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Thermal adaptation and acclimation in an intertidal goby (*Bathygobius cocosensis*)

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Thesis abstract

This thesis explores how short- and long-term thermal variability affects ecologically important performance traits and links how developmental traits alter juvenile acute thermal performance. Temperature is the most important abiotic factor that affects the functioning of ectothermic organism physiology and performance. Organisms experience variation in temperature at multiple time scales, from daily and seasonal fluctuations to among generation thermal changes. It is important to understand how thermal variability and developmental traits alter performance traits that are directly linked to survival and fitness. I aimed to assess if an organism can acclimate and maintain wide thermal performance curves in an environment that experiences equal daily and seasonal thermal variability; to assess if rate of luminance change for camouflage can acclimate with temperature; and to assess how larval traits such as growth rate and size affect juvenile acute thermal performance.

Daily and seasonal thermal variability plays an important role in the evolution of both the shape of thermal performance curves (TPC) and acclimation capacity. Acclimation occurs when an ectotherm changes their underlying physiology to maintain performance under changed environmental conditions. In theory, ectotherms in environments that experience small daily thermal fluctuations and large seasonal variation are predicted to have narrow TPCs and the capacity to acclimate. When daily thermal fluctuations are as great as seasonal thermal variability, however, ectotherms are expected to have wide TPCs and a limited capacity to acclimate. Few studies have assessed how the combination of daily and seasonal thermal variation affects the shape of TPCs and acclimation capacity in subtropical environments where there is relatively equal daily and seasonal thermal variability. I aimed to assess if an intertidal goby (*Bathygobius cocosensis*) that experiences equal magnitudes of daily and seasonal thermal variation has the capacity to acclimate. I found that although *B. cocosensis* experience large amounts of daily variability and have wide TPCs, *B. cocosensis* possess the ability to acclimate to seasonal conditions.

The responses of animals to temperature change have typically been explored in the context of energetics and locomotor performance, and these types of traits are likely to be important for individual fitness. The effect of temperature on physiological processes, however, can have broad reaching implications for other aspects of organismal behaviour and predator avoidance other than locomotion and energy expenditure. For example, intertidal environments are heterogeneous not only in terms of temperature but also substrate and background type/colour. Gobies have the ability to change luminance (perceived brightness) to match their backgrounds as a predator and prey avoidance mechanism. The rate at which animals can change luminance is acutely affected by temperature, and the rate of background matching may therefore be affected by climate change. No previous studies, however, have examined if rate of luminance change has the potential to acclimate to different longer-term thermal conditions. In this thesis, I demonstrate that rate of luminance change can acclimate with thermal change.

Many organisms, including marine fish, have complex life-cycles with distinct larval and post-metamorphic phases. Larval traits, such as size and growth rate, have the potential to affect juvenile thermal performance. *Bathygobius cocosensis* have a planktonic larval stage meaning that larvae are swept out to sea for 15-30 days. Both genetic and environmental sources of variation mean that individual larvae can grow at different rates and reach different settlement sizes for juvenile metamorphosis. No previous studies have assessed how wild larval growth rates and settlement sizes are correlated with post-metamorphic performance across a range of temperatures. While I found no effect of larval trait variation on the thermal sensitivity of post-metamorphic traits, I did find that larval growth rate and settlement size were negatively correlated with routine metabolic rate and burst swimming speed overall across test temperature. Therefore, slow growing larvae had faster post-metamorphic metabolic rates and burst swimming speeds than fast growing larvae, independently of the effect of temperature on those traits. I also found that juvenile body mass was positively correlated with their critical thermal maximum, but no larval traits were correlated with critical thermal tolerance.

I have explored how organisms in thermally variable environments respond to short- and long-term thermal change and have linked how developmental traits effect juvenile thermal performance. Interestingly, across studies, I found that thermal performance curve shape was not altered by thermal acclimation, or variation in larval growth rates. These results suggest that although performance is variable among individuals, and individuals can shift their thermal optimums with longer-term thermal change, it appears that the way that performance varies with short-term thermal variation may be constrained in *B. cocosensis*. These findings are important for improving the understanding of the co-evolution of thermal performance curve shape and acclimation capacity, and how ectotherms will respond to future changes in climate.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

Chapter 2: da Silva CRB, Riginos C, Wilson RS (2019) An intertidal fish shows thermal acclimation despite living in a rapidly fluctuating environment. Journal of Comparative Physiology B. DOI: 10.1007/s00360-019-01212-0

Chapter 4: da Silva CRB, Wilson RS, Riginos C (2019) Rapid larval growth is costly for post-metamorphic thermal performance in a Great Barrier Reef fish. Coral Reefs. DOI: 10.1007/s00338-019-01815-7

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None

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da Silva CRB, Groom SVC, Stevens MI, Schwarz MP (2016) Current status of the introduced allodapine bee *Branusapis puangensis* (Hymenoptera: Apidae) in Fiji. Austral Entomology, 55:43-48

da Silva CRB, Stevens MI, Schwarz (2016) Casteless sociality in an allodapine bee and evolutionary losses of social hierarchies. Insectes sociaux, 1: 67-78

Silva DP, Groom SVC, da Silva CRB, Stevens MI, Schwarz MP (2017) Potential pollination maintenance by an exotic allodapine bee under climate change scenarios in the Indo-Pacific region. Journal of Applied Entomology, 141: 122-132

Bousjein NS Stains M, Vo C, Puiu N, da Silva CRB, Harrington J, Wilkinson S, Pratt K, Schwarz MP (2017) Sex ratios in a socially parasitic bee and implications for host-parasite interactions. Journal of Insect Behaviour, 30: 130-137

Contribution by others to the thesis

	Contribution	Data Chapters
Robbie Wilson	Concept, design, funding, interpretation, review	2,3,4
Cynthia Riginos	Concept, design, funding, interpretation, review	2,3,4
Karen Cheney	Concept, design	3
Cedric Van den Burg	Piolet investigation, interpretation	3
Nicolas Condon	Wrote ImageJ script for photograph analysis	3
Nuria Raventos	Larval trait data extraction from otoliths	4

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Research involving human or animal subjects

For the purpose of this thesis individuals of the fish *Bathygobius cocosensis* were used. Collection of *B. cocosensis* were conducted at Point Lookout North Stradbroke Island, Point Cartwright North Stradbroke Island and in the scientific research zone of the intertidal area of Heron Island. Animal ethics was approved by The University of Queensland (SBS/425/15). Collection of *B. cocosensis* in Moreton Bay Marine Park (Point lookout North Stradbroke Island) was approved by the Queensland government Marine Parks Permits QS2015/MAN340. Approval for collecting *B. cocosensis* at Heron Island was given by The University of Queensland Limited Impact Research Accreditation UQ006/2015. Animal ethics permit is displayed in Appendix 1.

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Table of Contents

List of Figures & Tables

Table of figures and tables throughout the thesis. Figures and Tables are labelled by chapter number (e.g. 1.1 Chapter 1, Figure 1).

List of Abbreviations Used in the Thesis

1. TPC – Thermal performance curve
2. ROI – Region of interest
3. SS – Settlement size
4. GR – Growth rate
5. HS – Hatch size
6. PLD – Pelagic larval duration

Chapter 1
General introduction

1.1 Overview

Two central goals in biology are to understand how organismal performance and fitness are influenced by environmental variation and to be able to predict how organisms will respond to contemporary environmental change. In particular, it is important to understand how traits that are directly linked with survival, fitness, and interspecies behavioural interactions will be affected by climate change. In this thesis, I explore how short- and longer-term thermal change and developmental traits such as growth rate affect performance traits such as locomotion, metabolism and rate of camouflage. For example, the way different combinations of daily and seasonal thermal variation affect the capacity ectotherms have to acclimate (shift the thermal optima of performance traits with changes in temperature), and how thermal performance curve shape is altered by thermal variation remains a heavily debated topic in ecological physiology (Janzen 1967; Gilchrist 1995; Gabriel 1999; Wilson and Franklin 2000; Wilson and Franklin 2002; Gabriel 2005; Gabriel et al. 2005; Seebacher et al. 2005; Ghalambor et al. 2007; Angilletta 2009; Murren et al. 2015; Beaman et al. 2016; da Silva et al. 2019a). It is also important to determine how developmental traits that are linked with environmental temperature alter later life-stage thermal performance and fitness. By tying together how developmental traits alter performance and how magnitude of daily and seasonal thermal variation affect adult performance, we can gain an understanding of the multiple complex drivers of variation in organismal performance and begin to predict how ectothermic animals will respond to climate change.

Throughout this thesis, I explore these themes using an intertidal marine goby, *Bathygobius cocosensis*, as my study system. First, I will introduce temperature and plasticity as they are the running themes throughout my PhD. Then I will introduce my three main research questions: 1) can ectotherms maintain the capacity to acclimate while also exhibiting wide thermal performance curves in an environment that experiences equal daily and seasonal thermal variation? 2) Can rate of luminance change for camouflage acclimate to thermal conditions? And 3) how do larval traits such as growth rate and settlement size affect post-metamorphic thermal performance? Lastly, I will discuss why the intertidal zone and *B. cocosensis* provide a great opportunity to test these questions.

1.2 Thermal performance

Temperature is the most important environmental variable that affects every component of ectotherm function (Angilletta 2009). Temperature affects the rate of physiological reactions from the level of the enzyme to the whole organism level (locomotion, metabolism, growth, etc.) (Somero and Hochachka 1971; Hazel 1995; Somero 1995; Wilson and Franklin 1999; Angilletta 2009; Niehaus et al. 2012). Rates of physiological reactions underlying performance are dependent on temperature, and performance often increases with temperature until a thermal optimum is reached and then rapidly declines with further warming (Somero and Hochachka 1971; Huey and Kingsolver 1989; Kingsolver and Huey 2008; Angilletta 2009). The way in which performance is altered by acute changes in temperature is known as a thermal performance curve (Huey and Stevenson 1979; Huey and Kingsolver 1989; Gilchrist 1996; Angilletta Jr et al. 2002; Angilletta Jr 2006; Schulte et al. 2011) (Fig 1). The thermal performance curve depicts an organism's thermal minimum[a], optimum[b], maximum[c] (superscript letters correspond with annotations in Fig 1), and thermal breadth, which indicates the range of temperatures an organism can perform well in (Tattersall et al. 2012) (Fig 1.1). Thermal specialists are organisms that perform well over a narrow range of temperatures, and thermal generalists are organisms that can perform across a wide range of temperatures, but usually at a lower or reduced rate than thermal specialists (Angilletta 2009c; Tattersall et al. 2012) (Fig 1.1). Thermal specialists are thought to evolve in stable thermal conditions (e.g. tropical or polar locations) and thermal generalists are thought to evolve in variable thermal conditions (e.g. temperate regions) (Janzen 1967; Deutsch et al. 2008; Tewksbury et al. 2008).

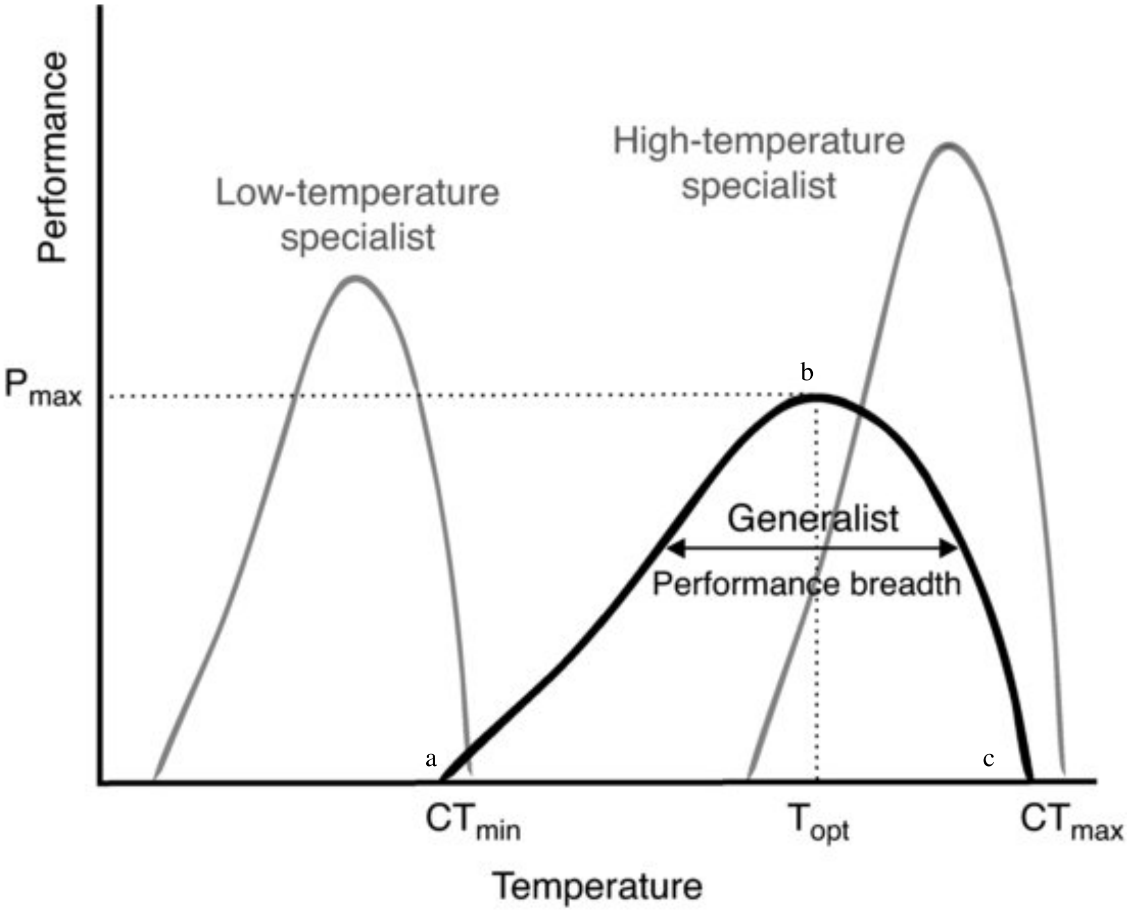


Figure 1.1. Thermal performance curves of low- and high-temperature specialists and thermal generalists (Adapted from Tattersall et al. (2012)).

1.3 Thermal acclimation

When environmental temperature changes, animals can respond by behaviourally thermoregulating (including migration, seeking shade or panting) (Walther et al. 2002; Sunday et al. 2010; Campbell et al. 2018), they can adapt over many generations (Fields et al. 1993; Hendry and Kinnison 1999; Kinnison and Hendry 2001; Parmesan and Yohe 2003; Root et al. 2003; Charmantier et al. 2004; Gienapp et al. 2008), or they can acclimate, by shifting their thermal performance curves to better match environmental temperatures (Wilson and Franklin 2002; Riddell et al. 2018; Rohr et al. 2018). Thermal acclimation, a form of reversible plasticity, occurs when an organism shifts their underlying physiology in response to a change in environmental temperature to maintain physiological function (Wilson and Franklin 2002; Beaman et al. 2016) (Fig 1.2). An example of thermal acclimation is shown by Hammill et al. (2004), where adult mosquito fish were exposed to cold or warm conditions for a period of many weeks. Post-thermal exposure, the warm-exposed fish showed longer sustained swimming at warm test temperatures compared to the cold-exposed fish, and the cold-exposed fish had longer sustained swimming at cold test temperatures compared to the warm-exposed fish (Hammill et al. 2004). This example shows how changes in long-term temperature alter underlying physiology and allow organisms to maintain optimal performance with changed conditions.

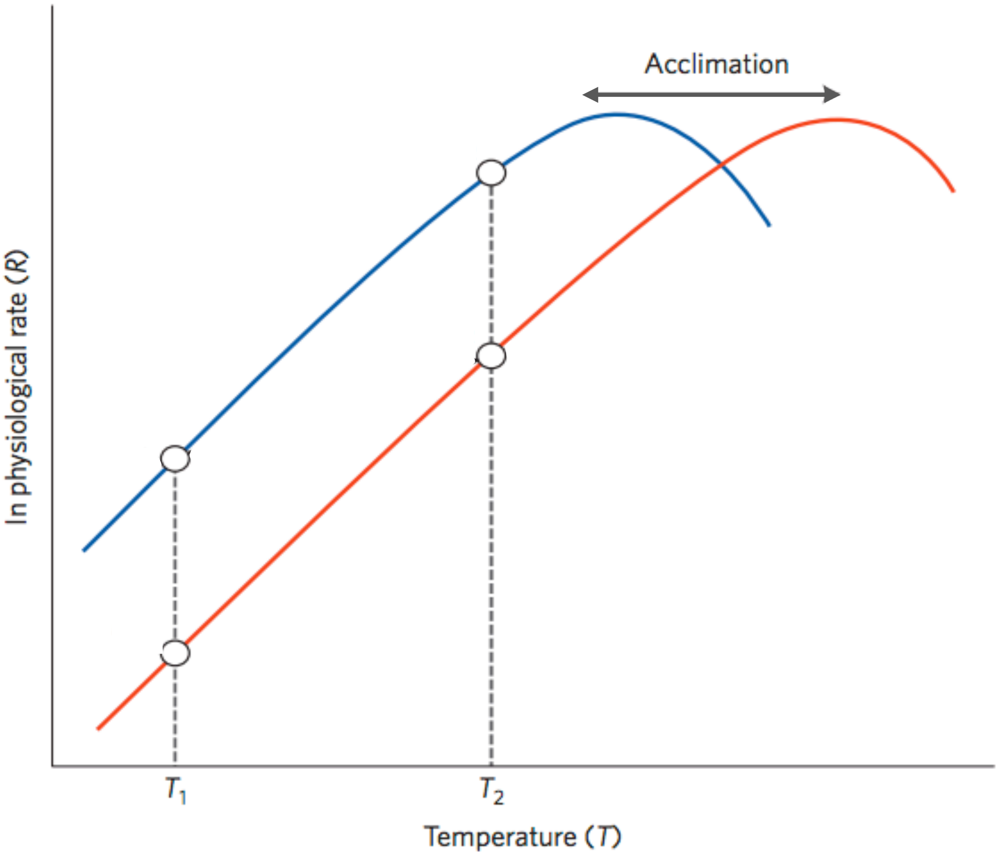


Figure 1.2. Example of thermal acclimation of a physiological trait to cold (blue) and warm (red) thermal conditions (adapted from Seebacher *et al*. 2014).

Thermal acclimation to seasonal conditions takes place within an individual's life time and is reversible with seasonal change (Angilletta 2009). The capacity to acclimate is hypothesised to evolve when environmental changes are predictable (e.g. seasonal change), enabling organisms to better match shifts in mean temperature (DeWitt et al. 1998). The capacity to acclimate is also hypothesised to evolve when short-term (daily) thermal fluctuations are smaller than long-term (seasonal) thermal variability (Gabriel 2005). This is because when daily thermal fluctuations are equal to or greater than seasonal thermal variation, large daily fluctuations are expected to mask seasonal thermal trends, making seasonal thermal change unpredictable (DeWitt et al. 1998). When thermal change is unpredictable, maintaining the ability to acclimate is predicted to become energetically costly (DeWitt et al. 1998). Species may not retain their capacity to acclimate in conditions that vary too rapidly for an effective acclimation response or in stable environmental conditions (DeWitt et al. 1998). Plasticity costs include those for: maintenance (energetic costs for sensory and regulatory mechanisms); production (having the correct machinery for acclimation to occur); information acquisition costs (energy invested in determining information about the environment and how it might shift); and genetic costs (pleiotropic and epistatic costs) (DeWitt et al. 1998). In addition, if an organism's phenotype is unable to change at the same rate as the environment, there is likely to be a lag between an organism's phenotype and the changed environmental conditions, which can be costly for fitness, especially if environments change rapidly and frequently (Murren et al. 2015). When these costs outweigh potential benefits of acclimation (maintaining thermal performance with changed thermal conditions), the capacity to acclimate may be lost (DeWitt et al. 1998; Gabriel 2005). For example, while some stenothermal Antarctic fish can adjust cardiac function to cope with warmer temperatures (Franklin et al. 2007), other species have lost the capacity to acclimate to increased temperatures (Somero 2005).

The magnitude of seasonal thermal variation a population experiences over evolutionary time is thought to influence the capacity individuals have to acclimate (how far they can shift their thermal optima with thermal change) (DeWitt et al. 1998; Wilson and Franklin 2000; Gabriel 2005). Generally, increases in the magnitude of thermal variability are thought to be positively correlated with the evolution of acclimation capacity (Janzen 1967; Johnson and Bennett 1995; Rohr et al. 2018). For example, organisms that inhabit higher latitudes and experience greater thermal variability have greater acclimation capacities than animals that live at low tropical latitudes (Rohr et al. 2018). In addition, experimental studies have shown that populations that have experienced greater thermal variability in past generations are likely to be more plastic than populations that experienced less thermal variability in past generations (Hallsson and Björklund 2012). The magnitude of daily thermal variability that organisms experience within their life-time may also influence acclimation capacity (Wilson and Franklin 2000; Scott and Johnston 2012; Beaman et al. 2016). For example, the conditions organisms experience during early development are hypothesised to affect the thermal acclimation capacity of juveniles and adults (Scott and Johnston 2012; Beaman et al. 2016).

Thermal variability not only influences the evolution of acclimation capacity but is also predicted to shape thermal performance breadth across acute temperature exposures (Janzen

1967; Gilchrist 1995; Wilson and Franklin 1999; Angilletta 2006; Healy and Schulte 2012). Importantly, it has been hypothesised that acute thermal performance breadth, in turn, influences whether acclimation to changed thermal conditions will be of benefit to organisms (Janzen 1967; Johnson and Bennett 1995; Wilson and Franklin 1999; Šamajová and Gvoždík 2010; Rohr et al. 2018). For example, individuals with narrow thermal performance breadths are thought to have greater acclimation capacities than individual with wide thermal performance curves (Wilson and Franklin 2000). This hypothesis was proposed because individuals with narrow thermal performance curves are likely to have very low performance when temperatures shift outside of their breadth, thus the costs (lowered performance and/or fitness) of not acclimating with environmental change is very high. Organisms with wide thermal performance curves have the ability to perform well over a wide range of temperature, and consequently are likely to perform adequately when the temperature changes without the need to invest energy in acclimation (Wilson and Franklin 2000). Since the benefit of maintaining the capacity to acclimate is likely to be low for organisms with wide performance curves (since they can already perform across a wide range of temperatures), the costs of maintaining the capacity to acclimate are likely to outweigh the benefits, and the capacity to acclimate may be lost (Huey and Slatkin 1976; Wilson and Franklin 2002). For example, terrestrial Antarctic mites experience greater daily thermal variability than marine mites and have wide thermal performance curves with no capacity to acclimate (Deere and Chown 2006). Marine mites that experience less daily thermal variability were found to have narrower thermal performance curves and had the capacity to acclimate (Deere and Chown 2006). These examples suggest that there might be feedback between thermal performance breadth and acclimation capacity and that this feedback is important for the co-evolution of phenotypic responses to short- and longer-term temperature change.

The way thermal variability is thought to affect the shape of the thermal performance curve and acclimation capacity has been debated. In contrast to the studies mentioned above, other studies suggest that animals with narrow thermal performance curves are unlikely to have the capacity to acclimate (Janzen 1967; Deutsch et al. 2008; Tewksbury et al. 2008). This is because animals with narrow thermal performance curves are thought to evolve in stable thermal conditions, where the capacity to acclimate is not required (Janzen 1967; Deutsch et al. 2008; Tewksbury et al. 2008). Thus, the costs of maintaining the capacity to acclimate when thermal change is rare is likely to outweigh the benefits of maintaining the capacity to acclimate (DeWitt et al. 1998). In addition, further studies suggest that animals that inhabit variable thermal conditions, such as those found in temperate regions, should have wide thermal performance curves and/or the capacity to acclimate to enable survival in a variable thermal environment (Janzen 1967; Huey and Kingsolver 1989; Gabriel 2005). The thermal conditions that promote the evolution of acclimation capacity and performance curve shape have been debated throughout the past half century, and many different hypotheses exist (Fig 1.3; Box 1). There is limited information, however, on how different combinations of daily and seasonal thermal variability affect the co-evolution of thermal performance curve shape and acclimation capacity in different taxa. It is also unclear whether thermal performance curve breadth is likely to alter acclimation capacity, whether acclimation capacity is likely to alter performance curve breath, whether the two traits are entirely co-dependent, or whether they evolve independently.

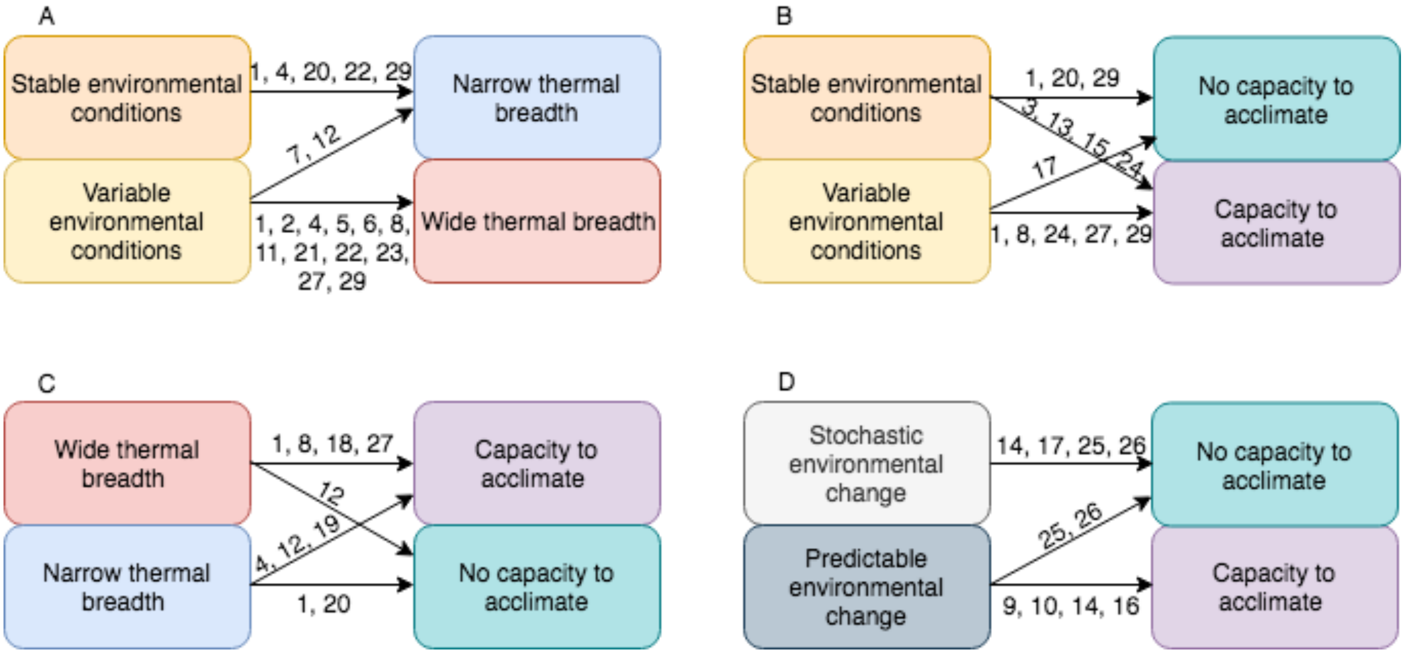


Figure 1.3. Network of competing findings on how thermal variability and predictability affects thermal performance breadth, acclimation capacity and the interaction between performance breadth width and acclimation capacity. The numbers along the arrowed lines correspond to the list of studies in Box 1. Studies are a subset of data and theory papers over the past 50 years that show the variation in findings on how thermal variability alters performance.

Box 1. Sample of studies through time that have conclusions regarding the effect thermal variation has on thermal performance breadth, acclimation capacity or both.

Studies with asterisks* are hypothesises and findings from model simulations. All other studies are based on experimental or meta-analysis findings.

1. **(Janzen 1967)*** – Animals in stable thermal environments should evolve narrow breadths and have no capacity to acclimate. Animals in variable environments should have wide breadths and the capacity to acclimate.
2. **(Levins 1968)*** – Diurnal and seasonal variation in temperate environments promotes wide tolerance breadths.
3. **(Brown and Feldmeth 1971)** – Capacity to acclimate can be conserved over generations and species even if populations do not experience thermal variability.
4. **(Huey and Slatkin 1976)** – Animals with narrow thermal breadths will thermoregulate more carefully than animals with wide thermal breadths. Environmental thermal variation affects thermal breadth.
5. **(Lynch and Gabriel 1987)*** – Great thermal variation within generations favours wide thermal breadths.
6. **(Huey and Kingsolver 1993)*** – Animals in variable environments should evolve wide thermal breadths.
7. **(Gilchrist 1995)*** – Narrow thermal breadths are favoured when within generation thermal variation increases, wide thermal breadths are favoured when among generation variation increases and within generation variation decreases.
8. **(Johnson and Bennett 1995)** – Temperate intertidal (large daily and seasonal thermal variation) animals have the capacity to acclimate burst swimming speed and have wide thermal performance curves.
9. **(Kingsolver and Huey 1998)*** – Acclimation depends on predictability of diel, daily and seasonal variations in temperature.
10. **(DeWitt et al. 1998)*** – Acclimation is beneficial when thermal variation is predictable.
11. **(Bennett and Lenski 1999)** – Daily and seasonal thermal variation promotes wide thermal breadths.
12. **(Wilson and Franklin 2000)** – Animals in environments where daily thermal fluctuation is small and seasonal thermal variations are large should evolve narrow thermal breadths and have the capacity to acclimate. Animals in environments with large daily variation and little seasonal variation should evolve wide thermal breadths and have no capacity to acclimate.
13. **(Cunningham and Read 2002)** – Tropical (stable condition) species had the capacity to acclimate.
14. **(Gabriel 2005)*** – Thermal acclimation should occur when thermal variation is predictable and experience “stress” periods that are shorter than the animal’s life span. As stochasticity increases, thermal breadth increases and acclimation becomes unlikely.
15. **(Glanville and Seebacher 2006)** – Found that a tropical (stable thermal environment) animal had the capacity to acclimate.
16. **(Sinclair et al. 2006)** – Neither wide or narrow thermal breadths are favoured in thermally variable environments, instead increased phenotypic plasticity and capacity to respond to environmental cues is favoured.
17. **(Deere and Chown 2006)** – Terrestrial (more variable environment and less predictable) animals did not show evidence of acclimation. Thermal acclimation had little effect on the shape of thermal performance curves within species.
18. **(Fangue et al. 2006)** – Latitude affects the critical thermal maximum of temperate intertidal fish and these species have the capacity to acclimate their upper thermal tolerance.
19. **(Franklin et al. 2007)** – Antarctic (stable environment) animals have the capacity to acclimate.
20. **(Deutsch et al. 2008)** – Tropical animals have narrow thermal breadths, live in environments close to their thermal maximum and have limited acclimation capacities.
21. **(Fangue et al. 2008)** – Temperate intertidal species have wide thermal performance curves for U_{crit}.
22. **(Tewksbury et al. 2008)*** – Tropical animals have narrow thermal breadths (stable thermal environment), temperate animals have wide thermal breadths (variable thermal environment).
23. **(Asbury and Angilletta Jr 2010)*** – When body temperature varies little within generations performance breadth should be proportional to among generation variation in body temperatures
24. **(Šamajová and Gvoždík 2010)** – Amphibians acclimate to mean thermal conditions in more stable thermal environments and acclimate to diel temperature fluctuations in variable thermal environments.
25. **(Niehaus et al. 2011)** – Thermal variability did not affect acclimation capacity.
26. **(Schuler et al. 2011)** – Thermal variation did not affect acclimation capacity.
27. **(Healy and Schulte 2012)** – Temperate intertidal fish have the capacity to acclimate aerobic scope and have wide thermal performance curves. Thermal acclimation had little effect on altering the shape of the acute thermal performance curve.
28. **(Pereira et al. 2017)** – Thermal variability (with latitude) did not correlate with plastic response.
29. **(Rohr et al. 2018)** – Acclimation capacity increases with latitude, body size and seasonality. Acclimated thermal performance breadth width increases with latitude and decreases with body size.

Assessment of the cross-over in findings and hypotheses in Figure 1.3 and Box 1 indicates that there is no definitive rule on how thermal variability or stochasticity alters thermal performance curve shape, acclimation capacity or both. Variables such as evolutionary history, organism size, life span and micro-habitat are all likely to influence organisms' responses to short and long-term changes in climate (Box 1). Future studies that test how the magnitude of daily and seasonal thermal variability alter species acclimation capacities and thermal performance curve shape across a range of microhabitats will improve our understanding of how reversible plasticity and performance breadth co-evolve.

1.4 Time scales and constraints

The time scales at which animals have to respond to changes in temperature are also important for understanding and predicting how species respond to variations in climate within generations and among generations. Slow thermal changes that occur over many generations often result in the evolution of differing thermal tolerances and acclimation capacities in populations that inhabit different thermal environments (Huey and Kingsolver 1989; Angilletta et al. 2002), where local adaptation allows animals to maintain performance and fitness within a particular environment. For example, populations of toad-headed lizards that evolve at different altitudes have different thermal tolerances and acclimation capacities based on the degree of thermal variability they are exposed to (Wu et al. 2018). The evolution of thermal specialisation (to stable or variable environments), however, is only likely to occur if the rate of evolutionary adaptation is greater than the rate of environmental change (Seebacher et al. 2005). This is because if the rate of environmental change is faster than the rate of evolutionary change, animals are unlikely to maintain function and survive with large shifts in temperature (depending on their performance curve breadth and acclimation capacity) and therefore will be unable to adapt to different thermal conditions. When the rate of evolutionary change is greater than rate of environmental change, populations and/or species at different latitudes or altitudes often have different thermal breadths and/or acclimation capacities (Rohr et al. 2018; Wu et al. 2018).

In contrast, other studies have found that latitude (or long-term exposure to certain environmental conditions, i.e. between generation thermal variability for low mobility species) does not correlate with acclimation capacity (van Heerwaarden et al. 2014; Gunderson and Stillman 2015; Pereira et al. 2017). In some cases, a lack of correlation between thermal variability (latitude or altitude) and plasticity can be a result of evolutionary constraints (e.g.

lack of genetic variation) (Brown and Feldmeth 1971). For example, some species have been observed to retain their capacity to acclimate even if their environment has become more stable over time (Brown and Feldmeth 1971; Feldmeth et al. 1974). In these cases, the capacity to acclimate is conserved even when thermal variability is minimal between generations, as the benefits of maintaining acclimation capacity could outweigh the costs if environments change in future generations (potential safety net mechanism) (Seebacher et al. 2005; Franklin et al. 2007). For example, desert pupfish found in variable ancestral freshwater streams have maintained their capacity to thermally acclimate despite living in stable freshwater streams for many generations (Brown and Feldmeth 1971). Species can also respond to very rapid thermal change (hours or days) by rapid acclimating, behaviourally thermoregulating, or by having wide thermal performance curves (Angilletta 2009). The rate at which different species can acclimate is variable, where smaller bodied organisms acclimate faster than larger bodied organisms on average with latitude and therefore increased thermal variability (Rohr et al. 2018). Yet, while smaller organisms acclimate faster, larger bodied organisms have greater overall capacities to acclimate than smaller bodied organisms (Rohr et al. 2018). Larger animals may have greater acclimation capacities because they are longer lived and therefore are likely to experience repeated seasonal variability, whereas smaller and shorter-lived species may not experience seasonal fluctuations or are likely to experience less annual thermal variability. If the rate of thermal change is faster than an organism's capacity to acclimate, reduced performance is likely to ensue, resulting in lower survival probabilities and fitness, and thus the costs of plasticity are likely to be higher than the benefits (DeWitt 1998; DeWitt et al. 1998) (Murren et al. 2015). Wide thermal performance curves can buffer the effects of rapid thermal change on organisms, as wide thermal performance curves allow organisms to maintain performance across a wide range of environmental temperatures (Janzen 1967; Levins 1968; Huey and Slatkin 1976; Lynch and Gabriel 1987; Healy and Schulte 2012). A model by Gilchrist (1995) provides an alternative explanation, suggesting that wide thermal performance curves evolve when among-generation thermal variation increases and within-generation thermal variation decreases. In this case, a wide thermal performance curve maintains fitness and survival in stochastic environments over generations.

1.5 Thermal performance knowledge gaps

How daily and seasonal thermal variability affect the co-evolution of the shape of thermal performance curves and acclimation capacity remains debated. The studies presented in Figure

3 highlight a range of hypotheses for the effect of thermal variability on performance curve shape, acclimation capacity, or both. Many of these studies do not discuss how the magnitude of daily and seasonal thermal variability together affect the co-evolution of performance breadth and acclimation capacity, or if thermal acclimation alters the shape of thermal performance curves. Many combinations of daily and seasonal thermal variability exist in nature. Therefore, studies that are more representative of natural thermal variability that assess acclimation capacity and performance breadth across a wide range of taxa at varying locations are needed.

Models predicting how short- and long-term thermal variability alter performance curve shape and acclimation capacity exist, but they often focus on organisms with short life spans (days to weeks), for which experience with repeated seasonal fluctuations are unlikely (Gilchrist 1995; Gabriel 1999; Gabriel 2005). Findings from these models suggest that organisms in environments with short-term thermal fluctuations that are equal to or greater than long-term variability are unlikely to have the capacity to acclimate. Testing these models with longer-lived species (that live in a variety of environments) will enable us to better understand how longer-lived organisms respond to thermal change.

In Chapter 2 of my thesis I investigate whether the capacity to acclimate can be retained in an intertidal goby (*Bathygobius cocosensis*) that experiences equal daily and seasonal thermal fluctuations (subtropical intertidal zone), and how thermal performance curves are altered with thermal acclimation to seasonal conditions. Previous studies have investigated whether temperate intertidal fish have the capacity to acclimate and maintain wide thermal performance curves despite living in a rapidly fluctuating environment (Fangue et al. 2006; Fangue et al. 2008; Healy and Schulte 2012). Temperate intertidal environments experience greater seasonal thermal variability than daily thermal variability, with seasonal thermal variability remaining predictable, thus the evolution of thermal acclimation is likely to occur (Fangue et al. 2006; Fangue et al. 2008; Healy and Schulte 2012). No previous studies have investigated whether organisms can maintain their capacity to acclimate when daily and seasonal thermal variability is equal, and how thermal acclimation to seasonal thermal conditions alters the shape of their thermal performance curves under these conditions. In an environment that experiences equal daily and seasonal thermal variability, I predict that organisms will have wide thermal performance curves and the capacity to acclimate if seasonal thermal trends remain predictable.

1.6 Colour change plasticity

The effects of temperature on physiological rate processes has implications not only for physiological performance, but also for traits such as colour change for camouflage, which influence behaviourally mediated species interactions (Cole 1939; King et al. 1994; Camargo et al. 1999; Lin et al. 2009). Changing colour to evade predation or to avoid being seen by prey is common across the animal kingdom (Sumner 1940; Stuart-Fox and Moussalli 2008; Sköld et al. 2013; Stevens 2016; Duarte et al. 2017). Colouration can be adjusted through morphological colour change (where the number and structure of chromatophores, or pigment cells, is altered), or physiological colour change, where pigment granules within chromatophores move (e.g. disperse or aggregate) through neuromuscular contraction or relaxation, which is fast acting. Animals can also change colour via hormonal signalling, which takes longer to occur (Sköld et al. 2013; Stevens 2016; Duarte et al. 2017). Due to the range of mechanisms used to change colour and among-species differences in physiology, the rate at which different species can change colour is quite variable. For example, cephalopods are known to change colour very quickly (in a matter of seconds), whereas Arctic hare can take months to change their colour phenotype (Caro et al. 2016; Stevens 2016; Duarte et al. 2017). Studies on rock-pool gobies in the United Kingdom show that they can change colour to match their backgrounds within one minute (Stevens et al. 2014; Smithers et al. 2017; Smithers et al. 2018). These rapid rates of colour change are often due to neuromuscular aggregation or dispersion of pigment granules within chromatophores (Duarte et al. 2017). Colour change has important implications for individual survival and reproduction (Sköld et al. 2013; Duarte et al. 2017b; Ruxton et al. 2018), and while many studies have focused on the mechanistic basis of colour change, relatively little is understood about the environmental drivers of variation in rate of colour change.

1.7 Rate of camouflage performance knowledge gaps

Metabolic rate, growth rate, locomotion and reproduction are commonly assessed in functional physiology (Angilletta 2009). While these traits are linked with survival and fitness, it is important to pair them with other ecologically relevant traits for a more holistic understanding of species responses to changes in climate. For example, rate of luminance change for background matching is an unexplored thermal performance trait, but it is likely to be important in defining predator-prey interactions. Rate of colour change is influenced by temperature, with acute test temperature being positively correlated with rate of colour change in fish and frogs (Cole 1939; King et al. 1994b; Camargo et al. 1999; Lin et al. 2009). However, no previous studies have assessed whether rate of colour change has the capacity to acclimate to long-term thermal change. The rate at which an animal can camouflage itself provides an ecologically relevant indicator of survival because it is a mediator of predator-prey interactions. In Chapter 3, I explore whether rate of luminance change (perceived brightness) for camouflage has the potential to acclimate to long-term seasonal change against black and white backgrounds. Luminance is an important component of colour change and camouflage ability because many animals use contrasts in luminance to detect motion, shape of objects and texture (Cronin et al. 2014; Ruxton et al. 2018). Therefore, the better an organism can match the luminance of its visual background, the better camouflaged it is likely to be to potential predators and prey. In this instance, I assessed rate of luminance change in *B. cocosensis* through the eye of a predatory fish, the coral trout (*Plectropmus leopardus*), known to prey upon benthic fish such as gobies and blennies. To do this, it is possible to model changing luminance (in goby skin) over time against different background colours through the eye of a coral trout. Modelling of predatory vision will be discussed in detail within Chapter 3.

1.8 Developmental experience and acute thermal performance

Many species with complex life-cycles disperse during their developmental phase where individuals develop in different locations to where they settle as adults (Emlet and Hoegh-Guldberg 1997; Marshall et al. 2003; Macpherson and Raventós 2005; Cowen and Sponaugle 2009; Gimenez 2010; Crean et al. 2011; Grorud-Colvert and Sponaugle 2011). For example, many marine fish species have a pelagic larval phase where oceanic currents determine the environmental conditions larvae experience as well as their settlement location (Campana et al. 1992; McCormick and Hoey 2004; Macpherson and Raventós 2005; Shima and Swearer 2010; Grorud-Colvert and Sponaugle 2011; Moody et al. 2015). During this period, larvae experience extreme predation pressure and a variety of environmental conditions (Meekan and Fortier 1996; Wilson and Meekan 2002; Cowen and Sponaugle 2009; Shima and Swearer 2009; Gimenez 2010). The conditions organisms experience during larval development likely influence thermal tolerance as juveniles and adults as well as thermal acclimation capacities (Scott and Johnston 2012). Very little is known about how wild marine developmental conditions affect post-metamorphic thermal performance as it is extraordinarily difficult to track the environmental conditions that individual larvae experienced throughout their pelagic dispersal. Therefore, it is important to assess how pelagic larval traits, which are influenced by both genetic and environmental factors (Thia et al. 2018), affect post-metamorphic performance and fitness.

Larval traits, such a pelagic larval duration (how long individuals spend in the pelagic phase), larval growth rate, hatch size and settlement size are measurable using otolith increment analysis (Campana et al. 1992; Jones 1992) (Fig 1.4). Otoliths are fish ear bones, and rings within otolith cross sections can infer information about larval traits. Hatch marks (transition from egg to larvae) and settlement marks (when the individual settled at an intertidal location) (Campana et al. 1992) can provide information on larval size and developmental strategy (fast or slow growth). Daily growth can also be measured by examining the distance between each otolith ring (Brothers et al. 1976; Swearer et al. 1999). Larval growth rate is influenced by environmental temperatures (Seikai et al. 1986; Green and Fisher 2004), where higher environmental temperatures are associated with faster growth rates (Seikai et al. 1986; Green and Fisher 2004). Larval traits are not always directly indicative of the environmental conditions larvae experienced, but traits such as growth rate can provide interesting information on larval survival strategies and energy partitioning for metabolism, development, locomotion and other energetically demanding activities. For example, as the pelagic larval stage is known to be an extremely risky period in the life-cycle, fast larval growth rates and short pelagic larval durations are predicted to minimise time spent in the pelagic zone to increase survival likelihood (Meekan and Fortier 1996; Wilson and Meekan 2002; Cowen and Sponaugle 2009; Shima and Swearer 2009; Gimenez 2010). Other individuals may have slower growth and longer pelagic larval duration to increase the size at which they settle in order to increase their competitive advantage for resources such as food and shelter at settlement (Carr and Hixon 1995; Gagliano and McCormick 2007). It is possible to assess how these developmental traits and strategies are linked with post-metamorphic (juvenile or adult) traits and performance. Often studies assess the traits and performance of juveniles and then they back calculate larval traits using their otoliths (Penney and Evans 1985; Meekan and Fortier 1996; Macpherson and Raventós 2005; Grorud-Colvert and Sponaugle 2011).

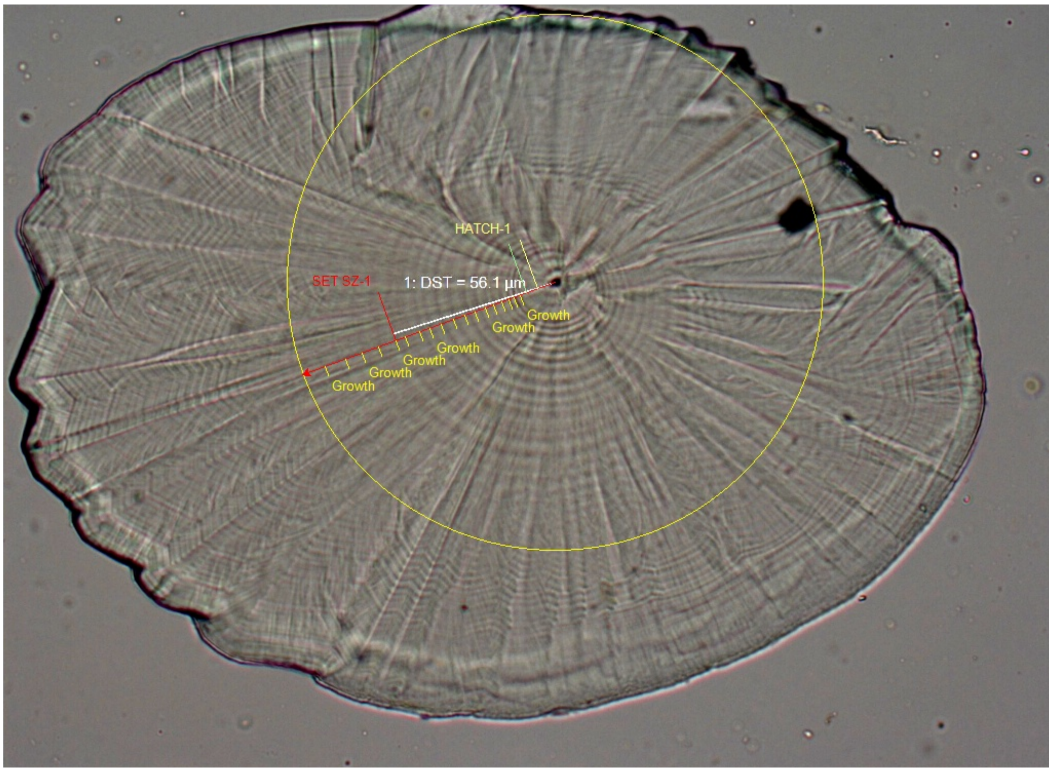


Figure 1.4 Cross section of an intertidal fish (*Bathygobius cocosensis*) otolith. The hatch mark (HATCH-1), settlement ring (SET SZ-1) and daily growth increments can be identified. Photograph taken by Nuria Raventos at the Spanish National Research Council, Advanced Research Centre of Blanes, Blanes, Spain.

1.9 Larval traits and post-metamorphic thermal performance knowledge gaps

How larval traits affect post-metamorphic traits and fitness has been a topic of debate over the past 50 years. Some studies suggest that larval traits are de-coupled with post-metamorphic traits because animals with complex life cycles inhabit different environments at each life stage and therefore need different phenotypic traits for survival in distinct habitats (Ebenman 1992; Moran 1994; Parichy 1998). Other studies argue that larval traits have significant links with post-metamorphic traits, known as carry-over effects (Pechenik et al. 1998; Marshall et al. 2003; Crean et al. 2011). Larval trait carry-over effects have been observed to have positive and negative implications for juvenile and adult performance or fitness. For example, large egg sizes are known to be linked with greater reproductive success and survival in marine invertebrates (positive carry-over effect) (Marshall et al. 2003). In contrast, fast larval growth rates in tadpoles are correlated with reduced jumping performance as adult frogs (negative carry-over effect) (Ficetola and De Bernardi 2006). No previous studies have assessed how wild marine fish larval traits are correlated with acute juvenile thermal performance. I hypothesise that fast larval growth rates (to avoid time spent in the dangerous pelagic phase) will be costly for post-metamorphic performance due to energetic trade-offs. Chapter 4 examines the correlations between larval traits and juvenile acute thermal performance in *B. cocosensis*.

1.10 Study system: the intertidal zone and *Bathygobius cocosensis*

The intertidal environment is thermally variable and thus provides an ideal system with which to address how thermal fluctuations on a short- and long-term basis alter the shape of organisms' thermal performance curves and acclimation capacities (da Silva et al. 2019a). I assess the acclimation capacity of *B. cocosensis* that experiences equal daily thermal fluctuations (~ 6°C) and changes in seasonal means (~ 6°C) in the subtropical intertidal system and how thermal acclimation alters their thermal performance curve shape in Chapter 2. Intertidal environments are not only heterogeneous in their thermal profiles, they also vary considerably in other abiotic variables such as salinity, oxygenation, nitrogen and the range of background colours that animals within rock-pools experience (Johnson 1975; Helmuth 1999; Helmuth et al. 2006). Therefore, a majority of the species that are residents within rock-pools must not only tolerate a range of environmental fluctuations but also have the capacity to maintain crypsis from predators or prey against a range of background types such as sand, rock or algae (Stevens et al. 2014b; Smithers et al. 2017; Smithers et al. 2018). Thus, the intertidal environment provides an excellent system to address how short- and long-term changes in temperature affect rate of background matching for crypsis. My study sites are located at intertidal environments in Queensland, Australia: Point Lookout on North Stradbroke Island, Point Cartwright on the Sunshine Coast, and Heron Island, in the southern Great Barrier Reef (Fig 1.5). All of these locations are characterised as subtropical; however, the Heron Island location is located at a lower latitude than the other study sites and is located further offshore. The Point Lookout and Point Cartwright study sites are rocky with distinct rock-pools, while the Heron Island site has some rock flat regions with a distinct lack of pools.

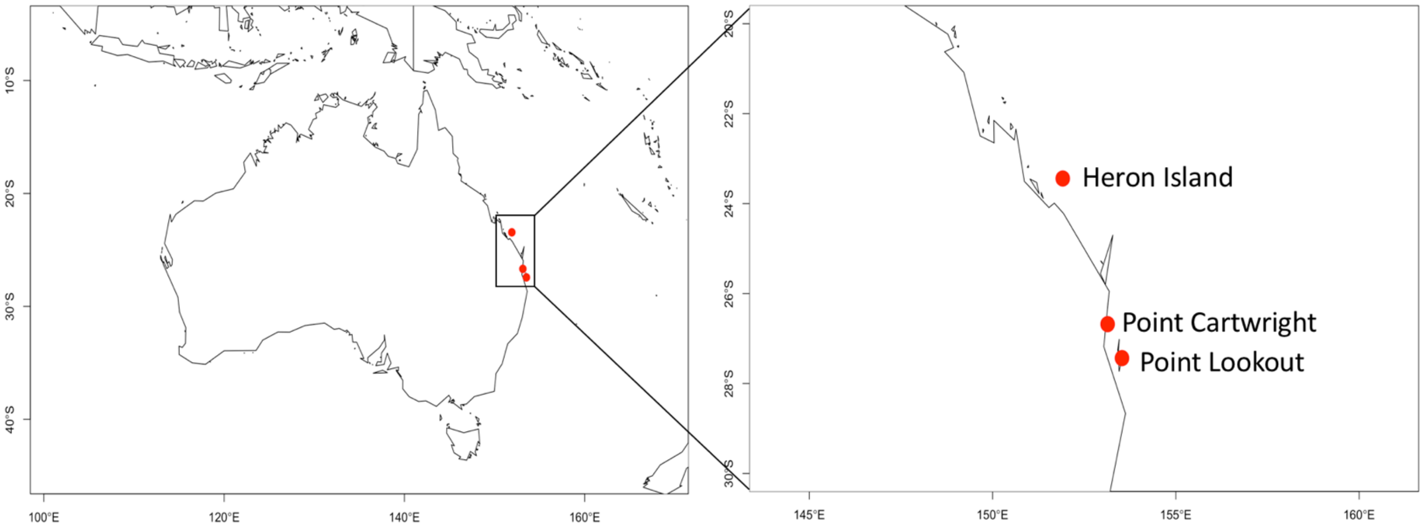


Figure 1.5 Map of study site locations along the east coast of Australia.

Bathygobius cocosensis (Fig 1.6) is the most common intertidal fish species off the east coast of Australia (Griffiths 2003). *Bathygobius cocosensis* have strong homing behaviour as adults (Griffiths 2003; White and Brown 2013; Malard et al. 2016), and studies have shown that individual gobies remain within the same rock-pools for many months (if not their entire adult lives) once they settle at a particular intertidal location (after their planktonic pelagic larval phase) (Griffiths 2003; White and Brown 2013; Malard et al. 2016). This strong homing behaviour is thought to facilitate rapid escape to known hiding places when a predator enters the pool. Their homing behaviour also means that once *B. cocosensis* settle at a particular intertidal location, they will experience thermal conditions in that location for the rest of their life (~ 3 years). *Bathygobius cocosensis* vary in their morphology depending on whether they inhabit the low-tide or high-tide zone, with those from high-tide rock-pools being smaller on average and with eyes further up their heads (potentially for better vision of avian predators) (Malard et al. 2016). *Bathygobius cocosensis* is an aggressive and territorial species (Paijmans and Wong 2017), and competition for food and habitat is intense particularly in the low-tide zone where there is better access to food and refuges.

Figure 1.6 *Bathygobius cocosensis* in a rock-pool eating a polychaete worm. Photographed by Carmen da Silva.

Bathygobius cocosensis morphology is also dependent on habitat background colour as they possess the ability to change their luminance to match background habitat. For example, *B. cocosensis* are often darker in colouration when observed against dark rocks or algae and conversely light and speckled in colouration when observed basking on sandy patches (see Fig 1.6). My preliminary observations suggest that individuals can rapidly change their luminance to match the background colour. Therefore, as *B. cocosensis* have the ability to change luminance and inhabit the thermally variable intertidal zone, they are an ideal species to assess if they can acclimate rate of luminance change to different thermal conditions. Rate of luminance change for background matching is a trait that is directly linked with survival and therefore fitness (Duarte et al. 2017; Ruxton et al. 2018). Hence, rate of luminance change is a good proxy with which to assess how well camouflage is maintained with long-term changes in temperature. I assess if *B. cocosensis* can acclimate their rate of background matching to different thermal conditions in Chapter 3.

Similar to other marine fish species, *B. cocosensis* have a planktonic pelagic larval phase and are carried by ocean currents for the first ~ 20 days of their life (Thia et al. 2018). Thus, the conditions that *B. cocosensis* experience during development are unlikely to be the same as the conditions they experience in later life stages. In addition, the thermal conditions that each generation experiences are likely to be different and potentially unpredictable. Therefore, *B. cocosensis* are unlikely to be highly adapted to the environment in which they settle. Currently, very little is understood about how wild larval traits and experience alter post-metamorphic thermal performance. *Bathygobius cocosensis* provide an excellent opportunity to assess how larval traits such as growth rate, hatch size and settlement size are linked with juvenile burst swimming speed, routine metabolic rate and critical thermal maximum. Changes in climate are likely to influence development, performance and fitness at all life stages and it is important to determine the knock-on effects that developmental traits may have on later life stages. I assess how larval traits are correlated with post-metamorphic thermal performance in Chapter 4.

1.11 Climate change

The unifying theme of this thesis is temperature and the effects of short- and long-term temperature variation on performance. Temperatures across the globe are changing at unprecedented rates, where thermal change is becoming more stochastic and thermal means are rising (Pachauri et al. 2014). Rapid changes in climate are likely to increase selection pressure on populations, especially those close to upper thermal maxima, such as tropically distributed ectotherms (Somero 2010; Hoffmann and Sgro 2011). Tropical ectotherms are predicted to be among the most sensitive species to climate change (Deutsch et al. 2008; Tewksbury et al. 2008; Somero 2010), as they experience relatively low thermal variation compared to temperate regions, and are likely to have limited capabilities to respond to thermal variation (Deutsch et al. 2008; Tewksbury et al. 2008; Kellermann et al. 2009; Somero 2010). In contrast, other studies suggest that temperate species are more likely to be negatively affected by climate change than tropical species as they experience greater thermal fluctuations and stochasticity compared to tropical species and thus are likely to experience local population extinctions when unable to respond to rapid thermal change (Helmuth et al. 2002).

A majority of studies that investigate how global warming will impact species focus on tropical species that experience warm temperatures on a regular basis or temperate species that experience larger seasonal and daily thermal fluctuations (Helmuth et al. 2002; Deutsch et al. 2008; Tewksbury et al. 2008; Somero 2010; Sheldon et al. 2011; Gunderson and Stillman 2015). Very few studies have investigated how subtropical species that experience moderate daily and seasonal thermal variability that are relatively equal in magnitude will respond to thermal change. In addition, few studies have assessed how thermal generalists will respond to climate change, as they are likely to be more robust than thermal specialists. Many thermal generalists, however, are keystone species for particular environments and loss of these species can have negative knock-on effects for whole ecosystems (Ellison et al. 2005). There is currently a great lack of sampling of species responses to climate change from different habitats, making predicting how a variety of species will cope to climate change difficult (Seebacher et al. 2015). For this reason, it is important for future studies to assess how a range of species (and traits) will respond to short- and long-term change in many different environments. I have made a contribution to progress in this field by addressing three important problems; how daily and seasonal thermal variability affect the acclimation capacity of a subtropical intertidal fish; whether colour change has the capacity to acclimate; and how developmental traits affect post-metamorphic thermal performance.

Chapter 2

An intertidal fish shows thermal acclimation despite living in a rapidly fluctuating environment

This thesis chapter has been published in the Journal of Comparative Physiology B. The full reference for this publication is: da Silva CRB, Riginos C, Wilson RS (2019) An intertidal fish shows thermal acclimation despite living in a rapidly fluctuating environment. Journal of Comparative Physiology B.

I contributed significantly to this publication. Robbie Wilson and I generated the initial research questions and experimental design and it was then refined by Cynthia Riginos. I collected the fish used for this project, conducted the thermal performance experiments, statistical analysis and manuscript writing. Both Robbie and Cynthia provided editorial advice.

Percentage of contribution towards da Silva *et al.* (2019a)

	da Silva	**Wilson**	**Riginos**
Concept and design	50	40	10
Field collection	100	0	0
Experiments	100	0	0
Statistical analysis	100	0	0
Writing of manuscript	80	15	5
Editing of manuscript	70	15	15

2.1 Abstract

The co-evolution of acclimation capacity and thermal performance breadth has been a contentious issue for decades, and little is known regarding the extent to which acclimation alters the shape of acute thermal performance curves. Current acclimation theory suggests that when daily variation is large and unpredictable ectotherms should not acclimate but should evolve wide performance breadths, allowing maintenance of performance across a wide thermal range. The subtropical intertidal zone, however, experiences a large amount of daily thermal variation, but daily means and ranges shift in predictable ways with season, where daily and seasonal variation is roughly equal. I predicted that animals in this habitat would maintain their capacity to acclimate, and that performance breadth would not be altered by acclimation to maintain function with rapidly fluctuating daily temperatures. I tested this prediction using a subtropical goby, *Bathygobius cocosensis*, which lives in tide pools that vary widely, over days and seasons. I exposed *B. cocosensis* to winter (12°C - 17°C) and summer (30°C - 35°C) thermal conditions for six weeks and then measured the thermal dependence of burst swimming speed, routine and maximum metabolic rate, and ventilation rate between 12°C - 36°C. *Bathygobius cocosensis* exhibited an acclimation response for burst swimming speed, maximum metabolic rate and metabolic scope, but acclimation did not alter the shape of acute thermal performance curves. These results indicate that thermal acclimation can occur when short-term thermal variability is large and equal to seasonal variation, and wide performance breadths can be maintained with acclimation in heterogeneous environments.

2.2 Introduction

Global climate change is increasing environmental thermal means, variability and stochasticity (IPCC 2014). Animals can respond to thermal change within their lifetime by moving to environments with more suitable temperatures (behavioural thermoregulation) (Walther et al. 2002; Sunday et al. 2011; Campbell et al. 2018) or they can acclimate (Wilson and Franklin 2002; Angilletta 2009; Riddell et al. 2018). Thermal acclimation, a form of reversible plasticity, is the modification of an organism's underlying physiology to allow maintenance of performance in changed environmental conditions (Huey et al. 1999; Wilson and Franklin 2002; Angilletta 2009; Beaman et al. 2016). Acclimation to changed thermal conditions has the potential to result in fitness benefits (Wilson and Franklin 2002; Ghalambor et al. 2007), because performance traits such as metabolism and locomotion affect growth, behaviour and development (Le Galliard et al. 2004; Wilson et al. 2007; Seebacher 2009). Fitness benefits associated with acclimation have the potential to occur when changes in environmental conditions are predictable and there are adequate environmental cues for future thermal conditions (Levins 1968; DeWitt 1998; Kingsolver and Huey 1998; Johnston and Temple 2002; Gabriel 2005). The environmental conditions that promote the evolution of thermal acclimation and thermal performance curve shape, however, has remained a controversial topic over the past 50 years (Janzen 1967; Lynch and Gabriel 1987; Huey and Kingsolver 1989, DeWitt 1998; Wilson and Franklin 2000; Gabriel 2005; Deere and Chown 2006; Ghalambor et al. 2007; Tewksbury et al. 2008; Deutsch et al. 2008; Healy and Schulte 2012; Rohr et al. 2018).

Thermal performance curves describe the way temperature affects animal performance, with the peak of the curve representing an organism's thermal performance optimum and the breadth of the curve representing the range of temperatures for adequate performance (Huey and Stevenson 1979; Huey and Kingsolver 1989; Gilchrist 1996; Angilletta et al. 2002; Angilletta 2006; Schulte et al. 2011). Thermal variability is believed to play an important role in shaping an organism's capacity to acclimate and the breadth of their thermal performance curve (Levins 1968; Huey and Kingsolver 1989; Wilson and Franklin 2000; Huey et al. 2012; Dillon et al. 2016). Many studies suggest that organisms in variable environments should evolve wide thermal performance breadths and the capacity to acclimate; conversely, animals in stable thermal environments should evolve narrow performance curves and little or no capacity to acclimate (Janzen 1967; Lynch and Gabriel 1987; Huey and Kingsolver 1993; Gilchrist 1995; Stillman 2003; Tewksbury et al. 2008; Deutsch et al. 2008; Rohr et al. 2018).

Many exceptions exist, however, including examples of animals in stable environments that have the capacity to acclimate (e.g. Brown and Feldmeth 1971; Cunningham and Read 2002; Glanville and Seebacher 2006; Franklin et al. 2007; Bilyk and DeVries 2011); organisms in thermally variable conditions with no capacity to acclimate (e.g. Wilson and Franklin 2000; Deere and Chown 2006); and organisms in variable environments having both the capacity to acclimate and having wide thermal performance curves (e.g. Johnson and Bennett 1995; Healy and Schulte 2012).

The way 'thermal variability' is defined is important as magnitude of daily and seasonal thermal variability together are likely to affect the co-evolution of thermal performance curve breadth and acclimation capacity, which often leads to different combinations of acclimation capacity and thermal performance curve shape (Wilson and Franklin 2000; Gabriel 2005; Healy and Schulte 2012; Dillon et al. 2016; Pörtner and Gutt 2016). In addition, many combinations of daily and seasonal thermal variability and predictability exist in nature, but we still have a limited understanding of how acclimation capacity and thermal performance breadth co-evolve in different environments, and how thermal acclimation might alter the shape of thermal performance curves. Gaining a deeper understanding of the relationships between thermal variability, acclimation capacity and performance curve shape will allow predictions of species responses to changing climates to be improved and for more specific and targeted conservation approaches to be implemented.

Some studies have investigated how both daily and seasonal thermal variability correlate with the shape of species thermal performance curves and their capacity to acclimate (Johnson and Bennett 1995; Wilson and Franklin 2000; Deere and Chown 2006; Niehaus et al. 2011; Schuler et al. 2011; Healy and Schulte 2012; Pereira et al. 2017; Rohr et al. 2018). Generally, most recent theory suggests that when seasonal thermal variation is predictable and greater than daily thermal fluctuations, animals should evolve the capacity to acclimate and have narrow thermal performance curves (Fig 2.1a) (Wilson and Franklin 2002; Gabriel 2005; Gabriel et al. 2005; Sinclair et al. 2006). For example, larval amphibians that inhabit aquatic environments where seasonal thermal variation is greater than daily thermal fluctuations often have capacity to acclimate to seasonal conditions and have narrow thermal performance curves (Wilson and Franklin 1999; 2000). When larval amphibians develop into adult frogs they transition into a predominantly terrestrial habitat where daily thermal fluctuations are greater than in aquatic environments, and these animals often have no capacity to acclimate but have wide thermal performance curves (Fig 2.1b) (Wilson and Franklin 2000). Large daily thermal fluctuations are often perceived to mask seasonal thermal change, making seasonal change unpredictable and costly, and therefore thermal acclimation unlikely to occur (DeWitt 1998; Gabriel 2005; Gabriel et al. 2005). Here I examine these expectations in the context of the marine intertidal zone where large daily thermal fluctuations occur but predictable seasonal variation is also present.

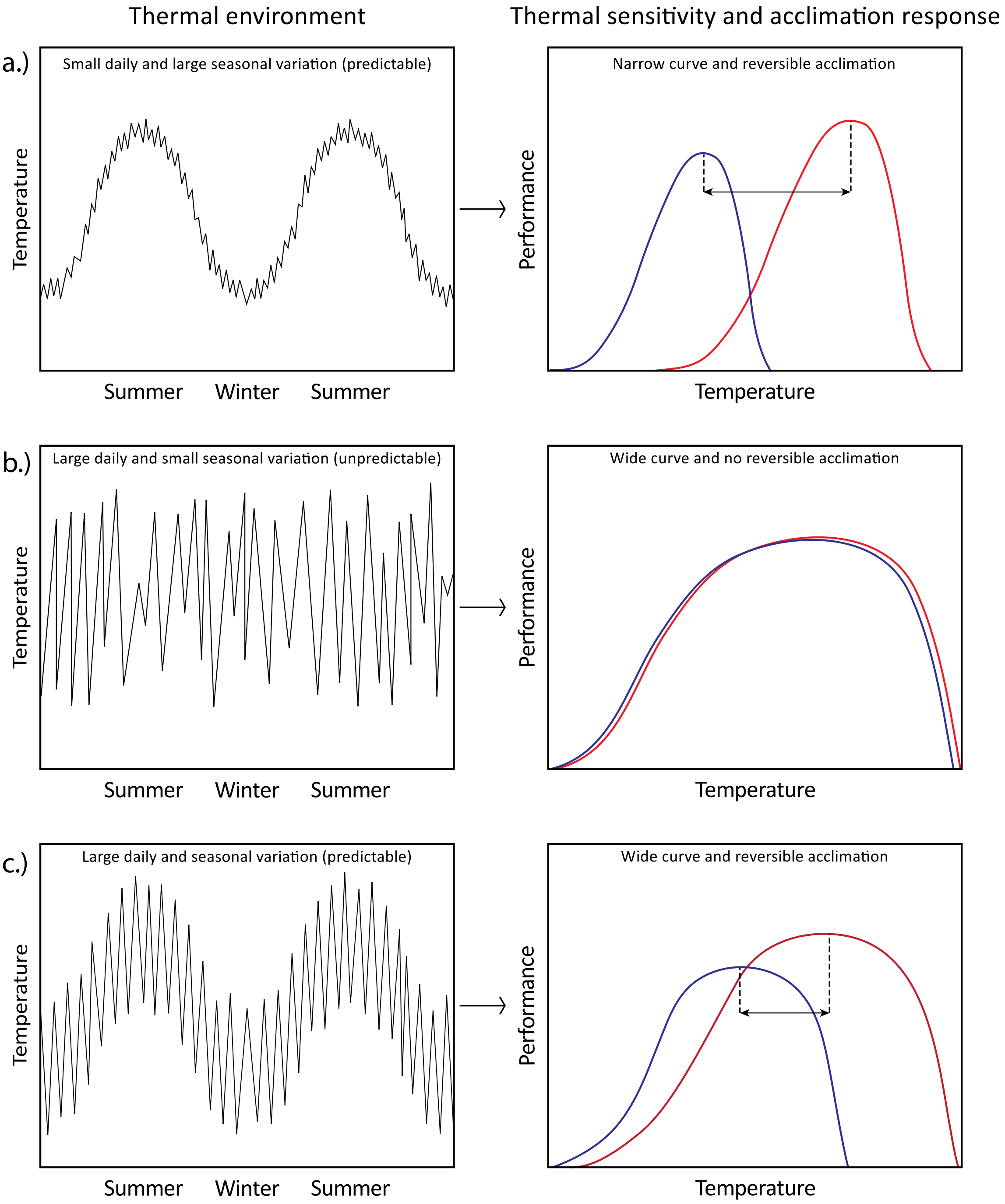


Figure 2.1. Conceptual illustration on how magnitude of daily thermal fluctuations and seasonal thermal variations affect thermal performance breadth and capacity for reversible

acclimation. Part a.) illustrates environments with small daily and large seasonal thermal variations: animals in those environments are predicted to have narrow thermal breadths and the capacity to acclimate. Part b.) illustrates an environment with large daily thermal fluctuations and little seasonal variation: animals in those environments are expected to have wide thermal performance curves and no capacity to acclimate. Part c.) illustrates an environment where magnitude of daily thermal fluctuations and mean seasonal thermal variation is equal but there is predictable seasonal change, animals in these environments are expected to have wide thermal performance curves and the capacity to acclimate.

The intertidal zone is known to experience extensive daily thermal fluctuations with changing tides, solar radiation and wind (Johnson 1975; Helmuth 1999; Helmuth et al. 2006), but the mean, maximum and minimum daily temperatures gradually shift with season, allowing seasonal changes to be predictable despite large daily fluctuations. Previous studies have found that temperate intertidal fish species, such as killifish (*Fundulus heteroclitus*) and sculpins (*Oligocottus maculosus* and *Myoxocephalus scorpius)*, that experience large fluctuations in daily and seasonal temperature have the capacity to thermally acclimate to seasonal temperatures and have wide thermal performance curves (Johnson & Bennett 1995; Temple and Johnston 1998; Fangue et al. 2006; Fangue et al. 2008; Fangue et al. 2011; Healy & Schulte 2012). In these conditions, thermal acclimation has little effect on the performance breadth of aerobic scope in killifish, where wide performance breadths are hypothesised to be required to maintain physiological function with rapid short-term thermal fluctuations (Healy and Schulte 2012). These temperate intertidal species, however, experience greater seasonal thermal variation (approx. 15-20°C) (Fangue et al. 2008) than daily thermal fluctuations (approx. 5-10°C) (Sidell et al. 1983; Fangue et al. 2008). Few studies have assessed the extent to which acclimation alters the shape of the acute thermal performance curves in organisms that experience roughly equal daily and seasonal thermal variation, such as subtropical ectotherms. I expect that acclimation capacity and performance breadth will be maintained in organisms that experience equal daily and seasonal thermal variation when seasonal variation remains predictable (Fig 2.1c), and that acclimation will have little effect on altering the shape of thermal performance curves.

I had two key aims. Firstly, I aimed to assess if an organism that experiences large but roughly equal daily and seasonal variation with predictable seasonal fluctuations had the capacity to acclimate to seasonal conditions; and finally, I aimed to assess the extent to which acclimation to seasonal conditions altered the shape of thermal performance curves. I used *Bathygobius cocosensis* (Bleeker 1854) (Fig 2.2), an intertidal fish commonly known as Cocos Frillgoby as my study organism. They are the most common intertidal fish on the east coast of Australia (Griffiths 2000) and have a widespread geographic distribution. The study site in south-east Queensland, Point Lookout North Stradbroke Island, experiences 6°C of thermal variation between seasonal *high tide* means (Supplementary Fig 1 & 2; and seasonal *high tide* (sea surface temerature) data collected by the navy meteorological and oceanography (METOC) harvester). Specifically, high tide rock pool temperatures are 26°C in the summer (January) and 20°C in the winter (July). On a daily basis, rock-pools fluctuate with diurnal tides, solar radiation and wind, where they have been observed to fluctuate by about 6°C daily (Supplementary Fig 2.S1 & 2.S2). Therefore, both daily fluctuations and seasonal means vary by about 6°C. As *B. cocosensis* experiences large daily thermal fluctuations and predictable seasonal changes, I hypothesised that *B. cocosensis* would possess the capacity to acclimate, have wide thermal performance breadths, and that thermal acclimation would have little effect on the shape of their thermal performance curve.

Figure 2.2 *Bathygobius cocosensis* in a rock-pool in south-east Queensland. Photographed by CRB da Silva.

2.3 Methods

Bathygobius cocosensis were collected from rock-pools at Point Lookout, North Stradbroke Island in southeast Queensland, Australia (GPS coordinates 27.4347°S, 153.5305°E). Fish were collected using a battery-operated bilge-pump and hand nets at low tide in April 2016. Fish were transported to the University of Queensland by vehicle in oxygen-saturated bags within insulated containers. Fish were anesthetized (0.3 x 10^{-3} mg L^{-1} of Aqui-S®) (Griffiths 2000; Malard et al. 2016) and tagged with Visible Implant Elastomer (VIE) florescent subdermal tags (Northwest Marine Technologies®, Inc.). Individual tags allowed unique recognition during experiments. VIE tags do not impact mortality or growth rates (FitzGerald et al. 2004). The sample sizes reported below were determined prior to field collection by using a power analysis for consideration of pre-specified effect sizes. Animal ethics was approved by The University of Queensland's Animal Ethics committee: Permit: SBS/425/2015. Collection in Moreton Bay was approved by the Fisheries Permit: QS2015/MAN340, and National Parks Permit: 18241.

I assessed if *B. cocosensis* had the capacity to acclimate to seasonal conditions by exposing them to extreme winter and summer thermal conditions that they experience at their thermal range limits. Cold treatment conditions mimicked winter (July) sea surface temperatures in (temperate) southern New South Wales (southern species range limit) and warm treatment conditions mimicked summer (January) sea surface temperatures in the (tropical) Northern Territory (northern Australian range limit) (Royal Australian Navy - sea surface temperature data base 2017). Fish were eased into thermal exposure conditions at a rate of 5°C per day from a starting temperature of 25°C. Warm fish (n = 32) were separated into six tanks (about five fish per tank) in a controlled temperature room set at 30°C, during the day aquarium heaters increased tank temperature to 35°C (heating started at 6am and stopped at 6pm). This daily variation in temperature mimicked changes in tide-pools with solar radiation, where the upper thermal experimental temperature was around 3°C above average summer rock-pool temperatures at Point Lookout QLD. Cold fish (n = 33) were separated into 6 tanks (about 5 fish per tank) in a controlled temperature room set at 12°C. Timed heaters increased tank temperature to 17°C between 6am and 6pm. Fish were exposed to the warm and cold thermal treatments for six weeks prior to testing. Both warm and cold treatment temperatures varied by 5°C on a daily basis so that magnitude of temperature fluctuation between treatments was identical. Both warm and cold treatments were set on a 12:12hr light-dark cycle, where

6am-6pm was light. Individuals were tested at random between the warm and cold treatments to account for any changes in metabolic rate based on circadian rhythm.

Fish were brought to acute testing temperatures at a rate of 3°C per hour (an appropriate rate to test the acute thermal performance of an intertidal fish (Schulte et al. 2011)). Only one performance trait (except maximum ventilation rate which was tested with maximum metabolic rate) was tested at one acute test temperature per day across all individuals. Test temperature and individual fish order was randomised for each performance measure.

Performance testing

Burst swimming speed was assessed at the test temperatures of 15°C, 22°C, 28°C and 33°C in a randomized order. Burst swimming responses were assessed in a 35 x 24 x 4 cm aquarium with a 20 cm measuring tape placed in the field of view for calibration. The aquarium was secured inside an arena made of plywood and a mirror angled at 45°. A Panasonic Lumix DMC-TZ40 camera recorded the image at 100 frames per second off the mirror. Burst swimming responses were elicited by gently tapping the fish's tail using a metal rod when fish were motionless on the bottom of the tank. Instantaneous swimming speed was extracted from videos using KINOVEA© (experimental version 0.8.25-x64 (www.kinovea.org)). Only swimming responses where the fish exhibited a C-start escape response were analysed. The point between the eyes of each fish was digitised to ensure a consistent point was tracked. A minimum of three burst responses was recorded for each fish and the maximum instantaneous speed for each escape response was determined. The maximum instantaneous speeds for each fish were averaged at each test temperature and used in statistical analysis.

I quantified the maximum ventilation and metabolic rate of each individual fish at 15°C, 22°C, 28°C, 33°C, and 36°C (and 12°C for ventilation rate only) in a randomized order. I tested maximum ventilation rate by chasing a fish (with a hand net) for a minute to elicit a sustained escape response. Fish were then immediately placed into a transparent petri dish and filmed ventrally for 60 seconds using a Panasonic Lumix DMC-TZ40 camera at a rate of 100 frames per second. Ventilation rate was then determined by visually counting the number of operculum ventilations over 60 seconds. Immediately after ventilation rate was filmed, fish were placed in a 133mL respirometer with a magnetic stirrer that produced a current within the respirometer for the fish to swim against (the current was turned up until fish could just maintain forward movement within the water column) to assess maximum metabolic rate. A PreSens Fibox 4 (POF-L2.5-1) with a polymer optical fiber and oxygen minisensor spots (batch ID – 140117-

001) was used to measure percentage air saturation (% a.s.) (one measurement/second) within the respirometer over five minutes of testing. After individual fish were tested they were then placed in an oxygen-saturated recovery bucket where the water temperature changed at a rate of 3°C per hour to bring the fish back to their treatment conditions. Three control (no fish) water samples were taken at each test temperature over five minutes to assess for any oxygen consumption or production within the bottle during testing. Metabolic rate (VO_2) was calculated using the following formula:

$$VO_2 = -1 \times \left((mf - mc) \div 100\right) \times V \times BO_2,$$

where *mf* was the slope of the percent air saturation consumed over time by *B. cocosensis* and *mc* was the slope of the percent air saturation consumed over time within the control respirometer (no fish). *V* was the volume of water within the respirometer and BO_2 represented the oxygen solubility of seawater at 35 ppt at each test temperature.

Routine metabolic rate of each fish was assessed at 15°C, 22°C, 28°C, 33°C and 36°C (in a randomised order) in darkened (black plastic covered) respirometers in a darkened laboratory. Fish were placed in 350 mL respirometers with open tops (covered with mesh to avoid fish escape) for one hour within a water bath prior to testing to allow oxygenated water flow to circulate the respirometers while the fish adjusted to test conditions and allowed metabolic rates to stabilize. Respirometer chambers were then sealed and placed on the magnetic stirrer at 100 rpm to create a weak current to ensure homogeneous oxygen saturation within the respirometer. Stirrers were necessary because bottom dwelling fish like gobies often sit still at the bottom of the respirometer and oxygen concentrations can become inconsistent throughout the respirometer. Oxygen consumption was measured every 15 minutes over one hour of testing using the PreSens Fibox 4. Aerobic scope was calculated by subtracting routine metabolic rate from maximum metabolic rate for each fish at each test temperature. I measured the mass (g) and standard length (mm) of each fish post thermal performance testing.

Statistics

I fitted linear mixed effect models using the nmle library (Pinheiro et al. 2018) in the R statistical package (R Core Team 2013) to assess if long-term warm or cold exposure had an effect on performance across a range of test temperatures. The acute effect of test temperature was modelled as a second-degree polynomial and set as a continuous variable (except for ventilation rate where it was set as a fixed factor due to the large amount of variance in the data set). The effect of acclimation was tested by assessing the interaction between test temperature and treatment. Fish length (burst swim speed only) or mass were set as covariates. Individual fish number was nested within tank number and these were set as random factors. Global model parameters were estimated according to Grueber et al. (2011). The library MuMIn (Bartoń 2013) was used to create a set of models from the global model using the dredge function. Akaike information criterion and the Akaike weight of each model was estimated to assess model fit probability. Multimodel averaging (Burnham and Anderson 2002) was used to calculate parameter averages from all models with Akaike weights above zero. Parameter values from the averaged models were used to calculate the most likely performance means for each treatment group across all test temperatures. I assessed the effect treatment (warm or cold-exposure) had across test temperatures on performance.

Individual thermal performance curve breadths were calculated using Gilchrist (1996) formula:

$$B = \sqrt{\sum_{i=1}^{N} \left[\frac{P_i(T_i - T_{opt)}}{P_{max}}\right]^2}$$

where N is the number of test temperatures, T_i is temperature i, P_i is performance at a certain test temperature, T_{opt} is the temperature at which performance is optimal, and P_{max} is the maximum performance rate. I compared average performance curve breadths between treatments for each performance measure using independent t-tests.

2.4 Results

Burst swimming speed

I found that long-term exposure to cold or warm thermal environments (treatment) affected the thermal sensitivity of burst swimming speed (Fig. 2.3a; Table 2.0 & 2.1) (test temperature × treatment (slope): estimate = -0.448 ± SE = 0.069, Z = 6.428, $P < 0.001$). The shape of the warm and cold exposed fish thermal performance curves, however, was not altered by thermal acclimation (test temperature × treatment (quadratic curvature): estimate = -0.018 ± SE = 0.073, Z = 0.236, $P = 0.814$). The cold-exposed fish swam 15% faster than warm-exposed fish when tested at 15°C on average, and warm-exposed fish swam 24% faster than cold-exposed fish when tested at 33°C on average. Differences in burst swimming speed were not apparent at test temperatures 22°C or 28°C (Fig 2.3a). Warm-exposed fish had thermal performance breadths of 11.63°C ± 0.55 and the cold-exposed fish had a mean thermal performance breadth of 12.43°C ± 0.76, differences in thermal performance breadth were not statistically significant (t_{40} = -0.870, P = 0.389). These results suggest that *B. cocosensis* have the capacity to acclimate burst swimming speed to extreme seasonal thermal conditions, however, the shape of their thermal performance curves is not altered by thermal acclimation.

Table 2.0. Top models of *B. cocosensis* burst swimming speed with fish length, treatment (thermal exposure) and test temperature.

Terms in the Model	***df***	**LogLik**	**AIC$_c$**	**ΔAIC$_c$**	***w*$_i$**
Length + poly(test temperature, 2) + treatment + poly(test temperature, 2) • treatment)	14	298.289	-565.8	0.00	0.923
Poly(test temperature, 2) + treatment + poly(test temperature, 2) • treatment)	13	294.616	-560.9	4.96	0.077

All models include fish number nested within tank number as random factors. Models are ranked according to AIC$_c$, and only models with w_i values greater than 0 are presented.

Table 2.1. Averaged linear mixed effect model summary of the response of burst swimming speed to fish length, treatment and test temperature.

Coefficients	**Estimate ± SE**	***Z***	***P***
Intercept	0.138 ± 0.036	3.837	< 0.001
Fish length	0.002 ± 0.0008	2.158	0.031
Poly(test temperature, 2) 1 (slope)	0.544 ± 0.055	9.872	< 0.001
Poly(test temperature, 2) 2 (quadratic curvature)	-0.176 ± 0.056	0.0568	0.0019
Treatment 2	-0.006 ± 0.017	0.290	0.772
Poly(test temperature, 2)1 • treatment (slope)	-0.448 ± 0.069	6.428	< 0.001
Poly(test temperature, 2)2 • treatment (quadratic curvature)	-0.018 ± 0.073	0.236	0.814

Maximum metabolic rate

Long-term exposure to cold or warm thermal conditions affected the thermal sensitivity of maximum metabolic rate (Fig 2.3b; Table 2.2 & 2.3) (test temperature × treatment (slope): estimate = -1.64 ± SE = 0.429, Z = 3.804, $P < 0.001$). Like burst swimming speed, the shape of the acute thermal performance curves for the warm and cold exposed fish was not altered by thermal acclimation (test temperature × treatment (quadratic curvature): estimate = -0.396 ± SE = 0.394, Z = 0.997, $P = 0.319$). At the test temperature 15°C cold-exposed fish had average maximum metabolic rates that were 33% greater than the average maximum metabolic rate of warm-exposed fish. At the test temperatures 33°C and 36°C the warm-exposed fish had average maximum metabolic rates that were 67% and 27% greater than the average cold-exposed fish maximum metabolic rates, respectively. There were no observable differences in maximum metabolic rate at 22°C or 28°C (Fig 2.3b). Cold-exposed fish had *predicted* (as we did not sample full performance curve) thermal performance breadths of 11.72°C ± 1.65, which was greater than the predicted thermal performance breadths of warm-exposed fish which was 6.95°C ± 0.8 ($t_{38} = -2.6$, $P = 0.013$).

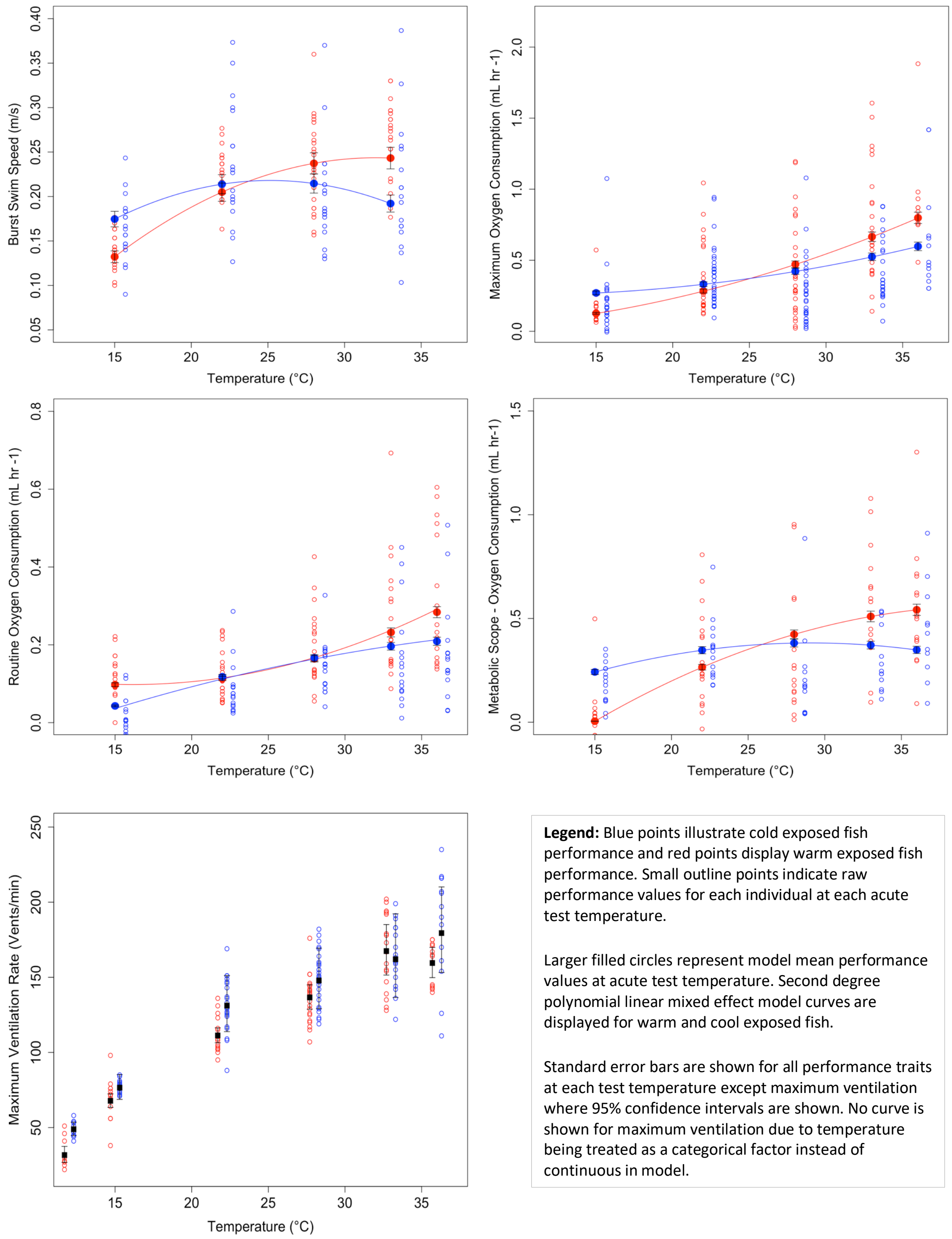
Table 2.2. Top models of *B. cocosensis* maximum metabolic rate (VO_2) with fish mass, treatment (thermal exposure) and test temperature.

Terms in the Model	***df***	**LogLik**	**AIC_c**	**ΔAIC_c**	**w_i**
Mass + treatment + test temperature + poly(test temperature, 2) • treatment)	15	47.167	-62.1	0.00	0.996
Mass + poly(test temperature, 2)	13	39.28	-50.9	11.22	0.004
Mass + treatment + poly(test temperature, 2)	12	36.69	-47.9	14.15	0.001

All models include fish number nested within tank number as random factors. Models are ranked according to AIC_c, and only models with w_i values greater than 0 are presented.

Table 2.3. Averaged linear mixed effect model summary of the response of maximum metabolic rate (VO_2) to fish mass, treatment and test temperature.

Coefficients	**Estimate ± SE**	***Z***	***P***
Intercept	0.168 ± 0.030	5.524	< 0.001
Mass	0.316 ± 0.026	11.686	< 0.001
Treatment 2	-0.026 ± 0.027	0.974	0.330
Poly(test temperature, 2)1 (slope)	2.75 ± 0.320	8.512	< 0.001
Poly(test temperature, 2)2 (quadratic curvature)	0.572 ± 0.299	1.961	0.049
Treatment • poly(test temperature, 2)1 (slope)	-1.64 ± 0.429	3.804	< 0.001
Treatment • poly(test temperature, 2) 2 (quadratic curvature)	-0.396 ± 0.394	0.997	0.319



Legend: Blue points illustrate cold exposed fish performance and red points display warm exposed fish performance. Small outline points indicate raw performance values for each individual at each acute test temperature.

Larger filled circles represent model mean performance values at acute test temperature. Second degree polynomial linear mixed effect model curves are displayed for warm and cool exposed fish.

Standard error bars are shown for all performance traits at each test temperature except maximum ventilation where 95% confidence intervals are shown. No curve is shown for maximum ventilation due to temperature being treated as a categorical factor instead of continuous in model.

Figure 2.3 Thermal dependence of warm and cold-exposed *Bathygobius cocosensis*. Part a.) Thermal dependence of burst swimming speed (warm exposed fish n = 25, cold exposed fish n = 20). Part b.) Thermal dependence of maximum metabolic rate (warm exposed fish n = 27, cold exposed fish n = 29). Part c.) Thermal dependence of routine metabolic rate (warm exposed fish n = 20, cold exposed fish n = 17). Part d.) Thermal dependence of metabolic scope (maximum-routine) (warm exposed fish n = 16, cold exposed fish n = 13). Part e.) Thermal dependence of maximum ventilation rate (warm exposed fish n = 23, cold exposed fish n = 28). Standard error bars are shown in black for each performance trait graph.

Routine metabolic rate

Long-term exposure to cold and warm thermal conditions did not affect the thermal sensitivity of routine metabolic rate (test temperature × treatment (slope): estimate = -0.082 ± 0.133, $Z = 0.608$, $P = 0.543$) (Fig 2.3c, Table 2.4 & 2.5). Thermal acclimation did not alter the shape of the acute thermal performance curve (test temperature × treatment (quadratic curvature): estimate = -0.338 ± 0.193, $Z = 1.742$, $P = 0.081$). At the test temperatures 15°C and 36°C, the warm-exposed fish had routine metabolic rates that were 704% and 65% greater than the average cold exposed fish routine metabolic rates, respectively. Routine metabolic rate did not differ between warm and cold-exposed fish at 22°C, 28°C, or 33°C. Warm-exposed fish had predicted thermal performance breadths of 11.35°C ± 1.0, which was greater than the predicted breadths of cold-exposed fish which was 7.39°C ± 0.53 ($t_{40} = 3.363$, $P = 0.002$).

Table 2.4. Top models of *B. cocosensis* routine metabolic rate (VO_2) with fish mass, treatment (thermal exposure) and test temperature.

Terms in the Model	***df***	**LogLik**	**AIC$_c$**	**ΔAIC$_c$**	***w*$_i$**
Mass + treatment + temperature + poly(test temperature, 2) • treatment	10	216.602	-411.9	0.00	0.846
Mass + treatment + poly(test temperature, 2)	8	212.218	-407.6	4.29	0.099
Mass + poly(test temperature, 2)	7	210.537	-406.4	5.46	0.055

All models include fish number nested within tank number as random factors. Models are ranked according to AIC$_c$, and only models with w_i values greater than 0 are presented.

Table 2.5. Averaged linear mixed effect model summary of the response of routine metabolic rate (VO_2) to fish mass, treatment and test temperature.

Coefficients	**Estimate ± SE**	***Z***	***P***
Intercept	0.056 ± 0.016	3.542	0.0004
Mass	0.125 ± 0.0104	11.919	< 0.001
Treatment 2	-0.029 ± 0.015	1.697	0.089
Poly(test temperature, 2)1 (slope)	0.87 ± 0.093	9.226	< 0.001
Poly(test temperature, 2)2 (quadratic curvature)	-0.252 ± 0.114	2.19	0.028
Treatment • poly(test temperature, 2)1 (slope)	-0.082 ± 0.133	0.608	0.543
Treatment • poly(test temperature, 2) 2 (quadratic curvature)	-0.338 ± 0.193	1.742	0.081

Aerobic Scope

Exposure to warm or cold thermal conditions affected the thermal sensitivity of aerobic scope (Fig 2.3d, Table 2.6 & 2.7) (test temperature × treatment (slope): estimate = -1.84 ± SE = 0.347, Z = 5.228, $P < 0.001$). Again, thermal acclimation did not alter the shape of the acute thermal performance curve (test temperature × treatment (quadratic curvature): estimate = 0.065 ± 0.314, Z = 0.205, $P = 0.837$). On average, at 15°C the cold-exposed fish had aerobic scopes that were 416% greater than the warm-exposed fish, and at 33°C and 36°C the warm-exposed fish had aerobic scopes that were 90% and 39% greater than the cold exposed fish, respectively. At 28°C there was no difference in aerobic scope between warm and cold-exposed fish. Cold-exposed fish had predicted thermal performance breadths of 9.83°C ± 1.46, which was greater than the predicted breadth of warm-exposed fish (6.26°C ± 0.9) ($t_{25} = -2.119$, $P = 0.268$).

Table 2.6. Top model of *B. cocosensis* metabolic scope (VO_2) with fish mass, treatment (thermal exposure) and test temperature.

Terms in the Model	***df***	**LogLik**	**AIC_c**	**ΔAIC_c**	w_i
Mass + treatment + poly(test temperature, 2) + poly(test temperature, 2) • treatment)	14	52.339	-73.4	0.00	1

All models include fish number nested within tank number as random factors. Models are ranked according to AIC_c, and only models with w_i values greater than 0 are presented.

Table 2.7. Linear mixed effect model summary of the response of metabolic scope (VO_2) to fish mass, treatment and test temperature.

Coefficients	**Estimate ± SE**	***Z***	***P***
Intercept	0.162 ± 0.037	4.33	<0.001
Mass	0.186 ± 0.022	8.346	< 0.001
Treatment 2	- 0.006 ± 0.039	0.145	0.885
Poly(test temperature, 2)1 (slope)	2.28 ± 0.237	9.523	< 0.001
Poly(test temperature, 2)2 (quadratic curvature)	-0.45 ± 0.215	2.07	0.0385
Treatment • poly(test temperature, 2)1 (slope)	-1.84 ± 0.347	5.228	< 0.001
Treatment • poly(test temperature, 2) 2 (quadratic curvature)	0.065 ± 0.314	0.205	0.837

Maximum ventilation rate

Exposure to warm or cold thermal conditions did not affect the thermal sensitivity of maximum ventilation rate (Fig 2.3e) (Table 2.8 & 2.9). Warm and cold-exposed fish had maximum ventilation rates that were significantly different from each other at 12°C ($F_{1,6}$= 8.105, P = 0.0293), 22°C ($F_{1,9}$= 8.44, P = 0.0174), 28°C ($F_{1,8}$= 8.824, P = 0.0179) and 36°C ($F_{1,9}$= 5.3703, p = 0.0457), however, there was no effect on the thermal sensitivity of performance as both warm and cold-exposed fish ventilations rates increased linearly at similar rates as temperature increased. There was no difference in maximum ventilation rate between warm and cold-exposed fish at 15°C ($F_{1,5}$= 3.507, P = 0.12) and 33°C ($F_{1,6}$= 0.3476, P = 0.577). There was no difference in the predicted thermal performance breadth for maximum ventilation rate between warm-exposed fish (10.62°C ± 0.5) and cold-exposed fish (11.49°C ± 0.56) (t_{27} = -1.132, P = 0.268).

Table 2.8. Top models of *B. cocosensis* maximum ventilation rate (vents/min) with fish mass, treatment (thermal exposure) and test temperature.

Terms in the Model	***df***	**LogLik**	**AIC_c**	**ΔAIC_c**	**w_i**
Mass + test temperature + treatment + poly(test temperature, 2) • treatment	8	22	-206.7	0	0.743
Test temperature + treatment + poly(test temperature, 2) • treatment	7	21	-204.6	2.13	0.257

All models include fish number nested within tank number as random factors. Models are ranked according to AIC_c, and only models with w_i values greater than 0 are presented.

Table 2.9. Averaged linear mixed effect model summary of the response of metabolic scope (VO_2) to fish mass, treatment and test temperature.

Coefficients	**Estimate ± SE**	***Z***	***P***
Intercept	3.485 ± 3.486	36.913	< 0.001
Mass	-0.046 ± 0.017	2.640	0.008
Test temperature 15°C	0.763 ± 0.010	7.513	< 0.001
Test temperature 22°C	1.257 ± 0.095	13.06	< 0.001
Test temperature 28°C	1.462 ± 0.092	15.801	< 0.001
Test temperature 33°C	1.667 ± 0.094	17.564	< 0.001
Test temperature 36°C	1.616 ± 0.103	15.575	< 0.001
Treatment	0.437 ± 0.096	3.962	< 0.001
Test temperature 15°C • treatment	-0.314 ± 0.110	2.827	0.005
Test temperature 22°C • treatment	- 0.269 ± 0.103	2.585	0.0097

Test temperature 28°C • treatment	-0.354 ± 0.099	3.549	< 0.001
Test temperature 33°C • treatment	-0.469 ± 0.105	4.427	< 0.001
Test temperature 36°C • treatment	-0.314 ± 0.116	2.685	0.00758

2.5 Discussion

Theory predicts that acclimation should not occur when daily thermal fluctuations are large or unpredictable and when animals have wide thermal performance curves (Tomanek and Somero 1999; Wilson and Franklin 2000; Gabriel 2005; Gabriel et al. 2005). Wide thermal performance curves, however, are likely to evolve when short-term thermal variability is large (Lynch and Gabriel 1987; Gabriel 2005; Wilson and Franklin 2000). Therefore, if long-term thermal varability remains predictable and short-term thermal fluctuations remain large, thermal performance curve shape should not be altered with thermal acclimation to seasonal change (see Healy and Schulte 2012). Thus, I predicted that *B. cocosensis* would possess the ability to thermally acclimate with seasonal change, despite inhabiting the thermally variable intertidal zone, and would have wide thermal breadths that would not be altered by thermal acclimation.

I found that *B. cocosensis* had the capacity to acclimate burst swimming speed, maximum metabolic rate and aerobic scope to seasonal thermal conditions and had wide thermal performance breadths for all performance traits, supporting my hypotheses. In addition, thermal acclimation did not alter the shape of thermal performance curves for any performance traits. These findings support the idea that animals are likely to evolve the capacity to acclimate when long-term (seasonal) variation is large but predictable, and maintain wide thermal breadths when short-term (daily) variation exists. Similar patterns have been observed in temperate intertidal fish such as killifish and sculpins, where they have the capacity to acclimate and possess wide thermal performance curves in variable daily and seasonal thermal conditions (Johnson & Bennett 1995; Temple and Johnston 1998; Fangue et al. 2006; Fangue et al. 2008; Fangue et al. 2011; Healy and Schulte 2012). Here I discuss the capacity *B. cocosensis* has to acclimate despite living in a rapidly fluctuating subtropical environment, and how thermal acclimation had little effect on altering the shape of thermal performance curves.

Bathygobius cocosensis possessed the ability to acclimate their burst swimming response to cold and warm thermal environments and had thermal breadths over a range of

11°C (exceeding the magnitude of average daily thermal variation they experience in nature). Maintenance of burst swimming speed with changing temperature is important for survival, as slower escape speeds can decrease the probability of escape from predators (Husak 2006a, b; Walker et al. 2005). Maintenance of broad thermal performance curves across acclimation treatments indicates that when organisms from variable thermal environments acclimate, their thermal optima and the location of thermal breadth may shift along the x-axis, but the curve itself is likely to retain its shape (also observed by Healy and Schulte (2012) in killifish aerobic scope). Again, these results are similar to those on other intertidal fish such as killifish and sculpins (Johnson and Bennett 1995; Temple and Johnston 1998), where they have the capacity to acclimate burst swimming speed and have relatively wide thermal performance curves. My findings support the idea that in *B. cocosensis* acclimation will occur even if both daily and seasonal thermal variations are large.

Bathygobius cocosensis also possessed the capacity to acclimate maximum metabolic rate to seasonal thermal conditions, where thermal performance curve shape remained wide and was not affected by seasonal acclimation. Acclimation of maximum metabolic rate allows physiological traits that are oxygen demanding (like sustained swimming and aerobic and anaerobic recovery) to be maintained under variable thermal conditions (Brett 1964). Although we found an acclimation response for maximum metabolic rate, routine metabolic rate did not show complete acclimation; the warm-exposed fish had higher metabolic rates on average than the cold-exposed fish at both warm and cold test temperatures. Aerobic scope, however, showed a similar acclimation response to maximum metabolic rate; this was not surprising because routine metabolic rate values were proportionally small compared to maximum metabolic rate values across all test temperatures. Like burst swimming speed and maximum metabolic rate, acclimation of aerobic scope also did not affect the shape of the thermal performance curve, like intertidal temperate killifish (Healy and Schulte 2012). The large aerobic scope observed shows that *B. cocosensis* have a large capacity to raise their metabolic rate for ecologically important activities (such as growth, maintaining body condition, escaping from predators and catching prey) aiding survival and reproduction. In addition, low aerobic scope at species thermal limits are known to constrict their geographical ranges (Wang & Overgaard 2007; Farrell 2009), hence, the maintenance of high aerobic scope across wide thermal breadths may facilitate the large geographic distribution observed in *B. cocosensis*. In contrast, we found no evidence of seasonal acclimation for maximum ventilation rate.

Potentially maximum metabolic rate and ventilation rate are not linked as tightly as one would expect with acclimation in *B. cocosensis*.

It was interesting that *B. cocosensis* had the capacity to acclimate to seasonal thermal conditions at the maximum and minimum of their thermal range (northern Northern Territory and southern New South Wales), which are likely to be outside of the usual thermal conditions that the Point Lookout population experiences in nature. As their thermal performance breadths were wide enough to encompass most of the seasonal thermal variation they experience in the subtropics, and curve shape did not change with long-term extreme thermal exposure, perhaps the Point Lookout population does not require acclimation to function and survive at Point Lookout. They may have retained their capacity to acclimate to seasonal species range extremes as an ancestral artefact, or perhaps it is a survival strategy for organisms with pelagic larval dispersal stages. *Bathygobius cocosensis* have a pelagic planktonic larval phase and have the potential to travel great distances from where they were hatched (Thia et al. 2018; da Silva et al. 2019b). Potentially organisms with dispersing larval phases are likely to maintain high levels of plasticity as their settlement location and therefore their juvenile and adult life stage thermal environment is unpredictable prior to settlement. In addition, the capacity to acclimate has also been observed, *B. fuscus*, a close relative of *B. cocosensis* in the tropical location of Sulawesi Indonesia, which would likely experience less daily and seasonal thermal variability than subtropical *B. cocosensis* populations (Eme and Bennett 2009). The ability *B. fuscus* had to acclimate was simultaneously surprising and intuitive, as small tropical species often have a limited capacity to acclimate (Rohr et al. 2018), but, *B. fuscus* inhabit the tidal flat region which experiences thermal variability despite being close to the equator. In addition, *B. fuscus* have species ranges that extend into the subtropics and have a planktonic pelagic larval phase. It would be interesting to compare the acclimation capacity between organisms with larval dispersal phases and organisms with limited dispersal in future studies.

The existing controversy on how thermal variability affects the co-evolution of thermal performance breadth and acclimation capacity has led us to ponder if thermal acclimation alters thermal performance curve shape; if thermal performance curve shape alters capacity to acclimate; or if once plasticity evolves it is retained (despite thermal performance curve shape). Our findings show that acclimation did not affect shape of thermal performance curve across many performance traits in a subtropical intertidal fish. Indeed, other studies have also shown evidence for thermal acclimation having little or no effect on altering the shape of thermal performance curves (i.e. Deere and Chown 2006; Healy and Schulte 2012; Pereira et al. 2017).

In addition, further studies have assessed if degree of thermal variability or stochasticity alters the shape of thermal performance curves in aquatic vertebrates or terrestrial isopods, however, thermal performance curve shape was not altered by increased thermal variability or stochasticity (Niehaus et al. 2011; Schuler et al. 2011). Perhaps thermal performance curve breadth determines the degree at which thermal optima can be shifted with thermal acclimation, rather than thermal acclimation shaping the acute thermal performance curve. Or maybe once thermal acclimation evolves (despite performance breadth) it is retained over many generations even if it is not required in a stable environment (see Brown and Feldmeth 1971). Sinclair et al. (2006) suggest an interesting alternative perspective that organisms in highly variable thermal environments are unlikely to favour a particular strategy of wide or narrow thermal breadths depending on thermal variability, but are more likely to evolve increased plasticity to allow efficient physiological responses to changes in environmental temperature.

The ability *B. cocosensis* has to acclimate may buffer them from increased temperatures associated with climate change, and some studies suggest that plasticity is already protecting some species from climate warming (Rohr et al. 2018; Riddell et al. 2018). Extreme heat events, however, are predicted to result in local population extinctions (Helmuth et al. 2006), as rate of thermal change is likely to be faster than rates of physiological change/acclimation (Peck et al. 2009). A meta-analysis that investigated the correlation between plasticity of upper thermal limits and latitude in terrestrial, aquatic and marine species, however, suggests that plasticity is unlikely to protect species from climate change as thermal plasticity in upper thermal limits is not correlated with latitude or seasonal variation across taxa (Gunderson and Stillman 2015). While upper thermal limits are important, many of the effects of climate change will occur through reductions in performance at sub-critical temperatures (Kingsolver et al. 2015). Interactions between acclimation capacity and thermal performance breadth will also be important in animal responses to climate change. For example, species that experience great thermal variation may not consistently have great acclimation capacities, but they might have wide thermal performance breadths (Wilson and Franklin 2000; Deere and Chown 2006), and thus may be buffered from climate warming through wide breadths rather than capacity to acclimate. Future climate change scenarios predict environments to become more thermally variable and stochastic (IPCC 2014; Vasseur et al. 2014), but predictions of species responses remain limited due to under sampling of physiological capabilities across taxa (Seebacher et al. 2015). The lack of understanding regarding the relationships between climatic variability and performance breadth and acclimation capacity will prove problematic when attempting to predict how different species and populations will respond with climatic change. Gaining a more comprehensive understanding of the interactions between fine-scale environmental variation, thermal breadths and acclimation capacities will help improve predictability of species responses to climate change. In addition, this knowledge will aid conservation biologists to determine which animals are likely to acclimate or adapt in a changing environment and which species should be the focus of conservation effort. Future studies should empirically test and model how the magnitude of thermal variability and predictability directly affects the co-evolution of thermal breadth, acclimation capacity, and fitness in changing environments through time.

Acknowledgments

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2.6 Supplementary material

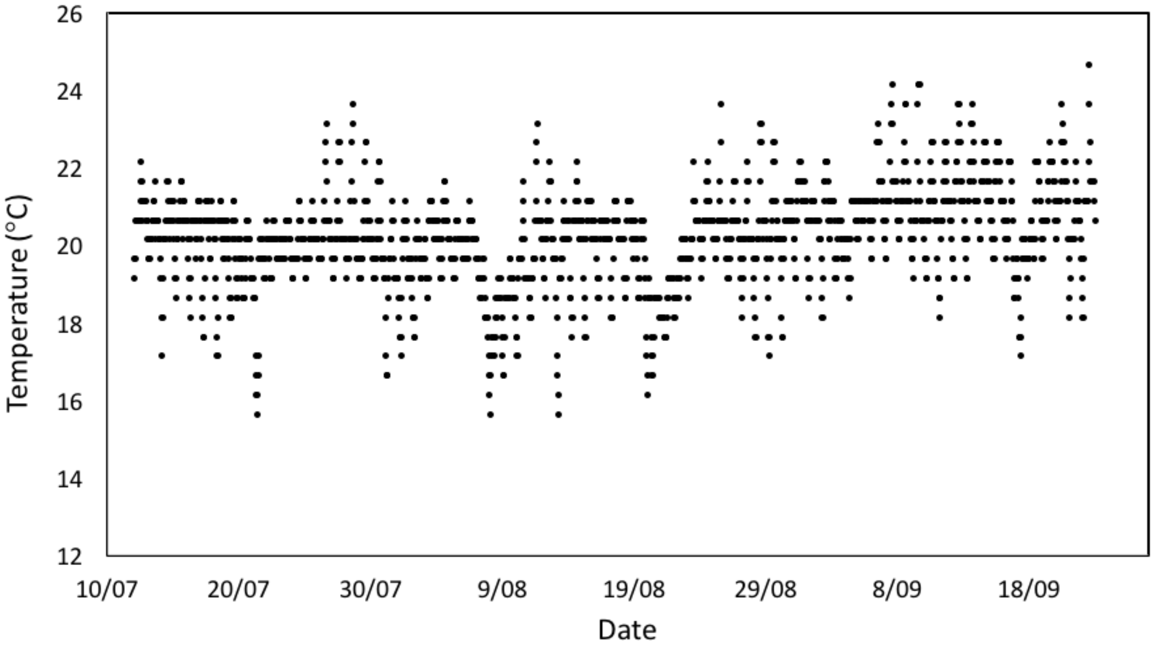


Figure 2.S1. Example of winter rock-pool temperature data from July to September 2018 at Point Lookout Queensland Australia (latitude = 27.43 ºS). Data logged in one hour intervals on 1-wire Thermochron iButtons®.

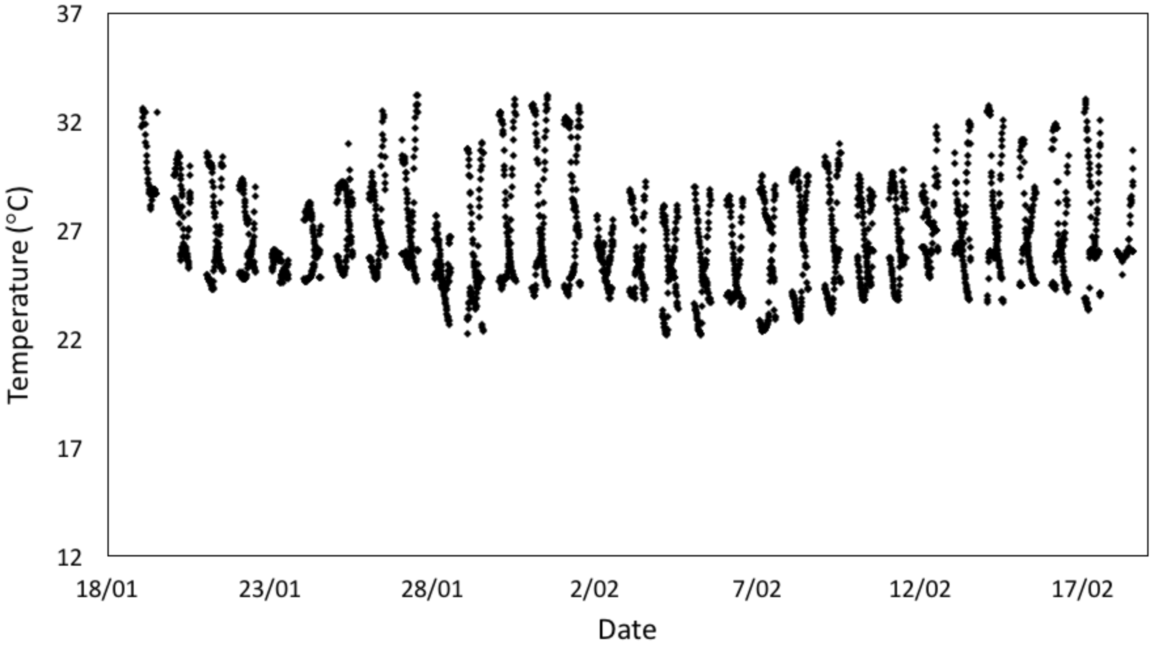


Figure 2.S2. Example of summer rock-pool temperature data from January to February (collected by L. Malard in 2015) one degree south of Point Lookout (latitude = 28.36 ºS). Data logged in 30 minute intervals on 1-wire Thermochron iButtons®.

Chapter 3

Intertidal gobies acclimate rate of luminance change with changes in long-term temperature to remain cryptic

3.1 Abstract

Many ectotherms have the ability to acclimate performance traits such as locomotion, metabolic rate and growth rate with changes in temperature. However, there is a need to understand how functional traits that are directly linked to survival, such as rate of colour change, respond to long-term changes in temperature. For example, acute changes in environmental temperature impact an animal's ability to change body colouration, affecting whether they are detected by predators when viewed against their background habitat. In this study, I assessed whether the rate of colour change is altered by long-term changes in temperature (such as seasonal variation) and whether rate of colour change can acclimate to seasonal thermal conditions. I used an intertidal rock-pool goby, *Bathygobius cocosensis,* to test this and exposed individuals to representative seasonal mean temperatures (16ºC or 31ºC, herein referred to cold and warm exposed fish, respectively) for nine weeks and then tested their rate of luminance change when placed on white and black backgrounds at acute test temperatures of 16ºC and 31ºC. When tested at 16ºC, the cold exposed fish changed luminance at faster rates than the warm exposed fish (against both black and white backgrounds), demonstrating that rate of luminance change can be adjusted to match long-term temperature exposure typical of seasonal temperature change. Similarly, when tested at 31ºC, the warm exposed fish changed luminance faster than the cold exposed fish when tested against the white background. Contrary to my predictions, warm exposed fish changed luminance at the same rate as cold exposed fish when placed against a black background at 31ºC, which was likely due to a stress darkening response in the cold exposed fish. I also found that *B. cocosensis* were able to match the black background better than the white background, which may be a stress response to quickly match crevices within rock-pools. I believe this is the first study to show that rate of colour change can acclimate to seasonal thermal conditions.

3.2 Introduction

Fluctuations in environmental temperatures impact the physiology of ectotherms and predicted changes in climate are likely to affect ectotherm survival, fitness and range distributions (Deutsch et al. 2008; Tewksbury et al. 2008; Peck et al. 2009; Hoffmann and Sgro 2011; Seebacher et al. 2015; Sinclair et al. 2016). Animals can respond to changing thermal environments within their lifetime by relocating (shifting their distribution or hiding in a cooler location) or by acclimating to the changed thermal conditions (Wilson and Franklin 2002; Angilletta 2009; Beaman et al. 2016). Acclimation, also known as reversible plasticity, occurs when an individual changes its underlying physiology to maintain its rate of performance in changed environmental conditions (Wilson and Franklin 2002; Beaman et al. 2016). We know that ectotherms can modify their physiological function in response to long-term changes in their thermal environment, including traits like metabolic rate, growth and locomotor performance, which allows maintenance of performance with changed thermal conditions (Angilletta 2009). For example, *Gambusia holbrooki* (mosquito fish) have the capacity to acclimate their muscle structure, swimming speeds, and mating success to seasonal thermal change (Hammill et al. 2004; Wilson et al. 2007). Although many studies have assessed thermal performance of locomotion and metabolism, we are yet to understand how long-term changes in temperature will affect behaviours such as physiologically-based anti-predator defences. For example, many animals use colour change to match the background against which it is viewed to prevent detection from predators or prey (camouflage), for thermoregulation and/or for communication (Cheney et al. 2017; Kodric-Brown 1998; Leclercq et al. 2010; Skold et al. 2013). The rate at which animals change colour can directly impact survival, as slow colour change may significantly increase the chance of being detected by predators (Stevens 2016; Duarte et al. 2017).

The rate at which animals change their body colouration is dependent on the mechanism used for colour change. Morphological colour change occurs over days or weeks during which the composition and number of chromatophores (skin pigment cells) is altered (Auerswald et al. 2008; Duarte et al. 2017). Physiological colour change occurs at a rapid rate (seconds/minutes) as pigment granules within chromatophores disperse or aggregate using neuromuscular movement, or more slowly via changes in hormone concentrations, such as melatonin (Skold et al. 2013; Caro et al. 2016; Stevens 2016; Duarte et al. 2017). Rapid changes in colour (hue and saturation), luminance (perceived brightness) or pattern can allow animals to match fine scale environmental heterogeneity, permitting animals to move more freely without suffering a mismatch with visual backgrounds (Duarte et al. 2017). Rates of physiological colour change in amphibians and fish are dependent on temperature, thus an animal's ability to match their background may be hindered by acute fluctuations in environmental temperature (Cole 1939; King et al. 1994; Camargo et al. 1999; Lin et al. 2009). It is unclear, however, how responses to longer-term temperature change might modulate the effects of acute temperature variation on the rate of colour change. For example, the depressive effects of cold temperature on the rate of colour change might be compensated for by phenotypic responses to longer-term exposure to cold temperatures (i.e. acclimation).

I tested these ideas by investigating an intertidal rock-pool fish, *Bathygobius cocosensis*, to assess whether they have the capacity to acclimate rate of luminance change to long-term seasonal change. *Bathygobius cocosensis* was chosen as my study organism because they are known to have the capacity to acclimate other physiological characteristics such as burst swimming speed, maximum metabolic rate, and metabolic scope, to seasonal thermal conditions (da Silva et al. 2019a). In addition, some goby species can alter their body colouration in terms of colour, luminance and pattern, to match their visual backgrounds and remain cryptic from predators within rock-pools (Stevens et al. 2014; Smithers et al. 2017). The capacity to rapidly alter colour and luminance in rock-pool fish is most likely mediated by physiological movement of pigment granules within melanophores (brown pigment cell) (Stevens et al. 2014; Smithers et al. 2017). Matching body luminance to that of the background luminance for camouflage is particularly important because motion, texture and shape of objects is largely conveyed by an animal's ability to perceive luminance contrast (Cronin et al. 2014; Ruxton et al. 2018).

Animals that live within the rocky-intertidal zone are ideal to assess how temperature alters short- and long-term thermal performance as they are exposed to diurnal tidal thermal fluctuations as well as seasonal thermal variation (Helmuth 1999; Helmuth and Hofmann 2001; Somero 2002). Intertidal organisms are often known as thermal generalists as they can withstand large fluctuations in temperature at rapid rates (Helmuth 1999; Helmuth and Hofmann 2001; Somero 2002; Griffiths 2003). For example, as the tide re-enters rock-pools after low-tide, rock-pools in temperate regions can change up to 10°C within a few minutes (Sidell et al. 1983; Fangue et al. 2008). In addition, rock-pools are comprised of many different background types (e.g. rock, sand or algae), where animals must change colour rapidly if they are to move and maintain crypsis in their environment (Stevens et al. 2014; Stevens 2016; Smithers et al. 2017). Rock-pool fish are exposed to a range of predator types; at low-tide fish in shallow pools are under predation pressure from birds whereas at high-tide fish are under predation pressure from larger teleost and cephalopods (Marsh 1986; Ellis et al. 2007; Stevens et al. 2014; Smithers et al. 2017).

I investigated whether *B. cocosensis* has the capacity to acclimate rate of luminance change to seasonal thermal conditions. I exposed half of the study population to warm conditions (31ºC) and the other half to cold (16ºC) conditions for nine weeks and then assessed rate of luminance change with each fish being tested against white and black backgrounds at both 16ºC and 31ºC acute test temperatures. To investigate how well *B. cocosensis* matched their background, I modelled how luminance changes might be perceived by a potential predatory fish using the visual sensitivities of the coral trout (*Plectropmus leopardus*) (Cortesi et al. 2016). I hypothesised that long-term exposure to different seasonal temperatures would enable *B. cocosensis* to acclimate rate of luminance change to different seasonal temperatures, when matching both darker and lighter background habitats colours. I predicted that fish exposed to warmer long-term temperatures would have a faster rate of change in warmer test temperatures compared to fish exposed to colder temperatures, and vice versa.

3.3 Methods

Bathygobius cocosensis (n = 80) were collected from the rocky inter-tidal zone at Point Cartwright, SE Queensland, Australia (26.6804°S, 153.1390°E) in November 2016. All fish were collected using a battery-operated bilge-pump and hand nets. Fish (22 – 47 mm standard length) were transported to The University of Queensland by vehicle in oxygen-saturated bags within an insulated container. Fish were anesthetized (0.3 x 10^{-3} mg L^{-1} of Aqui-S®) (Griffiths 2003b; Malard et al. 2016) and tagged dorsally between their head and first dorsal fin, with unique Visible Implant Elastomer (VIE) florescent subdermal tags (Northwest Marine Technologies®, Inc.) for individual recognition. VIE tags do not affect growth rates or survival in fish (FitzGerald et al. 2004). Fish were split into two treatments: warm fish (n = 40) were randomly allocated to 6 tanks (~ 7 fish per tank) set at 31°C and cold fish (n = 40) were randomly allocated to 6 tanks (~ 7 fish per tank) set at 16°C. Fish were held for nine weeks in treatment conditions before the start of luminance change testing, as it usually takes several weeks to acclimate to changed thermal conditions (Seebacher et al. 2005).

Before testing, fish were brought to test temperature at a rate of 5°C per hour, as deemed an appropriate rate for intertidal fish exposed to rapid fluctuations in temperature change (Schulte et al. 2011). Rate of change in dorsal body luminance was measured in a full factorial design where individuals from both warm and cold exposed treatment groups were tested against white and black backgrounds at both 16°C and 31°C. This resulted in four test groups: 16°C test temperature white background; 31°C test temperature white background; 16°C test temperature black background; 31°C test temperature black background. Fish from each treatment group were randomly assigned to test conditions (by temperature and colour) so order of test temperature or colour would not affect results (a fully crossed experimental design). Fish were tested in a controlled temperature room (16°C or 31°C) at The University of Queensland. Fish were tested under LED lights (Arlec 9 Watt Slim Bar Lights) to ensure environmental lighting conditions were consistent across trials.

Experimental preparation

Black and white containers were created by spray-painting matt black or white (MMP industrial Pty Ltd, Mulgrave, NSW, AUS) onto PVC plastic sheets to line the inside of testing containers. Once dry, the reflectance of the paint colour was measured using a spectrophotometer (USB2000 Ocean Optics©, Largo, FL USA) (https://oceanoptics.com/) with a 400µm bifurcated optical fiber cable and a PX-2 light source and calculated the average luminance of the white and black background. An intermediate grey background was produced by printing a grey 40% luminance background onto Kodak printing paper using an HP laser jet (HP LaserJet Pro 400 colour M451dn) printer and laminated it for waterproofing.

Experimental protocol

Fish were first placed into an 8cm x 15cm container with the intermediate grey background prior to testing for 10 min to ensure that all fish were exposed to the same conditions before testing. The fish were photographed with a calibrated Samsung NX1000 with Nikkor EL 80mm lens camera, with a fixed aperture, manual white balance settings, and with a colour standard (using Kodak colour squares) for image calibration within the photo frame. Photographs were taken in RAW format. Fish were swiftly (< 5 seconds) transferred with a small dip net into either the white or the black background container at either 16°C or 31°C and photographed immediately and once every 15 seconds for 10 minutes with the colour standard for image calibration within each photo. Each fish was tested separately to avoid stimulating a behavioural change in luminance. On the last day of testing, mass (g) and standard length (mm) of each fish was measured.

Image Analysis

I quantified body luminance of gobies through the eye of a potential predatory coral trout (*Plectropmus leopardus*). To do this, I used the Multispectral Image Analysis and Calibration (MICA) Toolbox plugin (Troscianko and Stevens 2015) in the program ImageJ (https://imagej.nih.gov/ij/). Images were calibrated and turned into 16-bit multispectral images using a grey standard with 73.3% and 5.1% reflectance (Fig. 3.1 & 3.2). Visual modelling using calibrated digital photography relies on detailed knowledge about the spectral sensitivities of both the potential animal viewer, the camera settings and the illumination within the image. Coral trout have single cones that contain a short wavelength sensitive pigment ($\lambda_{max} = 455$nm); and twin cones that exhibit broad absorbance spectra ranging from 507 to 532 nm (mean λ_{max} = 522 nm (Cortesi et al. 2016) so a mean λ_{max} = 522 nm for luminance perception was used. Although coral trout are unlikely to be the main predator of *B. cocosensis* along Australia's East coast, very few predatory fish spectral sensitivities are known, and their visual capacity is likely to be similar to other predatory teleosts (Losey et al. 2003).

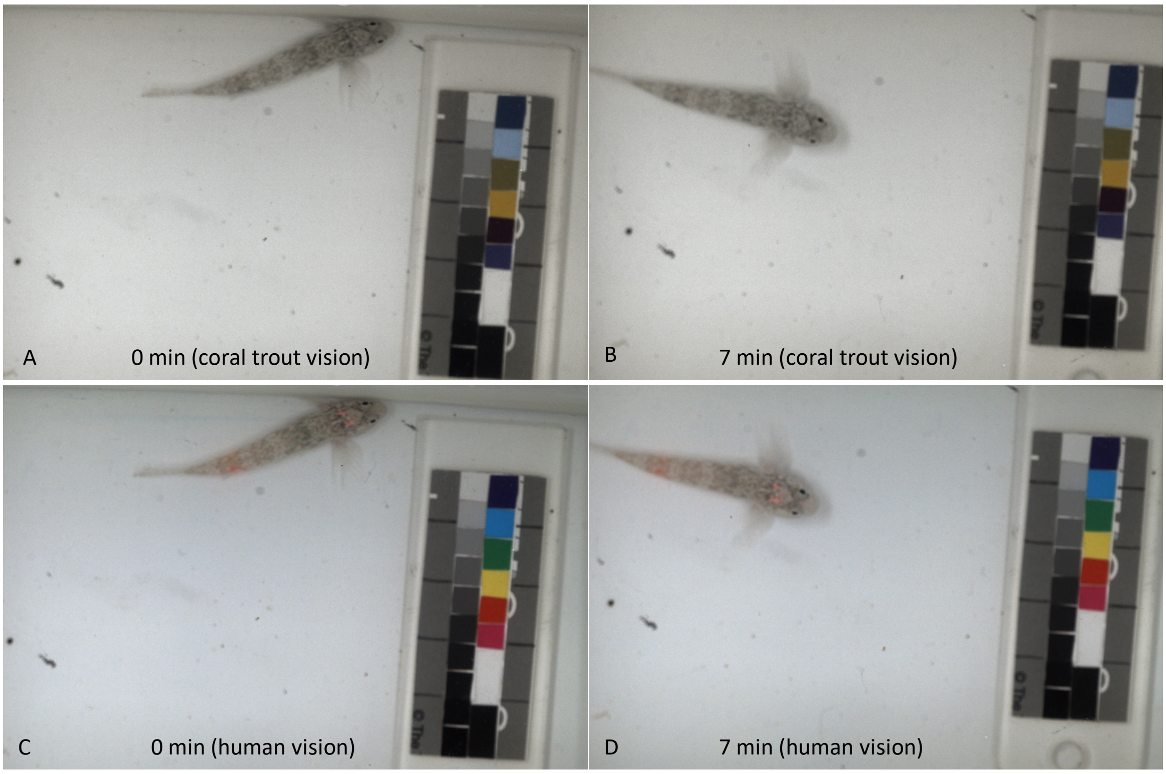


Figure 3.1 Panel A shows a modelled image of a goby from the cold exposure treatment at 16ºC immediately after it was placed in the testing chamber through the eye of a coral trout. Panel B shows how the goby has become slightly lighter over 7 minutes through the eye of a

coral trout (reconstructed RGB image). Panel C shows the same starting image but through the eye of a human and Panel D shows the goby after 7 minutes through the eye of a human.

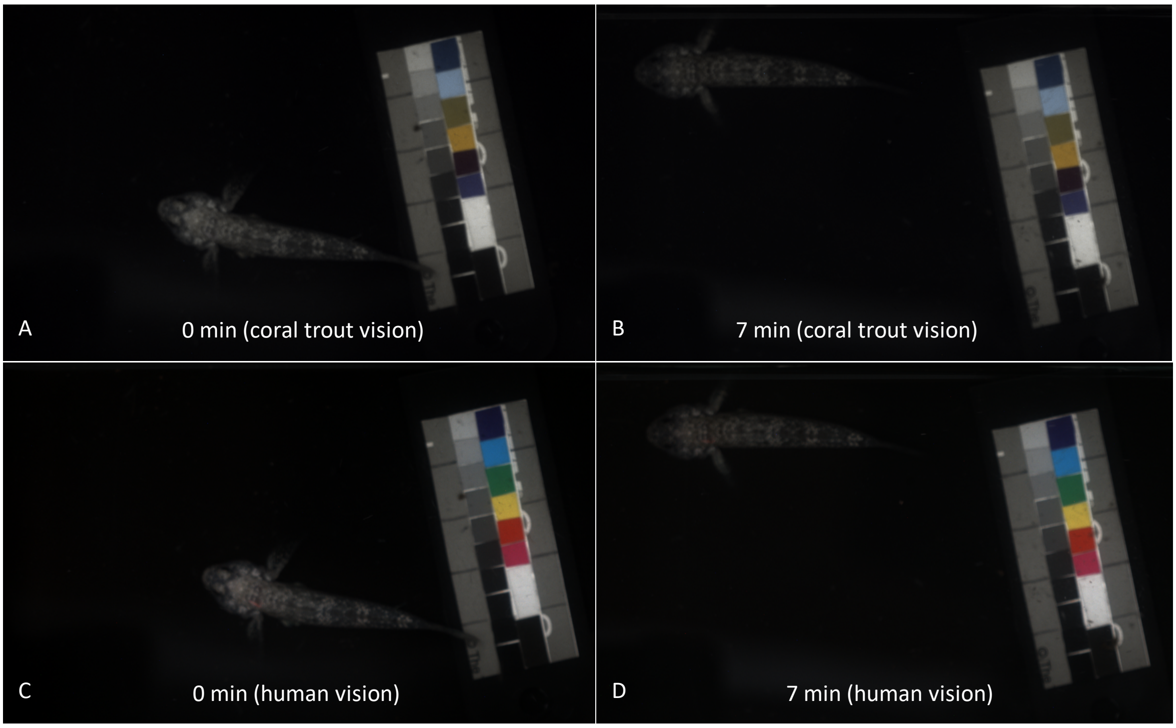


Figure 3.2 Panel A shows a modelled image of a goby from the warm exposure treatment at 31°C immediately after it has been placed in the testing chamber through the eye of a coral trout (reconstructed RGB image). Panel B shows how the goby has darkened itself over 7 minutes through the eye of a coral trout. Panel C shows the same starting image but through the eye of a human and Panel D shows the darkened goby after 7 minutes through the eye of a human.

Within the MICA toolbox I modelled photoreceptor stimulation. The visual model included information on the type of camera and lens we used to take the photos, the artificial lighting used to take the photographs, the coral trout visual sensitives, and the model illuminant (400-700nm daylight) (gobies are found in very shallow water so we modelled them being in a clear-sky daylight spectrum). Examples of modelled images of gobies against white and black backgrounds through coral trout vision in comparison to human vision are shown in Figures 3.1 and 3.2.

Using the visual models for each photograph, I assessed the luminance of each goby at each time point as the average coral trout double cone stimulation across our region of interest

(ROI). I drew an ROI around the inside edge of each goby (a triangle from their pectoral fins down their body and tail to avoid the elastomer tag) and calculated the median luminance. As thousands of photos were generated for this experiment (n > 6000), I used an ImageJ based script to partially automate this process for each photograph (ImageJ script in supplementary data).

I calculated the achromatic perceptual distance (ΔS) (how closely the goby matches its background through the eye of a coral trout), between each goby and its visual background using the receptor noise limited model (Vorobyev and Osorio 1998). The receptor noise limited model assumes the inherent noise in photoreceptors ultimately limits contrast perception. Animal-background contrast was measured at each time point and against white and black backgrounds to assess the achromatic contrast between the goby and its background throughout the duration of testing. Calculations of achromatic contrast (ΔS) are routinely conducted using equation 7 from Siddiqi et al. (2004) and dividing it by the noise to signal ratio (weber fraction ($\omega i = 0.05$)) (Stuart-Fox et al. 2003; Stevens et al. 2015) *(eq. 1)*

$$\Delta S = (\ln(l1) - \ln(l2)) \div \ \omega i) \qquad \text{(equation 1)}$$

where, *l1* is the background luminance (white or black background) and *l2* is the luminance of the goby polygon (ROI). The difference in achromatic perceptual distance was calculated at the start and the end of each rate of luminance test to assess if the total change in luminance over time would be noticeable to a coral trout. Threshold values that determine if a change in perceived achromatic distance are distinguishable (i.e. whether goby be distinguished from its background) have been argued, with studies suggesting values below 1 ΔS are indistinguishable (Siddiqi et al. 2004) and other studies suggesting values below 3 ΔS as indistinguishable (Stournaras et al. 2015; Abernathy et al. 2017). The higher the change in achromatic perceptual distance the more obvious the goby is against its background, increasing the likelihood of detection by a coral trout.

Statistical analysis

To assess whether long-term exposure to warm or cold thermal conditions altered the rate at which gobies changed their luminance against white and black backgrounds, I ran a linear mixed effect model using the nmle package (Pinherio et al. 2018) in the statistical program R version 1.1453 (R Core Team 2013). Long-term exposure treatment, background colour (white or black), and test temperature (16ºC or 31ºC) were set as fixed factors. Fish mass, body length and test time were included as covariates. Test time was also set as a random variable (slope) to identify variation in colour change rates, and fish number was set as a random variable. These random factors were generated for the slope and intercept to be correlated in the model, as the correlated random factor model had a better model fit than the model without a slope and intercept correlation (determined through AIC (Akaike information comparison)). I also tested interactions between treatment and test time, test temperature and background colour and treatment, test temperature and background temperature. All variables were left within the model as I believe that they are all ecologically and experimentally important.

The data was subdivided into four groups (Black 16ºC, White 16 ºC, Black 31 ºC, White 31ºC) and a slope was generated (rate of luminance change over time) for each warm and cold exposed fish in each of the experimental groups. The slopes from the first four minutes of testing were used (rather than the full ten minutes of testing) as the slopes were steepest over this time and then stabilized over the rest of the testing period. Linear models were used to assess the effect treatment and fish mass had on rate of luminance change against each background at each test temperature. Positive slopes indicate a brightening in fish skin pigment and negative slopes indicate darkening.

Mean change in achromatic perceptual distance values were calculated at the start and end of each luminance change trial, where the total change in mean change in achromatic distance over time was then calculated. I used a linear mixed effect model to assess if acclimation treatment altered total change in achromatic perceptual distance in each background colour and test temperature treatment.

3.4 Results

Long-term thermal exposure (warm or cold exposure) significantly impacted the rate of luminance change in *B. cocosensis* against white and black backgrounds (Fig 3.3 & 3.4) (Table 3.0) (Estimate = 0.027 ± 0.0035, $df = 5652$, $t = 7.942$, $P < 0.001$). Specifically, the warm exposed fish had faster rates of luminance change (within the first four minutes of testing) than the cold exposed fish when tested at 31ºC, and the cold exposed fish had faster rates of luminance change than the warm exposed fish when tested at 16ºC. These findings indicate that *B. cocosensis* acclimated rate of luminance change to their long-term thermal exposure conditions. I now explore each experimental group (test temperature and background colour) separately.

Table 3.0. Linear mixed effect model summary of the response luminance (% lumins) to the experimental coefficients (full model before post-hoc sub-setting to individual experimental groups).

Coefficients	***Estimate ± SE***	***df***	***t***	***P***
Intercept	0.098 ± 0.006	5652	17.12	0.000
Mass	-0.0004 ± 0.005	5652	-0.07877	0.937
Test time	-0.0018 ± 0.0003	5652	-6.228	< 0.000
Treatment (long-term exposure)	-0.0052 ± 0.0059	50	-0.8668	0.3902
Test temperature	-0.0197 ± 0.0009	5652	-20.535	< 0.000
Background colour	0.214 ± 0.0019	5652	109.60	< 0.000
Treatment * Test temperature	0.0116 ± 0.0014	5652	8.31445	< 0.000
Test temperature * background colour	0.0083 ± 0.0027	5652	3.07763	0.0021
Treatment * background colour	0.0165 ± 0.0025	5652	6.375	< 0.000
Treatment * test temperature * background colour	0.027 ± 0.0035	5652	7.942	< 0.000

16°C test temperature

The long-term cold (16°C) exposed fish turned lighter (against a white background) at a faster rate than the warm exposed fish when tested at the 16°C test temperature (over the first four minutes of testing) ($F_1 = 5.143$, $P = 0.029$) (Fig 3.3a & 3.4a) (Table 3.1). Fish mass had no effect on rate of luminance change (Table 3.1). Fish length was omitted from the model because it was correlated with fish mass. After four minutes of testing the cold exposed fish had luminance values of 33.3 ± 0.014% on average and the warm exposed fish had luminance values of 29.5 ± 0.017% on average, meaning the cold exposed fish became lighter than the warm exposed fish on average (when tested at 16°C against a white background).

Table 3.1. Linear model summary of the response of rate of luminance change to long-term thermal exposure and fish mass when tested at 16°C against a white background.

Coefficients	***df***	***F***	***P***
Mass	1	0.63	0.431
Exposure group	1	5.14	0.029
Residuals	37		

The cold exposed fish were also able to turn darker (against a black background) at a faster rate than the warm exposed fish when tested at the 16°C test temperature (F_1 = 10.74, *P* = 0.002) (Figure 3.3c & 3.4c) (Table 3.2). Fish mass had no effect on rate of luminance change (Table 3.2). Here, both warm and cold exposed fish showed a reduction in luminance over the first four minutes of testing. The cold exposed fish had luminance values of 7.6 ± 0.006% on average and the warm exposed fish had luminance values of 8.9 ± 0.006% on average, meaning the cold exposed fish were slightly darker after four minutes than the warm exposed fish.

Table 3.2. Linear model summary of the response of rate of luminance change to long-term thermal exposure and fish mass when tested at 16°C against a black background.

Coefficients	***df***	***F***	***P***
Mass	1	0.15	0.701
Exposure group	1	10.74	0.002
Residuals	35		

31°C test temperature

Long-term warm (31°C) exposed fish were able to turn lighter at a faster rate than the cold exposed fish against a white background when tested at 31°C, during the first four minutes of testing (F_1 = 4.19, *P* = 0.047) (Table 3.3) (Figure 3.3b & 3.4b). Although the warm exposed fish changed luminance at a faster rate than the cold exposed fish, the cold exposed fish had higher luminance values 33.6 ± 0.02% than the warm exposed fish, 32.0 ± 0.02%, after four minutes of testing. Again, fish mass had no effect on rate of luminance change (Table 3.3).

Table 3.3. Linear model summary of the response of rate of luminance change to long-term thermal exposure and fish mass when tested at 31°C against a white background.

Coefficients	***df***	***F***	***P***
Mass	1	0.15	0.699
Exposure group	1	4.19	0.047
Residuals	40		

The long-term warm exposed fish were able to turn darker at a slightly faster rate than the cold exposed fish when tested against a black background at 31°C (Figure 3.3d & 3.4d), however the difference was not statistically significant ($F_1 = 1.75$, $P = 0.193$) (Table 3.4). After the first four minutes of testing the warm exposed fish became darker than the cold exposed fish (Fig 3.4d) Mass had no effect on rate of luminance change (Table 3.4). On average, the warm exposed group was 6.6 ± 0.005% bright and the cold exposed group was 6.9 ± 0.007% bright after four minutes of testing, indicating that the warm exposed group was slightly darker than the cold exposed group.

Table 3.4. Linear model summary of the response of rate of luminance change to long-term thermal exposure and fish mass when tested at 31°C against a black background.

Coefficients	***df***	***F***	***P***
Mass	1	0.97	0.331
Exposure group	1	1.75	0.193
Residuals	40		

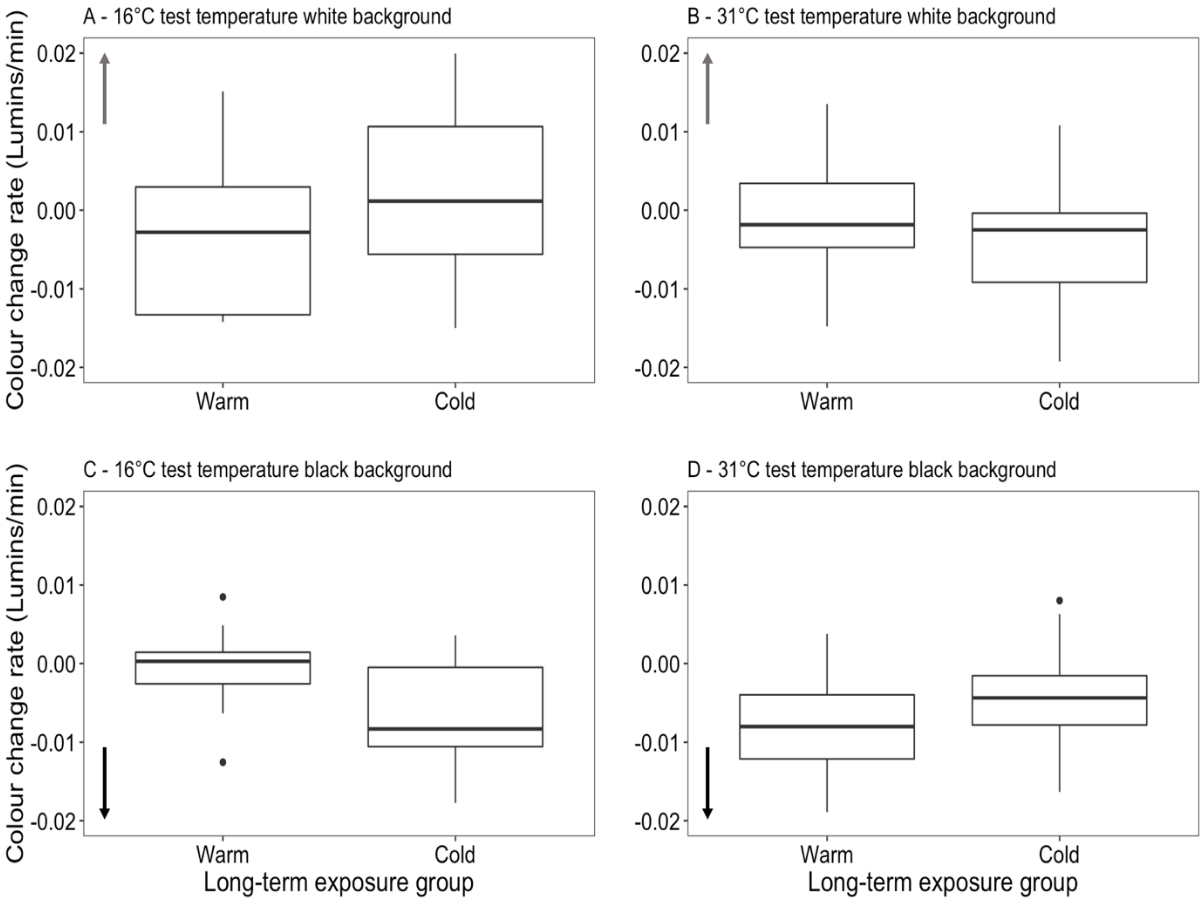


Figure 3.3 Rate of change in luminance for warm and cold exposed fish in A) against a white background in 16ºC; B) against a white background in 31ºC; C) against a black background in 16ºC; D) against a black background in 31ºC. Rates below zero indicate a darkening response and rates above zero indicate a pigment brightening. Arrows indicate if a positive or negative slope would be required for *B. cocosensis* to more closely match their background in terms of luminance.

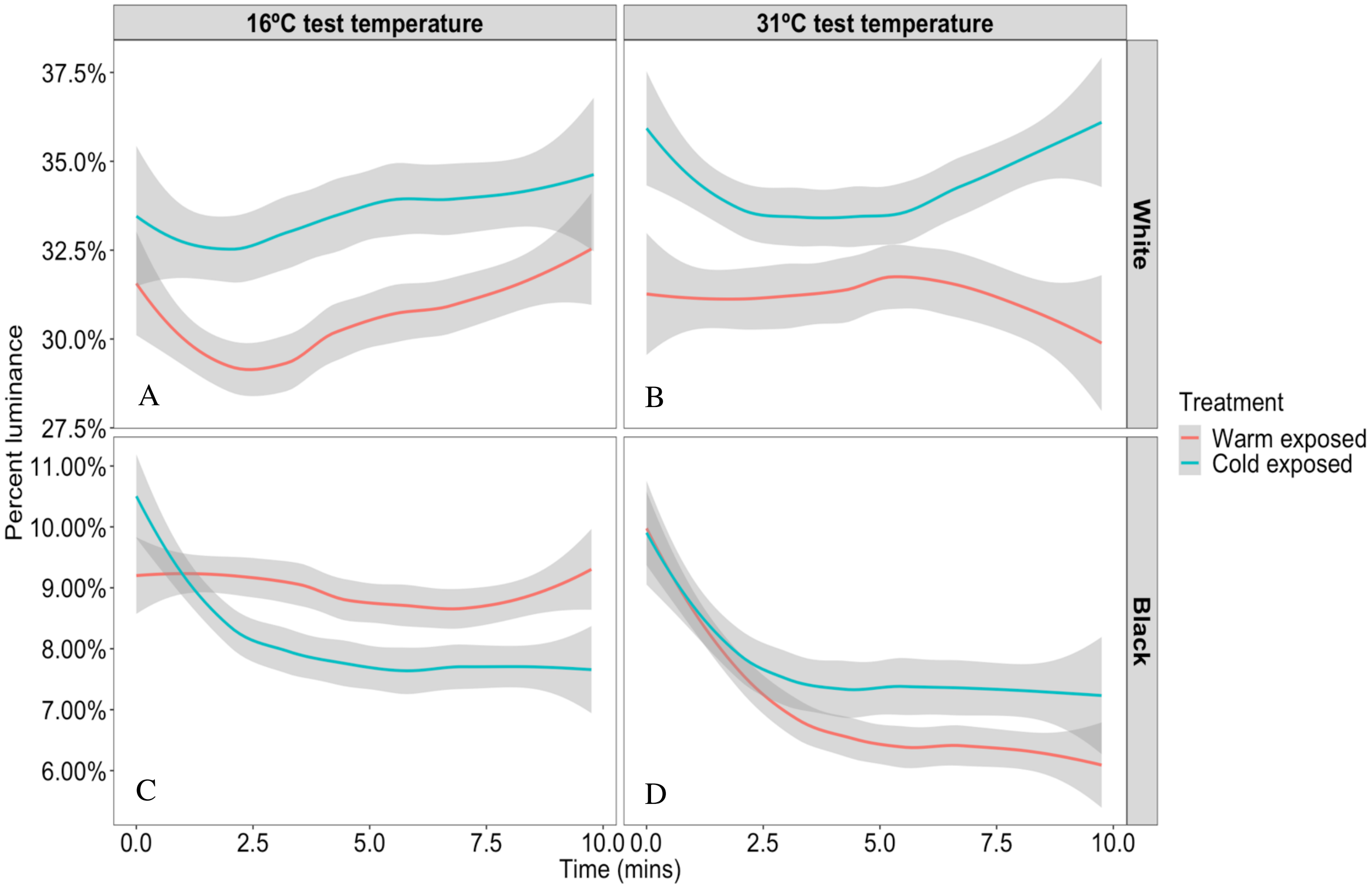


Figure 3.4 Average change in luminance (with standard error in grey) over 10 minutes for the long-term warm and cold exposed fish. A) against a white background in 16ºC; B) against a white background in 31ºC; C) against a black background in 16ºC; D) against a black background in 31ºC. Increases in luminance indicate a lightening in pigment and decreases in luminance indicate a darkening in pigment.

On average gobies were able to turn darker faster than lighter (Figure 3.4) (Table 3.5). In addition, gobies were able to change luminance (in the direction of the background luminance) faster at 31ºC than 16ºC (Fig 3.3 & 3.4) (Table 3.5).

Table 3.5. Average rates of colour change (lumins per minute) for each exposure group in each test treatment during the first four minutes of testing. * indicates correct colour change direction for background matching.

Test temperature	Colour	Warm exposed average rate of luminance change (lumins/min) ± SE	Cold exposed average rate of luminance change (lumins/min) ± SE
16°C	White	-0.0066 ± 0.003	0.0035* ± 0.003
31°C	White	-0.0033 ± 0.002	-0.0081 ± 0.003
16°C	Black	-0.0004* ± 0.001	-0.0068* ± 0.002
31°C	Black	-0.0084* ± 0.001	-0.0084* ± 0.002

Starting luminance

As the fish are partially translucent, their luminance was affected by the background colour (white or black) that they were tested on (Fig. 3.4). When placed on the white background, at the start of the 10 minute test period, the fish were 32.3 ± 0.006% luminance on average and when placed against a black background, fish were 9.9 ± 0.004% luminance on average and got darker over time (Fig. 3.4). The variance in starting luminance (time = 0 mins) against the white background (variance = 0.006) was greater than the starting luminance when tested against the black background (variance = 0.0009) irrespective of treatment. To assess if warm or cold exposure influenced starting luminance, we ran a linear model and found that exposure group (warm or cold) altered starting luminance when placed against a white background (Estimate = 0.04 ± 0.12, t = 3.34, *P* = 0.001), and had a small, but significant effect on starting luminance against black backgrounds (Estimate = 0.009 ± 0.005, t = 2.04, *P* = 0.043). We quantified the luminance of each fish when they were placed against the grey background prior to testing, here we found that the cold exposed fish had slightly higher luminance values on average, however, the difference between treatment groups was not quite statistically significant (Estimate = - 0.015 ± 0.008, t = -1.884, *P* = 0.063) (Supplementary Figure 3.S1).

Coral trout visual perception

The long-term exposure group (warm or cold) altered the total change in achromatic perceptual distance over time against white and black backgrounds (ΔS at 0 mins – ΔS time at 10mins) (Figure 3.5; Table 3.6). When tested against the black background, all experimental groups, except the warm exposed fish at 16ºC, showed an ecologically significant change in luminance through the eye of a coral trout (ΔS > 3) (Table 3.6). These results indicate that *B. cocosensis* are undergoing ecologically significant dark background matching in terms of decreasing luminance, which would likely make them less obvious to a coral trout. Their end change in achromatic perceptual distance values, however, remain greater than 3, meaning that they are still distinguishable from their background. An example of *B. cocosensis* showing very little change of luminance is shown in Figure 3.1 and an example of noticeable luminance change is shown in Figure 3.2.

Table 3.6. Average achromatic perceptual difference (ΔS, difference between goby luminance and background luminance) at the start, end, and total change in achromatic perceptual distance over test time against a **black** background over testing through the eye of a coral trout. ΔS above 3 indicate that a noticeable change in goby appearance would have occurred to the coral trout.

Treatment	**Start μ ΔS ± SE**	**End μ ΔS ± SE**	**Total Δ S**
Warm exposed 31ºC test temperature	31.80 ± 0.83	20.99 ± 1.45	**10.81***
Cold exposed 31ºC test temperature	32.23 ± 1.23	25.46 ± 1.29	**6.77***
Warm exposed 16ºC test temperature	30.9 ± 0.95	31.3 ± 0.82	**-0.4**
Cold exposed 16ºC test temperature	34.1 ± 0.93	26.40 ± 1.15	**7.7***

When tested against white backgrounds, *B. cocosensis* have less significant changes in achromatic perceptual distance between the start and end of testing than when tested against black backgrounds (Table 3.7). All of the total changes in achromatic perceptual distance against white backgrounds were less than one ΔS with no distinguishable change in luminance of the gobies against white backgrounds over the 10 minute testing period through the eye of the coral trout. Animal-background contrast in all treatments remained above 3 ΔS at all times.

Table 3.7. Average change in achromatic perceptual distance at the start and end of testing, and the total change in achromatic perceptual distance over test time against a **white** background through the eye of a coral trout.

Treatment	Start μ ΔS ± SE	End μ ΔS ± SE	Total ΔS
Warm exposed 31°C test temperature	17.05 ± 0.68	17.57 ± 0.55	**-0.52**
Cold exposed 31°C test temperature	14.08 ± 0.73	14.36 ± 0.60	**-0.28**
Warm exposed 16°C test temperature	17.01 ± 0.73	16.34 ± 0.47	**0.67**
Cold exposed 16°C test temperature	15.36 ± 0.99	14.93 ± 0.67	**0.43**

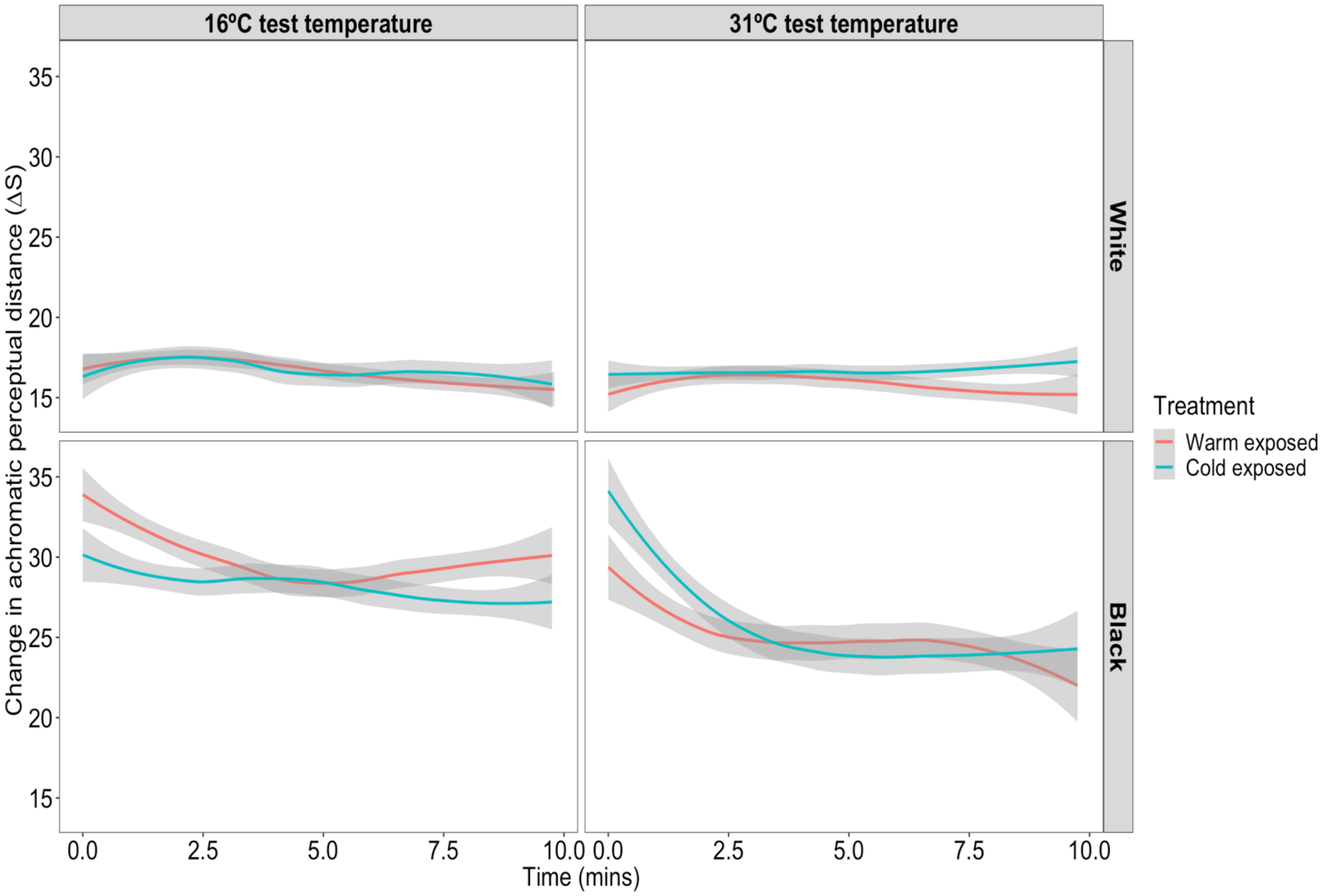


Figure 3.5 Change in achromatic perceptual distance over test time in the warm and cold exposed fish in each test treatment. Decreases in perceptual distance indicates improved background matching. Changes over ΔS 3 indicate that a change in luminance would be noticeable to the coral trout.

3.5 Discussion

Intertidal gobies can change luminance to match dark or light backgrounds, and here we have shown that the rate of luminance change can acclimate to long-term temperature change typical of that experienced across seasons. At 16ºC, cold exposed fish: 1) became lighter at a faster rate on a white background than warm exposed fish and 2) became darker at a faster rate when placed against a black background than the warm exposed fish (Fig 3.3a & c). Similarly, when tested at 31ºC, warm exposed fish: 3) became lighter at a faster rate than the cold exposed fish, and 4) became darker at a slightly faster rate than the cold exposed fish when tested against a black background (Fig 3.3b & d). In addition, total change in achromatic distance between the goby and the black background acclimated to seasonal temperatures, where the warm exposed fish better matched the black background when tested at 31ºC and the cold exposed fish better matched the black background when tested at 16ºC. Overall, these findings support the hypothesis that *B. cocosensis* have the capacity to acclimate rate of luminance change to seasonal temperatures, which was predicted based on their ability to acclimate other ecologically important traits such as burst swimming speed and maximum metabolic rate (da Silva et al. 2019).

This is the first study to examine if long-term thermal change (nine weeks) can alter the rate at which an animal is able to change its luminance. The phenotypic responses to longer-term temperature at least partly compensate for the potentially negative effects of short-term (acute) temperature change on the ability to change luminance for background matching. In other words, the capacity *B. cocosensis* have to acclimate rate of luminance change to seasonal temperatures is likely to be an adaptive response to remain inconspicuous against different background in heterogeneous rock-pools. Changes in seasonal thermal means in south-east Queensland are predictable (da Silva et al. 2019a), and these predictable shifts in seasonal temperature are likely to enable thermal acclimation of rate of luminance change for background matching to be beneficial to survival in this context. Short-term thermal fluctuations in rock-pool temperature, however, are likely to alter capacity to change luminance rapidly to match their visual background. For example, fish tested in temperatures they were not long-term exposed to had reduced background matching capabilities. On average, rate of background matching was more rapid in warm test temperatures than cold test temperatures. Thus, as the tide ebbs up the intertidal zone and rapidly fills warmed rock-pools with cool water with the rising tide, the capacity individuals have to match their background may be reduced. Therefore, the ability individuals have to respond to rapid thermal changes (short-term) is limited, but their capacity to shift their physiology to better perform with seasonal means (long-term) is likely to be beneficial to survival in the intertidal zone.

Interestingly, I found potentially maladaptive pigment darkening responses when fish were placed into test temperatures that they were not acclimated to (see Figs 3.3 & 3.4). The observed darkening responses (when an increase in luminance rather than decrease would improve background matching) may be explained by malfunctioning of chromatophore physiology at non-optimal temperatures. Aggregation of chromatophores through neuromuscular activity (rapid physiological colour change mechanism) requires ATP (Sköld et al. 2016) and is hypothesized to be more energetically taxing than dispersing them, as the contraction/aggregation of muscles requires more energy than relaxing/dispersing them (Curtin and Woledge 1974; Barclay 1994). This suggests that chromatophores are unable to aggregate their pigments optimally in temperatures that they have not thermally acclimated to, and as a result, lose control and disperse their pigments resulting in a darker phenotype.

I also found that *B. cocosensis* turn darker at a faster rate on average than brighter. These results are consistent with other studies on another goby species, *Gobius paganellus*, and a species of bullfrog, *Rana catesbeina*, where they also turn darker faster than lighter when placed against different background colours (Camargo et al. 1999; Smithers et al. 2018). The quick darkening response in bullfrogs is hypothesized to be due to increases in melanophore stimulating hormone (a darkening agonist), which induces chromatophore dispersion, whereas skin lightening is likely to result in reductions in the hormone concentration (Camargo et al. 1999). Rapid changes in colour (seconds), are more likely to occur from neuromuscular contraction or relaxation, or by a combination of both neural and hormonal signals in fish (Sköld et al. 2016; Duarte et al. 2017). Therefore, rapid darkening in *B. cocosensis,* may be due to chromatophores dispersing more rapidly than aggregating, especially if chromatophore dispersion is less energetically costly than aggregation. In addition, as rock-pool fish are likely to escape to rocky-crevices when threatened by predators, rapid darkening could be an adaptive response enabling rock-pool fish to become indistinguishable from rocky-hiding spots. Conversely, a study on *Fundulus* (killifish), a temperate intertidal fish, had a higher capacity to turn lighter at a faster rate than darker (Cole 1939). Perhaps killifish have evolved the capacity to turn light quickly in white sandy areas, to be less conspicuous to predators and prey while basking in the sun and hunting for small invertebrates. *Bathygobius cocosensis* have been observed to match sandy and pebbly rock-pool habitats well during field surveys, however, *B. cocosensis* are rarely exposed to very bright white backgrounds in their natural habitat. Mottled sandy coloured habitats are more common "light" habitats in the wild. Rock-pool gobies have been found to use behavioural preferences to choose backgrounds that they are better for camouflage (Smithers et al. 2018), suggesting that perhaps *B. cocosensis* avoid very light white backgrounds in nature as they have a limited capacity to turn light. In addition, *B. cocosensis* have been observed to take on a banded phenotype, which might help them camouflage in sandy or pebbly environments (Fig 3.6). The darkened band may also explain the average slight darkening response that some of the warm exposed gobies show when placed against white backgrounds in 31ºC. A light "sand coloured" or "mottled pattered" background, could be used instead of a white background to better simulate a natural "light" luminance change response in *B. cocosensis* in future experiments. It would also be beneficial to assess skin patterning in future studies to determine how patterning helps maintain crypsis within mottled patterned environments.

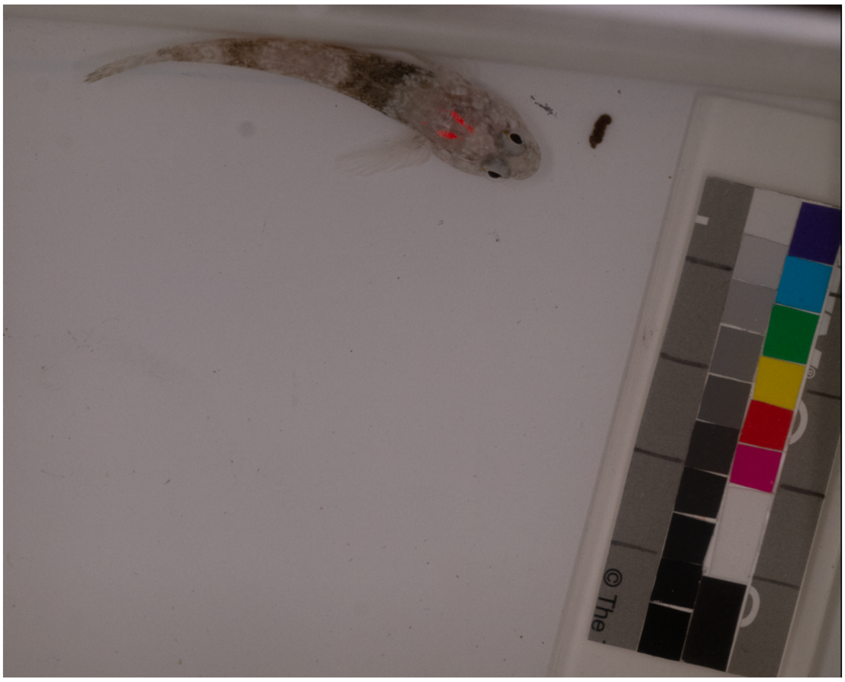


Figure 3.6 Example of a goby taking on a banded pattern. This image was taken of a warm exposed goby at 31ºC.

Overall, cold exposed fish were found to have higher luminance values at the end of the grey standardization period and start of testing when placed on both white and black backgrounds. This may be a result of long-term exposure to different seasonal thermal conditions and may be been driven by increase concentration of melatonin. The effect melatonin has on melanophore aggregation or dispersion has been shown to be seasonally dependent in a tropical reef fish *Rasbora daniconius* (Ovais et al. 2015). During summer months, increased concentrations of melatonin have an inverse aggregation response, whereas higher concentrations of melatonin correlates with lower melanophore aggregation and phenotype darkening. During winter months, however, increases in melatonin concentrations are positively correlated with melanophore aggregation, and therefore, phenotype brightening (Ovais et al. 2015). The average higher luminance values observed in the cold exposed fish may be explained by melatonin induced melanophore aggregation in cold temperatures, simulating winter. The effect melatonin has on the aggregation or dispersal of melanosomes however, is highly species dependent (Nishi and Fujii 1992; Ovais et al. 2015).

The capacity *B. cocosensis* has to acclimate rate of luminance change to seasonal thermal conditions may be a buffer from increasing temperatures associated with climate change. Thermal stochasticity and variability is increasing with climate change and these rapid changes are likely to have negative consequences on *B. cocosensis* thermal performance as thermal acclimation to changed temperatures usually occurs over a time scale of weeks in fish (Seebacher 2005); and *B. cocosensis* were observed to have reduced background matching responses when exposed to acute thermal conditions they were not acclimated to. Reduced capacity to background match for camouflage may result in fish being detected more easily by predators, resulting in reduced survival. This study provides a stepping stone to understanding a mechanistic basis for how climate change may alter behaviourally mediated species interactions. Malfunctioning of prey species chromatophore pigment aggregation is likely to increase the frequency of predator-prey interactions as prey species will be more obvious against their backgrounds to potential predators. Therefore, climate change may cause predator-prey interactions to more frequently favour predators than prey if prey species are unable to remain cryptic to predators when temperatures change quickly. Conversely, climate change might also make predators more conspicuous to prey species, meaning that the direction of the effect of thermal change on predator-prey interactions might be highly contingent of species differences and ecological context.

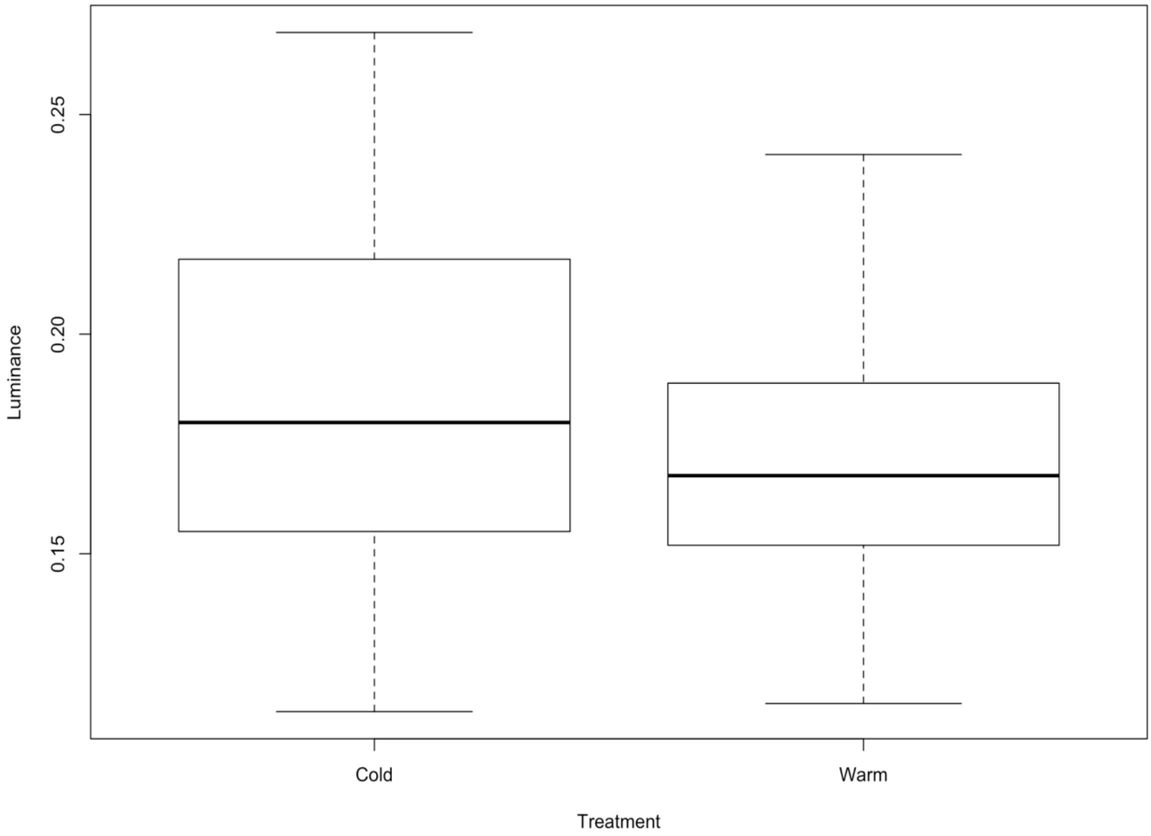
This is the first study to show that long-term exposure to different thermal conditions can affect rate of background matching, and that it is possible for rate of luminance change to thermally acclimate to seasonal conditions. Rate of luminance change for camouflage is an important ecological trait that affects survival and fitness, and it should be taken into account when modelling species responses to climate change, along with other traits such as metabolism and locomotion. In conclusion, I propose that gaining a more comprehensive understanding of how different functional traits, such as rate of background matching, have evolved to respond to short- and long-term temperature variation is important for understanding how changes in climate will affect species fitness and survival, as well as for understanding the physiological and behavioural basis of ecological interactions in variable environments more generally.

Note: This study provides an ImageJ based script (Supplementary material ImageJ code) to extract luminance values from the MicaToolbox plugin for future studies that wish to assess rate of luminance change.

Acknowledgements

We thank Natalie Freeman, Andrew Hunter and Julian Beaman for their help. Animal ethics was approved by The University of Queensland Animal Ethics Unit (permit number: QS2015/MAN340).

3.6 Supplementary material



Supplementary figure 3.S1 Differences in warm and cold exposed fish luminance (%) after exposure to a grey background for 10 minutes.

3.7 Multi-photograph luminance extraction code for MicaToolBox Plugin

ImageJ Macro code written by Dr. Nicholas Condon

Note: Running this macro in ImageJ will prompt you to select a folder of RAW format photographs. The script will execute the “Generate multispectral images” plugin for each photograph in the directory. The user is then prompted to select the light and dark colour standards to calibrate the photographs with the grey standards reflectance values (green highlight). It will then generate a visual model (factors can be altered – add customized factors into yellow highlighted region of code) of the photograph. Factor names can be found within “MICA Toolbox > Cone Mapping > Generate cone mapping model”. The double wave channel of the modelled image will be selected to measure luminance. Drawing around your region of interest (ROI) will allow you to calculate the luminance of the ROI. This data will then be automatically added to a “results” csv file. The macro will then loop for each photograph within the chosen directory.

ImageJ code

```
run("Clear Results");

path = getDirectory("Choose Source Directory ");
list = getFileList(path);

run("Set Measurements...", "area mean standard modal min centroid perimeter median display
redirect=None decimal=3");

for (i=0; i<list.length; i++) {
if (endsWith(list[i],".SRW")){
	//setTool("roundrect");

	run(" Generate Multispectral Image", "settings=Visible grey=[Same photo]
standard=5.06,73.31 alignment=None offset=16 scaling=5 scale_step_size=0.005
image=[Aligned Normalised 32-bit] image_0="+i+" select="+path+list[i]);
	name=getTitle;
print(name);
print(list[i]);
	run("Convert to Cone Catch", "model=[Samsung NX1000 Novoflex 35mm
Carmenlights to Coral Trout D65]");
	setSlice(4);
	//run("Wand Tool...", "tolerance=4650 mode=Legacy");
	waitForUser("Draw around fish");
	rename(list[i]);
	run("Measure");
	roiManager("reset");
	close();
	close();

}}

saveAs("Results", path+"Results.csv");
```

Chapter 4

Rapid larval growth is costly for post-metamorphic thermal performance in a Great Barrier Reef fish

This thesis chapter is published in the journal Coral Reefs: da Silva CRB, Wilson RS, Riginos C (2019) Rapid larval growth is costly for post-metamorphic thermal performance in a Great Barrier Reef fish. Coral Reefs. DOI: 10.1007/s00338-019-01815-7

I contributed significantly to this manuscript. I generated the initial project question and design and it was then refined by Cynthia Riginos and Robbie Wilson. I collected the fish used for this project, conducted the thermal performance experiments, statistical analysis and manuscript writing. Both Cynthia and Robbie provided editorial advice.

Percentage of contribution towards da Silva et al. (2019b)

	da Silva	**Wilson**	**Riginos**
Concept and design	70	10	20
Field collection	100	0	0
Experiments	100	0	0
Statistical analysis	100	0	0
Writing of manuscript	80	10	10
Editing of manuscript	60	10	30

4.1 Abstract

Most marine fish species disperse during a planktonic larval stage where individuals exhibit variation in pelagic duration, growth rate and settlement size. Extreme predation risk is predicted to select for rapid growth rates and decreased pelagic duration as a strategy for increasing survival to settlement. How larval traits affect post-metamorphic performance, however, has been a contentious topic over the past 50 years. Some studies suggest that larval traits have carry-over effects to later life stages, where larval traits can be positively or negatively correlated with post-metamorphic performance. For example, individuals with rapid larval growth rates may settle at larger sizes and have faster post-metamorphic locomotion than slow growing individuals. Alternatively, trade-offs between life stages might exist, where rapid larval growth rate may be negatively correlated with post-metamorphic locomotion, potentially due to energetic resource allocation trade-offs. In addition, other studies suggest that larval traits are de-coupled from later life stages to allow for a transition in morphology and habitat. I tested how *Bathygobius cocosensis* hatch size, larval growth rates, settlement size, and pelagic duration correlated with post-metamorphic thermal performance of burst swimming speed, routine metabolic rate and critical thermal maximum. I found that larval growth rate was negatively correlated with juvenile routine metabolic rate and burst swimming speed across a range of test temperatures. That is, fast growing larvae had slower burst swimming speeds and lower routine metabolic rates across temperature as juveniles compared to slower growing larvae. I also found that hatch size and pelagic larval duration were not correlated with post-metamorphic performance. Thus, I provide evidence both for larval traits having carry-over effects on later life stages and also for larval traits being de-coupled with thermal performance post-metamorphosis. This is the first study to show that rapid larval growth rate is costly for post-metamorphic thermal performance in a wild marine fish.

4.2 Introduction

Dispersal is one of the most important mechanisms for maintaining population connectivity, genetic diversity, and survival in changing environmental conditions (Lande 1988; Trakhtenbrot et al. 2005; Cowen and Sponaugle 2009). Many marine fish species disperse during their pelagic planktonic larval stage where they traverse the water column for many days and are exposed to varying environmental conditions (Meekan and Fortier 1996; Wilson and Meekan 2002; Shima and Swearer 2009; Gimenez 2010). Environmental conditions and variation in genotypes affect larval traits such as pelagic duration, growth rates, and settlement size (Gimenez 2010; Grorud-Colvert and Sponaugle 2011); the effect these traits have on later life stages has been a contentious issue for the past 50 years.

There is considerable debate as to whether larval traits (in any species with complex life-cycles) have carry-over effects to later life stages, or if larval traits are de-coupled from post-metamorphic traits (Wilbur 1980; Moran 1994; Meekan and Fortier 1996; Pechenik et al. 1998; Arendt 2003; Marshall et al. 2003; Crean et al. 2011). Some studies suggest that metamorphosis allows a de-coupling of traits for adaptation to particular environments for each life stage (Ebenman 1992; Moran 1994; Parichy 1998). This de-coupling hypothesis suggests that traits expressed during the larval stage are not correlated with traits in juvenile or adult life stages and that metamorphosis allows individuals to re-form for increased survivability in a new environment (Ebenman 1992; Moran 1994; Parichy 1998; Johansson et al. 2010).

In contrast, other empirical studies show that larval traits have significant effects (carry-over) on post-metamorphic performance (Pechenik et al. 1998; Crean et al. 2011). Larval traits that are carried over to later life stages can have either positive or negative effects on post-metamorphic traits. For example, larval size has been shown to have a positive effect on post-metamorphic growth in marine invertebrates (Marshall and Keough 2004), and larval frogs (tadpoles) reared with predatory pressure have longer hind limbs post metamorphosis than individuals reared without predators, showing that larval experience can affect later life stage performance (Relyea 2001). Rapid development, however, can also incur costs that have negative effects on post-metamorphic traits and performance (Arnold and Wassersug 1978; Wilbur 1980; Alvarez and Nicieza 2002). For example, some frogs with rapid larval growth have decreased jumping performance as juveniles (Ficetola and De Bernardi 2006). Physiological costs associated with fast growth rates (developmental or post metamorphic) can arise from allocating energetic resources to growth that could otherwise be used for cell maintenance or locomotion (Stevens et al. 1998; McCarthy 2000; Arendt et al. 2001). These physiological costs have the potential to transcend to later life stages once larvae settle as adults. Traits that are energetically demanding (such as rapid growth) during the larval phase are more likely to be associated with energy partitioning trade-offs and potentially costs for post-metamorphic traits. Larval traits such as size, however, are often shown to have positive cumulative positive knock-on effects to later life stages, where larger individuals have increased survivability and fitness (Vigliola and Meekan 2002; Marshall and Keough 2004). Although the aforementioned examples and many other studies document all three possible relationships between larval and post-metamorphic traits, most studies have assessed relationships between larval traits and post-metamorphic performance on laboratory reared animals (see review by Pechenik (2006)). There is, however, a need for research on how wild caught larval traits affect post-metamorphic performance to better understand wild population dynamics and survival strategies.

Another limitation of many empirical studies that assess trait correlations between wild larval and post-metamorphic life stages is that they compare traits such as growth rate, morphology or performance in a single environmental condition (Meekan and Fortier 1996; Pechenik et al. 1996; Alvarez and Nicieza 2002; Marshall et al. 2003; Fischer et al. 2004; Shima and Swearer 2010; Crean et al. 2011). What is missing is an understanding of how early life environmental variability (reflected in wild larval traits) affects thermal performance of traits such as metabolic rate and burst swimming speed, which are directly indicative of fitness and survival in dynamic natural environments. For example, maintenance of burst swimming speed across temperature aids escape from predation in variable thermal environments (Walker et al. 2005; Husak 2006a,b). Maintenance of metabolic rates is important for oxygen demanding activities such as sustained swimming, anaerobic recovery, digestion and growth (Brett 1964). In addition, critical thermal maximum can be indicative of geographic distribution and barriers to thermal limits. Here, I aim to examine correlations between larval traits and post-metamorphic thermal performance of routine metabolic rate, burst swimming speed and critical thermal maximum to gain an understanding of how wild larval traits affect juvenile thermal performance thereby gaining insight into how larval traits affect fitness and survival in a natural environment. Whereas such a correlative approach lacks the precision of controlled experiments, it allows me to examine fish whose prior experiences span a range of ecologically relevant environmental conditions.

I used wild juvenile *Bathygobois cocosensis* (Cocos Frillgoby) (Bleeker 1854) from Heron Island in the southern Great Barrier Reef as my study species. By assessing acute juvenile thermal performance and testing for correlations with larval traits (using otoliths to back calculate age, hatch size, growth rate and settlement size (Victor 1984; Wellington and Victor 1985,1992)), I can make insights on how larval traits and experience might affect post-metamorphic whole organism performance and fitness. By testing performance across a range of acute test temperatures I can also gain an understanding of how larval traits might influence adult performance across a range of environmental conditions.

Bathygobious cocosensis experience about 20 days as larvae in the pelagic zone, before settling at an intertidal location (this study; Thia et al. 2018). During this stage, larvae are exposed to extreme predation pressure and high mortality due to inability to find food or survive in physical environmental conditions (Meekan and Fortier 1996; Wilson and Meekan 2002; Cowen and Sponaugle 2009; Shima and Swearer 2009; Gimenez 2010; Shima and Swearer 2010). Rapid growth can reduce mortality rates, by reducing time spent in the pelagic larval phase, and large settlement sizes often increase post-metamorphic survival (Marshall et al. 2003) and potentially competitive ability. *Bathygobius cocosensis* are an aggressive species, and larger individuals have been observed to inhabit rock-pools in the low-tide region of the intertidal zone (Malard et al. 2016; Paijmans and Wong 2017), which generally provide greater access to food and refuges. Thia et al. (2018) found that individuals of *B. cocosensis* that settle at a small size remain small for at least four days post-settlement, and larger *B. cocosensis* adults have also been observed to have higher routine metabolic rates than smaller individuals and faster burst swimming speeds (da Silva et al. 2019a). Therefore, if individuals settle as large larvae, they are likely to benefit by gaining better access to high quality habitat, enabling larger growth as juveniles and adults, consequently having positive correlations with post-metamorphic performance beyond the immediate four days post settlement that are identifiable through otolith increment analysis.

Specifically, I tested whether pelagic larval duration, hatch size, larval growth rates, and settlement size correlated with juvenile thermal performance of routine metabolic rate and burst swimming speed across temperature and critical thermal maximum in juvenile *B. cocosensis*. I propose three sets of expectations based on competing hypotheses on how larval traits will be correlated with acute thermal performance: 1) **Life stage de-coupling hypothesis:** Some evidence suggests that larval traits are de-coupled with post-metamorphic traits as a method of life-stage partition for improved adaptation to each life stages habitat, under this scenario I predict that there will be no correlations between larval traits and post-metamorphic performance (Fig 1A). 2) **Bigger is better hypothesis:** Under the hypothesis that individuals that settle large stay large and have greater post-metamorphic performance, I predict that individuals with fast growth rates or long pelagic larval durations will have higher routine metabolic rates and burst swimming speeds across temperature than individuals with slow larval growth rates or short pelagic larval durations (Fig 1B). 3) **Life stage trade-off hypothesis:** Under the hypothesis that fast larval growth occurs to avoid predation during the pelagic stage but is negatively associated with post-metamorphic energetic trade-offs, I expect that individuals with short pelagic larval durations and fast growth rates will have lower metabolic rates and slower burst swimming speeds across acute test temperatures than individuals with longer pelagic larval durations and slow growth rates (Fig 1C). These competing hypotheses were evaluated in the context of whole organism performance across a range of temperatures.

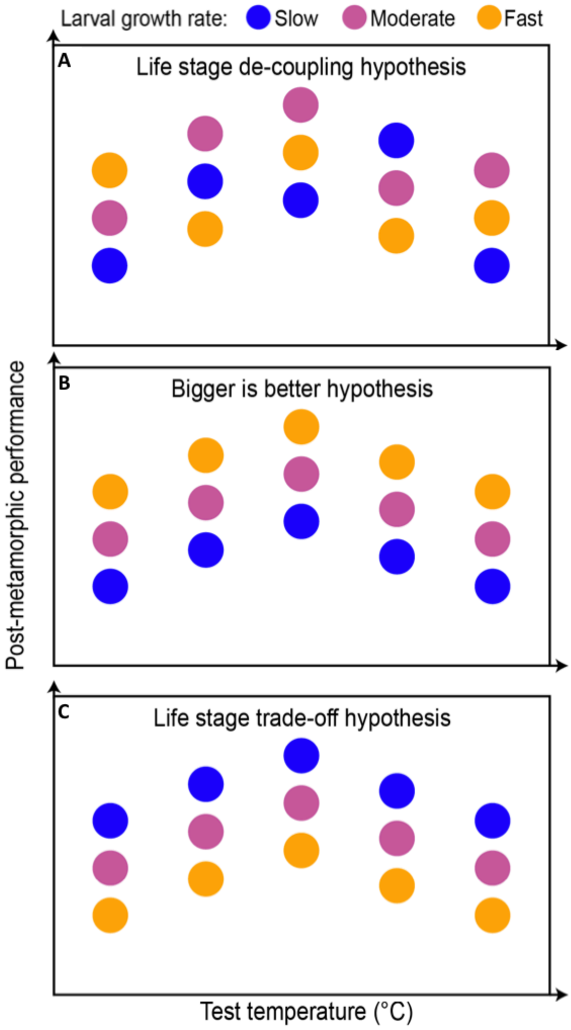


Figure 4.1. Conceptual illustration of three competing hypotheses for how larval traits (such as growth rate, as shown in the example) might correlate with post-metamorphic performance across acute test temperatures. A) The **life stage de-coupling hypothesis** predicts no

correlation between larval growth rate and post-metamorphic performance. B) The **bigger is better hypothesis** predicts that larval with fast larval growth rates will have the highest post-metamorphic thermal performance. C) The **life stage trade-off hypothesis** predicts that individuals with slow larval growth rates have the highest post-metamorphic performance.

4.3 Methods

Fish collection

Bathygobius cocosensis were collected in late March 2017 to allow for larval settlement, metamorphosis and some juvenile development (fish were aged 37 to 117 days old post hatching) (Griffiths 2003; Thia et al. 2018). Fish were collected from the intertidal area of the scientific research zone on Heron Island (23.4438°S, 151.9151°E) located in the Southern Great Barrier Reef, where ambient water temperatures were 27°C on average. Fish were collected by scraping dip-nets along the rocky intertidal flats. Only fish up to 3 cm in length were collected to ensure otolith readings for planktonic life history analysis could be accurately analysed (fish greater than 3 cm in length are usually too old for accurate daily larval otolith reading).

Bathygobius cocosensis (N = 96) were independently housed within numbered closed containers (15 x 8 x 7 cm) to allow for individual identification. Fish were kept in 27°C aerated seawater (35 ppt) at The University of Queensland's Heron Island Research Station facility on a 12:12 light-dark regime with daily water changes. Fish were left to rest in laboratory conditions for two days prior to the start of thermal performance testing and fish were fed three flakes of Sera marine GVG-Mix (Marine Treat Mix) per day (post performance testing).

Thermal performance of routine metabolic rate and burst swimming speed was tested over the course of 10 days (tank water was changed post performance testing each day). I tested routine metabolic rate during the first five days of testing where each fish was tested at acute test temperatures in the following randomised order to avoid effects of acclimation: 28°C, 15°C, 33°C, 22°C and 37°C (one test temperature per day). Individuals were tested in a randomised order. During the following five days burst swimming speed was tested at 28°C, 15°C, 33°C, 22°C and 37°C respectively. Fish were brought to test temperature at a rate of 3°C per hour (appropriate for intertidal organisms that experience rapid thermal fluctuations to test acute thermal performance (Schulte et al. 2011)).

Performance testing

Routine metabolic rate was assessed prior to burst swimming speed to minimise stress and to reduce error in metabolic rate samples. Fish were placed in darkened (black plastic covered) 350 mL respirometers for one hour within a water bath set at the daily test temperature prior to the start of testing to allow the fish's metabolic rates to stabilize. Oxygen consumption was then measured in the following hour using a PreSens Fibox 4 (POF-L2.5-1). A polymer optical fiber and oxygen minisensor spots (batch ID – 140117-001) were used to measure the decrease in percent air saturation within the respirometer. Control measures were taken from respirometers full of sea water (no fish) to detect for any microorganism metabolic activity or oxygen production occurring within the testing water that may affect results. A control water sample was taken during each batch of metabolic testing within the temperature-controlled water bath (therefore 18 fish respirometers + 1 control respirometer were tested per batch). Metabolic rate (VO_2) was calculated using the following formula:

$$VO_2 = -1 \times \left((mf - mc) \div 100\right) \times V \times BO_2,$$

where *mf* was the slope of the percent air saturation consumed over an hour and *mc* is the slope of the percent air saturation consumed over an hour within the control respirometer (no fish). *V* was the respirometer water volume and BO_2 was the oxygen solubility of ocean water at 35 ppt with test temperature.

Burst swimming speed was assessed to gain a whole organism measure of performance capacity at all test temperatures (see detailed methods in Seebacher et al. 2014). Fish were placed in an 8 cm x 10 cm testing aquarium with a fixed 10 cm measuring tape placed in the field of view for calibration. A high-speed camera (Panasonic Lumix DMC-TZ40) was set up directly above the middle of the testing aquarium and recorded the image. Burst swimming responses were promoted by tapping next to the fish's tail using a thin metal rod when fish were stationary. A minimum of three burst responses was recorded for each fish, and the maximum instantaneous speed for each escape response was determined and extracted from videos using the program KINOVEA© (experimental version 0.8.25-x64). Only swimming responses where the fish exhibited a C-start escape response (powerful escape response where fish bends body into C shape to flick tail for rapid burst movement) were analysed. I digitised the point between the eyes of each fish to ensure consistent tracking. Maximum instantaneous speeds for individual fish were averaged at each acute test temperature.

Critical thermal maximum was measured two days after burst swimming trials were complete. Each fish's critical thermal maximum was determined by placing each fish in a water bath at 27°C and ramping the water temperature up by 0.5°C every two minutes, an appropriate ramping speed for ectotherms that experience diurnal temperature fluctuations (Lutterschmidt and Hutchison 1997). The temperature at which *B. cocosensis* became unresponsive to a light touch with a thin metal rod was recorded as their critical thermal maximum. Fish were then euthanized with 0.3 x 10^{-3} mg L^{-1} of Aqui-S®and the standard length (mm) and wet mass of each fish (g) was measured.

Otolith analysis

As otoliths (fish ear bones) develop, growth increments form each day and are indicative of individual age, daily growth and settlement size and age (Sponaugle and Cowen 1997; Shima and Swearer 2010). Fish heads were removed and placed in 100% ethanol. Fish heads were sent to the Advanced Research Centre of Blanes in the Spanish National Research Council for otolith extraction and reading. They collected four larval life history traits: hatch size, pelagic larval duration, total pelagic growth and settlement size. Hatch size is the distance from the otolith nucleus to the hatch mark, pelagic larval duration is the number of days (rings) between hatching and settlement, pelagic growth is the distance between hatch and settlement otolith marks (total growth during the larval stage), and settlement size is the distance between the otolith nucleus and settlement mark (Thia et al. 2018). Growth rate was calculated by dividing total planktonic growth by number of days spent in the pelagic larval stage. Variance in daily larval growth was also determined by calculating the distance between each daily growth ring during the pelagic larval phase and then calculating variance in growth during the entire larval phase.

Statistical analysis

I assessed for pair-wise correlations between larval traits (hatch size, pelagic larval duration, growth rate, total growth, and settlement size) using type II linear regression models in the lmodel2 library (Legendre 2018) in the R statistical program (R Core Team 2013). I tested for correlations between planktonic life history traits and post metamorphic juvenile thermal sensitivity by fitting linear mixed effect models using the lme4 library (Bates et al. 2015). Routine metabolic rate and burst swimming speed data was log10 transformed to satisfy the conditions required for linear mixed effect models. The final models for routine metabolic rate and critical thermal maximum included the factors juvenile fish mass, test temperature and the larval traits: hatch size, pelagic larval duration and settlement size OR hatch size and growth rate. The fixed effect of temperature was modelled as a second-degree polynomial as this structure fitted the data more closely (shape of a thermal performance curve). The burst swimming speed models incorporated juvenile fish length instead of juvenile mass as fish length has been shown to be more important for estimating swimming speed than mass (Domenici and Blake 1997) but included the same predictive larval traits as the routine metabolic rate and critical thermal maximum models. I accounted for repeated measures (each fish tested at each test temperature) by modelling a separate random intercept and random slope across test temperature for each fish (Hickey et al. 2018). Here I take into account that performance within individual fish across test temperature is more correlated than between different fish (Hickey et al. 2018). The fixed effect of temperature gives the mean slope over temperature and the random intercept shows individual deviations from the mean slope. Models were produced using maximum likelihood. The linear mixed effect model formula with the random intercept and slope for each fish is shown below,

$$y_{ij} = \beta_0 + \beta_1 x_{ij1} + \beta_2 x_{ij2} + \beta x_{ij3} + \beta_3 x_{ij3}^2 + \beta_4 x_{ij4} + \beta_5 x_{ij5} + b_{i0} + b_{i1} x_{ij} + e_{ij}$$

where, y_{ij} is the performance of each individual fish (i = 1,....96) at each test temperature j. b_0 is the fixed intercept and β_1 is the fixed slope for the regression model of y on x_1, where other fixed factors (x's) are held constant (repeated for each fixed factor). x_{ij} is the predictor variable for each fish at each test temperature, where, x_1 = juvenile fish mass, x_2 = hatch size, x_3 = test temperature (second degree polynomial), x_4 = settlement size, x_5 = pelagic larval duration. b_{i0} is the random intercept for each fish and b_{i1} is the random slope across test temperature for each fish, and e_{ij} is the residual error term for each fish across test temperature.

Two separate models were made for each post-metamorphic performance trait, where one model incorporated pelagic larval duration and settlement size and the other model assessed correlations of larval growth rates on performance. The formula for the model that includes growth rate, therefore, would only have four fixed terms (x's), instead of five, like in the example model equation above. The two models for each performance trait could not be combined because growth rate is correlated with settlement size. Growth rate and settlement size, however, have different life strategy implications (explained in discussion), which is why it is important to assess for correlations of each on post-metamorphic performance.

I selected the final models for each performance measure by reducing factors within the model and comparing Akaike Information Criterion (AIC) values. Daily growth variance and the interaction term between growth rate and test temperature had no significant effect on performance (routine metabolic rate, burst swimming speed or critical thermal maximum), and inclusion of these factors increased model AIC values, meaning that model fit was reduced. Total larval growth was highly correlated with settlement size, so it was not included in the models as it would break assumptions required to run linear mixed effect models. Therefore, daily growth variance, total larval growth and the interaction terms were removed from the final models. I was only able to obtain fish ages for a subset of fish and therefore fish age was not included in the analyses, however, fish mass and length can also be indicative of age. Figures were produced in R library ggplot2 (Wickham 2016).

4.4 Results

Larval trait summary

Juvenile *B. cocosensis* ($N = 96$) collected at Heron Island had an average pelagic larval duration of 18.5 days (SE = ± 0.08), where pelagic larval durations ranged from 15 to 25 days. On average, hatch size was 6.13μm (SE = ± 0.094) and ranged from 4.39 to 9.49 μm. Otoliths had an average total pelagic growth of 50.02 μm (SE = ± 0.29) prior to settlement. Fish had an average larval otolith growth rate of 2.73 μm per day (SE = ± 0.01) with a range of growth rates between 2.2-3.4 μm per day and a variance of 0.052. Pelagic larval duration was correlated with larval settlement size ($R^2 = 0.419$) (Estimate = 0.19 ± 0.025, $t = 7.592$, $P < 0.000$), therefore, individuals that had short pelagic durations had somewhat smaller settlement sizes on average, and larval growth rate was weakly correlated with settlement size ($R^2 = 0.37$) (Estimate = 0.025 ± 0.004, $t = 6.873$, $P < 0.000$) (Fig 4.2). Interestingly, pelagic larval duration, was not correlated with larval growth rate ($R^2 = 0.0326$) (Estimate = -1.31 ± 0.798, $t = -1.64$, $P = 0.105$), but total larval growth was correlated with larval growth rate ($R^2 = 0.385$) (Estimate = 15.127 ± 2.135, $t = 7.084$, $P < 0.000$) (Fig 4.2). Total larval growth was strongly correlated with settlement size ($R^2 = 0.968$) (Estimate = 0.995 ± 0.02, $t = 49.257$, $P < 0.000$), which is intuitive because settlement size is determined by total larval growth, but total larval growth was not correlated with juvenile mass ($R^2 = 0.012$) (Estimate = -0.003 ± 0.003, $t = -0.998$, $P = 0.321$). In addition, hatch size was not correlated with settlement size ($R^2 = 0.0124$) (Estimate = -0.0014 ± 0.016, $t = -0.085$, $P = 0.932$), pelagic growth rate ($R^2 = 0.00083$) (Estimate = -0.416 ± 0.403, $t = -1.033$, $P = 0.305$), or total pelagic growth ($R^2 = 0.0132$) (Estimate = -0.024 ± 0.016, $t = -1.44$, $P = 0.153$). Therefore, individuals with small hatch size did not exhibit compensatory growth rates to reach the same settlement sizes as individuals with large hatch sizes. Juvenile fish had an average mass of 0.21g (SE = ± 0.01) and an average length of 20.56mm (SE = ± 0.24).

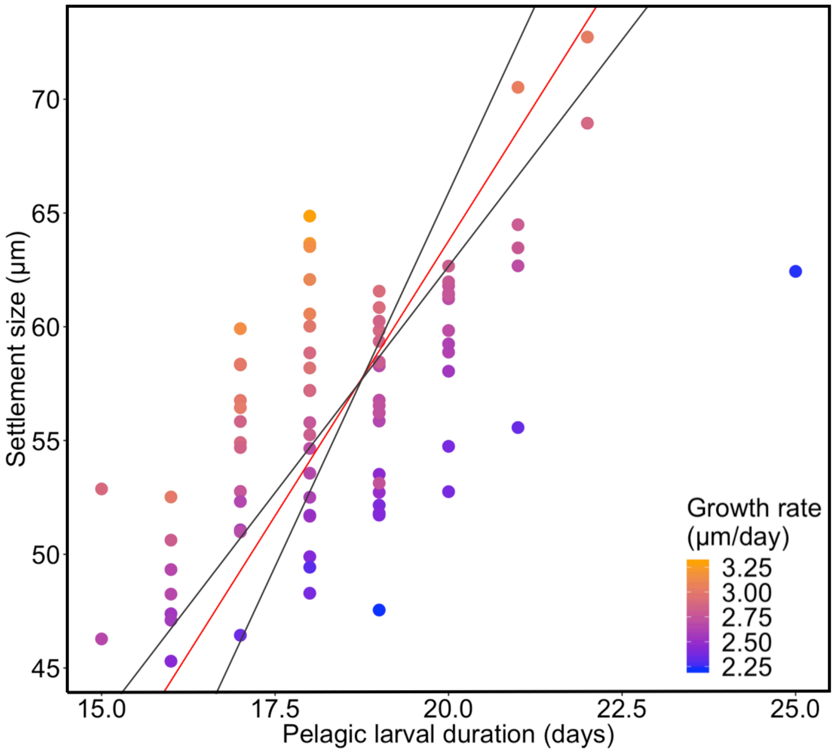


Figure 4.2. Type II regression between pelagic larval duration and settlement size ($R^2 = 0.419$). Coloured dots indicate growth rate (μm/day). The red line shows the correlation and grey lines show the error confidence limits.

Larval traits and juvenile thermal performance

I found that settlement size (SS) and larval growth rate (GR) was inversely correlated with post-metamorphic routine metabolic rate (SS: Estimate = -0.026 ± 0.010, $t_{77.12}$ = -2.48, $P = 0.015$; GR: Estimate = -0.444 ± 0.195, $t_{75.52}$ = -2.27, $P = 0.026$) (Fig 4.3) (Table 4.0 & 4.1). These data (Fig 4.3) show how larval growth rate correlates with juvenile routine metabolic rate when mass and test temperature are taken into consideration. Although fish mass surprisingly had no effect on routine metabolic rate (Estimate = 0.041 ± 0.32, $t_{172.27}$ = 1.273, $P = 0.204$) it was kept in the models as mass is a well-known ecologically relevant trait to metabolic rate (White and Kearney 2014). Perhaps mass had no significant effect because fish were sampled within a relatively small mass range (variance = 0.017) and often the effect body mass has on metabolism is more obvious over larger mass changes (White and Kearney 2014). As juvenile fish mass was not correlated with routine metabolic rate, I also tested if juvenile fish length was correlated with routine metabolic rate, and found that it also had no correlation with routine metabolic rate (Estimate = -0.015 ± 0.009, $t_{160.5}$= 1.694, P= 0.0922).

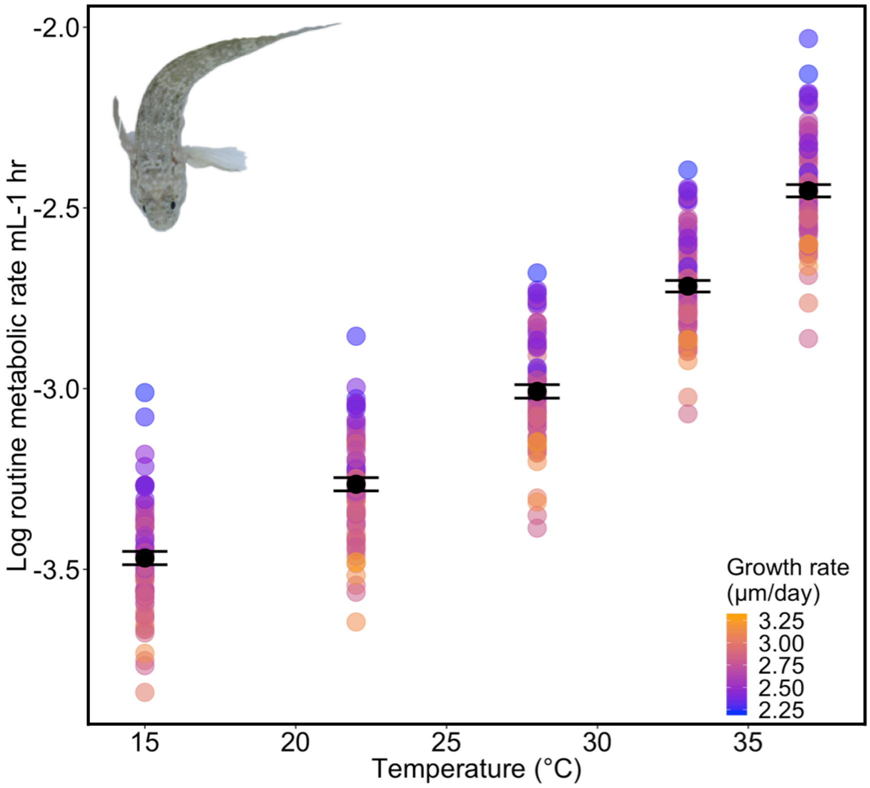


Figure 4.3. *Bathygobius cocosensis* routine metabolic rate across acute test temperature with individual larval otolith growth rate shown in coloured points (μm/day), when juvenile fish body mass, test temperature and hatch size are taken into consideration. Mean predicted routine metabolic rate and standard error bars are shown in black for each test temperature.

Table 4.0 Final model summary of the effects of hatch size, pelagic larval duration and settlement size on juvenile *Bathygobius cocosensis* routine metabolic rate.

Fixed effects	**Estimate ± SE**	***df***	***t***	***P***
Intercept	-2.22 ± 0.63	73.14	-3.53	0.0007
Juvenile fish mass	0.41± 0.32	172.27	1.273	0.204
Poly(test temperature)1	7.10 ± 0.76	276.43	9.36	<0.000
Poly(test temperature)2	1.01 ± 0.76	293.85	1.33	0.185
Hatch size	**-0.007 ± 0.052**	**68.33**	**-0.134**	**0.894**
PLD	**0.036 ± 0.036**	**76.35**	**0.996**	**0.322**
Settlement size	**-0.026 ± 0.010**	**77.12**	**-2.48**	**0.015**

Final model 4.1 = log10(VO_2) ~ juvenile mass + poly(test temperature, 2) + hatch size + PLD + settlement size + (1+test temperature|fish ID)

Table 4.1 Final model summary of the effect of hatch size and larval growth rate on juvenile *Bathygobius cocosensis* routine metabolic rate.

Fixed effects	**Estimate ± SE**	***df***	***t***	***P***
Intercept	-1.66 ± 0.67	76.71	-2.45	0.0152
Juvenile fish mass	0.379 ± 0.32	177.95	1.785	0.237
Poly(test temperature)1	7.10 ± 0.76	272.92	9.37	<0.000
Poly(test temperature)2	0.999 ± 0.76	292.95	1.318	0.189
Hatch size	**-0.03 ± 0.053**	**70.94**	**-0.524**	**0.602**
Larval growth rate	**-0.444 ± 0.195**	**75.52**	**-2.27**	**0.026**

Final model 4.2 = log10(VO_2) ~ juvenile mass + poly(test temperature, 2) + hatch size + growth rate + (1+test temperature|fish ID)

Settlement size was not correlated with juvenile burst swimming speed (SS: Estimate = -0.005 ± 0.0029, $t_{85.88}$= -1.74, *P* = 0.085) (Table 4.2), however larval growth rate was negatively correlated with burst swimming speed across test temperature (GR: Estimate = -0.12 ± 0.053, $t_{84.22}$= -2.24, *P* = 0.028) (Fig 4.4) (Table 4.3). Again, this data illustrates the negative correlation growth rate has with burst swimming speed when the effects of fish length and test temperature are taken into consideration. Fish length was positively correlated burst swimming speed (Estimate = 0.007 ± 0.0023, $t_{164.65}$= 3.397, *P* < 0.0008). These data show that larvae with faster growth rates had lower routine metabolic rates and burst swimming speeds across increasing temperature compared to fish with slower larval growth rates. Settlement size and growth rate, however, were not correlated with critical thermal maximum (SS: Estimate = -0.009 ± 0.013, $t_{89.73}$ = -0.77, $P = 0.44$; GR: Estimate = -0.039 ± 0.246, $t_{85.88}$ = -0.161, $P = 0.873$) (Tables 4.4 & 4.5). *Bathygobius cocosensis* had an average critical thermal maximum of 39.7°C (SE = ± 0.03) and juvenile fish mass was positively correlated with critical thermal maxima (Estimate = 2.08 ± 0.22, $t_{416.9}$ = 9.44, $P < 0.000$, $R^2 = 0.178$) (Fig 4.5).

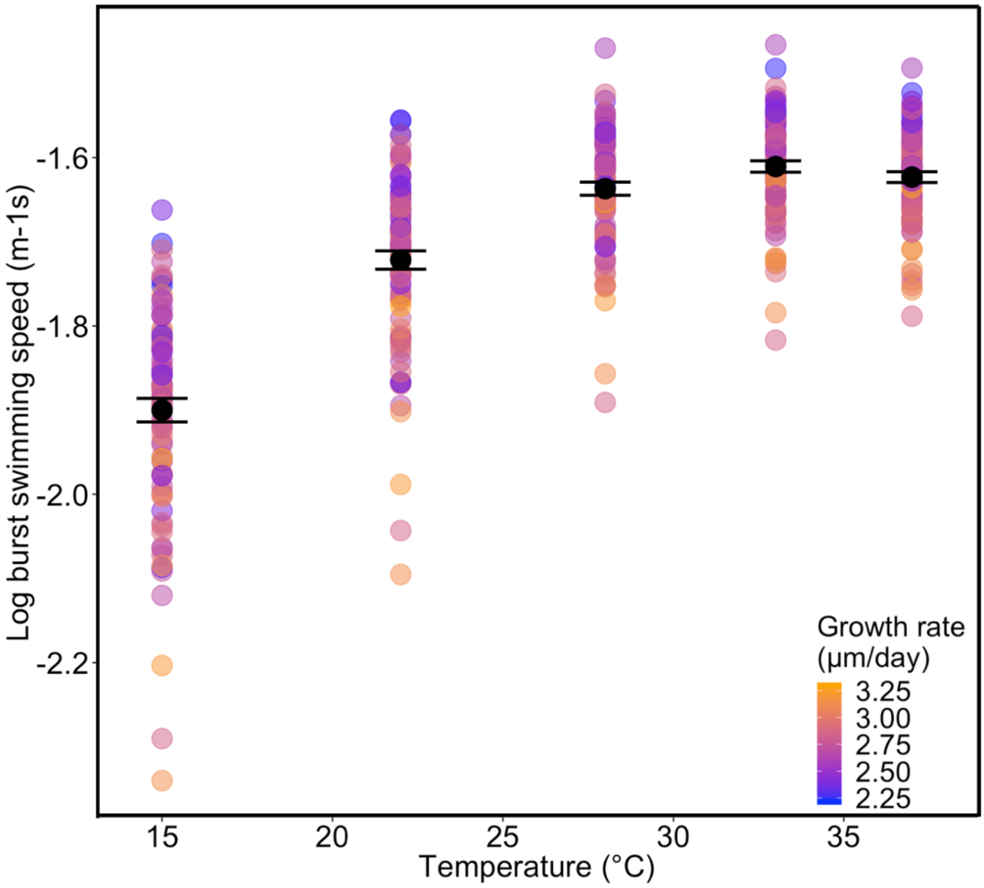


Figure 4.4. *Bathygobius cocosensis* burst swimming speed across acute test temperature with individual larval otolith growth rate shown in coloured points (µm/day), when juvenile fish length, test temperature and hatch size are taken into consideration. Mean predicted routine metabolic rate and standard error bars are shown in black for each test temperature.

Table 4.2 Final model summary of the effects of hatch size, pelagic larval duration and settlement size on juvenile *Bathygobius cocosensis* burst swimming speed.

Fixed effects	**Estimate ± SE**	***df***	***t***	***P***
Intercept	-1.91 ± 0.18	86.78	-10.47	<0.000
Juvenile fish length	0.0076 ± 0.0024	164.35	3.301	0.0011
Poly(test temperature)1	2.00 ± 0.22	83.74	9.13	<0.000
Poly(test temperature)2	-0.88 ± 0.19	260.10	-4.73	<0.000
Hatch size	**0.014 ± 0.015**	**82.47**	**0.918**	**0.362**
Settlement size	**-0.005 ± 0.0029**	**85.88**	**-1.74**	**0.085**
PLD	**0.013 ± 0.010**	**86.89**	**1.34**	**0.184**

Final model 4.3 = log10(Burst swimming speed) ~ juvenile length + poly(test temperature, 2) + hatch size + PLD + settlement size + (1+test temperature|fish ID)

Table 4.3 Final model summary of the effect of hatch size and larval growth rate on juvenile *Bathygobius cocosensis* burst swimming speed.

Fixed effects	**Estimate ± SE**	***df***	***t***	***P***
Intercept	-1.58 ± 0.19	91.14	-8.20	<0.000
Juvenile fish length	0.007 ± 0.0023	164.65	3.397	0.0008
Poly(test temperature, 2)1	2.00 ± 0.22	83.69	9.129	<0.000
Poly(test temperature, 2)2	-0.87 ± 0.19	260.32	-4.72	<0.000
Hatch size	**0.007 ± 0.015**	**83.69**	**0.52**	**0.599**
Larval growth rate	**-0.12 ± 0.053**	**84.22**	**-2.24**	**0.028**

Final model 4.4 = log10(Burst swimming speed) ~ juvenile length + poly(test temperature, 2) + hatch size + growth rate + (1+test temperature|fish ID)

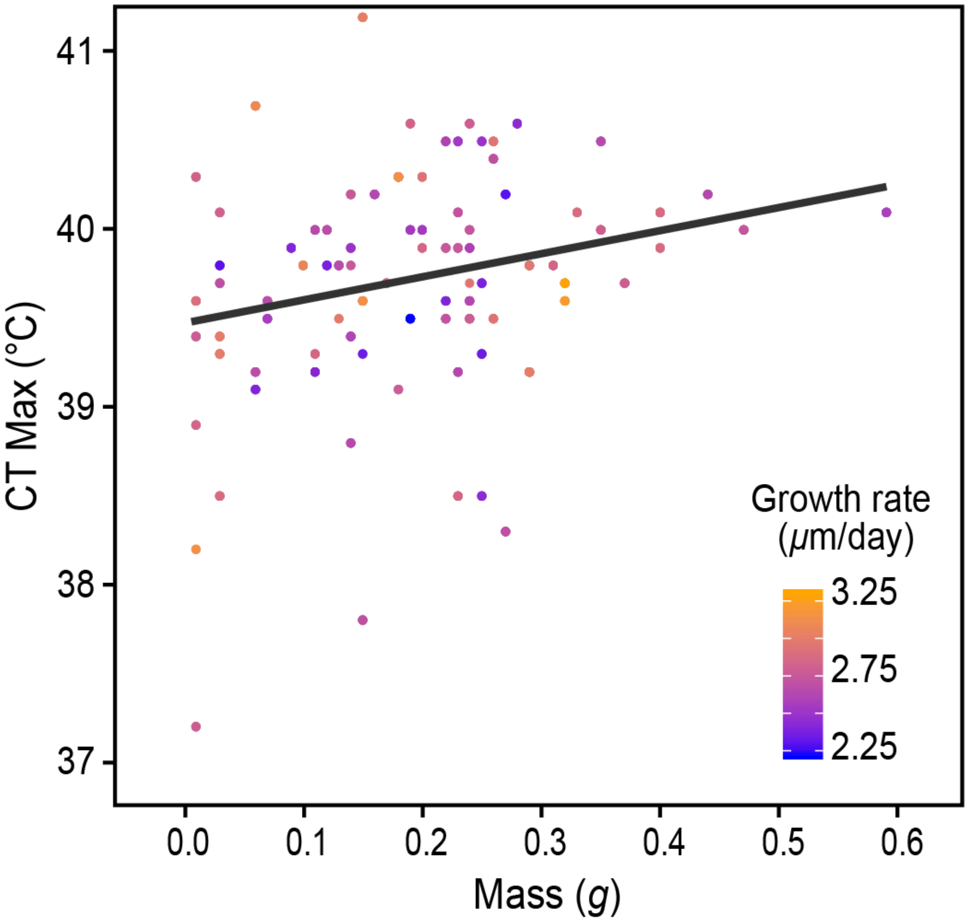


Figure 4.5. *Bathygobius cocosensis* critical thermal maximum and juvenile mass (g) (mass is positively correlated with critical thermal maximum (CT max). Coloured points indicate average daily larval otolith growth rate which has no correlation with body mass or CT max).

Hatch size (HS) and pelagic larval duration (PLD) were not correlated with post metamorphic routine metabolic rate (HS: Estimate = -0.007 ± 0.052, $t_{68.33}$= -0.134, *P* = 0.894; PLD: Estimate = -0.036 ± 0.036, $t_{76.35}$= 0.996, *P* = 0.322) (Table 4.0), burst swimming speed (HS: Estimate = -0.014 ± 0.015, $t_{82.47}$= 0.918, *P* = 0.362; PLD: Estimate = 0.013 ± 0.010, $t_{86.89}$= 1.34, *P* = 0.184) (Table 4.2), or critical thermal maximum (HS: Estimate = 0.065 ± 0.067, $t_{84.02}$= 0.963, *P* = 0.338; PLD: Estimate = -0.042 ± 0.044, $t_{89.15}$= -0.95, *P* = 0.342) (Table 4.4).

Table 4.4 Final model summary of the effects of hatch size, pelagic larval duration and settlement size on juvenile *Bathygobius cocosensis* critical thermal maximum.

Fixed effects	**Estimate ± SE**	***df***	***t***	***P***
Intercept	40.255 ± 0.79	84.64	51.364	<0.000
Juvenile fish mass	2.09 ± 0.22	415.88	9.494	<0.000
Hatch size	**0.065 ± 0.067**	**84.02**	**0.963**	**0.338**
PLD	**-0.042 ± 0.044**	**89.15**	**-0.95**	**0.342**
Settlement size	**-0.009 ± 0.013**	**89.73**	**-0.77**	**0.440**

Final model 4.5 = CTmax ~ juvenile mass + hatch size + PLD + settlement size + (1|fish ID)

Table 4.5. Final model summary of the effect of hatch size and larval growth rate on juvenile *Bathygobius cocosensis* critical thermal maximum.

Fixed effects	**Estimate ± SE**	***df***	***t***	***P***
Intercept	38.97 ± 0.84	86.43	46.3	<0.000
Juvenile fish mass	2.08 ± 0.22	416.9	9.44	<0.000
Hatch size	**0.07 ± 0.0.069**	**84.11**	**1.01**	**0.314**
Larval growth rate	**-0.039 ± 0.246**	**85.88**	**-0.161**	**0.873**

Final model 4.6 = CTmax ~ juvenile mass + hatch size + growth rate + (1|fish ID)

I removed factors that were less likely to be ecologically significant if they had no significant effect on performance, and models that excluded these factors had lower AIC values and therefor better fit. Average daily larval growth variance was removed from the final models as it had no effect on routine metabolic rate (Estimate = 0.002 ± 0.05, $t_{64.8}$= 0.039, *P* = 0.969), burst swimming speed (Estimate = 0.0098 ± 0.0055, $t_{77.08}$= 1.786, *P* = 0.078) or critical thermal maximum (Estimate = -0.0009 ± 0.079, $t_{83.8}$= -0.012, *P* = 0.991). Similarly, the interaction between test temperature and growth rate had no effect on routine metabolic rate (Estimate = -0.024 ± 0.023, $t_{300.58}$= -1.073, *P* = 0.284) or burst swimming speed (Estimate = -0.00005 ± 0.0009, $t_{82.1}$= -0.586, *P* = 0.560) and lowered model AIC values.

Figure 4.6 summarises the findings across the aforementioned multiple regression models. These results indicate that larval growth rate and settlement size are likely to be important traits for post-metamorphic thermal performance and that effects of larval growth rate are likely to be carried-over through metamorphosis, but pelagic larval duration may be de-coupled with post-metamorphic performance at metamorphosis.

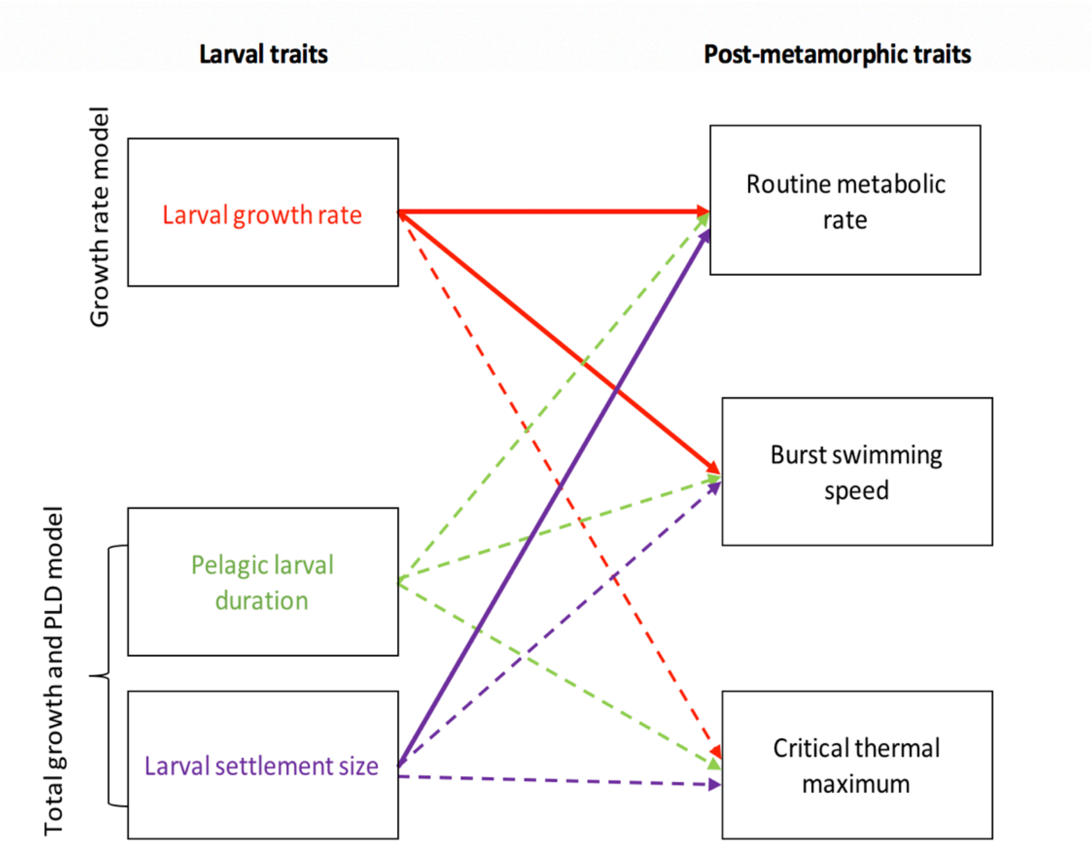


Figure 4.6. Results summary of correlations between larval traits and post-metamorphic traits. Thick lines indicate a significant *negative* effect ($P \leq 0.05$) of the larval trait on post-metamorphic performance and dashed lines indicate no correlation ($P > 0.05$) between larval traits and post-metamorphic performance

4.5 Discussion

To my knowledge, this is the first study to show that marine fish with fast larval growth rates are likely to have reduced post-metamorphic performance across temperature. Specifically, I found that fast larval growth rates correlate with slower burst swimming speeds and lower routine metabolic rates across temperature in a wild marine fish, supporting the **life stage trade-off hypothesis**. Larger settlement sizes also correlated with lower routine metabolic rates among individuals across test temperatures, but was not correlated with burst swimming speed. Thus, these results support the idea that settling large does not have a lasting advantage on juvenile performance and is costly for some post-settlement performance traits, rejecting the **bigger is better hypothesis**. In addition, hatch size and pelagic larval duration were not correlated with any post-metamorphic performance traits I assessed, and larval growth rate and settlement size were not correlated with critical thermal maximum suggesting that some larval traits may be de-coupled with post-metamorphic traits, supporting the **life stage de-coupling hypothesis**. These results are summarised in Figure 4.6.

I found that larval growth rate and settlement size were negatively correlated with post-metamorphic routine metabolic rate; and larval growth rate, but not settlement size, was negatively correlated with burst swimming speed. The rates at which larvae grow and the size individuals settle at, however, have different life strategy implications for individuals. For example, as growth rate was only weakly correlated with pelagic larval duration, an individual could grow slowly for a long period of time and reach the same settlement size as an individual that grew quickly over a short amount of time. Therefore, if settlement size was positively correlated with performance it would suggest that settlement size has long-lasting positive effects on fitness and survival, where individuals that settle large stay large post-metamorphosis and have better performance than smaller individuals. Larval growth rate, however, is linked with investment of energetic resources into different metabolic activities such as growth, locomotor function, and cell and body condition maintenance (Schluter 1995; Billerbeck et al. 2001; Arnott et al. 2006). Here, fast larval growth can either help individuals settle large and potentially stay large, or fast growth might be costly for other metabolically demanding activities post-metamorphosis. Since both larval growth rate and settlement size were correlated with each other, but were significantly *negatively* correlated with juvenile routine metabolic rate, I suggest that settlement size, larval growth rate and therefore metabolic investment, are likely to play a role in post-metamorphic performance and survival. In this case, larvae with slower growth rates have higher metabolic rates and faster burst swimming speeds, and larvae with smaller settlement sizes are likely to have higher post-metamorphic metabolic rates.

I found no evidence suggesting that settling large has any long term positive effects on performance or survival. This is surprising given that several other have studies found that large settlement size is advantageous to many invertebrate species (Marshall et al. 2003; Marshall and Keough 2004; Marshall et al. 2006). Many of these previous studies, however, were on non-feeding larvae, whereas *B. cocosensis* feeds during their larval stage. Feeding during development is likely to affect growth rates (Strathmann 1985), and therefore is likely to increase the amount of variation in larval growth rate within a population depending on feeding success. I found that variance in larval growth rates were limited within the sample population, however, where otoliths only grew 2.2 – 3.4 µm on average per day. This could be due to larval growth rates being evolutionarily constrained, or the lack of variance could be explained by sampling bias of collecting individuals that survived past larval settlement. Greater larval growth variation may have existed prior to settlement at Heron Island, but only individuals that made it past juvenile metamorphosis were able to be collected for this study, and of that sample group phenotypic variance was limited.

Interestingly, hatch size was not correlated with larval growth rates or settlement size, but larval growth rates and total larval growth were correlated with settlement size. I expected to observe compensatory growth in individuals that hatched small, however, other variables, such as predation pressure, water temperature, food availability or genetics may have influenced variation in larval growth rate, rather than hatch size. My findings, however, are consistent with another study on temperate Australian *B. cocosensis* that assessed how larval traits correlate with post-settlement traits across three cohorts, and indicates that hatch size does not predict settlement size (Thia et al. 2018). As rapid larval growth rates were negatively associated with post-metamorphic thermal performance of burst swimming speed and routine metabolic rate, perhaps *B. cocosensis* have evolved to avoid compensatory growth for small hatching individuals to evade post-metamorphic performance costs that may be associated with fast larval growth rates.

This study shows that individuals with slow larval growth rates have faster burst swimming speeds and higher metabolic rates than individuals with fast larval growth rates. It is likely that fast larval growth is a strategy to avoid mortality associated with the dangerous pelagic larval phase, but rapid growth during this stage is costly and trade-offs between growth rate and post-metamorphic performance are likely to exist. Fast larval growth rates have been observed in other species as a survival strategy such as larval frogs, where fast growth can allow improved escape from predators in drying ponds (Travis et al. 1985; Banks and Beebee 1988; Newman 1988) but has also been observed to have negative effects on post-metamorphic locomotion (Ficetola and De Bernardi 2006). Hatch date has also been shown to effect growth rate in male common triplefins (*Forsterygion lapillum*), where individuals that hatched later had greater compensatory growth rates, and individuals that had earlier hatch dates had greater reproductive success (Moginie and Shima 2018). Trade-offs between rapid growth and performance/fitness may allow population wide genetic variation for growth rate to be maintained, for example, the fitness of individuals with slow larval growth rates and fast post-metamorphic swimming speeds may be similar to fast larval growth rates and slow post-metamorphic swimming speeds (Mangel and Stamps 2001; Arendt 2003). Thus, individuals with both strategies could be equally likely to be found in nature.

I found no relationship between hatch size and pelagic larval duration and post-metamorphic performance. I suspect that this lack of correlation may be an example of trait decoupling with metamorphosis. As metamorphosis from larval to juvenile life stages is usually accompanied with a shift in habitat niche, it is intuitive that some larval traits would change with metamorphosis to allow for adjustment to a new habitat. Considering *B. cocosensis,* developmental traits such as settlement size and growth rate were correlated with post-metamorphic performance across temperature and are therefore likely to have carry-over effects to later life stages. Therefore, I believe that some larval traits are likely to transcend to later life stages while other traits may be de-coupled.

In addition, it was interesting that acute thermal performance curve shape was not altered by larval growth rate or settlement size (as there was no significant interaction between growth rate and test temperature or settlement size and test temperature on post-metamorphic thermal performance). In other words, the shape of their thermal performance curve was not correlated with either larval growth rate or settlement size. In another study, adult *B. cocosensis* did not alter the shape of acute thermal performance curves with acclimation to seasonal thermal temperatures (da Silva et al. 2019). Therefore, perhaps thermal performance curve shape is constrained in *B. cocosensis*. I hypothesise that wide thermal performance curves are maintained in both populations of *B. cocosensis* as a mechanism to survive in the thermally variable intertidal zone.

I also investigated effects of juvenile morphology on critical thermal maxima and found that juvenile individuals with greater body masses had higher critical thermal maxima (regardless of larval experiences). This result is interesting because it indicates that individuals with lower body masses are more prone to negative effects of extreme heat days, which are becoming more frequent with global climate change. Individuals with shorter standard lengths also had slower burst swimming speeds, therefore, if temperatures throughout the Great Barrier Reef continue to rise smaller fish may have slower escape speeds from predators and have lower maximum temperature thresholds. These small individuals will therefore be under the most physiological thermal stress and are likely to have higher mortality rates due to predation (as a result of slow swimming speeds) prior to reproductive age. Other studies, however, have found that reef fish and rainbow trout body mass and critical thermal maximum either have no significant relationship or an inverse relationship (Ospina and Mora 2004; Recsetar et al. 2012; Messmer et al. 2017), rather than a positive relationship as observed in our data. Therefore, future experiments on more species are required to understand the relationship between mass and critical thermal maximum in marine fish.

In conclusion, this study is the first to show that developmental traits can affect post-metamorphic performance across temperature in a marine fish with a planktonic larval stage. In addition, I provide evidence both for larval traits having carry-over effects on later life stages and also for larval traits being de-coupled with thermal performance post-metamorphosis. Larval traits such as growth are important for individual body condition and energy portioning in later life stages and are consistently found to play an important role in post-metamorphic performance and fitness across many different taxa with complex life-stages (Pechenik et al. 1996; Marshall et al. 2003; Ficetola and De Bernardi 2006; Careau et al. 2008; Grorud-Colvert and Sponaugle 2011). Rapid growth during the larval phase is likely to be a strategy to avoid predation during the risky planktonic stage to survive to settlement but appears to be associated with negative performance trade-offs post-metamorphosis for *B. cocosensis*. Thermal performance is an important indicator of organismal fitness, however, there is a need for future studies to test the direct effects of larval traits and environmental variation on life-time fitness.

Chapter 5

General discussion

5.1 Overview

Organisms living in intertidal environments offer the opportunity to address how short- and longer-term thermal fluctuations affect ectotherm thermal performance. In variable thermal environments, selection is likely to favour individuals with wide thermal breadths, the capacity to acclimate, or both (Gilchrist 1995; Gabriel 2005; Gabriel et al. 2005; Angilletta 2009c; Lande 2009; Healy and Schulte 2012). The way in which daily and seasonal thermal variability affect the co-evolution of thermal performance curve shape and acclimation capacity and how larval traits affect post-metamorphic thermal performance remain relatively unknown. I used marine gobies in intertidal environments to assess how a variety of performance traits are altered by short- and long-term thermal change. In addition, I used the complex life-cycle of *Bathygobius cocosensis* to assess how larval traits and survival strategies are likely to be linked with post-metamorphic performance. In this thesis, I show that: 1) thermal acclimation and wide thermal performance curves can be maintained when equal daily and seasonal thermal fluctuations are experienced; 2) the rate of luminance change (for camouflage) can acclimate with thermal change on a scale of weeks; and 3) fast larval growth rates are costly in that they are negatively correlated with post-metamorphic thermal performance, but larval traits do not alter thermal performance curve shape.

5.2 Thermal acclimation and performance curve shape

In Chapter 2, I show that subtropical intertidal fish have the capacity to acclimate despite experiencing equal daily and seasonal thermal fluctuations and possess wide thermal performance breadths. Thermal acclimation is predicted to be costly and unlikely to evolve when daily thermal variability is equal to or greater than seasonal thermal variability, or when thermal performance curves are wide (Gabriel 1999; Wilson and Franklin 2000; Gabriel 2005). This is because large daily thermal fluctuations are predicted to mask small changes in seasonal thermal variation, making seasonal thermal change unpredictable, and therefore costly (Gabriel 2005; DeWitt et al. 1998). In addition, the costs of maintaining the ability to acclimate are likely to outweigh the benefits of thermal acclimation when performance curves are wide as performance improvements with acclimation are likely to be minimal (Wilson and Franklin 2000; Angilletta 2009). I suggest that *B. cocosensis* maintain their ability to acclimate in the subtropical intertidal environment because gradual changes in daily thermal means and range with season allow seasonal thermal variation to remain predictable, and therefore thermal acclimation is likely to be beneficial despite large daily thermal fluctuations, hence favouring the evolution or retention of acclimation capacity within a population (DeWitt et al. 1998; Gabriel et al. 2005). It is also likely that *B. cocosensis* maintains wide thermal performance curves as an adaptation to an environment with rapid diurnal change (Healy and Schulte 2012). Wide thermal performance curves allow ectotherms to maintain performance across a wide range of temperatures and permit organisms to survive in environments that fluctuate too quickly for thermal acclimation to occur (i.e. on a scale of hours) (Angilletta et al. 2002; Angilletta 2006; Angilletta 2009; Asbury and Angilletta 2010).

Although seasonal thermal change remains predictable in the subtropical intertidal environment, it is surprising that *B. cocosensis* have the capacity to acclimate to extreme seasonal thermal conditions (I exposed them to their thermal range extremes: tropical summer conditions that occur in the Northern Territory, and temperate winter thermal conditions that occur in southern New South Wales) that they are unlikely to experience at subtropical Point Lookout. It is likely that intertidal fish with planktonic pelagic larval phases maintain high degrees of plasticity throughout their life-time and between generations because their parent's thermal environment, their larval environment, and the environment that they settle at are all likely to be different. Therefore, animals that experience high thermal uncertainty within and among life-stages may retain high degrees of plasticity to avoid phenotype-environment mismatches and decreases in thermal performance and fitness throughout their lifetime and between generations. For example, a model by Scheiner and Holt (2012) shows that increases in dispersal rates select for increased plasticity when spatial and temporal environmental variation is high to increase the likelihood of offspring survival in changed environmental conditions.

It is commonly argued that the frequency and predictability of temperature change imposes selection pressure on acclimation capacity (Wilson and Franklin 2000; Gabriel 2005; Angilletta 2009). These processes are likely to be important for organisms that experience temporal variation, however, they may not explain how species that experience a large range of temporal and spatial thermal variation develop responses to thermal change (Chevin and

Lande 2011). For example, a model by de Jong (1999) shows that variation in population phenotypes (such as performance) among habitats are caused by a combination of phenotypic plasticity and local adaptation in an organism that has a life-cycle with unpredictable selection and spatial variation. High degrees of plasticity are likely to occur due to the low predictability of future selective environments, especially when dispersal occurs between development and selection (de Jong 1999; de Jong and Behera 2002). The high degree of thermal variability and unpredictability that *B. cocosensis* experience between their larval and adult life stages and between generations may explain their ability to acclimate to seasonal temperatures that they have not previously experienced in their juvenile and adult life stages. This suggests that thermal acclimation can remain beneficial when environmental change is not predictable. Gilchrist (1995) suggests that wide performance curves are more likely to evolve (than narrow thermal performance curves) when between generation thermal variation is greater than within generation thermal variation, because wide performance breadths act as a safety net for future thermal change. A similar hypothesis can be posed for organisms, such as those with dispersing larval phases, that are likely to experience different generational thermal means and increases in thermal variability. Previous studies have shown that plasticity is dependent on the degree of thermal variability a population has experienced in past generations, where populations that experience more past thermal variability are likely to be more plastic (Hallsson and Björklund 2012). Therefore, perhaps a large degree of plasticity evolves or is retained when among-generation thermal variation is greater than within-generation thermal variation. The ability to acclimate to seasonal change and survive in different thermal environments each generation may therefore be governed by a combination of within- and among-generation thermal variability.

The dispersal patterns of larval *B. cocosensis* remain unclear, but it is likely that each generation moves in a relatively southerly direction with currents off Australia's east coast (Fig 5.1 & Fig 5.2). Direction of larval flow is complex and not well understood, and small nearshore counter currents along Australia's east coast are likely to increase larval retention at lower latitudes. However, it is likely that a large proportion of the larvae are pushed south along Australia's east coast (Figure 5.2) (Hock et al. 2017). Each generation is then likely to experience a decrease in mean temperature and increased daily and seasonal thermal variability with dispersal to higher latitudes (Fig 5.1). Along Australia's east coast each generation is likely to experience predictable seasonal thermal change (as seasonal thermal fluctuations are greater than daily thermal fluctuations in the tropics and temperate regions, and I show that seasonal thermal change remains predictable in the subtropical intertidal zone). Therefore, if the capacity to acclimate is driven by within-generation daily and seasonal thermal variability alone, then each generation would not have the ability to survive in average cooler and more variable thermal conditions. An explanation that that involves how unpredictable spatial and temporal thermal variation between generations alters acclimation responses may be more conducive to explaining how organisms with high dispersal maintain plasticity (de Jong 1999; de Jong and Behera 2002; Chevin and Lande 2011). Previous studies have argued that developmental conditions can influence post-metamorphic acclimation capacities (Scott and Johnston 2012; Seebacher et al. 2014; Beaman et al. 2016). I believe that *B. cocosensis* are likely to retain their capacity to acclimate irrespective of the conditions they experience during development. Maintaining a high level of plasticity will allow individuals to respond to a large degree of among generation and between life-cycle thermal variability and unpredictability. In addition, if developmental conditions dictate post-metamorphic acclimation capacity, adult individual are likely to experience a phenotype-environment mismatch if their settlement location experience different thermal regimes to their early developmental conditions.

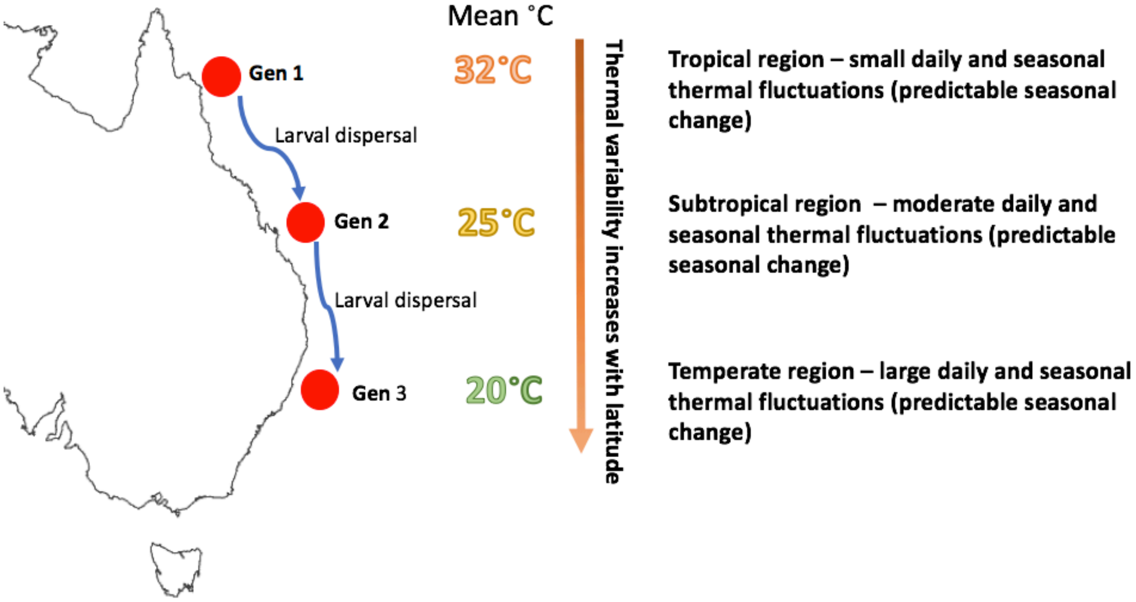


Figure 5.1. Conceptual illustration showing how each generation of *Bathygobius cocosensis* is likely to experience increased thermal variability and decreased generational thermal means. Seasonal thermal change is likely to remain predictable along all of these locations, despite the magnitude of thermal change becoming greater with latitude. Between generation thermal variation is likely to be greater than within generation thermal variation. Temperature information was collected from the Australian Governments Bureau of Meteorology.

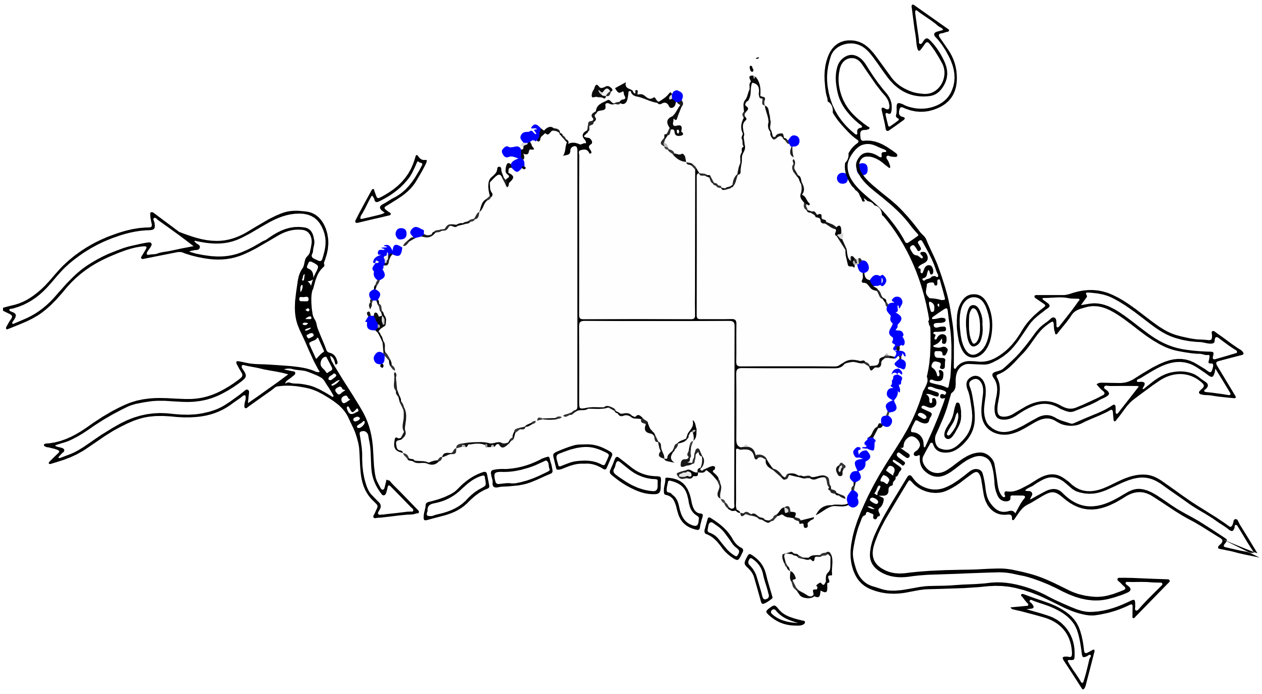


Figure 5.2. Main oceanic currents around Australia, both the East Australian Current and the Leeuwin current move in a southerly direction down Australia's coastlines. The blue dots indicate *Bathygobius cocosensis* locality points collected from the Global Biodiversity Information System (https://www.gbif.org/).

It would be interesting to assess differences in acclimation capacities among populations of *B. cocosensis* along Australia's east coast in the future. If all populations of *B. cocosensis* have the same capacity to shift their thermal sensitivity (despite inhabiting environments that experience different degrees of daily and seasonal thermal variability) perhaps their ability to acclimate is driven by between-generation changes in thermal environment rather than within-generation thermal variability. If acclimation capacity is driven only by within-generation thermal variability, then populations at higher latitudes that experience greater thermal variability would be expected to have greater acclimation capacities (if there was local adaptation). It would also be interesting to compare the acclimation capacities of species with and without larval dispersal. Data from these studies could be used to develop a model to assess how within-generation (daily and seasonal thermal variability) and between-generation thermal variability drive the co-evolution of thermal performance curve shape.

Predicting how thermal generalists, like *B. cocosensis* and other intertidal species, will respond to future climate change scenarios is an under-studied area of thermal biology. Most studies that predict organism's responses to climate change are conducted on thermal specialists (organisms with narrow thermal performance curves) as they are likely to be the most sensitive to thermal change (Deutsch et al. 2008; Tewksbury et al. 2008; Somero 2010). Thermal generalists (organisms with wide thermal performance curves), however, are extraordinarily important in ecosystem processes (Richmond et al. 2005), and are often very abundant within their ecosystem. Loss of thermal generalists, and potentially key-stone species, may lead to ecosystem collapses (Ellison et al. 2005). It would be interesting to assess how a range of thermal generalist species respond to future climate change scenarios in a future study. For example, assessment of whether range size correlates with percent range change under predicted future environmental conditions in thermal generalists would be interesting. Species with greater thermal performance breadths and latitudinal ranges would be predicted to be more robust than species with more narrow curves and smaller geographical ranges. This has yet to be explicitly shown in previous studies.

5.3 Acclimation of luminance change

Camouflage is a fascinating predator avoidance mechanism that is used among many cryptic animals (Stuart-Fox et al. 2003; Stuart-Fox and Moussalli 2008; Sköld et al. 2013; Sköld et al. 2016; Stevens 2016; Cheney et al. 2017; Duarte et al. 2017b). The rate at which animals can change colour for background matching is affected by environmental temperature, however, no previous studies have assessed how long-term changes in temperature affect rate of background matching. Most previous studies that have assessed how performance is altered by long-term temperature have assessed physiological traits such as metabolism, locomotion or growth rate (Wilson and Franklin 1999,2000; Hammill et al. 2004; Fangue et al. 2008; Angilletta 2009c; Healy and Schulte 2012). However, it is important to determine how traits that influence predator-prey dynamics and other behavioural interactions will be impacted by changes in environmental temperature. In Chapter 3, I show for the first time, that rate of luminance change, for background matching can acclimate to different thermal conditions. Thermal acclimation was observed when warm-exposed fish showed faster rates of luminance change than the cold-exposed fish when tested at 31ºC, and the cold exposed-fish had faster rates of luminance change than the warm-exposed fish when tested at 16ºC. The ability to acclimate rate of camouflage is a highly adaptive trait, as the ability to remain cryptic from prey and predators in shifting climates will improve survival probabilities and fitness. As this was the first study to investigate how long-term changes in temperature alter rate of luminance change, no previous studies have assessed the physiological mechanism that allows chromatophore function to acclimate or the energetic costs involved in changing colour.

The energetic demands of luminance change, or the effect rate of colour change has on metabolism is still not well understood, however, faster rates of colour change are hypothesized to be more metabolically demanding than slower rates of colour change (Duarte et al. 2017). I found that on average rate of luminance change was faster in the warm acute test temperature than the cold acute test temperature, and that routine metabolic rate increases with test temperature in Chapter 2. Both rate of luminance change and metabolism are likely to increase with temperature because biochemical reactions proceed more quickly at higher temperatures (Angilletta 2009). However, I did not test how rate of luminance change affects metabolism directly, and there may be an additional cost of rapid luminance change in addition to the thermodynamic effects of temperature on metabolism. One study has shown that guppies increase their food consumption when they change colour to match their visual backgrounds (Rodgers et al. 2013), suggesting an increase in metabolic demand. Rapid changes in colour often occur through neuro-muscular action (Duarte et al. 2017), and rate of muscular movement, which has significant metabolic demands, is known to be directly related to temperature (Racinais and Oksa 2010). Therefore, it is likely that faster rates of luminance change are positively correlated with higher metabolic rates and test temperature. However, experimental tests that assess metabolic rate before and while an animal is conforming to its background at different test temperatures are required to understand the energetic demands of colour or luminance change.

Bathygobius cocosensis altered their phenotypes to become darker over time to match the black background they were tested against, however, they remained distinguishable from the background to the coral trout. Birds are the other largest predators of *B. cocosensis*, especially to individuals that inhabit shallow high tide pools. Birds are tetrachromates, rather than trichromates like fish, meaning that birds can see wavelengths in the UV spectrum (Endler and Mielke 2005). Therefore, the capacity *B. cocosensis* have to background match may be perceived differently to an avian predator. It is important to understand how changes in phenotype will influence predator-prey dynamics among many species in an ecosystem to gain a more holistic view on how changes in temperature will alter survival and fitness. By assessing the affect temperature has on prey background matching ability we can begin to understand how climate change will affect a physiological mechanism that influences predator-prey behavioural interactions. Rapid stochastic changes in temperature are likely to affect how quickly cryptic species can match their backgrounds, where decreases in rate of camouflage are likely to result in detection by a predator which is likely to reduce survival and fitness. Therefore, climate change is likely to directly increase the frequency of predator-prey interactions, by reducing prey species background matching abilities. Future studies are required to understand how climate change is altering predator avoidance mechanisms in other taxa that use camouflage as an anti-predator mechanism.

5.4 Developmental traits and post-metamorphic thermal performance

In my fourth chapter, I investigated how larval traits are linked with juvenile thermal performance. I found fast larval growth rates are correlated with slower burst swimming speeds and lower routine metabolic rates. This finding is interesting because it shines light on how different larval survival strategies can have knock-on effects for juvenile thermal performance. No previous studies have identified how wild marine larval traits are linked with the thermal performance of post-metamorphic fish.

Fast larval growth rates are predicted to be a strategy to reduce the time spent in the dangerous pelagic zone where mortality rates are extraordinarily high (Meekan and Fortier 1996; Wilson and Meekan 2002; Cowen and Sponaugle 2009; Shima and Swearer 2009; Gimenez 2010). Temperature is also known to affect larval growth rates, where increases in temperature are often correlated with increased larval growth rates (Green and Fisher 2004). In line with my findings, rapid larval growth rates have been shown to be costly for post-metamorphic performance in amphibians (Ficetola and De Bernardi 2006), and it is predicted that energetic costs involved with rapid larval growth are likely to be costly for other metabolically demanding activities such as muscular development and locomotion (Stevens et al. 1998; McCarthy 2000; Arendt et al. 2001). If increases in developmental temperature are driving rapid larval growth rates, then warmer oceanic temperatures associated with climate change are likely to have serious implications on the performance of post-metamorphic individuals. For example, increases in oceanic temperatures with climate change are likely to drive faster larval growth rates (Pankhurst and Munday 2011), which are correlated with reduced post-metamorphic performance in fish and frogs (Beck and Congdon 2000; Ficetola and De Bernardi 2006; Tejedo et al. 2010; da Silva et al. 2019b). Therefore, fitness in populations that experience warmer environmental conditions is likely to be reduced, and therefore, genetic variation at low latitudes (where warm water temperatures are experienced) may also be reduced.

While slower burst swimming speeds are an obvious cost of rapid larval growth rates, as slower individuals are likely to have reduced predator avoidance capabilities, it is less clear whether fast or slow metabolic rates are "better" for fitness. Some studies suggest that individuals with slow resting metabolic rates are likely to have higher fitness than individuals with fast metabolic rates (Steyermark 2002; Krams et al. 2013). This is because individuals with low metabolic rates are able to allocate more energy to demanding activities such as reproduction and cellular maintenance (Beck and Congdon 2000). For example, low metabolic rates have been observed to be correlated with higher survival rates in meal worm beetles (Krams et al. 2013). In contrast, other studies predict that faster metabolic rates are likely to be linked with higher fitness (Garamszegi et al. 2006; Kaseloo et al. 2012), as high metabolic rates are also likely to facilitate increased energy for other energetically demanding activities. For example, male mice with higher basal metabolic rates have larger testes and thus are likely to have greater reproductive success (Kaseloo et al. 2012), and high metabolic rates in birds are correlated with greater song complexity, which is known to be linked with mate quality and fitness (Garamszegi et al. 2006). A large meta-analysis that compares metabolic rates with fitness across taxa is required to gain a better understanding on how metabolic rate affects fitness.

I was able to determine how larval traits such as growth rates, hatch size and settlement size are correlated with post-metamorphic thermal performance in this study; however, it was disappointing that I was unable to determine the environmental conditions that *B. cocosensis* experienced during their larval dispersal. Initially I aimed to test how thermal conditions during development altered post-metamorphic thermal performance. For example, I hypothesised that individuals that experienced greater thermal variability during larval dispersal would have wider thermal performance curves and greater acclimation capacities. It is possible to determine the natal origins of fish using Nitrogen, Carbon or Strontium isotope signatures at the hatch mark within otoliths (Thorrold et al. 2001; Rachel et al. 2008; Walther et al. 2008; Schloesser et al. 2010). Isotope analysis requires a calibrated isotope library for the study site latitude water chemistry, and is quite expensive, and thus was outside the scope of my thesis (Thorrold et al. 2001; Rachel et al. 2008; Walther et al. 2008; Schloesser et al. 2010). In addition, it is possible to back-calculate larval dispersal patterns using their pelagic larval durations and current velocities that likely carried larvae to their settlement locations (Condie et al. 2005; Pfeiffer-Herbert et al. 2007). This technique allows estimation of the distance and direction that larvae dispersed prior to arriving at their settlement location. Oceanic temperatures along the dispersal path of larvae can then be estimated at different locations using marine environmental variable data bases such as BIO-ORICLE (Tyberghein et al. 2012), which provides information on marine temperature, salinity, oxygenation and chlorophyll *a* concentrations at different depths. However, many marine larvae migrate vertically with changes in photoperiod and age (Paris and Cowen 2004), and the depth that *B. cocosensis* larvae migrate to is unknown but is likely to influence the environmental temperatures that they are exposed to. Future studies could attempt to use these techniques to determine the range of thermal conditions larvae experience and how these conditions might drive differences in their post-metamorphic thermal performance. The conditions pelagic larvae experience and how their traits alter post-metamorphic performance is a fascinating and relatively unexplored topic and further research in the field is likely to uncover links between developmental conditions and experience, on the one hand, and adult fitness and survival, on the other.

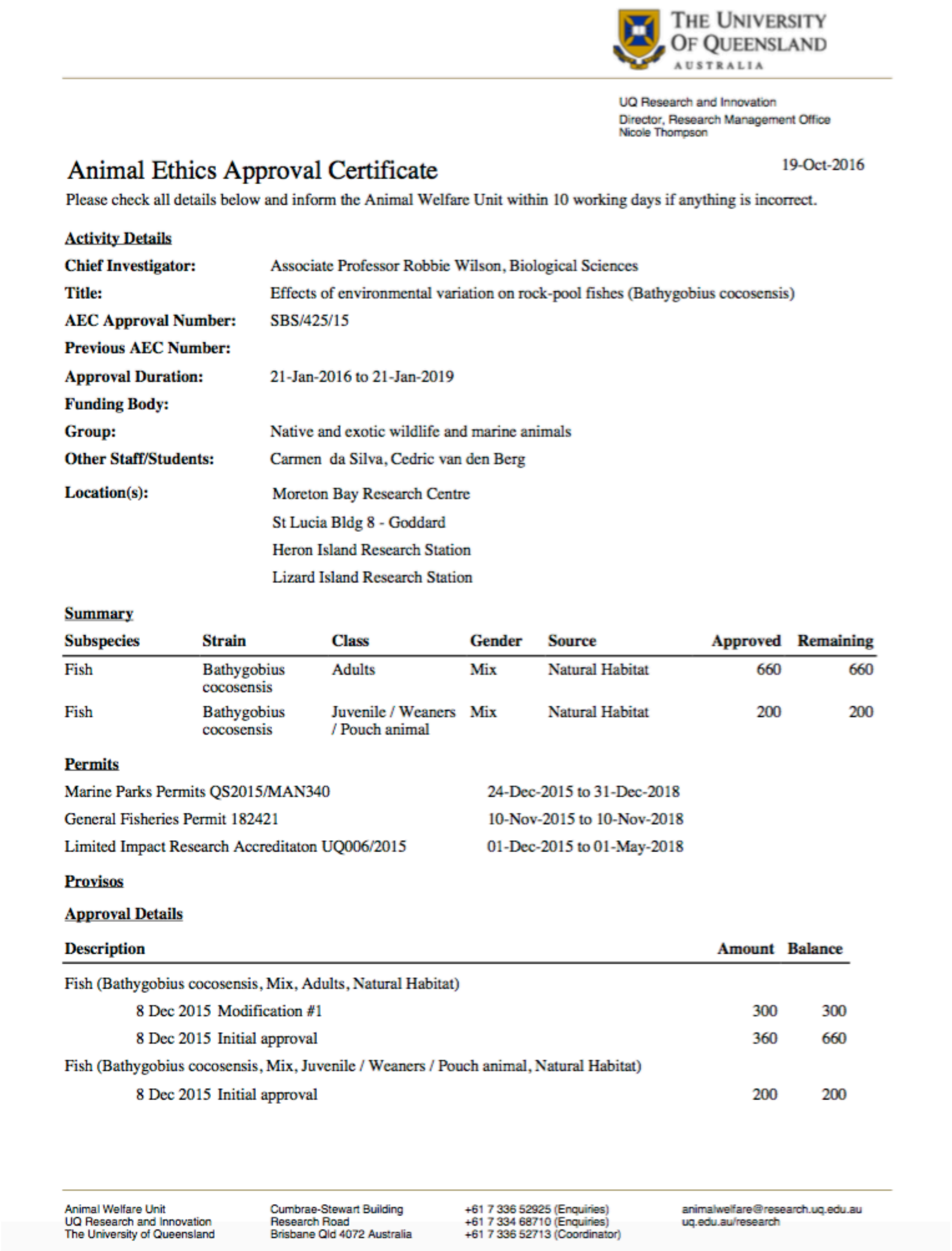
5.5 Concluding remarks

The way in which daily, seasonal and intergenerational thermal variability affects ectotherm performance is extraordinarily complex. The time scales, magnitude and predictability of thermal variation affects the evolution of thermal performance curve shape and acclimation capacity (Gabriel 1999; Angilletta Jr et al. 2002; Gabriel 2005; Gabriel et al. 2005; Angilletta 2006; Angilletta 2009; Asbury and Angilletta Jr 2010; Healy and Schulte 2012; Beaman et al. 2016; Rohr et al. 2018). Developmental traits such as growth rate and size can also influence post-metamorphic acute thermal performance (Chapter 3). To understand how animals respond to changes in temperature it is important to assess ecologically relevant traits that are linked to survival and fitness. Measuring traits such as rate of luminance change for background matching can shine light on the physiological mechanisms that drive predator-prey interactions. In addition, it is important to compare how temperature affects the thermal sensitivity of performance among populations and different life-stages.

I found an interesting commonality between the Point Lookout adult population and the Heron Island juvenile population. Thermal performance curve shape was not altered by thermal acclimation to seasonal thermal conditions in adult fish from Point Lookout (i.e. curve shape stayed the same but the curve itself shifted with changes in temperature) or variation in larval growth rate and settlement size in juvenile fish from Heron Island. This finding is interesting because there is a large degree of variation in performance between individuals in both studies, and performance thermal optima’s can be shifted with changes in temperature or larval growth rate, but variation in performance curve shape is relatively small in both studies. These findings provide evidence to suggest that the shape of thermal performance curves may be constrained in *B. cocosensis*, or animals that experience rapid daily thermal fluctuations. Similar findings by Healy and Schulte (2012) show that thermal performance curve shape is not altered by thermal acclimation in temperate killifish; and other studies by Niehaus et al. (2011) and Schuler et al. (2011) show that thermal performance curve shape does not change with exposure to different degrees of thermal variability or stochasticity. Therefore, in environments that experience rapid daily thermal fluctuations, thermal performance curve shape may be constrained, but selection may favour a high degree of reversible plasticity for species that experience seasonal thermal variation or changes in thermal environment between life-cycles.

In this thesis I contribute to the understanding of the evolution of reversible plasticity and performance curve shape by showing that thermal acclimation can be maintained in an environment where daily and seasonal thermal variability is equal; by adding evidence to suggest that acclimation capacity can be retained when thermal performance curves are wide; that animals that experience great intergenerational thermal variability are likely to retain high levels of plasticity; that rate of luminance change for background matching has the potential to acclimate to changed thermal conditions; and that rapid developmental growth rates can be costly for juvenile thermal performance. I have combined concepts across a broad range of biological disciplines including thermal physiology, thermal adaption, evolutionary ecology, visual ecology, animal behaviour, larval biology, and developmental physiology. By combing concepts and techniques from these disciplines I have been able to contribute knowledge on how temperature affects a species that experiences a high degree of environmental uncertainty from larval dispersal and a large degree of daily and seasonal thermal variability.

Appendix: Animal ethics approval



THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

UQ Research and Innovation
Director, Research Management Office
Nicole Thompson

Animal Ethics Approval Certificate

19-Oct-2016

Please check all details below and inform the Animal Welfare Unit within 10 working days if anything is incorrect.

Activity Details

Chief Investigator:	Associate Professor Robbie Wilson, Biological Sciences
Title:	Effects of environmental variation on rock-pool fishes (Bathygobius cocosensis)
AEC Approval Number:	SBS/425/15
Previous AEC Number:	
Approval Duration:	21-Jan-2016 to 21-Jan-2019
Funding Body:	
Group:	Native and exotic wildlife and marine animals
Other Staff/Students:	Carmen da Silva, Cedric van den Berg
Location(s):	Moreton Bay Research Centre St Lucia Bldg 8 - Goddard Heron Island Research Station Lizard Island Research Station

Summary

Subspecies	Strain	Class	Gender	Source	Approved	Remaining
Fish	Bathygobius cocosensis	Adults	Mix	Natural Habitat	660	660
Fish	Bathygobius cocosensis	Juvenile / Weaners / Pouch animal	Mix	Natural Habitat	200	200

Permits

Marine Parks Permits QS2015/MAN340	24-Dec-2015 to 31-Dec-2018
General Fisheries Permit 182421	10-Nov-2015 to 10-Nov-2018
Limited Impact Research Accreditaton UQ006/2015	01-Dec-2015 to 01-May-2018

Provisos

Approval Details

Description	Amount	Balance
Fish (Bathygobius cocosensis, Mix, Adults, Natural Habitat)		
8 Dec 2015 Modification #1	300	300
8 Dec 2015 Initial approval	360	660
Fish (Bathygobius cocosensis, Mix, Juvenile / Weaners / Pouch animal, Natural Habitat)		
8 Dec 2015 Initial approval	200	200

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