to achieve partial or full compensation acclimation much faster than the rate observed for massive *Porites* spp. in this study.

2.5.3 Cold and heat responses

Consistent with results of Roth et al. (2012), my study shows that the thermal responses differ between exposure to cold and heat. Holobiont (Pnet and R) and symbiont performance (F_v/F_m and $\Delta F/F_m$') initially declined when exposed to cold, but reached a steady state within ~2 weeks, whereas heat exposure induced a more gradual but continuous decline of each of these traits until the end of the experiment. Variation in the response to cold and heat is likely due to strong temperature dependence of biochemical processes that

drive acclimation (Somero & Hochachka 1971, Prosser 1991). For instance, cold constrains performance by slowing the rates of biochemical reaction (Arrhenius 1915), while heat constrains performance due to the denaturation of proteins. Additionally, denaturation of proteins due to heat is an irreversible and time-dependent process (Prosser 1991), meaning that longer exposure at high temperature results in more proteins that denature assuming the temperature is high enough. Consequently, performance during heat exposure may decline gradually over time with a more deleterious outcome compared to cold exposure, as observed in my study.

Cold exposure did not affect any of the symbiont dominated traits during my study, although I note that the dynamics at the onset of the temperature change were not fully captured. At the cellular level, the antioxidant and protein content doubled during the first two weeks of cold exposure, suggesting a strong initial response to cold stress, consistent with previous studies (Saxby et al. 2003, Roth et al. 2012). In these studies, F_v/F_m and chlorophyll concentrations declined in response to cold, which was related to photodamage and oxidative stress. However, the temperatures of this study (21 and 31 °C) were within the range that massive *Porites* spp. experience in the field and were not, therefore, extremely stressful. Indeed, fluorometry results did not suggest photoinhibition or damage of PSII during cold or heat exposure, as Q_m remained relatively constant and F_v/F_m decreased gradually, but only in the heated treatment. This contrasts with the study of Roth et al. (2012) who observed a sharp increase of Q_m after ~10 days exposure to 31 °C accompanied with a strong decrease of F_v/F_m. Such discrepancy cannot be attributed to methodology, as acclimation time and temperature treatment were similar between studies, but might be due to different inherent characteristics of the coral species (*Acropora yongei* is bleaching susceptible; Hoegh-Guldberg 1999) and associated Symbiodiniaceae genera and/or its thermal history.

2.5.4 Fluctuations versus constant temperature

Although the temperatures used in this experiment were within the range of temperatures experienced by the study species in its natural environment, it is possible that the combination of a rapid change of temperature followed by a consistently high or low temperature constrained the coral's ability to acclimate. A previous study that investigated the impact of slow (0.5 °C day^{-1}) or fast (1 °C day^{-1}) heating rate on *Acropora formosa* fragments collected around Orpheus Island showed no impact of heating rate on net photosynthesis rates at 30°C nor on the onset of bleaching at 33 °C (Middlebrook et al. 2010). Although the heating (or cooling) rate in my study was faster than that used by Middlebrook et al. (2010), the magnitude of total increase (and decrease) in temperature was smaller (5 °C compared to 7 °C) suggesting that heating rate alone is unlikely to explain the lack of compensation acclimation observed in my study.

In contrast, the exposure to a constant temperature after the initial change may have hindered the coral's potential for acclimation. Previous studies have shown that tolerance to withstand lethal temperatures increases when corals are pre-exposed to sublethal temperatures (Jokiel & Coles 1990). Also, short (2 days) exposure to elevated temperatures provided corals with more photoprotective mechanisms to resist bleaching temperatures (Middlebrook et al. 2008), and corals from highly fluctuating environments were more heat tolerant than those from stable environments (Barshis et al. 2010, Oliver & Palumbi 2011). It is plausible that exposure to fluctuating temperature regimes with a mean temperature similar to the steady temperature treatments during this study, would have resulted in acclimation trajectories with higher compensation outcomes, as brief periods with less 'extreme' high or low temperatures could serve as temporary refuges to repair cellular damage. However, Putnam and Edmunds (2011) showed that diel fluctuations of 4°C resulted in a decline of symbiont density similar to a treatment with a constant (high) temperature. This suggests that

prior exposure to high temperatures leads to increased tolerance to stressful temperatures, rather than increased thermal acclimation capacity. Further research is necessary to investigate whether fluctuating temperature regimes results in a different acclimation trajectory.

2.6 Conclusion

The ability for coral reefs to survive through this era of rapid climate change will be influenced by their capacity for acclimatization and adaptation to new environmental conditions. The thermally tolerant massive *Porites* spp. make an important contribution to the topographic complexity of reefs, and are among the most widespread corals on reefs globally (Veron 1995). This study showed that massive *Porites* spp. cope with changes in the thermal environment by tolerance rather than by acclimation, as acclimation did not occur during exposure to heat and resulted in 'no compensation' during exposure to cold. This suggests that massive *Porites* spp. do not rapidly change their physiology to suit the new temperature regime but, rather seems to use mechanisms that minimize the loss of performance until the temperature returns to normal. The symbionts were less sensitive to thermal changes, but the chlorophyll concentration decreased indicating a disrupted symbiosis despite no apparent damage of the photosystems. Furthermore, antioxidants and fluorescent proteins were upregulated which may have helped the coral holobiont to tolerate a stressful temperatures until they return back to normal.

CHAPTER 3

Seasonal acclimation of reef-building corals[3]

Holding tank (raceway), Orpheus Island Research Station, winter 2015

Coral fragments suspended in raceway

P. cylindrica fragment

[3] This chapter is accepted pending revisions by Marine Ecology Progress Series, revisions are currently underway.

3.1 Abstract

Thermal performance curves (TPCs) describe the relation between temperature and the rate of biological processes. These relationships can vary among species and environments, allowing organisms to acclimatize to their local thermal regime. This study quantified the seasonal variation in the thermal performance of several coral and symbiont dominated physiological traits for the thermally tolerant coral species, *Porites cylindrica*, and thermally sensitive coral species, *Acropora valenciennesi*. Photosynthesis rates, respiration rates, maximum PSII quantum yield and electron transport rates were measured in winter and summer on coral fragments exposed to an acute temperature increase and decrease up to 5 ºC above and below the average seawater temperature in each season. Results showed that colonies of *A. valenciennesi* acclimated primarily by shifting their optimal temperature to a higher temperature in summer whereas colonies of *P. cylindrica* had broader thermal breadth during summer. For symbionts within both species, performance was higher at all temperatures in summer, while the thermal optima and performance breadth remained unchanged. Despite these changes in thermal performance, the thermal optima of most traits did not match the ambient environmental temperature, but fell between the summer and winter temperatures. Overall, these results showed that both coral species were physiologically plastic in response to temperature change, but that there are constraints on the rate or capacity for acclimation that prevent a perfect match between the average temperature of the environment and the thermal optimum of the species.

3.2 Introduction

Temperature influences the biology and ecology of all organisms as it determines the rate of biochemical and physiological reactions. Extremely high and low temperatures are lethal

and, therefore, influence the geographic limits of the distributions of species (Pörtner 2002). Within the lethal temperature limits however, most physiological functions, such as metabolism, locomotion and growth, perform optimally at specific temperatures (Angilletta 2009). However, the relationship between temperature and physiological functioning varies among and within species due to differences in body size, life-history traits and/or genotype-specific patterns of gene expression. For example, the maximum sprinting speed varied among 13 species of lizards when they were exposed to the same temperature, due to morphological and physiological differences (Bauwens et al. 1995). In addition, temperature is highly variable over time and space, meaning that physiological functions must often occur under conditions that are suboptimal for performance. To cope with these changes, organisms can adapt and/or acclimate through altering their physiology and/or morphology in order to optimize performance in the new environment (Pörtner 2002).

Thermal sensitivity, defined as the degree to which an organism's performance depends on its temperature (Angilletta 2009), can be understood by measuring how the rates of various physiological functions change over a temperature gradient. In general, these investigations produce and analyse a thermal performance curve (TPC) (Huey & Stevenson 1979), or a 'reaction norm'. Typically, TPCs capture: the rate of increase of performance as temperature begins to increase; the maximum performance (Pf_{max}) which occurs at the optimal temperature (T_{opt}); and the decline in performance at temperatures above the optimum. The breadth of the curve (T_{br}) encompasses the temperature range at which performance is positive. The height and breadth of the TPC (as determined by Pf_{max} and T_{br}), and the position of the TPC along a temperature gradient (as determined by T_{opt}) varies between species from different thermal environments. For example, when comparing the jumping performance of five populations of the striped marsh frog *Limnodynastes peronil* from different latitudes, T_{opt} increased with increasing environmental temperature because

populations from cooler climates performed better at cooler temperatures and vice versa (Wilson 2001). Similarly, among lizard species from different altitudes, T_{opt} for sprinting speed was lower for species restricted to high altitudes and higher for species restricted to low altitudes (Berkum 1986).

The capacity to change the shape and position of the TPC depends on the phenotypic plasticity of the organism, defined as the ability of a genotype to express different phenotypes of a trait when exposed to changes in environmental conditions (Whitman & Agrawal 2009). In theory, an organism with infinite thermal plasticity would have the smallest possible thermal breadth and a T_{opt} that constantly shifts to correspond to the environmental temperature. In contrast, an organism with limited thermal plasticity would have a thermal breadth proportional to the variance of the thermal environment and a fixed T_{opt} that corresponds to the median environmental temperature (Angilletta 2009). In reality, however, the shape and position of the TPC are influenced by the variability and predictability of the thermal environment, and the time required to adjust to the new environment (Gabriel 2005). Therefore, T_{opt} is likely to be a compromise between the past, current and future environments. Moreover, if the time required for acclimation to a new environment exceeds the time spent in the new environment (e.g., an organism requires more time to adjust its performance to correspond to winter temperatures than the actual duration of winter) T_{opt} will be closer to the future temperature. In addition, T_{br} is likely to vary depending on the predictability of the environmental change such that if environmental stochasticity increases, T_{br} should also increase (Gabriel 2005). While these concepts can explain why certain species show a mismatch between the environmental temperature and their T_{opt}, few empirical studies tested these predictions directly.

Coral reefs ecosystems are particularly vulnerable to rising sea surface temperatures associated with climate change (Pörtner et al. 2014). Reef-building corals live within a

relatively narrow temperature range close to their upper thermal threshold (Jokiel & Coles 1990, Berkelmans & Willis 1999). Heat stress is linked to coral bleaching (Brown 1997), a process that disrupts the relationship between the coral host and its algal symbiont (Symbiodiniaceae) and can lead to coral mortality. However, the response to heat stress differs among coral species (e.g. Loya et al. 2001). Various mechanisms can enhance coral tolerance to heat stress, including physiological acclimation (Coles & Brown 2003, Oliver & Palumbi 2011), genetic adaptation of the coral host (Howells et al. 2016) and symbionts (Csaszar et al. 2010, Howells et al. 2012), as well as changes in Symbiodiniaceae genera (Berkelmans & Van Oppen 2006, Silverstein et al. 2015). However, increased heat tolerance of coral-algal symbioses can result in cold intolerance (Howells et al. 2013). This means that physiological acclimation to summer warming needs to be reversed during winter in order to maintain the fitness of coral colonies and populations. Depending on the duration and temperature range experienced during different seasons, adopting the "wrong" thermal strategy will have consequences for the productivity and survival of coral reefs. Hence, understanding plasticity of coral thermal performance provides insight as to whether or how corals can adapt and acclimatise to global warming (Logan et al. 2014).

On coral reefs, temperatures naturally vary over seasonal and diurnal cycles. At present, however, it is unknown whether corals cope with these temperature fluctuations via high thermal plasticity or via broad thermal tolerance. Previous research on corals demonstrates that physiological acclimation can occur on short time scales, from several weeks (Anthony & Hoegh-Guldberg 2003, Hoogenboom et al. 2010) to 2 days (Middlebrook et al. 2008) and can be reversible, which is likely to be beneficial in fluctuating environments. Evidence for certain coral species to increase the upper thermal threshold in summer (Berkelmans & Willis 1999) shows that the thermal sensitivity of some corals changes between seasons, but does not reveal whether it is the shape or position of the TPC, or a combination of both, that varies

between seasons. Additionally, studies demonstrated seasonal variation in the average or maximal performance for a variety of physiological traits, such as symbiont density (Fitt et al. 2000), photosynthesis rate (Scheufen et al. 2017), and calcification rate (Falter et al. 2012). Ulstrup et al. (2011) showed seasonal variation in photosynthesis and respiration rates for two coral species on the Great Barrier Reef, but this variation could have been caused by changes in photoperiod in addition to, or instead of, changes in temperature. To my knowledge, only one study has quantified the thermal performance of a coral using thermal performance curves (Rodolfo-Metalpa et al. 2014), but this was on a temperate coral species with colonies collected from different local populations with different thermal environments. Hence, it remains unknown whether and how tropical coral species adjust the shape and position of their thermal performance curves to cope with seasonal fluctuations in temperature.

This study aimed to determine whether corals use thermal plasticity to cope with seasonal differences in temperature. By quantifying coral thermal performance curves in in summer and winter, I assessed whether the shape of the TPC (thermal breadth and maximum performance) and the position of the TPC (optimal temperature) changed between seasons. In addition, I investigated whether any shifts of the optimal temperature could optimize coral performance as environmental temperature varied seasonally. I hypothesized that if corals are able to acclimate their thermal performance following seasonal variation in temperature, their maximum performance will be at a higher temperature in summer, and/or the thermal breadth will be larger in summer than in winter. Finally, I assessed whether plasticity in the shape and position of the TPC was species-specific by comparing a coral species known to be tolerant to high temperatures with a coral species known to be sensitive to high temperatures. I hypothesized that the bleaching resistant species would have a higher upper thermal threshold than the bleaching sensitive species and that this would be the result of a wider thermal breadth. As thermal sensitivity is variable within a species, I additionally compared

performance curves between colonies for within-population (i.e. among genotype) variation. Finally, to investigate whether variation in the thermal performance between season and between species was due to plasticity of the holobiont or the symbiont, I measured performance traits dominated by the holobiont physiology and performance traits that were symbiont specific. Overall, this study provided new insight into the physiological plasticity of corals, which will improve our understanding of how temperature change is likely to influence the fitness of coral populations.

3.3 Material and Methods

3.3.1 Experimental design

The physiological performance of two stony coral species was assessed over a range of temperatures in both summer and winter to determine whether their TPCs were fixed or plastic, and to investigate whether plasticity of the TPC could benefit corals by matching their optimal temperature for performance to the seasonal average temperature. Winter thermal performance was measured during the last week of August and first week of September 2015 (austral winter). Summer thermal performance was measured during the last two weeks of January 2016 (austral summer). Fragments of *Acropora valenciennesi* and *Porites cylindrica* were collected from a consistent depth at several sites around the Palm Islands, central Great Barrier Reef, in April 2015. Seawater temperatures are on average 29 °C in summer and 24 °C in winter, with daily fluctuations of around 0.5 °C (obtained from the Australian Institute of Marine Science data-portal; AIMS 2017). The species were identified based on morphological characteristics and chosen for their abundance throughout the Great Barrier Reef, but also for their contrasting thermal sensitivity with *A. valenciennesi* being more bleaching susceptible then *P. cylindrica* (Loya et al. 2001, Visram & Douglas

2007). Previous studies commonly showed *A valenciennesi* harbours *Cladocopium* C3 (formerly, Clade C sub-clade C3) and *P. cylindrica Cladocopium* C15 (formerly, Clade C sub-clade C15) (LaJeunesse et al. 2003, Madin et al. 2016; LaJeunesse et al. 2018). Between these two *Cladocopium* species, C3 is more sensitive to heating than C15 (Fisher et al. 2012). Therefore, differences in the shape and position of the performance curves between species and seasons were expected. A total of 50 fragments of each coral species, ~8 cm length, 5 per colony, were collected at depths between 4 to 6 m. Fragments were transported to Orpheus Island Research Station, attached to nylon string, labelled by source colony identity, and placed in a large (1500 l) shaded outdoor aquarium ('raceway'). The raceway functioned as a holding tank where the fragments were maintained prior to and between the winter and summer thermal experiment. Due to unexpected high mortality mid-September, which was caused by several days of abnormally low temperatures in the raceway (<20 ºC) caused by cool air temperatures, fragments (25 per species, five per colony) of *A. valenciennesi* were re-collected in November 2015 and *P. cylindrica* in December 2015. The recollected fragments were treated the same way as previously described and were used for the summer thermal experiment.

3.3.2 Raceway conditions

The raceway (holding tank) was supplied with seawater pumped from the reef slope in front of the station with a flow through rate of approximately 250 l h^{-1}. Light levels in the raceway were measured hourly during two days every month with a LI-193 spherical underwater quantum sensor (LI-COR) at different spots in the raceway. Maximum light levels averaged 1172 (± 697) µmol m^{-2} s^{-1} in summer and 1132 (± 572) µmol m^{-2} s^{-1} in winter. Water temperature in the raceway varied naturally according to the ambient temperature on the reef, and was recorded with a HOBO data logger (Onset Computer

Corporation, Bourne, USA) set to a one hour interval. During the summer months, starting in December 2015, two chiller/heater units (TK-2000, TECO, Ravenna, Italy) were placed next to the raceway to prevent the water temperature from increasing above 31 °C (± 0.5 °C) which could occur at the research station as seawater was pumped across the shallow reef flat and held temporarily in large storage tanks. During these months, the water flow through rate was also increased to 500 l h^{-1} to help maintain a consistent water temperature. It was assumed that the seawater supplied adequate nutrition for the coral fragments, but every two weeks additional *Artemia nauplii* was given as a supplementary food source, during which water flow was interrupted for 4 hours to allow feeding. Every month the raceway and fragments were cleaned to minimize algal proliferation. Temperature in the raceway was compared with the ambient temperature on the reef slope at 5.8 m depth (Orpheus Island Relay Pole 1) measured by temperature loggers of the Australian Institute of Marine Science (AIMS 2017).

3.3.3 Experimental conditions

To quantify seasonal variation in thermal performance, twenty-five fragments of each species, five fragments from each of five colonies, were selected from the raceway and distributed between two additional tanks (50 l each) placed directly adjacent to the raceway. This design enabled manipulation of water temperature for thermal performance measurements without any change in the light environment to which the corals were naturally acclimated. The same water was supplied to the smaller tanks and the raceway, except that the flowrate in the 50 l tanks was lowered to 36 l h^{-1} to facilitate experimental temperature manipulation. The corals were maintained at ambient temperature in the 50 l tanks for one week of acclimation before starting with the measurements. Temperature in each tank was recorded using HOBO data loggers (Onset Computer Corporation, Bourne, USA). After

measuring the response variables (see below) at ambient temperature, one fragment of each colony was frozen at -80 °C (n = 5 per species) for later tissue analyses. Subsequently, the water temperature in one 50 l tank was progressively increased while the water temperature in the other 50 l tank was progressively decreased using a chiller/heater unit (TK-2000, TECO, Ravenna, Italy) connected to a pump (Aquapro AP1050, Aquatec, Perth, Australia) that circulated the water through the temperature control unit at a rate of 500 l h^{-1}. Every morning, the water temperature was increased or decreased by 0.5 °C over the course of 12 days, until the 50 l tanks reached either 5 °C above or below ambient temperature (the winter experiment was extended to 31 °C). At every 1 °C increment, five physiological response variables (see below) were measured. At the end of the thermal experiment, fragments were frozen at -80 °C and transported, frozen on dry ice, to laboratory facilities at James Cook University for tissue analyses.

3.3.4 Response variables

Although the coral holobiont is a symbiosis between coral host and symbiont, the thermal response of the symbiont might differ from that of the holobiont (colony). Therefore, net photosynthesis rates and respiration rates (predominantly a coral host response) were measured using oxygen respirometry to provide information about the plasticity of the thermal performance curve of the holobiont, and maximum PSII quantum yield and electron transport rate (predominantly a symbiont response) were measured to provide information about the plasticity of the thermal performance curve of the symbiont. All measurements were performed daily at the same time to allow for comparison of the physiological response between days. Net photosynthesis rates were measured in the morning, followed by electron transport rate around midday. Dark respirometry measurements were done in the afternoon and finished with measurements of the maximum PSII quantum yield.

3.3.4.1 **Holobiont response variables**

For each coral fragment, rates of net photosynthesis (Pnet) and respiration (R) were measured in transparent experimental cells of Plexiglass (six cells, approximately 7 cm in diameter and 15 cm high, ~550 ml) for one hour. Five cells were filled with filtered seawater (15 µm) in which a coral fragment was suspended and one cell contained only seawater to control for background oxygen production and/or consumption. The cells were placed in a water bath connected to a chiller/heater unit (TK-2000, TECO, Ravenna, Italy) to control the water temperature inside the cells. During 1 h, the dissolved oxygen concentration inside each cell was measured using oxygen probes (LDO101, Hach, Loveland, USA) connected to a meter device (HQ40D, Hach) at 1 min interval. The cells were placed on a submersible magnetic stirrer plate (MIXdrive 6, 2mag, Muenchen, Germany) and a magnetic stirrer bar inside each cell ensured continued mixing of the water. Pnet rates were measured at a light intensity of 350 µmol photons m^{-2} s^{-1} provided by two wide beam lamps (Oracle, Sylvania, Padstow, Australia) with 150W metal halide light. R rates were measured in the dark directly after the photosynthesis measurements. At the end of the respirometry measurements, corals were returned to their experimental tanks (50 l tanks, see above). Pnet and R rates of each coral were corrected by subtracting the differential oxygen concentration of the empty control cell and multiplying by the net water volume of the cell, which was measured for each cell after every respirometry measurement and accounted for the displacement volume of the coral fragment, oxygen probe and magnetic stirrer.

3.3.4.2 **Symbiont response variables**

At the end of the dark respiration measurement, the maximum quantum yield (F_v/F_m) of photosystem (PS) II of each fragment was measured using a pulse-amplitude modulated (PAM) fluorometer (DIVING-PAM, Walz, Germany). F_v/F_m describes the proportion of light energy used for photochemistry by the (dark-adapted) symbionts. On dark-adapted fragments,

chlorophyll fluorescence was measured using a fiberoptic probe kept at a fixed distance (3 mm) from the coral surface by a flexible piece of tubing placed around the probe tip. First, the minimal fluorescence (F_0) was measured by applying a weak light pulse (<1 mol photon m^{-2} s^{-1}) that did not induce photosynthesis and determined the proportion of open reaction centres of PSII. Subsequently, the maximum fluorescence (F_m) was measured by applying a saturating light pulse (>5,000 µmol photons m^{-2} s^{-1}) that closed all PSII reaction centres and resulted in a greater fluorescence emission. F_v/F_m was calculated as $[F_m – F_0] / F_m$ (Schreiber 2004). Five measurements, evenly distributed over the coral surface, were made on each coral fragment of which an average F_v/F_m was taken. PAM settings were as follows: Measuring light intensity = 8; Saturation intensity = 6; Saturation pulse width = 0.8 s; Gain = 2; Damping = 2.

Immediately after the light photosynthesis measurement, rapid light curves (RLCs) were measured on the light-adapted fragments using the DIVING-PAM. RLCs provide information on the saturation characteristics of PSII electron transport (Ralph & Gademann 2005) and were used to assess the photosynthetic capacity of PSII as a function of instantaneous irradiance under different temperatures. RLCs were measured using an internal program of the DIVING-PAM that provided a sequence of nine actinic light steps, with light intensities increasing from 5 to 1800 µmol photons m^{-2} s^{-1}. Each illumination period lasted 10 s and finished with a saturating pulse after each step that measured the effective PSII quantum yield ($\Delta F/F_m'$), calculated as $[F_m' – F] /F_m'$, where F_m' is the maximum fluorescence of the light adapted sample and F is the instant fluorescence emission. The relative electron transport rate (rETR) was then calculated as:

$$rETR = \Delta F/F_m' * PAR * 0.84 * 0.5 \quad \text{(Eq. 3.1)}$$

where PAR was the photosynthetically active radiation, 0.84 was the assumed light absorbance of the sample, and 0.5 corrected for 2 photons of light required for the transport of 1 electron. RLCs were then created by plotting rETR against instant irradiance, from which the maximum rETR ($rETR_m$) was calculated after fitting the model of Platt et al. (1980).

3.3.4.3 **Chlorophyll concentration**

Chlorophyll concentrations were determined for control fragments that were frozen at the start of the experiment at ambient temperature (ambient group, $n = 5$), and for fragments that were frozen at the end of the experiment after exposure to 5 °C above or below ambient temperature (consecutively, heated and chilled group, $n = 10$ per group). Coral tissue was removed from the skeleton using an airbrush and 15 ml filtered seawater. The tissue slurry was homogenized using a homogenizer (T 25 Ultra-Turrax, IKA, Germany) after which 5 ml was centrifuged (Rotina 380R, Hettich Lab Technology, Germany) for 10 min at 5,000 *g*. The supernatant was discarded and 5 ml of 90% acetone was added to the pellet and left at 4 °C in darkness for 24 h to extract the chlorophyll. The extract was then centrifuged once more for 10 min at 5,000 *g*, after which 200 µl of the supernatant was added in triplicates to a multiwell plate. Absorbance was measured at 630, 663 and 750 nm using a spectrophotometer (Spectramax M2 Reader, Molecular Devices, Sunnyvale, USA). Chlorophyll *a* and c_2 concentrations were calculated using the equations of Jeffrey and Humphrey (1975a).

3.3.5 Data normalization

To allow for comparison between fragments, the respirometry rates and chlorophyll concentrations were normalized by coral skeletal surface area. Coral skeletal surface area was determined following the single wax dipping method described by Veal et al. (2010). Briefly,

the weight of the coral skeleton was recorded before and after being dipped in melted paraffin. The mass increase of the coated skeleton was calibrated to surface area using a standardized curve, which was plotted as the surface area versus mass increments of wooden cylinders of varying sizes.

3.3.6 Data analyses

Data were analysed using the statistical software R version 3.0.3 (The R Foundation for Statistical Computing) and graphed with Prism GraphPad Software version 7.03.

To assess whether the temperature response of *P. cylindrica* and *A. valenciennesi* varied between seasons and species, nonlinear least-squares regression models were fitted to the data of the response variables photosynthesis rates, $rETR_m$ and F_v/F_m. Symmetrical (Gaussian and Quadratic) and asymmetrical functions (Modified Gaussian and Weibull) were compared using the Akaike information criterion (AIC; Appendix **Table B.1**). The following Gaussian function (Rodolfo-Metalpa et al. 2014) was used to fit to the data:

$$P = Pf_{max} \exp [-0.5 (abs (T - T_{opt})) / T_{br})^2] \quad \text{(Eq. 3.2)}$$

where P is the temperature (T) dependent physiological response, Pf_{max} is the maximum value of that response, T_{opt} is the temperature at which the response value is optimal (i.e. the mean value) and T_{br} is the breadth of the response curve (i.e. the standard deviation). For each response variable, the function was first fitted to all the data pooled together regardless of season and species, and then fitted to the data separated by either species or season, and finally fitted to the data separated by both species and season. The Akaike information criterion (AIC) test was used to identify the model that was best supported by the data, which was the one with the lowest AIC value. Parameter estimates (Pf_{max}, T_{opt} and T_{br}) were calculated as the average of the colony responses for each species per season. Simple Welch

t-tests were used to detect differences in the parameter estimations between seasons that could indicate reversible acclimation. P-values were considered significant when $p < 0.05$.

Respiration data were grouped by species and season and examined by linear regression with temperature as covariate. Since the parameters $Pf_{max,}$ T_{opt} and T_{br} could not be calculated as with non-linear regression, T_{opt} was assumed to be the temperature at which the respiration rate was the lowest, and thermal sensitivity was measured using the temperature coefficient Q_{10}, which was calculated as

$$Q_{10} = (R_2 / R_1) \exp 10 / (T_2\text{-}T_1) \quad \text{(Eq. 3.3)}$$

where Q_{10} is the ratio of the respiration rates R_1 and R_2 measured at temperatures T_1 and T_2. Since the temperature gradient was different between seasons (19 – 31 °C in winter and 23 – 34 °C in summer), T_1 was set at 23 °C and T_2 at 31 °C. The Q_{10} is typically around 2 for physiological processes (Withers 1992), and a lower Q_{10} indicates that respiration rates were less sensitive to increasing temperatures, whereas a higher Q_{10} indicates higher thermal sensitivity. In addition, a mixed effect Analysis of Variance (ANOVA) was used, with species and season as independent variables, temperature as the covariate and colony as random effect, to test whether the effect of temperature on the respiration rates differed between the seasons or between species. For this analysis, data were log transformed to meet the assumptions of homogeneity of variance and normality of the residuals. Similar to the model selection procedure for the non-linear regression, factors were sequentially added to the linear model and the fit of the model to the data was compared. To do so, the effect of temperature as the only predictor for the respiration rates was estimated first, then species or season were added as main effects in the model and finally the interaction of species and season with temperature was added. For consistency with the non-linear regression model selection procedure, AIC values were calculated and assessed at each step of the model as

well to see if the added variable improved the overall fit of the model to the data. In similar fashion was assessed whether colony as random effect improved the model fit.

Chlorophyll data were tested for assumptions of normality (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variance (Levene's test, $p > 0.05$) and log transformed when assumptions were not met (chlorophyll data for *P. cylindrica*). To account for repeated measures of fragments from the same colonies, data were analysed using mixed effects ANOVA's per species with treatment (heated and chilled) and season as fixed effect and colony as random effect, to detect variation in mean chlorophyll concentrations between treatments and season. Chlorophyll concentrations of the fragments collected at the start of the experiment at ambient temperature were assessed separately, using a two-way ANOVA with species and season as fixed effect, to detect differences in the chlorophyll concentration in summer and winter and across species.

All data are averages ± standard deviation, if not reported otherwise.

3.4 Results

3.4.1 Seasonal variation in temperature

The seawater temperature in the raceway followed the seasonal trend of the seawater temperature *in situ* (**Figure 3.1**). The lowest recorded temperature was in July (19.1 °C and 21.7 °C in the raceway and on the reef slope respectively) and the highest in January (30.1 °C and 29.9 °C in the raceway and on reef slope respectively). Mean (winter) seawater temperature in the raceway during the last two weeks of August was 23.9 °C ± 0.7, hence 24 °C was set as ambient temperature during the winter thermal experiment. Mean (summer) seawater temperature in the raceway during the last two weeks of January was 29.3 °C ± 0.4, therefore the ambient temperature during the summer thermal experiment was set at 29 °C.

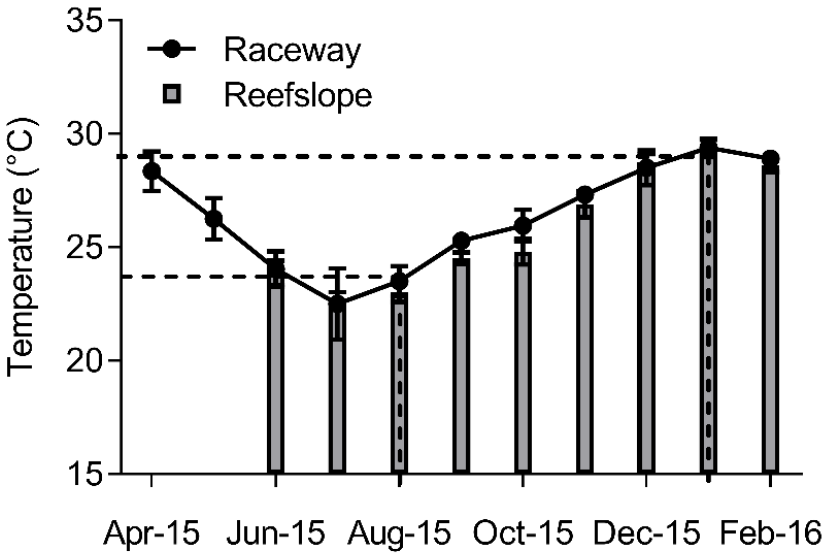


Figure 3.1 Mean monthly temperature variation measured at Orpheus Island on the reef at 5.8 m depth (columns) and in the raceway (line). Dashed lines represent the temperature at the start of the winter thermal experiment in August 2015 (24 ^{0}C) and summer thermal experiment in January 2016 (29 ^{0}C). Seawater temperature data were recorded by in-situ data loggers of the Australian Institute of Marine Science (AIMS 2017).

3.4.2 Holobiont response

Photosynthesis (Pnet) rate showed a Gaussian relation with temperature (**Figure 3.2 a-b**), whereas respiration (R) rates increased linearly with increasing temperatures (**Figure 3.2 c-d**). There was strong model support for seasonal and among-species variation (**Table 3.1**) for all of the measured response variables, indicating that the thermal performance varied between species and season.

The thermal optimum for photosynthesis of *A. valenciennesi* corresponded to the environmental temperature in winter (24.4 ± 0.4 °C; **Figure 3.2 a** and **Table 3.2**), and significantly increased in summer (Welch t-test, p = 0.002; **Table 3-3**) but remained ~ 1 °C below the ambient temperature in summer (27.9 ± 1.5 °C; **Table 3.2**). However, in summer,

Table 3-1 Comparison of thermal performance curves with different combinations of data selection for different physiological responses. Nonlinear regression models were fitted to the data for net photosynthesis rate, F_v/F_m and $rETR_m$; mixed linear regression models were fitted to respiration rate. Models were fitted as follows: 1) seasonal and species variation pooled together, referred to as "all data"; 2) only seasonal variation; 3) only species variation; 4) species and seasonal variation. K is number of estimated parameters in the model, delta AIC is the difference between the AIC value of the model and the minimum AIC value among all the models of the thermal response and the AIC weight represents the relative likelihood of the model.

Thermal response	Data selection	K	Cumulative AIC	Δ AIC	AIC weight
Pnet	All data	3	-699.95	419.34	8.72×10^{-92}
	Season	6	-901.03	218.26	4.02×10^{-48}
	Species	6	-821.19	298.10	1.85×10^{-65}
	Season * Species	12	-1119.30	0.00	1.00
R	All data	2	561.20	-1114.40	1.42×10^{-24}
	Season	4	601.74	-1191.48	7.76×10^{-8}
	Species	4	575.64	-1139.28	3.59×10^{-19}
	Season * Species	8	622.11	-1224.22	1.00
F_v/F_m	All data	3	-1387.04	373.94	6.31×10^{-82}
	Season	6	-1682.97	78.01	1.15×10^{-17}
	Species	6	-1424.96	336.02	1.08×10^{-73}
	Season * Species	12	-1760.98	0.00	1.00
$rETR_m$	All data	3	4543.37	223.95	2.34×10^{-49}
	Season	6	4377.10	57.68	2.99×10^{-13}
	Species	6	4517.47	198.05	9.87×10^{-44}
	Season * Species	12	4319.42	0.00	1.00

photosynthetic rate (0.51 ± 0.04 µmol O_2 h^{-1} cm^{-2} versus 0.30 ± 0.06 µmol O_2 h^{-1} cm^{-2} in winter and summer respectively). For *P. cylindrica*, the thermal optimum temperature for net photosynthesis was similar between seasons (Welch t-test, p = 0.414; **Table 3-3**) which was also below the ambient temperature in both seasons (T_{opt} of 21.4 ± 2.4 °C and 22.8 ± 3.3 °C in winter and summer respectively; **Figure 3.2 b** and **Table 3.2**). Similar to *A. valenciennesi*, the breadth of the curve significantly increased in summer (12.2 ± 3.5 °C and 18.5 ± 4.7 °C in winter and summer respectively; **Figure 3.2 b**), and the maximum photosynthetic rate decreased (Pf_{max} of 0.41 ± 0.06 µmol O_2 h^{-1} cm^{-2} in winter and 0.25 ± 0.02 µmol O_2 h^{-1} cm^{-2} in summer).

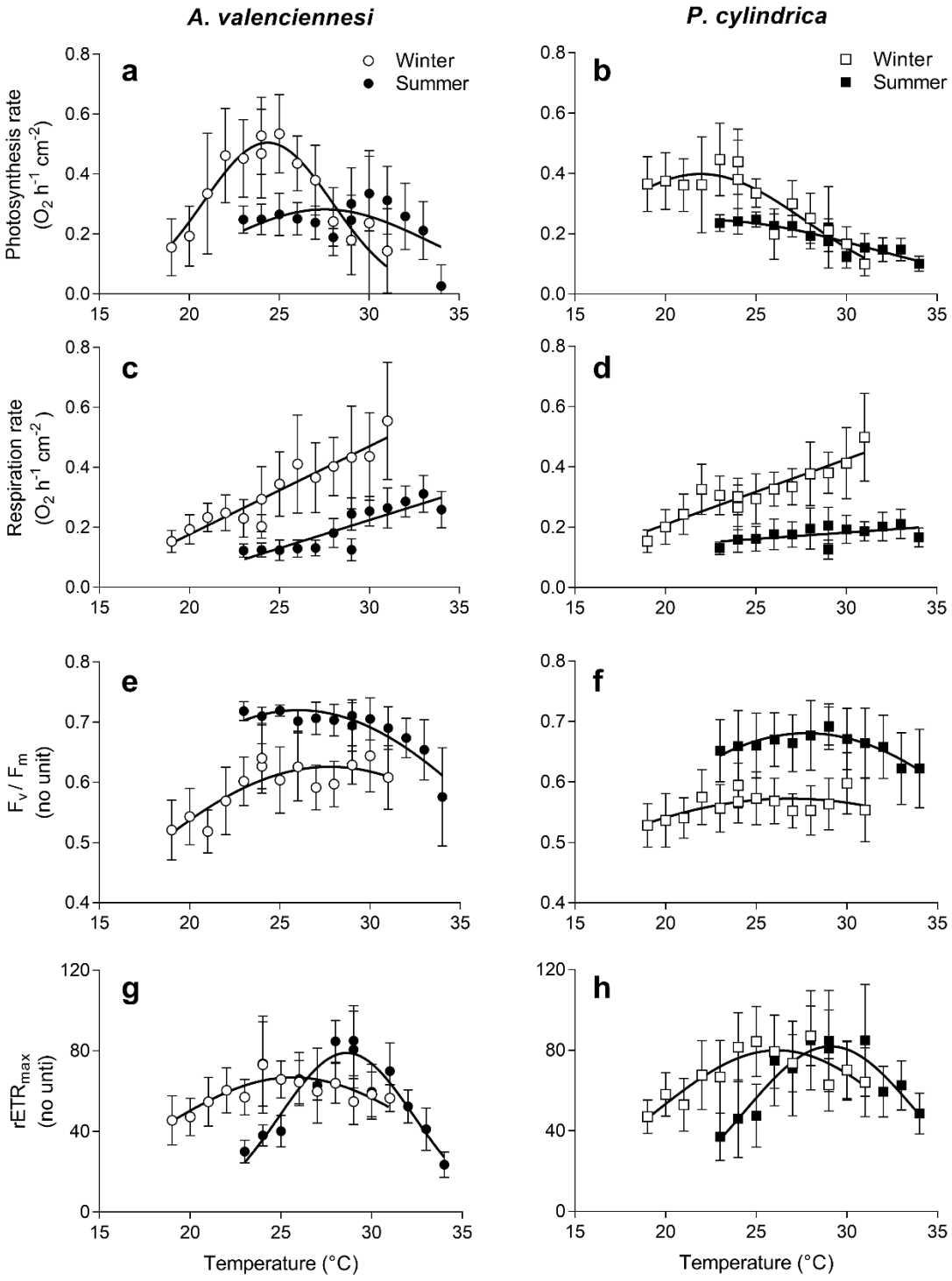


Figure 3.2 Thermal performance curves of net photosynthesis rate (**a-b**), respiration rate (**c-d**), maximum PSII quantum yield (**e-f**) and maximum electron transport rate (**g-h**) measured on *Acropora valenciennesi* (left panels) and *Porites cylindrica* (right panels) during summer (dots) and winter (squares). Point are the mean values ± s.d, n = 10. Curves are fitted using least square non-linear regressions for all variables except respiration rate, which are linear regressions.

Table 3-2 Mean (± s.d.) of the parameter estimates for the net photosynthesis rate (Pnet), maximum quantum yield (Fv/Fm) and maximum electron transport rate (rETRm) of *Acropora valenciennesi* and *Porites cylindrica* colonies. *Topt for respiration rate (R) corresponds to the lowest respiration rate, which was at the lowest experimental temperature.

Thermal response	Parameter estimate	*A. valenciennesi* Winter	*A. valenciennesi* Summer	*P. cylindrica* Winter	*P. cylindrica* Summer
Pnet	Pf_{max} (O_2 h^{-1} cm^{-2})	0.51 ± 0.04	0.30 ± 0.06	0.41 ± 0.06	0.25 ± 0.02
	T_{opt} (ºC)	24.4 ± 0.4	27.9 ± 1.5	21.4 ± 2.4	22.8 ± 3.3
	T_{br} (ºC)	7.1 ± 1.7	10.5 ± 0.8	12.2 ± 3.5	18.5 ± 4.7
R	*T_{opt} (ºC)	*19	*23	*19	*23
F_v/F_m	Pf_{max} (no unit)	0.62 ± 0.04	0.72 ± 0.01	0.58 ± 0.02	0.67 ± 0.03
	T_{opt} (ºC)	27.6 ± 1.0	26.0 ± 0.8	28.0 ± 2.7	27.7 ± 0.9
	T_{br} (ºC)	28.2 ± 4.4	30.9 ± 7.6	51.3 ± 22.7	30.3 ± 10.4
$rETR_m$	Pf_{max} (no unit)	68.69 ± 7.72	78.05 ± 7.12	80.75 ± 7.23	83.59 ± 10.76
	T_{opt} (ºC)	25.6 ± 0.6	28.6 ± 0.4	26.2 ± 1.1	29.2 ± 0.9
	T_{br} (ºC)	16.8 ± 6.3	7.3 ± 0.5	13.7 ± 1.6	9.3 ± 1.4

Table 3-3 Results of Welch t-tests to detect variability between seasons in the parameter estimates of the thermal performance curves for net photosynthesis, maximum quantum yield and maximum electron transport rate.

		A. valenciennesi			*P. cylindrica*		
Thermal response	Parameter estimate	t-value	df	p-value	t-value	df	p-value
Pnet	Pf_{max}	-7.242	8.639	0.000	-5.417	4.792	0.003
	T_{opt}	5.645	5.678	0.002	0.852	9.988	0.414
	T_{br}	4.111	5.408	0.008	2.628	9.958	0.025
F_v/F_m	Pf_{max}	6.033	4.492	0.003	5.756	9.942	0.000
	T_{opt}	-2.272	7.364	0.056	-0.217	4.583	0.837
	T_{br}	0.733	8.199	0.484	-1.928	5.216	0.109
$rETR_m$	Pf_{max}	2.073	8.329	0.071	0.547	9.994	0.596
	T_{opt}	10.119	6.560	0.000	5.147	7.505	0.001
	T_{br}	-3.373	4.041	0.028	-4.853	7.716	0.001

the breadth of the curve became wider (T_{br} of 7.1 ± 1.7 °C and 10.5 ± 0.8 °C in winter and summer respectively) which coincided with a significant decrease of the maximum net Including colony as a random effect improved the fit of the linear model for the respiration rates (AIC value for model without and with random effect, respectively -1184 and -1224). There was a positive linear relationship between temperature and the respiration rates of both species (**Figure 3.2 c-d**; mixed effects ANOVA, effect of temperature, ($F(1,513) = 336.57$, $p < 0.001$). In both seasons, the temperature at which the respiration rate was lowest corresponded to the lowest experimental temperature, which was 19 ºC in winter and 23 ºC in summer (**Table 3.2**). Furthermore, the effect of temperature on the respiration rates was stronger in winter than in summer (mixed effects ANOVA, interaction between temperature and season, $F(1,513) = 55.63$, $p < 0.001$). This was also evident by the higher Q_{10} values in winter (3.4 ± 1.8 for *A. valenciennesi* and 2.1 ± 0.9 for *P. cylindrica*) than in summer (2.7 ± 1.0 for *A. valenciennesi* and 1.6 ± 0.3 for *P. cylindrica*). While for *A. valenciennesi* in summer there was a significant effect of temperature on the respiration rates ($Q_{10} > 2.0$), for *P. cylindrica*, this effect was absent, which might be related to the wide thermal breadth of Pnet in summer (**Figure 3.2 b**).

3.4.3 Symbiont response

Both symbiont response variables, maximum PSII quantum yield (F_v/F_m) and maximum electron transport rate ($rETR_m$), showed a Gaussian relation with temperature (**Figure 3.2 e-h**). Furthermore, similar to the response variables at holobiont level, there was strong model support for seasonal and among-species variation in both traits (**Table 3.1**).

The performance curves for F_v/F_m of both species responded in similar fashion to the seasonal environmental temperature (**Figure 3.2 e-f**): Pf_{max} increased when acclimated to the summer temperatures, but there was no change in the thermal optimum and performance

breadth (**Table 3-3**). Although some parameter estimates appear higher (T_{opt} of *A. valenciennesi*, T_{br} of *P. cylindrica*) in winter than in summer (**Table 3.2**), the standard error and 95% confidence intervals around these parameter estimates were large (large error bars, Appendix **Figure B.1**), caused by substantial variation in the T_{opt} and T_{br} of symbionts within different coral colonies.

The optimal temperature for performance of the $rETR_m$ was around 26 °C for both species in winter (**Table 3.2** and **Figure 3.2 g-h**), which was ~ 2 °C higher than the environmental temperature. However, in summer, both species increased their T_{opt} to correspond to the environmental temperature (28.6 ± 0.4 °C and 29.2 ± 0.9 °C for *A. valenciennesi* and *P. cylindrica* respectively). Likewise, both species had equally wide thermal breadths in winter but this significantly decreased in summer by nearly 30% for *P. cylindrica* (from 13.7 ± 1.6 °C in winter to 9.3 ± 1.4 °C in summer) and by more than 50% for *A. valenciennesi* (from 16.8 ± 6.3 °C in winter to 7.3 ± 0.5 in summer). Lastly, the height of the curves did not significantly change between winter and summer for either species (**Table 3-3**).

3.4.4 Within-population variability

Since the objective of this study was to compare thermal plasticity among populations, data were aggregated across colonies and ignored the within-population (among-colony) variability in thermal performance. However, when non-linear and linear regressions were fitted to data separated by season, species as well as individually for each colony, AIC tests showed that this improved the fit of the model for every response variable (Appendix **Table B.2**). This indicates significant variability in the performance of colonies within local populations (see Chapter 6 General Discussion). Variability in T_{opt} was particularly large for the holobiont response between *A. valenciennesi* colonies in summer (ranging from 25.8 – 29.5 ºC), while in winter the magnitude of variation was reduced to less than 1 ºC (**Figure 3.3 a**). Similarly for *P. cylindrica* (**Figure 3.3 b**), the variability in T_{opt} between colonies for the holobiont response was more than double in summer compared with winter.

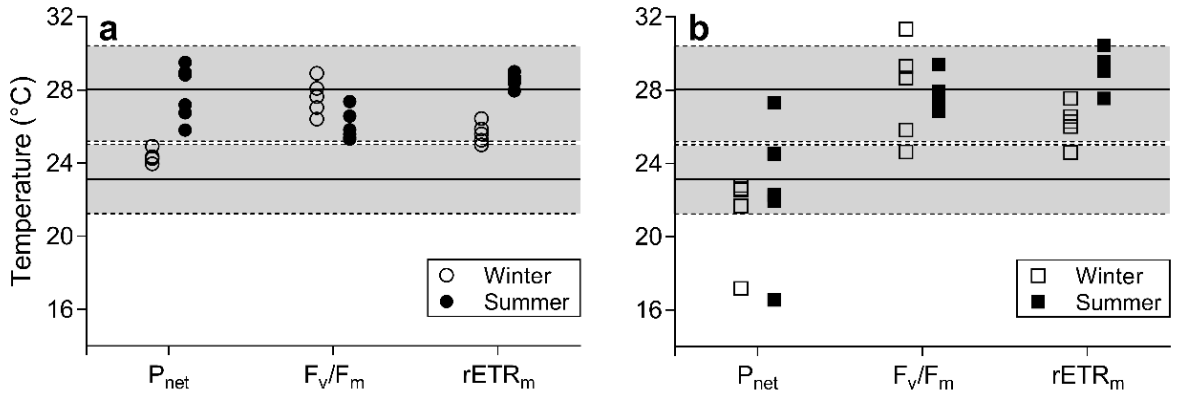


Figure 3.3 Variation in optimal temperature between colonies of *Acropora valenciennesi* (**a**) and *Porites cylindrica* (**b**) during summer (filled points) and winter (open points) for net photosynthesis, maximum PSII quantum yield and maximum electron transport rate. Datapoints show T_{opt} derived by non-linear regression of 2 or 4 fragments from the same colony. Horizontal lines represent the average seawater temperature measured over 90 days prior to the start of the winter experiment (July – August 2015) and summer experiment (October 2015 – January 2016). Dashed lines show the minimum and maximum temperature during those time intervals.

3.4.5 Chlorophyll concentration

Chlorophyll concentrations were similar between seasons for *A. valenciennesi* colonies (**Figure 3.4 a**), but lower in winter for *P. cylindrica* colonies (**Figure 3.4 b**; mixed effects ANOVA with main effect of season, $F(1,10) = 22.47$, $p < 0.001$). The effect of thermal exposure on the chlorophyll concentration was not apparent in *A. valenciennesi* colonies (mixed effects ANOVA with main effect of treatment, $F(1,13) = 4.64$, $p > 0.05$), but in *P. cylindrica*, chlorophyll concentrations were lower in colonies exposed to the increased thermal gradient (main effect of treatment, $F(1,17) = 13.81$, $p < 0.01$). However, this effect was only significant in summer (main effect of treatment on chlorophyll concentration analysed separately for *P. cylindrica* colonies in summer, $F(1,12) = 14.21$, $p = 0.01$). In addition, chlorophyll concentrations were higher in the heated than the chilled *A. valenciennesi* colonies, whereas the opposite was observed in *P. cylindrica* colonies. Lastly, the chlorophyll concentration in fragments at ambient temperature was similar across seasons and between species (two-way ANOVA with season and species as main effects, respectively $F(1,20) = 3.19$, $p = 0.09$ and $F(1,20) = 1.12$, $p = 0.30$).

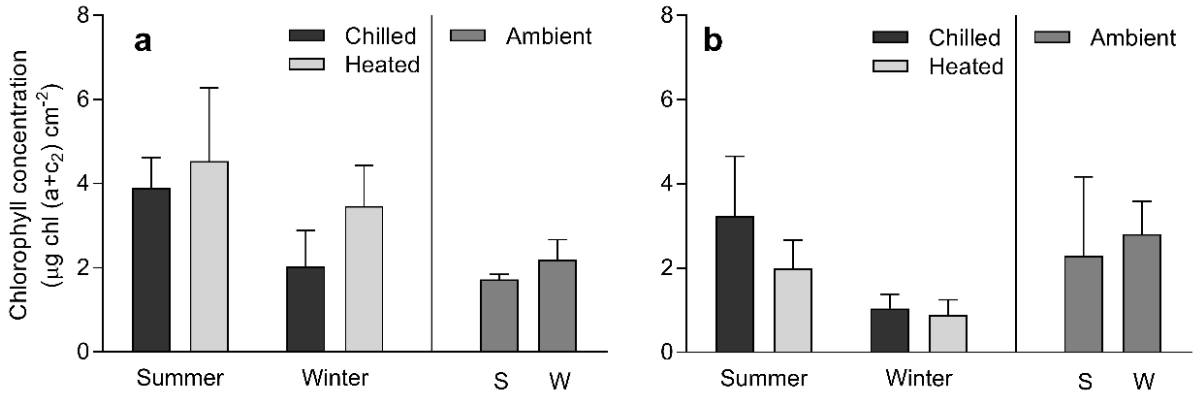


Figure 3.4 Chlorophyll concentrations of *Acropora valenciennesi* (**a**) and *Porites cylindrica* (**b**) measured on fragments before (ambient, $n = 5$) and after (chilled and heated, $n = 10$) completion of the thermal summer and winter experiment. Mean values ± s.d. are shown.

3.5 Discussion

This study is the first to quantify seasonal variation in the thermal optimum and thermal breadth of several holobiont and symbiont physiological traits for two coral species that differ in their responses to heat stress (bleaching tolerance). I found that at holobiont level, the species that is sensitive to heat stress, *Acropora valenciennesi*, acclimated to changing temperatures primarily by shifting the position of the performance curve. In contrast, the species that is more tolerant to heat stress, *Porites cylindrica*, acclimated by altering the performance breadth. However, at symbiont level, the performance curve only changed through variation in the height of the curve, while the thermal optimum and performance breadth remained unchanged. Furthermore, T_{opt} of most traits did not correspond to the ambient environmental temperature, but fell between the summer and winter temperatures. Lastly, there was significant within-population variability implying among genotype variation, which was particularly large for the holobiont traits. Overall, these results showed that both species were physiologically plastic, but this plasticity was colony- and species-specific at the holobiont level, whereas the symbiont plasticity was limited and uniform across both coral species.

The holobiont performance breadth of *P. cylindrica* was nearly twice as wide as that of *A. valenciennesi*. To my knowledge, this study is the first to identify such differences in thermal acclimation strategies of coral species. A large thermal performance breadth implies that the holobiont physiology is relatively insensitive to changes in temperature. This is consistent with previous studies that reported that *P. cylindrica* is a resistant to thermal stress (Visram & Douglas 2007, Fitt et al. 2009). In contrast, a small thermal performance breadth reflects a sharp peaked TPC and greater thermal sensitivity. Indeed, *Acropora* spp. in general, and branching morphologies such as *A. valenciennesi* in particular, have been frequently reported to rapidly respond to small changes in the temperature of the environment, often with

detrimental effects including bleaching-related mortality (e.g. Loya et al. 2001, Hoogenboom et al. 2017).

My study also showed that, in response to seasonal variation in the environmental temperature, *A. valenciennesi* shifted its thermal optimum for performance and changed the performance breadth, whereas *P. cylindrica* only varied the performance breadth. Such differences in thermal strategy despite sharing an identical thermal history supports that species do not perceive, or respond to, their thermal environment the same way (Angilletta et al. 2006). Such differences could simply be due to genetic divergence among species, but could also be related to species generation times. Species with longer generations are likely to experience greater thermal variation within generations than species with shorter generation times. In this case, *Acropora* species are generally fast-growing and reach reproductive maturity early (short generation time) in comparison with *Porites* species that are generally slow-growing, reach reproductive maturity later but have longer generation times (Darling et al. 2012, Pratchett et al. 2015, Madin et al. 2016). Consequently, *P. cylindrica* is more likely to experience thermal extremes in summer and winter within generations and, therefore, selection is likely to have favoured more of a thermal generalist strategy (i.e. wide performance breadth) compared with *A. valenciennesi*. Although a two-species comparison is limited in distinguishing between environmentally induced selection from phylogenetic constraint (e.g. Garland and Adolph, 1994), it is adequate to generate hypotheses for future research with a multispecies approach. Further study of thermal acclimation strategies for corals with different generation times is required to test this hypothesis. Alternatively, several other physiological and metabolic mechanisms also vary between the two species that may have influenced the thermal acclimation strategy. For example, *A. valenciennesi* released substantial amounts of mucus during respirometry, while no mucus release was observed with *P. cylindrica*. Coral mucus protects the coral from invasive microbes (Ritchie 2006), but

synthesis is highly costly and nearly half of the total energy acquired through carbon fixation is lost due to mucus release (Crossland et al. 1980) and this energy use might affect acclimation capacity. In addition, studies have suggested that corals with high metabolic rates acclimatize more effectively than those with high growth rates and low metabolic rates (Gates & Edmunds 1999, Loya et al. 2001). Here, a difference in the thermal strategy of *A. valenciennesi* and *P. cylindrica* might be the result of differences in patterns of energy allocation.

Respiration and net photosynthesis rates were higher in winter than in summer, as observed by a vertical shift of the performance curve of both species. This shift of the photosynthetic performance cannot be explained by changes in the chlorophyll concentration, because the chlorophyll concentration at ambient temperature was constant between seasons and lower in winter than in summer after exposure to the thermal experiment. Likewise, the maximum PSII quantum yield of both species was lower in winter and the electron transport rate was constant between seasons for *P. cylindrica* and slightly enhanced in summer for *A. valenciennesi*. These results suggest that the symbionts had higher capacity for photosynthesis in summer, which is inconsistent with the downwards shift of the net photosynthetic performance curve of the holobiont. A possible explanation for the absence of enhanced net photosynthesis in summer might be due to costs associated with changing the shape (*P. cylindrica*) and position (*A. valenciennesi*) of the performance curve, i.e. costs of thermal acclimation. Angilletta et al. (2003) identified several tradeoffs that constrain performance curves due to mechanisms that underlie the expression of phenotypes. The allocation tradeoff dictates that increased performance of one trait at a certain temperature occurs at the expense of decreased performance of another trait at that temperature. For instance, acclimation to high summer temperatures might necessitate the production of protective mechanisms, such as heat-shock proteins (Feder & Hofmann 1999). Hence, despite

an enhanced photosynthetic capacity in summer at symbiont level, photosynthetic performance at holobiont level was compromised due to costs associated with thermal acclimation to summer temperatures. Reduced photosynthesis rates and synthesis of heat shock proteins have been frequently reported as consequences of elevated temperatures (Fitt et al. 2001).

The Gaussian curve is frequently applied to model the relationship between temperature and performance (Angilletta 2006). However, in this study, linear regression provided a better fit to model the respiration performance, even though linear approximations should be avoided since they fundamentally differ from the dynamics of the biological and physiological processes that underlie thermal performance (Bulte & Blouin-Demers 2006). Nevertheless, a linear relationship between coral respiration and temperature is reported previously by Coles and Jokiel (1977). The most likely explanation for this is that the performance curve for respiration is highly asymmetrical (skewed to the left), with the optimum (i.e. temperature at which the respiration rate is highest) very close to the upper critical threshold temperature. Hence, I expect that exposure of corals to higher (more extreme) temperatures than the ±5 ºC range used in this study would show a rapid decline of the respiration performance. In a previous study on the metabolic rate of the killifish *Fundulus heteroclitus*, the authors were also unable to capture the falling phase of the curve because the organism's critical thermal maximum was at temperatures slightly higher than those measured (Healy & Schulte 2012). In addition, interpretation of thermal optima for respiration rates in corals is complex, due to multiple, competing energy requiring processes. For instance, changes in the respiration rate may be due to changes in the energy expenditure for calcification (Al-Horani et al. 2003) which would be beneficial for colony growth, but changes in respiration can also reflect increases in the metabolic rates of the host and symbiont in response to stress. Increased respiration rates at elevated temperatures has been

demonstrated for numerous organisms, including terrestrial plants (Berry & Bjorkman 1980), anemones (Goulet et al. 2005) and corals (Coles & Jokiel 1977), and has been attributed to increased oxygen consumption at mitochondrial level (Pörtner 2002, Schulte 2015). Additionally, oxidative stress related to elevated temperatures induces the synthesis of protective mechanisms, such as superoxide dismutase and antioxidants (Fitt et al. 2009), which results in a linear relationship of respiration rate with temperature.

Results showed that coral thermal acclimation rarely resulted in a perfect match between the thermal optima and average environmental temperatures. These results indicate that, as predicted by Gabriel (2005), the variability of the thermal environment, the time required for adjusting the physiology and the costs associated with acclimation constrain acclimation rates and magnitudes. At my study location, the mean environmental temperature calculated over 2 weeks prior to the start of the experiment was 29 ºC in summer and 24 ºC in winter, but this mean varied by more than 1 ºC when calculated over the 4 week period prior to the start of the experiment. Such rapid fluctuations mean that the environment is less predictable and this reduces the benefits of acclimation. This is especially true for holobiont acclimation that involves restructuring or synthesizing of proteins and pigments (Black et al. 1995, Fitt et al. 2009), uptake or expulsion of symbionts (Hoegh-Guldberg & Smith 1989, Muscatine et al. 1991, Fitt et al. 2009), and changes in the mitochondrial density (Pörtner 2002). Consequently, holobiont acclimation is likely to be more time-consuming and energetically costly than acclimation of the photosynthetic apparatus at symbiont level, which may explain why the only perfectly acclimated trait to summer temperature was the electron transport rate.

Lastly, the within-population variability of T_{opt} showed that thermal acclimation varied between coral colonies. This could be due to variation in the symbiont species composition between colonies, or genetic variation in both the thermal tolerance and thermal plasticity. Such variation could be related to differences in the ‘age’ of the sampled coral colonies that

could drive a divergence between the current environment and the selective environment at the time of development (De Jong 1999). Irreversible acclimation of some phenotypic traits might have been established during the early developmental stages of the coral larvae and juveniles, and this could constrain the thermal performance and plasticity of adult colonies. Alternatively, small differences in the microhabitat of the sampled coral colonies might have resulted in differences in the thermal environment experienced by colonies, despite their being collected within a narrow depth and habitat range. Although further research is needed to determine the mechanisms underlying differences in thermal acclimation among colonies, my results indicate that such differences are primarily driven by the coral host, as the thermal performance of Symbiodiniaceae living within the two coral species was less plastic than that of the coral hosts.

3.6 Conclusion

The unprecedented global coral bleaching event of 2017 (Hughes et al. 2017b) has highlighted the need to understand the capacity of corals to acclimate to elevated temperatures. Current models that project coral population dynamics in climate change scenarios (e.g. Hoegh-Guldberg et al. 2007) do not incorporate the reversible acclimation capacity of corals, or their performance at sub lethal temperatures, although both processes influence coral survival and fitness. This study showed that the thermally sensitive *A. valenciennesi* maximized performance between seasons by shifting the thermal optimum, whereas the thermally tolerant *P. cylindrica* maintained performance through widening the performance breadth. Such differences in thermal strategy imply that during summer warming, *A. valenciennesi* is likely to maintain high performance until a threshold temperature, after which performance will decline rapidly. In contrast, the performance of *P. cylindrica* is less affected by temperature change and therefore will decline less dramatically

at summer extremes. Additionally, the symbiont response to seasonal warming and cooling was generally consistent among the study species, and the electron transport rate was perfectly acclimated to the ambient temperature in both seasons. These results suggest that the capacity for physiological acclimation of the coral host, rather than the symbionts, will limit coral performance as ocean temperatures increase in the future.

CHAPTER 4

Thermal acclimation strategies of scleractinian corals along a latitudinal gradient on the GBR[4]

Corals bleaching around Lizard Island (March 2016)
Acropora sp. around Orpheus Island (Dec 2015)
Healthy reef around Heron Island (Feb 2017)

[4] This chapter is prepared for a theme issue of Phil Trans of the Royal Society entitled 'Physiological diversity across latitudinal and depth gradients: testing key hypotheses involving temperature and oxygen'

4.1 Abstract

Species have evolved different thermal strategies, associated with a wide range of physiological, morphological and behavioural responses, to cope with spatial and temporal thermal heterogeneity. Species with broad spatial distributions may be thermal generalists that perform well across a broad range of temperatures, or they might contain subpopulations of locally-adapted thermal specialists. Here I quantified the variation in thermal performance of two coral species that both have broad geographic distributions along a latitudinal temperature gradient on the Great Barrier Reef. The thermal performance of a bleaching tolerant coral species, *Porites cylindrica*, was compared with that of a bleaching sensitive coral species, *Acropora* spp., at Lizard Island (northern GBR, 14°S), Orpheus Island (central GBR, 18°S) and Heron Island (southern GBR, 23°S). Photosynthesis rates, respiration rates, maximum quantum yield and maximum electron transport rates were measured on coral fragments exposed to an acute temperature increase and decrease up to 5 °C above and below the local environmental temperature. Results showed that despite the geographic variation in the performance curves of both species at holobiont and symbiont level, this did not lead to an alignment of the optimal temperature for performance with the average temperature of the local environment, suggesting that the capacity for local thermal acclimation of the coral populations was constrained. Furthermore, symbiont thermal performance generally had an optimum closer to the average environmental temperature than did holobiont performance, suggesting that symbionts have a higher capacity for acclimation than the coral host, potentially aiding the coral host when temperatures are unfavourable.

4.2 Introduction

Many species have wide geographic distributions that cover broad latitudinal gradients and a correspondingly broad range of environmental conditions. For instance, populations that reside at higher latitudes are exposed to colder environments than populations that occur around the equator (Sunday et al. 2012), and the thermal environment is generally more variable at higher latitudes compared with at the equator (Stevens 1989). To cope with the thermal heterogeneity along latitudinal gradients, species have evolved to have different thermal strategies associated with a wide range of physiologic, morphologic and behavioural responses (e.g., Angilletta 2009). For instance, some species rely on heat absorption for optimal functioning, while others have evolved a morphology that facilitates heat dissipation (such as larger limbs or ears; Allen 1877), and yet others use behavioural strategies to escape the heat of the day by being active at night. Consequently, two species may tolerate a similar range of temperatures, and occupy the same geographic range, using very different strategies to cope with temperature variation.

The relationship between temperature and a trait can be fixed or (more or less) plastic along a temperature gradient (Angilletta 2009, Schulte et al. 2011). Plasticity of this relationship may lead to thermal acclimation, defined here as the adjustment of a physiological trait in response to changes in the environmental temperature that alters the performance to enhance fitness. When acclimation in response to temperature occurs during early life (known as developmental acclimation), the changes in the specific trait can become fixed during the life of the organism (Kinne 1962). Alternatively or simultaneously, acclimation can occur constantly throughout an organism's life in a process known as reversible acclimation (Whitman & Agrawal 2009). As it results in a fixed response to temperature, developmental acclimation only maximises fitness if that fixed response is optimal under the spatial and temporal heterogeneity of the thermal environment (Berrigan &

Scheiner 2004). For instance, if the environment varies over a long temporal scale that exceeds the lifetime of an individual (coarse-grained, *sensu* Levins 1968), then the individual experiences a largely homogeneous thermal environment that makes a plastic response unnecessary. Similarly, if the thermal environment varies among sites, but not within sites, a fixed temperature response would be expected at each site with variation in that fixed response observed among sites. In contrast, if the thermal environment varies during the lifetime of an individual and also varies among locations (fine-grained, *sensu* Levins 1968), reversible acclimation is required (e.g. Chapter 3), with different and plastic temperature responses expected at different sites. Through mathematical modelling, there is extensive theoretical literature on the optimal acclimation strategies given a certain thermal environment (e.g. Gabriel & Lynch 1992, Gilchrist 1995, Gabriel 1999, Gabriel 2005), but empirical evidence is ambiguous, with numerous studies showing contradicting patterns (see review by Angilletta 2009). Reasons for this can be maladapted genotypes or phenotypes that enter the local populations (Gilchrist and Kingsolver, 2001), or constraints that inhibit the phenotype to reach the optimum, such as trade-offs on the energy-budget (Angilletta et al. 2003).

According to the scenarios mentioned above, three general thermal strategies could arise within species that have broad geographic distributions (Angilletta 2009). First, the relationship between temperature and performance could be fixed requiring a broad thermal tolerance according to the entire temperature gradient the species encounters throughout its geographic distribution (**Figure 4.1 a**). This 'non-plastic thermal generalist' strategy is likely to occur if gene flow among local populations prevents local adaptation (Slatkin 1987), if temperature fluctuations are rapid and unpredictable making acclimation ineffective (Gilchrist 1995), or if the costs of plasticity outweigh the benefits (DeWitt et al. 1998). Alternatively, a species might select specific thermal microhabitats within its geographic

range, by way of behaviour or through habitat selection at settlement, such that it experiences a homogenous thermal environment and reversible acclimation is not required. Such "non-plastic thermal specialist" species (**Figure 4.1 b**) can be expected to have higher maximal performance than non-plastic generalists (Huey & Hertz 1984). Lastly, a species could

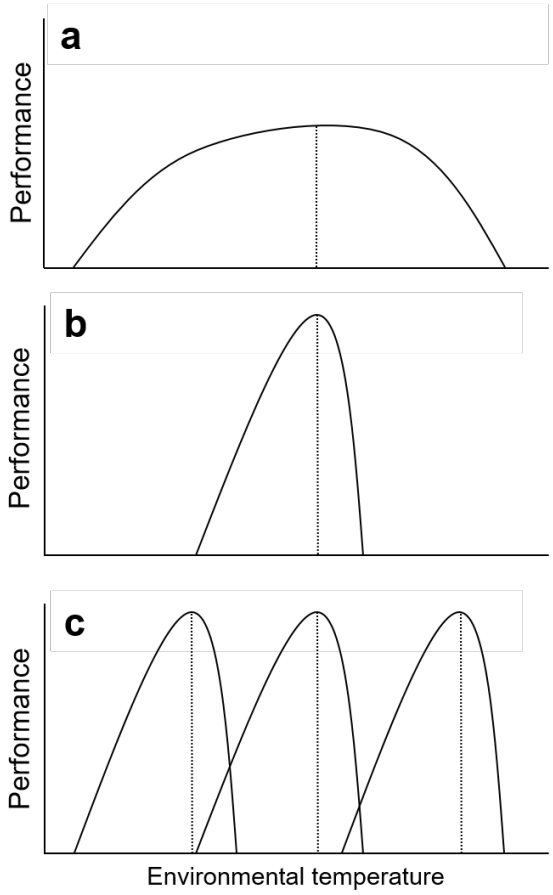


Figure 4.1 Hypothetical thermal strategies of a species over the temperature gradient along its distribution range. A species may be a non-plastic thermal generalist (a) when it experiences a heterogeneous thermal environment but lacks the capacity for acclimation. Alternatively, a species can be a non-plastic thermal specialist (b) when it experiences a homogeneous thermal environment thus does not need the capacity for acclimation. Or, a species can be a plastic thermal specialist (c) as developmental acclimation enables subpopulations to maximize performance within homogeneous thermal sites thereby allowing the species to have an overall geographic distribution similar to that of a non-plastic thermal generalist.

perceive the thermal environment as heterogeneous *among* populations, but homogenous *within* populations (Gilchrist 1995). Such species can maximize performance within each population through developmental acclimation, and/or local adaptation in cases where populations are isolated, and can be referred to as plastic thermal specialists (**Figure 4.1 c**). Consequently, a plastic thermal specialist species can survive under a similar range of temperatures to that of a non-plastic thermal generalist species, but uses a very different strategy to do so.

Thermal performance curves (TPCs) are widely used to quantify the thermal sensitivity of species (see review by Angilletta 2009). TPCs show the instantaneous performance of an organism in response to short-term (acute) environmental fluctuations along a temperature gradient (Huey and Stephenson, 1979). Typically this produces a curve from which three important parameters can be derived (Huey & Kingsolver 1989): the maximal performance (Pf_{max}), the temperature for optimal performance (T_{opt}), and the temperature range over which the performance is positive, known as the thermal breadth (T_{br}). Through developmental and reversible acclimation, the shape and position of the curve can change in response to changes in the thermal environment (Kingsolver et al. 2001). Each shift represents a trade-off between the cost of acclimation and the benefit gained from enhancing performance in the changed environment (Angilletta et al. 2003). For instance, increasing T_{opt} will enhance performance in warm environments, but can be costly if the environmental temperature decreases unpredictably. Likewise, increasing T_{br} will enhance performance in thermally heterogeneous environments, but comes at the cost of decreased Pf_{max} (Huey & Hertz 1984). Thus, to maximize performance it is important to select a thermal strategy that corresponds to the present and future thermal environment.

A complicating factor to the idealised thermal strategies described previously (**Figure 4.1**) is that nearly all environments vary both within and among populations, particularly for

long-lived species that have wide geographic distributions. Theory predicts that in such scenarios, shifting T_{opt} through developmental acclimation is only beneficial if the thermal heterogeneity among sites is greater than within sites (Gabriel & Lynch 1992; Kassen 2002), and environmental cues are accurate (Moran 1992). Additionally, increasing T_{br} is only beneficial if the temperature does indeed fluctuate during the organism's lifetime, because increased thermal breadth comes at the cost of reduced Pf_{max} (Huey & Hertz 1984). In summary, the thermal generalist strategy enables species to have positive performance across a wide temperature range, but allows for misinterpretation of environmental cues (Kassen 2002). For instance, a large meta-study involving a global data set of vertebrate species showed that high seasonal temperature variability and low diurnal temperature variability both favour thermal generalist species over thermal specialists (Chan et al. 2016). In contrast, developmental acclimation allows a plastic thermal specialist to maximize performance within a narrow temperature range but comes at the costs of poor performance when environmental cues are not accurate (Kassen 2002). Accordingly, studies proposed that global warming provides thermal generalists with greater advantages compared to thermal specialists (Stillman 2003, Dillon et al. 2010, Tewksbury et al. 2008, Huey et al. 2012). Lastly, TPCs vary between traits due to different proximate mechanisms that underlie the phenotypic expression (Angilletta et al. 2003). Therefore, the performance of multiple traits at various levels of biological organization should be measured when comparing TPCs of populations along a latitudinal cline (e.g., see review by Chown & Gaston 2016).

Global warming threatens many ecosystems, and coral reefs in particular (Pörtner et al. 2014). Corals reefs are among the most productive and biologically diverse ecosystems on Earth and provide valuable goods and services, such as fisheries, tourism, aesthetic and cultural values to vast numbers of people (Moberg & Folke 1999). However, reefs are known to be negatively affected by temperature change (Hoegh-Guldberg 1999). The Great Barrier

Reef (GBR) off the coast in northeastern Australia is the world's largest coral reef ecosystem containing ~3,000 individual reefs that extends over 14 degrees of latitude (10°40`S to 24°30`S). Accordingly, there is a thermal gradient along the GBR with a cooler and more variable thermal environment in the southern GBR and a warmer and more stable thermal environment towards the northern GBR (AIMS 2017). However, most of the ~450 hard coral species that are found in the GBR have distribution ranges throughout the entire GBR, and many are broadly distributed throughout the Indo-Pacific (Hughes et al. 2013). Consequently, the thermal environment these species experience varies significantly across space and through time. However, it is unknown whether these species are thermal generalists, non-plastic thermal specialists or plastic thermal specialists.

Despite their broad geographic distributions, corals are sensitive to temperature change, with both hot and cold leading to breakdown of the symbiosis between corals and their photosynthetic algae (i.e., coral bleaching). To date, studies of coral thermal biology have mostly focused on identifying the upper thermal thresholds for coral bleaching and survival (e.g., see Berkelmans & Willis 1999, Fitt et al. 2001, Loya et al. 2001). The few studies that investigated coral performance over a temperature gradient are ambiguous about the species-specific and environmental controls on coral thermal tolerance. For instance, T_{opt} for growth of the tropical species *Pocillopora damicornis* varied between populations with different thermal environments (Clausen & Roth 1975) suggesting a plastic thermal specialist strategy whereas another study showed no difference in T_{opt} for net productivity of *Montastraea annularis* among populations (Castillo & Helmuth 2005). Moreover, a recent study showed that there was no variation in the T_{opt}, for multiple coral host and symbiont related performance traits for Mediterranean corals from populations with different thermal environments (Rodolfo-Metalpa et al. 2014), suggesting a thermal generalist strategy. Studies comparing the thermal performance of multiple coral species, and across multiple

physiological traits, are required to assess whether and how thermal tolerance strategies differ among species.

The overarching aim of this study was to determine whether and how the coral thermal physiology varies between species and among populations distributed over a latitudinal gradient in the GBR, and thereby assess their thermal tolerance strategy. Therefore, I investigated the shape and position of the thermal performance curve of two coral species from three populations with different thermal environments. This method allowed me to answer whether the corals from these populations were acclimated to their specific thermal environment, suggesting a plastic thermal specialist strategy, or if they shared a common thermal performance curve, suggesting a non-plastic thermal generalist strategy. Aditionally, I investigated how the thermal strategy varied between species that experience a similar thermal range acros their geographic distribution. Assuming that there was limited gene flow between reefs (Ayre & Hughes 2000), the thermal performance curves should vary predictably along the latitudinal gradient. I hypothesized that the corals from the southern reef have their T_{opt} at a lower temperature than the corals from the central or northern reefs, and that the T_{br} increases with increasing thermal heterogeneity. Knowledge of the plasticity of the thermal performance of coral species and their thermal tolerance strategies will provide insight into how global warming might shape coral reefs, as a plastic thermal specialist strategy may result in higher fitness under global warming than a non-plastic thermal generalist strategy.

4.3 Material and Methods

4.3.1 Experimental design

Thermal performance curves were quantified for two stony coral species at three different locations on the Great Barrier Reef (GBR) to assess the species' thermal tolerance strategy and determine whether the populations were acclimated to the local thermal environment. The study locations (**Figure 4.2 a**) occurred along a latitudinal thermal gradient between Lizard Island (LI) situated in the northern GBR (14° 40′ 08″ S 145° 27′ 34″ E), Orpheus Island (OI) in the central GBR (18° 37′ 06″ S 146° 29′ 37″ E) and Heron Island (HI) in the southern GBR (23°26'18.71" S 151°54'30.23" E). LI and HI are both further offshore (~30 km and ~80 km respectively) compared with OI (~17 km), and the latter generally experiences higher turbidity. Seawater temperature data were recorded by *in-situ* data loggers deployed by the Australian Institute of Marine Science (AIMS 2017) at LI, OI and HI at a

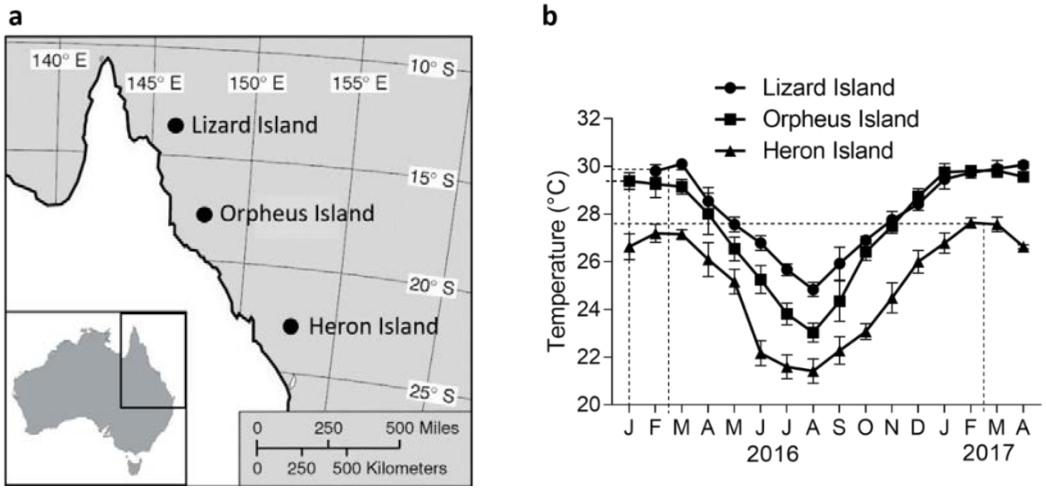


Figure 4.2 Coral collection and experimental study sites (a) located along a latitudinal gradient in Great Barrier Reef and monthly average seawater temperatures from January 2016 to April 2017 at Lizard Island at 10.1 meters depth, Orpheus Island at 5.8 m depth and Heron Island at 5.4 m depth (b). Dashed lines indicate the average ambient temperatures at the start of the thermal experiment for each location. Data sourced from the Australian Institute of Marine Science (AIMS 2017).

depth of 10.1 m, 5.8 m and 5.4 m respectively. Temperatures were recorded at an interval of 30 minutes year-round, but for this study, data collected from December 2015 to March 2017 were analysed

Fragments of *Acropora valenciennesi* (at OI), *Acropora intermedia* (at HI and LI) and *Porites cylindrica* (at LI, OI and HI) were collected (identification was based on morphological characteristics). Two species of *Acropora* were sampled due to their local abundances at the study locations but both *A. valenciennesi* and *A. intermedia* have similar morphologies (arborescent branching), symbiont communities (*Cladocopium* C3; LaJeunesse et al. 2003, Madin et al. 2016; LaJeunesse et al. 2018) and both are sensitive to high temperatures (Hoogenboom et al. 2017), whereas *P. cylindrica* contains *Cladocopium* C15 (LaJeunesse et al. 2003, Madin et al. 2016; LaJeunesse et al. 2018) and is more tolerant to high temperatures (Loya et al. 2001). Between-genus differences in the shape and position of the thermal performance curves were therefore expected. A total of twenty-five fragments of each species, five per colony, were collected at depths between 4 to 6 m by SCUBA diving on reefs adjacent to each island research station. Around Orpheus Island Research Station, coral fragments were collected in November and December 2015, with the thermal experiment starting on January 25 2016. Around Lizard Island Research Station, coral fragments were collected mid-February 2016, with the thermal experiment starting on March 2 2016. Around Heron Island Research Station, fragments were collected mid-February 2017, with the thermal experiment starting on March 6 2017. The duration of the thermal experiments meant that data collection could not be collected at all locations in the same season in a single year.

After collection, fragments were directly transported to the research station, attached to nylon string, labelled to keep track of colony identity, and randomly distributed among two large (50 L) shaded outdoor tanks. The tanks received a constant supply of new seawater at

ambient temperature pumped from the adjacent reef flat. The average (and maximum) seawater temperature measured over two weeks prior to the start of the thermal experiment on the reef flat was 29.9 (30.7) ºC at LI, 29.3 (30.2) ºC at OI and 27.6 (28.8) ºC at HI (**Figure 4.2 b**). Hence, the experimental temperature (T_{exp}) at the start of the thermal experiment, representing ambient conditions, was set at 28 ºC for HI, 29 ºC for OI and 30 ºC for LI. Fragments were given at least 1 week to recover from collection and acclimate to the tank conditions before starting the measurements.

Care was taken to minimize variation in the experimental procedure at each research station and the following description of the thermal experiment applies to each location, unless specified. The summer thermal experiment of **Chapter 3** was used to represent the thermal performance of the corals at OI. Briefly, corals were divided into two groups (two fragments of each colony per group); one group was exposed to progressively lower temperatures, while the other group was exposed to progressively higher temperatures. This design enabled calculation of two TPCs for each colony over the entire temperature gradient. After at least one week of acclimation to tank conditions, various physiological response variables (see below) were measured for each colony at ambient temperature, after which one fragment of each colony was immediately frozen at -80 °C (n = 5) for subsequent tissue analyses. After that, every day, the water temperature in each tank was increased, or decreased, by 0.5 °C using a chiller/heater unit (TK-2000, TECO, Ravenna, Italy) connected to a pump (Aquapro AP1050, Aquatec, Perth, Australia) that circulated the water at a rate of 500 l h^{-1}. This continued during 10 days, resulting in a total temperature change of 5 °C above and below ambient temperature. After every 1 °C increment, each response variable was measured, as an indicator for the performance of the coral fragment at that temperature. Qualitative observations were made about the coral colour (paleness) and tentacle expansion of the fragments in the holding tank twice per day (morning and evening). At the end of the

thermal experiment, fragments were frozen at -80 °C and subsequently transported to laboratory facilities at James Cook University for tissue analyses.

4.3.2 Response variables

Different response variables were measured in order to differentiate between the thermal responses of the holobiont versus the photosynthetic symbionts specifically. Photosynthesis and respiration rates, measured using oxygen respirometry, are mostly dominated by the coral host physiology because the biomass of the coral tissue is much larger than the biomass of the symbionts (Muscatine et al. 1981). Maximum quantum yield and electron transport rate were measured using fluorometry, as a proxy for the symbiont response, because this measuring technique quantifies the fluorescence signal from the photosynthetic pigments within the symbionts specifically.

4.3.2.1 **Holobiont response variables**

For each coral fragment, rates of net photosynthesis (Pn) and respiration (R) were measured in transparent experimental cells (6 cells, 550 ml ± 5 ml) for 1 h. Five cells were filled with filtered seawater (15 µm) and included one coral fragment, and a separate control cell contained only filtered seawater (15 µm) to account for background respiration of microorganisms in the seawater. The cells were placed on a submersible magnetic stirrer plate (MIXdrive 6, 2mag, Munich, Germany) in a water bath that controlled the water temperature inside the cells. A magnetic stirrer bar inside each cell ensured continued mixing of the water. The temperature of the water bath was controlled by a chiller/heater unit (TK-2000, TECO, Ravenna, Italy). The dissolved oxygen concentration inside each cell was measured at 1 min intervals for a duration of 1 h using oxygen probes (LDO101, Hach, Loveland, USA) connected to a meter device (HQ40D, Hach). Pnet rates were measured at a light intensity of 350 µmol photons m^{-2} s^{-1} provided by led lights (R420r, 180 W, Maxspect

Razor). At OI, for logistical reasons, 2 wide beam lamps (Oracle, Sylvania, Padstow, Australia) with 150W metal halide bulbs were used and provided a similar light intensity. R rates were measured in the dark directly after the photosynthesis measurements for a duration of 1 h. At the end of the respirometry measurements, corals were returned to their original tanks. Pnet and R rates of each coral were corrected for background oxygen consumption/production by subtracting the differential oxygen concentration of the empty control cell, and multiplying by the water volume of the cell. Data were normalized by coral skeletal surface area using the wax dipping method described by Veal et al. (2010). Briefly, the weight of the coral skeleton was recorded before and after being dipped in melted paraffin. The mass increase of the coated skeleton was calibrated to surface area using a standardized curve, which was generated using the surface area versus mass increments of wax-dipped wooden cylinders of varying (known) sizes.

4.3.2.2 **Symbiont response variables**

After finishing the dark respirometry, the maximum quantum yield (F_v/F_m) of photosystem (PS) II was measured on the dark-adapted fragments using a pulse-amplitude modulated fluorometer (DIVING-PAM, Walz, Germany). F_v/F_m describes the maximum capacity of open PS II reaction centres (within the symbiont) to capture light energy which is used for photosynthesis (Suggett et al. 2010). Quantification of F_v/F_m over a temperature gradient provides an indication of the PS II activity, or 'performance', of the symbiont at each temperature increment. Chlorophyll fluorescence was measured on dark-adapted coral fragments using a fiberoptic probe that was at a fixed distance (~3 mm) from the coral surface with a flexible piece of tubing placed around the probe tip. First, a weak light pulse (<1 mol photon m^{-2} s^{-1}) was emitted to determine the minimum fluorescence (F_0), which represents the proportion of open reaction centres of PSII (Suggett et al. 2010). Subsequently, a saturating light pulse (> 5,000 µmol photons m^{-2} s^{-1}) was applied that closed all PSII

reaction centres and was used to determine the maximum fluorescence (F_m). The maximum quantum yield, here referred to as maximum quantum yield (F_v/F_m) was calculated as [F_m – F_0] / F_m (Schreiber 2004). On each coral fragment, five measurements, evenly distributed over the coral surface, were made from which an average F_v/F_m was calculated.

In addition, after finishing the light respirometry, rapid light curves (RLCs) were measured on the light-adapted coral fragments using the DIVING-PAM. RLCs provide information on the saturation characteristics of the electron transport and the photosynthetic performance of the symbiont (Ralph & Gademann 2005). Here, RLCs were used to assess the photosynthetic capacity of PSII at different temperatures as a function of instantaneous irradiance after illumination for a fixed time period. RLCs were measured using an internal program of the DIVING-PAM that provided a sequence of 9 light steps, with light intensities increasing from 5 to 1800 µmol photons m^{-2} s^{-1} (Light-Curve Intensity 2). Each illumination period lasted 10 s and finished with a saturating pulse that measured the effective quantum yield ($\Delta F/F_m'$), calculated as [F_m' – F] /F_m', where F_m' is the maximum fluorescence of the light adapted sample and F is the instant fluorescence emission. The relative electron transport rate (rETR) was then calculated as:

$$rETR = \Delta F/F_m' * PAR * 0.84 * 0.5 \quad \text{(Eqn. 4.1)}$$

where PAR is the photosynthetically active radiation, 0.84 is the assumed light absorbance of the sample, and 0.5 corrects for 2 photons of light required for the transport of 1 electron. RLCs were created by plotting rETR against instant irradiance, from which the maximum rETR ($rETR_m$) was taken from the plot.

4.3.2.3 **Chlorophyll concentration**

Chlorophyll concentrations were determined as an indication for the total photosynthetic capacity of the corals at each site. Heat and cold can lead to damage of the photosystems,

which affects the photosynthetic capacity of the symbiont and holobiont (Roth et al. 2012). Therefore, chlorophyll concentrations were measured in fragments sampled at the start of the experiment at ambient temperature (ambient group, $N = 5$) and in fragments sampled at the end of the experiment after exposure to 5 °C above or below ambient temperature (consecutively, heated and chilled group, $N = 10$ per group). Coral tissue was removed from the skeleton using an airbrush and 15 ml filtered (15 µm) seawater. The tissue slurry was homogenized using a homogenizer (T 25 Ultra-Turrax, IKA, Germany) and centrifuged for 10 min at 5,000 k (Rotina 380R, Hettich Lab Technology, Germany). The supernatant was discarded and 5 ml of 90% acetone was added to the pellet and left at 4 °C in darkness overnight to extract the chlorophyll. The solution was then centrifuged once more for 10 min at 5,000 k, after which 200 µl of the supernatant was added in triplicates to a multiplate well. Absorbance was measured at 630, 663 and 750 nm using a spectrophotometer (Spectramax M2 Reader, Molecular Devices, Sunnyvale, USA). Chlorophyll *a* and *c2* concentrations were calculated using the equations of Jeffrey and Humphrey (1975b) and normalized by skeletal surface area, measured as described above.

4.3.3 Data analyses

Data were analysed using the statistical software R version 3.0.3 (The R Foundation for Statistical Computing) and graphed with Prism GraphPad Software version 7.03.

To assess whether the temperature response of *Porites cylindrica* and *Acropora* spp. varied between locations and species, nonlinear least-squares regression models were fitted to the data for each response variable (Pnet, R, F_v/F_m and $rETR_m$). A symmetrical Gaussian function was chosen over an asymmetrical function as this provided a better fit with fewer parameters (Angilletta 2006, see Appendix **Table C.1**). The following Gaussian function (Rodolfo-Metalpa et al. 2014) was used:

$$P = Pf_{max} \exp [-0.5 (\text{abs} (T - T_{opt})) / T_{br})^2] \quad \text{(Eq. 4.2)}$$

where P is the temperature (T) dependent physiological response, Pf_{max} is the maximum value of that response, T_{opt} is the temperature at which the response value is optimal (i.e. the mean value) and T_{br} provides a measure of the breadth of the response curve (i.e., the standard deviation).

For each response variable, the function was first fitted to all the data pooled together regardless of location and species, then fitted to the data separated by either species or location, then to the data separated by both species and location, and finally to the data separately for each coral colony of each species and at each location. The Akaike Information Criterion (AIC) was used to assess whether the shape of the thermal performance curve differed significantly between species and among locations. To do this, I summed the AIC values over the multiple fits of the equation to different divisions of the data, and chose the division of the data with the lowest summed AIC value as the model that was most strongly supported by the data.

As the overall aim of this study was to determine whether coral populations are acclimated and/or adapted to the thermal regime of their local environment, I focused primarily on the average responses of the species at each site. Therefore, the population response was calculated for each parameter of the TPC (P_{max}, T_{opt} and T_{br}) by averaging the colony responses at every location (per species). A one-way analyses of variance (ANOVA) was used to detect differences in the parameter estimations between the populations. When there were significant differences, Tukey post-hoc analyses were performed. P-values were considered significant when $p < 0.05$.

Chlorophyll data were tested for assumptions of normality using the Shapiro-Wilk test and Levene's test for homogeneity of variance. Data were log transformed (for *Acropora*) or square root transformed (for *Porites*) when the assumption of homogeneity was violated.

Considering the repeated measurement of multiple coral fragments from individual coral colonies, data were analysed separately for each species using mixed effects ANOVAs with treatment (heated and chilled) and location as fixed effects and colony as random effect, to detect differences in mean chlorophyll concentrations within species across location and treatment. Chlorophyll concentrations of the fragments collected at the start of the experiment at ambient temperature were analysed separately, using a two-way ANOVA with species and location as main effect, to detect differences in the chlorophyll concentration between locations and species and a post-hoc Tukey test to detect which locations differed.

4.4 Results

4.4.1 Thermal environment at sampling sites

During the thermal experiments at each site, the average (and maximum) seawater temperature was 29.4 (30.2) ºC at OI in January 2016, 29.7 (30.7) ºC at LI in February 2016 and 27.6 (29.1) ºC at HI in February 2017. Temperature data were not available for LI during December 2015 and January 2016, but overall, seawater temperatures were distinctly lower at HI compared with OI and LI, with the latter two sites having similar summer temperatures (**Table 4.1**). However, in winter, OI experienced cooler temperatures than LI and therefore the annual variability in temperature was larger at OI than at LI (minimum and maximum temperature in 2016/2017 at OI was 22.2 ºC to 31.0 ºC and at LI 24.2 ºC to 30.8 ºC). The annual temperature variability was even greater at HI, where temperature fluctuated from 18.1 ºC to 29.1 ºC in 2016/2017, which was 1.7 times larger than the fluctuation at LI and 1.3 times larger than that at OI. Additionally, LI experienced higher sustained temperatures than OI in February and March 2016, with 100% of hourly data above 29 ºC and 44% above 30 ºC at LI compared with 71% of the hours above 29 ºC and only 3% above 30 ºC at OI).

Table 4-1 Summary of the seawater temperatures at Heron Island (at 5.4 m depth), Orpheus Island (at 5.8 m depth) and Lizard Island (at 10.1 m depth) analysed over the period December 1 to March 31 in the years 2015 – 2016 and 2016 – 2017. Underlined text shows the data that defined the thermal environment prior to the thermal experiment at each location. At Lizard Island in 2015 – 2016, data were not recorded during December and January. Data sourced from the Australian Institute of Marine Science (AIMS 2017).

		2015 - 2016			2016 - 2017		
		HI	OI	LI	HI	OI	LI
Average T	Dec	25.6 ± 0.4	28.7 ± 0.2	n.a	26.0 ± 0.3	28.7 ± 0.2	28.4 ± 0.1
± st. dev	Jan	26.6 ± 0.3	29.4 ± 0.2	n.a	26.8 ± 0.3	29.8 ± 0.2	29.5 ± 0.1
	Feb	27.2 ± 0.3	29.3 ± 0.3	29.8 ± 0.1	27.6 ± 0.3	29.8 ± 0.1	29.7 ± 0.1
	Mar	27.2 ± 0.4	29.2 ± 0.1	30.1 ± 0.1	27.6 ± 0.3	29.8 ± 0.2	29.9 ± 0.1
Min – Max T	Dec	22.8 - 27.1	27.3 - 29.6	n.a	24.0 - 27.7	26.5 - 29.7	27.8 - 29.2
	Jan	**24.2 - 28.8**	28.4 - 30.4	n.a	25.2 - 28.5	**29.0 - 31.0**	28.6 - 30.5
	Feb	25.5 - 28.8	**26.7 - 30.6**	29.3 - 30.7	**25.7 - 29.1**	29.4 - 30.8	29.0 - 30.6
	Mar	25.6 - 28.5	28.3 - 30.1	**29.5 - 30.8**	26.3 - 28.9	29.2 - 30.9	**29.2 - 30.8**
Variability (min-max)		10.5	9.4	8.0	11.0	8.7	6.5
% hrs > 28 ºC	Dec	0	97	n.a	0	99	94
	Jan	2	100	n.a	0	100	100
	Feb	2	93	100	13	100	100
	Mar	1	100	100	15	100	100
% hrs > 29 ºC	Dec	0	29	n.a	0	24	1
	Jan	0	83	n.a	0	100	82
	Feb	0	77	100	0	100	100
	Mar	0	65	100	0	100	100
% hrs > 30 ºC	Dec	0	0	n.a	0	0	0
	Jan	0	4	n.a	0	30	12
	Feb	0	6	24	0	13	13
	Mar	0	0	68	0	21	39

4.4.2 Thermal performance

The experimental coral fragments showed high survival during the experiments, with 93% of fragments remaining alive at the end of the thermal experiment. During the experiment at LI, all 4 fragments from one *Acropora* colony showed tissue necrosis after T_{exp} +3 ºC and T_{exp} - 4 ºC. These fragments were excluded from the experiment because the cause of the tissue necrosis could not be reliably determined. Some paling of tissues was observed

for *Acropora* fragments at both HI and LI, when the experimental temperature reached T_{exp} + 5 ºC and tentacle expansion was no longer observed at those temperatures. Overall, the response variables generally showed non-linear relationships with temperature for both *Acropora* (**Figure 4.3**) and *Porites* (**Figure 4.4**). Each response variable is considered in more detail in the following paragraphs, but for several variables there was a relatively broad temperature range over which responses were consistent followed by a steep drop in the response at the highest temperatures (e.g. net photosynthesis rates and photosynthetic efficiency of both species, **Figure 4.3 a-c, g-i** and **Figure 4.4 a-c, g-i**). The data for respiration rates did not show a strong curvature with increasing temperature and linear regression through the data fitted the data equally well as the Gaussian curve for the data from OI. However, linear approximations of thermal performance curves should be avoided since they fundamentally differ from the physiology of thermal performance, thus Eq. 4.2 was fitted to the respiration rates at all three locations. Finally, model selection based on AIC revealed that data divided by location, by species and by individual coral colony provided the best fit to the host and symbiont response variables (Appendix **Table C.2**). Dividing the data by location and by species provided the next best fit to the data and the model selection technique did not support pooling data across locations or across species. Therefore, these data indicate that the thermal performance varied among locations and between species. A stylised presentation of the performance curves (**Figure 4.5**) facilitates direct comparison in the change in position and shape of the curves among locations for each species.

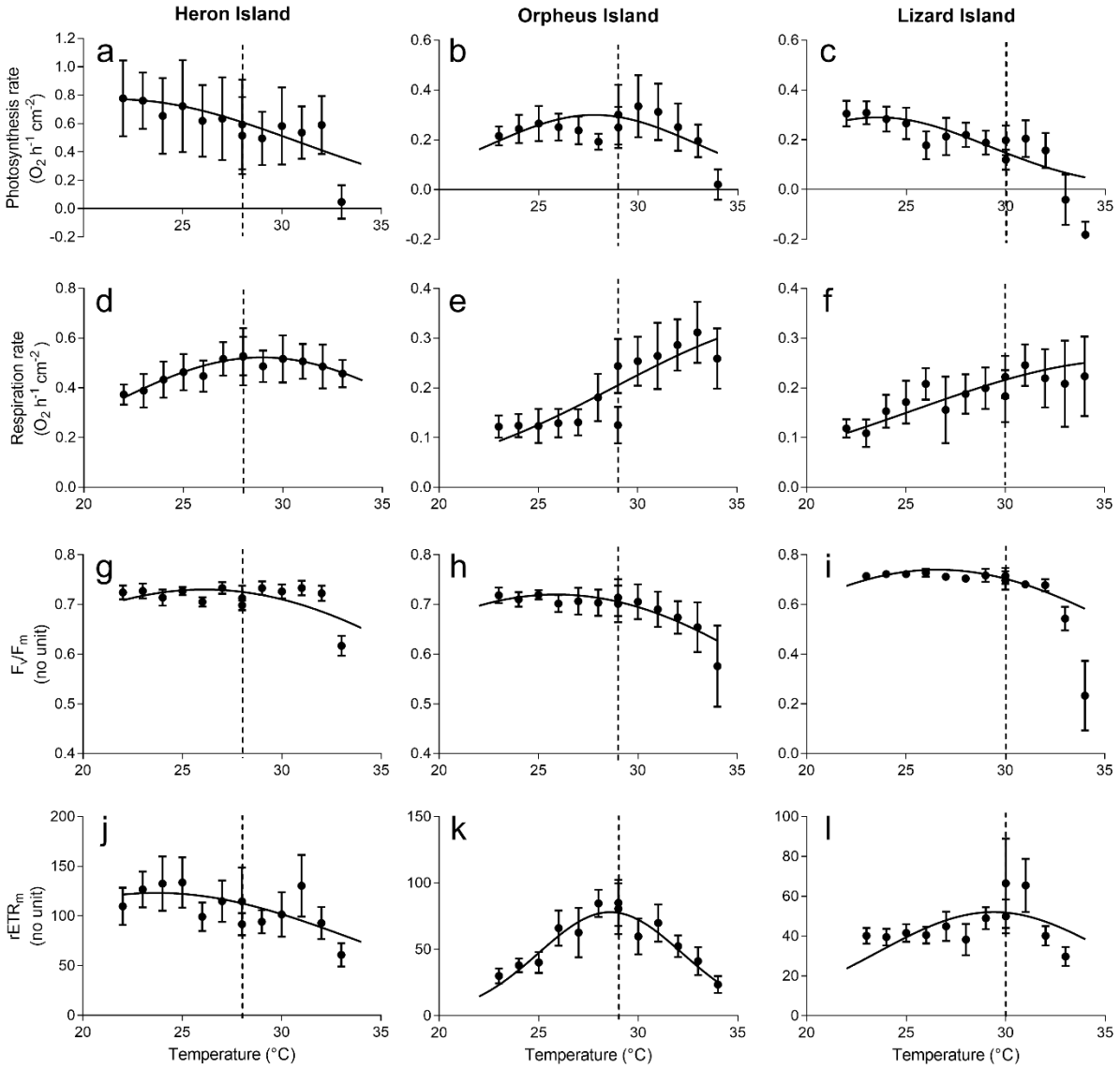


Figure 4.3 Thermal performance curves of *Acropora* intermedia measured at Heron Island (first column) and Lizard Island (last column) and *Acropora valenciennesi* at Orpheus Island (middle column). Thermal responses are net photosynthesis rate (a-c), respiration rate (d-f), maximum quantum yield (g-i) and maximum electron transport rate (j-l). Data points are the mean values ± s.d (n = 10). Curves are mean thermal performance curve of individual colonies (n = 5), fitted with least square non-linear regressions. Vertical dashed lines represent the environmental temperature at the start of the thermal experiment.

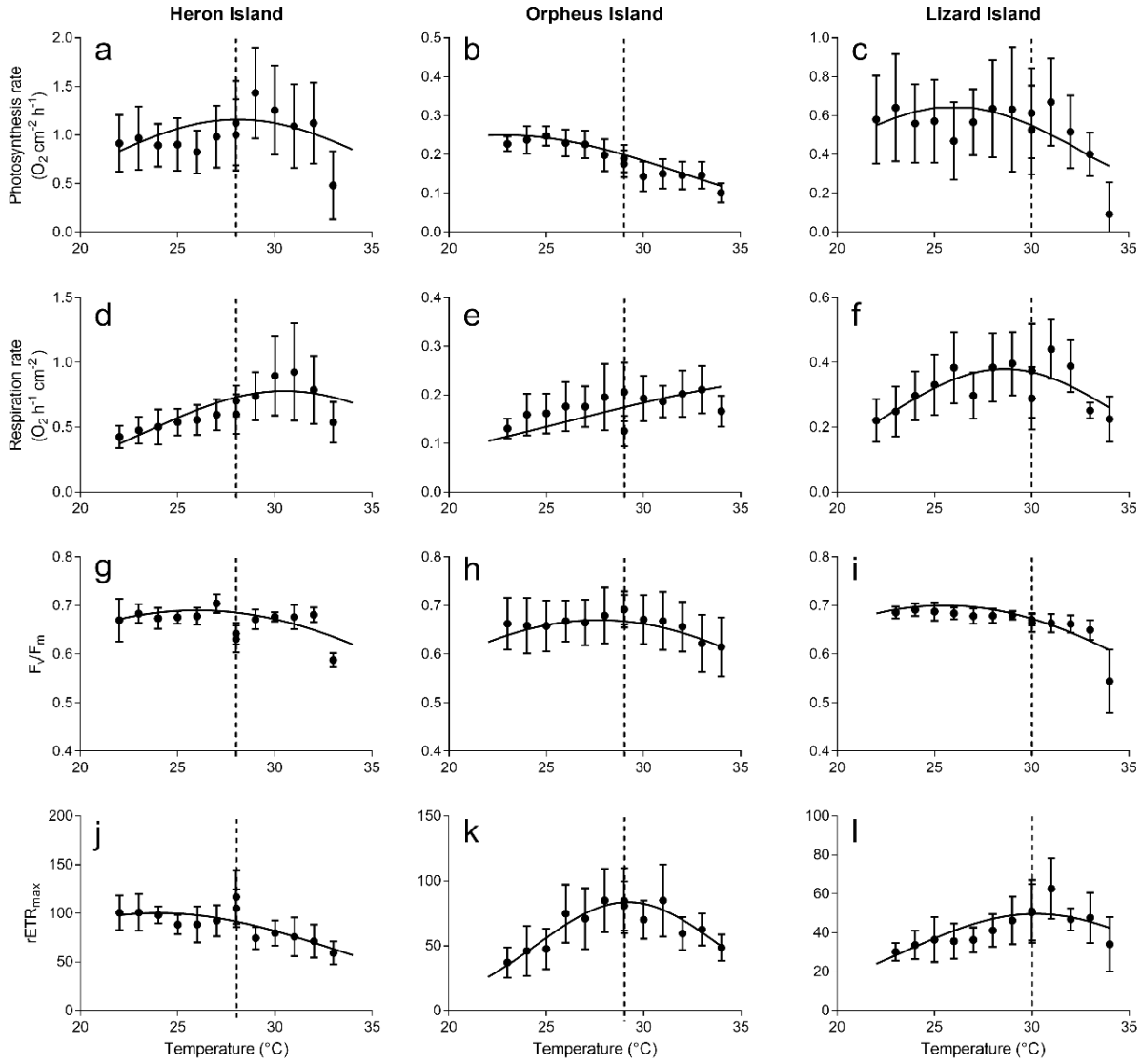


Figure 4.4 Thermal performance curves of *Porites cylindrica* measured at Heron Island (first column), Orpheus Island (middle column) and Lizard Island (last column). Thermal responses are net photosynthesis rate (a-c), respiration rate (d-f), maximum quantum yield (g-i) and maximum electron transport rate (j-l). Data points are the mean values ± s.d (n = 10). Curves are mean thermal performance curve of individual colonies (n = 5), fitted with least square non-linear regressions. Vertical dashed lines represent the environmental temperature at the start of the thermal experiment.

4.4.2.1 **Holobiont response**

The temperature at which the net photosynthesis rate was maximum, T_{opt}, was below the environmental temperature at all three locations for both species, except for the *Porites* population at HI where the optimal temperature was approximately the same as the environmental temperature (**Figure 4.4 a**). There was no clear trend of increasing T_{opt} corresponding to increasing environmental temperature for either species; for *Acropora* the highest T_{opt} was observed at OI (27.8 ± 1.5 ºC, **Table 4.2**), whereas for *Porites*, the highest T_{opt} was observed at HI (28.1 ± 2.4 ºC, **Table 4.2** and see Appendix **Table C.3** for post-hoc comparisons of the parameter estimates between populations). The breadth of the curve (T_{br}) for *Acropora* was significantly larger at HI (T_{br} = 18.4 ± 5.4 ºC) than at the other two locations (T_{br} = 10.4 ± 0.8 ºC and 11.0 ± 4.2 ºC; ANOVA, p = 0.012, **Table 4.3**), consistent with the greater variability in temperature at HI. For the *Porites* populations, there was no significant variation in T_{br} among locations (**Table 4.3**). The maximum net photosynthesis rate was significantly higher at HI than at the other two locations for both *Acropora* and *Porites* (respectively, ANOVA, p = 0.012 and p < 0.0001, **Table 4.3**). Overall, the performance curves of the *Acropora* populations (**Figure 4.5 a**) shifted vertically (through increased Pf_{max} at HI), horizontally (through increased T_{opt} at OI), and by changing the performance breadth (through increased T_{br} at HI). For the *Porites* populations (**Figure 4.4**), the performance curve shifted vertically (highest Pf_{max} at HI and lowest at OI) and horizontally (lowest T_{opt} at OI) but there was no change in the performance breadth.

The respiration rates of the *Acropora* (**Figure 4.3 d-f**) and *Porites* (**Figure 4.4 d-f**) populations at HI and LI increased with increasing temperature and then decreased at approximately T_{exp} +3 ºC. In contrast, at OI, the respiration rates of the *Acropora* population increased linearly with temperature without a decrease at high temperatures, while the respiration rates of the *Porites* population were not strongly influenced by temperature.

Table 4-2 Average ± standard deviation of the parameter estimates for each physiological thermal response variable of *Acropora* spp. at Heron Island, Orpheus Island and Lizard Island (first three columns) and *Porites cylindrica* at Heron Island, Orpheus Island and Lizard Island (last three columns) computed through least square non-linear regression for individual colonies (*n* = 5).

Thermal response	Parameter estimate	*Acropora* spp.			*P. cylindrica*		
		Heron Island	Orpheus Island	Lizard Island	Heron Island	Orpheus Island	Lizard Island
Pnet	P_{max} (O_2 h^{-1} cm^{-2})	0.77 ± 0.16	0.30 ± 0.06	0.29 ± 0.07	1.16 ± 0.11	0.25 ± 0.02	0.64 ± 0.22
	T_{opt} (ºC)	21.7 ± 2.0	27.8 ± 1.5	23.6 ± 3.9	28.1 ± 2.4	22.8 ± 3.3	26.0 ± 1.8
	T_{br} (ºC)	18.4 ± 5.4	10.4 ± 0.8	11.0 ± 4.2	15.0 ± 4.8	18.4 ± 4.8	14.2 ± 2.8
R	P_{max} (O_2 h^{-1} cm^{-2})	0.52 ± 0.03	0.44 ± 0.20	0.26 ± 0.08	0.78 ± 0.19	0.24 ± 0.12	0.38 ± 0.08
	T_{opt} (ºC)	29.0 ± 0.5	39.6 ± 6.2	37.0 ± 10.3	30.5 ± 1.9	40.5 ± 22.8	28.6 ± 0.5
	T_{br} (ºC)	16.4 ± 1.2	20.6 ± 8.8	23.0 ± 12.2	14.0 ± 3.0	28.8 ± 22.8	12.4 ± 1.4
F_v/F_m	P_{max} (no unit)	0.73 ± 0.01	0.72 ± 0.01	0.74 ± 0.03	0.69 ± 0.01	0.67 ± 0.03	0.70 ± 0.01
	T_{opt} (ºC)	26.1 ± 0.4	25.9 ± 1.3	26.6 ± 0.7	26.0 ± 1.0	27.7 ± 0.9	25.5 ± 2.1
	T_{br} (ºC)	33.4 ± 3.6	30.8 ± 8.4	21.4 ± 9.0	34.6 ± 2.8	30.4 ± 10.4	32.0 ± 13.0
$rETR_m$	P_{max} (no unit)	123.3 ± 4.9	78.1 ± 7.1	52.1 ± 5.0	100.3 ± 5.7	83.6 ± 10.8	49.7 ± 8.3
	T_{opt} (ºC)	23.7 ± 1.7	28.6 ± 0.4	29.4 ± 0.6	24.0 ± 2.7	29.2 ± 0.9	30.2 ± 0.6
	T_{br} (ºC)	20.4 ± 3.4	7.2 ± 0.4	11.8 ± 2.2	18.4 ± 6.6	9.4 ± 1.4	13.6 ± 3.4

Overall, this resulted in parameter estimates for T_{opt} that were relatively high, ranging from 28.6 ± 0.5 ºC for the *Porites* population at HI up to 39.6 ± 6.2 ºC for the *Acropora* population at OI (**Table 4.2**). I note that T_{opt} corresponds to the highest respiration rate which is generally interpreted to reflect metabolic costs (e.g. tissue maintenance, stress) rather than metabolic processes that contribute to growth. Caution must also be taken when interpreting the respiration rates, as abrupt declines in respiration at temperatures beyond T_{opt} are likely due to impairment of the enzyme-driven reactions rather than a decrease in metabolic costs. The breadth of each of the performance curves for respiration was relatively broad and not significantly different across locations for either species (**Table 4.3**). However, the respiration rates of the *Porites* population at HI was more than two-fold higher compared with LI, and three-fold higher compared with OI, but for the *Acropora* populations was the variation in P_{max} not significant (**Table 4.3**). Overall, the performance curve of the *Acropora* populations did not show any significant shift (either vertically or horizontally; **Figure 4.5 c**), while among *Porites* populations (**Figure 4.5 d**), the curve only shifted vertically (highest T_{opt} at HI).

4.4.2.2 **Symbiont thermal response**

Temperature did not have a strong effect on the maximum quantum yield (F_v/F_m) of the *Acropora* populations (**Figure 4.3 g-i**) or *Porites* populations (**Figure 4.4 g-i**) at any of the study locations, which resulted in flattened performance curves, even though the high experimental temperatures (T_{exp} +4 ºC and T_{exp} +5 ºC) caused a strong decline in F_v/F_m. For the *Acropora* fragments at LI, it was not possible to measure a reliable photosynthetic yield at 34 °C (T_{exp} +5 ºC), or F_v/F_m was < 0.30. Hence, data points at 34 °C were not included when fitting the non-linear regressions for the *Acropora* colonies at LI. Nevertheless, the T_{opt} for F_v/F_m of both species was below the environmental summer temperature at every location.

Table 4-3 Results of the statistical analyses to detect population variability (at Lizard Island, Orpheus Island and Heron Island) in the parameter estimates (Pmax, Topt and Tbr) of the thermal performance curves for four physiological response variables. Parameter estimates were calculated using non-linear regression for 5 colonies at each location for *Acropora* spp. and *Porites cylindrica*.

Thermal response	Parameter estimate	*Acropora* spp.			*P. cylindrica*		
		df	F-value	*p-value*	df	F-value	*p-value*
Pnet rate	P_{max}	2, 12	35.26	**0.000**	2, 14	68.61	**0.000**
	T_{opt}	2, 12	7.21	**0.009**	2, 14	5.87	**0.014**
	T_{br}	2, 12	6.51	**0.012**	2, 14	1.69	0.220
R rate	P_{max}	2, 11	3.70	0.059	2, 14	23.99	**0.000**
	T_{opt}	2, 11	3.00	0.091	2, 14	1.12	0.354
	T_{br}	2, 11	0.59	0.572	2, 14	2.20	0.148
F_v/F_m	P_{max}	2, 12	1.27	0.315	2, 14	1.55	0.247
	T_{opt}	2, 12	0.71	0.511	2, 14	4.32	**0.034**
	T_{br}	2, 12	3.21	0.077	2, 14	0.28	0.763
rETRm	P_{max}	2, 12	160.83	**0.000**	2, 14	42.48	**0.000**
	T_{opt}	2, 12	45.96	**0.000**	2, 14	22.27	**0.000**
	T_{br}	2, 12	46.12	**0.000**	2, 14	8.13	**0.004**

The variation in T_{opt} among the *Acropora* populations was less than 1 ºC and not significantly different, ranging from 25.9 ± 1.3 ºC at OI to 26.6 ± 0.7 ºC at LI (**Table 4.2** and **Table 4.3**). There was slightly more variability in T_{opt} among the *Porites* populations, with a T_{opt} at OI significantly higher than at LI (T_{opt} = 27.7 ± 0.9 ºC and 25.5 ± 2.1 ºC respectively; Tukey post-hoc, p = 0.038). The breadths of the curves of both species were broad but became narrower with decreasing environmental variability (**Table 4-1**), although this trend was not significant (**Table 4.3**). Lastly, the maximum quantum yield was higher in *Acropora* (**Figure 4.3 g-i**) than in *Porites* (**Figure 4.4** g-i). Overall, the performance curve of *Acropora* did not change significantly among locations (**Figure 4.5 e**), while the performance curve of *Porites* only shifted horizontally (T_{opt} at OI the highest; **Figure 4.5 f**).

For the maximal relative electron transport rate ($rETR_m$), T_{opt} significantly increased with environmental temperature for *Acropora* (**Figure 4.3 j-l**) and *Porites* (**Figure 4.4 j-l**). In addition, T_{opt} was also close to the environmental summer temperature for the populations at OI and LI (for example, the environmental temperature at OI was ~29 ºC and the T_{opt} of the *Acropora* population was 28.6 ± 0.4 ºC and that of the *Porites* population was 29.2 ± 0.9 ºC; **Table 4.2**), suggesting that acclimation to the local temperature environment occurred at symbiont level for this particular photosynthesis trait. Likewise, the parameter estimates for T_{br} of both species were significantly larger at HI and smaller at OI and LI (**Table 4.2** and **Table 4.3**), which was similar to the trend observed for F_v/F_m and likely to be associated with the larger variability in environmental temperatures at HI (**Table 4-1**). Lastly, $rETR_m$ was highest at HI and lowest at LI, a trend observed with the holobiont responses as well (**Table 4.2**). Overall, (**Figure 4.5 g-h**), the performance curves of both species shifted vertically (highest Pf_{max} at HI), horizontally (lowest T_{opt} at HI) and in the performance breadth (widest T_{br} at HI).

4.4.3 Within-population variability

There was strong model support for different thermal responses among locations, species and colonies (Appendix **Table C.2**), indicating that the thermal performance varied considerably among colonies within species across all locations (see Chapter 6 General Discussion). All three parameter estimates (T_{opt}, T_{br} and P_{max}) for the holobiont and symbiont response variables varied among colonies (Appendix **Table C.4** - **Table C.7**), and among-colony variation in T_{opt} is visualised in **Figure 4.6 a-c.** Regarding T_{opt}, variability between colonies was generally larger for the holobiont responses compared to the symbiont responses (**Figure 4.6 a-c**). For instance, the lowest and highest optimal temperature for net photosynthesis within the *Acropora* population at LI ranged from 17.9 ºC to 29.0 ºC, while

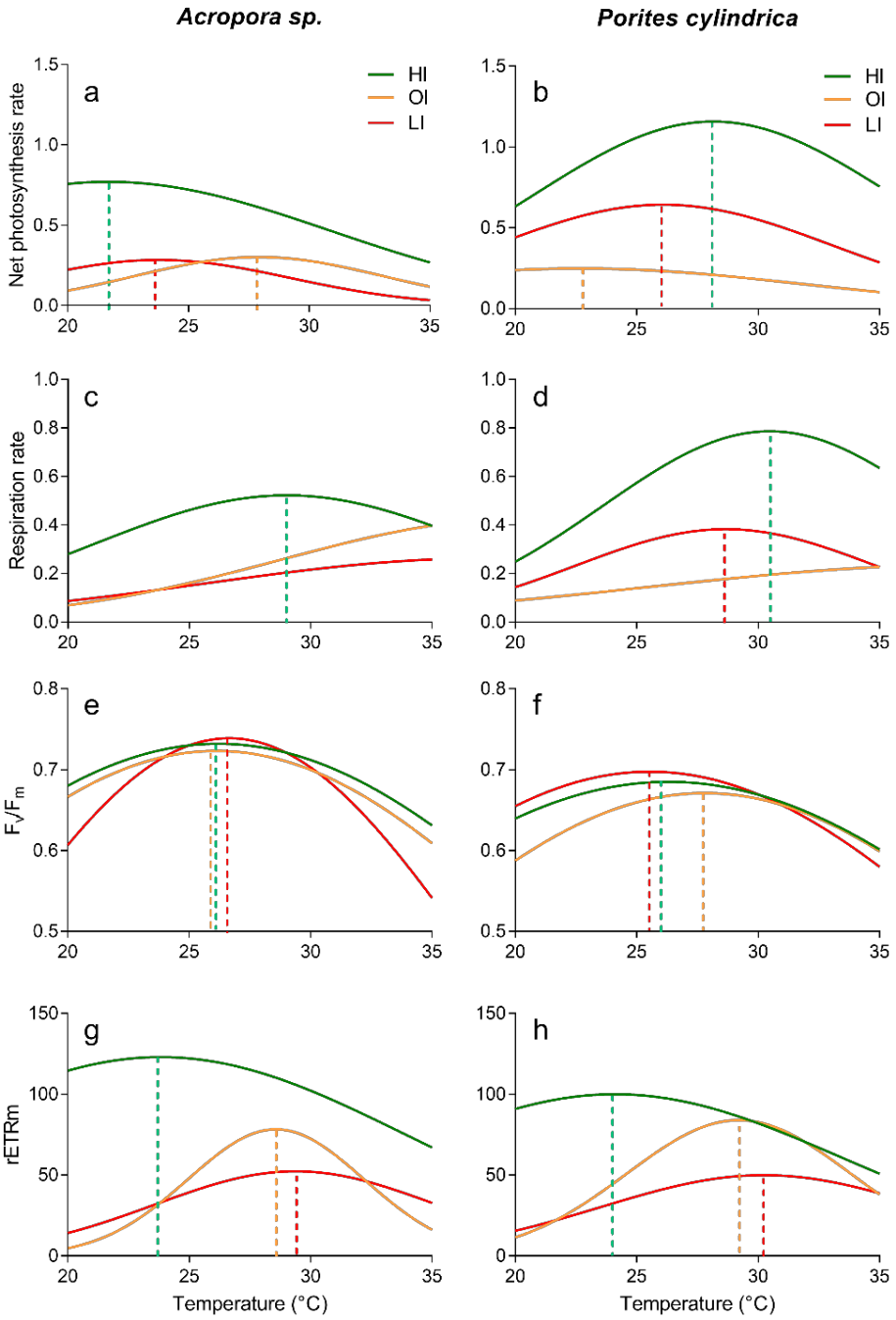


Figure 4.5 Stylised presentation of the thermal performance curves of *Acropora* spp. (left column) and *Porites cylindrica* (right column) at Heron Island (green line), Orpheus Island (orange line) and Lizard Island (red line) to visualize the change in the position and shape of the curves among locations. Thermal responses displayed are net photosynthesis rate (a-b), respiration rate (c-d), maximum quantum yield (e-f) and maximum electron transport rate (g-h). Dashed lines represent the temperature at which the performance was optimal (Topt) at each location. Curves were fitted with least square non-linear regressions using Equation 2 to individual colonies (n = 5), of which the average per species are displayed.

the T_{opt} for F_v/F_m within same population ranged only from 25.9 to 26.8 ºC. Similarly for *Porites,* the range in T_{opt} for net photosynthesis of the population at HI (**Figure 4.6 a**) was 6.2 ºC, while for F_v/F_m within the same population, this range was only 2.3 ºC. Although these ranges are within the environmental range (11.0 ºC at HI for 2016/2017), there were several *Porites* colonies with a T_{opt} (for the holobiont responses) higher than the maximum annual temperature (**Figure 4.6 a**, open squares are around and above the upper dashed line).

The variability in T_{opt} among *Porites* colonies was slightly larger than that observed for *Acropora*. Interestingly, the variability for Pnet among *Porites* colonies was greatest at OI (10.8 ºC; **Figure 4.6 b**) compared to the other locations, while this was the smallest among *Acropora* colonies (3.7 °C). Vice versa, the greatest variability for Pnet among *Acropora* colonies was observed at LI (11.1 °C; **Figure 4.6 c**) while this was the smallest among *Porites* colonies (4.3 °C).

For most colonies of both species were the T_{opt} within the range of the environmental variability (ignoring the T_{opt} for respiration, since that requires a different interpretation, as mentioned above). Generally, the T_{opt} of the holobiont performance were closer to the lower thermal threshold (only at HI was the T_{opt} of several *Porites* colonies above the upper threshold; Fig 6a), while the T_{opt} for the symbiont performances were closer to the average environmental temperature of the weeks prior to the thermal experiment (solid line in **Figure 4.6 a-c**), suggesting a higher capacity of acclimation at symbiont level and poor performance of most colonies at holobiont level at their current environmental temperature.

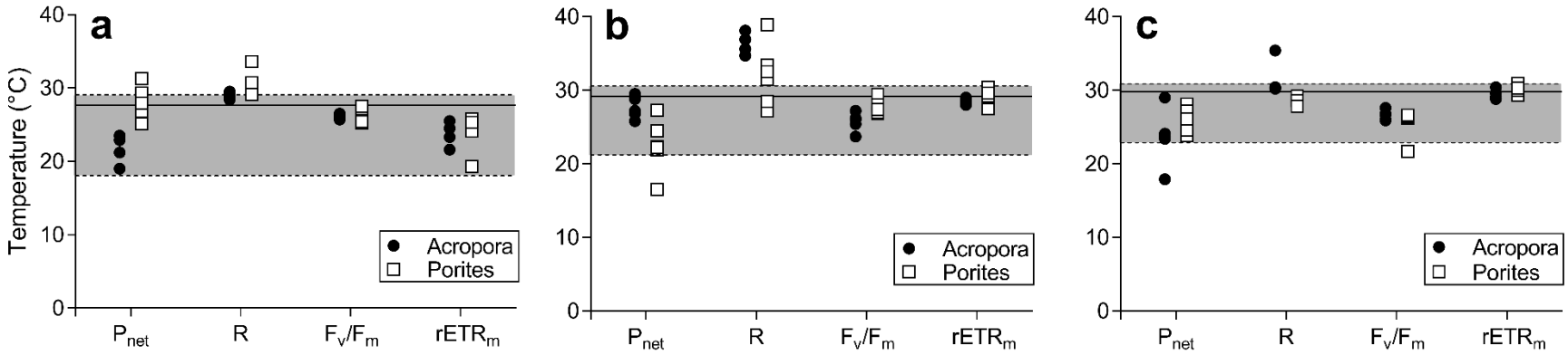


Figure 4.6 Variation in optimal temperature between colonies of *Acropora* spp. (closed circles) and *Porites cylindrica* (open squares) at Heron Island (a), Orpheus Island (b) and Lizard Island (c) for net photosynthesis rate (Pnet), respiration rate (R), maximum quantum yield (F_v/F_m) and maximum electron transport rate (rETRm). Data points show mean Topt derived by non-linear regression of 2 or 4 fragments from the same colony. Horizontal lines represent the average seawater temperature measured over 14 days prior to the start of the thermal experiment at each location, with dashed lines the minimum and maximum temperature recorded over 2015/2016. Seawater temperature data recorded by in-situ data loggers of the Australian Institute of Marine Science (AIMS 2017).

4.4.4 Chlorophyll concentration

The thermal experiment affected the chlorophyll concentration in both species (mixed effect model with main effect of treatment for *Acropora* and *Porites* respectively, $F(1,38) = 61.59$, $p < 0.001$ and $F(1,40) = 24.95$, $p < 0.001$; Appendix **Table C.7**), with generally a higher chlorophyll concentration in the fragments that were exposed to the chilled treatment than those exposed to the heated treatment (**Figure 4.7 a-b**). Only among the *Acropora* populations I observed variation in the chlorophyll concentration between locations (mixed effect model with main effect of location for *Acropora*, $F(2,12) = 112.22$, $p < 0.001$), with a higher concentration at OI possibly due to the different *Acropora* species at this site (*A. valenciennesi* instead of *A. intermedia*). Lastly, the chlorophyll concentration in fragments at ambient temperature was higher in *Porites* (**Figure 4.7 b**) than in *Acropora* (two-way ANOVA with main effect of species, $F(1,27) = 11.65$, $p = 0.002$; Appendix **Table C.8**), which corresponds to the higher net photosynthetic performance observed with *Porites* fragments compared with *Acropora* fragments. The chlorophyll concentration was also higher in fragments of *Porites* at LI than in fragments at the other two locations (two-way ANOVA with main effect of location, $F(2,27) = 31.21$, $p < 0.001$; Appendix **Table C.8**).

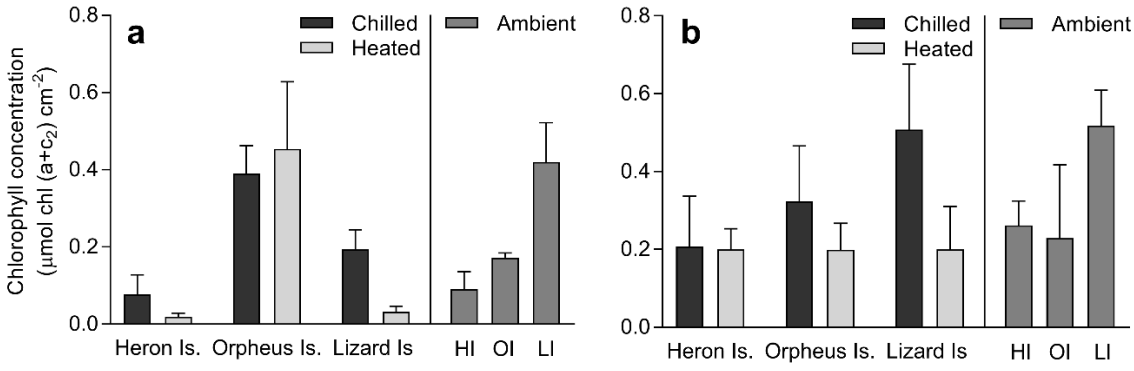


Figure 4.7 Chlorophyll concentration in *Acropora* spp. (a) and *Porites cylindrica* (b) after (chilled and heated, n = 10) and before (ambient, n = 5) exposure to a thermal gradient at Heron Island, Orpheus Island and Lizard Island.

4.5 Discussion

Our current understanding about coral thermal sensitivity is primarily based on the tolerance of different species to abnormally high temperatures; surprisingly little is known about the strategies that corals use to cope with temperature variation across their often broad geographic ranges. This study showed that the thermal performance varied between two coral species that occur across the same latitudinal temperature gradient along the Great Barrier Reef, and that have broadly similar Indo-Pacific geographic distributions (Wallace 1999, Veron 2000). Moreover, my results indicate that both species are plastic thermal specialists, rather than non-plastic thermal generalists, because the thermal performance differed within species among locations. Nevertheless, the observed differences in thermal performance among populations did not lead to an alignment of the optimal temperature for performance with the average temperature of the local environment, suggesting that the capacity for thermal acclimation of coral populations is constrained.

I hypothesized that the thermal performance curves of subpopulations of plastic thermal specialist species would change shape and position according to the thermal variability and mean environmental temperature of their local environment (e.g., Gabriel & Lynch 1992). Specifically, I expected increasing thermal breadth with increasing latitude due to greater thermal heterogeneity at high latitudes, but decreasing thermal optima with increasing latitude due to lower mean environmental temperatures. Results for the thermal performance of symbiont traits showed a general trend consistent with these hypotheses, but not for the thermal performance of holobiont traits. In fact, although the optimal temperature for holobiont performance (net photosynthesis and respiration rate) varied among coral populations, it did not consistently match the (recent) average environmental temperatures at each site. Instead, T_{opt} was below the environmental temperature at all three locations (T_{opt} for the respiration rate excluded), except for the *Porites* population at HI. Thermal

acclimation along a latitudinal cline of the photosynthetic performance specifically has been observed for a variety of organisms. For instance, a positive correlation between latitude and T_{opt} for photosynthesis has been observed for macrophytes (Santamaría & van Vierssen 1997). Similarly, T_{opt} for net photosynthesis was higher in tropical tree species than in temperate tree species (Cunningham & Read 2002). However, the absence of a correlation between latitude and T_{opt} for photosynthesis for corals has now been reported in three studies (Coles & Jokiel 1977, Rodolfo-Metalpa et al. 2014). Collectively, these findings suggest the presence of factors that constrain thermal acclimation of local coral populations more so than for other taxa.

In contrast to the responses of coral colonies, thermal acclimation at the symbiont level led to a closer alignment of thermal performance with local environmental conditions, which was apparent by the performance curves fitted to the data segregated by location and species, as well as those fitted to the data segregated by colony. For $rETR_m$, the thermal optima and performance breadths increased with average environmental temperature and variability, according to my hypotheses. For F_v/F_m, the performance breadths increased with environmental thermal variability, while the thermal optima were below the average environmental temperatures and remarkably similar between locations for both coral species. These different results for different symbiont traits suggest that the effect of temperature on photosynthesis is sequential instead of simultaneous: where the $rETR_m$ is reduced at increased temperature, this could prevent inhibition of F_v/F_m. This interpretation is based on other studies that showed that during the early stages of thermal stress, the enzyme activity in the Calvin-Benson cycle is slower, which directly influences the rate of electron transport but does not directly damage the photosystems (see review by Allakhverdiev et al. 2008).

Despite the observed mismatch between thermal optima and local mean environmental temperatures, the performance breadth of each population was wide and generally

encompassed the range of temperatures experienced at each location. This means that corals live at suboptimal conditions for performance, but declines in performance at temperatures above and below the optima are relatively small. Similarly wide performance breadths are observed in other studies on corals (e.g., Jokiel & Coles 1977a, Carricart-Ganivet et al. 2012, Rodolfo-Metalpa et al. 2014) suggesting that this finding is not specific to the species studied here. Such broad performance breadth could explain why T_{opt} did not consistently match the average environmental temperatures because the small increase in performance achieved through 'perfect' acclimation of the thermal response might not outweigh the costs of acclimation. However, the observed performance breadth (presented as the average across multiple coral colonies at each location) also reflects the high level of variation in performance among colonies. A likely explanation for this high among-colony variation is dispersal of coral larvae across large distances, and among sub-populations with different thermal histories. Coral recruits can be sourced from the local reef (Sammarco & Andrews 1988), but many spawning species (including the species studied here) produce larvae with a relatively long planktonic stage that can disperse to maintain moderate to high levels of gene flow along the GBR (Ayre & Hughes 2000). Hence, the influx of maladapted genotypes or phenotypes on reefs around LI, OI and HI may have prevented perfect acclimation of each population. Moreover, despite collection of coral fragments from colonies that were approximately the same size, these colonies potentially settled onto the reef in different years with different environmental conditions. Strong developmental acclimation to the temperature environment at the time of settlement could also drive high variation in thermal responses later observed among adult colonies. Lastly, the variation in T_{opt} for holobiont dominated responses between *Acropora* colonies was larger than the thermal variation they experience annually. Although this negates successful acclimation of the overall population performance, the silver lining is that this high level of natural variation in thermal performance provides raw material for natural selection

and adaptation and can therefore promote survival under climate change. In addition, the notion that the performance curves at symbiont level appear better acclimated to the local environment supports the idea that maladapted immigrated colonies are able to take up well-acclimated/adapted symbionts from the local environment.

In addition to larval dispersal and development acclimation, there are other plausible reasons for the observed mismatch of holobiont performance and the local thermal regime. First, measurement of the local thermal environment was based on data from the preceding two weeks whereas acclimation might occur over longer or shorter time frames (Chapter 2). In addition, the thermal experiments at LI and OI were executed in the summer of 2016 during an El Niño event that brought unusual high seawater temperatures causing severe bleaching of the northern reefs of the GBR (Hughes et al. 2017b). Therefore, the assumed thermal environments at LI and OI during this study (calculated as the average water temperature of two weeks prior to the thermal experiment) were above the local annual average (Appendix **Table C.9**), perhaps leading to a greater mismatch in T_{opt} and the environmental temperature during this particular summer. Indeed, for the *Porites* population at LI, the T_{opt} was closer to the annual average temperature than the local environmental temperature. In contrast, the T_{opt} of the *Porites* population at HI was closer to the local environmental temperature, corresponding to the hypothesis that organisms from a more variable thermal environment have a higher capacity for acclimation (Gabriel & Lynch 1992).

Trade-offs in the energy balance might also have constrained the acclimation of the photosynthetic performance to local temperatures. Under light saturating conditions, the photosynthesis rate is generally limited by the amount or activity of Rubisco, the enzyme involved in CO_2 fixation. Rubisco Activase switches Rubisco from an inactive to an active form (required for CO_2 fixation) through an energy-consuming step influenced by

temperature. Studies on plants showed that heat-stress reduced the activity of Activase (Crafts-Brandner & Salvucci 2000) and constrained shifts of the optimal temperature for photosynthesis (Hikosaka et al. 2005). Hence, the photosynthetic performance of the populations at OI and LI might have been constrained by thermal stress and other more urgent cellular processes may have demanded energy, such as changes in the composition of membrane lipids or synthesis of reactive oxygen species (ROS) and heat shock proteins (HSPs). High concentrations of ROS and HSPs may lead to interference with normal cell functioning and deplete energy reserves (see review by Sørensen et al. 2003). Lastly, since OI is an inshore reef with higher sedimentation and nutrient levels than the mid-shelf reefs around HI and LI, I cannot exclude that the observed patterns may have been (partly) driven by factors other than temperature.

The respiration, or oxygen consumption, rate represents the whole-organism metabolism, including energy requiring processes of both host and symbiont for maintenance, repair and growth. However, symbiont consumption is considered to be negligible, as the symbiont:coral ratio generally ranges somewhere between 0.03 and 0.1 depending on the coral species (Muscatine et al. 1981, Falkowski et al. 1984). Thus, the observed changes in the respiration rate in this study were mostly due to changes in the host physiology. For corals, interpretation of the thermal optima for respiration rate is complicated. For instance, the T_{opt} was well above 30 ºC for most populations considered here, a temperature that is only rarely experienced in the environment. Generally, linear approximations of thermal performance should be avoided, as they fundamentally differ with the biological justification of thermal performance (Angilletta 2006). However, within the temperature range that corals can tolerate, respiration rates are often found to increase with increasing temperature (Muthiga & Szmant 1987). Therefore, it is likely that the performance curve for respiration is asymmetrical, with a sharp sudden decrease of the respiration rate close to the upper thermal

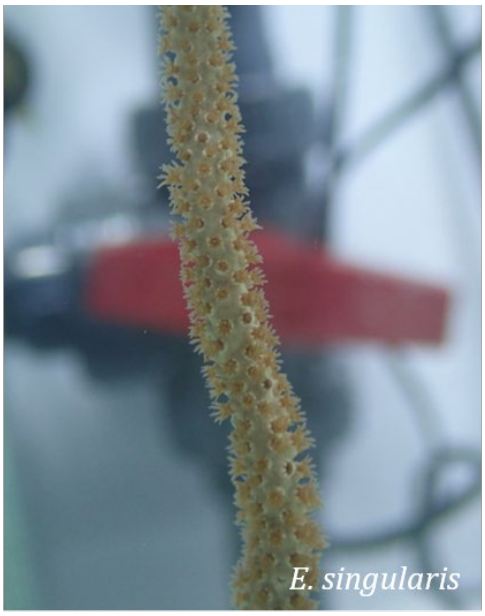
threshold. Additionally, high respiration rates are generally associated with high levels of stress and metabolic costs (e.g., Withers 1992), suggesting that the parameter estimation for T_{opt} signified the temperature at which metabolic costs were highest rather than the temperature at which performance was maximized. I observed declined respiration rates for the populations at HI after ~30 ºC, but at OI and LI, respiration declined only at the highest two temperatures measured (> 33 ºC). This suggests that the latitudinal thermal cline influenced the thermal acclimation to some extent. Further research encompassing a wider temperature scope, and during which cellular responses are monitored in addition to whole-organism respiration rates will provide more insight into the true shape of the curve.

4.6 Conclusion

My findings show that the holobiont thermal performance varied among locations and between species, therefore excluding a non-plastic thermal generalist strategy. However, thermal specialization through acclimated T_{opt} and narrow T_{br} was not observed. Instead, populations of both species, across all locations, generally lived at temperatures above their optima, constraining their performance nearly all year round. While these temperatures may not be lethal to the corals on short term, they are suboptimal for fitness and suggest that corals are maladapted to present-day seawater temperatures.

CHAPTER 5

Thermal performance of two temperate and tropical coral species[5]



[5] This chapter is prepared for submission to Coral Reefs.

5.1 Abstract

Temperate organisms are generally exposed to a more variable and cooler climate than tropical organisms, and are therefore expected to have a broader thermal tolerance and a higher capacity for acclimation than tropical organisms. In this chapter I investigated this hypothesis by comparing the thermal performance of the coral populations at Heron Island (**Chapter 4**) with two temperate coral species from the Mediterranean Sea. The respiration rates and photosynthetic efficiency showed a linear relation with temperature rather than a curve, suggesting a broad thermal tolerance. The photosynthesis rates and electron transport rates showed a curve-shaped response to temperature with relatively broad performance breadths that were similar between the tropical and temperate species, rejecting the hypothesis that temperate organisms have broader tolerance than tropical organisms. The thermal optimum for holobiont performance was generally below the local environmental temperature, which is likely driven by physiological constraints on the coral host than on the symbionts. The large thermal tolerance for photosynthesis displayed in this study supports previous observations that corals can survive short periods of abnormally warm temperatures, and suggests that corals, in general, adopt thermal generalist strategies to cope with temperature variation in the environment.

5.2 Introduction

Environmental conditions such as temperature, solar insolation and rainfall, are generally more variable in temperate regions than in the tropics. For example, the seasonal variation in temperature can be more than four-fold larger at temperate latitudes than at tropical latitudes, where annual and daily temperature regimes are relatively uniform (Clarke & Gaston 2006). Decades of research demonstrate that these contrasting climate regimes fundamentally

constrain the physiology, ecology and evolution of temperate, tropical and cosmopolitan species (e.g., Dobzhansky 1950, Stevens 1989, Eller et al. 2017). For instance, in an early influential paper, Janzen (1967) hypothesized that the physiological tolerance of each individual organism to climate variation should be large enough to encompass the entire gradient of conditions experienced throughout its life. As a result, organisms in less variable environments (i.e. tropical regions) should have smaller tolerance ranges than organisms in more variable environments (i.e. temperate regions) (Janzen 1967). This hypothesis has been widely tested and is supported by studies showing that the range of body temperatures for salamanders was smallest in the tropics and increased with latitude (Feder & Lynch 1982), and likewise for lizards (van Berkum 1988). However, there are many studies that do not support Janzen's hypothesis (see review by Angilletta 2009), or show opposite trends. For instance, a meta-analysis across taxonomic groups and geographic regions showed that the organisms from stable environments had a higher capacity for acclimation, and that this capacity increased with decreasing latitude (Seebacher et al. 2015).

Physiological tolerance curves quantify the tolerance of an organism to an environmental variable, and quantify the mode (defining the optimal environment) and the width (defining the range of 'tolerable' environmental conditions). The thermal performance curve (TPC) is frequently used to show the relation of organismal performance with temperature (Angilletta 2009), including a thermal optimum (i.e. the organism's optimum temperature for performance) and a performance breadth (i.e. temperature range over which performance is positive) (Huey & Stevenson 1979). The shape and position of TPCs can vary between and within species depending on the acclimation capacity, environmental conditions and thermal history of the organism (Angilletta 2009). Another approach to understanding organismal tolerance to climatic variability are optimality models that predict the optimal position and shape of tolerance curves given the local thermal environment using mathematical equations

(Lynch & Gabriel 1987, Gabriel & Lynch 1992, Gilchrist 1995). Consistent with Janzen's hypothesis, optimality models show that the thermal optimum of an organism depends on the long term mean environmental temperature, whereas the performance breadth depends on the spatial and temporal variation in temperature within and between generations (Lynch & Gabriel 1987).

TPCs have been used to predict the impact of global warming on the species' performance (Deutsch et al. 2008, Bozinovic et al. 2011, Clusella-Trullas et al. 2011, Kingsolver et al. 2013). The capacity of species to persist under global warming depends on their thermal sensitivity, i.e. the degree to which the performance of an organism is influenced by a change in temperature (Calosi et al. 2008). If organisms in more variable environments (e.g. temperate) have broader TPCs than organisms in stable environments (e.g. tropical), then global warming is likely to affect tropical organisms more severely than temperate ones (Tewksbury et al. 2008). Indeed, several tropical taxa, including terrestrial insects, amphibians and marine invertebrates, are currently living close to their upper thermal limits (e.g. Stillman 2003, Deutsch et al. 2008, Tewksbury et al. 2008, Duarte et al. 2012). However, the impact of global warming on organismal performance also depends on the degree of warming itself, which varies geographically and is predicted to be increase faster in temperate than in tropical regions (Pörtner et al. 2014). This in turn may make temperate species more vulnerable than tropical organisms. Quantification of the thermal performance over the entire temperature gradient is necessary to predict how temperate versus tropical species will respond to global warming.

Coral reefs have been identified as one of the most sensitive ecosystems to global climate change (Pörtner et al. 2014) and are already impacted by anthropogenic stressors, such as global warming, ocean acidification, pollution and overfishing (Hoegh-Guldberg 1999, Pandolfi et al. 2003, De'ath et al. 2012). Although coral reefs show their highest biodiversity

in the tropics (Veron 2000, Hughes et al. 2002, Roberts et al. 2002), numerous octocorals (i.e. gorgonians) and scleractinian corals occur in temperate regions. All together, these temperate corals form three dimensional structures, and sometimes reef-like structures, that function as shade and shelter for numerous other species in a similar way to tropical reefs (Weinberg & Weinberg 1979). Some species of the scleractinian corals and octocorals in the Mediterranean harbour photosynthetic dinoflagellate symbionts, such as the scleractinian *Cladocora caespitosa* and the gorgonian *Eunicella singularis*. Their physiology is therefore most similar to that of tropical reef-building corals (Schiller 1993a), and both species can thus serve as good temperate models, in comparison to the tropical ones.

The symbiosis between the coral host and algal symbiont (family Symbiodiniaceae) is easily disrupted by hot and cold temperature extremes resulting in coral bleaching (Brown 1997). In the past decades, several global mass bleaching events have occurred on tropical reefs due to above average sea surface temperatures (Heron et al. 2016), often resulting in significant coral mortality. Likewise, in the Mediterranean Sea, mass bleaching events and mortality of symbiotic organisms has been associated with abnormal warming (Cerrano et al. 2000, Perez et al. 2000, Rodolfo-Metalpa et al. 2000, Garrabou et al. 2009). To date, research on coral thermal tolerance has focused most strongly on the quantification of the upper thermal threshold for bleaching and survival (e.g., Fitt et al. 2001, Oliver & Palumbi 2011, Howells et al. 2012). Consequently, little is known about the shape of the thermal performance curve of corals in general. Therefore, it is currently unclear if the performance breadth of temperate corals is indeed larger than that of tropical corals following Janzen's hypothesis, or if the performance curves of temperate corals match the mean and variance of local environmental temperature consistent with optimality models. Thus far, only few studies specifically quantified coral thermal performance curves (see **Chapter 3** and **4**) that showed some variation in the thermal breadth and thermal optima between populations from

different thermal environments, but not for the Mediterranean coral *Oculina patagonica* (Rodolfo-Metalpa et al. 2014). However, inconsistency in the acclimation period between these studies prior to the thermal response experiments prevents a direct comparison of the performance breadth and thermal optimum between corals from temperate and tropical regions. Additionally, quantification of TPCs of corals with different geographic origins will provide insight into the thermal strategies of these corals more broadly, and identify whether corals are adapted/acclimatized to their local thermal environments.

In this study, I test the hypotheses that temperate corals have broad performance curves with thermal optima close to the environmental mean temperature, and that tropical corals have narrow performance curves with poorly defined thermal optima since temperature does not change significantly with season. To investigate these hypotheses, thermal performance curves were measured for samples of the Mediterranean Sea endemic coral species *Cladocora caespitosa*, and the Mediterranean symbiotic gorgonian *Eunicella singularis*. For the tropical corals, I assessed thermal performance curves for *Acropora intermedia* and *Porites cylindrica* on the Great Barrier Reef (**Chapter 4**). For both temperate and tropical corals, I measured a range of physiological traits at temperatures ranging to 5 degrees above and below the environmental temperature, which resulted in a temperature range of 12 – 22 °C for the temperate corals and 23 – 33 °C for the tropical corals. This study is the first to directly compare the thermal performance of tropical and temperate coral species. These data will improve our understanding of the thermal strategies that corals use to cope with heterogeneity in thermal environments, and of the role that temperature plays in limiting the geographic distributions of different species.

5.3 Material and Methods

The thermal performance was measured for two temperate corals from the Mediterranean Sea, and two tropical corals from the Indo-Pacific Ocean. The temperate species consisted of the symbiotic stony coral *Cladocora caespitosa* and the symbiotic gorgonian *Eunicella singularis*; previous studies commonly found that both corals harbour '*Symbiodinium*' Temperate A (Visram et al. 2006, Forcioli et al. 2011, Casado-Amezúa et al. 2014; LaJeunesse et al. 2018). Five mother colonies of each coral were randomly collected by SCUBA diving at two locations in the northwest Mediterranean Sea (hereafter NWM; **Figure 5.1 a**). *C. caespitosa* colonies were collected off the coast of La Spezia in shallow water (ca. 10 m) in September 2016 and *E. singularis* colonies in Cassis at 10-15m depth in October 2016. Colonies were maintained in aerated cool boxes and transferred to the laboratory in Monaco, where they were placed in culture aquaria. Subsequently, corals were maintained at a light intensity of 80-100 µmol photons m^{-2} s^{-1} provided by 400 W metal halide lamps (HPIT, Philips) and continuously supplied with Mediterranean seawater at a renewal rate of 20% h^{-1} and at ambient temperature (**Figure 5.1 c**). All colonies were fed twice weekly with *Artemia nauplii* during the culture period. Two weeks prior to the start of the thermal experiment, five gorgonian tips (8 to 10 cm long, referred to as fragments hereafter) or five coral fragments (between 5 to 10 nubbins per fragment) were cut from five mother colonies (n = 25 fragments per species), suspended with nylon string, labelled and distributed over four experimental tanks (25 L) placed under controlled conditions similar to that of the mother colonies.

The tropical species included the symbiotic stony corals *Acropora intermedia* and *Porites cylindrica*, both harbouring Symbiodiniaceae from the same genera: *Cladocopium* C3 (formerly, Clade C sub-clade C3) in *A. intermedia* and *Cladocopium* C15 (formerly, Clade C sub-clade C15) in *P. cylindrica* (LaJeunesse et al. 2003, Madin et al. 2016; LaJeunesse et al.

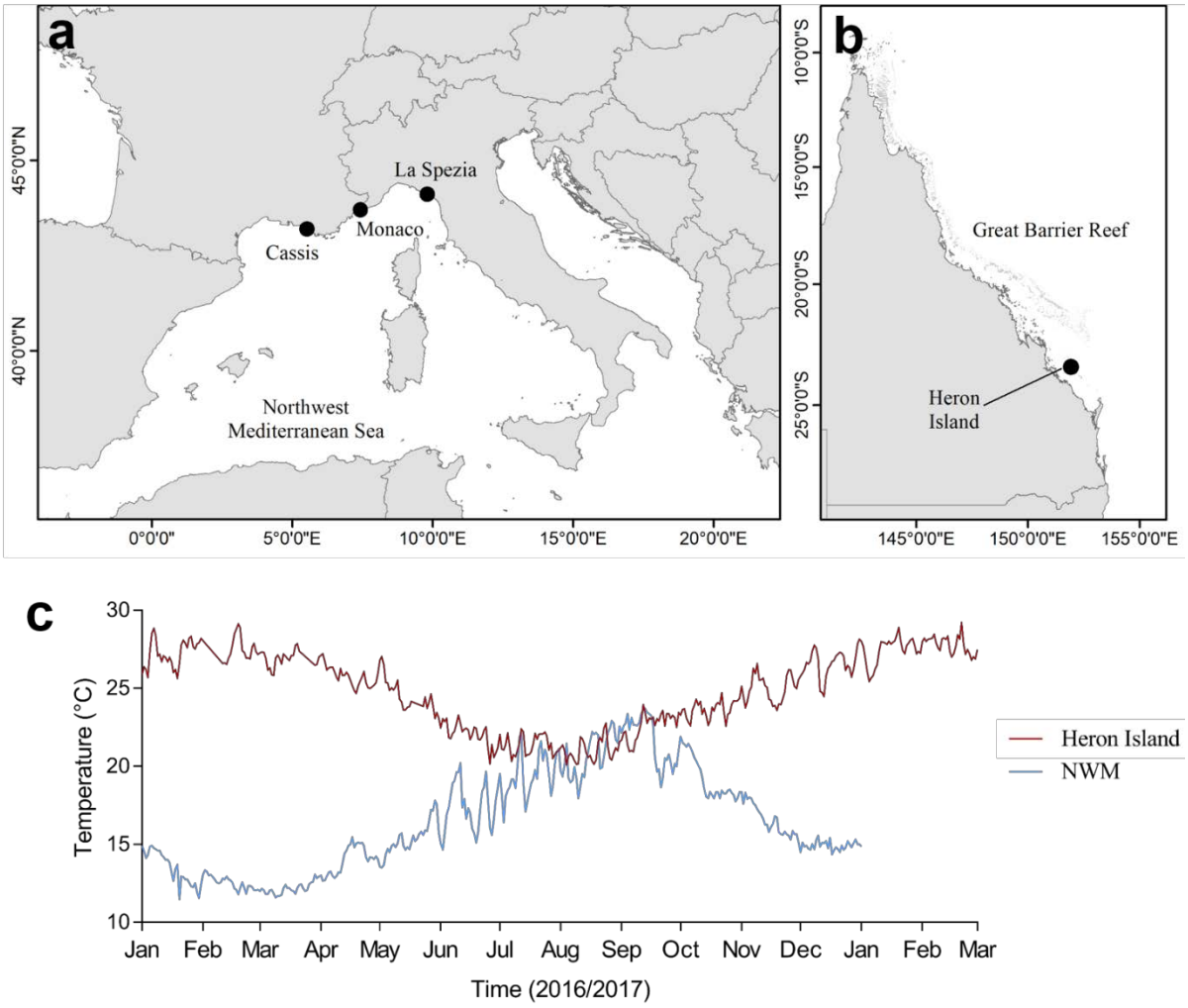


Figure 5.1 Temperate corals were collected at Cassis and La Spezia in the north-western Mediterranean Sea and maintained at the laboratory facilities in Monaco (**a**), tropical corals were collected and maintained at Heron Island in the Great Barrier Reef (**b**), and daily average seawater temperatures at Heron Island and North West Mediterranean Sea (NWM) for 2016 and start of 2017 (**c**). For Heron Island, data was recorded by temperature loggers of the Australian Institute for Marine Science (AIMS 2018) and for NWM by Coriolis Data Centre for Operational Oceanography (CORIOLIS 2018).

2018). These species were selected because of their branching morphology, local abundance in the Great Barrier Reef, and different thermal sensitivities: *A. intermedia* is generally more bleaching sensitive and *P. cylindrica* more bleaching tolerant (Loya et al. 2001). For each

species, five coral fragments from five colonies (n = 25) were collected by SCUBA diving on reefs around Heron Island, Australia (**Figure 5.1 b**), in shallow water (< 10 m) in February 2017. Seawater temperature at the time of collection was ~ 28 °C. Fragments were directly transported to the research station on Heron Island, attached to nylon string, labelled and distributed over two experimental tanks (50 L) placed in the shade outdoors. Daily average irradiance was 150-200 µmol photons m^{-2} s^{-1} with a peak irradiance of 850-900 µmol photons m^{-2} s^{-1} measured with a LI-193 spherical underwater quantum sensor (LI-COR) every hour on two cloudless days. Tanks were continuously supplied with seawater pumped from the adjacent reef flat at a renewal rate of 20% h^{-1} at ambient temperature (**Figure 5.1 c**). Although water supply was assumed to provide the corals with adequate nutrients, freshly hatched *Artemia nauplii* was given twice weekly. At least one week of recovery from collection was allowed before the start of the first measurements.

Temperature records of the thermal environment around Monaco for the year 2016 were accessed through the database of CORIOLIS (IFREMER) on February 2018. On the same day I accessed the temperature records of the thermal environment around Heron Island for 2016 through the database of AIMS (Australian Institute of Marine Science). These records contained *in-situ* hourly measurements of the seawater temperature measured at 1.5 m depth and were used to determine the daily minimum, maximum and average temperature, and calculate the mean summer, winter and annual temperature, and the annual temperature range and variance.

5.3.1 Thermal experiment

The thermal experiments with the temperate corals and gorgonians were conducted at the Centre Scientifique of Monaco (CSM) in November 2016, followed by the thermal experiments with the tropical corals at Heron Island Research Station (HIRS) in March 2017.

Coral performance (described below) was initially measured at ambient temperature (18 °C for the temperate species and 28 °C for the tropical species, **Figure 5.1 c**), after which the water temperature was increased or decreased by 0.5 °C day^{-1} until 5 °C above or below ambient temperature (thus 10 days total). At CSM, temperature was modified using bar heaters (Visi-Therm, 300 W) connected to electronic controllers (ElliWell PC 902/T). At HIRS, temperature was controlled with two chiller/heater units (TK-2000, TECO) connected to a submersible pump (Aquapro AP1050, Aquatec) that circulated the water at a rate of 500 l h^{-1}. Thermal performance of the corals was measured every second day at 1 °C increments. Qualitative observations were made twice daily (morning and evening) to check the colour (paleness) and tentacle expansion of the fragments in the holding tanks. Control fragments, one of each colony, were frozen at -80 °C after the initial performance measurements at ambient temperature for tissue analyses, and the remaining fragments were frozen at the end of the thermal experiment. Coral fragments at HIRS were transported on dry ice to the laboratory facilities at James Cook University for tissue analyses.

5.3.2 Response variables

A whole-organism response to temperature such as that of a coral (holobiont) is naturally influenced by the temperature dependent response of the symbiont. However, the symbiont can have a different thermal sensitivity than the coral (**Chapter 3** and **4**), which can influence the shape of the holobiont thermal performance curve. Therefore, performance traits specific to the symbiont were measured in addition to the performance that is dominated by the physiology of whole-organism. Holobiont performance traits in this study were net photosynthesis (Pnet) and respiration (R) rate, because these processes are dominated by whole-organism physiology due to the larger biomass of the coral tissue compared to that of the symbionts, although photosynthesis itself is driven by the symbiont. Symbiont specific

performance included maximum quantum yield (F_v/F_m) and maximum relative electron transport rate ($rETR_m$), because these processes occur at the photosystem level within the symbionts.

5.3.3 Holobiont performance

Pnet and R rates for each fragment were quantified by measuring the change in the dissolved oxygen concentration in the water during 1 hour incubations. After 10 min light or dark acclimation period, oxygen production (Pnet) was measured at an irradiance of 200 µmol photons m^{-2} s^{-1} at CSM provided by a metal halide lamp (Philips, HPIT 400W), and at 350 µmol photons m^{-2} s^{-1} at HIRS provided by led lights (R420r, 180 W, Maxspect Razor). Light levels during respirometry corresponded to the subsaturation light intensity for the temperate species (Rodolfo-Metalpa et al. 2008) and tropical species (Anthony & Hoegh-Guldberg 2003). Oxygen consumption (R) in the dark was subsequently measured after the illumination period during 1 hour, after chambers were flushed with new seawater. Fragments were suspended in Plexiglas respirometry chambers with lids (6 chambers, 200 ml at CSM and 550 ml at HIRS) filled with filtered seawater (0.45 µm at CSM and 1 µm at HIRS) and stirred with magnetic stirrers. Each chamber contained an individual fragment and one chamber without a fragment served as a blank to account for background respiration of microorganisms naturally present in seawater. The chambers were placed on a submersible magnetic stirrer plate (MIXdrive 6, 2mag) inside a water bath that controlled the temperature inside the chambers (Polystat 36 Circulating Bath, Fisher Scientific at CSM, TK-2000 chiller/heater unit, TECO at HIRS). Oxygen production and consumption was measured using oxygen probes (LD101, Hach) connected to a meter device (HQ40D, Hach) at one minute intervals. Oxygen probes measure the oxygen saturation of the water and therefore respiration rates are likely an underestimation of the absolute respiration rates as measured

with higher resolution equipment such as micro sensors (Kühl et al. 1995). However, oxygen probes provide enough accuracy for comparison of differences in respiration at different temperatures, and among species. Furthermore, to reduce measuring error between CSM and HIRS, the same oxygen probes and meter were used. Pnet and R rates were estimated by regressing oxygen evolution over incubation time, taking into account the water volume in the chambers and background respiration measured in the blank chamber. Pnet and R data were normalised by surface area (as described below). At the end of the respirometry measurements, fragments were returned to their holding tanks.

5.3.4 Symbiont performance

Symbiont performance was measured using Pulse Amplitude Modulated (PAM) fluorometry (DIVING-PAM, Walz GmbH). PAM fluorometry measures the chlorophyll fluorescence emitted by photosynthetic units in response to illumination with a series of signal pulses. Changes in chlorophyll fluorescence are used to make inferences about the photosynthetic state of the sample (Suggett et al. 2010). Immediately after dark respirometry, a weak measuring light (< 1 µmol photons m^{-2} s^{-1}) followed by a saturating light pulse (> 8000 µmol photons m^{-2} s^{-1}) was applied to the dark-adapted fragments using a fiberoptic probe at a fixed distance (~3 mm) from the sample. The measuring light induced minimal fluorescence (F_o) which indicates the proportion of open reaction centres in photosystem (PS) II. The subsequent saturating light pulse induced maximal fluorescence (F_m) by closing all reaction centres. The maximum quantum yield of PSII (F_v/F_m) was calculated as (F_m - F_o) / F_m, and provided information about the state of PSII at each measuring temperature, which was used as an indicator for the physiological thermal performance of the symbionts. On each fragment and at each temperature, an average F_v/F_m of at least 3 measurements was taken.

In addition, immediately after light respirometry, rapid light curves (RLCs) were measured on light-adapted fragments as described by Ralph and Gademann (2005) to assess $rETR_m$ as a function of instant irradiance at different temperatures. For this, a series of nine saturating light pulses was given at 10 s intervals, while an actinic light source progressively increased the light intensity from 5 to 1800 µmol photons m^{-2} s^{-1} during each interval (Light-Curve Intensity 2). After each saturating pulse, the light-adapted effective quantum yield ($\Delta F / F_m'$) was recorded and relative electron transport rate (rETR) was calculated as follows

$$rETR = 0.84 * 0.5 * \Delta F/F_m' * PAR \quad \text{(Eq. 5.1)}$$

where 0.84 is the assumed light absorption ratio, 0.5 is the assumed ratio of PSII reaction centres to PSI reaction centres, and PAR is the variable actinic light intensity. For every fragment, RLCs were drawn by plotting rETR across the light intensity from which the $rETR_m$ was calculated, which was used as an indicator for saturation capacity of PSII.

5.3.5 Chlorophyll concentration, symbiont density and surface area

Chlorophyll concentrations were determined at the end of the thermal experiment to detect changes in the chlorophyll concentration that could have influenced the photosynthetic performance. Fragments were grouped according to their thermal exposure: chilled (after exposure to the decreasing thermal gradient, n = 10), heated (after exposure to the increasing thermal gradient, n = 10) or ambient (fragments that were frozen at the start of the thermal experiment, n = 5). Tissue was removed from the skeleton using an air-pick (*C. caespitosa*), scalpel (*E. singularis*) or airbrush (*A. intermedia* and *P. cylindrica*), collected in 15 mL of seawater and homogenized using a Potter grinder (*C. caespitosa* and *E. singularis*) or tissue homogenizer (T 25 Ultra-Turrax, IKA). The slurry (5 mL) was centrifuged (Rotina 380R, Hettich Lab Technology) at 5,000 *g* for 10 min after which 5 mL of 90% acetone was added to the pellet and left at 4 ºC in darkness overnight. The solution was then centrifuged once

more at 5,000 *g* for 10 min, after which the chlorophyll absorption was measured at 630, 663 and 750 nm using a spectrofluorometer (Xenius, SAFAS at CSM, and Spectramax M2 Reader, Molecular Devices at JCU). Chlorophyll *a* and *c2* concentrations were calculated using the equations of Jeffrey and Humphrey (1975a).

Respirometry rates, chlorophyll concentrations and symbiont density were normalized by skeletal surface area. For *C. caespitosa*, the polyp surface was measured using a calliper and surface area was calculated following Rodolfo-Metalpa et al. (2006a). For *E. singularis*, the length and the width of the fragment (tip) were measured using a calliper and surface area calculated according to the geometric formula for area of a cylinder. For *A. intermedia* and *P. cylindrica*, surface area was calculated using the wax dipped method following Veal et al. (2010).

5.3.6 Data analyses

All data were analysed using R version 3.3.2 (R Foundation for Statistical Computing, 2016) and graphed with Prism GraphPad Software version 7.03. All data reported in the results are averages ± standard deviation, unless otherwise specified. Thermal performance curves were fitted using non-linear least squared regressions (function 'nls' in R) to the physiological response variables measured (Pnet, R, F_v/F_m and $rETR_m$). A symmetrical Gaussian function with only three parameter estimates provided a better fit than an asymmetrical function with more parameters (Appendix **Table D.1**) and has been used in other studies to model the thermal responses of temperate and tropical corals (Marshall & Clode 2004, Rodolfo-Metalpa et al. 2014). The following equation was used (Rodolfo-Metalpa et al. 2014):

$$P = Pf_{max} \exp [-0.5 (\text{abs} (T - T_{opt})) / T_{br})^2] \quad \text{(Eq. 5.2)}$$

where P is the physiological response variable measured at temperature T, Pf_{max} is the maximum value of this response, T_{opt} is the temperature at which the response variable is maximal (i.e. the mean value) and T_{br} is the breadth of the response curve (i.e. the standard deviation).

First, Eq. 5.2 was fitted to all data pooled together, then to the data separated by region (i.e., temperate or tropical), then to the data separated by species within regions and, lastly, to the individual colony responses. The Akaike Information Criterion (AIC) was used to find the best fit of the model to the data, thereby providing information about the major sources of variation in the non-linear functional relationships between performance and temperature. For this, the AIC values for each model fit were summed up according to the above described separation of the data. For instance, AIC value of the model fitted to the temperate responses were added to the AIC value of the model fitted to the tropical responses, etc. The division of the data with the lowest summed AIC value corresponded to the model that was most strongly supported by the data. Parameter estimates (Pf_{max}, T_{opt} and T_{br}) for the thermal performance curve of each species were calculated as averages of the parameter estimates of the thermal performance curves fitted to each colony and a one-way analysis of variance (ANOVA) was used to detect differences between species for these parameters. Tukey post-hoc analysis were performed when *p*-values were significant (< 0.05).

If the physiological response to temperature was not bell-shaped, a generalized linear mixed-effects model was fitted (package 'lme4'). To account for the repeated measure of colonies and between colony variations, 'colony' was included as a random effect and 'temperature' and 'species' were added as fixed effects. For consistency in model selection procedure for the non-linear responses, mixed-effects models were fitted to the data segregated in similar fashion as with the non-linear models (i.e., the linear model first only included 'temperate as fixed effect, then 'region' was added and lastly 'region' was replaced

by ‘species’) and AIC values were calculated to find the model that most strongly supported the data. If the interaction of species and temperature was significant ($p < 0.05$), post-hoc comparisons were analysed to find out which species differed.

Lastly, to investigate whether the thermal experiment affected the chlorophyll concentration (as an indicator of temperature stress leading to photoinhibition and/or damage), I compared the chlorophyll concentration of the fragments exposed to the increasing temperature with those exposed to the decreasing temperature and with those that remained at ambient temperature. For this, I used a linear model with species and treatment (i.e., heated, chilled or ambient) as categorical variables and chlorophyll concentration as dependent variable. I first analysed the significance of ‘colony’ as nested variable by comparing a nested model (colony nested within species) to a model where colony identity was not included using a likelihood test. After selecting the best model, an ANOVA was used to detect differences in the chlorophyll concentrations between species and treatments and Tukey post-hoc comparisons were made if a variable was significant.

5.4 Results

The average seawater temperature in the North West Mediterranean (NWM) in 2016 was 16.4 ± 3.4°C, with a minimum of 11.2 °C recorded on January 21 and maximum of 26.4 °C on August 4. Hence, the total temperature range that the temperate corals were exposed to was 15.2 °C in 2016, which was greater than the thermal gradient of the experiment (13 °C - 23 °C). The mean summer temperature was 19.2 ± 2.0 °C (June – August 2016) and mean winter temperature was 13.4 ± 1.2 °C (December 2016, January – February 2016). At Heron Island for the same year, the average seawater temperature was 24.9 ± 2.4 °C, with a minimum of 18.1 °C on August 27 and a maximum of 30.5 °C on February 18. Consequently, the highest temperatures during the thermal experiment (30-33 °C) exceeded

the temperature that the corals experienced in the field. Furthermore, the temperature range that the tropical corals experienced was 12.4 °C, which is only 2.7 °C smaller than the total variability in the NWM. The mean summer temperature at Heron Island was 27.0 ± 0.9 °C (December 2016, January – February 2016) and mean winter temperature was 21.5 ± 0.8 °C (June – September 2016), which is a similar mean seasonal variability (~ 6 °C) compared to NWM. However, the daily variability was around two-fold greater at NWM than at Heron, particularly during the summer months (June to August, **Figure 5.1**).

5.4.1 Holobiont response

The relationship between the net photosynthesis rate (Pnet) and temperature was bell-shaped for all corals (**Figure 5.2 a-d**), although this effect was more pronounced in *E. singularis* and *P. cylindrica* than in *C. caespitosa* and *A. intermedia*. Model selection showed that the response varied both between species (Appendix **Table D.2**). There was also substantial variation in the shape of the TPC among colonies of each species.

For the temperate species (**Figure 5.2 a-b**), the thermal optimum for the photosynthesis was around 15.0 °C for both species (**Table 5.1**), which was 3 °C lower than the ambient temperature at the start of the experiment (~18 °C) and also lower than annual average temperature (16.4 ± 3.4°C °C), although within one standard deviation of the average temperature. The performance breadth (T_{br}) was more than 1.5 times larger in *C. caespitosa* than in *E. singularis* (ANOVA, $F(3,15) = 3.906$, $p = 0.030$, see Appendix **Table D.** for post-hoc comparisons). The smaller T_{br} in *E. singularis* (10.6 ± 1.2 °C) was caused by a strong decrease in the Pnet rate when exposed to > 20 °C. For comparison with their natural habitat, in 2016 corals in the NWM experienced 64 days at which the temperature reached at least 20 °C. Furthermore, Pnet rates per unit tissue surface area were more than double in *C. caespitosa* than in *E. singularis* resulting in a significantly higher Pf_{max} (0.78 ± 0.09 μmol O_2

Table 5-1 Average ± s.d. of the parameter estimates for the performance curves of *Cladocora caespitosa* and *Eunicella singularis* and *Acropora* intermedia and *Porites cylindrica* (n = 5). Parameter estimates for respiration were only calculated for the two tropical species, because linear regressions were used for temperate species.

Response variable	Parameter estimate	*C. caespitosa*	*E. singularis*	*A. intermedia*	*P. cylindrica*
Pnet rate	Pf_{max} (O_2 h^{-1} cm^{-2})	0.78 ± 0.09	0.33 ± 0.04	0.77 ± 0.16	1.16 ± 0.11
	T_{opt} (ºC)	15.0 ± 2.2	15.3 ± 0.6	21.7 ± 2.0	28.1 ± 2.4
	T_{br} (ºC)	18.3 ± 4.1	10.6 ± 1.2	18.4 ± 5.4	15.1 ± 4.7
R rate	Pf_{max} (O_2 h^{-1} cm^{-2})			0.52 ± 0.03	0.78 ± 0.19
	T_{opt} (ºC)			29.0 ± 0.5	30.5 ± 1.9
	T_{br} (ºC)			16.4 ± 1.2	14.0 ± 3.0
$rETR_m$	Pf_{max} (no unit)	44.9 ± 4.3	32.1 ± 2.6	123.3 ± 4.9	100.3 ± 5.7
	T_{opt} (ºC)	14.0 ± 3.7	13.9 ± 3.1	23.7 ± 1.7	24.0 ± 2.7
	T_{br} (ºC)	26.3 ± 6.4	25.6 ± 6.9	20.4 ± 3.4	18.9 ± 6.5

h^{-1} cm^{-2} and 0.33 ± 0.04 µmol O_2 h^{-1} cm^{-2} in *C. caespitosa* and *E. singularis* respectively; **Table 5.1** and **Table 5.2**), which is attributed to a higher efficiency of the symbiont within *C. caespitosa*. Indeed, Pnet rate per symbiont was around 4 times higher in *C. caespitosa* than in *E. singularis*, which was attributed to the higher Pnet performance and reduced chlorophyll concentration (see section 5.4.4) in *C. caespitosa* compared to *E. singularis* (see Appendix **Figure D.1** for Pnet rates per symbiont).

Between the tropical species (**Figure 5.2 c-d**), T_{opt} was the lowest in *A. intermedia* at 21.7 °C, which was well below the environmental temperature of 28 °C at the time of collection and also below the yearly average of 24.9 ± 2.4 °C). In contrast, the T_{opt} for the Pnet rates of *P. cylindrica* was at 28.1 °C (**Table 5.1**), which corresponded perfectly to the environmental temperature at the time of collection. The performance breadth was slightly larger in *A. intermedia* (18.4 ± 5.4 °C) than in *P. cylindrica* (15.1 ± 4.7 °C), but not significantly different (Appendix **Table D.**), and was wide enough to encompass the annual temperature range (12.8 °C). Furthermore, the overall Pnet rates were significantly higher in *P. cylindrica* than

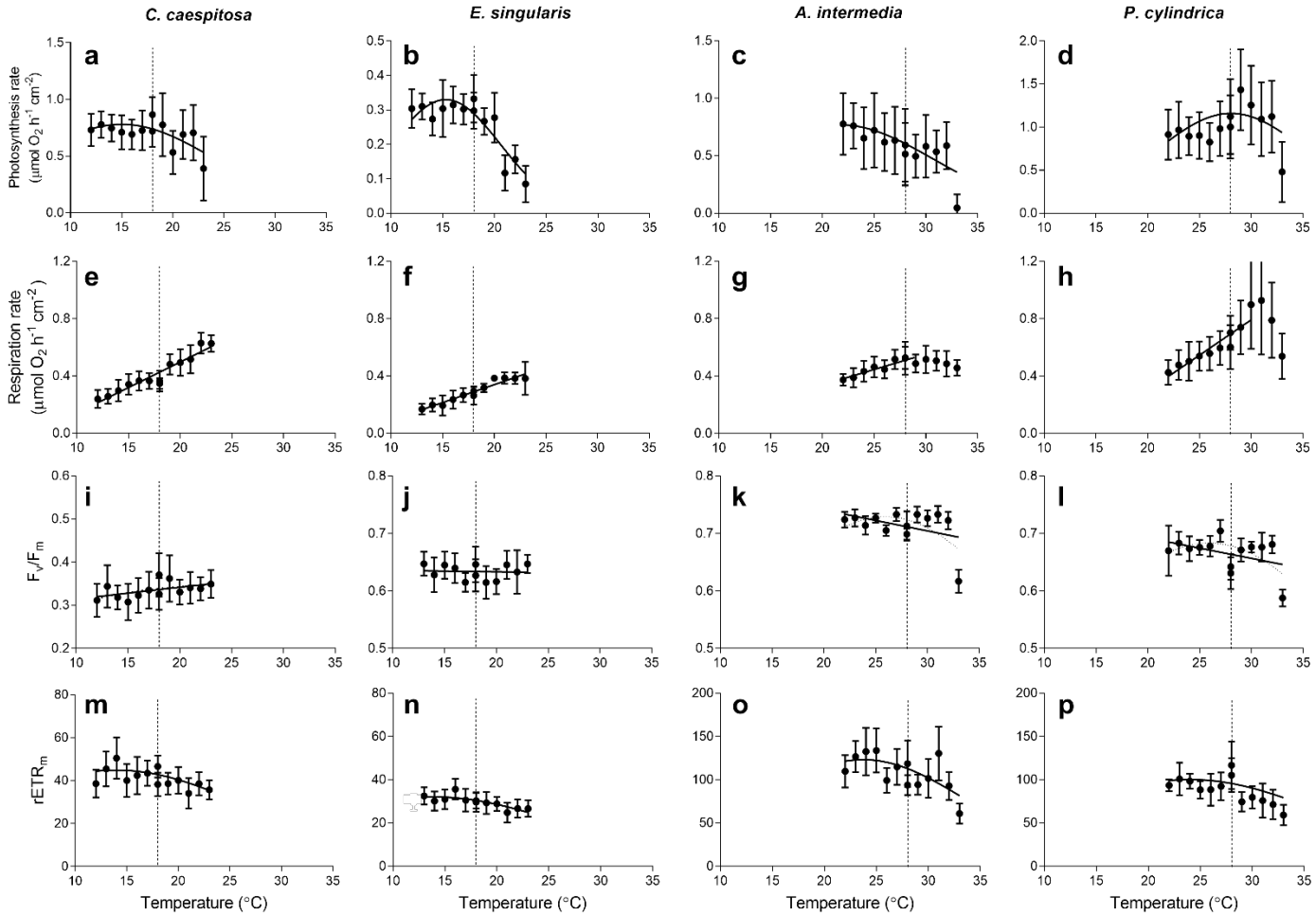


Figure 5.2 Thermal performance curves and linear regressions for the temperate corals *Cladocora caespitosa* and *Eunicella singularis* and the tropical corals *Acropora intermedia* and *Porites cylindrica*. Holobiont performance (top two rows) are net photosynthesis and respiration rate and symbiont performance (bottom two rows) are maximum quantum yield and maximum electron transport rate. In panels **k** and **l** are both the linear and non-linear regression (solid slope and dotted curve respectively) shown. Data points are mean values ± s.d., n = 10.

Table 5-2 ANOVA results to detect variability between species in the parameter estimates (P_{max}, T_{opt} and T_{br}) of the thermal performance curves for net photosynthesis and maximal electron transport rate.

Thermal response	Parameter estimate	df	F-value	*p-value*
Pnet rate	Pf_{max}	3, 15	52.661	0.000
	T_{opt}	3, 15	51.427	0.009
	T_{br}	3, 15	3.906	0.030
rETRm	Pf_{max}	3, 15	429.71	0.000
	T_{opt}	3, 15	18.089	0.000
	T_{br}	3, 15	1.787	0.193

in *A. intermedia* (respectively, 1.16 ± 0.11 μmol O_2 h^{-1} cm^{-2} and 0.77 ± 0.16 μmol O_2 h^{-1} cm^{-2}, **Table 5.1** and **Table 5.2**), which was mostly driven by a higher performance of *P. cylindrica* at temperatures above 28 °C. Lastly, in contrast with my hypothesis, the T_{br} of the tropical species not significantly smaller than that of the temperate species (Appendix **Table D.**). In fact, the T_{br} of *A. intermedia* was exactly the same as that of *C. caespitosa* (**Table 5.1**).

The respiration rates increased linearly with temperature for all species (**Figure 5.2 e-h**), although the tropical species also showed decreased R rates at the highest temperatures (> 30 °C). To allow for comparison between all four species, non-linear regressions were initially fitted to the R rates of the colony responses of the tropical species to quantify the specific T_{opt} for each colony (average T_{opt} of 29.0 °C for *A. intermedia* and 30.5 °C for *P. cylindrica*; **Table 5.1** and Appendix **Table D.** for the parameter estimates of each colony), after which linear regressions were fitted to the R rates until the colony specific T_{opt}. Also regarding the linear regressions, there was pronounced variation in R rates between colonies of the same species, as including colony as random effect in the analysis significantly improved the fit of the model (Appendix **Table D.**). The R rates increased more strongly in response to

Table 5-3 Average ± standard deviation of the respiration rates and maximum quantum yield for the temperate species *Cladocora caespitosa* and *Eunicella singularis* and the tropical species *Acropora intermedia* and *Porites cylindrica* computed using mixed linear effects models, with posthoc comparisons to detect variation between species. For the tropical species, linear regressions were fitted to data up to the optimal temperature for respiration that was calculated using non-linear regression.

Response variable	Species	Linear regression coefficient	*p* – value comparison with *C. caespitosa*	*p* – value comparison with *E. singularis*	*p* – value comparison with *A. intermedia*
Resp rate	*C. caespitosa*	0.036 ± 0.005			
	E. singularis	0.025 ± 0.004	0.002		
	A. intermedia	0.022 ± 0.004	0.003	0.489	
	P. cylindrica	0.049 ± 0.017	0.001	0.000	0.000
F_v/F_m	*C. caespitosa*	0.003 ± 0.002			
	E. singularis	0.000 ± 0.001	0.025		
	A. intermedia	0.001 ± 0.001	0.050	0.892	
	P. cylindrica	0.000 ± 0.002	0.012	0.675	0.601

increasing temperature in *P. cylindrica* and *C. caespitosa*, which corresponds to the higher Pnet rates observed for these species. In contrast, the response to temperature was weaker in *E. singularis* and *A. intermedia* and very similar between the two species (**Table 5.3**). If R rates are interpreted as reflecting baseline metabolic costs for maintaining tissues, then the lowest R rates should be considered to be 'optimal performance'. In that case, the linear relationship with temperature suggests that T_{opt} occurred at the lowest measured temperatures, which was 12 °C for the temperate species and 22 °C for the tropical species.

5.4.2 Symbiont response

The symbiont performance (maximum quantum yield and electron transport rate, **Figure 5.2 i-p**) was notably less affected by temperature than the holobiont performance, and the responses were also more uniform among all four coral species. This resulted in linear responses with temperature for F_v/F_m, and very broad performance curves for the $rETR_m$.

However, model selection showed that regressions fitted to each species separately improved the fit of the model to the data (Appendix **Table D.**), which is most likely due to the variation between species in the overall performance rates (i.e. the height of the line or curve) rather than variation in the response to temperature (i.e. shape/slope or position of the line or curve within tropical and temperate species, see Appendix **Figure D.2**).

For F_v/F_m (**Figure 5.2i-l**), there was significant variation between the colonies (Appendix **Table D.**, adding colony as random effect improved the model). However, this was mostly driven by variation between colony intercept as the response to temperature was very similar between colonies (see Appendix **Table D.4** for the colony coefficients). The F_v/F_m of *C. caespitosa* (**Figure 5.2 i**) was unusually low at all temperatures (average F_v/F_m was 0.335 ± 0.042), but this did not compromise the photosynthetic performances of *C. caespitosa*, since Pnet rates were generally high at all temperatures (**Figure 5.2 a**). *C. caespitosa* was also the only species that showed a small but significant increase in F_v/F_m with temperature (average slope was 0.003 ± 0.000, which was significantly different from *E. singularis* and *P. cylindrica*; **Table 5.3**). For *E. singularis*, the average F_v/F_m was 0.632 ± 0.028 and did not change in response to temperature (**Figure 5.2 j** and **Table 5.3**). The average F_v/F_m of *A. intermedia* (**Figure 5.2 k**) and *P. cylindrica* (**Figure 5.2 l**) were respectively 0.713 ± 0.03 and 0.666 ± 0.036, and showed no response with temperature until 33 °C, after which the performance dropped substantially to 0.617 ± 0.020 and 0.588 ± 0.015 for *A. intermedia* and *P. cylindrica* respectively. However, seawater temperatures around 33 °C did not occur at reefs around Heron Island in 2016.

The performance curves for $rETR_m$ of the two temperate species (**Figure 5.2 m-n**) were identical regarding the position (T_{opt}) and shape (T_{br}) (Tukey posthoc comparison, $p = 0.997$ and $p = 0.999$, Appendix **Table D.**). The T_{opt} for $rETR_m$ was around 14 °C, which is 1 °C lower than their T_{opt} for Pnet and therefore also well below the annual average seawater

temperature at NWM. Curiously, the overall performance at all temperatures, as well as Pf_{max}, was higher for *C. caespitosa* than for *E. singularis* despite the lower F_v/F_m. A similar trend was observed for the tropical species (**Figure 5.2 o-p**), with closely related values for T_{opt} and T_{br} (Tukey posthoc comparison, $p = 0.999$ and $p = 0.982$, **Table D.**). For the tropical species, the T_{opt} was approximately 24 °C (**Table 5.1**), which was below the local temperature at the time of collection and execution of the thermal experiment, but approximated the yearly average seawater temperature around Heron Island. The T_{br} was broad (~ 20 °C, **Table 5.1**), which largely encompasses the range of the annual thermal variability. The overall performance and Pf_{max} was higher in *A. intermedia* than in *P. cylindrica*, which was reversed to the overall performance for Pnet. Lastly, the thermal breadth of the tropical corals were similar to that of the temperate corals ($F(3,15) = 1.787$, $p = 0.193$), which was also observed for Pnet and once more contrasts with my hypothesis.

5.4.3 Within-species variation

There was considerable variation in the thermal performance between the colonies of each species for all four performance traits (**Figure 5.3** and **Figure 5.4**). For colonies of *C. caespitosa*, the optimal temperature for Pnet ranged from 11.5 °C to 17.5 °C, which was below the annual average temperature for most colonies, but within the range of environmental temperatures experienced in the field (**Figure 5.3 a** and **Figure 5.4 a**). The variation in T_{opt} between colonies of *E. singularis* was the smallest of all species, ranging from 14.2 °C to 15.9 °C, which was also closer to the annual average temperature. For $rETR_m$ the variation in T_{opt} was greater for both species, ranging from 9.7 °C to 18.9 °C for *C. caespitosa* and 9.0 °C to 16.3 °C for *E. singularis*. However, the lowest T_{opt} were estimated with the greatest uncertainty for both species (standard deviations of colony C.4 and E.3 were particularly large, Appendix **Table D.**), indicating generally low thermal sensitivity for

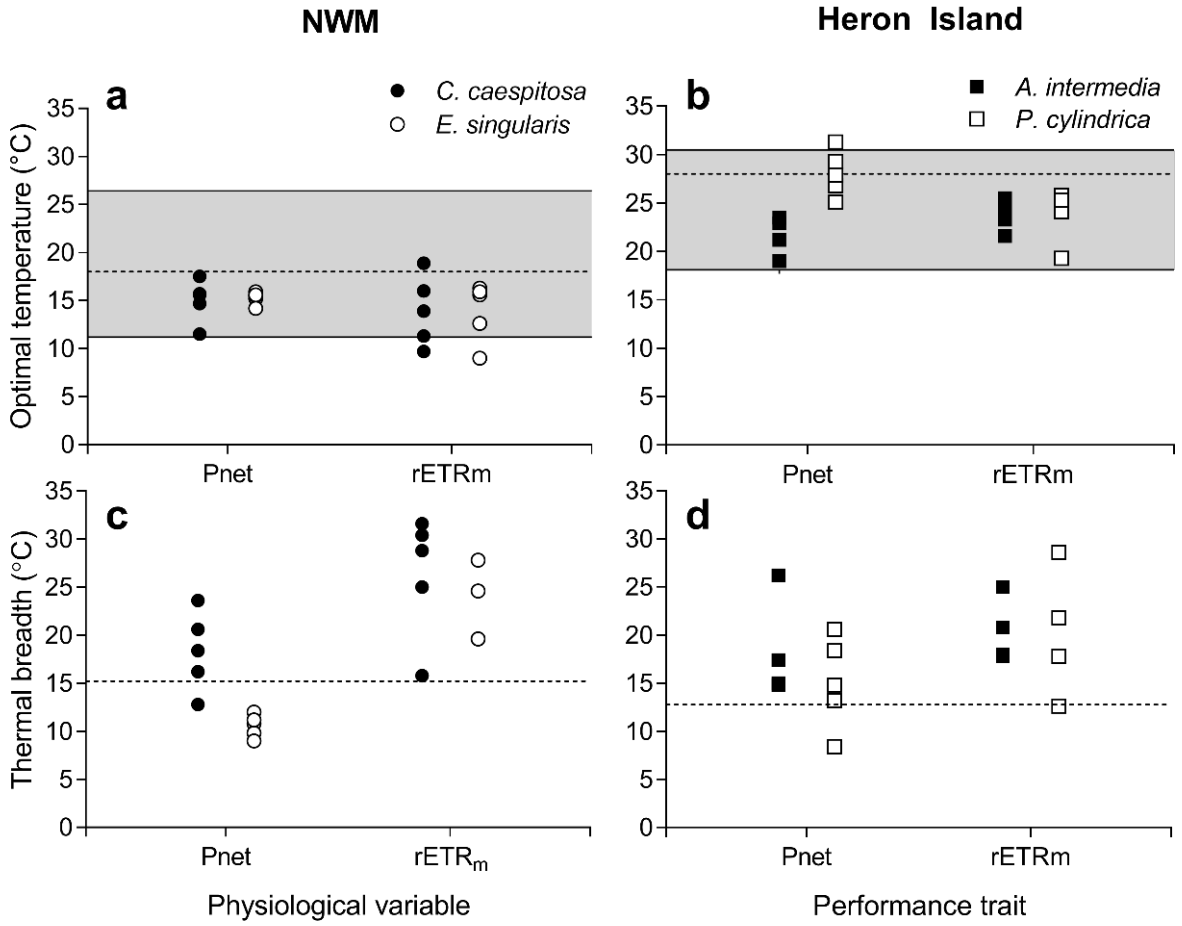


Figure 5.3 Optimal temperature and thermal breadth for net photosynthesis and maximum electron transport rate for individual colonies of *Cladocora caespitosa*, *Eunicella singularis*, *Acropora intermedia* and *Porites cylindrica*. Top panels show the variation in thermal optimum for the temperate corals (**a)** and tropical corals (**b**) derived by fitting non-linear regressions to the thermal response of fragments from the same colony (n = 4). Shaded grey area shows the annual temperature range (minimum and maximum temperature) for 2016 and dashed line the local temperature at the start of the thermal experiment in the North West Mediterranean and around Heron Island. In similar fashion show the bottom panels the variation in thermal breadth between colonies of the temperate species (**c**) and tropical species (**d**). The dashed line indicates the annual temperature range at these locations.

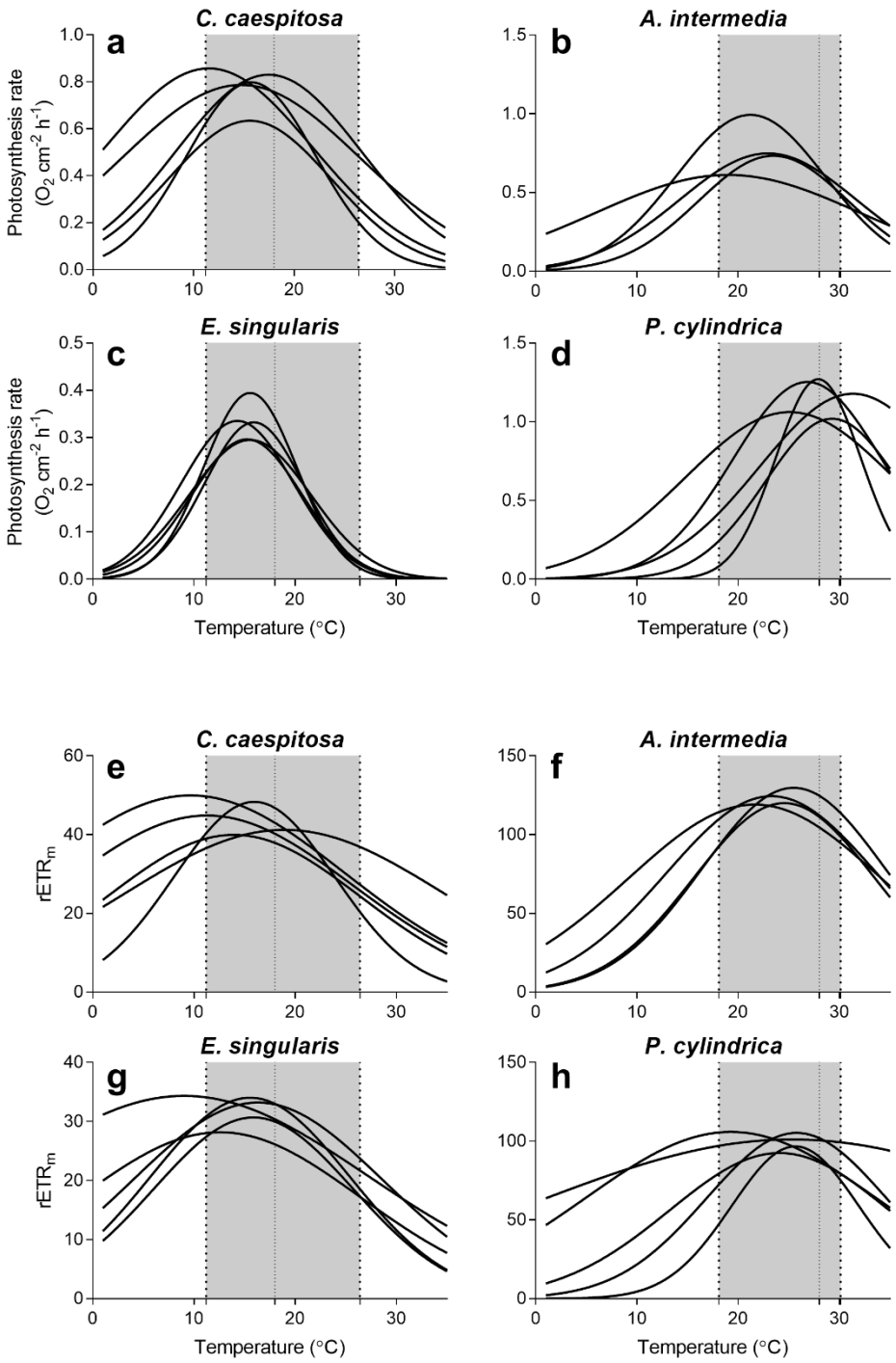


Figure 5.4 Thermal performance curves for net photosynthesis rate (a-d) and maximum electron transport rate (e-h) of colonies of *Cladocora caespitosa* and *Eunicella singularis* (left column), and *Acropora intermedia* and *Porites cylindrica* (right column). Least square non-linear regressions were fitted to the responses of fragments (n = 4) from the same colony. The shaded area shows the annual temperature range in the North West Mediterranean and around Heron Island. The dotted line indicates the ambient seawater temperature at the time of the thermal experiment.

these colonies. For the tropical species (**Figure 5.3 b**) the among-colony variation in T_{opt} was slightly larger for the holobiont related trait (Pnet) than for the symbiont related trait ($rETR_m$), and for *P. cylindrica* compared with *A. intermedia*. Furthermore, the T_{opt} for Pnet of *A. intermedia* colonies ranged below the average annual environmental temperature (from 19.0 °C to 23.5 °C**; Figure 5.4 b**), while for *P. cylindrica* colonies this ranged mostly above the annual average temperature (from 25.1 °C to 31.3 °C; **Figure 5.4 d**). In contrast, the variation of T_{opt} for $rETR_m$ was smaller and closer to the environmental temperature for both species.

Among-colony variation in thermal breadth for Pnet was more than 3 times greater in *C. caespitosa* compared with *E. singularis* (**Figure 5.3 c**), and was broader than the environmental variability of 15.2 °C for all but one colony (**Figure 5.4 a**). Interestingly, none of the colonies of *E. singularis* had a thermal breadth large enough to encompass the annual temperature range (**Figure 5.4 c**), suggesting that this species requires a more plastic response between seasons than *C. caespitosa.* For $rETR_m$, there was greater variation in thermal breadth between colonies compared to that Pnet, and the thermal breadth for all colonies encompassed the annual thermal variability, indicating a wide and uniform thermal tolerance at symbiont level. The variation in thermal breadth between colonies of the tropical corals was relatively similar between species for both of the performance traits (**Figure 5.3 d**), and also broad enough to encompass the annual thermal variability for nearly all colonies **Figure 5.4 e-h**).

5.4.4 Chlorophyll concentration

There was no significant difference in the chlorophyll concentrations between colonies and, therefore, this factor was not included in the model (likelihood ratio test, $p = 0.066$ for the temperate species and $p = 0.156$ for the tropical species). Regarding the temperate corals,

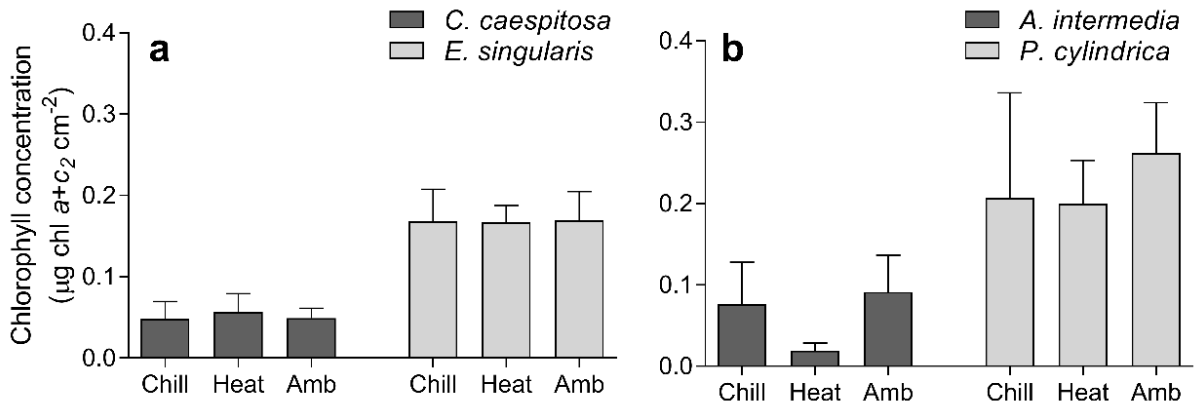


Figure 5.5 Chlorophyll concentrations of the temperate corals (**a**) and tropical corals (**b**), measured on fragments exposed to a decreasing or increasing thermal gradient (Chill and Heat respectively, *n* = 10), or on fragments that remained at ambient (Amb) temperature (n = 5). Mean values ± s.d. are shown.

E. singularis contained three times more chlorophyll than *C. caespitosa* (**Figure 5.5 a**; ANOVA, $F(1,46) = 243.916$, $p < 0.001$, Appendix **Table D.**), which is remarkable given that the Pnet rates per surface area were almost two-fold lower. There was no variation in the chlorophyll concentrations between treatments for either species, indicating that the thermal experiment did not result in degradation of the pigments or expulsion of symbionts (ANOVA, treatment effect, $F(2,46) = 0.106$, $p = 0.899$). For the tropical corals, the chlorophyll concentration was significantly lower in *A. intermedia* compared with *P. cylindrica* (**Figure 5.5 b**; ANOVA, species effect, $F(1,49) = 67.701$, $p < 0.001$) and also lower in the heated treatment compared with the ambient treatment (main effect of treatment, ANOVA, treatment effect, $F(2,49) = 3.614$, $p = 0.035$; post hoc heated versus chilled, $p = 0.027$, **Table D.**), most likely related to heat stress during the highest temperature incubation (33°C).

5.5 Discussion

The results of this study are inconsistent with both Janzen's hypothesis and optimality models that predict that temperate organisms with greater seasonality have broader performance curves than tropical organisms' with smaller seasonal and daily variations in temperature. Instead, the performance breadth for the holobiont related trait (net photosynthesis) and symbiont related trait (maximum electron transport rate) were broad and similar between two tropical and two temperate coral species. My results are also inconsistent with the second aspect of Janzen's hypothesis stating that temperate organisms from more variable environments have a higher acclimation capacity than tropical organisms from more constant environments. If this was true, then the optimum temperature for performance of the temperate species would have approximated the local environment at the time of the thermal experiment. Instead, the T_{opt} was consistently lower than the local and yearly average seawater temperature, suggesting a limited capacity of the temperate species to match its photosynthetic performance to the environmental temperature.

The holobiont performance of the tropical corals varied between species; the T_{opt} for net photosynthesis of *P. cylindrica* corresponded accurately to the environmental temperature while that of *A. intermedia* was more than 6 °C lower. This suggests that certain tropical coral species have greater capacity for thermal acclimation of photosynthesis than that of the temperate corals, although additional studies on a wider range of species are required to confirm this interpretation. Additionally, the thermal response of the symbionts of the tropical corals was very similar across the different coral species, indicating that the observed differences in holobiont performance are mostly driven by the coral host physiology, consistent with the results of **Chapter 3**. However, overall, the physiological response to temperature was comparable between the temperate and tropical corals, suggesting similar

thermal strategies of these species despite their occurrence in distinctively different geographic regions.

The absolute temperature range that the temperate coral populations experienced in 2016 was only 23% greater than that of the tropical corals. This general similarity in temperature range may be responsible for the absence of clear differences in thermal breadth among the temperate and tropical corals investigated here. However, the magnitude of short-term temperature fluctuations in spring and early summer at NWM were more than twice that at reefs around Heron Island (**Figure 5.1**), which indicates that a major difference in the thermal environment between these two regions was the short-term fluctuations within seasons rather than the longer-term variability between seasons. Nevertheless, the absence of short-term fluctuations in temperature in the tropics did not result in smaller thermal breadths for the tropical corals. Remarkably, of all species, the smallest thermal breadth was observed for the photosynthesis rates of *E. singularis*, a species known for high resistance to elevated temperatures (Linares et al. 2013). Yet, the thermal breadth for net photosynthesis was too small to allow high performance at high temperatures, indicating that colonies of this species need to be plastic in their performance between seasons (see **Chapter 3**). It is also likely that *E. singularis* relies less on photosynthetically fixed carbon during summer, because it maintains a heterotrophic feeding mode in summer and winter (Cocito et al. 2013) and it requires less autotrophic carbon than other corals (Ferrier-Pagès et al. 2015). Therefore, *E. singularis* may not need a thermal breadth for photosynthesis as broad as the other corals in this study.

Optimality models predict that strong seasonality should result in a higher capacity for acclimation (Gabriel 2005), meaning that the thermal optima for performance should be closer to the *in-situ* environmental temperature for temperate compared with tropical species. Yet, this alignment was only observed for the photosynthesis rate of the *P. cylindrica*

population at Heron Island. This finding could be related to the particular Symbiodiniaceae species found in *P. cylindrica*. Although I did not assess symbiont species in this study, multiple studies have demonstrated that *P. cylindrica* at Heron Island associates with *Cladocopium* C15 (e.g. LaJeunesse et al. 2003, Stat et al. 2009, Fisher et al. 2012). The resilience to thermal stress and bleaching of *P. cylindrica* has been attributed to its association with this species (LaJeunesse et al. 2003) and a heat stress experiment at Heron Island showed that *P. cylindrica* harbouring *Cladocopium* C15 had greater photosynthetic stability than other coral- Symbiodiniaceae associations (Fisher et al. 2012). In contrast, the *A. intermedia* colonies in my study most likely harboured *Cladocopium* C3 (LaJeunesse et al. 2003; LaJeunesse et al. 2018), which is reported to be more sensitive to heating than *Cladocopium* C15, irrespective of host species (Fisher et al. 2012). Nonetheless, the performance curves for the symbionts within *P. cylindrica* and *A. intermedia* were similar to each other, and showed low sensitivity to temperature in both cases. Therefore, my study suggests that the difference in T_{opt} between *P. cylindrica* and *A. intermedia* for net photosynthesis was influenced by the coral host. This could be related to different morphological characteristics. For instance, tissue thickness has been related to thermal stress (Hoegh-Guldberg 1999). Corals of the genera *Porites* generally have thicker tissue than corals of the genera *Acropora* (Loya et al. 2001), which can support a higher metabolic demand through stored energy reserves (Glynn & D'croz 1990, Fitt et al. 2000). Therefore, *P. cylindrica* may have a greater range of physiological plasticity that enables the coral to acclimate more accurately to the thermal environment. Likewise, differences in the expression of heat-shock proteins and antioxidants (Lesser 2006), energy reserve utilization (Porter et al. 1989) and heterotrophic plasticity (Grottoli et al. 2006) may result in differences in thermal sensitivity (Fitt et al. 2009) and thus thermal optimum.

The thermal optimum for photosynthesis of the temperate corals was only 1 °C below the annual average temperature, but more than 3 °C below the environmental temperature at the start of the experiment. This mismatch is could be due to the depth distribution ranges of *C. caespitosa* and *E. singularis* that both are most abundant at 20 -30 m depth, with sightings as deep as 50 m for *C. caespitosa* (Schiller 1993b) and 67 m for *E. singularis* (Gori et al. 2011). This suggests that the optimal performance temperature of these temperate corals corresponds to the cooler deeper waters, even though the colonies in this study were collected at shallower depths ranging between 10 and 15 m. This interpretation is also consistent with the high among-colony variability in thermal performance curves observed in this study, and in previous chapters, because different colonies at a given collection site are likely to come from mother colonies from different (deeper) depths, and thus different thermal regimes that could constrain acclimation to warmer temperatures. Experiments comparing the thermal performance curves of adults, larvae and juveniles grown under different temperature environments are needed to test these concepts.

The temperate thermal experiments were executed at a time when the environmental temperature was decreasing rapidly, which could explain the mismatch between T_{opt} and the environmental temperature. Inability to acclimate among seasons (see **Chapter 3**) can result in poor performance in summer, which was observed both for *E. singularis* (Ferrier-Pagès et al. 2015) and other coral species, such as the temperate coral *Oculina patagonica* (Rodolfo-Metalpa et al. 2014) and multiple tropical corals (e.g. Warner et al. 2002, Scheufen et al. 2017). However, if acclimation of T_{opt} was lagging behind the environmental temperature because of this rapid decrease, the optimal performance of the temperate corals should be closer to the mean summer temperatures than observed here. These results therefore contradict the hypothesis that temperate corals have a high capacity for acclimation of their photosynthetic performance. Instead, it likely that during summer these corals are close to

their upper thermal threshold and suffer poor performance (Rodolfo-Metalpa et al. 2006a, Rodolfo-Metalpa et al. 2014), especially colonies in shallow water.

Due to logistical constraints, the temperate and tropical thermal experiments conducted for this study took place in different seasons: for the temperate corals in late autumn/early winter and for the tropical corals at the end of summer. This may have influenced the study species' thermal acclimation capacity, because energy-demanding physiological processes vary with seasons (Hinrichs et al. 2013). For instance, rapid growth rates and reproduction usually occur during spring and summer (Ribes et al. 2007), which implies that the photosynthetic performance of the tropical species (measured during summer) may have been constrained by secondary physiological processes such as tissue synthesis. However, such constraints would be expected to increase the thermal sensitivity because they would reduce the resources available for synthesis and repair of damaged proteins. In contrast, I observed an increase of the thermal breadth during summer (Chapter 3), which suggests reduced thermal sensitivity. Alternatively, differences in the relationship between temperature and respiration for the temperate and tropical species could be related to differences in the reliance of species on autotrophic compared with heterotrophic energy intake, as mentioned previously for *E. singularis*. For instance, heterotrophic feeding rates of *C. caespitosa* were more than 3 times higher than that of the tropical coral *Turbinaria reniformis* (Tremblay et al. 2011). This heterotrophic acquired carbon accounted for more than 60% of the carbon budget of *C. caespitosa* and was enough to completely sustain its respiratory requirements. Similarly, low autotrophic carbon acquisition was demonstrated for *E. singularis* that was even lower than that of *C. caespitosa* (respectively, 48 µg C cm^{-2} d^{-1} versus 150 µg C cm^{-2} d^{-1}) (Ferrier-Pagès et al. 2015). In comparison, the autotrophic carbon acquisition for tropical corals is generally >150 µg C cm^{-2} d^{-1} (Muscatine et al. 1981, Anthony & Hoegh-Guldberg 2003, Yakovleva & Hidaka 2004). For instance, the tropical *Stylophora pistillata* obtains

78% of its carbon from the symbiont, of which 48% is used for respiration (Tremblay et al. 2012, Tremblay et al. 2014). Together this suggests that the energy budget of the temperate corals relies less strong on photosynthesis than that of the tropical corals and therefore there is a reduced need to acclimate this performance accurately.

The temperate *E. singularis* had the smallest thermal breadth for net photosynthesis of all four species. This was mostly driven by a significant reduction of the photosynthesis rates at temperatures above 20 °C and is consistent with observations from other studies that showed reduced net photosynthesis and respiration rates in *E. singularis* at temperatures above 20 °C (Previati et al. 2010, Ezzat et al. 2013). However, *E. singularis* is generally described as a species with a relatively high thermal tolerance (Previati et al. 2010, Pey et al. 2011, Linares et al. 2013), and a thermal threshold for survival ranging between 28 and 29 °C (Linares et al. 2013), while *C. caespitosa* is reported as relatively sensitive to temperature (Rodolfo-Metalpa et al. 2005, Rodolfo-Metalpa et al. 2006b). Although my study did not investigate the threshold for survival, the photosynthetic capacity of *E. singularis* was less resistant to high temperatures than that of *C. caespitosa*. This was apparent by the broad performance breadth of *C. caespitosa* with photosynthesis rates maintained close to maximum performance over a wide range of temperatures. Most studies that investigated the effect of temperature on the physiology of *C. caespitosa* used relatively high temperatures and showed increased respiration rates at >24 °C, decreased chlorophyll and symbiont concentrations and photosynthesis rates at >26 °C (Rodolfo-Metalpa et al. 2006b), and decreased F_v/F_m and ETR at >29 °C (Rodolfo-Metalpa et al. 2006a). My study shows that *C. caespitosa* has a low thermal sensitivity at lower temperatures (13-23 °C). This suggests that *C. caespitosa* has a very asymmetrical performance curve, with a sharp drop in performance at temperatures above the temperatures measured in this study.

In none of the species can the reduction of the photosynthesis rates at higher temperatures be attributed to photoinhibition, because the quantum yield and chlorophyll concentration did not change during or after exposure to these temperatures (except for the chlorophyll content of *A. intermedia*). Since the respiration rates increased linearly with temperature for the temperate corals, this could partly explain the reduced net photosynthesis rates at high temperatures. However, the respiration rates of the tropical corals stabilized (*A. intermedia*) or decreased (*P. cylindrica*) at temperatures above 30 °C, thus mechanisms other than increased metabolic rates related to thermal stress must be causing the reduced photosynthesis rates as well. Additionally, the maximum electron transport rate also showed a curve-shaped response with temperature corresponding to that of the photosynthesis rates, suggesting that a diminished electron flow capacity reduced the oxygen production. A similar pattern where oxygen production or electron flow and photosynthetic yield were disconnected has been observed previously in plants, microalgae (Kromkamp et al. 1998) and tropical and temperate corals (Jones et al. 1998, Ezzat et al. 2013) and related to high mitochondrial respiration (Beardall et al. 1994), chlororespiration (Peltier & Cournac 2002), the Mehler reaction (Ort & Baker 2002) and plastoquinol oxidase (Zehr & Kudela 2009). Any of these four processes could explain the observed pattern of my study. For instance, with plastoquinol oxidase, electrons are siphoned away from PSI, resulting in reduced electron flow and oxygen production while the ATP production remains high (Zehr & Kudela 2009), which means that this energy can be used for metabolic processes related to thermal stress that results in increased respiration rates, such as the synthesis of heat shock proteins (Feder & Hofmann 1999).

5.6 Conclusion

The impact of climate change is predicted to be greater at tropical latitudes than temperate latitudes, because tropical organisms have narrower thermal tolerances, and live closer to their upper thermal threshold (Tewksbury et al. 2008). Coral biodiversity is highest in the tropics (Hughes et al. 2002), and coral bleaching episodes related to above average sea surface temperatures have occurred more frequently in the past decades (Heron et al. 2016), demonstrating that temperatures during these events have been repeatedly above the corals' upper thermal thresholds. The results from this study support the notion that corals live above their thermal optimum, as the optimal temperature for coral performance was below the local and mean annual environmental temperature, regardless of coral species. However, the thermal breadth for photosynthesis was broad, and generally encompassed the thermal variability between seasons. Furthermore, the symbiont performance showed low sensitivity to thermal change regardless of symbiont subtype or coral host, indicating that the mismatch of the thermal optimum with the local environment is likely driven by physiological constraints on the coral host rather than on the symbionts. The large thermal tolerance for photosynthesis displayed in this study supports previous observations that corals can survive short periods of abnormally warm temperatures, however survival of coral populations under long term climate change will depend upon the coral's ability to also increase its thermal optimum for performance.

CHAPTER 6

General discussion

This thesis provides insight into the strategies that corals use to cope with spatial and temporal variation in temperature. My thesis shows, for the first time, that the thermal optimum for coral photosynthesis is generally well below the average environmental temperature experienced by corals, although there was considerable variation between colonies with some thriving at higher temperatures. Thermal acclimation in corals is complex because the coral host and algal symbiont can respond independently or synergistically to temperature (Gates & Edmunds 1999). The literature demonstrates that some Symbiodiniaceae species are more thermally tolerant than others which can enhance the coral's capacity to tolerate higher temperatures (Bhagooli & Hidaka 2003, Rowan 2004), while other corals can increase their thermal tolerance through symbiont shuffling (Berkelmans & Van Oppen 2006, Silverstein et al. 2015, Boulotte et al. 2016). The tropical corals in my study harboured *Cladocopium* species (formerly Clade C, LaJeunesse et al. 2018). Members of this genus are not known for high thermal tolerance (Rowan 2004), such as *Durusdinium* species (formerly Clade D, LaJeunesse et al. 2018). Nonetheless, my results show that the symbiont performance generally was better acclimated to the local environment than the holobiont performance, and that symbionts had broad thermal breadths. Furthermore, results indicate that the physiology of the coral host strongly influences the photosynthetic performance and possibly constrains the capacity of corals to acclimatize to environmental change.

In **Chapter 2** I investigated the acclimation trajectory of massive *Porites* spp. during cold and heat exposure. The thermal acclimation trajectory is generally unknown for most organisms (Somero 2015) which confounds interpretation of optimum performance in a changing thermal environment. Furthermore, there can be considerable variation between the acclimation trajectories of physiological traits within the same organism, for instance between those measured at tissue level, or those measured at whole-organism level (Schulte et al. 2011). In a previous study, Roth et al. (2012) showed that during 20 days exposure to cold, the maximum quantum yield of the symbionts did not significantly decrease while the symbiont density of the coral decreased by ~40%. The same study also showed that heat exposure induced a delayed but more deleterious response than cold exposure. However, no previous study has identified the acclimation trajectory, or specifically tested if and when the holobiont or symbiont performance reached a new steady state following a change in temperature. In **Chapter 2**, I demonstrated that during cold exposure the holobiont and symbiont performance of massive *Porites* spp. reached a steady state after ~2 weeks with 'no' or 'inverse' compensation of the performance. Conversely, heat exposure induced a gradual decline of the holobiont and symbiont performance, but neither reached a steady state after 30 days, thus no acclimation was observed. These results show that there is no rapid compensatory acclimation response when massive *Porites* spp. are exposed to an immediate change in the thermal environment, and that compensation of the performance is unlikely to occur in response to short-term variations in temperature. Lastly, the results of **Chapter 2** showed that massive *Porites* spp. are both resistant and resilient to thermal stress, and has likely adopted a thermal generalist strategy.

Building on these findings, I investigated whether and how seasonal variation of the environmental temperature influenced the thermal performance of corals. Previous studies observed seasonal fluctuations in several physiological traits of corals, such as symbiont

density (Fitt et al. 2000), photosynthesis rate (Scheufen et al. 2017), and calcification rate (Falter et al. 2012). In these studies, results generally indicated variation in the average or maximal performance of these physiological traits and in the upper thermal threshold for bleaching (Berkelmans & Willis 1999). While these studies demonstrate that corals have a capacity for reversible acclimation of their performance, they do not elucidate whether seasonal variation of the thermal performance was driven by a horizontal shift of the thermal performance curve through a change in thermal optimum, or by change of the thermal breadth. In **Chapter 3** I demonstrated that for the bleaching sensitive *Acropora* spp., seasonal variation of the thermal performance was driven by an increase of the thermal optimum in summer, while for the bleaching tolerant *Porites cylindrica*, seasonal variation was driven by a change of the thermal breadth. These results reveal, for the first time, that there is species-specific variation in the strategies of reversible thermal acclimation for corals.

Subsequently, in **Chapter 4** I investigated the thermal performance of these same two coral species living in different thermal environments. To date, there is only limited evidence in the literature to show that coral performance varies between colonies from contrasting thermal environments. These previous studies suggested that calcification rates were higher in colonies living in warmer environments compared to colonies in cooler environments (Lough & Barnes 2000), and that growth patterns varied between colonies from variable or more stable thermal environments (Smith et al. 2007). Such studies indicated that corals from environments with frequent thermal fluctuations or elevated temperatures are better able to withstand temperature extremes (Oliver & Palumbi 2011). However, the few previous studies that have specifically investigated whether the optimal temperature for performance varied between colonies from different thermal environments show conflicting results. For instance, the thermal optimum for coral growth varied between populations of *Pocillopora damicornis* (Clausen & Roth 1975), but not for net productivity of *Montastrea annularis* despite

differences in the ambient temperature regimes (Castillo & Helmuth 2005). Likewise, Rodolfo-Metalpa et al. (2014) demonstrated that populations of *Oculina patagonica* living in different thermal environments shared a similar relatively low thermal optimum for a variety of holobiont and symbiont performance traits.

In **Chapter 4** I used the latitudinal gradient along the Great Barrier Reef to compare the thermal performance of coral populations living in different thermal regimes. I specifically assessed if coral species with wide geographic distributions are phenotypically plastic in their performance or rather conform to a thermal generalist strategy. Results clearly showed geographic variation in the thermal performance among populations, which is indicative of physiological plasticity in thermal performance traits along environmental gradients. However, this plasticity was greater among physiological processes dominated by the coral host than among processes dominated by the symbiont, whereas acclimation to the local environment was more accurate for the symbiont performance. These findings suggest that environmental conditions experienced during early development may constrain the coral's capacity to acclimate to higher summer temperatures, while the symbiont's capacity for acclimation remains broader, possibly due to its shorter generation times (Wilkerson et al. 1988).

Lastly, I compared thermal performance of tropical corals with that of corals from the Mediterranean Sea (**Chapter 5**). Central to this chapter was the hypothesis that temperate organisms have a broader performance breadth (*sensu* Janzen 1967) and higher acclimation capacity (Gabriel 2005) than tropical organisms as a result of greater variations in the thermal environment at temperate latitudes than in the tropics. For corals, previous studies indicate that the thermal tolerance was higher if colonies lived in environments with greater and more frequent temperature fluctuations (Middlebrook et al. 2008, Barshis et al. 2010, Oliver & Palumbi 2011), but a direct comparison between the thermal tolerance of temperate and

tropical corals had not previously been made. The results of **Chapter 5** showed that performance breadths of the temperate and tropical corals were equivalent. This was likely due to the large temperature range that all four coral species experience in their local environment, in contrast to the generalisation in the literature that temperate environments have higher variability. For the study species investigated here, the thermal breadths observed were large enough to encompass the local annual thermal range suggesting that these corals adopted a thermal generalist strategy to cope with temporal heterogeneity. My study also showed that both the temperate and tropical corals generally live at temperatures above their thermal optimum for photosynthesis, despite considerable variation in thermal performance among colonies. In fact, the only significant difference between the temperate and tropical corals was the thermal optimum and maximal performance, which was lower in the temperate corals corresponding to the lower local and annual mean environmental temperature.

6.1 Corals as thermal generalists

All organisms possess some capacity to modify their behavioural, physiological or morphological characteristics in response to environmental temperature through developmental and/or reversible acclimation (Angilletta 2009). For corals, this ability is assumed to be ubiquitous (Edmunds & Gates 2008), although acclimation in thermally heterogeneous environments can be physiologically challenging and result in a variety of compensatory responses due to different costs and benefits to the individual (Prosser 1991). These costs and benefits, and time lags in acclimation, mean that a thermal generalist strategy can be more advantageous than a plastic thermal specialist strategy. My thesis showed that massive *Porites* spp. were unable to acclimate their photosynthetic physiology to compensate for reduced performance induced by cold and warm shifts in the thermal environment (**Chapter 2**). This supports the results of Edmunds (2014) who similarly did not observe

beneficial acclimation of coral growth and maximum photosynthetic efficiency for this species. Thermal acclimation is costly because it involves protein synthesis to repair and stabilize the physiological processes impacted by changes in temperature (Somero & Hochachka 1971, Gates & Edmunds 1999). This imposes a physiological trade-off in which the energy spent on acclimation can no longer be allocated to other processes such as reproduction or growth (Jones & Berkelmans 2011). Minor changes in temperature may only incur a small energetic cost enabling the coral to keep up with protein synthesis and repair thereby compensating for its performance during acclimation, while more extreme thermal changes may result in energy constraints that hamper the acclimation response (Kirkwood 1981). Additionally, acclimation is costly because it requires time for developmental or physiological changes to occur (Angilletta 2009). If acclimation occurs slowly relative to the rate of change of the environmental temperature, then the net benefit of acclimation may also be marginal. As observed in this thesis **(Chapters 3, 4 and 5**), these processes can result in a mismatch between optimal performance and local environmental temperature for a variety of coral populations and species.

If corals are thermal specialists, then slow acclimation rates may result in large declines in performance if the environment changes. In contrast, if corals are thermal generalists (e.g. exhibiting a broad and relatively flat thermal performance curve) then a mismatch between the optimal performance and the environmental temperature would not result in a substantial loss of performance. Visualizing the thermal performance of each colony measured in this thesis (Figure 6.1) shows that both *Acropora* spp. and *Porites cylindrica* have a relatively broad thermal tolerance (median 10.5 °C and 14.5 °C respectively), suggesting that they both are thermal generalists. This finding seems to challenge studies previous reports that the performance of corals, in terms of photosynthesis and growth, is strongly dependent on temperature (e.g. Warner et al. 1996, Lesser 1997). Thermal performance curves measured

here used increments of only 2 days between increasing/decreasing temperatures for a total of 10 days of exposure to altered temperature in order to capture instantaneous thermal performance. Consequently, my thesis measures thermal performance over a relatively shorter time frame in comparison with those reported earlier, particularly with regard to the time required for performance traits to reach a new steady state (> 10 days) in **Chapter 2**. Although the thermal performance curves measured here were generally consistent with those measured for another coral species using increments of 2 weeks between increasing/decreasing temperatures (Rodolfo-Metalpa et al. 2014), increasing time increments would likely result in larger cumulative effects of elevated/decreased temperature and 'steeper' thermal performance curves.

Previous studies showing thermal sensitivity of corals (e.g. bleaching) often refer to the sensitivity of the performance at elevated temperatures and not necessarily to the performance breadth. My study shows that the photosynthetic performance indeed decreases at elevated temperatures (> ~25 °C), but additionally demonstrates that this is linked to the low thermal optimum identified for virtually all colonies (**Figure 6.1**). Indeed, the loss of performance at elevated temperatures (Tm in **Figure 6.2**) will be small if the optimal performance is at a temperature close to this elevated temperate (**Figure 6.2 a**), whereas the loss of performance will be greater if the thermal optimum is further away from this elevated temperature (**Figure 6.2 b**). This was the case for virtually every coral colony regardless of location or species (**Figure 6.1**). Thus, corals may be thermal generalists that still live well above their thermal optimum, but suffer reduced performance during prolonged periods of high temperatures during summer (Heron et al. 2016, Hughes et al. 2018). Note that the rate at which the temperature changes may influence the loss of performance, that is, if the temperature changes at a slow enough rate, the loss of performance may be reduced, and vice versa. Also of importance is that the term 'thermal generalist' is used relative to the thermal

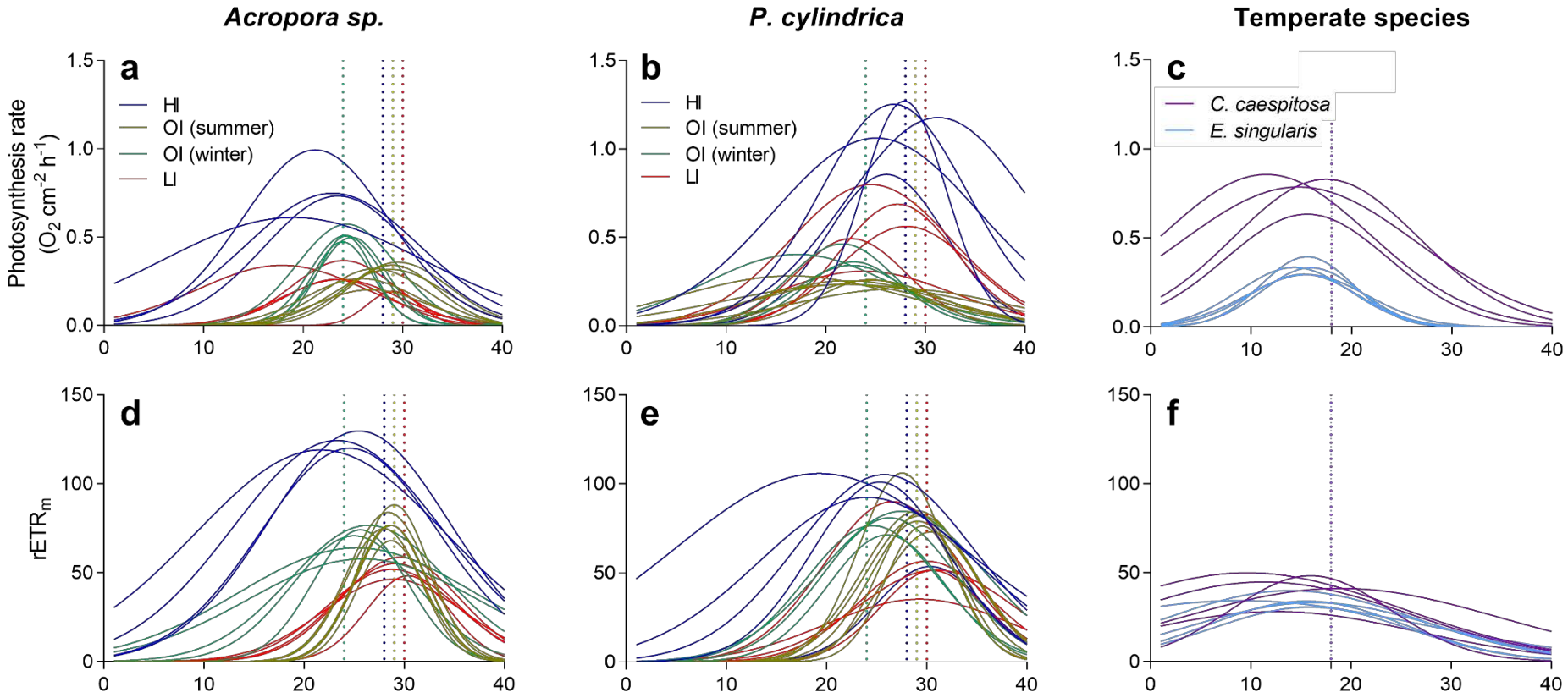


Figure 6.1 Colony specific performance curves of *Acropora* spp., *Porites cylindrica*, *Cladocora caespitosa* and *Eunicella singularis*, measured in populations at Heron Island (HI), Orpheus Island (OI, in summer and winter), Lizard Island (LI) and North West Mediterranean Sea. Displayed are non-linear regressions of net photosynthesis rate (**a-c**) and maximum electron transport rate (**d-f**). The dotted lines are the local environmental temperatures at the time of the thermal experiment.

heterogeneity of the environment that corals experience. Marine mammals, for example, are thermal generalists over a much wider range of temperatures and occur across latitudes between polar and tropical seas (Pörtner 2002). Therefore, the results from my thesis suggests that corals are not strict thermal specialists that need to shift their performance curves when the environmental temperature changes, but that they are thermal generalists that can perform across the range of temperatures that encompasses their annual environmental variability.

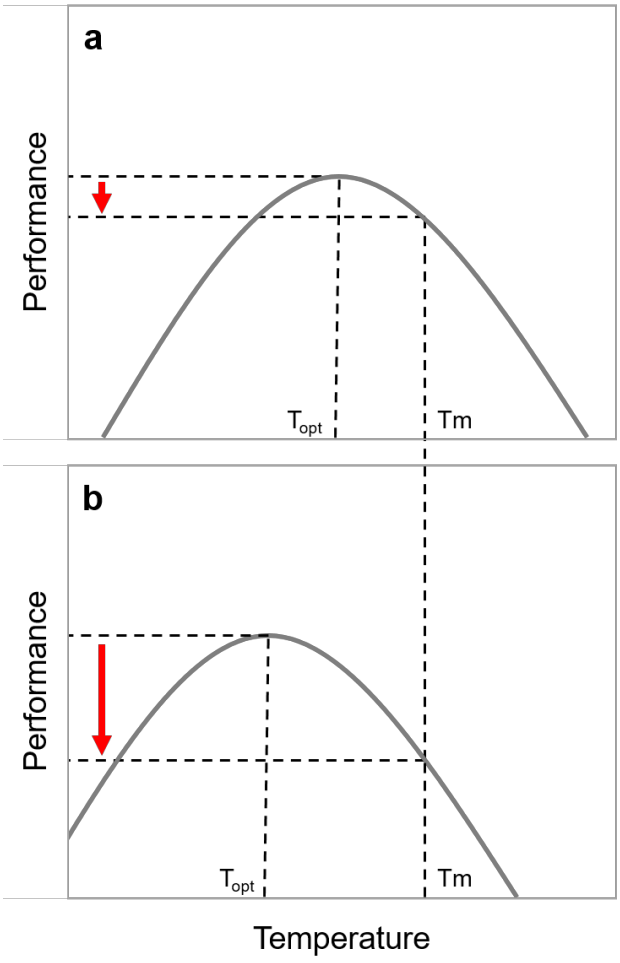


Figure 6.2 Hypothetical performance curves with a thermal optimum (Topt) at a high temperature (a) and low temperature (b). If performance is measured at certain temperature (Tm), then is the loss of performance smaller when the thermal optimum is closer to this measuring temperature.

6.2 Thermal acclimation in a heterogeneous environment

Numerous studies have demonstrated that the coral physiology responds to seasonal variation, for instance through changes in the symbiont density and chlorophyll concentration (Fitt et al. 2000), calcification rates (Kayanne et al. 2005, Falter et al. 2012) and photosynthesis rates (Kayanne et al. 2005, Scheufen et al. 2017). Likewise, the results presented in **Chapter 3** showed that the coral performance varied between seasons and that acclimation occurred through species-specific changes in the performance curve. Interestingly, the symbiont performance was less plastic between seasons than the holobiont performance, suggesting that seasonal acclimation occurred mostly by the coral host and less by the symbiont. This lack of response in the symbiont may be the result of a less tolerant symbiont type. The thermal sensitivities of the symbiont varies between Symbiodiniaceae species and associations with less sensitive symbionts can increase the thermal tolerance of the coral (Berkelmans & Van Oppen 2006, Howells et al. 2012). For instance, members of *Durusdinium* (formerly Clade D) have a higher thermal tolerance than those of *Cladocopium* (formerly Clade C) (Rowan 2004), and corals that associated with *Durusdinium* spp. therefore have increased resistance to elevated sea surface temperatures (Baker et al. 2004, Stat & Gates 2011). Similarly for species within of the same genus, corals associated with *Cladocopium* C78 and *Cladocopium* C8 were more bleaching resistant than corals associated with *Cladocopium* C79 and *Cladocopium* C35 (Sampayo et al. 2008). It is possible therefore, that the lack of symbiont plasticity found in my study may be due to associations with less tolerant species. While I did not empirically test the symbiont species, evidence of common coral-symbiont associations suggests this is unlikely. Previous studies have commonly found that the tropical corals investigated here harboured *Cladocopium* C15 in *P. cylindrica* and *Cladocopium* C3 in *A. intermedia* and *A. valenciennesi* (LaJeunesse et al. 2004, Madin et al. 2016), and the temperate corals harboured '*Symbiodinium*' Temperate A

(Visram et al. 2006, Forcioli et al. 2011, Casado-Amezúa et al. 2014). Between the *Cladocopium* species, C3 is more sensitive to heating than C15 (Fisher et al. 2012). However, the symbiont responses during my summer and winter thermal experiments were relatively uniform between season and coral species, and showed limited variation in thermal optimum or thermal breath for F_v/F_m and $rETR_m$ (**Chapter 3**). This suggests that the symbiont did not directly assist the coral to increase its tolerance in summer, as suggested by previous studies (e.g. Fisher et al. 2012).

The results of this thesis raise the question whether symbiosis promotes or constrains reversible acclimation of the corals. Previous studies show that both the coral host and symbiont shape the coral response to stress (see review by Baird et al. 2009), but it is unclear what their roles are during acclimation. It is possible that some symbionts confer plasticity that accelerates coral acclimation, while others are less plastic and constrain the acclimation capacity of the coral. Logically, corals that harbour multiple symbiont types that each have different physiological optima, or corals that harbour symbionts with broad thermal performances, are likely to have stable performance over a broad range of temperatures. However, these stabilising (compensatory) processes could prevent the need for acclimation and lead to widespread occurrence of thermal generalist strategies among symbiotic corals.

6.3 Deviation from predicted optimal performance?

The results from this thesis reveal geographic variation in the thermal optimum among populations of the same species in the Great Barrier Reef (**Chapter 4**), but did not support the hypothesis that populations from warm environments (Lizard Island) have higher thermal optima than populations from cooler environments (Heron Island). Instead, the thermal optima were below the environmental temperature for nearly every population. In temperate corals were the thermal optima, as expected, significantly lower than that of the tropical

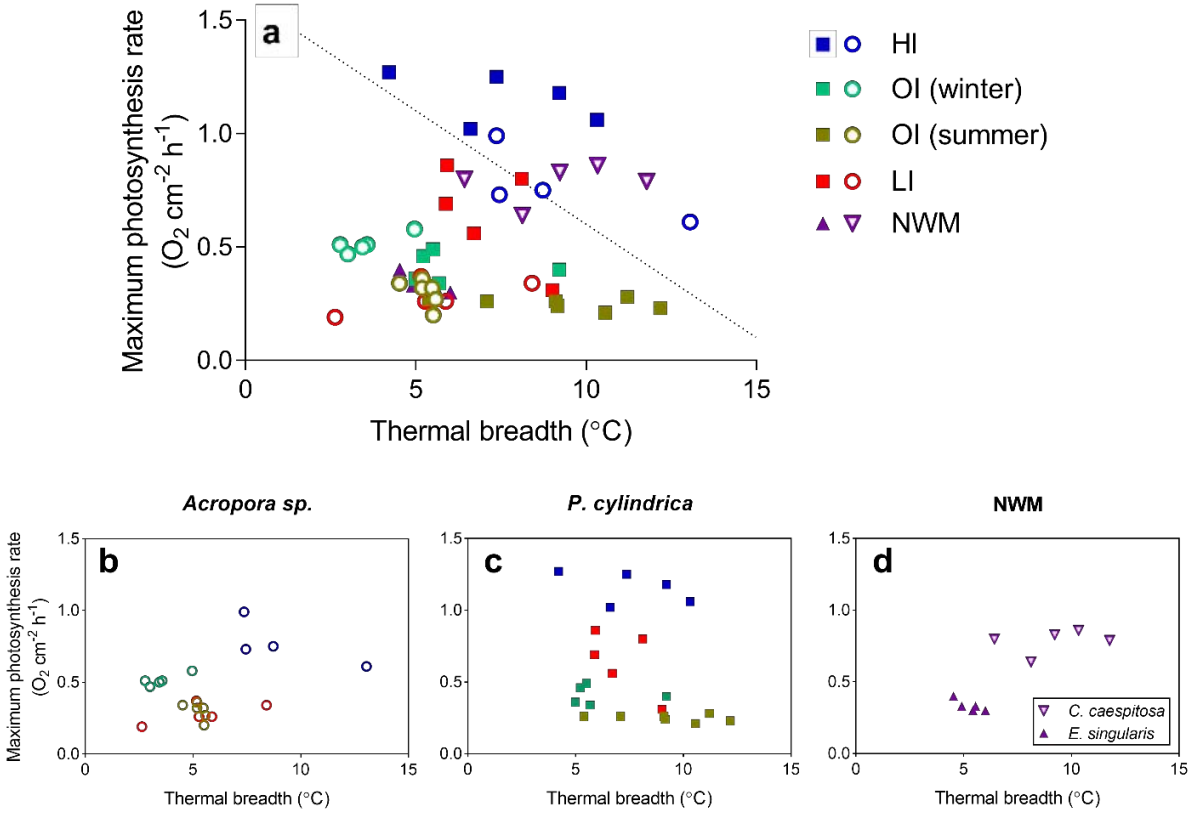


Figure 6.3 Maximum photosynthesis rate of the colony performance curves in relation to the thermal breadth, measured on *Acropora* spp. (circles), *Porites cylindrica* (squares), *Cladocora caespitosa* (upwards triangle) and E. singularis (downwards triangle) in populations at Heron Island (blue), Orpheus Island (green), Lizard Island (red) and North West Mediterranean (NWM - purple). Top panel (a) shows data pooled together with a hypothetical trend line, bottom panels show the same data but grouped by populations of *Acropora* spp. (b), P. cylindrica (c) and in the North West Mediterranean (d).

corals over the range of temperatures tested. Interestingly, I found little variation in thermal breadth among the tropical species (**Chapter 4**) or between the tropical and temperate species (**Chapter 5**), which also contrasted with the hypothesis that temperate species have a broader performance breadth than tropical species (e.g. Janzen 1967). Optimality models predict that acclimation of the thermal optimum provides a greater advantage than acclimation of the thermal breadth, and thus should occur more readily within generations while acclimation of the thermal breadth should reflect the variation of temperatures within generations (Gabriel &

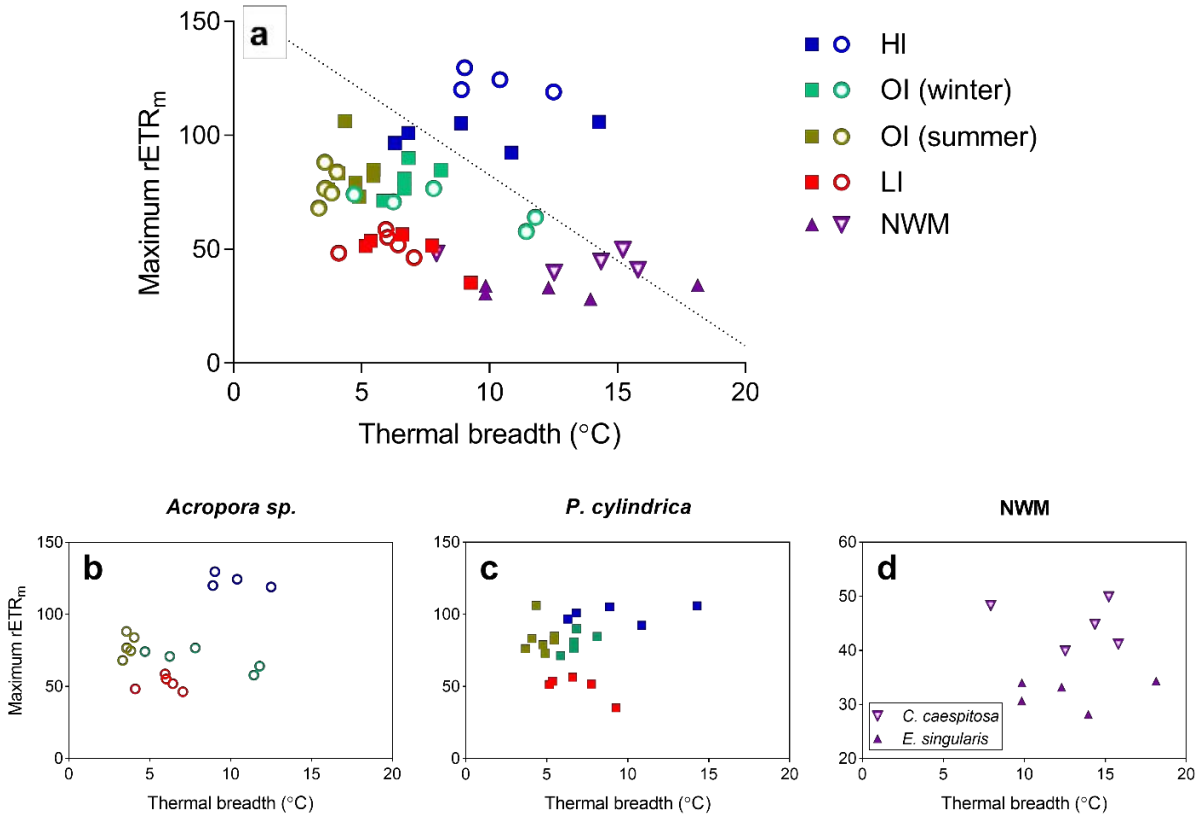


Figure 6.4 Maximal maximum electron transport rate of the colony performance curves in relation to the thermal breadth, measured on *Acropora* spp. (circles), *Porites cylindrica* (squares), *Cladocora caespitosa* (upwards triangle) and *Eunicella singularis* (downwards triangle) in populations at Heron Island (blue), Orpheus Island (green), Lizard Island (red) and North West Mediterranean (NWM - purple). Top panel (a) shows all species together with a hypothetical trend line, bottom panels show the same data but grouped by populations of *Acropora* spp. (b), P. cylindrica (c) and in the North West Mediterranean (d).

Lynch 1992, Angilletta 2009). Yet, in my thesis the thermal optimum was generally lower than the environmental temperature, and the thermal breadth was often wider than the thermal heterogeneity. One of the core assumptions of these models is that the thermal performance is constrained by a generalist-specialist tradeoff: the maximal performance of a specialist exceeds that of a generalist and thus greater maximal performance generates a smaller thermal breadth (dotted line in **Figure 6.3** and **Figure 6.4**). Interestingly, I found no strong

correlation between the maximal performance and performance breadth at the holobiont (**Figure 6.3**) or symbiont level (**Figure 6.4**), regardless of species or location. Thus, the coral species examined here appear to be masters of all temperatures rather than 'a master of none' (Huey & Hertz 1984). Previous studies demonstrated similar deviations from the predicted patterns for various taxa, for instance, for photosynthesis and tuber production of aquatic plants (Pilon & Santamaría 2002, Santamaría et al. 2003), clutch size of *Daphnia pulicaria* (Palaima & Spitze 2004) and several metabolic processes of Atlantic halibut (Imsland et al. 2000). Thus, although the generalist-specialist tradeoff is an important assumption of optimality models, it does not always correctly predict patterns of empirical data.

While this study endeavoured to monitor the *in situ* thermal environment as accurately as possible, coral reefs are complex environments and additional variables which were not accounted for may also have affected acclimation (**Chapter 3, 4** and **5**). Temperature records were obtained from sites as close to the coral collection sites as possible, but these temperature observation stations (AIMS for the tropical sites and CORILOS for the Mediterranean sites) may not accurately represent the thermal environment that the corals experience *in situ*. This includes variation of the *in situ* light environment compared to that of the experimental setting. Effort was made to match the experimental light environment with the ambient light environment, but small differences in light levels may have induced photoacclimation and temporal changes in physiological processes during thermal performance experiments influencing the photosynthetic performance of the corals measured.The state of the environment may also have contributed to deviation from the optimal performance from the environmental temperature. For instance, Orpheus Island is an inshore reef with high sedimentation and nutrient input (Walther et al. 2013) that may have led to differences in coral performance compared with the other locations. Lizard Island and Heron Island are mid shelf reef that suffer less turbidity than Orpheus Island, but the sea

surface temperatures at the reefs around Lizard Island were above average for numerous consecutive weeks during the time frame of experimental work at this location. This may have resulted in significant thermal stress and coral bleaching, potentially constraining the photosynthetic performance of the corals during my experiment (**Chapter 4**). The reefs around Heron Island were in better health than the central and northern reefs at the time of experimentation (Hughes et al. 2017b), which may have resulted in higher maximal performance rates and improved acclimation. Furthermore, the thermal experiment at Heron was performed a year later (2017) than those in Orpheus and Lizard (2016), with environmental differences between the summer seasons also potentially affecting performance and acclimation.

6.4 Variability in thermal performance among colonies

I observed considerable variation between individual colonies from the same population in their response to changes in environmental temperature (**Chapters 3-5**; **Figure 6.1**). Inter-colony variation can be driven by genetic variation between colonies (Meyer et al. 2009, Csaszar et al. 2010), or by other differences in the coral holobiont such as differences in microbial communities (Ainsworth et al. 2010, Gates & Ainsworth 2011). The variation in thermal optimum and performance breadth between colonies highlighted that despite an overall low thermal optimum at the local population level, there are individuals with high thermal optima. This is an important finding, because these are colonies with the potential to perform well under future climate change scenarios. If these individual differences in thermal performance are also translated in differences in genotype, then this provides additional substrate for natural selection to act upon (Careau et al. 2014). Therefore, the implications of individual coral variability is significant in light of climate change and the search for ‘super corals’, to advance studies directed at assisted evolution (van Oppen et al. 2015)

The variation in the thermal optimum of the performance curves of individual colonies was generally larger for holobiont related traits than for symbiont related traits. There are several reasons that may explain this. First, the extent to which the coral experiences the spatial and temporal heterogeneity of the thermal environment is different to that of the symbiont and this can influence the amount of physiological variation that is present at a given time (Ghalambor et al. 2015). For instance, the significantly shorter generation time of the symbiont may result in a more rapid evolution that converges to match the present thermal environment with smaller individual variation (Howells et al. 2012). For example, under laboratory conditions it took only 2.5 years (~80 generations) for symbionts to adapt to an increased upper thermal threshold and temperature tolerance range (Chakravarti et al. 2017). Second, corals may select and retain symbionts that perform well and expel symbionts that perform poorly during or after coral bleaching (Baker 2003), obscuring the physiological variation in symbiont types that is naturally present in the environment. Third, coral larvae are likely to disperse over larger distances than symbionts (Howells et al. 2009), with the presence of poorly-adapted coral genotypes from different thermal environments resulting in greater within-population variation among corals than among symbionts.

Reef-building coral are generally recognized as highly vulnerable to even slight increases in water temperature, and therefore perhaps the most familiar example of dangers faced by tropical marine species under climate change (Hoegh-Guldberg et al. 2007). However, interpreting my empirical data in light of climate change, gives a glimmer of hope that there is a future with corals reefs. IPCC models predict that summers will become hotter and this will occur more frequently and for longer periods of time (IPCC, 2014). Although my results show that the optimal performance temperature of most coral populations is drastically below these summer extremes, at individual level, there are colonies that have their optimal performance temperature at elevated temperatures, and thus these colonies might be able to

persist. Consequently, it is possible that the presence of these 'super' corals allows for a more rapid adaptation of corals to climate change than current models predict, as the thermally-tolerant genotypes are already present in current populations.

6.5 Directions for future research

The concepts investigated in this thesis contribute new insights into coral thermal performance and builds on theories well explored in thermal biology in general, but have previously only been applied to corals in a limited way. Corals are particularly interesting because of their symbiosis with algal symbionts that can respond differently to changes in temperature which can facilitate or constrain the coral performance. Although this thesis examined the performance of several coral species with contrasting life-history strategies (*A. intermedia, A. valenciennesi, C. caespitosa, E. singularis, P. cylindrica,* and massive *Porites* spp.), it did not quantify the potential for different symbiont types to ameliorate holobiont performance. Further research should be directed towards further differentiating the influence of the symbiont's thermal optimum compared to that of the coral host's, and importantly how this impacts the process of thermal acclimation in heterogeneous environments. Corals with the capacity to reshuffle symbiont types may ameliorate the impact of poor symbiont performance at higher temperatures (Baker 2003). Therefore, future experimental work on coral thermal performance should include coral species known to associate with multiple symbiont types, and sampling designs structured to determine if reshuffling dominant symbiont strains results in changes to thermal optima for symbiont performance and the performance of the coral. To more clearly determine the influence of the coral-algal symbiosis on thermal performance of the holobiont, comparisons of performance curves between symbiotic corals and asymbiotic corals from similar thermal environments should be considered.

The observed mismatch between thermal optimum for photosynthesis with thermal environment in this thesis warrants further exploration. Imprecise thermal acclimation can be due to time lags in the process of acclimation versus the time during which temperature change can occur, and may be an ideal thermal strategy to reduce the loss of performance during thermal change. However, the length of time required for acclimation to new thermal regimes remains unclear. Determination of the time required for acclimation should involve investigating physiological mechanisms at the cellular level, by quantifying how gene expression and/or metabolite concentrations present in coral tissues impact performance at the symbiont and holobiont level. The findings from my thesis showed that cold and heat exposure resulted in poor coral performance where beneficial acclimation was undetectable. Future work should focus on identifying acclimation trajectories and time frames at different levels of biological organization (i.e. holobiont, symbiont, cellular and biochemical level), in order to improve our mechanistic understanding of coral thermal acclimation.

Additionally, other performance traits more directly linked to coral fitness, such as fecundity and growth, should be included in future studies on coral thermal acclimation which may be achieved through rearing corals at varying developmental temperatures or through investigations of thermal performance of corals from contrasting thermal environments. For instance, corals that inhabit intertidal habitats which experience significant diurnal variation in temperature (Oliver & Palumbi, 2011), or corals living in the Red Sea that are exposed to consistently high average and maximal temperatures (Fine et al. 2013). A comparison of the thermal performance of coral species in these types of extreme environments with those in less extreme environments will clarify how corals deal with thermal stress and test hypotheses about possible physiological trade-offs. Similarly, investigating corals with different geographic distribution ranges (e.g. temperate compared

with tropical endemics) could provide insight into the specialist-generalist trade-off highlighted in this thesis.

This thesis relied on symmetrical thermal performance curves (Gaussian function) to describe the responses of the coral holobiont and symbiont to temperature change despite the use of asymmetrical curves being prevalent in the literature (Angilletta 2009). Other studies indicate that thermal performance curves tend to be asymmetric, often with a steep decline in performance at high temperatures (Huey & Kingsolver 1989). However, model selection consistently favoured the symmetrical Gaussian equation over asymmetrical modified Gaussian and Weibull equations and was therefore consequently used to estimate the shape of the performance curves. The use of this function is also consistent with the broader literature, because theorists often use a Gaussian function to model the evolution of thermal performance curves (e.g. Lynch & Gabriel 1987, Huey & Kingsolver 1993, Pfab et al. 2016). Although the conclusions are robust, because the symmetrical curve provided the best fit to the data, the shape of the thermal performance curve can substantially influence the predictions of the impact of climate change on corals. It is therefore important for future studies to explore utilisation of asymmetric curves in measurements of coral thermal performance. Similarly, I relied on linear regressions for comparisons of some of the traits in several chapters of this thesis. The absence of a curve-shaped thermal responses in these analyses indicate that the measured performances never reached their optimal temperature. Expansion of temperature ranges during thermal experiments should improve upon the findings here.

6.6 Conclusions and implications

Coral reefs are rapidly changing in response to anthropogenic climate change, which means we urgently need to improve our understanding of the responses and trajectories of

corals to rising temperatures. My thesis shows that thermal acclimation in corals is a slow process. Physiological constraints on the coral host appear to hinder accurate acclimation of corals to local environmental temperature, which was demonstrated by a mismatch of the thermal optimum for photosynthesis and the local environmental temperature. Therefore, the coral populations investigated in this study lived at temperatures above their thermal optimum, some even all year round. As such, most of the time they suffer poor performance, which may contribute towards reduced resilience during thermal stress events. The performance of *P. cylindrica* was generally higher than that of *Acropora* spp., including a thermal optimum closer to the environmental temperature and a wider performance breadth (**Figure 6.1**). Consequently, *P. cylindrica* colonies performed better at high temperatures than *Acropora* spp., and further temperature increases may therefore result in greater loss of performance for *Acropora* spp. than for *P. cylindrica* (**Figure 6.2**). This is a disturbing observation, because corals of the genus *Acropora* add structural complexity to the reef which, in turn, promotes diversity of fishes and the reef ecosystem overall (Graham & Nash 2013). However, corals are not completely at the mercy of environmental temperature. Considerable variation among colonies highlighted that some colonies perform better at high temperatures than others, providing a glimpse of hope for the future of coral reefs.

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APPENDICES

Appendix A: Supplementary Tables and Figures Chapter 2

Table A.1 Results of the likelihood ratio test that compared the fit of a simple linear regression with the fit of piecewise linear regressions to the physiological responses. When piecewise regressions proved to be a better fit to the data (p-value < 0.05), this indicated that the physiological response varied between 10 days intervals.

		Chilled					Heated					Ambient				
Response	Regression	df	AIC	logLik	L.ratio	p-value	df	AIC	logLik	L.ratio	p-value	df	AIC	logLik	L.ratio	p-value
Pnet	Simple	3	-284	145			3	-197	101			-	-	-		
	Piecewise	7	-288	151	12.82	0.012	7	-198	106	8.89	0.064	-	-	-	-	-
R	Simple	3	-222	114			3	-197	101			-	-	-		
	Piecewise	7	-227	121	13.88	0.008	7	-208	111	19.59	0.000	-	-	-	-	-
F_v/F_m	Simple	4	-1605	807			4	-1170	589			4	-797	403		
	Piecewise	8	-1620	818	23.39	0.000	8	-1193	605	31.08	0.000	8	-818	417	28.34	0.000
$\Delta F/F_m'$	Simple	4	-369	189			4	-271	139			4	-156	82		
	Piecewise	6	-368	190	2.89	0.236	6	-267	140	0.31	0.858	6	-153	83	1.16	0.561
Q_m	Simple	3	-302	154			3	-200	103			3	-124	65		
	Piecewise	5	-301	156	3.29	0.193	5	-195	103	0.40	0.819	5	-120	65	0.43	0.807
$rETR_m$	Simple	4	1029	-511			4	1048	-520			4	322	-514		
	Piecewise	8	1035	-509	3.07	0.547	8	1050	-516	6.98	0.137	8	324	-157	6.14	0.189

Table A.2 Tukey posthoc comparisons between the slopes of the physiological responses to temperature during three time intervals (days 0-10, 11-20 and 21-30).

Response	Comparison slope	Chilled				Heated				Ambient			
		Difference	S.E.	t-value	*p*-value	Difference	S.E.	t-value	*p*-value	Difference	S.E.	t-value	*p*-value
Pnet	0-10 vs 11-20	0.015	0.006	2.754	0.007	0.005	0.008	0.567	0.572	-	-	-	-
	0-10 vs 21-30	-0.001	0.006	-0.250	0.803	0.019	0.008	2.301	0.023	-	-	-	-
	11-20 vs 21-30	-0.017	0.006	-2.820	0.006	0.015	0.008	1.793	0.076	-	-	-	-
Resp	0-10 vs 11-20	0.008	0.007	1.018	0.311	-0.012	0.008	-1.500	0.136	-	-	-	-
	0-10 vs 21-30	-0.013	0.007	-1.753	0.082	0.006	0.008	0.734	0.461	-	-	-	-
	11-20 vs 21-30	-0.020	0.008	-2.485	0.014	0.018	0.008	2.416	0.017	-	-	-	-
F_v/F_m	0-10 vs 11-20	0.003	0.002	2.130	0.034	0.011	0.002	4.875	0.000	0.002	0.002	0.757	0.450
	0-10 vs 21-30	0.005	0.002	2.945	0.003	0.002	0.003	0.769	0.443	0.004	0.002	1.540	0.125
	11-20 vs 21-30	0.002	0.002	0.788	0.431	-0.009	0.003	-2.965	0.003	0.002	0.003	0.817	0.415
$\Delta F/F_m'$	9-20 vs 21-30	0.001	0.002	0.603	0.548	-0.001	0.003	-0.378	0.707	0.004	0.004	1.005	0.321
Q_m	9-20 vs 21-30	0.002	0.003	0.788	0.433	0.002	0.005	0.416	0.679	0.003	0.006	0.455	0.651

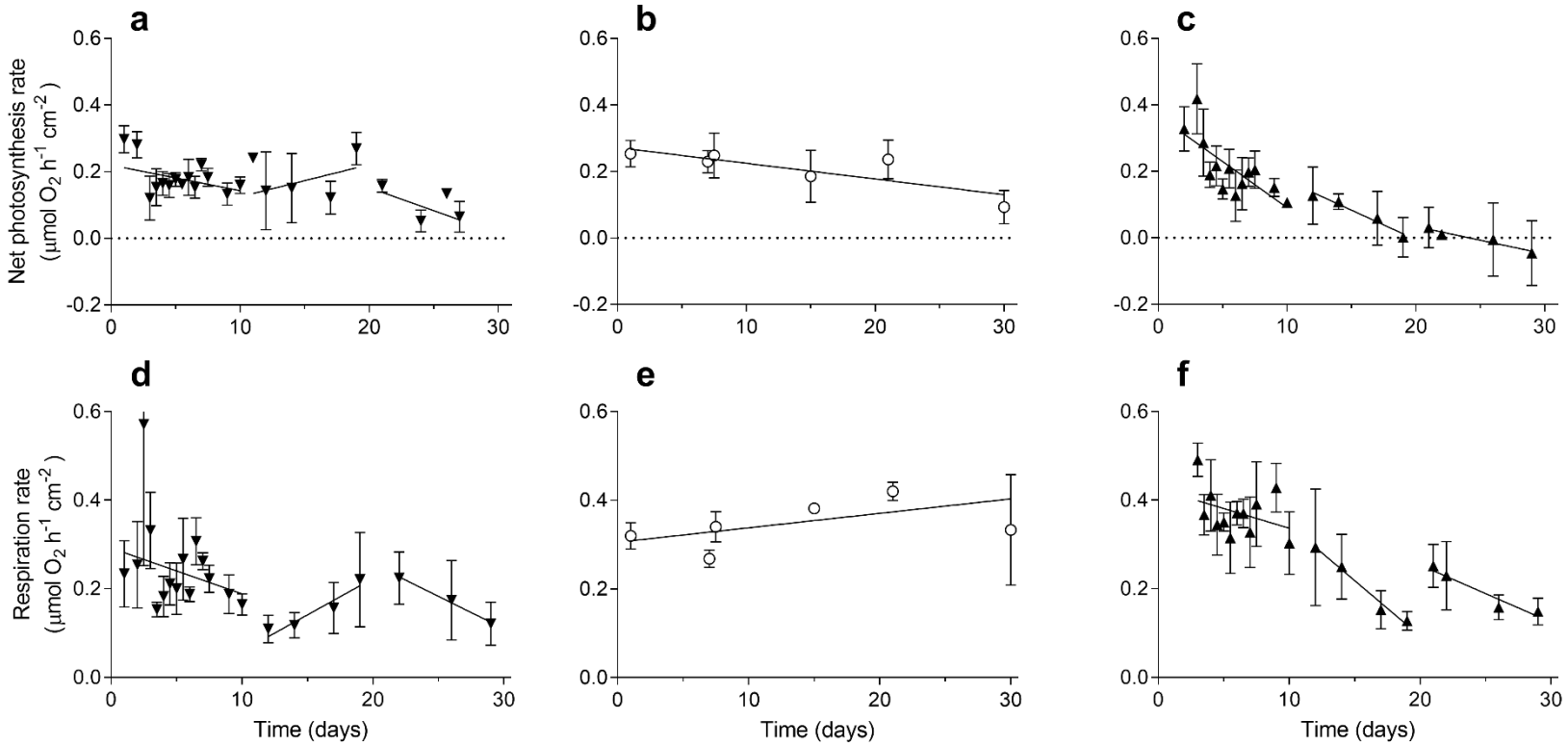

Figure A. 1 Mean net photosynthesis rates (top panels) and absolute respiration rates (bottom panels) of massive *Porites* spp. during 30 days exposed to chilled (21 ^{0}C; **a,d**), ambient (26 ^{0}C; **b,e**) or heated (31 ^{0}C; **c,f**) seawater. During the first 7 days, measurements were taken every morning and evening, the remaining days measurements were taken in evening**. Repeated measured fragments are excluded from regression**. Fragments at ambient seawater were measured after 1, 7, 15, 21 and 30 days of exposure. Data points represent averages (excluding repeated measured individuals, hence *N* is variable), error bars are standard error of the mean and line shows the linear regression that was fitted to data.

Appendix B: Supplementary Tables and Figures Chapter 3

Table B.1 AIC values when symmetrical (Gaussian, Quadratic) and asymmetrical (Mod. Gaussian, Weibull) functions were fitted to different combinations of data selection for different physiological responses. Functions were fitted as follows: 1) seasonal and species variation pooled together, referred to as "all data"; 2) only seasonal variation; 3) only species variation; 4) species and seasonal variation; 5) species, seasonal and within-population variability. When a function could not be fitted, it is displayed as 'No fit' and was not selected for the model.

Thermal response	Data selection	Gaussian	Quadratic	Mod. Gaussian	Weibull
Pnet	All data	-699.95	-688.37	-707.33	-711.22
	Season	-901.03	-896.43	No fit	-898.60
	Species	-821.19	-812.31	-825.40	-817.94
	Season * Species	-1119.30	-1110.98	-1152.69	-1102.72
	Season * Species * Colony	-1238.12	1229.08	No fit	1237.98
F_v/F_m	All data	4543.37	4548.74	4523.89	4574.89
	Season	4377.10	4385.22	4380.01	4397.31
	Species	4517.47	4521.13	4507.23	4551.80
	Season * Species	4319.42	4331.76	4323.77	4351.38
	Season * Species * Colony	4299.04	4329.78	No fit	4311.86
$rETR_m$	All data	-1387.04	-1387.94	-1388.22	-1387.59
	Season	-1682.97	-1684.23	-1692.52	-1677.57
	Species	-1424.96	-1426.08	-1425.39	-1425.66
	Season * Species	-1760.98	-1763.02	-1286.10	-1753.35
	Season * Species * Colony	-2044.85	-2042.66	No fit	-2030.26

Table B.2 Comparison of thermal performance curves with different combinations of data selection for different physiological responses. Nonlinear regression models were fitted to the data for net photosynthesis rate, F_v/F_m and $rETR_m$ data; linear regression models were fitted to respiration rate. Models were fitted as follows: 1) seasonal and species variation pooled together, referred to as "all data"; 2) only seasonal variation; 3) only species variation; 4) species and seasonal variation; 5) species, seasonal and within-population variability. K is number of estimated parameters in the model, delta AIC is the difference between the AIC value of the model and the minimum AIC value among all the models of the thermal response and the AIC weight is the weighted average of the model and represent the relative likelihood.

Thermal response	Data selection	K	Cumulative AIC	Δ AIC	AIC weight
Pnet	All data	3	-699.95	538.17	1.37×10^{-92}
	Season	6	-901.03	337.09	6.34×10^{-74}
	Species	6	-821.19	416.93	2.92×10^{-91}
	Season * Species	12	-1119.30	118.83	1.57×10^{-26}
	Season * Species * Colony	60	-1238.12	0.00	1.00
R	All data	4	561.20	-1114.40	1.42×10^{-24}
	Season	6	601.74	-1191.48	7.76×10^{-8}
	Species	6	575.64	-1139.28	3.59×10^{-19}
	Season * Species	10	622.11	-1224.22	1.00
	Without colony (gls)	9	601.20	-1184.40	2.26×10^{-9}
F_v/F_m	All data	3	-1387.039	652.816	1.75×10^{-142}
	Season	6	-1682.970	356.884	3.19×10^{-78}
	Species	6	-1424.962	614.893	3.00×10^{-134}
	Season * Species	12	-1760.978	278.877	2.77×10^{-61}
	Season * Species * Colony	60	-2039.854	0.000	1.00
$rETR_m$	All data	3	4543.372	244.337	8.77×10^{-54}
	Season	6	4377.098	78.063	1.12×10^{-17}
	Species	6	4517.468	218.433	3.70×10^{-48}
	Season * Species	12	4319.420	20.385	3.74×10^{-5}
	Season * Species * Colony	60	4299.035	0.000	1.00

Table B.3 Best fit and 95% confidence interval around the coefficient estimates for data pooled together (i.e. ignoring species and season variation) for each physiological thermal response variable of *Acropora valenciennesi* and *Porites cylindrica* computed through least square non-linear regression for net photosynthesis rate, F_v/F_m and rETRm and linear regression for respiration rate.

Thermal response	Parameter estimate	All data Coefficient estimate	All data 95% c.i.
Net photosynthesis rate	Pf_{max} (O_2 h^{-1} cm^{-2})	0.35	0.33 – 0.37
	T_{opt} (ºC)	22.9	21.9 – 23.6
	T_{br} (ºC)	13.6	12.0 – 15.8
Respiration rate	Intercept	-0.27	-0.35 – -0.19
	Temp	0.02	0.02 – 0.02
F_v/F_m	Pf_{max} (no unit)	0.66	0.65 – 0.66
	T_{opt} (ºC)	28.6	28.0 – 29.3
	T_{br} (ºC)	28.2	25.6 – 32.0
$rETR_m$	Pf_{max} (no unit)	71.24	68.81 – 73.70
	T_{opt} (ºC)	27.4	26.9 – 27.9
	T_{br} (ºC)	15.2	13.6 – 17.2

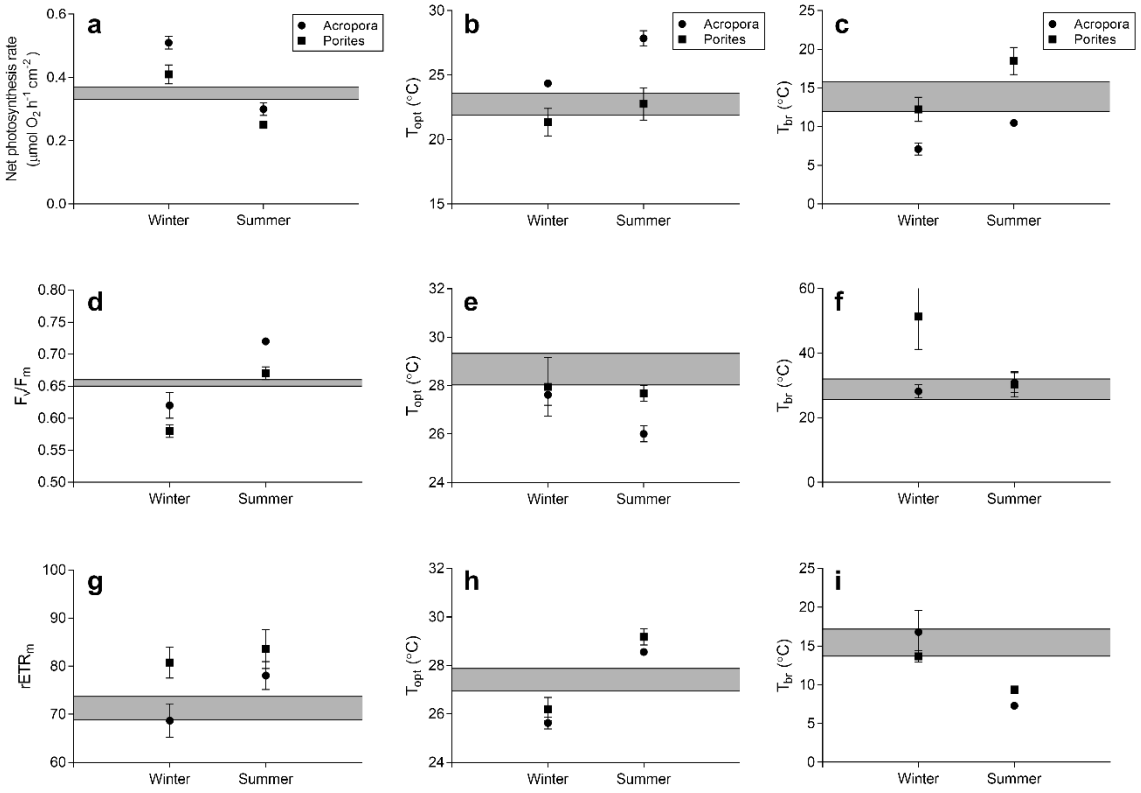

Figure B.1 Variation in thermal performance according to summer and winter temperature in *Acropora valenciennesi* (circles) and *Porites cylindrica* (squares). Data points are the parameter estimates of the performance curves with in the first column maximum performance (Pfmax), in the middle column thermal optimum (Topt) and in the last column thermal breadth (Tbr) for the net photosynthesis rate (a-c), maximum PSII quantum yield (d-f) and maximum electron transport rate (g-i). The shaded regions show the 95% confidence interval for each estimated parameter when data were pooled across species and season (Table B.3).

Appendix C: Supplementary Tables and Figures Chapter 4

Table C.1 AIC values when symmetrical (Gaussian, Quadratic) and asymmetrical (Mod. Gaussian, Weibull) functions were fitted to different combinations of data selection for different physiological responses. Functions were fitted as follows: 1) location, species and colony variability pooled together, referred to as "all data"; 2) only variability by location; 3) only variability by species; 4) variability by species and location; 5) species, location and within-population variability. When a function could not be fitted, it is displayed as 'No fit' and was not selected for the model.

Thermal response	Data selection	Gaussian	Quadratic	Mod. Gaussian	Weibull
Pnet	All data	650.23	657.33	No fit	664.49
	Location	-281.92	-268.93	No fit	-254.82
	Species	451.65	461.25	No fit	470.82
	Location * Species	-849.21	-815.31	No fit	-791.30
	Loc. * Spec. * Colony	-1071.61	-1021.57	No fit	-1040.56
Resp	All data	-241.40	-239.88	-243.88	-236.25
	Location	-1304.05	-1300.75	No fit	-1298.57
	Species	-360.57	-359.32	No fit	-355.07
	Location * Species	-1764.20	-1759.79	No fit	-1743.86
	Loc. * Spec. * Colony	-1890.88	-1876.51	No fit	-1834.10
F_v/F_m	All data	-2654.58	-2660.56	-2730.44	-2622.88
	Location	-2698.01	-2705.28	No fit	-2665.45
	Species	-2803.05	-2810.83	-2905.54	-2765.15
	Location * Species	-2879.31	-2889.52	No fit	-2837.93
	Loc. * Spec. * Colony	-3102.99	-3086.73	No fit	-3082.85
$rETR_m$	All data	7328.03	7325.43	No fit	7340.98
	Location	6490.26	6500.77	No fit	6504.11
	Species	7298.87	7296.31	No fit	7312.17
	Location * Species	6397.89	6412.15	No fit	6439.81
	Loc. * Spec. * Colony	6324.43	6358.60	No fit	6324.01

Table C.2 Comparison of thermal performance curves with different combinations of data selection for different physiological responses. Nonlinear regression models were fitted to the data as follows: 1) location, species and colony variability pooled together, referred to as "all data"; 2) only variability by location; 3) only variability by species; 4) variability by species and location; 5) species, location and within-population variability. K is number of estimated parameters in the model, delta AIC is the difference between the AIC value of the model and the minimum AIC value among all the models of the thermal response and the AIC weight is the weighted average of the model and represent the relative likelihood.

Thermal response	Data selection	K	Cumulative AIC	Δ AIC
Pnet	All data	3	657.33	1678.90
	Location	9	-268.93	752.64
	Species	6	461.25	1482.82
	Location * Species	18	-477.24	544.33
	Location * Species * Colony	93	-1021.57	0.00
R	All data	3	-241.40	1649.48
	Location	9	-1304.05	586.83
	Species	6	-360.57	1530.31
	Location * Species	18	-1764.21	126.68
	Location * Species * Colony	90	-1890.88	0.00
F_v/F_m	All data	3	-2654.58	432.15
	Location	9	-2698.01	388.72
	Species	6	-2803.05	283.68
	Location * Species	18	-2879.31	207.42
	Location * Species * Colony	93	-3086.73	0.00
$rETR_m$	All data	3	7328.03	2487.53
	Location	9	6490.26	1649.75
	Species	6	7298.87	2458.37
	Location * Species	18	6397.89	1557.38
	Location * Species * Colony	93	4840.51	0.00

Table C.3 Tukey post-hoc p-values to specifically compare the parameter estimates of *Acropora* spp. and *Porites cylindrica* populations at Heron Island with Orpheus Island, Heron Island with Lizard Island and Orpheus Island with Lizard Island.

Thermal response	Parameter estimate	*Acropora* spp.			*P. cylindrica*		
		HI - OI	HI - LI	OI - LI	HI - OI	HI - LI	OI – LI
Pnet rate	P_{max}	0.000	0.000	0.952	0.000	0.000	0.000
	T_{opt}	0.009	0.530	0.055	0.012	0.454	0.143
	T_{br}	0.015	0.026	0.977	0.388	0.949	0.243
R rate	P_{max}	0.700	0.058	0.146	0.000	0.001	0.219
	T_{opt}	0.081	0.257	0.835	0.505	0.979	0.390
	T_{br}	0.755	0.550	0.895	0.250	0.986	0.191
F_v/F_m	P_{max}	0.748	0.750	0.286	0.607	0.760	0.226
	T_{opt}	0.972	0.697	0.500	0.134	0.796	0.038
	T_{br}	0.867	0.091	0.149	0.743	0.909	0.954
$rETR_m$	P_{max}	0.000	0.000	0.000	0.016	0.000	0.000
	T_{opt}	0.000	0.000	0.367	0.000	0.000	0.521
	T_{br}	0.000	0.000	0.011	0.003	0.140	0.197

Table C.4 Parameter estimates (P_{max}, T_{opt} and T_{br}) for the individual *Acropora* and *Porites* colonies around **Heron Island** for four physiological response variables (net photosynthesis rate, respiration rate, maximum quantum yield and electron transport rate). Non-linear regressions were fitted to the data of 4 fragments from the same colony. *Acropora* colony A.51 was excluded at the start of the experiment due to paleness and replaced by the same amount of extra fragments of colony A.52.

Thermal response	Parameter estimate	**Heron Island *Acropora* population**					**Heron Island *Porites* population**				
		A.51	A.52	A.53	A.54	A.55	P.51	P.52	P.53	P.54	P.55
Pnet rate	P_{max} (O_2 h^{-1} cm^{-2})	Excluded	0.73 ± 0.08	0.61 ± 0.26	0.75 ± 0.09	0.99 ± 0.19	1.02 ± 0.11	1.25 ± 0.14	1.27 ± 0.13	1.18 ± 0.11	1.06 ± 0.08
	T_{opt} (ºC)	Excluded	23.5 ± 3.4	19.0 ± 19.7	22.9 ± 4.8	21.2 ± 4.7	29.3 ± 1.7	26.8 ± 1.5	27.9 ± 0.6	31.3 ± 4.1	25.1 ± 3.4
	T_{br} (ºC)	Excluded	15.0 ± 7.0	26.2 ± 31.4	19.4 ± 10.0	14.8 ± 6.6	13.2 ± 5.2	14.8 ± 6.6	8.4 ± 1.6	18.4 ± 10.2	20.6 ± 13.0
R rate	P_{max} (O_2 h^{-1} cm^{-2})	Excluded	0.54 ± 0.02	0.48 ± 0.02	0.55 ± 0.03	0.51 ± 0.02	0.59 ± 0.03	1.09 ± 0.20	0.70 ±0.04	0.81 ± 0.05	0.73 ± 0.03
	T_{opt} (ºC)	Excluded	29.1 ± 0.5	28.8 ± 0.9	29.5 ± 1.1	28.4 ± 0.6	29.6 ± 0.8	33.6 ± 5.6	29.3 ± 0.6	30.7 ± 1.2	29.1 ± 1.1
	T_{br} (ºC)	Excluded	15.8 ± 2.0	18.2 ± 4.2	15.4 ± 3.6	16.2 ± 2.6	13.4 ± 2.4	16.8 ± 8.6	10.0 ± 1.6	12.6 ± 2.6	17.4 ± 4.4
F_v/F_m	P_{max} (no unit)	Excluded	0.72 ± 0.01	0.74 ± 0.01	0.73 ± 0.01	0.74 ± 0.01	0.68 ± 0.01	0.67 ± 0.01	0.69 ± 0.01	0.69 ± 0.01	0.70 ± 0.01
	T_{opt} (ºC)	Excluded	25.9 ± 0.7	25.7 ± 1.0	26.2 ± 0.7	26.5 ± 0.8	26.5 ± 1.4	27.5 ± 1.0	25.2 ± 1.7	25.6 ± 0.8	25.4 ± 1.3
	T_{br} (ºC)	Excluded	34.8 ± 5.4	37.8 ± 7.6	30.2 ± 5.6	30.8 ± 6.4	37.8 ± 13.6	35.9 ± 6.6	36.0 ± 11.0	30.8 ± 5.2	32.8 ± 8.4
$rETR_m$	P_{max} (no unit)	Excluded	120.0 ± 6.3	119.1 ± 10.6	124.4 ± 6.1	129.7 ± 9.7	96.7 ± 4.9	105.3 ± 4.8	92.5 ± 6.4	101.1 ± 6.1	105.9 ± 14.8
	T_{opt} (ºC)	Excluded	24.5 ± 2.0	21.6 ± 5.9	23.3 ± 2.8	25.5 ± 2.2	25.6 ± 0.7	25.8 ± 1.2	24.1 ± 4.2	25.3 ± 1.1	19.3 ± 8.1
	T_{br} (ºC)	Excluded	17.8 ± 5.8	25.0 ± 13.0	20.8 ± 6.6	18.0 ± 8.4	12.6 ± 2.2	17.8 ± 4.8	21.8 ± 13.2	13.6 ± 3.2	28.6 ± 14.6

Table C.5 Parameter estimates (P_{max}, T_{opt} and T_{br}) for the individual *Acropora* and *Porites* colonies around **Orpheus Island** for four physiological response variables (net photosynthesis rate, respiration rate, maximum quantum yield and electron transport rate). Non-linear regressions were fitted to the data of 4 fragments from the same colony (for colonies A.11 & A.18 of the *Acropora* population, and P.12 &P.16 of the *Porites* population, regressions were fitted to only 2 fragments of the same colony).

Thermal response		**Orpheus Island *Acropora* population**						**Orpheus Island *Porites* population**					
		A.5	A.6	A.8	A.11	A.15	A.18	P.12	P.16	P.20	P.31	P.32	P.33
Pnet rate	P_{max}	0.27 ± 0.02	0.20 ± 0.01	0.34 ± 0.03	0.36 ± 0.04	0.32 ± 0.03	0.32 ± 0.03	0.26 ± 0.02	0.21 ± 0.01	0.23 ± 0.03	0.26 ± 0.01	0.24 ± 0.03	0.28 ± 0.10
	T_{opt}	25.8 ± 1.1	26.8 ± 0.6	29.0 ± 0.6	29.5 ± 1.0	27.2 ± 0.8	28.8 ± 0.9	24.5 ± 1.2	27.3 ± 0.8	22.3 ± 6.8	24.5 ± 1.0	21.9 ± 3.9	16.5 ± 8.7
	T_{br}	11.2 ± 2.6	11.0 ± 1.8	9.0 ± 1.8	10.4 ± 3.0	10.4 ± 2.2	11.0 ± 3.0	10.8 ± 2.0	21.2 ± 4.4	24.4 ± 13.8	14.2 ± 2.0	18.4 ± 6.0	22.4 ± 8.8
R rate	P_{max}	0.28 ± 0.09	0.84 ± 0.21	0.38 ± 0.07	0.37 ± 0.07	0.44 ± 1.12	0.36 ± 0.25	0.15 ± 0.01	0.24 ± 0.08	0.17 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.21 ± 0.02
	T_{opt}	38.1 ± 8.3	36.9 ± 7.0	35.6 ± 3.2	34.7 ± 3.8	51.5 ± 78.7	40.6 ± 14.5	27.2 ± 1.5	38.9 ± 10.0	33.4 ± 3.1	28.5 ± 0.7	31.5 ± 5.0	32.5 ± 5.2
	T_{br}	20.6 ± 9.6	11.4 ± 8.8	13.0 ± 3.8	12.4 ± 4.8	36.8 ± 64.8	20.6 ± 13.4	17.0 ± 6.6	23.2 ± 11.8	18.2 ± 6.2	15.0 ± 3.4	25.6 ± 19.6	22.6 ± 14.8
F_v/F_m	P_{max}	0.72 ± 0.01	0.71 ± 0.01	0.73 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.74 ± 0.01	0.66 ± 0.01	0.63 ± 0.02	0.66 ± 0.01	0.70 ± 0.01	0.71 ± 0.01	0.64 ± 0.02
	T_{opt}	26.2 ± 0.7	25.4 ± 0.7	27.2 ± 0.6	23.7 ± 4.0	26.0 ± 1.3	27.2 ± 0.7	27.8 ± 0.6	29.4 ± 2.0	28.0 ± 0.3	26.8 ± 1.3	27.3 ± 1.1	27.4 ± 0.7
	T_{br}	20.0 ± 2.6	25.2 ± 2.6	30.0 ± 4.6	43.2 ± 17.6	57.6 ± 8.4	29.2 ± 5.4	25.8 ± 4.6	33.2 ± 18.0	17.0 ± 1.6	40.6 ± 11.6	45.6 ± 12.6	19.2 ± 3.6
$rETR_m$	P_{max}	84.0 ± 4.4	88.2 ± 3.6	76.8 ± 4.2	68.0 ± 5.2	76.6 ± 4.4	74.7 ± 6.2	106 ± 9.0	73.1 ± 4.7	82.2 ± 8.4	79.1 ± 5.2	84.9 ± 6.2	76.4 ± 3.8
	T_{opt}	28.4 ± 0.3	29.0 ± 0.2	28.6 ± 0.3	28.7 ± 0.3	28.7 ± 0.3	28.0 ± 0.4	27.5 ± 0.5	30.4 ± 0.6	29.4 ± 0.9	29.1 ± 0.5	29.0 ± 0.6	29.6 ± 0.3
	T_{br}	8.0 ± 0.8	7.2 ± 0.4	7.2 ± 0.6	6.6 ± 0.8	7.2 ± 0.6	7.6 ± 1.0	8.8 ± 1.4	9.8 ± 1.4	11.0 ± 2.8	9.6 ± 1.4	11.0 ± 2.0	7.4 ± 0.6

Table C.6 Parameter estimates (P_{max}, T_{opt} and T_{br}) for the individual *Acropora* and *Porites* colonies around **Lizard Island** for four physiological response variables (net photosynthesis rate, respiration rate, maximum quantum yield and electron transport rate). Non-linear regressions were fitted to the data of 4 fragments from the same colony. There are no parameter estimates for the respiration rate of *Acropora* colony A.43, because the Gaussian distribution did not fit the data.

Thermal response	Parameter estimate	**Lizard Island *Acropora* population**					**Lizard Island *Porites* population**				
		A.41	A.42	A.43	A.44	A.45	P.41	P.42	P.43	P.44	P.45
Pnet rate	P_{max} (O_2 h^{-1} cm^{-2})	0.26 ± 0.02	0.34 ± 0.16	0.19 ± 0.03	0.26 ± 0.03	0.37 ± 0.04	0.31 ± 0.02	0.56 ± 0.03	0.69 ± 0.06	0.80 ± 0.06	0.86 ± 0.05
	T_{opt} (ºC)	23.4 ± 1.4	17.9 ± 8.7	29.0 ± 0.6	23.7 ± 2.5	24.1 ± 1.4	23.8 ± 3.3	28.1 ± 0.5	27.2 ± 1.0	24.6 ± 2.1	26.1 ± 0.7
	T_{br} (ºC)	10.6 ± 2.4	16.8 ± 8.6	5.2 ± 1.8	11.8 ± 4.6	10.4 ± 2.6	18.0 ± 7.6	13.4 ± 2.0	11.8 ± 3.2	16.2 ± 5.4	11.8 ± 2.0
R rate	P_{max} (O_2 h^{-1} cm^{-2})	0.20 ± 0.01	0.24 ± 0.01	n.a.	0.22 ± 0.09	0.37 ± 0.79	0.26 ± 0.02	0.37 ± 0.02	0.39 ± 0.03	0.44 ± 0.03	0.46 ± 0.03
	T_{opt} (ºC)	30.2 ± 0.7	30.4 ± 2.2	n.a.	35.4 ± 11.6	52.1 ± 77.3	28.7 ± 0.9	28.6 ± 0.5	29.2 ± 0.9	28.6 ± 0.5	27.8 ± 0.5
	T_{br} (ºC)	13.2 ± 1.8	18.4 ± 6.6	n.a.	19.8 ± 15.6	40.8 ± 66.2	15.0 ± 3.8	11.8 ± 1.6	12.6 ± 2.8	11.6 ± 1.6	11.4 ± 1.6
F_v/F_m	P_{max} (no unit)	0.78 ± 0.02	0.72 ± 0.01	0.72 ± 0.01	0.72 ± 0.00	0.77 ± 0.02	0.70 ± 0.01	0.68 ± 0.01	0.70 ± 0.01	0.70 ± 0.01	0.69 ± 0.01
	T_{opt} (ºC)	26.8 ± 0.5	25.9 ± 0.6	27.6 ± 1.9	26.0 ± 0.8	26.7 ± 0.5	26.2 ± 0.7	26.3 ± 1.0	26.5 ± 0.6	21.7 ± 5.0	26.6 ± 0.5
	T_{br} (ºC)	14.2 ± 1.8	20.6 ± 2.8	22.4 ± 12.4	35.8 ± 7.6	14.0 ± 1.8	26.8 ± 3.8	31.4 ± 6.0	20.2 ± 2.4	54.2 ± 20.0	27.6 ± 3.0
$rETR_m$	P_{max} (no unit)	46.4 ± 2.5	51.9 ± 3.7	48.3 ± 3.0	58.7 ± 5.8	55.2 ± 3.6	35.3 ± 2.3	56.6 ± 3.9	51.4 ± 3.3	51.9 ± 1.9	53.7 ± 3.8
	T_{opt} (ºC)	28.9 ± 0.8	28.8 ± 0.9	30.4 ± 0.8	29.6 ± 1.6	29.1 ± 0.9	29.3 ± 1.4	30.0 ± 1.0	30.6 ± 0.7	30.9 ± 1.0	30.3 ± 0.8
	T_{br} (ºC)	14.0 ± 3.4	12.8 ± 3.4	8.2 ± 2.6	12.0 ± 4.6	12.0 ± 3.0	18.6 ± 7.6	13.2 ± 3.2	10.4 ± 1.8	15.6 ± 2.8	10.8 ± 2.0

Table C.7 Results of the mixed effect models to detect variation in the chlorophyll concentration in *Acropora* spp. and *P. cylindrica* between locations and treatments (main effects) taking into account colony variation (as random effect).

Species	Parameter	df	F-value	*p* -value
Acropora spp.	Location	2, 12	112.22	< .001
	Treatment	1, 38	61.59	< .001
	Location * Treatment	2, 38	14.60	< .001
P. cylindrica	Location	2, 14	3.08	0.078
	Treatment	1, 40	24.95	< .001
	Location * Treatment	2, 40	11.86	0.001

Table C.8 Results of a two-way ANOVA to detect differences in the chlorophyll concentration after exposure to ambient temperature between species (*Acropora* spp. and *Porites cylindrica*) and locations.

Parameter	Degrees of Freedom	Sum of squares	Mean square	F-value	*p* -value
Location	2	0.574	0.287	31.21	< .001
Species	1	0.107	0.107	11.65	0.002
Location * Species	2	0.019	0.010	1.04	0.368
Residuals	27	0.248	0.009		

Table C.9 Annual average (and monthly minimum) seawater temperatures ranging from April to March of the corresponding years at reefs around Heron Island, Orpheus Island and Lizard Island (AIMS 2017), and the thermal optimum (and standard deviation) for net photosynthesis rate in *Acropora* spp. and *Porites cylindrica*.

	T (°C) 2014/2015	T (°C) 2015/2016	T (°C) 2016/2017	T_{opt} (°C) *Acropora*	T_{opt} (°C) *Porites*
Heron Isl.	24.2 (20.8)	24.5 (21.3)	24.6 (21.1)	21.7 ± 2.0	28.1 ± 2.4
Orpheus Isl.	26.2 (22.3)	26.4 (22.6)	Post exp.	27.8 ± 1.5	22.8 ± 3.3
Lizard Isl.	26.3 (23.6)	26.6 (23.8)	Post exp.	23.6 ± 3.9	26.0 ± 1.8

Appendix D: Supplementary Tables and Figures Chapter 5

Table D.1 AIC values when symmetrical (Gaussian, Quadratic) and asymmetrical (Mod. Gaussian, Weibull) functions were fitted to different combinations of data selection for net photosynthesis rate and $rETR_m$. When a function could not be fitted, it is displayed as 'No fit' and was not selected for the model.

Thermal response	Data selection	Gaussian	Quadratic	Mod. Gaussian	Weibull
Pnet	All data	451.02	448.37	429.69	451.08
	Region	321.89	324.49	No fit	330.53
	Species	-238.55	-229.37	No fit	-196.98
	Colony	-266.96	-257.91	No fit	-263.90
$rETR_m$	All data	4882.09	4949.53	4835.52	4873.60
	Region	4195.01	4193.99	No fit	4194.41
	Species	3950.33	3949.88	No fit	3948.92
	Colony	3901.37	3901.07	No fit	3899.07

Table D.2 Model selection of thermal performance curves with different combinations of data selection for the physiological responses. Nonlinear regression models were fitted to the data for net photosynthesis rate and $rETR_m$; mixed linear regression models were fitted to respiration rate and F_v/F_m. K is number of estimated parameters in the model, ΔAIC is the difference between the AIC value of the model and the minimum AIC value among all the models of the thermal response.

Thermal response	Data selection	K/df	Cumulative AIC	Δ AIC
Net photosynthesis rate	All data	3	448.37	705.15
	Region	6	324.49	581.27
	Species	12	-229.37	27.41
	Colony	60	-256.782	0.00
$rETR_m$	All data	3	4882.09	980.72
	Region	6	4195.01	293.64
	Species	12	3950.33	48.96
	Colony	60	3901.373	0.00
Respiration rate	All data	4	-807.27	58.68
	Region	6	-814.01	51.94
	Species	10	-865.95	0.00
F_v/F_m	All data	4	-1991.55	91.71
	Region	6	-2002.99	80.27
	Species	10	-2083.26	0.00

Table D.3 Tukey posthoc *p*-values comparing the parameter estimates (Pf_{max}, T_{opt} and T_{br}) for the thermal performance curves of Pnet (shaded) and $rETR_m$ (clear) between the four coral species.

		C. caespitosa	*E. singularis*	*A. intermedia*	*P. cylindrica*
C. caespitosa	Pf_{max}	-	0.000	0.997	0.000
	T_{opt}	-	0.996	0.001	0.000
	T_{br}	-	0.039	0.999	0.603
E. singularis	Pf_{max}	0.002	-	0.000	0.000
	T_{opt}	0.999	-	0.001	0.000
	T_{br}	0.997	-	0.053	0.333
A. intermedia	Pf_{max}	0.000	0.000	-	0.000
	T_{opt}	0.001	0.001	-	0.001
	T_{br}	0.492	0.600	-	0.639
P. cylindrica	Pf_{max}	0.000	0.000	0.000	-
	T_{opt}	0.000	0.000	0.999	-
	T_{br}	0.258	0.343	0.982	-

Table D.4 Parameter estimates (P_{max}, T_{opt} and T_{br}) for the individual colonies of the temperate corals *Cladocora caespitosa* and *Eunicella singularis* for two physiological response variables (net photosynthesis rate and electron transport rate).

Thermal response	Parameter estimate	*C. caespitosa*					*E. singularis*				
		C.1	C.2	C.3	C.4	C.5	E.1	E.2	E.3	E.4	E.5
Pnet rate	Pf_{max}	0.86 ± 0.11	0.64 ± 0.04	0.79 ± 0.04	0.83 ± 0.03	0.80 ± 0.09	0.30 ± 0.01	0.33 ± 0.02	0.30 ± 0.02	0.40 ± 0.02	0.33 ± 0.02
	T_{opt} (ºC)	11.5 ± 5.7	15.6 ± 1.6	14.7 ± 3.0	17.5 ± 0.8	15.7 ± 2.3	15.2 ± 0.5	15.9 ± 0.4	15.5 ± 0.9	15.6 ± 0.4	14.2 ± 0.9
	T_{br} (ºC)	20.6 ± 10.6	16.2 ± 5.8	23.6 ± 11.4	18.4 ± 4.6	12.8 ± 5.0	10.8 ± 1.4	9.8 ± 1.0	12.0 ± 2.6	9.0 ± 1.0	11.2 ± 2.0
$rETR_m$	Pf_{max}	44.85 ± 3.92	48.33 ± 2.56	41.22 ± 1.52	49.96 ± 7.81	39.95 ± 2.08	34.03 ± 1.19	33.21 ± 1.21	34.34 ± 5.71	30.68 ± 0.94	28.18 ± 1.41
	T_{opt} (ºC)	11.3 ± 6.9	16.0 ± 1.0	18.9 ± 2.8	9.7 ± 10.7	13.9 ± 4.2	15.6 ± 1.2	16.3 ± 1.5	9.0 ± 14.5	15.9 ± 0.9	12.6 ± 4.6
	T_{br} (ºC)	28.8 ± 16.4	15.8 ± 4.0	31.6 ± 20.6	30.4 ± 21.2	25.0 ± 14.2	19.6 ± 5.0	24.6 ± 9.6	36.2 ± 31.6	19.6 ± 4.4	27.8 ± 13.0

Thermal response	Regression coefficient	*C. caespitosa*					*E. singularis*				
		C.1	C.2	C.3	C.4	C.5	E.1	E.2	E.3	E.4	E.5
R rate	Intercept	-0.15 ± 0.06	-0.15 ± 0.07	-0.20 ± 0.10	-0.30 ± 0.07	-0.28 ± 0.09	-0.19 ± 0.04	-0.25 ± 0.04	-0.16 ± 0.06	-0.02 ± 0.05	-0.18 ± 0.05
	Slope	0.032 ± 0.003	0.030 ± 0.004	0.036 ± 0.005	0.040 ± 0.004	0.040 ± 0.005	0.025 ± 0.002	0.030 ± 0.002	0.024 ± 0.004	0.020 ± 0.003	0.027 ± 0.003
FvFm	Intercept	0.34 ± 0.05	0.34 ± 0.03	0.25 ± 0.04	0.27 ± 0.04	0.23 ± 0.03	0.60 ± 0.03	0.66 ± 0.03	0.60 ± 0.03	0.63 ± 0.02	0.63 ± 0.03
	Slope	0.000 ± 0.003	0.001 ± 0.002	0.003 ± 0.002	0.003 ± 0.002	0.005 ± 0.002	0.000 ± 0.002	-0.002 ± 0.001	0.001 ± 0.002	0.001 ± 0.001	0.001 ± 0.001

Table D.5 Results ANOVA and Tukey post-hoc tests to detect variability in the chlorophyll concentration between the treatments (chilled, heated or ambient) and species (*C. caespitosa, E. singularis, A. intermedia* and *P. cylindrica*).

Variable	df	F-value	*p-value*
Treatment	2, 97	2.6501	0.076
Species	3, 97	58.092	**0.000**

Tukey post-hoc	*C. caespitosa*	*E. singularis*	*A. intermedia*	*P. cylindrica*
C. caespitosa	-			
E. singularis	**0.000**	-		
A. intermedia	0.981	**0.000**	-	
P. cylindrica	**0.000**	**0.000**	**0.000**	-

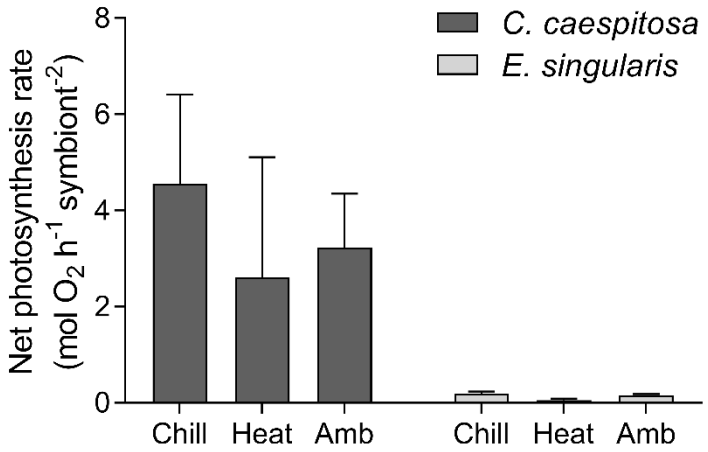

Figure D.1 Mean (with s.d.) net photosynthesis rates per symbiont for the temperate corals *Cladocora caespitosa* and *Eunicella singularis* at the end of the experiment after exposure to the decreased and increased temperature gradient (respectively, chill and heat, *N* = 10), and at the start of the experiment when measured at ambient (amb) temperature (18 °C; *N* = 5).

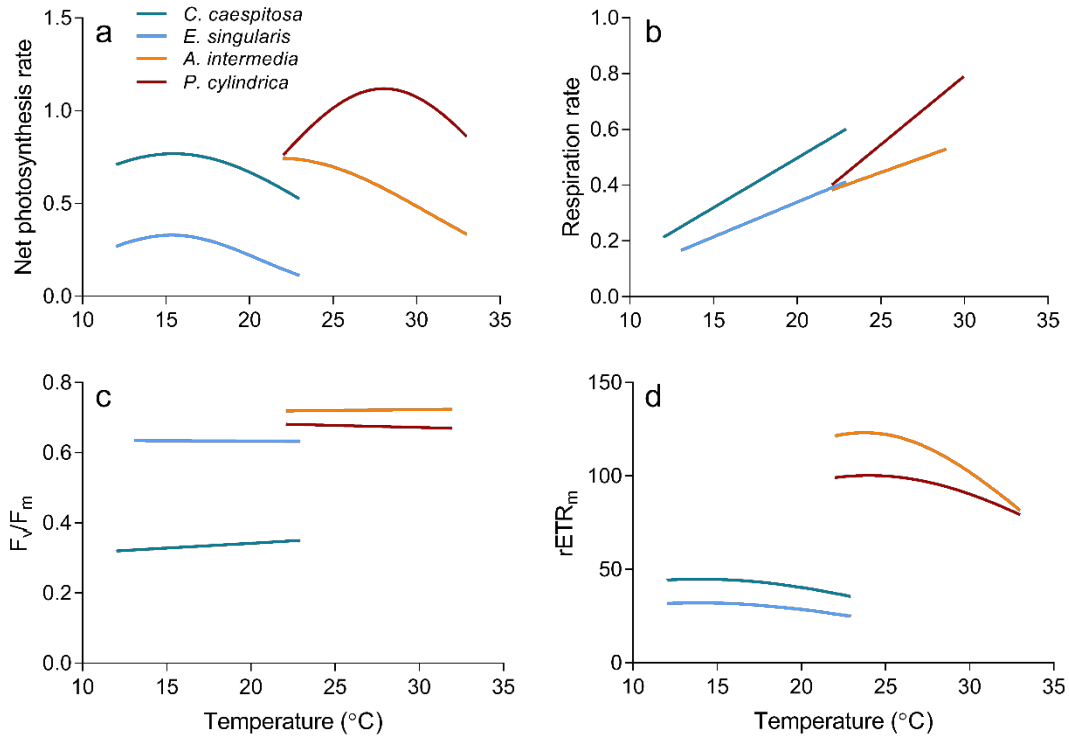

Figure D.2 Stylised presentation of the thermal responses of *Cladocora caespitosa*, *Eunicella singularis*, *Acropora intermedia* and *Porites cylindrica* to visualize the change in the position and shape of the regressions among species. Thermal responses displayed are net photosynthesis rate (**a**), respiration rate (**b**), maximum quantum yield (**c**) and maximum electron transport rate (**d**).