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Temperature preferences of tropical fishes and the influence of local abiotic and biotic factors

Thesis submitted by

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Statement of Contribution of Others

This work is the result of collaborations with my supervisors A/Prof Andrew Hoey and Prof Morgan Pratchett as well as collaborations with A/Prof Jodie Rummer who provided intellectual and editorial guidance and financial support. A/Prof Jacob Johansen and Prof John Steffensen provided intellectual and editorial guidance as well as technical support for all chapters. Dr. Connor Gervais provided intellectual and editorial guidance as well as data collection for Chapters 2 and 3. Rohan Longbottom assisted in the collection and animal husbandry of epaulette sharks required for Chapter 3 and Michael Meehan provided statistical advice for environmental temperature data for Chapter 3. Research funding was provided by the Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University. Research funding for Chapter 3 was provided through the #WalkingSharks crowdfunding campaign organised through James Cook University. I received a stipend through the Postgraduate Research Scholarship from James Cook University.

Declaration of ethics

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care of Use and Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research project received animal ethics approval from the JCU Animal Ethics Committee Approval Numbers A2316 and A2089.

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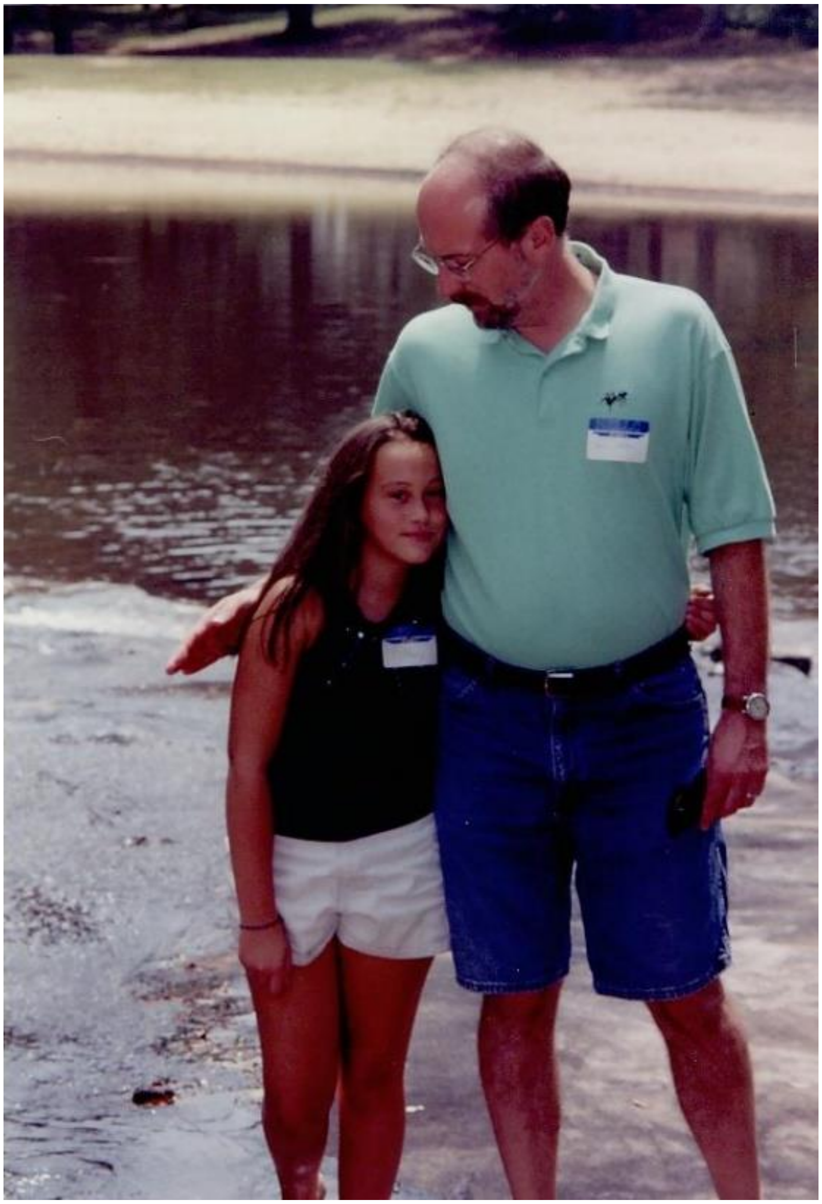

I love you Dad.

General Abstract

Temperature is one of the most important abiotic conditions for ectothermic organisms as it dictates the rates of many physiological and biochemical processes and is a large determinant of the distribution of ectotherms across a range of spatial scales. Within an organism's thermal tolerance range, the relationship between temperature and a range of performance traits (e.g., metabolic rate, fecundity, and growth) typically resemble a left-skewed distribution; with performance increasing with increasing temperature until it reaches a maximum (or thermal optimum), beyond which performance rapidly declines toward their critical thermal maximum. While thermal performance curves have traditionally been used in studies of adaptative evolution and phenotypic plasticity, there has been increasing interest in their use to understand and predict the responses of populations, species, and assemblages to global warming. For example, those species that have evolved in relatively thermally stable environments (i.e., low latitudes) are thought to have a narrower thermal tolerance range and their thermal optimum closer to their thermal maximum, making them more susceptible to increases in temperature than those that have evolved in more thermally variable environments (i.e., high latitudes). Predicting the likely effects of increasing temperatures on populations and species, and the potential for individuals to use movement to occupy favoured thermal environments requires a clearer understanding of their optimal temperature, and how these are influenced by the thermal variability of the environment and of local biotic and abiotic factors. The selection of preferred temperatures (as a proxy for optimum temperature) has been studied in many taxa, but has been largely overlooked in tropical fish species.

Therefore, the overall objective of this thesis is to investigate the thermal preferences of tropical reef fish species and how local abiotic and biotic factors affect their thermal preference. To do this, the thesis is divided into two sections based on the thermal variability of the environment. The first two chapters focus on the thermal preferences of two species that inhabit thermally variable intertidal and reef flat environments and the behavioural strategies they use to cope with such variability. In chapter 2, I aimed to establish the physiological (i.e., oxygen uptake rate) and behavioural (i.e., emergence and thermal preference) responses of the barred mudskipper (*Periophthalmus argentilineatus*), a common inhabitant of tropical intertidal habitats, when exposed to a range of temperatures for a prolonged period of time. In chapter 3, I determined the thermal preference of a coral reef flat resident, the epaulette shark (*Hemiscyllium ocellatum*), and investigated whether this species was using movement to occupy thermally favourable microhabitats or was able to tolerate a broad range of temperatures. The final two data chapters focus on the effect of biotic and abiotic factors on the thermal preferences of a common inhabitant of relatively thermally stable reef crest and reef slope environments, the black-axil chromis (*Chromis atripectoralis*). In chapter 4, I specifically aimed to investigate the influence of the presence of conspecifics, heterospecifics (*Neopomacentrus bankieri*), and a predator (*Cephalopholis spiloparaea*) on the thermal preference and threshold temperatures of the black-axil chromis. In the final data chapter (Chapter 5), I investigated the effect of habitat complexity on the thermal preferences and threshold temperatures of the black-axil chromis.

Tropical species are thought to have evolved in relatively thermally-stable environments and thus, are predicted to have a narrow thermal tolerance range. Some tropical species, such as the amphibious mudskipper fishes (f. Oxudecidae), however, occupy thermally-variable tropical intertidal habitats that experience a wide variation in temperatures throughout the day and tidal cycle. As such, mudskippers have a variety of strategies, both physiological and behavioural, to cope with a broad range of thermal conditions. This chapter examined the relationship between prolonged (5 weeks) exposure to a range of temperatures (22, 25, 28, or 32°C) on oxygen uptake and movement behaviours (i.e., thermoregulation and emergence) in a common amphibious fish, the barred mudskipper (*Periophthalmus argentilineatus*). At the highest temperature examined (32°C, approximately 5°C above their summer average temperatures), barred mudskippers exhibited 33.7-97.7% greater oxygen uptake rates at rest ($\dot{M}O_{2Rest}$) and emerged at a higher temperature (CT_e; i.e., a modified critical thermal maxima (CT_{Max}) methodology) of 41.3±0.3°C relative to those maintained at 28, 25, or 22°C. The 32°C-maintained fish also ceased movement activity at the highest holding temperature suggesting that prolonged submergence at elevated temperatures is physiologically and energetically stressful to the individual. Using exhaustive exercise protocols with and without air exposure to simulate a predatory chase, the time to recovery was examined for all individuals. When submerged, mudskippers required 2.5x longer recovery time to return to resting oxygen uptake from exhaustive exercise than those fully emerged in air. Oxygen uptake data revealed that air exposure did not accrue oxygen debt, thereby allowing faster return to resting oxygen consumption rates. If the option to emerge was not available, mudskippers preferentially sought more

benign water temperatures (26.7 ± 2.1 °C), resembling those experienced by these fish during the Austral autumn, regardless of prolonged exposure to higher or lower temperatures. These results add to our understanding of the strategies that amphibious fishes may use to mitigate extra costs associated with living in warm waters, and could be the key to understanding how such species will cope with increasing temperatures in the future.

The epaulette shark (*Hemiscyllium ocellatum*), similar to the mudskipper investigated in chapter 2, is a common inhabitant of coral reef flats, a tropical habitat known for its extreme variation in environmental conditions. While epaulette sharks are known to have strategies to deal with low levels of oxygen within their environment, it is unknown how this species copes with the extreme temperature variation experienced on coral reef flats. To investigate whether the epaulette shark uses movement to occupy thermally favourable microhabitats or tolerate the broad range of temperatures experience on the reef flat, I firstly determined their thermal preference under controlled conditions, and then compared this to environmental temperatures and body temperatures of individual sharks across the Heron Island reef flat. The thermal preference of epaulette sharks, was established in the laboratory setting using an automated shuttlebox system. Spatial and temporal variation in environmental temperatures were quantified using temperature data loggers placed within open and concealed microhabitats at 50m and 100m intervals across the reef flat that recorded temperature every 30 minutes for two years. The body temperature of 7 epaulette sharks was quantified by surgically implanting iButton temperature loggers into the abdominal cavity and recapturing individuals after 6 months. By comparing all three components, the use of movement as a strategy in epaulette sharks was calculated. Preferred temperatures in the laboratory (20.7±1.3°C) were most similar to environmental temperatures during winter months. The coral reef flat on Heron Island ranged from 15-34°C during 2016 and 2017 with statistical differences between two locations, Coral Gardens and Shark Bay. However, both locations lacked any spatial heterogeneity of temperatures, across the reef flat and between microhabitats, creating a low-quality thermal habitat for behavioural thermoregulation. Thus, epaulette shark body temperatures mirrored that of environmental temperatures. Behavioural thermoregulation is assumed in many shark species, but this highly specialized species may utilize other physiological strategies to cope with extreme temperature fluctuations on coral reef flats. As ocean warming continues, it is crucial to understand how species will respond to future changes and which strategy, or combination of strategies, species will utilize to deal with changing environmental conditions.

While the thermal variability of an environment can have important implications for the behavioural and physiological strategies used by organisms occupying these environments, local biotic and abiotic conditions may also influence an organism's thermal preference. For example, both intra- and inter-specific interactions can be important determinants of a species distribution and as such, may be expected to influence their preferred temperature. The aim of this chapter was to investigate the influence of the presence of potential competitors (i.e., conspecifics and heterospecifics, *Neopomacentrus bankieri*) and a predator (the strawberry rockcod, *Cephalopholis spiloparaea*) on the thermal preference of the black-axil chromis, *Chromis atripectoralis*. To do this, a modified shuttlebox system was used in which a clear Plexiglas tube was

added to the centre of the two shuttlebox chambers. Conspecifics, heterospecifics, or a predator were added to either the warm chamber, cold chamber, or both chambers to quantify their effects on thermal preference based on position. The black-axil chromis preferred to remain with conspecifics and heterospecifics at upper temperatures similar to control, 27.5±1.0°C, and decreased their lower thermal preference to 21.1±1.1°C to 21.2±1.4°C and lower threshold temperatures to 21.7±1.1°C to 23.1±0.8°C. On the other hand, when predators were added to the shuttlebox system, focal fish actively avoided predators, only moving toward the predator when extreme temperatures were reached, 19.6±0.5°C to 34.6±0.4°C. This study demonstrates the associated trade-off between physiological costs and the associated benefits of species interactions; however, this effect appears limited as it was only present when treatment fish were present at cooler temperatures. The lack of effect nearing warmer temperatures suggests that populations nearing upper thermal limits may resort to benefits associated with thermal optima than benefits associated with group membership. Understanding the relationship between species interactions and temperature preference is critical to our understanding of how and where fish will coexist under future ocean warming scenarios.

Habitat complexity in coral reef ecosystems is a major driver of species richness and population abundance; however, large scale coral mortality and the subsequent declines in structural complexity are threatening the functioning of coral reef ecosystems. Understanding how changes in the structural complexity of reef habitats influence the thermal preferences of reef fish are critical to predict the likely impacts of ongoing climate change on populations and species. The aim of this chapter, therefore

was to investigate the effect of habitat complexity on the thermal preferences of the black-axil chromis, *C. atripectoralis*, a branching coral specialist. To do this, a complex habitat structure (i.e., dead branching *Acropora nasuta*) or a degraded habitat structure (i.e., coral rubble) was added to the centre of each chamber of the shuttlebox system. In the absence of any habitat, *C. atripectoralis* exhibited a thermal preference of 28.1 ± 0.9°C. Upper thermal preferences remained similar to control, but lower thermal preferences decreased to 18.9 ± 0.9°C. The black-axil chromis spent over half of each trial with complex habitat structure; however, ultimately moved away from complex habitats at upper threshold temperatures of 30.9 ± 0.4°C and lower threshold temperatures of 17.6 ± 0.7°C. Complex coral reef structures consistently retained fish better than rubble or no habitat, but only within the thermal tolerance range of these fishes. Our results highlight that as the climate continues to warm, the availability of complex habitat may help to retain some fishes on degraded reefs; however, when temperatures are pushed above thermal preference limits, fish are likely to vacate settled habitats in search of more benign thermal conditions regardless of habitat availability and condition.

Increasing ocean temperature has highlighted the importance of understanding an organism's thermal range and the factors that impact this range. Additionally, understanding temperature preference can provide insight not only to the temperature(s) selected by an individual or species, but also can provide insight into the temperatures avoided. The first two chapters highlight the importance of understanding species specific movements and responses, and the factors that may influence the use of temperature within natural environments. Within chapter 2, mudskipper species were able to utilise movement to avoid deleterious water temperatures. However, in chapter 3, epaulette shark species, although preferring temperatures in the laboratory, did not follow preferred temperatures in the wild suggesting alternative physiological strategies may be in use to deal with fluctuations in temperature. Furthermore, thermal preference is not a rigid trait and fish may preferentially select sub-optimal temperatures in favour of other factors, such as group membership or habitat. Chapters 4 and 5 demonstrate the influence of both biotic and abiotic variables on temperature selection. The presence of potential competitors, predators, and complex coral habitat largely influenced the selection of particular temperatures. This relationship revealed preferred thermal limits to ecological interactions suggesting strong trade-offs between optimal physiological performance and the benefits associated with group membership or habitat complexity. In sum, this thesis investigated not only temperature exposure as a driving factor of behavioural strategies, but also hot temperature can be selected as a resource, similar to food or shelter. The importance of understanding both aspects of temperature use cannot be understated given the deleterious conditions predicted under future ocean warming scenarios. As the climate changes, species may be driven to select more physiologically preferred temperatures; however, the availability of other resources such as group membership or habitat availability will likely affect these choices. With many tropical species shifting their distributional range to occupy cooler, deeper waters, understanding how thermal preferences may be utilised within environments and the factors that may influence this use is essential to our understanding of how and where future fish populations will reside in the future.

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Chapter 1: General Introduction

Temperature has a profound effect on all life, from influencing the rates of biochemical processes at the cellular level, to the physiological performance of individuals, and the geographic distribution of species (Tewksbury *et al.,* 2008; Pörtner & Peck 2010). Indeed the effect of temperature on metabolic rates is a central tenet of the Metabolic Theory of Ecology (MTE) (Brown *et al.,* 2004; Gillooly *et al.,* 2001). Although not without its criticisms, MTE highlights the potential importance of temperature in linking the performance of individuals to the ecology of populations, communities, and ecosystems (Brown *et al.,* 2004). For example, a species upper and lower thermal limits can dictate the range of thermal environments it may occupy (Angilletta, 2009; Payne *et al.,* 2016), and within these limits its abundance may be highest at the temperature in which individual performance is maximised (e.g., Brown, Mehlman, & Stevens, 1995; Munday *et al.,* 2008; Pironon *et al.,* 2017; Waldock *et al.,* 2019). Understanding the effects of temperature on individual performance and the distribution of populations and species is becoming increasingly important due to the effects of ongoing climate change (Angilletta, 2009; Payne *et al.,* 2016; Pinsky *et al.,* 2019).

For ectotherms (i.e., those species whose internal body temperature, and hence rates of biochemical and cellular processes are largely governed by environmental temperature) the relationship between temperature and the rates of physiological performance (e.g., oxygen uptake or growth) or behavioural traits (e.g., speed of movement) may be described by a thermal performance curve (figure 1.1; Pörtner & Farrell, 2008)). Thermal performance curves are being increasingly used to understand how species perform under different thermal environments and to predict the response of species to climate change (Monaco, McQuaid, & Marshall, 2017). Thermal performance curves are typically negatively (or left) skewed curves, are defined by a set of common parameters (e.g., critical thermal minima and maxima, thermal optima; figure 1) that describe the temperatures at which a species can thrive, withstand, or no longer perform (Pörtner & Farrell, 2008; Pörtner & Peck, 2010). The thermal performance curve is bounded by the critical thermal minima and maxima, beyond which aerobic performance is no longer sufficient for survival (figure 1.1; Pörtner & Peck 2010). Within this thermal tolerance range physiological performance gradually increases with increasing temperature until the thermal optimum (T_{opt}) is reached (the temperature at which performance is maximised), beyond which performance declines rapidly until the thermal maximum is reached (figure 1.1; Pörtner & Farrell, 2008). The difference between the thermal optimum and critical thermal maxima is the thermal safety margin and is being increasingly used to compare the sensitivity of populations and/or species to climate change (Pinsky *et al.,* 2019; Tewksbury *et al.,* 2008).

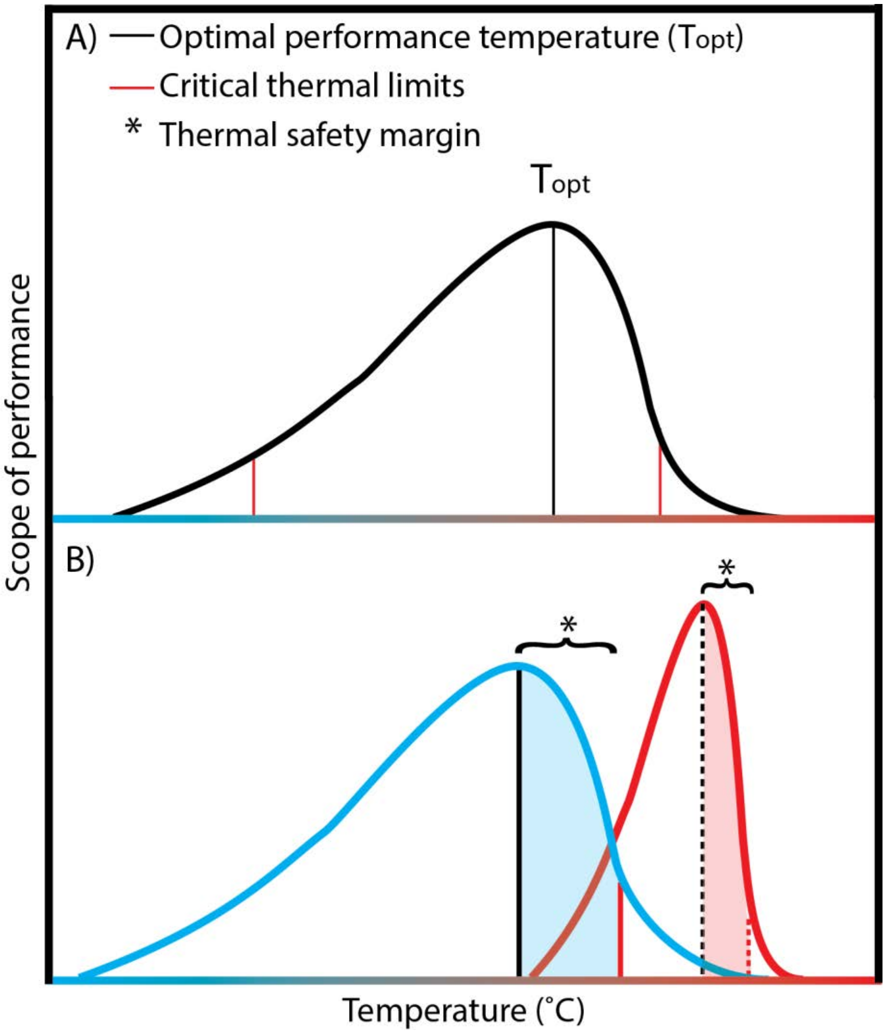

Figure 1.1. (A) A representative thermal performance curve, showing the hypothesised relationship between temperature and a performance trait for an ectotherm. (B) The comparison of thermal performance curves of a temperate (blue) species and a tropical (red) species. Both figures display the optima (black), critical temperatures (red), and thermal safety margins (*). Adapted from Pörtner & Farrell (2008), Pörtner & Peck (2010), and Tewksbury *et al.* (2008).

The shape and position of a species thermal performance curve is a useful tool in predicting a species response to different temperatures and is typically related to their thermal history (Angilletta, 2009). Most notably species that have evolved in and/or inhabit thermally stable environments are hypothesised to have a narrower thermal tolerance range, steeper thermal performance curve, and smaller thermal safety margin than species that have evolved in and/or occupy thermally variable environments (Rezende *et al.,* 2014; Tewksbury *et al.,* 2008). This is perhaps best illustrated through the comparison of thermal performance curves of low latitude (i.e., tropical) versus high latitude (i.e., temperate) taxa (Pinsky *et al.,* 2019; Tewksbury *et al.,* 2008; Vinagre *et al.,* 2016). The reduced seasonal variation in temperature at lower latitudes, compared to higher latitudes, has been suggested to reduce both thermal tolerance range and thermal safety margin of tropical taxa, and thereby making them more vulnerable to relatively small increases in environmental temperature compared to those at higher latitudes (Pinsky *et al.,* 2019; Tewksbury *et al.,* 2008). Equally, differences in thermal variability across relatively small spatial scales may be expected to influence the thermal performance curves of resident species in similar ways (e.g., Harborne, 2013; Madeira *et al.,* 2012; Vinagre *et al.,* 2016). Understanding how different thermal environments relate to the thermal performance (e.g., thermal tolerance range, thermal optima, and thermal safety margin) of resident species is critical to predict the likely impacts of increasing temperatures on populations and communities.

A species tolerance of environmental temperatures is a key determinant of their geographic range (Angilletta, 2009; Payne *et al.,* 2016), however, a range of biotic and abiotic factors may moderate distributions within this range (Araújo & Luoto, 2007;

Gilman *et al.,* 2010), leading to potential trade-offs between physiological and ecological processes. For example, many studies have shown that the availability of refugia, dietary resources, species interactions and/or other environmental factors (e.g., oxygen availability) can influence the abundances of fishes within a habitat (Bolin *et al.,* 2018; Chittka *et al.,* 2003; Creel, 2018; Hawlena & Schmitz, 2010; Heithaus & Dill, 2002; Hodge *et al.,* 2018; Khater, Murariu, & Gras, 2016). Selecting for these other factors may lead to occupying sub-optimal thermal conditions and associated physiological costs (Pörtner & Farrell, 2008). Recognising the importance of ecological interactions, such as habitat quality or biotic interactions, together with thermal preferences is critical to understand current and predict future species distributions.

Tropical fishes, like other tropical species, are thought to have a relative narrow thermal tolerance range and limited thermal safety margin (Habary *et al.,* 2017; Jokiel & Coles, 1977; Tewksbury *et al.,* 2008). Moreover, tropical species, unlike their temperate counterparts, are thought to have a limited ability to acclimate to increasing temperatures and although tropical oceans are warming at 70% of the global average are at greater risk to chronic ocean warming (Rummer *et al.,* 2014; Stillman, 2003; Stillman & Somero, 2000; Tewksbury *et al.,* 2008; Vinagre *et al.,* 2016). Predicting likely impacts of increasing ocean temperatures on tropical marine fishes requires a clearer understanding of how their thermal environment and ecological factors influences their preferred and threshold temperatures, and the behavioural strategies (if any) they use to reduce thermal stress.

Thesis objective and aims

The overall objective of this thesis was to establish the influence of thermal variation and biotic factors on the thermal performance of tropical marine fish. To do this, this thesis is divided into two components; the first (chapters 2 and 3) investigated the thermal performance of two species that are residents of thermally-variable intertidal environments and the second (chapters 4 and 5) investigated the influence of local abiotic and biotic factors on the thermal preferences of a species from a thermally-stable subtidal environment.

Residents of thermally-variable intertidal habitats typically exhibit wider thermal tolerance ranges than those who occupy adjacent and thermally-stable subtidal environments (Madeira *et al.,* 2012; Vinagre *et al.,* 2016), however, it is unknown what, if any, behavioural strategies these species may use to moderate the effects of extreme temperatures on their physiology. Strategies, such as behavioural thermoregulation and/or reducing activity, have been widely studied across many taxa (Beever *et al.,* 2017; Carrascal *et al.,* 1992; Dubois *et al.,* 2009; Kelley *et al.,* 2016; Nice & Fordyce, 2006; Vesterdorf, Blache, & Maloney, 2011); however, these strategies have been largely overlooked in tropical fish species.

Specifically, the aim of chapter 2 was to determine how exposure to different thermal conditions influences the oxygen uptake rates, preferred temperatures, and emergence behaviours of barred mudskippers (*Periophthalmus argentilineatus*). Mudskippers are well known for their unique morphological and physiological adaptations allowing species to thrive in extremely thermally variable mangrove habitats (Gordon *et al.,* 1968; Tytler & Vaughan, 1983); however, little is known about their tolerance to a range of temperatures and what strategies may be used to mitigate exposure to extreme temperatures. To establish the effects of exposure to a range of temperatures, commonly found within tropical mangrove ecosystems (i.e., 22, 25, 28, or 32°C), on physiological performance, I used intermittent flow respirometry to establish resting and maximum oxygen uptake rates as proxies for metabolic rates. Emergence temperatures were estimated using a modified critical thermal maxima protocol where the fish were allowed to emerge out of the water following increases in temperatures. Furthermore, thermal preference was established as the most time spent at a particular temperature using an automated shuttlebox system.

The aim of chapter 3 was to determine if the epaulette shark (*Hemiscyllium ocellatum*) uses movement to regulate body temperature within its natural environment. The epaulette shark is a common inhabitant of coral reef flat environments, but little is known about the strategies used when exposed to large fluctuations in temperature within this environment. Within this chapter, I use an automated shuttlebox to determine the thermal preference of *H. ocellatum* and then compare this to the environmental and body temperatures of *H. ocellatum* in the field to determine if they use movement to regulate their internal body temperatures.

The second component of this thesis (chapters 4 and 5) focuses on the potential trade-offs between temperature and biotic (species interactions) and abiotic factors (habitat structure) for a common coral reef fish, the black-axil chromis (*Chromis atripectoralis*). Species interactions, specifically group membership, are common among many taxa, including tropical fish species (Krause, 2002), with benefits being related to predator protection (Roberts, 1996; Ward *et al.,* 2011) and food acquisition (Pitcher, Magurran, & Winfield, 1982). Conversely, interactions with potential predators, can have both consumptive and non-consumptive effects and consequently predator avoidance is of most importance (Brown, Krause, & Laland, 2011). Further, many coral reef fishes are closely linked to the physical structure of their habitat, and experience rapid declines in abundance following the loss of corals and associated structural complexity (Jones *et al.,* 2004; Pratchett *et al.,* 2008).

The aim of chapter 4 was to determine how species interactions (i.e., the presence of conspecifics, heterospecifics, or a predator) influence thermal preferences and thresholds of the black-axil chromis, *Chromis atripectoralis*. I first established the preferred and threshold temperatures of *C. atripectoralis* using an automated shuttlebox system, and then investigated how the presence of conspecifics, heterospecifics, or a potential predator affected these temperatures.

The aim of chapter 5 was to determine if habitat complexity influences the thermal preference and thresholds of *C. atripectoralis*. While considered a facultative coral dwelling species, *C. atripectoralis* has been shown to increase in abundance following coral mortality (Pratchett, Hoey, & Wilson, 2016) suggesting it may be responding to the physical structure of the coral, rather than the live coral per se. To investigate the effect of habitat structure on thermal preferences of *C. atripectoralis*, I compared how the presence of complex branching coral skeleton vs. coral rubble influenced preferred and threshold temperatures in an automated shuttlebox system.

Chapter 2: The emergence emergency: a mudskipper's response to temperature

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2.1 Introduction

Temperature has a profound effect on all life, and a particularly influential effect on ectotherms, as the rate of their biochemical and physiological processes are largely governed by the temperature of their external environment (Tewksbury *et al.,* 2008; Pörtner & Peck, 2010). For example, fishes have been shown to increase resting oxygen uptake rates when exposed to acute or chronic increases in water temperatures (e.g., Lefevre, 2016; Gillooly *et al.,* 2001; Clarke & Johnston, 1999; Brown, 1989). This increased oxygen demand suggests that more energy will be required to maintain daily performance at higher temperatures, potentially reducing the energy available for key processes, such as growth and reproduction (Fry, 1947; Pörtner & Peck 2010). As global temperatures continue to increase, many tropical ectotherms are thought to be at risk as they are already experiencing temperatures close to their upper thermal limits (Rummer *et al.,* 2014). Most of the fishes, as obligate aquatic ectotherms, can utilize strategies such as acclimation, adaptation, or behaviour to cope with changes in temperatures (Fry, 1947; Pörtner & Peck 2010); whereas amphibious fishes (i.e., those who spend part of their life on land) are uniquely adapted to life in two different environments and may use a range of strategies (e.g., emergence) to cope with changing temperatures.

Mudskippers (family Gobiidae, subfamily Oxudercinae) are amphibious fishes that are common inhabitants of mudflat and mangrove environments, regularly emerging from the water (e.g., to forage, evade predation; Gordon *et al.,* 1968). Mudskipper species have the ability to take in oxygen through gills, other brachial surfaces (i.e., buccal, pharyngeal, branchial, and opercular cavities; Graham, 1997), and cutaneous surfaces, and although all species have this ability, the ratio at which they utilise a particular oxygen uptake method differs between species (Graham, 1997). For example, Tamura and colleagues (1976) found that *Boleophthalmus chinensis* relied more heavily on gills (59%) than skin (43%) for oxygen uptake. In contrast, *Periophthalmus cantonensis* relied more heavily on skin (76%) than gills (27%) for oxygen uptake. They concluded that the differences between oxygen uptake sources were related to the transition from aquatic to terrestrial air breathing. These adaptations in mudskipper fishes allow them to use several behaviours that can be used with changes in tide, temperature, light (day vs. night), and salinity (Baeck *et al.,* 2013; Clayton, 1993). For instance, juvenile mudskippers in Kuwait Bay have been observed to regulate their body temperature by ‘basking’, specifically by orientating their body at a right angle to the sun to increase body temperature (Clayton & Vaughan, 1988; Tytler & Vaughan, 1983). Conversely, several amphibious fishes, *Pseudogobius sp.* and *Favonigobius exquisitus* (Ford *et al.,* 2004), and *Kryptolebias marmoratus* (Gibson *et al.,* 2015) are known to leave the water in response to elevated temperatures. This emergence behaviour has been linked to the use of evaporative cooling (Tytler & Vaughan, 1983). Although mudskippers may be well equipped to cope with their environmental conditions, predicted changes in sea surface temperatures, including those in marginal habitats (i.e., habitats supporting relatively few species because of limited environmental conditions) may challenge the mudskippers' present adaptations.

Physiological responses and behavioural strategies can differ greatly over the temporal scale at which fish can utilize them. Physiological responses can last from a couple of minutes to acclimation or adaptation processes over months, years, and across generations (Atkins & Travis 2010; Buckley *et al.,* 2015; Donelson *et al.,* 2012). Behavioural strategies can support more immediate thermoregulation over short time scales. By moving away (e.g., jumping, 'skipping', or 'crutching' in amphibious species, see Harris, 1960) from thermally stressful conditions into more benign conditions closer to preferred temperatures, species can mitigate some of the physiological costs that sub-optimal temperature present, such as increased maintenance costs. To date, the use of movement to select a preferred temperature has been studied across many taxa (Buckley *et al.,* 2015; Coggan *et al.,* 2011; Killen, 2014; Medvick & Miller, 1979; Speed *et al.,* 2012); however, this has yet to be examined for amphibious fishes. Previous studies have suggested the use behaviour to occupy a thermal preference (Tytler & Vaughan, 1983); however, such preferences have not been established in the laboratory.

Determining oxygen uptake rates and understanding how movement (i.e., emergence and thermoregulation) is used to mitigate the effects of suboptimal temperatures could help explain how amphibious fishes, such as mudskippers, are able to live under a range of thermal conditions. The objective of this study was to establish how exposure to different thermal conditions influences oxygen uptake rates, preferred water temperatures and emergence behaviours of a common amphibious fish, the barred mudskipper, *Periophthalmus argentilineatus*. Additionally, as temperatures can vary greatly between day and night-time periods, I aimed to investigate the differences in thermal preferences between day and night-time periods. As mudskipper species exhibit emergence behaviours, I also aimed to establish the upper thermal emergence limits to gain a better understanding of other behavioural strategies this and perhaps other amphibious fish species may utilize to mitigate future increases in temperature.

2.2 Materials and Methods

Animal care

Adult barred mudskipper (*Periophthalmus argentilineatus*) of similar size (mean±SE; mass: 6.12±1.43g, standard length: 6.85±0.55cm) were collected in March 2015 from a mangrove forest in Cockle Bay on Magnetic Island, Queensland, Australia (19°10'32.4"S 146°49'45.8"E) using hand nets. To quantify temporal variation in environmental temperature, a HOBO Pendant® Temperature/Light Data Logger (Onetemp, Australia) was deployed in March 2015 at low tide in a 10cm deep pool at the site of collection, and it was retrieved in January 2016. Following collection, animals were transported in aerated bags partially filled with seawater to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Queensland, Australia. All individuals were placed into two 68L (64.5x41.3x39.7cm), aquaria with flow-through filtered 28°C seawater. Each aquaria contained 10L of water and submerged PVC shelters and brick platforms. Following a two-week habituation period to ensure that fish were feeding appropriately, each individual was randomly assigned to one of five temperature treatments (22, 25, 28, or 32°C) and tagged with a unique subcutaneous visible elastomer (Northwest Marine Technology, Washington, USA) to allow individuals to be identified. Each treatment consisted of four 8.5L aquaria (22x23x22cm), each housing 2-3 fish of similar body size (weight: one way ANOVA $F_{3,26}$=1.137, P=0.36; length: one way ANOVA $F_{3,26}$=0.08, P=0.97). Each aquarium consisted of submerged PVC shelters; however, these did not allow for emergence. Animals were isolated in the aquatic environment during acclimation to ensure that the acclimation conditions were constant, given that emergence would create inconsistent temperature, humidity, and osmotic conditions. Throughout the study, a 12:12 hour photoperiod was maintained. Fish were fed daily commercial pellets and marine green frozen fish food (Fish Fuel Co., South Australia, Australia). Prior to experiments, fish were fasted for 24h to ensure post-absorptive state that would maximize energy available for performance (Niimi & Beamish, 1974).

Temperature Treatments

The treatment temperatures were chosen to reflect a range of ecologically relevant temperatures experienced throughout the year in an adjacent intertidal seagrass meadows in Cockle Bay, Magnetic Island; winter minimum (22°C), temperatures experienced throughout the year (25°C, and 28°C, respectively), and summer average (32°C) (Collier & Waycott, 2014). Treatment temperatures were reached either by decreasing the ambient water temperature (28°C) by 0.5°C per day or by increasing it by 0.3°C per day until the target temperature was attained. Rates of temperature increase were slower than rates of decrease, as pilot trials showed that daily increases of 0.5°C over several days resulted in prolonged erratic behaviours (e.g., continuous rapid, burst swimming behaviours) until mudskippers became unresponsive. These behaviours were not observed with a daily increase of 0.3°C.

Upon reaching treatment temperature, fish were maintained at treatment temperatures for five-weeks (prior to any experiments). This acclimation time was chosen as it is follows best practices and is thought long enough for critical acclimation processes to be complete (Nilsson *et al.,* 2010).

Oxygen Uptake Rates

Fish were placed individually into 0.46L intermittent-flow respirometry chambers inside a temperature-controlled water bath (following Svendsen *et al.,* 2016). Each chamber was connected to a flush pump and recirculating pump to maintain water circulation and homogenous oxygen levels within the system throughout the trial. A digital relay timer controlled the flush pumps so that the water in the respirometry chambers was flushed with well-aerated, filtered seawater for 5-mins every 10 minutes throughout the 20-hour trial. The flush cycle was determined such that O_2 levels did not fall below 80% air saturation (Clark *et al.,* 2013). Temperature-compensated oxygen concentrations were recorded every 2s using contactless spots (2mm) with O_2-sensitive REDFLASH dye attached to the inside of glass tubes in line with each recirculating pump. The spots were linked to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany) via 2m fibre-optic cables.

The established method incorporating a 3-min chase followed by 1-min air exposure (see Clark *et al.,* 2012, 2013; Roche *et al.,* 2013) was used to determine maximum oxygen uptake rates ($\dot{M}O_{2Max}$) for fish from all temperature treatments (22°C: n=6, 25°C: n=7, 28°C: n=4, and 32°C: n=4). Immediately following the exercise protocol, fish were placed into respirometry chambers. Each chamber was sealed, and the measurement period began within 10 seconds. Following each trial, all equipment was rinsed in a 10% bleach solution, rinsed in freshwater, and then sun-dried to reduce microbial background oxygen uptake.

Given mudskippers are able to withstand extended periods of air exposure (Gordon *et al.,* 1968), the standard chase protocol consisting of 3min chase and 1min air exposure (see Clark *et al.,* 2012, 2013; Roche *et al.,* 2013) might not elicit maximal exhaustion typically seen in species less resilient to air exposure. Therefore, I assessed the utility of the standard protocol by comparing it to maximum oxygen uptake rate elicited by three complimentary protocols adjusted for their unique physiology. Individuals within the 25°C treatment group were exposed to either: (i) 3-min chase at a high water level without any air exposure, (ii) 3-min chase at a low water level to induce a jumping behaviour followed by a 1-min air exposure, and (iii) 3-min air exposure. In total, these four different chase protocols allowed for direct comparison between the standard protocol and different "exhaustive treatments" such as jumping, swimming or complete removal from water. Following each protocol, individuals were immediately placed into respirometry chambers in the aquatic environment to establish recovery time. Each individual from the 25°C treatment was given 48 hours to recover from experimental trials before tested under alternate protocols, and the sequence of chase protocols were randomised among individuals.

Oxygen uptake rates (as a proxy for energetic costs) during the measurement period (i.e., non-flushing) were calculated using linear least square regression of oxygen concentration over time in LabChart v.6.1.3 (AD Instruments, Colorado Springs, CO, USA). The highest rate of change for every 30-sec period, during the initial three closed respirometry phases following the placement of individuals inside the chamber, were used to determine the maximum O_2 uptake (i.e., $\dot{M}O_{2Max}$). This method was deemed appropriate as R^2 values for the closed respirometry phases were above 0.95. Resting oxygen uptake rate (i.e., $\dot{M}O_{2Rest}$) was calculated as the 'mean of the lowest normal distribution' method (MLND) as described by Chabot *et al.* (2016). Recovery time was defined as the time difference between $\dot{M}O_{2Max}$ until the first oxygen uptake rate equivalent to $\dot{M}O_{2Rest}$. Aerobic scope was defined as the absolute difference between $\dot{M}O_{2Max}$ and $\dot{M}O_{2Rest}$. Background O_2 uptake in the system was determined prior to the fish being placed into the chambers and again concluding the trial after fish were removed. The background O_2 uptake was assumed linear (Clark *et al.,* 2013) and was determined to be less than 5% of mudskipper oxygen uptake rates. Given that this value was established as low, background O_2 uptake was incrementally subtracted from each slope.

Critical Thermal Emergence (CT_e)

The critical emergence temperature (CT_e) for barred mudskippers was estimated using a modified critical thermal maxima (CT_{Max}) methodology (Beitinger & Bennett, 2000) in which fish were allowed to emerge from the water (22°C: n=4, 25°C: n=7, 28°C: n=5, and 32°C: n=5). A 125L (64.5x64.5x30.15cm) square, glass tank was fitted with a 71.2cm x 64.5cm PVC ramp with evenly spaced holes (10mm wide diameter and separate by approximately 5cm). The ramp was positioned at a 30° angle from the base of the tank to the far top corner. A 2000 W heater (Omega 2000W, Full gauge TIC-17RGT Thermostat) and submersible mixing pump (WH-500, Weipro®, Guangdong, China) were placed below the ramp away from the experimental compartment to ensure homogeneous temperature throughout the entire aquarium. An air stone was placed inside the experimental compartment to ensure sufficient aeration. First, water was heated or chilled to the fish's treatment temperature. Then, the fish was placed inside the experimental compartment, and a plastic sheet was placed on the surface of the water to ensure the fish could not emerge prematurely. Fish were observed inside the compartment for 2 minutes, and the time spent actively moving (i.e., henceforth 'activity') was recorded. Following the initial 2-min observation period, the plastic sheet was moved along the water's surface towards the back of the tank opening a 5cm gap that allowed for emergence. The water temperature was then increased at a rate of 0.27 ± 0.01°C min^{-1} until the fish emerged onto the ramp. Emergence temperatures were established as the temperatures at which the fish first emerged from the water (i.e., the temperature at which the eyes were completely above the water) and at which the entire body emerged from the water (i.e., the temperature at which the caudal peduncle was out of the water). These endpoints were selected to represent the point at which the mudskipper may evaluate the aerial environment prior to full emergence. Air temperatures were maintained at 25°C, and humidity was constant between 40-60% throughout all trials.

Preferred Temperature (T_{pref})

A shuttlebox system (figure 2.1), designed by Schurmann and Steffensen (1991) and Petersen and Steffensen (2003), was used to determine the preferred temperature (T_{pref}) for each fish from each treatment temperature (22°C: n=6, 25°C: n=7, 28°C: n=7, and 32°C: n=4). A detailed description of the shuttlebox system is provided in Nay et al. (2015). Briefly, the system is composed of two chambers joined by a 50mm wide opening allowing the fish to travel freely between the two chambers. One chamber was established as the ‘warm’ chamber and the other as a ‘cool’ chamber, with a 1°C difference maintained between the chambers throughout the trial (Killen, 2014). When the fish entered the ‘warm’ chamber, the temperature of the entire system would increase at a rate of 6°C hr^{-1}. Conversely, when the fish entered the ‘cool’ chamber, the temperature of the system would decrease at a rate of 6°C hr^{-1}. By moving between chambers the fish can control their thermal environment and therefore their internal body temperature. The ‘warm’ and ‘cool’ chamber assignments were switched halfway through trials to ensure there was no bias toward a particular chamber.

An individual fish was placed inside the chamber set to the individual’s treatment temperature, and a plexiglass cover was placed on the surface of the water to prevent the fish from emerging. Fish were allowed a 1.5hr adjustment time prior to turning on the system. This time period was used as fish were observed entering both chambers of the shuttlebox system. Water within one chamber flowed clockwise while water in the other chamber flowed counter-clockwise to prevent the water from mixing and allow for the 1°C temperature difference to be maintained.

A custom program was written using Labtech Notebook Pro (Laboratories Technology Corp., Andover MA) to track the position of the fish and automate the activation/deactivation of the appropriate pumps based on the position of the fish. From each trial I recorded preferred temperature, number of chamber movements, and the selected temperature range. The preferred temperature (T_{pref}) was defined as the temperature at which the fish spent the largest proportion of time (i.e., modal temperature), while the chamber movements were the number of movements made between the chambers during diurnal and nocturnal periods. Selected temperature ranges were represented as the difference between maximum and minimum temperatures chosen by each fish.

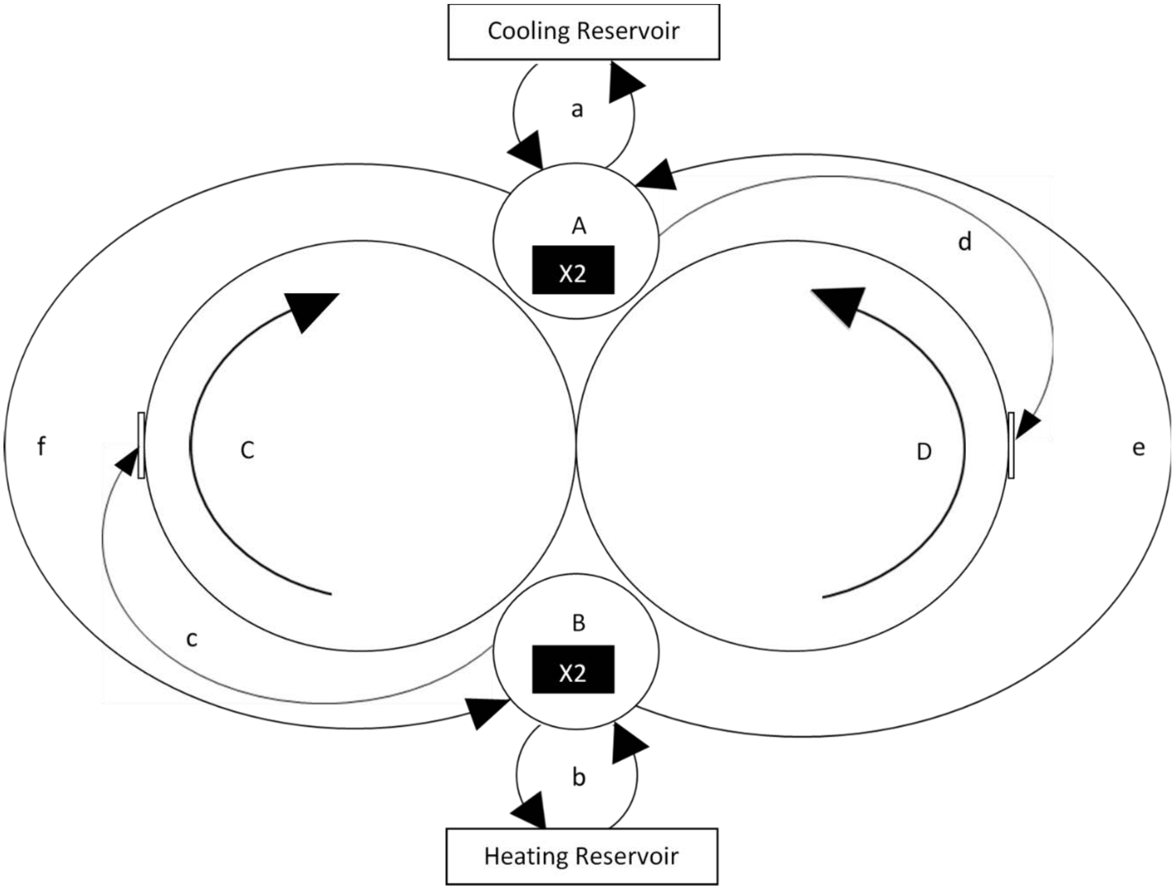


Figure 2.1. A diagram of the automated shuttlebox system. Uppercase letters represent chambers and buffer tanks while lowercase letters represent water flow. (A) and (B) are buffer tanks that receive water from heating or cooling reservoirs. The fish is placed in chamber (C) or (D) and are able to move freely between to actively select the temperature. (a) and (b) represent the flow of water between the buffer tanks (A or B) and heating or cooling reservoirs. The flow of water from the buffer tanks (A or B) to the main chambers (C or D) are represented by (c) or (d). Furthermore, if the temperature differential between the two chambers (C or D) is too great, the flow of water (e or f) will activate to return the temperature differential to the appropriate level.

Data Analyses

To test the effect of treatment temperature on minimum and maximum oxygen uptake rates as well as initial and final emergence temperatures, one-way ANOVAs were used. To test the effect of chase protocols for fish maintained at 25°C, $\dot{M}O_{2Max}$, $\dot{M}O_{2Rest}$, and recovery time were analysed using one-way ANOVAs. The $\dot{M}O_{2Max}$, $\dot{M}O_{2Rest}$, and recovery time data were $\log_{10}$ transformed prior to analysis to conform to requirements for normality and homogeneity of variance. Activity was analysed with Kruskal-Wallis test given the data were not normally distributed. Temperature preference, chamber movements, and temperature range were analysed using a two-way ANOVA (treatment temperature and diel period [night-time/day-time]). All values were log transformed to meet assumptions of analysis (homogeneous variance and normal distribution). Holm-Sidak post-hoc tests were performed when significant differences were observed. To account for running multiple tests on the same individuals within experiments, an FDR correction was used post-hoc (Benjamini & Hochberg, 1995). All values are presented as mean ± SE.

2.3 Results

The field data logger revealed the average temperatures within the tide pool at Cockle Bay, Magnetic Island to be 25.48±3.23°C during 2015 (mean±SD; Fig. 2.2). Temperatures ranged from 23.29°C to 39.50°C during Austral summer (i.e., November to January) and from 12.40°C to 32.60°C during Austral winter (i.e., June to August).

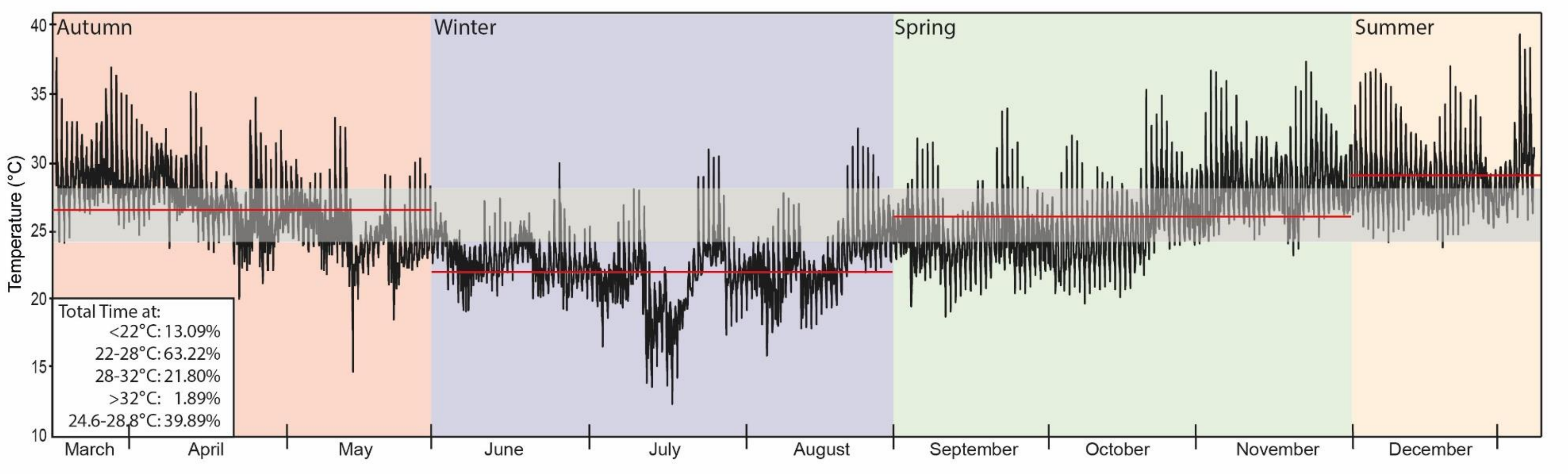


Figure 2.2. Temperature data collected with HOBO data loggers in Cockle Bay, Magnetic Island, Queensland, Australia from March 2015-January 2016. The data logger was placed within a 10cm deep tide pool at low tide at the base of mangrove, areas commonly frequented by the barred mudskipper, *Periophthalmus argentilineatus*.

Oxygen Uptake Rates

The resting oxygen uptake rate ($\dot{M}O_{2Rest}$) of *P. argentilineatus* differed among temperature treatments ($F_{3,17}$=3.40, P=0.04), with individuals maintained at 32°C having higher $\dot{M}O_{2Rest}$ (225.88±23.44 $mgO_2kg^{-1}h^{-1}$) than those maintained at 22°C (119.06±23.76 $mgO_2kg^{-1}h^{-1}$), 25°C (114.21±16.19 $mgO_2kg^{-1}h^{-1}$), and 28°C (169.00±41.68 $mgO_2kg^{-1}h^{-1}$, Fig. 2.3). In contrast, there was no difference in maximum oxygen uptake rates ($\dot{M}O_{2Max}$) following standard chase protocols among fish from each temperature treatment group (22°C: 739.61±142.07 $mgO_2kg^{-1}h^{-1}$, 25°C: 631.42±90.00 $mgO_2kg^{-1}h^{-1}$, 28°C: 522.92±66.70 $mgO_2kg^{-1}h^{-1}$, 32°C: 534.85±80.45 $mgO_2kg^{-1}h^{-1}$, $F_{3,17}$=0.53, P=0.67). Although aerobic scope appears to be decreasing across treatments, it did not differ significantly across temperature treatments (22°C: 649.91±142.71 mgO2kg-1h-1, 25°C: 548.17±89.87 mgO2kg-1h-1, 28°C: 444.94±70.29 mgO2kg-1h-1, 32°C: 377.74±82.12 mgO2kg-1h-1, $F_{3,17}$=0.91, P=0.46). Comparing chase protocols among fish maintained at 25°C revealed that recovery time was significantly shorter when fish were exposed to air and then allowed to recover in the aquatic environment (1.53±0.41h) compared to fish exposed to the standard chase protocol (3.93±0.71h) or modified chase protocol (3.37±0.67h; F3,16=7.22, P=0.003; Fig. 2.2) and then placed into water for recovery. Neither maximum oxygen uptake rates ($F_{3,32}$=0.64, P=0.59), resting oxygen uptake rates ($F_{3,32}$=2.07, P=0.12), nor aerobic scope ($F_{3,32}$=1.14, P=0.35) differed among protocols.

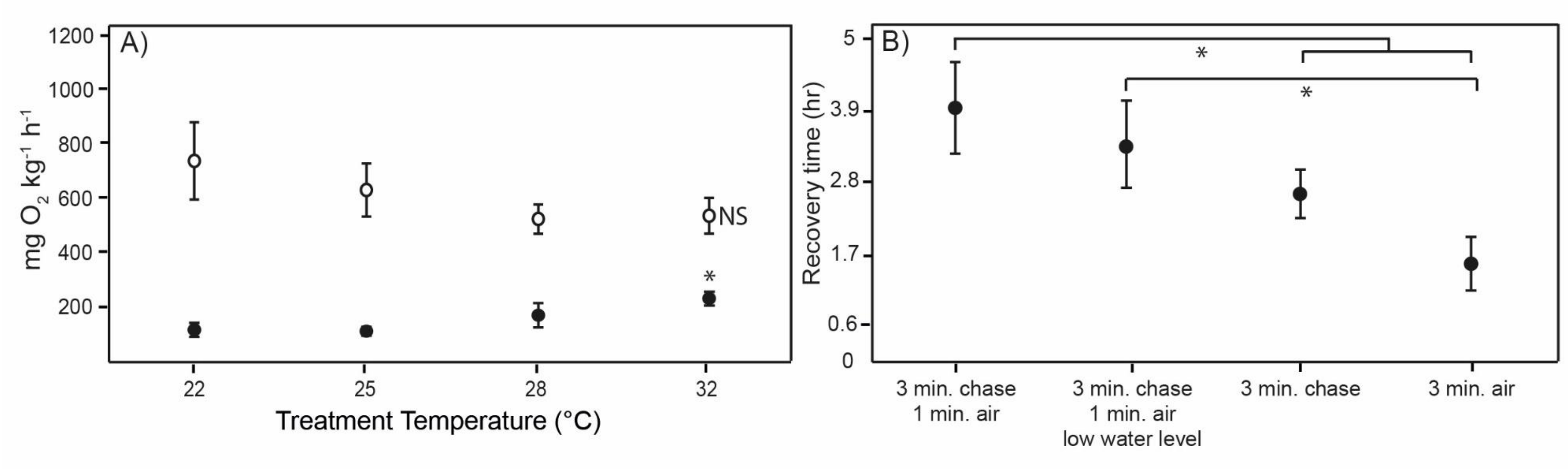


Figure 2.3. Oxygen uptake rates and recovery time for treatment temperatures and the different protocols for 25°C maintained barred mudskippers, *Periophthalmus argentilineatus*. A) Maximum oxygen uptake rates ($\dot{M}O_{2Max}$) represented as open circles along with resting oxygen uptake ($\dot{M}O_{2Rest}$) represented as closed circles for fish from each treatment temperature (22°C: n=6, 25°C: n=7, 28°C: n=4, and 32°C: n=4). Maximum oxygen uptake rates ($\dot{M}O_{2Max}$) were established using a 3 minute chase and a 1 minute air exposure. B) Recovery time required for each protocol within the 25°C treatment group. All points are represented as means with standard error of the mean. Significant values are indicated as an asterisk with α=0.05.

Critical Thermal Emergence (CT_e)

The fish's initial and final emergence temperatures as well as the duration of activity prior to emergence differed among temperature treatments (Fig. 2.4). The temperature at which individuals initially emerged from the water (i.e., eyes are fully above the water) was higher for fish that had been maintained at 32°C (37.79±1.16°C) when compared to those maintained at 22°C (28.34±2.33°C; $F_{3,19}$=6.69, P=0.003). Fish that were maintained at 32°C and 25°C completely emerged themselves (i.e., withdrew caudal peduncle fully above the water) at significantly warmer temperatures (41.31±0.36°C and 40.89±0.78°C respectively) than fish maintained at 28°C (37.94±1.96°C; $F_{3,17}$=4.15, P=0.02). The time spent actively moving was greatest for fish that had been maintained at 28°C (19.40±7.10s of the 2-min observation period), and decreased for fish from both lower (25°C: 11.25±5.93s; 22°C: 6.33±2.03s) and higher (32°C: 0±0s) temperatures ($F_{3,19}$=14.32, P<0.05).

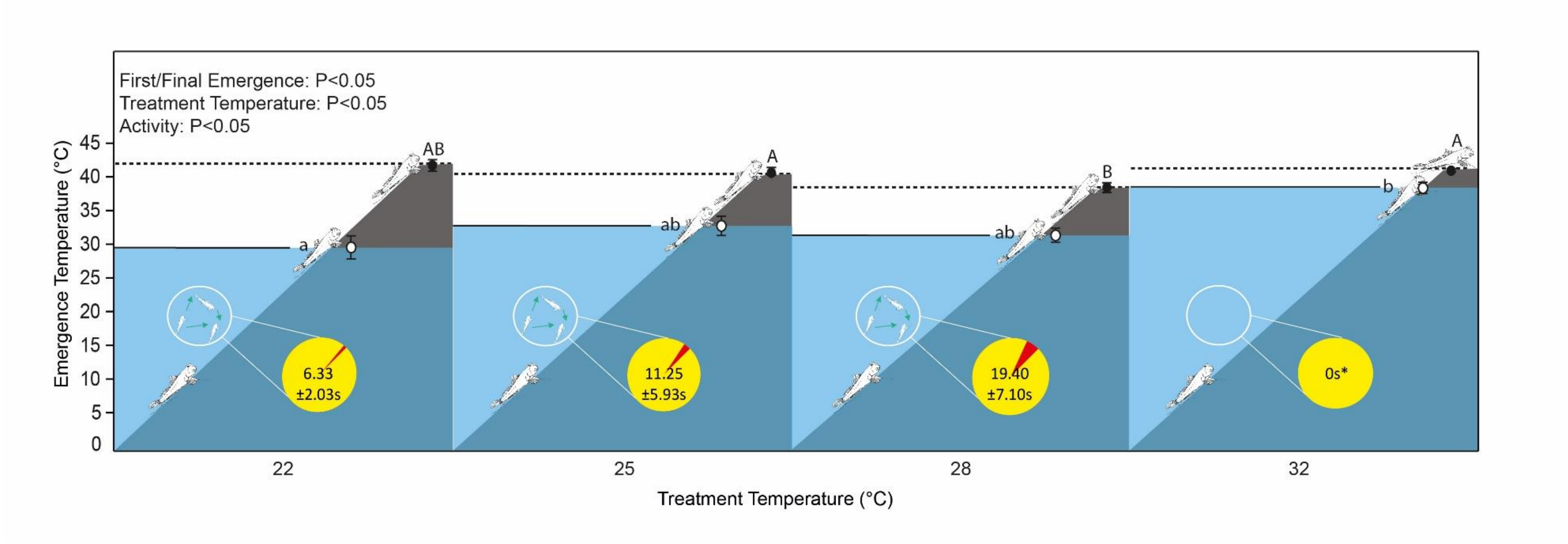


Figure 2.4. Emergence temperatures for the barred mudskipper, *Periophthalmus argentilineatus*. Treatment groups include 22°C: n=4, 25°C: n=7, 28°C: n=5, and 32°C: n=5. Initial emergence temperatures represented as a solid line and open circle, while final emergence temperatures are represented as a dotted line and closed circle. The swim time observed during the two-minute period prior to CT_e trials is represented with a yellow and red pie chart. Red sections represent the time spent active during the two-minute activity period prior to trials. Points and error bars are means with standard deviation. Significant differences are indicated between uppercase letters (final emergence temperature), lowercase letters (initial emergence temperature), and an asterisk for activity levels, all with α=0.05.

Preferred Temperature (T_{pref})

Despite five weeks of continuous exposure to either 22, 25, 28, or 32°C, *P. argentilineatus* preferred approximately the same temperature (26.7±2.1°C; $F_{3,43}$=2.20, P=0.10), which also did not differ between time periods (i.e., nocturnal vs. diurnal: $F_{1,43}$=1.62, P=0.21; Fig. 2.5a). There was, however, considerable variation in preferred temperature among all individuals, as evidence by the variation around the mean. Additionally, fish from some groups made more chamber movements to maintain their preferred temperature than others. For example, fish maintained at 28°C moved more frequently to maintain their preferred temperature than 25°C-maintained fish ($F_{3,43}$=3.11, P=0.02). During nighttime periods, fish moved more frequently to maintain their preferred temperatures than they did during the daytime periods ($F_{1,43}$=18.99, P<0.001). However, during daytime hours, fish selected a wider range of temperatures regardless of treatment temperature ($F_{1,43}$=22.50, P<0.05). There were no interactions between treatment, time period, preferred temperature, movements, and selected temperature ranges ($F_{3,43}$=0.91, P=0.45; $F_{3,43}$=2.67, P=0.06; $F_{3,43}$=0.90, P=0.45).

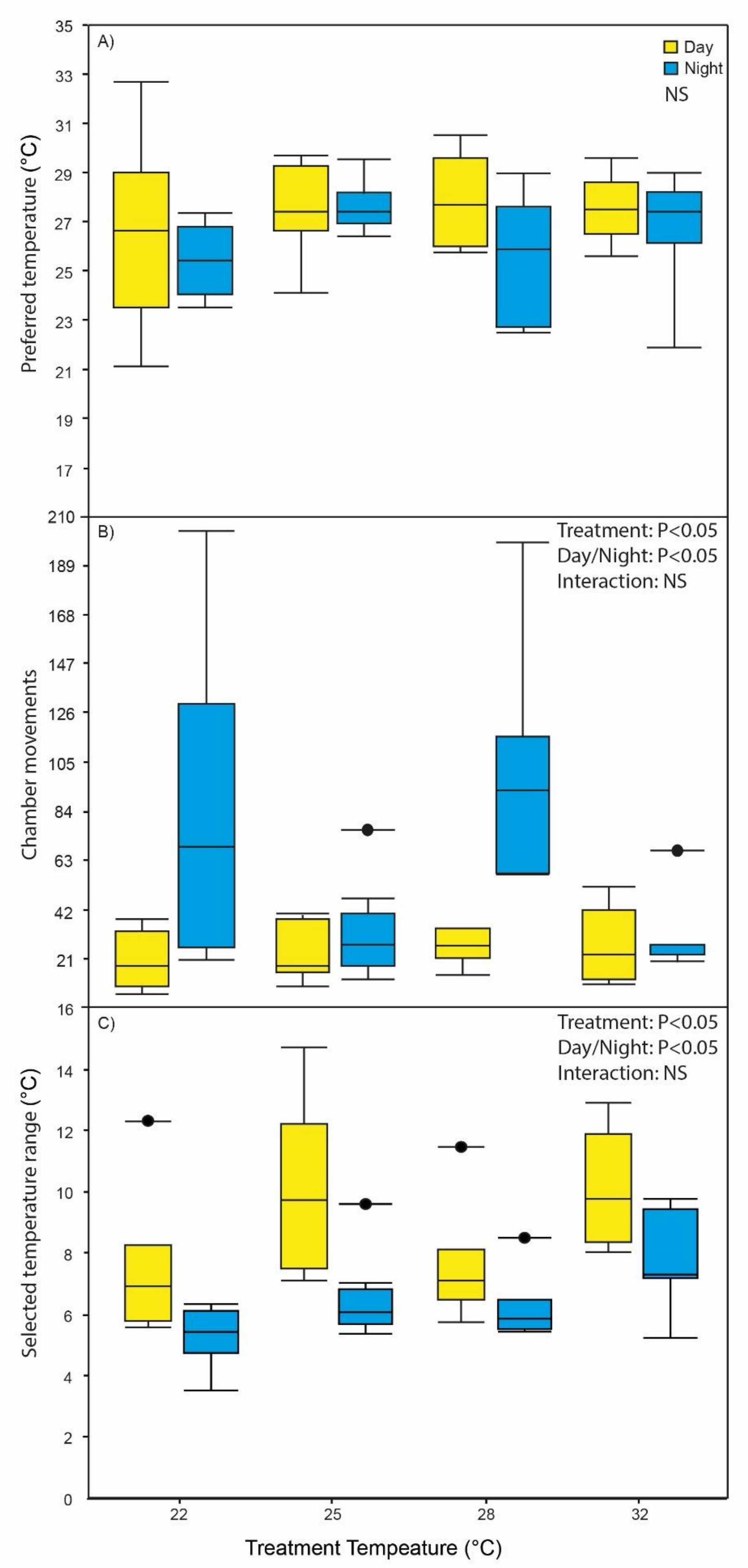

A)
🟨 Day
🟦 Night
NS
Preferred temperature (°C)
35
33
31
29
27
25
23
21
19
17
B)
Treatment: P<0.05
Day/Night: P<0.05
Interaction: NS
Chamber movements
210
189
168
147
126
105
84
63
42
21
C)
Treatment: P<0.05
Day/Night: P<0.05
Interaction: NS
Selected temperature range (°C)
16
14
12
10
8
6
4
2
0
22
25
28
32
Treatment Tempeature (°C)

Figure 2.5. A) Temperature preference (T_{pref}), B) chamber movements, and C) the selected temperature ranges of all treatment groups per 5-hour trial. Treatment groups included are 22°C: n=6, 25°C: n=7, 28°C: n=7, and 32°C: n=4. The boxes represent first and third quartiles, and the whiskers (errors) represent the minimum and maximum values outside of outliers. Outliers are solid lines with a closed circle. Within each box, median (solid line) values are included. Daytime values are in light (yellow) boxplots. Nighttime values are in dark (blue) boxplots.

2.4 Discussion

Amphibious fishes may use behavioural strategies to minimise their exposure to unfavourable environmental temperatures. Maintaining barred mudskippers, *Periophthalmus argentilineatus*, for 5 weeks at constant temperatures that they naturally experience (22-32°C) in the wild had no effect on their preferred water temperatures (26.7°C). Fishes that had been maintained at 28°C were also the most active compared to those maintained at either lower (22 and 25°C) or higher water temperatures (32°C). Despite the similarities in preferred water temperatures, fish that had been maintained at 32°C emerged from the water at higher water temperatures, exhibited an increase in their resting oxygen uptake rates, and selected a wider range of water temperatures than their counterparts that had been maintained at cooler temperatures. Exposure to air prior to submersion provided the shortest recovery time of any of the exercise protocols (1.53±0.41h) likely due to their ability to uptake oxygen in air. The longest recovery time was required by those who were exercised for 3 minutes in water and allowed a 1 minute air exposure (3.93±0.71h) followed by submersion. Although I recognize that the barred mudskipper occupies naturally thermally variable environments (12.40-39.50°C), when they are maintained long-term at constant water temperatures closer to the upper limits of what they currently experience (32°C), their oxygen uptake rates may increase and behaviour altered. Therefore, prolonged exposure to elevated temperatures (e.g., 32°C) may force mudskipper species to either move or risk elevated energetic costs.

Mudskippers, similar to many intertidal species, have the ability to leave the water when water conditions become suboptimal, and several factors can alter this emergence temperature threshold. For example, *Favonigobius exquisitus* and *Pseudogobius sp.*, two gobiid fish species common to the intertidal area of Moreton Bay, Australia, emerge from the water when exposed to high thermal stress (Ford *et al.,* 2004). Similarly, *Kryptolebias marmoratus* emerge when exposed to elevated temperatures (Gibson *et al.,* 2015). Local air temperatures experienced across daily and seasonal cycles, together with maximum daily water temperatures appear to play an important role in initiating emergence of amphibious fishes. Along the coast of central Chile, *Sicyases sanguineus* emerged at 17.8°C when water temperatures mimicked maximum daily air temperatures (Ebeling *et al.,* 1970). Similarly, barred mudskippers in this study maintained at cooler temperatures exhibited initial emergence at 32.63°C reflecting average maximum daily air temperatures recorded at Magnetic Island (~31.4°C). Humidity, along with temperature, can also play an important role in emergence temperatures. Gibson et al. (2015) found that *K. marmoratus* individuals lost more body heat in low humidity environments than those in high humidity scenarios. As relative humidity increased, the amount of body heat lost by *K. marmoratus* decreased.

Given that relative humidity in this study was between 40-60%, emergence behaviours could have led to beneficial evaporative cooling mechanisms. Although I did not examine the specific physiological mechanisms underpinning emergence behaviours that occur at higher temperatures, previous studies have suggested potential mechanisms such as anaerobic metabolic pathways, antioxidative mechanisms, or heat-shock protein responses (Pörtner & Peck, 2010). These mechanisms may allow more time to be spent at elevated temperatures during a period of time where temperatures are increasing. A thorough investigation into these mechanisms is an avenue for future studies. Emergence behaviour can be a useful response for mudskipper species to escape extreme conditions; however, such behaviours come with associated trade-offs.

Under scenarios where emergence behaviours are too risky and/or energetically costly, it may be necessary to regulate body temperature while submerged. Barred mudskippers in this study showed a preference for relocating to thermal conditions similar to those experienced in Austral autumn months. The mangrove habitat on Magnetic Island experiences temperatures between 24.6-28.8°C (i.e., the range of preferred temperatures determined for mudskippers in this study) for nearly 5 months of the year during both high and low tidal cycles. These temperatures are also suggested as the preferred temperature ranges for other amphibious species, although selected using alternative thermoregulatory behaviours such as body placement next to cooler tide pools (e.g., *Mnierpes microcephalus*; Graham, 1973). Furthermore, the temperature range throughout the year between low and high tides is much greater, fluctuating by 10-18°C, than has been previously noted (e.g., 12°C) in other intertidal habitats (Potts &

Swart, 1984). Unlike fishes that are fully aquatic, amphibious fishes, such as mudskippers, may require more frequent movements to occupy preferred temperatures, given the dynamic nature of temperature within their habitats; however, such movements may expose these fish to new threats, including aerial or aquatic predation (Sayer & Davenport, 1991).

Ultimately using behaviours to regulate temperature can have several associated risks including but not limited to attracting predation, missing opportunities, and the energy trade-offs required for movement (Sayer & Davenport, 1991). For example, movement may draw the attention of predators and/or scare off potential prey (i.e., missed opportunity for foraging) (Angiletta, 2009). Furthermore, the energy conserved as part of moving to a preferred temperature needs to be greater than the energy required to relocate (Angiletta, 2009). Here, barred mudskippers increased movements during nocturnal periods, which may reflect a reduced predation risk at night. However, as temperatures approach the upper thermal limits of resident species, occupying thermally beneficial areas could become essential in order to reduce energetic costs associated with elevated temperatures.

Elevated temperatures, above an organism's natural range, can be associated with increased energetic costs, which in turn can affect a species' behavioural patterns. Many fully aquatic/marine fish species have exhibited elevated resting oxygen uptake rates at higher temperatures causing declines in traits such as aerobic scope and swimming performance (Johansen & Jones, 2011; Munday *et al.,* 2008; Rummer *et al.,* 2014). These declines can, although not always, coincide with declines in growth and reproductive output, as energy may be allocated to more essential life-sustaining processes (Lefevre, 2016; Pörtner & Peck, 2010). In contrast, recent studies have suggested that the decline in aerobic scope seen in some species may not be universal and may not align with other performance traits such as growth (Lefevre, 2016; Jutfelt et. al., 2018). Aerobic scope in barred mudskippers in the present study did not decline as exposure temperatures were increased or decreased, which suggests that this metric may not align with all performance traits of this, and other amphibious fish, species. For example, unlike aerobic scope, emergence temperatures did follow the trend of increasing as exposure temperature increased. A previous study on *Blennius pholis* suggests that emergence depends on the oxygen demand of the fish (Davenport & Woolmington, 1981) and therefore, as oxygen demand increases upon exposure to elevated temperatures, emergence may be a valid strategy to alleviate negative effects of temperatures (e.g., evaporative cooling and/or oxygen uptake). Indeed, some mudskipper species are thought to have the ability to uptake enough oxygen in air to satisfy resting metabolic demands (Teal & Carey, 1967; Gordon *et al.,* 1968; Tamura *et al.,* 1976; Kok *et al.,* 1998; Takeda *et al.,* 1999) and therefore could use air exposure as a valid strategy when recovering from aquatic exercise. Aquatic exercise in this study required a maximum 3.93±0.71h of recovery time before returning to resting levels. Similarly, Jew et al. (2013) suggested that 4.87±2.19h was required to recover from terrestrial exercise. Given mudskippers are uniquely able to utilise both aquatic and aerial environments, selecting preferred temperatures or emergence may allow these species to occupy thermally forgiving areas thus optimizing performance.

Conclusions

Maintaining barred mudskippers at constant elevated temperatures led to increases in their emergence temperatures and resting oxygen uptake rates, however had no detectable effect on their preferred water temperature of 27°C. The shift in emergence temperature suggests that barred mudskippers may have the capacity to acclimate and shift their upper thermal limits with prolonged exposure to constant temperatures. Amphibious fishes, such as mudskippers, have the ability to occupy aerial and aquatic environment, and as such are exposed to changing temperatures in both environments. When exposed to treatment conditions in the aquatic environment, resting oxygen uptake increased with elevated treatment temperatures. This is consistent with the response of many strictly aquatic fishes (Nilsson et al. 2009; Rummer et al. 2014) and suggests as ocean warming continues, elevated temperatures may be energetically expensive for amphibious species. Understanding the responses to elevated temperatures as well as the potential avoidance behaviours (i.e., emergence and thermoregulation) used by amphibious fishes are key to understanding how these fish will cope with future climate change scenarios.

Chapter 3: Regulate or tolerate: Thermal strategies of a coral reef flat resident, the epaulette shark, *Hemiscyllium ocellatum*.

3.1 Introduction

The distribution and abundance of species are shaped by the combined effects of abiotic and biotic environmental factors (e.g., Brown, 1984). Tolerance to abiotic factors (e.g., temperature, rainfall, hydrodynamics) typically dictate the range of habitats a species it may occupy, while biotic interactions (e.g., competition, predation) influence the abundance of a species within these habitats (Brown, 1984; Tewksbury *et al.,* 2008). Of the abiotic factors, environmental temperature is one of the most important in shaping a species distribution, especially for ectotherms as their rates of biochemical and physiological processes for ectotherms are tightly linked to environmental temperature (Angilletta, 2009; Pörtner & Peck, 2010). Importantly, it is not just the mean environmental temperature that influences a species distribution, but also the variability of environmental temperatures (Tewksbury *et al.,* 2008; Vinagre *et al.,* 2016). Ectothermic species that occupy thermally variable environments must, therefore, be able to either tolerate a broad range of temperatures, reduce activity and hence energy demands at extreme temperatures (chapter 2; Johansen *et al.,* 2013), and/or use movement to influence their internal body temperature (i.e., behavioural thermoregulation: Casterlin & Reynolds, 1979; Holland *et al.,* 1992; Stevenson, 1985; van de Ven, McKechnie, & Cunningham, 2019). Of these, using movement to regulate body temperatures is a short-term strategy that many taxa use to avoid sub-optimal temperatures (reptiles/fish: Angilletta , 2009; lizard: Carrascal *et al.,* 1992; turtles: Dubois *et al.,* 2009; insect: Nice & Fordyce, 2006), and by doing so influence specific physiological processes and/or the performance of the individual as a whole (Angilletta, 2009; Fry, 1947). For example, pregnant female Aspic vipers (*Vipera aspis*) are known to occupy warmer areas than non-pregnant conspecifics, a strategy that is thought to speed up gestation and decrease development times (Ladyman *et al.,* 2003). Similarly, Gila monsters (*Heloderma suspectum*) have been shown to select warmer temperatures after feeding to optimize digestion rates (Gienger, Tracy, & Zimmerman, 2013). Despite the prevalence of behavioural thermoregulation among ectotherms, few studies have examined the potential use of behavioural thermoregulation by tropical fish species (for exceptions see: Reyes *et al.,* 2011; Reynolds & Casterlin, 1981).

Tropical ecosystems are often characterized as having relatively stable thermal environments compared to those at higher latitudes (Tewksbury *et al.,* 2008). Some tropical environments, however, experience variations in temperature that are comparable to more temperate ecosystems. Within the coral reef ecosystems, for example, shallow reef flats are known for their extreme thermal variability (Harborne, 2013). Coral reef flats experience dramatic changes in environmental conditions over relatively short temporal scales due to tidal movement and are considered one of the most extremely variable thermal habitats within tropical marine ecosystems (Harborne, 2013; Kinsey & Kinsey, 1967; Nilsson *et al.,* 2007; Potts & Swart, 1984). Although, water temperatures on coral reef flats may mirror that of adjacent deeper habitats (e.g., reef slope) during high tide, they can reach temperatures 4°C warmer than these deeper habitats during periods of low tide due to the heating and pooling of shallow water (Chisholm, Barnes, & Devereux, 1996; Harborne, 2013; Potts & Swart, 1984). These elevated temperatures during low tides coupled with daily variation in temperature can lead to daily temperature fluctuations in excess of 12°C (Potts & Swart, 1984). While many species move onto and off coral reef flats with the incoming and outgoing tides, respectively (Harborne, 2013; Vivien, 1973), some resident species (e.g., the epaulette shark, *Hemiscyllium ocellatum*) remain on the reef flat during the entire tidal cycle and are thus exposed to these variable temperatures. The mechanisms that these resident species use to cope with such variable temperatures is largely unknown.

Using a common reef flat resident, the epaulette shark, *Hemiscyllium ocellatum*, as a model species, I investigate the potential use of behavioural thermoregulation or thermal tolerance as mechanisms to occupy this thermally variable environment. Specifically, I established the preferred temperatures *of H. ocellatum*, under controlled laboratory conditions, and compare this to the environmental temperatures and internal body temperatures of individuals on the reef flat of Heron Island, Queensland, Australia (23.4423° S, 151.9148° E) over a 6-month period.

3.2 Materials and Methods

Heron Island, Queensland, Australia (23.4423° S, 151.9148° E) is a low island within the Capricorn-Bunker group at the southern end of the Great Barrier Reef. It has a large reef flat (~27km^2) that ranges from 0.3-1m depth (Chen & Krol, 2004) and has a semi-diurnal tidal cycle with a 1.1m neap tidal range to a 2.3m spring tidal range (McGowan *et al.,* 2010). Heron Island was selected as it is known to support a large population of *H. ocellatum*, estimated to be in excess of 2,200 individuals (1994-1997;

Heupel & Bennett, 2007). To investigate the potential for *H. ocellatum* to behaviourally thermoregulate and/or tolerate the thermally variable environments on the reef flat, I determined the preferred temperatures of individual *H. ocellatum* under controlled laboratory conditions (T_{pref}), and compared this to the availability of environmental temperatures (T_e) and internal body temperatures of *H. ocellatum* (T_b) across the reef flat of Heron Island.

A*nimal husbandry and preferred temperatures*

The preferred temperature, and upper and lower threshold temperatures of *H. ocellatum* were established using an automated shuttlebox system (see chapter 2, section 2.2 materials and methods; Schurmann, Steffensen, & Lomholt, 1991). Epaulette sharks (*Hemiscyllium ocellatum*; n=12 total) were collected by hand during low tide on the reef flat on Heron Island in June 2017 (n=5) and January 2018 (n=7). Individual *H. ocellatum* were transported to a 2000L holding tank at Heron Island Research Station within 60 minutes of collection and held a minimum of three days prior to being placed inside 10L plastic bags filled with seawater and oxygen and transported, via car, to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Queensland, Australia. Upon arrival, sharks were randomly placed into one of five 500L tanks supplied with 27±1°C (mean ± SD) filtered seawater. Individual *H. ocellatum* were fed ~4% of their body mass of fresh prawn every second day. *H. ocellatum* were of similar length (57.7±0.7cm; mean ± SEM) and mass (431.8±24.7g) regardless of collection season (length: z=-0.26 P=0.79; mass: z=-1.58 P=0.12; generalized linear model (GLM)) or sex (length: z=-0.47 P=0.64; mass: z=-0.74

P=0.46; GLM). Prior to shuttlebox trials, individuals were fasted for 48hr to ensure a post-absorptive state (Niimi & Beamish, 1974).

Preferred temperature (T_{pref}) of *H. ocellatum* was established using an automated shuttlebox system (refer to chapter 2, section 2.2 Materials and Methods; see Petersen & Steffensen, 2003; Schurmann *et al.,* 1991) with the size of the chambers, pumps and heaters/chillers increased to accommodate the larger body size of *H. ocellatum*. Briefly, the shuttlebox comprised of two adjoining chambers (120cm Ø) that had a 15cm opening that allowed the shark to travel freely between each chamber. To aid in active tracking of *H. ocellatum* within the shuttlebox system each individual was fitted with a small elastic harness with a piece of reflective tape (4cm x 2cm), with a web camera (Microsoft LifeCam HD-3000 Webcam) and four small LED lights positioned directly above the system. The web camera was connected to a custom program (Labtech Notebook Pro, Laboratories Technology Corp., Andover MA) to actively track the position of individual sharks within the chambers.

For each trial, an individual *H. ocellatum* was randomly selected from the holding tanks, placed into the shuttlebox system at 1400h and the shuttlebox system was activated. Individuals were allowed 19h to familiarize with the system. At 0900h, data collection began and continued for 5h. The preferred temperature was determined as the temperature at which the shark spent the most time during the 5h trial (i.e., modal temperature). The lower and upper threshold temperatures were defined as the minimum and maximum temperatures experienced by an individual during the 5h trial, respectively.

Environmental and body temperature

Environmental temperature (T_e) and body temperatures of individual *H. ocellatum* were collected on the coral reef flat at Heron Island. Environmental temperature was measured using 19 HOBO Pendant® Temperature Data Loggers (OneTemp, Australia) positioned along 2 transects on the Heron Island reef flat in January 2017 for 13 months. Each transect was perpendicular to the shoreline, starting ~50m from the low water mark and extending to the reef crest (approximately 350-400m from the shoreline). Temperature loggers were placed both in open (i.e., over sand with no overlying structure) and concealed (i.e., within the coral matrix) microhabitats to investigate potential differences in local thermal environments across the reef flat. Temperature loggers in open microhabitats were placed at 50m intervals and in concealed microhabitats at 100m intervals along each transect. Each logger recorded temperature every 30 minutes and the data downloaded every 6 months. The first transect, Coral Gardens (T_{e1}) was approximately 300m long and had 9 HOBO data loggers (i.e., 6 open microhabitats, 3 concealed microhabitats) and the second transect, Shark Bay (T_{e2}) was approximately 350m long, had 10 data loggers (7 open microhabitats, 3 concealed microhabitats). To protect and secure the loggers they were placed inside a small PVC tube (length: 10cm, diameter: 2.5cm) with numerous 2-3cm diameter holes to allow for water movement, and cable tied to a concrete block.

To quantify the internal body temperatures (T_b) of *H. ocellatum*, 40 individual sharks were collected by hand during low tide on the reef flat on Heron Island. Sharks were held in a 2000L holding tank for a minimum of 24h prior to being transferred to individual 100L tanks where they remained for ~10h prior to tagging. Sharks were anaesthetized in a bath comprised of 100mg/L tricaine methanesulfonate (MS-222) buffered with 100mg/L sodium bicarbonate for 4min (Smith *et al.,* 2004). Immediately following, a small ventro-lateral incision (~2cm) was made anterior to the pelvic fins and an ibutton data logger (DS1922L iButton Temperature Loggers, Maxim Integrated, USA) placed into the abdominal cavity. The incision was sutured closed, and a dart tag (spaghetti tag, Hallprint, SA) inserted into the dorsal muscle tissue immediately below the anterior dorsal fin to allow for later identification (Heupel, 1997). The surgical procedure took no longer than 6.5 minutes. Immediately following surgery, sharks were placed into recovery tanks and monitored for a minimum of 24h prior to being released onto the reef flat at the approximate site of capture. Ibutton temperature loggers were set to record body temperatures every 30 minutes for the following 6 months. In total 30 individuals were tagged in June 2017 and 10 individuals in January 2018.

Extensive searches for tagged *H. ocellatum* were conducted on the Heron Island reef flat over a 9-day period in July 2018. During these searches, seven tagged sharks were identified and recaptured by hand (five of the 30 that had been tagged in June 2017, and two of 10 that had been tagged in January 2018). Although only 17.5% of tagged *H. ocellatum* (n=7) were recaptured, this was directly comparable to previously reported recapture rates of *H. ocellatum* at Heron Island (Heupel & Bennett, 2007) and broadly comparable to the sample sizes in previous studies investigating the movements of shark and pelagic fish species (Holland *et al.,* 1992; Payne *et al.,* 2018; Thums *et al.,* 2013).

Recaptured sharks were transferred to a holding tank within 2h of collection where they were held for a minimum of 24hrs prior to surgery to remove the temperature loggers. Individual sharks were anaesthetized as described above, the implanted ibutton temperature loggers removed, and the incision suture closed. Sharks were placed into recovery tanks for a minimum of 24h prior to being released on the reef flat.

Data analyses

Preferred and threshold temperatures of *H. ocellatum* under controlled (i.e., lab) conditions were compared between collection season and sex using generalized linear models (GLM) with a gamma distribution. Collection season and sex were fixed effects, and holding tank was included as a random effect. Environmental temperatures were compared between transects (i.e., Coral Gardens or Shark Bay; fixed factor) using a generalized additive mixed model (GAMM) with a gaussian distribution. Day and hour were included with smoother functions to incorporate natural temporal variation (i.e., tidal and diurnal changes). Location (i.e., distance along each transect) was used as the random factor. Model assumptions (homogeneity of variance, collinearity, variance inflation) were assessed using diagnostic plots. Tukey post hoc tests were used to compare among groups. All values reported as means ± SEM unless specifically stated. All analyses were performed in R (Version 3.4.1, R Core Development Team 2013) using the *lme4* and *mcgv* packages. Due to the nature of the environmental and body temperature data (i.e., non-normal), a Wilcox test was used to compare the environmental and body temperatures of *H. ocellatum*. The analysis was restricted to data from July 2017 to December 2017 as this was the only period for which both environmental and body temperature data were available.

3.3 Results

Under controlled conditions the preferred temperature of *H. ocellatum* was 20.7±1.5°C (figure 3.1), with upper and lower threshold temperatures being 27.9±0.8°C and 17.7±0.6°C, respectively (figure 3.2). The preferred and threshold temperatures were consistent between sexes (T_{pref}: z=1.60 P=0.11; upper threshold: z=-0.86 P=0.39; lower threshold: z=0.99 P=0.32) and collection season (T_{pref}: z=-1.55 P=0.12; upper threshold: z=-1.84 P=0.07; lower threshold: z=0.35 P=0.73).

There was a small, but significant difference in environmental temperatures between the two transects, with Coral Gardens transect being 0.1±0.01°C warmer than the Shark Bay transect (t=-259, P<0.001; figure 3.3). There were, however, no differences between microhabitats (i.e., open vs. concealed; t=-0.516, P=0.61). Overall, environmental temperatures across the Coral Gardens transect ranged from 15.9-33.8°C with a mean temperature of 24.9°C, while Shark Bay transect ranged from 15.9-33.6°C with a mean temperature of 24.8°C.

There was no statistical difference between the environmental temperatures and the body temperatures of *H. ocellatum* on the reef flat over the 6 month period (P=0.09; figure 3.4). The mean deviance of body temperatures from environmental temperature was 0.01±0.34°C (mean ± SD; range: 0.00-2.46°C). Additionally, when the frequency of temperature was examined per month (figure 3.5), body temperatures mirror that of the environmental temperatures. Overall, body temperatures remained similar to environmental temperatures throughout the year and only approached temperatures similar to the laboratory established thermal preferences during winter months.

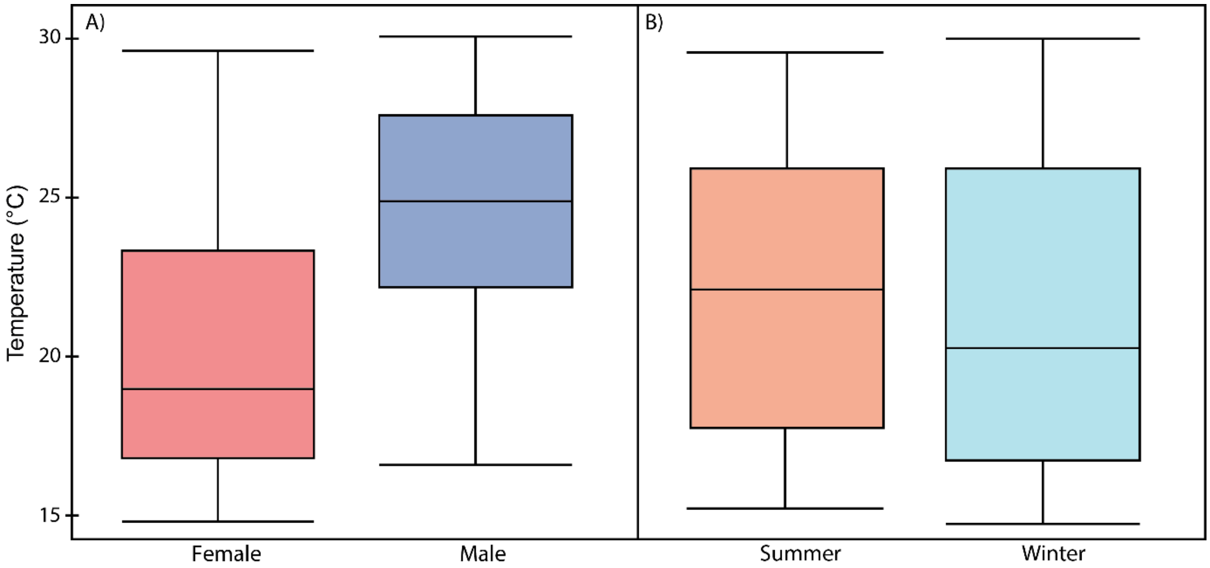


Figure 3.1. The effect of sex (A; female: n=8, male: n=4) and collection season (B; summer: n=7, winter: n=5) on temperature preference of the epaulette shark, *Hemiscyllium ocellatum*. Data are based on the modal temperature over a 5h trial in an automated shuttlebox system.

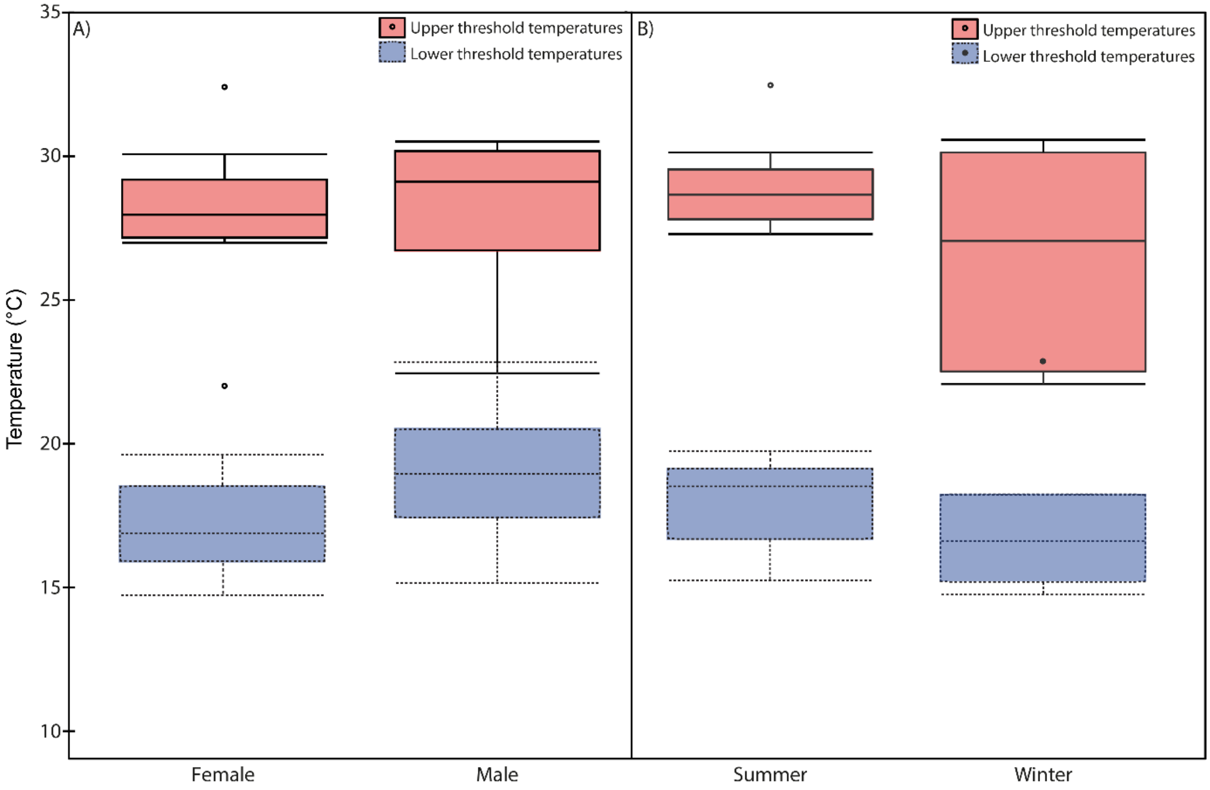
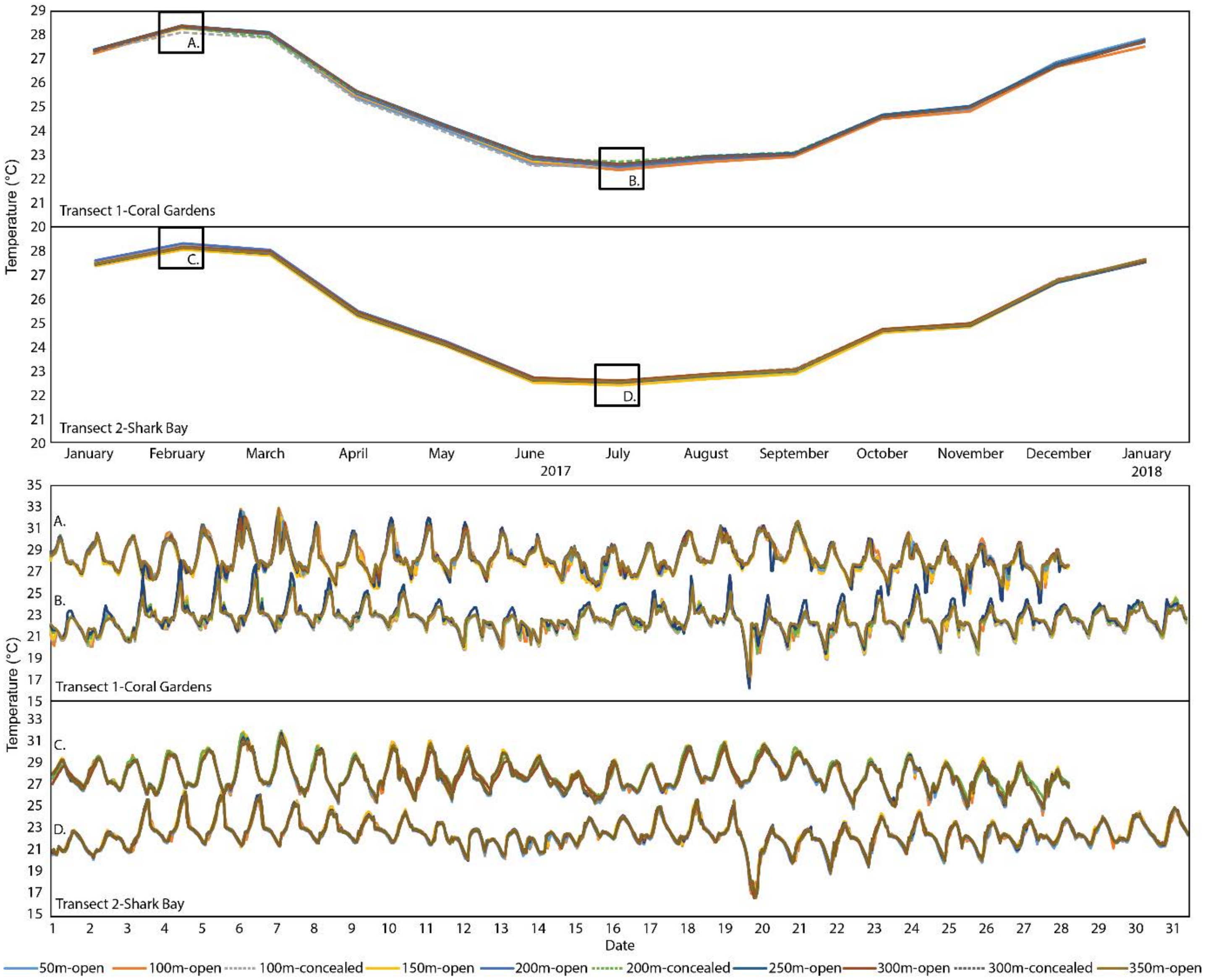


Figure 3.2. The effect of sex (A; female: n=8, male: n=4) and collection season (B; summer: n=7, winter: n=5) on upper and lower threshold temperatures of the epaulette shark, *Hemiscyllium ocellatum*. Data are based on the maximum (i.e., upper threshold) and minimum temperature (i.e., lower threshold) experienced by each individual during a 5h trial in an automated shuttlebox system.


Temperature (°C)
29
28
27
26
25
24
23
22
21
20
A.
B.
Transect 1-Coral Gardens
C.
D.
Transect 2-Shark Bay
January
February
March
April
May
June
2017
July
August
September
October
November
December
January
2018
35
33
31
29
27
25
23
21
19
17
15
A.
B.
Transect 1-Coral Gardens
C.
D.
Transect 2-Shark Bay
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26
27
28
29
30
31
Date
50m-open
100m-open
100m-concealed
150m-open
200m-open
200m-concealed
250m-open
300m-open
300m-concealed
350m-open

Figure 3.3. Variation in environmental temperature between location (Coral Gardens and Shark Bay) and microhabitats (open and concealed) on Heron Island between January 2017 and January 2018. The top panels are the temperatures for both transects, Coral Gardens and Shark Bay. The lower panels are monthly temperatures for the warmest (February 2017) and coolest (July 2017) recorded months. Highlighted areas of A and C are the temperatures for every 1h in August 2016 for both transects (i.e., Coral Gardens and Shark Bay). Highlighted areas of B and D are the monthly temperatures for every 1h in July 2017 for both transects.

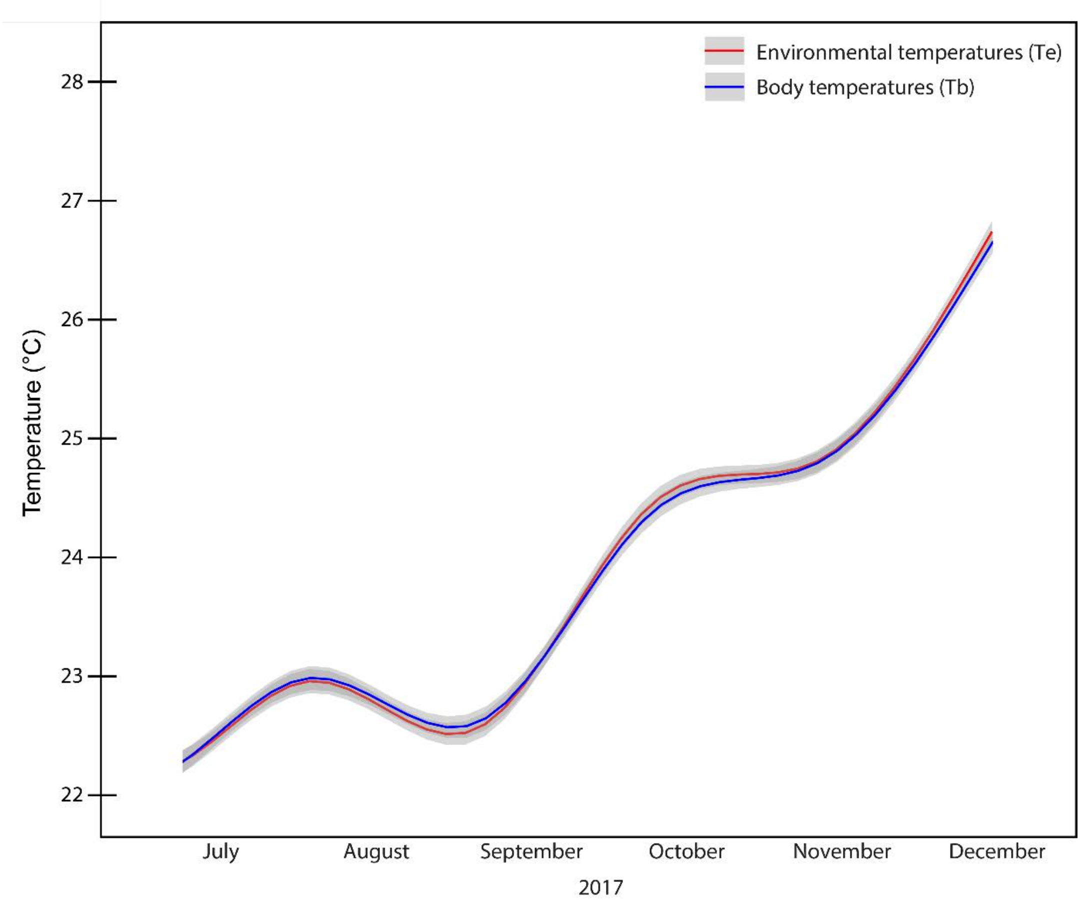

Figure 3.4. Comparison of mean body temperatures (T_b) of five *H. ocellatum*, and environmental (T_e) temperatures on the reef flat of Heron Island, Australia from July to December of 2017. T_e is represented as a solid red line and T_b is represented as a solid blue line. The grey shading represents 95% confidence intervals. Temperature data were selected at 1h increments.

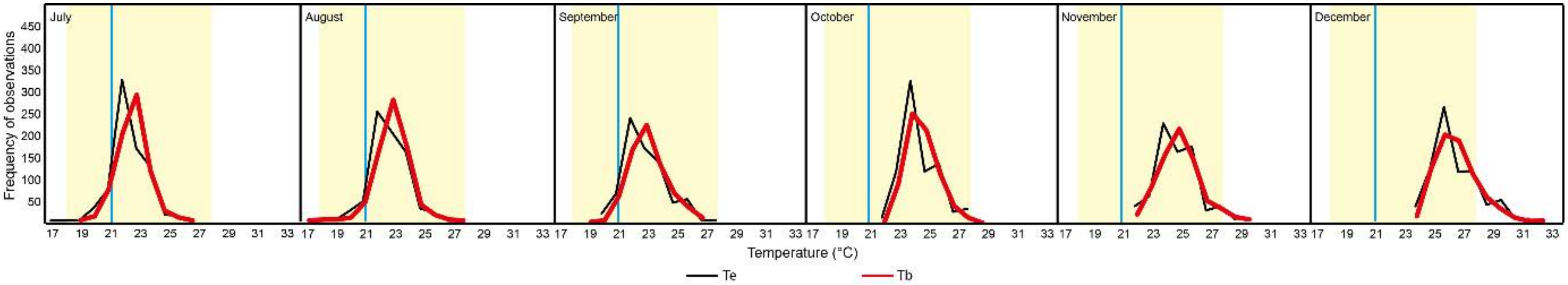

Figure 3.5. Comparison of the distribution of environmental temperatures (T_e) and body temperatures (T_b) of five *H. ocellatum* on the Heron Island reef flat. The red line shows the distribution of body temperatures, and the thinner black line the distribution of environmental temperatures. The blue line represented the preferred temperature under controlled conditions (i.e., 20.7°C) and the yellow shading represents the threshold temperature range (i.e., the temperatures from lower to upper threshold temperatures).

3.4 Discussion

Coral reef flats are thought to be thermally challenging for resident species, however, the mechanisms these species use to cope with such variable temperatures is largely unknown. Despite mean water temperatures on the Heron Island reef flat exceeding the preferred temperature of *H. ocellatum* (i.e., 20.7±1.5°C) 98% of the time on the reef flat, and exceeding their upper threshold temperature (i.e., 27.9±0.8°C) 11% of the time, the body temperatures of tagged *H. ocellatum* mirrored that of environmental temperatures. The confluence of environmental and body temperatures suggests that *H. ocellatum* are not moving off the reef flat to more favourable thermal environments (e.g., reef slope) during high temperatures, and rather than behaviourally thermoregulate, *H. ocellatum* are able to endure a wide range of temperatures (15.9-33.8°C). The lack of evidence for behavioural thermoregulation was perhaps not surprising given the spatial homogeneity of environmental temperature across and within the reef flat on Heron Island. This is supported by estimates of high efficiency (following Hertz *et al.,* 1993) that show that the body temperatures of tagged *H. ocellatum* only approached their preferred temperatures during the cooler months (21.3-24.2°C) when environmental temperatures approximated their preferred temperature (appendix I). *H. ocellatum* are specialized reef flat residents that possess physiological adaptations to deal with hypoxia etc., and it appears they are also to tolerate the wide range of temperatures experienced on the reef flat.

Coral reef flats, like other intertidal and shallow subtidal habitats, are known to be more thermally variable than adjacent deeper habitats due to the heating and pooling of shallow water during low tides (Harborne, 2013). Although this variation in temperature is primarily temporal, some spatial variation may be expected due to differences in water depth, pooling, and shading (Davis *et al.,* 2011; Hearn, 2011). Despite considerable temporal variation in water temperature on the Heron Island reef flat (15.9-33.8°C) there was little or no spatial variation between sites or microhabitats, or across the reef flat. Given the spatially homogeneity of temperature across the reef flat, resident epaulette sharks did not have opportunity to access to more thermally favourable microhabitats without leaving the reef flat. Indeed, many coral reef fishes are known to migrate from the reef flat to adjacent reef crest and slope habitats during low tides (Gibson, 2003; Harborne, 2013; Unsworth, Bell, & Smith, 2007), presumably due to the high temperatures on the reef flat. I found no evidence to suggest that *H. ocellatum* were moving from the reef crest to the more favourable thermal environment of the reef slope, despite its proximity. This apparent reluctance to move to the reef slope suggest that any physiological benefit of occupying a potentially more favourable thermal environment may be outweighed by the costs associated with other biotic or abiotic factors.

An individuals' selection of a habitat typically reflects a trade-off between the physiological benefits of selecting a favourable thermal environment and the physiological and/or ecological costs of associated abiotic and biotic factors (Angilletta, 2009). Moreover, moving to a more favourable thermally environment is only beneficial if the movement does not increase the risk of predation, the likelihood of missed opportunities (i.e., allocation of time away from foraging or reproducing), or the energetic costs as a result of moving (Angilletta, 2009). For example, the giant shovelnose ray, *Glaucostegus typus*, and reticulated whipray, *Himantura uarnak*, have been shown to occupy suboptimal thermal conditions to avoid areas of increased predation pressure (Vaudo & Heithaus, 2013). In the present study, *H. ocellatum* may have realized physiological benefits by moving to the more thermally favourable reef slope, however doing so may have exposed individuals to greater risk of predation as many large, mobile predators are unable to inhabit the shallow coral reef flats, especially during periods of low tide (Harborne, 2013; Rizzari, Frisch, & Magnenat, 2014; Vivien, 1973). Additionally, moving to a different habitat such as the reef slope could potentially limit access to prey (e.g., Villén-Pérez, Carrascal, & Seoane, 2013) and/or shelter (e.g., Nielsen & McGaw, 2016), or lead to increased competition for resources (e.g., Rusch, Angilletta, & Herrel, 2017). Although the mechanism is unclear and warrants further investigation, it appears that factors other than temperature are influencing habitat selection by *H. ocellatum*.

H. ocellatum are a known resident of coral reef flats, spending both high and low tide in coral reef flat habitats, and despite having a relatively low preferred temperature of 20.7±1.5°C under controlled conditions, *H. ocellatum* were able to endure a wide range of temperatures in their environment (15.9-33.8°C), are known for their unique physiological strategies to cope with extreme hypoxic conditions (e.g., metabolic depression and neuronal hypometabolism; see Devaux, Hickey, & Renshaw, 2019; Mulvey & Renshaw, 2000; Stensløkken, Milton, Lutz, Sundin, Renshaw *et al.,* 2008), and although epaulette sharks experienced temperatures above their upper threshold temperatures for ~11% of 2017, it is still unknown how epaulette sharks mitigate the effects of temperature exposure within their natural environment. A previous study using a similar shuttlebox system has shown that, juvenile *H. ocellatum* move to select their thermal environment during summer months, however, during winter months, individuals did not move in response to increasing temperatures even when approaching their critical thermal limits (Gervais *et al.,* 2018). The ability to occupy such a thermally variable environment indicate *H. ocellatum* may possess other physiological mechanisms (e.g., heat-shock proteins in fish or gastropods; Roberts *et al.,* 2010), Tomanek & Somero 1999; Tomanek 2010), that allow them to tolerate suboptimal temperatures.

Occupying thermally variable environments, such as coral reef flats, is assumed to be challenging for ectotherms. While behavioral thermoregulation appears common among larger mobile marine fishes and sharks (e.g., Bigeye tuna: Holland *et al.,* 1992; Whale shark: Thums *et al.,* 2013; Tiger shark: Payne *et al.,* 2018), this might not be the case for small-bodied site-attached species, such as *H. ocellatum*. Rather, such species may rely on physiological adaptations to endure such extreme thermal environments. Irrespective of the adaptations, increasing temperature under ongoing climate change are likely to be challenging for reef flat residents. *H. ocellatum* were exposed to temperatures upwards of 35°C on the Heron Island reef flat, a temperature that is approaching their critical thermal limit of 38°C (Gervais *et al.,* 2018). Exposure to such high temperatures are expected to increase in frequency and duration under future ocean warming scenarios (Collins *et al.,* 2013). Understanding epaulette sharks and other reef flat residents cope with extreme temperatures, and their capacity to cope with even higher temperatures will be key to predicting future populations and distributions.

Chapter 4: Species interactions alter the selection of thermal environment in a coral reef fish.

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4.1 Introduction

Ocean warming is one of the most pervasive stressors affecting marine ecosystems. Increases in ocean temperatures have caused shifts in the geographic distribution (e.g., Feary *et al.,* 2014; Pitt, Poloczanska, & Hobday, 2010), phenology (e.g., Beever *et al.,* 2017), behaviour and performance of marine species (e.g., Beever *et al.,* 2017; Nagelkerken & Munday, 2016), and consequently impacted the composition of communities and species interactions (e.g., Vergés *et al.,* 2016). Predicted future increases in mean ocean temperatures, coupled with the increasing frequency and intensity of marine heatwaves (Kerr, 2011), will be particularly challenging for ectothermic species, as their body temperatures mirror that of their environment and thus regulate the rate of many key physiological and biochemical processes. Moreover, most tropical ectotherms (e.g., coral reef fish) have evolved in relatively stable thermal environments and have a narrower thermal tolerance range than temperate ectotherms (Tewksbury *et al.,* 2008), and as such are predicted to be susceptible to even relatively small changes in temperature (Pörtner & Farrell, 2008; Pörtner & Peck, 2010).

While a species' thermal tolerance limits may dictate the range of thermal environments it may occupy, individuals are hypothesized to select a temperature, or range of temperatures, that optimize certain physiological processes or performance traits (Angilletta, 2009; Clark, Sandblom, & Jutfelt, 2013; Fry, 1947; Payne *et al.,* 2016). Such thermal optimum typically occur towards a species' maximum thermal limit, such that the relationship between temperature and the rate of physiological process (i.e., thermal performance curve) resembles a left-skewed distribution (Schulte, Healy, & Fangue, 2011). Although thermal optima have been historically viewed from the perspective of the whole organism (Fry, 1947), it is increasingly recognized that different thermal optima may exist for different physiological processes (multiple optima theory see Clark *et al.,* 2013; e.g., Brett, 1971; Matern *et al.,* 2000).

A species thermal optimum is, however, only one of several factors that may shape its distribution patterns across a variety of spatial scales. Indeed, a range of abiotic and biotic factors have been shown to affect the distribution of fish species among habitats (e.g., Harborne, 2013; Matern *et al.,* 2000) and thermal environments (e.g., Booth, Figueira, Gregson, Brown, & Beretta, 2007). For instance, abiotic factors such as hypoxia (Petersen & Steffensen, 2003), hydrodynamics (Bellwood *et al.,* 2002), structural complexity (Roberts & Ormond, 1987), water depth (Friedlander & Parrish, 1998), and habitat condition (Richardson *et al.,* 2018) can largely influence a species distribution. Similarly, positive and negative species interactions are also important determinants of habitat use (Travis, Brooker, & Dytham, 2005). For example, many fish species exploit monospecific or heterospecific schools to increase swimming efficiency (Hemelrijk *et al.,* 2015), increase foraging performance, decrease the risk of predation (Hoare *et al.,* 2000; Wisenden *et al.,* 2003), or avoid areas in which predators and/or competitors are present, or frequently use (Boaden & Kingsford, 2015; Bonin *et al.,* 2015; Chase *et al.,* 2002; Hixon & Beets, 1993; Hoey & Bellwood, 2011; Larson, 1980). Importantly, these species interactions are likely to influence the distribution of species among thermal environments. For example, subtropical and tropical rays (*Glaucostegus* *typus* and *Himantura uarnak*) have been shown to use a suboptimal thermal environment (i.e., shallow, warm water, especially during low tide) presumably due to high predator abundance in deeper, cooler waters— their preferred thermal environment (Vaudo & Heithaus, 2013). For most species, however, the exact effect of species interactions on preferred temperature and threshold temperatures is largely unknown.

Numerous laboratory studies have documented the preferred temperatures of temperate (Johnson & Kelsch, 1998; Killen, 2014; Ward *et al.,* 2010) and tropical (Habary *et al.,* 2017; Nay *et al.,* 2015) fish species under controlled conditions, however, such studies have yet to investigate how species interactions may affect a fish's thermal preference and thermal thresholds. Using a common coral reef fish, the black-axil chromis (*Chromis atripectoralis*) as a model species, I investigated how interactions with conspecifics, heterospecifics (*Neopomacentrus bankieri*), or a potential predator (*Cephalopholis spiloparaea*) influences thermal preferences and threshold temperatures. *Chromis atripectoralis* is a common Indo-Pacific schooling species (Randall, Allen, & Steene, 1997) that frequently form mixed species schools with other planktivorous coral reef fishes (e.g., *N. bankieri*) above branching corals (Quattrini, Bshary, & Roche, 2018).

4.2 Materials and Methods

Study species and animal husbandry

The black-axil chromis (*Chromis atripectoralis*, Pomacentridae, maximum total length (TL): 11cm; Randall *et al.,* 1997) was selected as our model species, as it is a common inhabitant on Indo-Pacific coral reefs and occupies a wide range of latitudes, and therefore temperatures, from the Ryukyu Islands to the subtropical reefs of Eastern Australia (32°N-32°S, Froese & Pauly, 2018). *Chromis atripectoralis* is a planktivorous fish and typically occurs in both monospecific and heterospecific schools associated with live branching corals (primarily *Acropora* spp.) on shallow reefs across much of its range (Randall *et al.,* 1997). The Chinese demoiselle (*Neopomacentrus bankieri*, Pomacentridae, max TL: 8cm*)* was selected as the heterospecific, as this species is commonly found co-inhabiting small coral bommies with *C. atripectoralis* across its distributional range, and shares a similar diet (Breder & Rosen, 1966; Quattrini *et al.,* 2018; Smith, 2016). The Strawberry rock cod (*Cephalopholis spiloparaea*, Serranidae, max TL: 30cm) was selected as the predator, as it is a small piscivorous fish that is common on coral reefs throughout the Indo-Pacific (Kuiter & Tonozuka, 2001).

A total of 70 *C. atripectoralis* and 50 *N. bankieri* were collected from Pioneer Bay, Orpheus Island, Queensland, Australia (18.6161° S, 146.4972° E; mean water temperature ± SD: 25.1±3.2°C; AIMS, 2019) in May and June 2017 using hand nets and small barrier nets. Similar sized *C. atripectoralis* (4.7±0.2cm mean total length (TL) ± SE; hereafter 'focal' fish) were haphazardly selected and placed into ten 100L aquaria, while smaller individuals (<4.0cm TL) were placed into two 100L aquaria for later use as 'conspecifics'. All *N. bankieri* individuals (<5.0cm TL) were housed in a single 100L aquarium for later use as heterospecifics. The piscivorous *Ce. spiloparaea* were sourced from a commercial aquarium fish collector (Cairns Marine, Australia), and each individual was held in a separate 100L aquarium. All aquaria were supplied with flow-through filtered seawater at ambient temperature (~26°C) and held under a 12:12 hour photoperiod.

Following a two-week adjustment period, focal *C. atripectoralis* were tagged with unique visible elastomer (Northwest Marine Technology, Washington, USA) to allow for individual identification. Both *C. atripectoralis* and *N. bankieri* were fed commercial pellets (NRD 2mm, ProAqua, Brisbane, Australia) to satiation twice daily, while each *Ce. spiloparaea* was fed ~5% of its body mass in frozen pilchard once every two days. Focal fish were fasted for 24h prior to experiments to ensure a post-absorptive state that maximized the energy available for performance (Niimi & Beamish, 1974) and to ensure thermal preferences were not altered by food quantity or quality (Killen, 2014; van Dijk, Staaks, & Hardewig, 2002). The wet weight and total length of each focal fish were measured at the end of each experimental trial.

Temperature preference

Preferred water temperature (T_{pref}) for each focal *C. atripectoralis* was determined using an automated shuttlebox system as described in chapter 2, section 2.2 Materials and Methods. In addition to the standard shuttlebox system design, a transparent plexiglass container (Ø 15cm) with a series of 0.5cm holes was placed in the center of each chamber. This inner plexiglass chamber was in place to contain the treatment fish (i.e., conspecifics, heterospecifics, or predator) in either the ‘warming’ or ‘cooling’ chamber, thereby allowing the focal fish to see and smell the treatment fish without directly interacting with it. Treatment fish assignment was randomized prior to beginning experiments and assignments of ‘warming’ and ‘cooling’ chambers were switched halfway through all trials to allow for the detection of any potential chamber bias.

A focal fish was haphazardly selected and placed into the shuttlebox system at 1430h and allowed to adjust to the static system (i.e., no warming or cooling) for 90 minutes. At 1600h the shuttlebox system was activated and fish allowed an additional 17hr to adjust to the system prior to data collection. At 0700h, three *C. atripectoralis* (i.e., conspecifics), three *N. bankieri* (i.e., heterospecifics), or a single *Ce. spiloparaea* (i.e., predator) were haphazardly selected and added to the plexiglass chamber either in the ‘warming’ or the ‘cooling’ chamber. The system would continue to heat or cool both chambers depending on the location of the focal fish, not the treatment fish. Focal fish were given 2hr (0700h-0900h) to adjust to the additional treatment fish prior to data collection. To establish the effects of positive interactions (i.e., where the focal fish selectively stayed in the same chamber as the treatment fish), the upper preferred temperature and the upper threshold temperatures were determined by the placement of conspecifics or heterospecifics in the ‘warming’ chamber, while lower preferred temperatures and lower threshold temperatures were established by the placement of conspecifics or heterospecifics in the ‘cooling’ chamber. Conversely, as I expected the focal fish to avoid the predator by staying in the opposite chamber of the treatment fish (i.e., a negative interaction), the upper threshold temperatures were established by the placement of a predator in the ‘cooling’ chamber and lower threshold temperatures were established by the placement of a predator in the ‘warming’ chamber. Each focal individual was used only once. A total of 60 focal fish were tested this way, n=10 for each treatment (i.e., conspecific, heterospecifics, or predator) x chamber (i.e., ‘warming’ or ‘cooling’) combination. An additional set of ‘control’ treatments was also conducted, whereby three conspecifics were placed in each of the plexiglass containers inside the ‘warming’ and the ‘cooling’ chambers simultaneously (n=10). All data collection periods began at 0900h and ended at 1400h for the conspecifics, heterospecifics, and control trials. For the predator treatments, pilot trials revealed that focal fish would not move into the chamber that housed the predator until extreme temperatures were reached. As such, the predator trials were ended following the first movement toward and then away from the predator, and both temperatures were used to calculate the lower and upper threshold temperatures by focal fish.

For each of the control, conspecific, and heterospecific trials, the preferred temperature and threshold temperature were calculated. Preferred temperature (T_{pref}) was defined as the temperature at which the focal fish spent the greatest proportion of time (i.e., modal temperature) during each 5h trial. The lower and upper threshold temperatures were established as the maximum and minimum temperature experienced by the focal fish within each 5hr trial, respectively. Lower and upper threshold temperatures were the only parameters calculated for predator trials as predator trials were ended following the first movement toward and away from the predator.

Data Analyses

All analyses were performed using R statistical software (Version 3.4.1, R Core Development Team 2013). Generalized linear models (GLM) were used from the package *lme4* to compare preferred temperature and threshold temperatures between treatments. Treatment fish (i.e., conspecific, heterospecific, predator) and placement (i.e., ‘warming’, ‘cooling’, or control) were combined and included as a fixed effect. Holding tank was included as a random effect. A Gamma distribution with a log link was used for all models. All assumptions were checked by visual inspection of residuals and

Q-Q plots. Tukey *post hoc* tests were used for all analyses. All values are reported as means ± SEM.

4.3 Results

The preferred temperature of *C. atripectoralis* under control conditions (i.e., conspecifics in both the ‘cooling’ and ‘warming’ chamber) was 27.5±1.0°C. When either conspecifics or heterospecifics (*N. bankieri*) were present in the cooler environment (i.e., ‘cooling’ chamber of the shuttlebox) the preferred temperature of *C. atripectoralis* decreased by ~ 6°C (conspecifics: 21.2±1.4°C, z=-3.52 p=0.01; heterospecifics 21.1±1.1°C, z=-4.24 p<0.001; figure 4.1). In contrast, the presence of either conspecifics or heterospecifics in the warmer environment (i.e., ‘warming’ chamber of the shuttlebox) had no effect on the upper preferred temperature of *C. atripectoralis* (conspecifics: 28.9±1.2°C, z=1.16 p=0.86; heterospecifics: 29.7±1.1°C, z=0.59 p=0.99; figure 4.1).

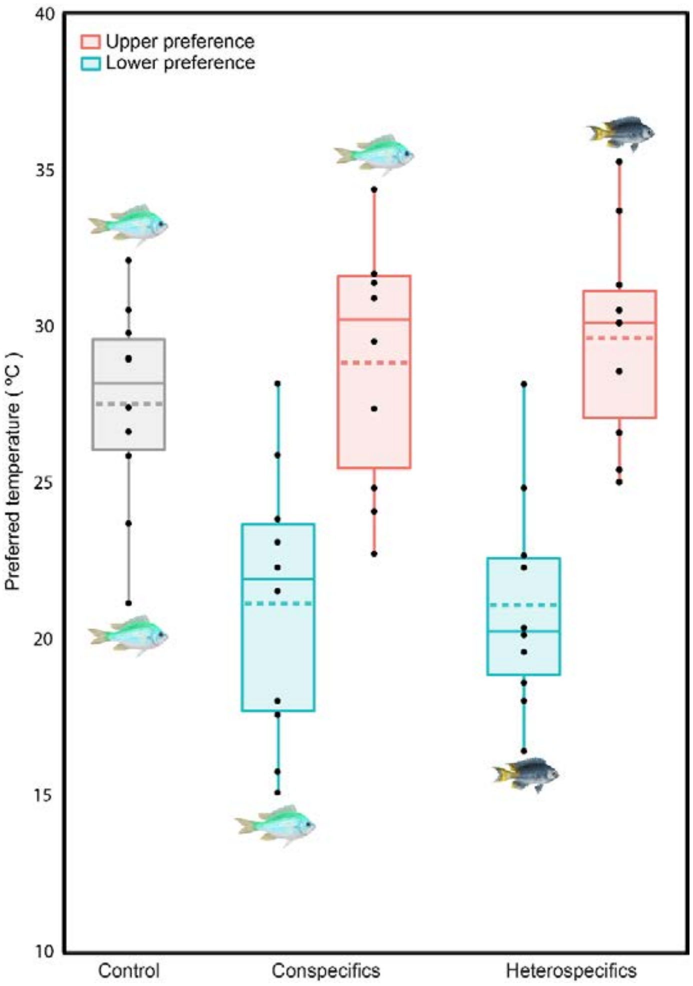

Figure 4.1. The effect of conspecific and heterospecific association on the preferred temperatures of *Chromis atripectoralis*. Lower preferred temperatures (blue) were established when conspecifics or heterospecifics were placed into the 'cooling' chamber, while upper preferred temperatures (red) were established from trials where conspecifics and heterospecifics were present in the 'warming' chamber. The placement of the fish image represents the chamber location within the shuttlebox system. Within the boxes, mean values are represented by dashed lines, and median values are represented by solid lines. The boxes represent the first and third quartiles, and the whiskers represent the minimum and maximum values outside of outliers. Outliers are points found outside of the box and whisker range.

The lower and upper threshold temperatures for *C. atripectoralis* were 23.5±0.9°C and 29.7±0.7°C under control conditions (figure 4.2). When focal *C. atripectoralis* were associating with either conspecifics or heterospecifics they tolerated significant lower (conspecifics: 18.1±0.8°C; z=-4.74 p<0.001; heterospecifics: 19.2±0.9°C; z=-3.73 p=0.003), but not higher threshold temperatures (conspecifics: 30.8±0.9°C; z=1.14 p=0.91; heterospecifics: 31.4±0.8°C; z=0.49 p=0.99; figure 4.2) before moving away from the treatment fish. The presence of the potential predator, *Ce. spiloparaea*, in the shuttlebox system, had the largest effect on threshold temperature of *C. atripectoralis*, with focal fish on average tolerating temperatures down to 18.6±0.5°C (z=-4.32 P<0.001) and as high as 35.2±0.5°C before giving up on avoiding the predator and moving into the same chamber as the predator (z=3.39 P=0.01; figure 4.2).

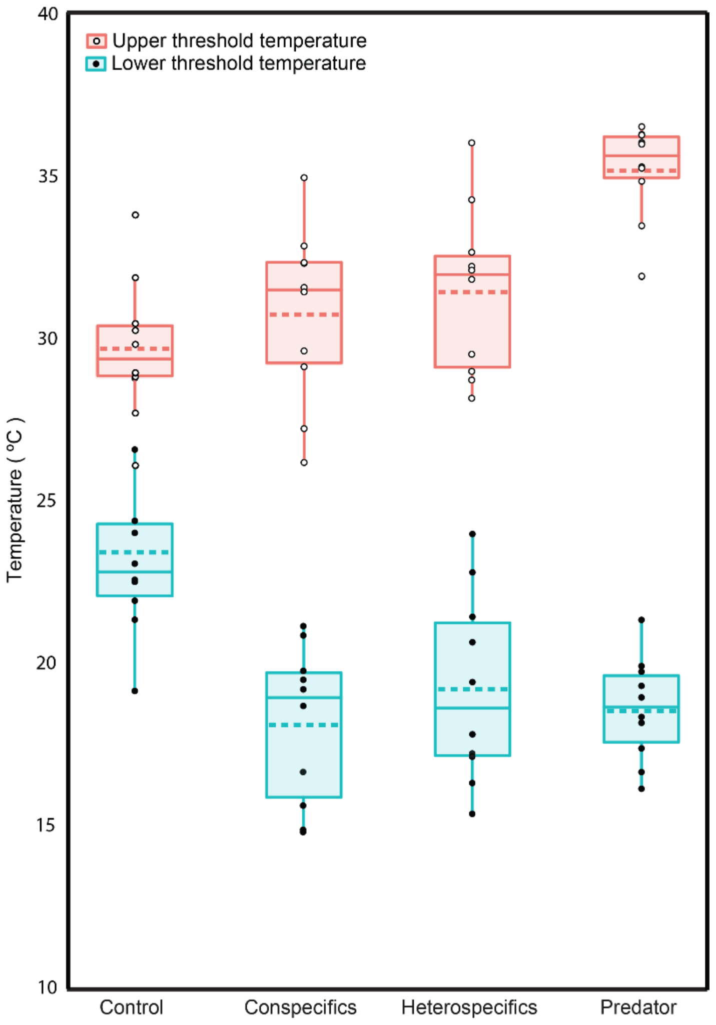

Figure 4.2. The effect of conspecifics, heterospecifics, or a predator on lower and upper threshold temperatures of *Chromis atripectoralis*. The lower and upper threshold temperatures are the minimum and maximum temperature experienced by a focal *C. atripectoralis* for each of the four treatments. Mean values are represented by dashed lines and median values represented by solid lines. The boxes represent the first and third quartiles and the whiskers represent the minimum and maximum values outside of outliers. Outliers are points found outside of the box and whisker range.

4.4 Discussion

Fishes, and other ectotherms, are hypothesized to select temperatures that optimise one or more physiological processes. However, an individual's choice of thermal environment will depend on a range of biotic factors, in particular interactions with other taxa. Using a common coral reef fish as our model species, I show that intra- and inter-specific interactions had a significant effect on the preferred and threshold temperatures, with focal fish selecting cooler, but not warmer, preferred and threshold temperatures. The preferred temperature decreased by ~6°C and threshold temperature decreased by ~4-5.5°C when associated with either conspecifics or heterospecifics (*N. bankieri*) compared to those under control conditions. In contrast, *C. atripectoralis* associated with conspecifics or heterospecifics did not select or tolerate temperatures higher than control focal fish. Collectively, these results suggest that there was a trade-off between the potential benefits of group membership and the physiological cost of being exposed to non-preferred temperatures. However, there appeared to be limited scope for *C. atripectoralis* to occupy environments with temperatures higher than preference levels, suggesting that the benefit of group membership was not as great as the cost of being exposed to elevated temperatures. The greatest effect, however, was due to predator (*Ce. spiloparaea*) avoidance, with *C. atripectoralis* tolerating temperatures ~5.5°C cooler or warmer than control fish before moving toward the predator. These findings highlight the importance of species interactions in shaping the distributions of coral reef fishes as well as the trade-offs with temperature-dependent physiological processes.

Increasing global temperatures and the resultant poleward shifts in the distribution of many species (Booth, Bond, & Macreadie, 2011; Feary *et al.,* 2014; Sunday *et al.,* 2015) has highlighted the importance of environmental temperature in shaping the distribution of ectotherms, and led to an emerging body of research investigating thermal preference of species (e.g., Beever *et al.,* 2017; Nay *et al.,* 2015; Payne *et al.,* 2016). Most previous attempts to quantify thermal preferences have either done so under laboratory conditions in the absence of other factors (e.g., Habary *et al.,* 2017; Johnson & Kelsch, 1998; Killen, 2014; Schurmann *et al.,* 1991), or inferred the relative importance of temperature and other abiotic and/or biotic factors from correlative field studies. Here I show there is a trade-off between exposure to sub-optimal temperatures and the benefits group membership for *C. atripectoralis.* Group membership has been shown to enhance predator evasion (Roberts, 1996; Ward *et al.,* 2011), energy conservation (Hemelrijk *et al.,* 2015; Nadler *et al.,* 2016; Cooper, Adriaensses, Killen, 2018), foraging efficiency (Pitcher, Magurran, & Winfield, 1982), and swimming efficiency (Marras *et al.,* 2015). Group membership has also been shown to decrease oxygen uptake rates in *C. viridis*, a close sister species to *C. atripectoralis*, presumably due to a calming effect (Nadler *et al.,* 2016). While the lower preferred and threshold temperatures of *C. atripectoralis* in the presence of both conspecifics and heterospecifics indicates that the benefits of group membership were greater than the costs of being exposed to cooler, and presumable suboptimal, temperatures, such an effect was not evident at higher temperatures. This suggests that the costs of selecting a higher temperature outweighs the benefits of group membership which is consistent with theoretical predictions of thermal performance curves for other tropical species

(Angilletta, 2009; Tewksbury *et al.,* 2008). Indeed, studies have shown lower latitude populations to be living near their upper thermal limits, while higher latitude populations have more scope to select temperatures (Johansen & Jones, 2011; Rummer *et al.,* 2014; Stuart-Smith *et al.,* 2015).

The greatest effect on the thermal thresholds of *C. atripectoralis* was the presence of the predator, *Ce. spiloparaea,* with *C. atripectoralis* tolerating temperatures up to ~5.5°C cooler or warmer than control fish before risking moving into the same area as the predator. While the predator in this study could not access the focal *C. atripectoralis*, the visual and olfactory cues appear sufficient to pose a perceived predation risk to the focal fish. C*hromis atripectoralis* tolerated temperatures of up to 35°C before moving toward the predator. Such extreme temperature is approaching the critical thermal maxima (CT_{Max}) of its sister species, *C. viridis* (~37°C; Habary *et al.,* 2016) and will likely be similar for *C. atripectoralis*. Exceeding critical thermal maxima elicits extreme declines in physiological performance at which point an organism is unable to appropriately respond to its environment and thus risks fatal endpoints. However, given the disproportionately high consequences of predation, focal fish may risk exposure to potentially detrimental conditions (i.e., acute exposure to extreme temperatures) and risk temporary declines in performance to ultimately allow for continued survival.

This study is one of the first to demonstrate the importance of both positive and negative species interactions in modulating the preferred and threshold temperatures of a tropical fish, and has direct implications for understanding current and predicting future species distributions. Many marine ecosystems around the globe are under threat from ocean warming (Collins *et al.,* 2013; Walther *et al.,* 2002), and an increased frequency and intensity of marine heatwaves (Hughes *et al.,* 2018; Wernberg *et al.,* 2012). The spatial distribution of ocean warming and heatwaves is projected to impact the future geographic distribution of species, populations, and communities (Collins *et al.,* 2013). Populations at lower latitudes were traditionally thought to be limited by biotic interactions (i.e., species interactions; Brown, Stevens, & Kaufman, 1996; Dobzhansky, 1950), however, recent theoretical (Cahill *et al.,* 2014) and empirical literature (Gardiner, Munday, & Nilsson, 2010; Rummer *et al.,* 2014) suggests that low latitude populations may be primarily shaped by their tolerance to abiotic conditions, in particular temperature (Cahill *et al.,* 2014). Many low-latitude populations are already living closer to their thermal optimum and therefore, may have limited ability to cope with further increases in temperature, even under favorable biotic conditions. Higher latitude populations, however, may be shaped by positive species interactions over a range of temperatures, allowing them to occupy a broad range of habitats. This is particularly relevant for the many species that have already begun to redistribute into cooler/deeper waters as a result of continued ocean warming (Feary *et al.,* 2014). Within these expanding ranges, many vagrant species have begun to associate with native fish species gaining the benefits from group membership (Smith *et al.,* 2018). Understanding how species interactions influence a species preferred and threshold temperature will be imperative in predicting how and where species will coexist into the future.

Chapter 5: Habitat complexity results in high preference of lower, but not higher temperature by a common coral reef fish, *Chromis atripectoralis*.

5.1 Introduction

Changing environmental conditions, and most notably increasing temperatures, are having important direct and indirect effects on marine species (Hoegh-Guldberg & Bruno, 2010; Pecl *et al.,* 2017), and are being compounded by local anthropogenic stressors. The direct effects of increasing temperature on an organisms physiology are driving shifts in individual behaviour (e.g., Beever *et al.,* 2017), phenology (Nagelkerken & Munday, 2016), and species distributions (e.g., Feary *et al.,* 2014), and are especially pronounced in marine ecosystems as marine ectotherms generally occur in environments that are closer to their upper thermal maxima and have fewer thermal refugia than their terrestrial counterparts (Pinsky *et al.,* 2019). The direct effects of increasing temperature are also occurring alongside indirect effects changing environmental conditions, mostly due to degradation and loss of habitat (Robinson *et al.,* 2019). For example, across tropical and temperate reef systems, climate-induced changes in environmental and biological conditions are causing massive reductions in the abundance of key habitat-forming organisms (Hughes *et al.,* 2018b; Ling *et al.,* 2009; Madin *et al.,* 2018; Vergés *et al.,* 2016; Wernberg *et al.,* 2010). Declines in the abundance of formerly dominant habitat-forming organisms (reef-building corals and kelp forests, respectively), and corresponding declines in habitat complexity, can have a profound influence on the biodiversity and functioning of these ecosystems (Graham *et* *al.,* 2006). Our ability to predict and manage populations under ongoing climate change will require a greater understanding of both the indirect and direct effects of changing temperature in shaping species' distributions.

Coral reefs are extremely vulnerable to climate change (Walther *et al.,* 2002), due largely to the thermal sensitivities of the dominant habitat forming organisms, the reef-building corals (e.g., Baird & Marshall, 2002; Hughes, D Anderson, Connolly, Heron, T Kerry *et al.,* 2018a). The increased frequency and intensity of thermal bleaching events over the past few decades (Hughes *et al.,* 2018a) has contributed to widespread and sustained declines in the abundance of corals, and a corresponding loss of structural complexity (Bento *et al.,* 2016; Berumen & Pratchett, 2006; Hughes *et al.,* 2018b; Hughes *et al.,* 2017; Loya *et al.,* 2001; McClanahan *et al.,* 2007). Coral loss and topographic collapse of reef habitats is having a dramatic effect on reef associated organisms (Pratchett *et al.,* 2008; Stella *et al.,* 2011). Those species that rely on live corals for food and/or shelter are the most rapidly and adversely affected by declines in live coral cover (e.g., Pratchett *et al.,* 2008; Stuart-Smith *et al.,* 2018; Wilson *et al.,* 2006), while those species that rely on the physical structure of corals typically exhibit protracted declines as the coral skeletons erode and the physical structure is lost (Graham *et al.,* 2006; Pratchett *et al.,* 2011; Wilson *et al.,* 2006).

Marine fish, like other ectotherms, are particular sensitive to increasing temperatures as their rates of physiological and biochemical processes are largely determined by environmental temperature (Fry, 1947), and generally occupy environments that are close to their upper thermal limits (Pinsky *et al.,* 2019). Moreover, tropical marine species tend to have a narrower thermal tolerance range than temperate species as they evolved in relatively stable thermal environments (Tewksbury, Huey, & Deutsch, 2008), and hence exhibit smaller thermal safety margins (Pinsky *et al.,* 2019). Indeed, many low latitude populations of tropical fishes are already living in thermal environments that are near or above their thermal optima (Gardiner, Munday, & Nilsson, 2010; Nguyen *et al.,* 2011; Rummer *et al.,* 2014) limiting their capacity to cope with local increases in temperatures (Collins *et al.,* 2013; Hughes *et al.,* 2018a; Kerr, 2011). Indeed, Stuart-Smith et al. (2018) reported a restructuring of fish and invertebrate communities following the 2016 coral bleaching event on the Great Barrier Reef that were independent of coral loss, and suggested the observed changes were due to the direct effects of temperature.

Given the predicted increases in ocean temperatures with ongoing climate change, a greater understanding of the preferred and threshold temperatures of coral reef fishes, and the ecological factors that may influence these temperatures is urgently needed. The objective of this chapter was to investigate the relative importance of physical structure versus thermal environment in shaping habitat choice in a common coral reef fish, *Chromis atripectoralis*. Specifically, this study uses an automated shuttlebox system to determine how availability of a complex habitat influences the preferred and threshold temperatures of *C. atripectoralis*.

5.2 Materials and Methods

Animal husbandry

Black-axil chromis (*Chromis atripectoralis*, Pomacentridae) was selected as my model species as they are common across a wide range of latitudes (32°N-32°S, from the Ryukyu Islands to Northern Australia), and hence temperatures on Indo-Pacific coral reefs (Froese & Pauly, 2018). *C. atripectoralis* are relatively small bodied (maximum total length, TL: 12cm) and closely associate with complex coral structures (Lieske & Myers, 1996), making them an ideal species for examining the impact of habitat complexity on thermal preference. *C. atripectoralis* were collected from Pioneer Bay, Orpheus Island, Queensland, Australia (18.6161° S, 146.4972° E) using small barrier nets and hand nets in May and June 2017. Following collection, fish were held at the Orpheus Island Research Station with fresh flow-through seawater for 48h, then transported in aerated bags filled with seawater to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Queensland, Australia. Forty-five similar sized *C. atripectoralis* (mean ± SEM; TL: 5.91±0.16cm; mass: 11.28±0.74g) were randomly selected and held in 100L aquaria, with a maximum of 10 fish per aquaria. All aquaria were continuously supplied with filtered seawater maintained at 26±1°C. Fish were fed commercial pellets twice daily and held under a 12:12 hour photoperiod. Fish were held for two weeks prior to tagging with a subcutaneous coloured elastomer (Northwest Marine Technology, Washington, USA) in the dorsal musculature for individual identification and allowed to recover for a minimum of 2 weeks.

Preferred and threshold temperatures

To establish the influence of habitat structure on the preferred temperature of *C. atripectoralis* a modified shuttlebox design was used (refer to chapter 2, section 2.2 Materials and Methods) in which structurally complex habitat was added to the centre of one chamber and a structurally simple habitat of equal volume was added to the other chamber. A skeleton of the branching coral *Acropora nasuta* (~15cm diameter, ~25cm height) was used as the ‘complex’ habitat and rubble (~15cm diameter, ~5cm height) created by breaking up an *Acropora nasuta skeleton of similar size, was used as the ‘simple’* habitat. The skeleton branching coral was used instead of live coral as I aimed to establish the effects of structural complexity, independent of the health and condition of the coral habitat. The *A. nasuta* skeleton allowed fish to occupy space under, above, and between coral branches, while the rubble structure was of similar volume but provided fish with limited refugia. The preferred and threshold temperatures of *C. atripectoralis* were determined: (i) in the absence of any habitat in either chamber, i.e., ‘control’, (ii) with the complex structure in the ‘warmer’ chamber and rubble structure in the ‘cooler’ chamber, and (iii) with the complex structure in the ‘cooler’ chamber and rubble structure was placed into the ‘warmer’ chamber. Fifteen *C. atripectoralis* were used for each treatment, with a different individual being used for each trial (total n=45).

Individual fish were haphazardly selected and allocated to one of the three treatments. All fish were fasted for 24h prior to trials to maximize the energy available for performance (Niimi & Beamish, 1974). For each trial, the fish and habitat were placed into the system at 1430h and given 1.5hr to familiarize with the system prior to the heating and cooling pumps being turned on. Fish were given an additional 17hr learning period prior to data collection. Data collection began at 0900hr the next morning and continued for 5h until 1400hr.

For each treatment, the position of the fish along with the corresponding temperature was used to calculate preferred temperature (T_{pref}) and the lower and upper threshold temperatures. T_{pref} was defined as the temperature where the fish spent the most time (i.e., modal temperature) within each trial. The lower and upper threshold temperatures were defined as the lowest and highest absolute temperature, respectively, each individual fish experienced when associated with a either a complex or rubble habitat. For the control, the lower and upper threshold temperatures were defined as the lowest and highest temperature experienced by each fish during a trial.

Data Analysis

All analyses were performed in R (Version 3.4.1, R Core Development Team 2013) using '*lme4*'. Generalized linear models (GLM) using the gamma distribution and 'log' link function were used to compare T_{pref} and upper and lower threshold temperatures between control and complex or rubble habitats. Treatment was used as a fixed effect and holding tank was included as a random effect. All assumptions were checked by visual inspection of residuals, variance inflation factors and Q-Q plots. Tukey *post hoc* tests were used for all a priori analyses. To account for running multiple tests on the same individuals within experiments, an false discovery rate correction was used post-hoc (Benjamini & Hochberg, 1995). All values reported as mean ± SEM.

5.3 Results

In the absence of any habitat (i.e., control), *C. atripectoralis* tolerated threshold temperatures of 22.9±0.8°C and up to 31.9±0.6°C, with a preferred temperature of 28.1±0.9°C (figures 5.1 and 5.2). When the alternative habitat types (rubble versus intact colony of *A. nasuta*) were added to the shuttlebox, fish preferentially associated with the complex habitat spending 62.7% and 78.8% of each trial associating with the intact coral skeleton, as opposed to rubble (figure 5.3).

When associated with the complex habitat, *C. atripectoralis* would tolerate lower (18.6±0.7°C, z=4.37 p<0.001), but not higher (31.7±0.4°C, z=0.27 p=0.79; figure 5.2) threshold temperatures than control fish, with a preferred temperature being 18.9±1.0°C (z=8.27 p<0.001) or 28.3±0.7°C (z=-0.752 p=0.73) depending on the placement of the complex habitat in the ‘cooler’ or ‘warmer’ chamber, respectively (figure 5.1).

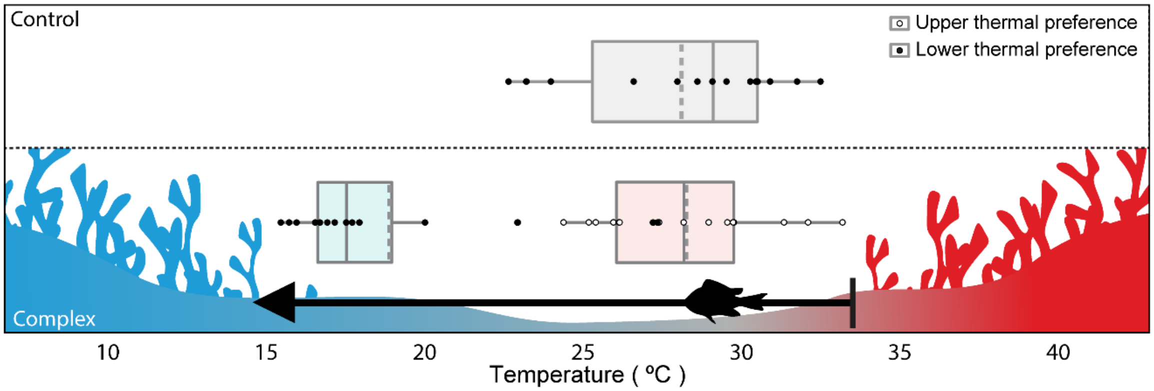

Figure 5.1. The effect of complex habitat structure on the thermal preference of *Chromis atripectoralis*. Lower preferred temperatures (blue) were established when complex habitat structure was placed into the 'cooler' chamber, while upper preferred temperatures (red) were established from trials where complex habitat structure was placed in the 'warmer' chamber. Dashed lines are means; solid lines are medians.

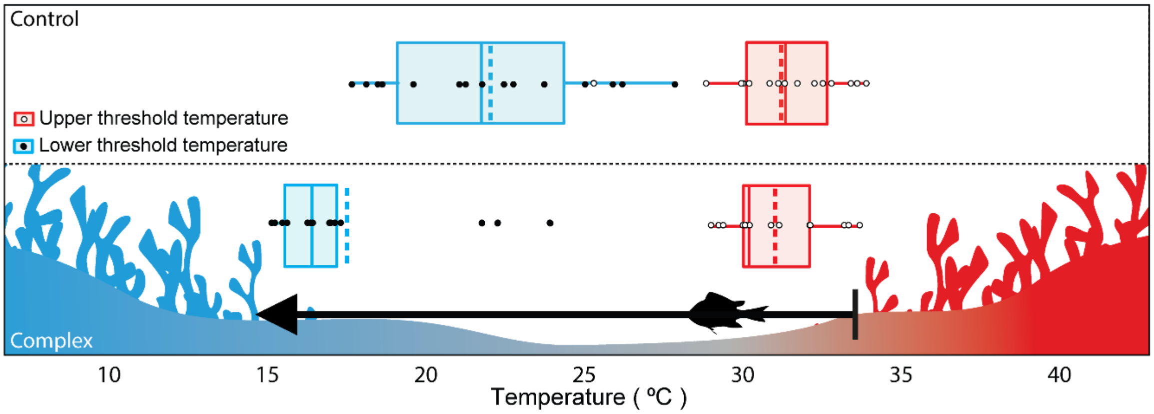

Figure 5.2. The effect of complex and rubble habitat structure on the upper and lower threshold temperatures of *Chromis atripectoralis*. The lower (blue) and upper (red) threshold temperatures are the minimum and maximum temperature experienced by a focal *C. atripectoralis* for each of the treatments. Dashed lines are means; solid lines are medians.

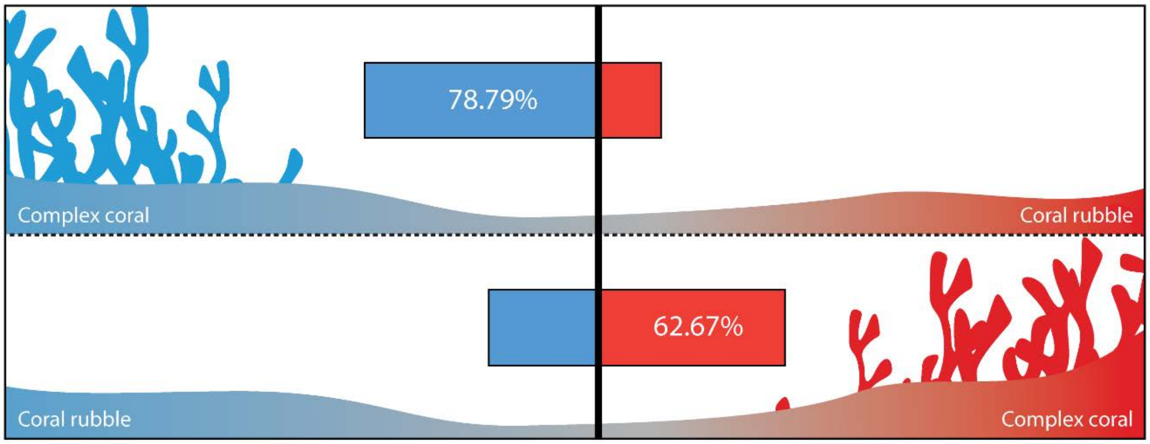

Figure 5.3. The proportion of time spent with complex (vs. rubble) structure of *Chromis atripectoralis*. The upper panel displays the proportion of time when the complex coral was placed in the ‘cooler’ changer; and the lower panel is the proportion of time when the complex coral was placed in the ‘warmer’ chamber.

5.4 Discussion

As oceans continue to warm and coral reefs degrade globally, species distribution patterns will increasingly be driven by the availability of suitable thermal environments and structurally-complex habitats. Here, I demonstrate that a common coral reef fish, *C. atripectoralis*, appears to trade-off between ecological benefits of associating with a preferred habitat and physiological costs of occupying a suboptimal thermal environment, but only at lower temperatures. In the absence of any habitat, *C. atripectoralis* tolerated temperatures between ca. 23°C and 32°C, with a preferred temperature of 28.1°C. When associated with the complex habitat, *C. atripectoralis* tolerated temperatures 4.5°C lower than in the absence of any habitat, resulting in a 9.2°C decrease in their preferred temperature. In contrast, I found no evidence that *C.*

atripectoralis would tolerate higher temperatures when associated with complex habitat. This is supported by previous studies that have shown several tropical damselfishes and cardinalfishes (including *C. atripectoralis*) are occupying thermal environments that are close to their upper thermal limits (Gardiner *et al.,* 2010; Rummer *et al.,* 2014). While numerous studies have investigated the effects of habitat degradation and loss of structural complexity (e.g., Richardson *et al.,* 2018; Roberts & Ormond, 1987) or changing temperatures (e.g., Donelson *et al.,* 2010; Habary *et al.,* 2017) on reef fish, few, if any, have considered how habitat availability may affect temperature choice and vice-versa (see Matis *et al.,* 2018) for exception). Understanding the nature and magnitude of the costs and benefits of associating with different habitat/s and thermal environments is crucial to predict how populations and distributions of coral reef fishes will respond to future conditions under ongoing ocean warming.

Reductions in live coral and the consequent loss of structural complexity are known to reduce the abundance and diversity of coral reef fish assemblages, with those species that rely directly on corals for food and/or shelter being the most adversely affected (Caley & John, 1996; Coker, Pratchett, & L. Munday, 2009; Pratchett *et al.,* 2008). While *C. atripectoralis* is considered a facultative coral dweller, a meta-analysis has shown they are not sensitive to the loss of live coral (Pratchett, Hoey, & Wilson, 2016). The results of the present study suggest that preference of *C. atripectoralis* for the complex habitat, although important, may be lesser than the effects of temperature on physiological function and survival. The unwillingness or inability of *C. atripectoralis* to tolerate temperatures greater than 31.9°C even when the preferred complex habitat was available is likely due to increased physiological costs, although this response may have been different if the complex habitat was a live coral colony or if there was a threat of predation. This is supported by a previous study that demonstrated a decline in physiological performance (i.e., oxygen uptake rate) of *Chromis viridis*, the sister species to *C. atripectoralis* with similar preferred temperature (28.9°C), at temperatures above 31°C (Habary *et al.,* 2017). The lack of change in upper threshold temperatures when associating with complex coral structure suggests that thermal thresholds for this species may be close to upper thermal limits as seen in other tropical taxa (e.g., ~37°C for C. viridis, (Habary *et al.,* 2017).

Seawater temperature and habitat structure are widely recognized as two of the major drivers of reef fish communities (Pratchett *et al.,* 2008; Robinson *et al.,* 2019; Stuart-Smith *et al.,* 2009; Waldock *et al.,* 2019), yet are often viewed at different spatial scales. Increasing ocean temperatures have typically been related to shifts in the geographic distribution of reef fishes (e.g., Feary *et al.,* 2014; Sunday, Bates, & Dulvy, 2012) while changes in habitat structure have been related to changes in fish communities within or among proximal locations (e.g., Darling *et al.,* 2017; Messmer *et al.,* 2011). The results of this study, however, highlight the need to consider both the thermal conditions and physical structure when considering the preferred habitat of reef fishes. Indeed, the lack of suitable habitat has been suggested to constrain the poleward expansion of some reef fish species (Feary *et al.,* 2014; Munday *et al.,* 2008). The only other study I am aware of investigated the effect of temperature on the habitat choice of coral reef fishes suggested that temperature exposure to 22, 28, or 31°C influenced habitat selectivity of 3 species of juvenile damselfish, and although some changes were reported, the effect sizes were small (Matis *et al.,* 2018).

Global declines in coral cover, and the subsequent reductions in the goods and services they provide has led to an increased emphasis on coral reef restoration projects to aid in coral reef recovery (Fox *et al.,* 2019; Hein *et al.,* 2017; Rinkevich, 2015). While there are a growing number of approaches to coral restoration (e.g., enhanced larval supply: Cruz & Harrison, 2017); assisted evolution of thermally tolerated corals; Oppen, Gates, Blackall, Cantin, Chakravarti *et al.,* 2017); growth and outplanting of coral nubbins: Suggett, Camp, Edmondson, Boström-Einarsson, Ramler *et al.,* 2019); structural complexity enhancement: Yanovski & Abelson, 2019), all are aimed at increasing the cover of live coral and the physical structure they provide on the recipient reefs. It is often assumed, either implicitly or explicitly, that the provisioning of physical structure will facilitate the recovery of reef fish assemblages (Ladd, Burkepile, & Shantz, 2019). However, the physiological tolerances of reef fishes to increasing temperature are rarely considered. The results of the present study suggest that the provisioning of habitat structure alone may not be sufficient to restore or maintain fish populations, especially at their lower latitude boundaries, under ongoing climate change.

Changes in the abundance, diversity, and composition of reef fish assemblages have typically been related to changes in coral cover and/or the physical structure of the habitat (Pratchett *et al.,* 2008; Wilson *et al.,* 2006). However, the results of this study suggest that as oceans continue to warm the physiological effects of local environmental temperatures are likely to overwhelm any benefit of associating with their preferred habitat. This is particularly important as both theoretical predictions and empirical evidence suggest that many coral reef fish species have limited thermal safety margins as their preferred, and often realized, temperatures are close to the thermal maximum (Gardiner *et al.,* 2010; Pinsky *et al.,* 2019; Rummer *et al.,* 2014; Tewksbury *et al.,* 2008). Understanding the relative importance of temperature, habitat structure, and potentially other biotic factors (e.g., species interactions) are critical to predict how fishes will respond to future ocean warming and the potential of restorative techniques for maintaining future fish populations.

Chapter 6: General Discussion

Global warming is one of the most pervasive stressors affecting the structure and functioning of the world's ecosystems (e.g., Pecl *et al.,* 2017; Poloczanska *et al.,* 2013; Wernberg *et al.,* 2011). In the marine environment, increasing sea surface temperatures have led to the degradation of habitats (e.g., Hughes *et al.,* 2018; Wernberg *et al.,* 2012), shifts in the geographic distribution of species (e.g., Feary *et al.,* 2014; Pitt, Poloczanska, & Hobday, 2010), changes in phenology and growth (e.g., Beever *et al.,* 2017), behaviour (e.g., Nagelkerken & Munday, 2016), strength and nature of trophic interactions (e.g., Johansen *et al.,* 2015; Vergés *et al.,* 2016), and community composition (e.g., Hughes *et al.,* 2018; Stuart-Smith *et al.,* 2018). Despite the importance of environmental temperature in shaping the composition and functioning of marine ecosystems, few studies have quantified the preferred and threshold temperatures of tropical marine fishes (for exceptions see Habary *et al.,* 2017; Nay *et al.,* 2015; Reyes *et al.,* 2011) or how these temperatures may be influenced by their thermal environment, the strategies used to cope with exposure to extreme temperatures (for exceptions see Donelson *et al.,* 2011; Donelson *et al.,* 2011; Habary *et al.,* 2017), or the effect of biotic or abiotic factors on these selected temperatures.

Research presented in this thesis represents the most comprehensive assessment of the thermal preferences of tropical reef fishes to date, and highlights the importance of considering the influence of a species 'natural' thermal environment and a range of ecological factors in determining threshold and, to a lesser extent, preferred temperatures of tropical marine fishes.

Differences in thermal environments across latitudes have previously been associated with the breadth of thermal tolerance ranges, with the magnitude of thermal variability of the environment being positively related to the thermal tolerance ranges of species occupying that environment (Angilletta, 2009; Tewksbury *et al.,* 2008). The results of this thesis provide support for the relationship between the thermal variability of the environment and the thermal tolerance range of resident species. Although the threshold temperatures recorded in the shuttlebox do not reflect critical thermal limits, the range of temperatures experienced was generally greater for the two species from thermally variable intertidal environments (*Hemiscyllium ocellatum*, ~10°C; *Periophthalmus argentilineatus* ~7-8°C) than the species from the more thermally stable subtidal environment (*Chromis atripectoralis* ~6°C; figure 6.1). Contrary to expectations, however, the broader ranges of *H. ocellatum* and *P. argentilineatus* were related to exposure to cooler, rather than warmer water. While the underlying mechanisms are unclear, it suggests that these species may rely on other behavioural and/or physiological mechanisms to cope with exposure to high temperatures within their natural environments. For example, *P. argentilineatus*, like other mudskipper species, have the ability to exploit the aerial environment and as such, may use this alternative habitat to avoid high water temperatures and rely less on thermal preferences within the marine environment. Similarly, other intertidal species, such as the gastropods *Nodilittorina peruviana* and *Littorina sitkana*, change their position in the intertidal zone depending on aerial or marine conditions and may therefore be able to more accurately select thermally beneficial temperatures, given the availability of additional habitats (Jones & Boulding, 1999; Soto & Bozinovic, 1998). Interestingly, *H. ocellatum* does not appear to use movement to influence its internal body temperature and likely relies on other physiological specialisations to enable it to withstand temperatures ~34°C (~6°C above their upper threshold) in their natural environment.

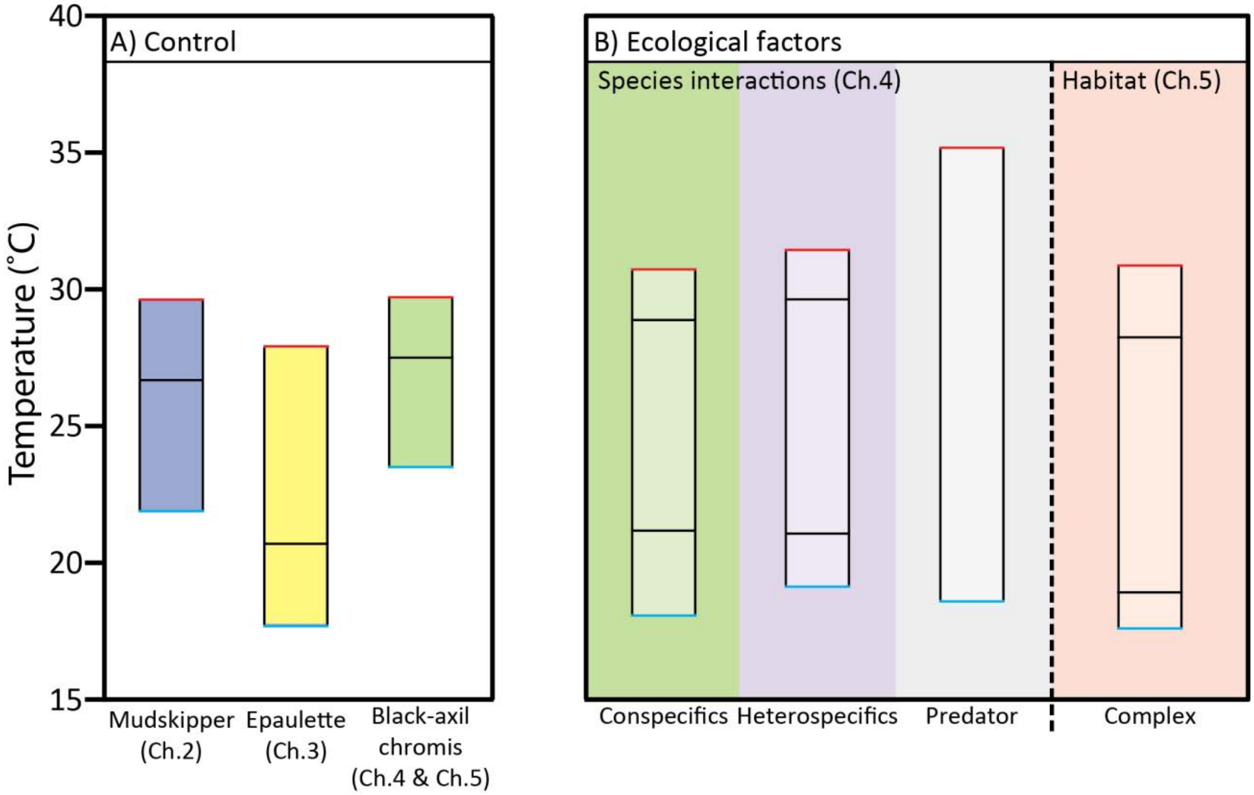

Figure 6.1. Summary of preferred and threshold temperatures for three species of tropical fish examined in this thesis. Boxes are represented by upper (red) and lower (blue) threshold temperatures with preferred temperatures represented by the solid black line. (A) Comparison of preferred and threshold temperatures of two fish species that occupy thermally variable environments, the barred mudskipper (*Periophthalmus argentilineatus*) and epaulette shark (*Hemiscyllium ocellatum*), and one species that occupies a more thermally stable environment, the black-axil chromis (*Chromis atripectoralis*) under controlled conditions. (B) The effects of species interactions (conspecifics, heterospecifics, and a predator) and habitat association (complex habitat) on the preferred and threshold temperatures of *C. atripectoralis*.

Within a species thermal tolerance range, rates of physiological performance generally increase gradually with increasing temperatures until the thermal optimum (T_{opt}) is reached, beyond which performance declines rapidly until the thermal maximum is reached (Pörtner & Peck, 2010); figure 1.1). As such, a species thermal optimum is hypothesised to be closer to upper than the lower thermal limits, resulting in a left-skewed thermal performance curve (Pörtner & Farrell, 2008; Pörtner & Peck, 2010). Such proximity of the thermal optimum to the upper thermal threshold held for both *P. argentilineatus* and *C. atripectoralis* (chapters 2 and 4, respectively), but not for the epaulette shark *H. ocellatum* (chapter 3). The preferred temperature of *H. ocellatum* (~21°C) was closer to its lower temperature, suggesting the thermal performance curve of *H. ocellatum* may be less skewed with performance being optimised at cooler than expected temperatures. While the mechanisms underlying this apparent preference for relatively low temperatures is unknown, it is an exciting avenue for future research.

Together with the effect of a species thermal environment on preferred and threshold temperatures, this thesis demonstrates the potential influence of species interactions and habitat structure on choice of thermal environment by a common coral reef damselfish, *C. atripectoralis*. The effect of associating with conspecifics, heterospecifics and complex habitat had remarkably similar effects on the choice of thermal environment by focal *C. atripectoralis*, with individuals exposing themselves to temperatures ~4-5°C cooler, but not warmer, than control fish (figure 6.1). The apparent inflexibility of the upper temperature threshold suggests that it, similar to critical thermal maxima, may be constrained by physiological processes regardless of exposure to other ecological factors. The only exception was the presence of the potential predator, *Cephalopholis spiloparaea* which lead to a 5.5°C increase in the upper thermal threshold. The reluctance of *C. atripectoralis* to move toward the predator was likely driven by a perceived lethal threat and it is unclear if *C. atripectoralis* could tolerate such high temperatures (35°C) for an extended period of time. Indeed, the upper temperatures experienced when avoiding moving toward the predator were approaching the critical thermal maximum (~37°C) of *C. viridis* (Habary *et al.,* 2017), the sister taxon to *C. atripectoralis*. In contrast, lower threshold temperatures of C. atripectoralis appeared to be much more plastic suggesting there are likely trade-offs between physiological costs and ecological benefits. Collectively, these findings highlight the importance of examining not only physiological tolerance of species, but also how ecological factors influence these tolerances. Such information will be critical for predicting the future distribution of species and assemblages.

This thesis provides essential baseline information on the thermal preferences of three tropical fish species, yet how a species preferred temperature under controlled conditions relates to its thermal performance is largely unknown. Thermal preferences have been studied for many years in a wide array of taxa, and although often assumed, either explicitly, or implicitly, to mirror thermal optimal (Angilletta, 2009; Fry, 1947), there have been few, if any, comparisons of thermal preference and thermal optima in marine fish species (for exception see Habary *et al.,* 2017). Further, several recent studies have highlighted that a species is likely to have multiple thermal optima, each relating to a different physiological process or performance trait. It has also been hypothesised that the abundance of a species will be greatest at, or near, their thermal optima and decline at both higher and lower temperatures (Munday *et al.,* 2008; Waldock *et al.,* 2019), however such spatial comparisons have yet to include data on the thermal preference of individual species. There is a clear need to understand how thermal preferences and threshold temperatures derived from experimental approaches, such as the automated shuttlebox used throughout this thesis, relate to a species thermal performance curve, and physiological process to which it relates. Future research should also consider how these parameters differ among populations and the potential for acclimation or adaptation of such preferences or if these strategies could be utilised in unison with behavioural strategies.

The research conducted in this thesis has not only provided novel thermal preference data for tropical fishes, but it is also hoped it will initial a larger body of work aimed at linking thermal performance to the ecology and distribution of tropical fishes. While, the influence of ecological factors on thermal preferences has been widely studied in many terrestrial taxa (e.g., Carr & Lima, 2011; Downes, 2001; Mason *et al.,* 2017; Podhorna *et al.,* 2018; Savagian & Fernandez-Duque, 2017; van de Ven, McKechnie, & Cunningham, 2019), it has been largely overlooked in marine taxa. As such it presents an exciting avenue for future research. For example, examining the thermal preferences of an individual when exposed to a combination of both complex habitat structure and predator presence would provide a more realistic understanding of the trade-offs between shelter and the selection of a thermal environment. Furthermore, it is critical that studies examine how thermal preferences relate to a suite of physiological performance traits and how these may be altered under different ecological scenarios. As the climate continues to warm, populations are likely to continue shifting towards more favourable thermal conditions (Feary *et al.,* 2014).

Identifying ecological factors that may influence a species' selection of a thermal environment is critical to understand current and predict the future redistribution of species under ongoing climate change.

Temperature and ecological factors influence where and how organisms are distributed within their natural environments (Araújo & Luoto, 2007; Gilman *et al.,* 2010; Tewksbury *et al.,* 2008; Waldock *et al.,* 2019), but are often examined independently. However, with the growing threat of climate change, understanding how these factors influence the distribution of species is critical in predicting future tropical marine fish assemblages and the goods and services they provide. This thesis provided an insight into the thermal preferences and thresholds of three tropical marine fish species, and how they are shaped by the thermal variability of their natural environment, and how ecological factors (i.e., species interactions and habitat quality) influence these preferences. It is hoped this will initiate a large body of work into the thermal performance and preferences of tropical fishes so that we may better understand the likely impacts of continued warming and manage them into the future.

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Appendix I: Methodology and data analysis of effectiveness of thermoregulation for Chapter 3

Materials and Methods for epaulette shark thermoregulation

The quality of thermal habitat and effectiveness of thermoregulation were calculated based on established protocols by Hertz et al. (1993). All calculations were established for two transects, Coral Gardens (T_{e1}) and Shark Bay (T_{e2}), each at two-time scales; monthly between February-December 2017 and weekly at four weeks throughout the year. Weeks (i.e., 8-14 February 2017, 6-12 June 2017, 6-12 July 2017, 1-7 December 2017) were selected as they were during the full moon of their respective months and would thus be times where the most extreme tides would be exhibited. Therefore, thermoregulatory movement may be necessary during these periods.

To establish the thermal quality of the habitat or the degree to which the habitat temperatures were within the T_{pref} range, the deviations (d_e) between T_e and T_{pref} were calculated. If d_e were close to 0, the habitat was optimally suited for thermoregulation (i.e., environmental temperatures thus body temperatures would always be within the preferred range); however, as the value of d_e increases, the habitat became less optimal for thermoregulatory strategies (i.e., epaulette sharks would have to actively thermoregulate to achieve temperatures within their preferred range).

To establish the effectiveness of thermoregulation, T_b was compared to T_{pref}. If T_b values were within the T_{pref} range (i.e., defined here as the central 68% or 1 standard deviation of all temperatures selected during T_{pref} trials; (DeWitt & Friedman, 1979), the deviations (d_b) were equal zero and may suggest active thermoregulation. If T_b values were above or below the T_{pref} range, d_b would be the absolute difference between the two and may suggest that body temperatures would regularly fall outside of preferred temperature ranges. This value was used to establish the average degree that the sharks experienced temperatures outside of their T_{pref}. Ultimately to establish the effectiveness of thermoregulation, the equation: ($E = 1 - (\overline{d}_b / \overline{d}_e$) was used. If the d_b and d_e values were similar, *E* approached zero and suggested a non-thermoregulating organism. If d_b was smaller than d_e, *E* approached one and suggested a carefully thermoregulating organism.

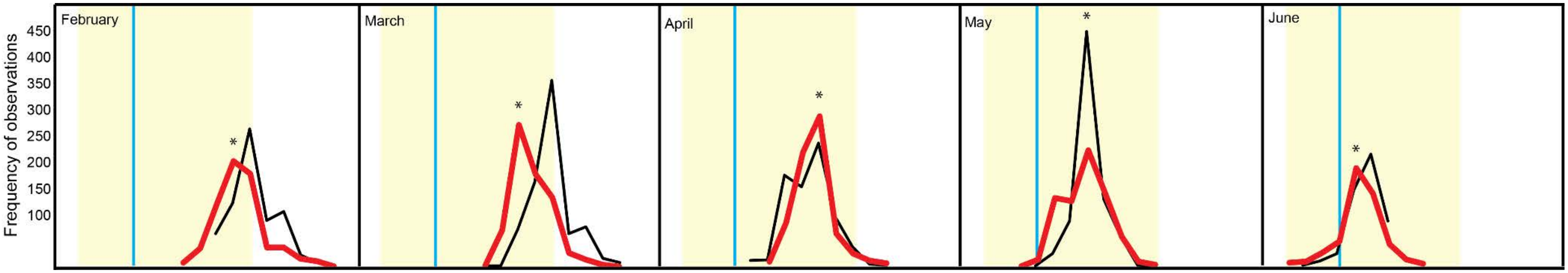
Results

Efficiency calculations demonstrated that epaulette sharks were effective at thermoregulation during the majority of the year with the exception of summer months (i.e., February and December). Finer scale analyses (i.e., week duration) mirrored that of broad scale analyses (i.e., yearly), such that body temperatures resembled preferred temperatures more closely during the weeks in June and July, and less so during weeks in February and December. Although efficiency calculations suggest highest thermoregulation abilities during winter months, upon closer examination, body temperatures of all sharks mirrored that of environmental temperatures during all time periods.

Despite the apparent lack of spatial variation there was considerable variation in temperature across both short (i.e., tidal cycle), and long (i.e., seasonal) temporal scales. Winter months (i.e., June and July) provided the most optimal conditions for effective thermoregulation, while summer conditions were least optimal for thermoregulation. As such, body temperatures more closely aligned with preferred temperatures between the months of May and October 2017.

Appendix table 1. Body temperatures (T_b), environmental temperatures for Coral Gardens (T_{e1}) and Shark Bay (T_{e2}), and preferred temperature (T_{pref}) values for epaulette sharks, *Hemiscyllium ocellatum,* from Heron Island, Queensland, Australia. T_{pref} min and T_{pref} max represent 68% of the central temperatures chosen by epaulette sharks in the laboratory. All values are represented as monthly mean values. d_b represents the degree at which body temperatures fell within T_{pref} ranges. D_e values represent the thermal quality of the habitat for each transect and E is the effectiveness of thermoregulation, with 1 being the value of a perfect thermoregulator.

Month	T_{set}/T_{pref}	T_{set} min	T_{set} max	T_b	T_{e1}	T_{e2}	E1	E2
	20.75	19.79	24.59	25.16	24.57	24.49	0.97	0.97
February				27.46	28.26	28.16	0.52	0.52
March				26.82	27.98	27.93	0.54	0.54
April				25.66	25.52	25.34	0.84	0.84
May				23.85	24.16	24.09	1	1
June				22.03	22.80	22.58	1	1
July				22.53	21.75	21.75	1	1
August				22.82	21.37	21.27	1	1
September				22.99	22.84	22.82	1	1
October				24.56	23.82	23.80	1	1
November				24.89	25.12	25.07	0.89	0.89
December				26.65	26.66	26.61	0.66	0.66



Appendix figure 1. Comparison of the distribution of environmental temperatures (T_e) and body temperatures (T_b) of five *H. ocellatum* on the Heron Island reef flat. The red line shows the distribution of body temperatures, and the thinner black line the distribution of environmental temperatures. The blue line represented the preferred temperature under controlled conditions (i.e., 20.7°C) and the yellow shading represents the threshold temperature range (i.e., the temperatures from lower to upper threshold temperatures). As T_b data were not collected for February to June 2017, T_b data were used from February to June 2018 to correspond with T_e data of February to June 2017 and are indicated by an asterisk. Temperature data were selected at 1h increments.

Appendix II: Resulting publications

Publications resulting from this thesis

Nay TJ, Gervais CR, Hoey AS, Johansen JL, Steffensen JF, Rummer JL. (2018) The emergence emergency: a mudskipper's response to temperature. *Journal of Thermal Biology* 78: 65-72.

Nay TJ, Johansen JL, Rummer JL, Steffensen JF, Hoey AS. Species interactions alter the preferred and threshold temperatures in a coral reef fish. (Submitted October 2019 to *Proceedings of the Royal Society B*)

Publications resulting from collaborations

Spady BL, **Nay TJ**, Rummer JL, Munday PL, Watson SA. (2019) Aerobic scope of two tropical cephalopod species unaltered by projected near-future CO_2 levels. *Conservation Physiology* 7 (1)

Gervais C, **Nay T**, Brown C. Friend or Foe? Odour detection, differentiation, and anti-predator response in an embryonic elasmobranch, *Heterodontus portusjacksoni* (Submitted September 2019 to *Proceedings of the Royal Society B*)

Gervais C, **Nay T**, Renshaw G, Johansen JL, Steffensen JF, Rummer JL. (2018) Too hot to handle? Using movement to alleviate effects of elevated temperatures in a benthic elasmobranch, *Hemiscyllium ocellatum*. *Marine Biology* 165(11): 162.

Journal of Thermal Biology 78 (2018) 65–72

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The emergence emergency: A mudskipper's response to temperatures

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ABSTRACT

Temperature has a profound effect on all life and a particularly influential effect on ectotherms, such as fishes. Amphibious fishes have a variety of strategies, both physiological and/or behavioural, to cope with a broad range of thermal conditions. This study examined the relationship between prolonged (5 weeks) exposure to a range of temperatures (22, 25, 28, or 32 °C) on oxygen uptake rate and movement behaviours (i.e., thermoregulation and emergence) in a common amphibious fish, the barred mudskipper (*Periophthalmus argentilneatuis*). At the highest temperature examined (32 °C, approximately 5 °C above their summer average temperatures), barred mudskippers exhibited 33.7–97.7% greater oxygen uptake rates at rest ($\dot{M}O_{2Rest}$), emerged at a higher temperature (CT_e; i.e., a modified critical thermal maxima (CT_{Max}) methodology) of 41.3 ± 0.3 °C relative to those maintained at 28, 25, or 22 °C. The 32 °C-maintained fish also ceased movement activity at the highest holding temperature suggesting that prolonged submergence at elevated temperatures is physiologically and energetically stressful to the individual. Using exhaustive exercise protocols with and without air exposure to simulate a predatory chase, the time to recovery was examined for all individuals. When submerged, mudskippers required 2.5x longer recovery time to return to resting oxygen uptake from exhaustive exercise than those fully emerged in air. Oxygen uptake data revealed that air exposure did not accrue oxygen debt, thereby allowing faster return to resting oxygen consumption rates. If the option to emerge was not available, mudskippers preferentially sought more benign water temperatures (26.7 ± 2.1 °C), resembling those experienced by these fish during the Austral autumn, regardless of prolonged exposure higher or lower temperatures. These results add to our understanding of the strategies that amphibious fishes may use to mitigate extra costs associated with living in warm waters, and could be the key to understanding how such species will cope with increasing temperatures in the future.

1. Introduction

Temperature has a profound effect on all life, and a particularly influential effect on ectotherms, as the rate of their biochemical and physiological processes are largely governed by the temperature of their external environment (Tewksbury et al., 2008; Pörtner and Peck, 2010). For example, fishes have been shown to increase resting oxygen uptake rates when exposed to acute or chronic increases in water temperatures (e.g., Lefevre, 2016; Gillooly et al., 2001; Clarke and Johnston, 1999; Brown, 1989). This increased oxygen demand suggests that more energy will be required to maintain daily performance at higher temperatures, potentially reducing the energy available for key processes, such as growth and reproduction (Fry, 1947; Pörtner and Peck, 2010). As global temperatures continue to increase, many tropical ectotherms are thought to be at risk as they are already experiencing temperatures close to their upper thermal limits (Rummer et al., 2014). Most of the fishes, as obligate aquatic ectotherms, can utilize strategies such as acclimation, adaptation, or behaviour to cope with changes in temperatures (Fry, 1947; Pörtner and Peck, 2010); whereas amphibious fishes (i.e., those who spend part of their life on land) are uniquely adapted to life in two different environments and may use a range of strategies (e.g., emergence) to cope with changing temperatures.

Mudskippers (family Gobiidae, subfamily Oxudercinae) are amphibious fishes that are common inhabitants of mudflat and mangrove environments, regularly emerging from the water (e.g., to forage, evade predation; Gordon et al., 1968). Mudskipper species have the ability to

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take in oxygen through gills, other brachial surfaces (i.e., buccal, pharyngeal, branchial, and opercular cavities; Graham, 1997), and cutaneous surfaces, and although all species have this ability, the ratio at which they utilize a particular oxygen uptake method differs between species (Graham, 1997). For example, Tamura and colleagues (1976) found that *Boleophthalmus chinensis* relied more heavily on gills (59%) than skin (43%) for oxygen uptake. In contrast, *Periophthalmus cantonensis* relied more heavily on skin (76%) than gills (27%) for oxygen uptake. They concluded that the differences between oxygen uptake sources were related to the transition from aquatic to terrestrial air breathing. These adaptations in mudskipper fishes allow them to use several behaviours that can be used with changes in tide, temperature, light (day vs. night), and salinity (Baeck et al., 2013; Clayton, 1993). For instance, juvenile mudskippers in Kuwait Bay have been observed to regulate their body temperature by ‘basking’, specifically by orientating their body at a right angle to the sun to increase body temperature (Clayton and Vaughan, 1988; Tytler and Vaughan, 1983). Conversely, several amphibious fishes, *Pseudogobius* sp. and *Favonigobius exquisitus* (Ford et al., 2004), and *Kryptolebias marmoratus* (Gibson et al., 2015) are known to leave the water in response to elevated temperatures. This emergence behaviour has been linked to the use of evaporative cooling (Tytler and Vaughan, 1983). Although mudskippers may be well equipped to cope with their environmental conditions, predicted changes in sea surface temperatures, including those in marginal habitats (i.e., habitats supporting relatively few species because of limited environmental conditions) may challenge the mudskippers’ present adaptations.

Physiological responses and behavioural strategies can differ greatly over the temporal scale at which fish can utilize them. Physiological responses can last from a couple of minutes to acclimation or adaptation processes over months, years, and across generations (Atkins and Travis, 2010; Buckley et al., 2015; Donelson et al., 2012). Behavioural strategies can support more immediate thermoregulation over short time scales. By moving away (e.g., jumping, ‘skipping’, or ‘crutching’ in amphibious species, see Harris, 1960) from thermally stressful conditions into more benign conditions closer to preferred temperatures, species can mitigate some of the physiological costs that sub-optimal temperature present, such as increased maintenance costs. To date, the use of movement to select a preferred temperature has been studied across many taxa (Buckley et al., 2015; Coggan et al., 2011; Killen, 2014; Medvick and Miller, 1979; Speed et al., 2012); however, this has yet to be examined for amphibious fishes. Previous studies have suggested the use behaviour to occupy a thermal preference (Tytler and Vaughan, 1983); however, such preferences have not been established in the laboratory.

Determining oxygen uptake rates and understanding how movement (i.e., emergence and thermoregulation) is used to mitigate the effects of suboptimal temperatures could help explain how amphibious fishes, such as mudskippers, are able to live under a range of thermal conditions. The objective of this study was to establish how exposure to different thermal conditions influences oxygen uptake rates, preferred water temperatures and emergence behaviours of a common amphibious fish, the barred mudskipper, *Periophthalmus argentilneatuis*. Additionally, as temperatures can vary greatly between day and night-time periods, we aimed to investigate the differences in thermal preferences between day and night-time periods. As mudskipper species exhibit emergence behaviours, we also aimed to establish the upper thermal emergence limits to gain a better understanding of other behavioural strategies this and perhaps other amphibious fish species may utilize to mitigate future increases in temperature.

2. Materials and methods

2.1. Animal care

Adult barred mudskipper (*Periophthalmus argentilneatuis*) of similar size (mean ± SE; mass: 6.12 ± 1.43 g, standard length: 6.85 ± 0.55 cm) were collected in March 2015 from a mangrove forest in Cockle Bay on Magnetic Island, Queensland, Australia (19°10'32.4"S 146°49'45.8"E) using hand nets. To quantify temporal variation in environmental temperature, a HOBO Pendant® Temperature/Light Data Logger (Onetemp, Australia) was deployed in March 2015 at low tide in a 10 cm deep pool at the site of collection, and it was retrieved in January 2016. Following collection, animals were transported in aerated bags partially filled with seawater to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Queensland, Australia. All individuals were placed into two 68 L (64.5 × 41.3 × 39.7 cm), aquaria with flow-through filtered 28 °C seawater. Each aquaria contained 10 L of water and submerged PVC shelters and brick platforms. Following a two-week habituation period to ensure that fish were feeding appropriately, each individual was randomly assigned to one of five temperature treatments (22, 25, 28, or 32 °C) and tagged with a unique subcutaneous visible elastomer (Northwest Marine Technology, Washington, USA) to allow individuals to be identified. Each treatment consisted of four 8.5 L aquaria (22 × 23 × 22 cm), each housing 2–3 fish of similar body size (weight: one way ANOVA $F_{3,26} = 1.137$, $P = 0.36$; length: one way ANOVA $F_{3,26} = 0.08$, $P = 0.97$). Each aquarium consisted of submerged PVC shelters; however, these did not allow for emergence. Animals were isolated in the aquatic environment during acclimation to ensure that the acclimation conditions were constant, given that emergence would create inconsistent temperature, humidity, and osmotic conditions. Throughout the study, a 12:12 h photoperiod was maintained. Fish were fed daily commercial pellets and marine green frozen fish food (Fish Fuel Co., South Australia, Australia). Prior to experiments, fish were fasted for 24 h to ensure post-absorptive state that would maximize energy available for performance (Niimi and Beamish, 1974).

2.2. Temperature treatments

The treatment temperatures were chosen to reflect a range of ecologically relevant temperatures experienced throughout the year in an adjacent intertidal seagrass meadows in Cockle Bay, Magnetic Island; winter minimum (22 °C), temperatures experienced throughout the year (25 °C, and 28 °C, respectively), and summer average (32 °C) (Collier and Waycott, 2014). Treatment temperatures were reached either by decreasing the ambient water temperature (28 °C) by 0.5 °C per day or by increasing it by 0.3 °C per day until the target temperature was attained. Rates of temperature increase were slower than rates of decrease, as pilot trials showed that daily increases of 0.5 °C over several days resulted in prolonged erratic behaviours (e.g., continuous rapid, burst swimming behaviours) until mudskippers became unresponsive. These behaviours were not observed with a daily increase of 0.3 °C. Upon reaching treatment temperature, fish were maintained at treatment temperatures for five-weeks (prior to any experiments). This acclimation time was chosen as it is follows best practices and is thought long enough for critical acclimation processes to be complete (Nilsson et al., 2010).

2.3. Oxygen uptake rates

Fish were placed individually into 0.46 L intermittent-flow respirometry chambers inside a temperature-controlled water bath (following Svendsen et al., 2016). Each chamber was connected to a flush pump and recirculating pump to maintain water circulation and homogenous oxygen levels within the system throughout the trial. A digital relay timer controlled the flush pumps so that the water in the respirometry chambers was flushed with well-aerated, filtered seawater for 5 mins every 10 min throughout the 20-h trial. The flush cycle was determined such that O_2 levels did not fall below 80% air saturation (Clark et al., 2013). Temperature-compensated oxygen concentrations were recorded every 2 s using contactless spots (2 mm) with O_2-

sensitive REDFLASH dye attached to the inside of glass tubes in line with each recirculating pump. The spots were linked to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany) via 2 m fibre-optic cables.

The established method incorporating a 3-min chase followed by 1-min air exposure (see Clark et al., 2012, 2013; Roche et al., 2013) was used to determine maximum oxygen uptake rates ($\dot{M}O_{2Max}$) for fish from all temperature treatments (22 °C: n = 6, 25 °C: n = 7, 28 °C: n = 4, and 32 °C: n = 4). Immediately following the exercise protocol, fish were placed into respirometry chambers. Each chamber was sealed, and the measurement period began within 10 s. Following each trial, all equipment was rinsed in a 10% bleach solution, rinsed in freshwater, and then sun-dried to reduce microbial background oxygen uptake.

Given mudskippers are able to withstand extended periods of air exposure (Gordon et al., 1968), the standard chase protocol consisting of 3 min chase and 1 min air exposure (see Clark et al., 2012, 2013; Roche et al., 2013) might not elicit maximal exhaustion typically seen in species less resilient to air exposure. Therefore, we assessed the utility of the standard protocol by comparing it to maximum oxygen uptake rate elicited by three complimentary protocols adjusted for their unique physiology. Individuals within the 25 °C treatment group were exposed to either: (i) 3-min chase at a high water level without any air exposure, (ii) 3-min chase at a low water level to induce a jumping behaviour followed by a 1-min air exposure, and (iii) 3-min air exposure. In total, these four different chase protocols allowed for direct comparison between the standard protocol and different "exhaustive treatments" such as jumping, swimming or complete removal from water. Following each protocol, individuals were immediately placed into respirometry chambers in the aquatic environment to establish recovery time. Each individual from the 25 °C treatment was given 48 h to recover from experimental trials before tested under alternate protocols, and the sequence of chase protocols were randomised among individuals.

Oxygen uptake rates (as a proxy for energetic costs) during the measurement period (i.e., non-flushing) were calculated using linear least square regression of oxygen concentration over time in LabChart v.6.1.3 (AD Instruments, Colorado Springs, CO, USA). The highest rate of change for every 30-sec period, during the initial three closed respirometry phases following the placement of individuals inside the chamber, were used to determine the maximum O_2 uptake (i.e., $\dot{M}O_{2Max}$). This method was deemed appropriate as R^2 values for the closed respirometry phases were above 0.95. Resting oxygen uptake rate (i.e., $\dot{M}O_{2Rest}$) was calculated as the 'mean of the lowest normal distribution' method (MLND) as described by Chabot et al. (2016). Recovery time was defined as the time difference between $\dot{M}O_{2Max}$ until the first oxygen uptake rate equivalent to $\dot{M}O_{2Rest}$. Aerobic scope was defined as the absolute difference between $\dot{M}O_{2Max}$ and $\dot{M}O_{2Rest}$. Background O_2 uptake in the system was determined prior to the fish being placed into the chambers and again concluding the trial after fish were removed. The background O_2 uptake was assumed linear (Clark et al., 2013) and was determined to be less than 5% of mudskipper oxygen uptake rates. Given that this value was established as low, background O_2 uptake was incrementally subtracted from each slope.

2.4. Critical thermal emergence (CT_e)

The critical emergence temperature (CT_e) for barred mudskippers was estimated using a modified critical thermal maxima (CT_{Max}) methodology (Beitinger and Bennett, 2000) in which fish were allowed to emerge from the water (22 °C: n = 4, 25 °C: n = 7, 28 °C: n = 5, and 32 °C: n = 5). A 125 L (64.5 × 64.5 × 30.15 cm) square, glass tank was fitted with a 71.2 cm x 64.5 cm PVC ramp with evenly spaced holes (10 mm wide diameter and separate by approximately 5 cm). The ramp was positioned at a 30° angle from the base of the tank to the far top corner. A 2000 W heater (Omega 2000 W, Full gauge TIC-17RGT Thermostat) and submersible mixing pump (WH-500, Weipro®, Guangdong, China) were placed below the ramp away from the experimental compartment to ensure homogeneous temperature throughout the entire aquarium. An air stone was placed inside the experimental compartment to ensure sufficient aeration. First, water was heated or chilled to the fish's treatment temperature. Then, the fish was placed inside the experimental compartment, and a plastic sheet was placed on the surface of the water to ensure the fish could not emerge prematurely. Fish were observed inside the compartment for 2 min, and the time spent actively moving (i.e., henceforth 'activity') was recorded. Following the initial 2-min observation period, the plastic sheet was moved along the water's surface towards the back of the tank opening a 5 cm gap that allowed for emergence. The water temperature was then increased at a rate of 0.27 ± 0.01 °C min^{-1} until the fish emerged onto the ramp. Emergence temperatures were established as the temperatures at which the fish first emerged from the water (i.e., the temperature at which the eyes were completely above the water) and at which the entire body emerged from the water (i.e., the temperature at which the caudal peduncle was out of the water). These endpoints were selected to represent the point at which the mudskipper may evaluate the aerial environment prior to full emergence. Air temperatures were maintained at 25 °C, and humidity was constant between 40% and 60% throughout all trials.

2.5. Preferred temperature (T_{pref})

A shuttlebox system, designed by Schurmann and Steffensen (1991) and Petersen and Steffensen (2003), was used to determine the preferred temperature (T_{pref}) for each fish from each treatment temperature (22 °C: n = 6, 25 °C: n = 7, 28 °C: n = 7, and 32 °C: n = 4). A detailed description of the shuttlebox system is provided in Nay et al. (2015). Briefly, the system is composed of two chambers joined by a 50 mm wide opening allowing the fish to travel freely between the two chambers. One chamber was established as the 'warm' chamber and the other as a 'cool' chamber, with a 1 °C difference maintained between the chambers throughout the trial (Killen, 2014). When the fish entered the 'warm' chamber, the temperature of the entire system would increase at a rate of 6 °C h^{-1}. Conversely, when the fish entered the 'cool' chamber, the temperature of the system would decrease at a rate of 6 °C h^{-1}. By moving between chambers the fish can control their thermal environment and therefore their internal body temperature. The 'warm' and 'cool' chamber assignments were switched halfway through trials to ensure there was no bias toward a particular chamber.

An individual fish was placed inside the chamber set to the individual's treatment temperature, and a plexiglass cover was placed on the surface of the water to prevent the fish from emerging. Fish were allowed a 1.5 h adjustment time prior to turning on the system. This time period was used as fish were observed entering both chambers of the shuttlebox system. Water within one chamber flowed clockwise while water in the other chamber flowed counter-clockwise to prevent the water from mixing and allow for the 1 °C temperature difference to be maintained.

A custom program was written using Labtech Notebook Pro (Laboratories Technology Corp., Andover MA) to track the position of the fish and automate the activation/deactivation of the appropriate pumps based on the position of the fish. From each trial we recorded preferred temperature, number of chamber movements, and the selected temperature range. The preferred temperature (T_{pref}) was defined as the temperature at which the fish spent the largest proportion of time (i.e., modal temperature), while the chamber movements were the number of movements made between the chambers during diurnal and nocturnal periods. Selected temperature ranges were represented as the difference between maximum and minimum temperatures chosen by each fish.

2.6. Data analyses

To test the effect of treatment temperature on minimum and maximum oxygen uptake rates as well as initial and final emergence temperatures, one-way ANOVAs were used. To test the effect of chase protocols for fish maintained at 25 °C, $\dot{M}O_{2Max}$, $\dot{M}O_{2Rest}$, and recovery time were analysed using one-way ANOVAs. The $\dot{M}O_{2Max}$, $\dot{M}O_{2Rest}$, and recovery time data were $\log_{10}$ transformed prior to analysis to conform to requirements for normality and homogeneity of variance. Activity was analysed with Kruskal-Wallis test given the data were not normally distributed. Temperature preference, chamber movements, and temperature range were analysed using a two-way ANOVA (treatment temperature and diel period [night-time/day-time]). All values were log transformed to meet assumptions of analysis (homogeneous variance and normal distribution). Holm-Sidak post-hoc tests were performed when significant differences were observed. To account for running multiple tests on the same individuals within experiments, an FDR correction was used post-hoc (Benjamini and Hochberg, 1995). All values are presented as mean ± SE.

3. Results

The field data logger revealed the average temperatures within the tide pool at Cockle Bay, Magnetic Island to be 25.48 ± 3.23 °C during 2015 (mean ± SD; Fig. 1). Temperatures ranged from 23.29 °C to 39.50 °C during Austral summer (i.e., November to January) and from 12.40 °C to 32.60 °C during Austral winter (i.e., June to August).

3.1. Oxygen uptake rates

The resting oxygen uptake rate ($\dot{M}O_{2Rest}$) of *P. argentilineatus* differed among temperature treatments ($F_{3,17}$ = 3.40, P = 0.04), with individuals maintained at 32 °C having higher $\dot{M}O_{2Rest}$ (225.88 ± 23.44 mgO$_2$ kg^{-1} h^{-1}) than those maintained at 22 °C (119.06 ± 23.76 mgO$_2$ kg^{-1} h^{-1}), 25 °C (114.21 ± 16.19 mgO$_2$ kg^{-1} h^{-1}), and 28 °C (169.00 ± 41.68 mgO$_2$kg^{-1} h^{-1}, Fig. 2). In contrast, there was no difference in maximum oxygen uptake rates ($\dot{M}O_{2Max}$) following standard chase protocols among fish from each temperature treatment group (22 °C: 739.61 ± 142.07 mgO$_2$ kg^{-1} h^{-1}, 25 °C: 631.42 ± 90.00 mgO$_2$ kg^{-1} h^{-1}, 28 °C: 522.92 ± 66.70 mgO$_2$ kg^{-1} h^{-1}, 32 °C: 534.85 ± 80.45 mgO$_2$ kg^{-1} h^{-1}, $F_{3,17}$ = 0.53, P = 0.67). Although aerobic scope appears to be decreasing across treatments, it did not differ significantly across temperature treatments (22 °C: 649.91 ± 142.71 mgO2 kg-1 h-1, 25 °C: 548.17 ± 89.87 mgO2 kg-1 h-1, 28 °C: 444.94 ± 70.29 mgO2 kg-1 h-1, 32 °C: 377.74 ± 82.12 mgO2 kg-1 h-1, $F_{3,17}$ = 0.91, P = 0.46). Comparing chase protocols among fish maintained at 25 °C revealed that recovery time was significantly shorter when fish were exposed to air and then allowed to recover in the aquatic environment (1.53 ± 0.41 h) compared to fish exposed to the standard chase protocol (3.93 ± 0.71 h) or modified chase protocol (3.37 ± 0.67 h; F3,16 = 7.22, P = 0.003; Fig. 2) and then placed into water for recovery. Neither maximum oxygen uptake rates ($F_{3,32}$ = 0.64, P 0.59), resting oxygen uptake rates ($F_{3,32}$ = 2.07, P = 0.12), nor aerobic scope ($F_{3,32}$ = 1.14, P = 0.35) differed among protocols.

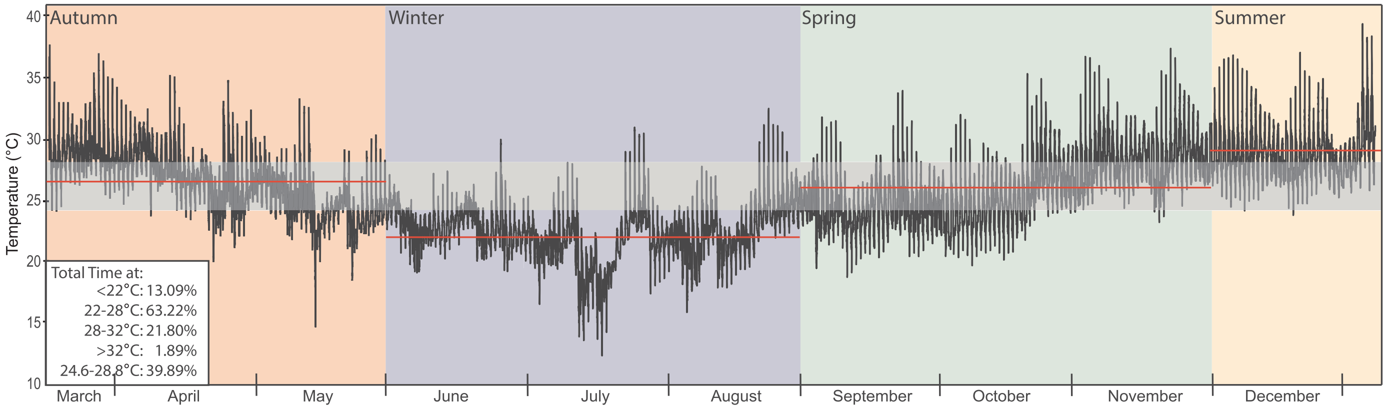

Fig. 1. Temperature data collected with HOBO data loggers in Cockle Bay, Magnetic Island, Queensland, Australia from March 2015-January 2016. The data logger was placed within a 10 cm deep tide pool at low tide at the base of mangrove, areas commonly frequented by the barred mudskipper, *Periophthalmus argentilneatuis*.

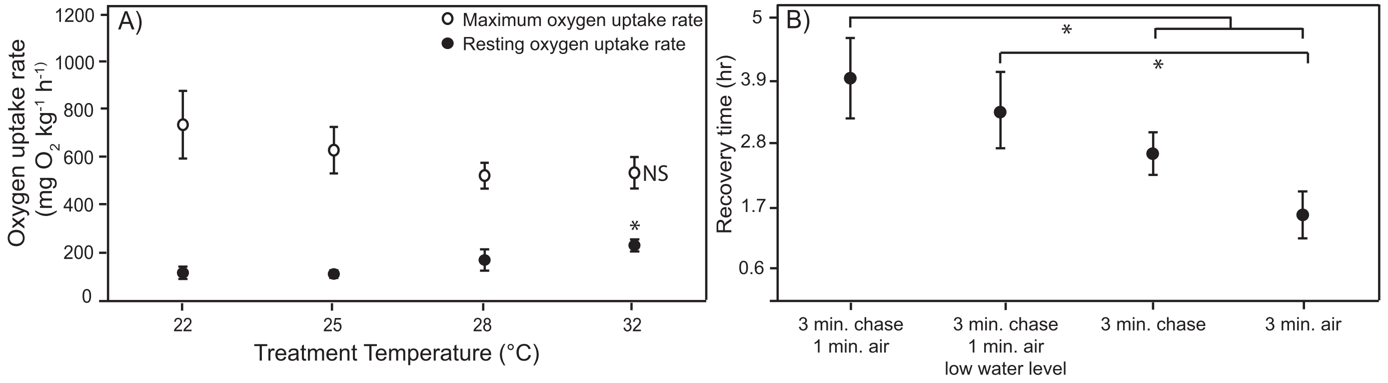

Fig. 2. A) Maximum oxygen uptake rates ($\dot{M}O_{2Max}$) represented as open circles along with resting oxygen uptake ($\dot{M}O_{2Rest}$) represented as closed circles for fish from each treatment temperature (22 °C: n = 6, 25 °C: n = 7, 28 °C: n = 4, and 32 °C: n = 4). Maximum oxygen uptake rates ($\dot{M}O_{2Max}$) were established using a 3 min chase and a 1 min air exposure. B) Recovery time required for each protocol within the 25 °C treatment group. All points are represented as means with standard error of the mean. Significant values are indicated as an asterisk with α = 0.05.

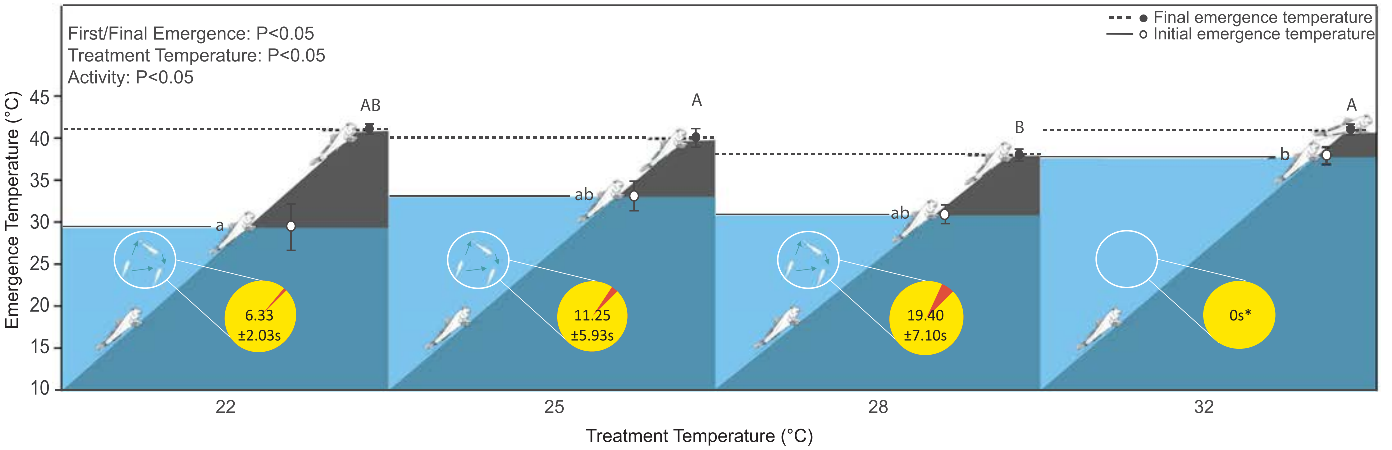

Fig. 3. Emergence temperatures for all treatment groups (22 °C: n = 4, 25 °C: n = 7, 28 °C: n = 5, and 32 °C: n = 5). Initial emergence temperatures represented as a solid line and open circle, while final emergence temperatures are represented as a dotted line and closed circle. The swim time observed during the two-minute period prior to CT_e trials is represented with a yellow and red pie chart. Red sections represent the time spent active during the two-minute activity period prior to trials. Points and error bars are means with standard deviation. Significant differences are indicated between uppercase letters (final emergence temperature), lowercase letters (initial emergence temperature), and an asterisk for activity levels, all with α = 0.05.

3.2. Critical thermal emergence (CT_e)

The fish's initial and final emergence temperatures as well as the duration of activity prior to emergence differed among temperature treatments (Fig. 3). The temperature at which individuals initially emerged from the water (i.e., eyes are fully above the water) was higher for fish that had been maintained at 32 °C (37.79 ± 1.16 °C) when compared to those maintained at 22 °C (28.34 ± 2.33 °C; $F_{3,19} = 6.69$, $P = 0.003$). Fish that were maintained at 32 °C and 25 °C completely emerged themselves (i.e., withdrew caudal peduncle fully above the water) at significantly warmer temperatures (41.31 ± 0.36 °C and 40.89 ± 0.78 °C respectively) than fish maintained at 28 °C (37.94 ± 1.96 °C; $F_{3,17} = 4.15$, $P = 0.02$). The time spent actively moving was greatest for fish that had been maintained at 28 °C (19.40 ± 7.10 s of the 2-min observation period), and decreased for fish from both lower (25 °C: 11.25 ± 5.93 s; 22 °C: 6.33 ± 2.03 s) and higher (32 °C: 0 ± 0 s) temperatures ($F_{3,19} = 14.32$, $P < 0.05$).

3.3. Preferred temperature (T_{pref})

Despite five weeks of continuous exposure to either 22, 25, 28, or 32 °C, *P. argentilineatus* preferred approximately the same temperature (26.7 ± 2.1 °C; $F_{3,43} = 2.20$, $P = 0.10$), which also did not differ between time periods (i.e., nocturnal vs. diurnal: $F_{1,43} = 1.62$, $P = 0.21$; Fig. 4a). There was, however, considerable variation in preferred temperature among all individuals, as evidence by the variation around the mean. Additionally, fish from some groups made more chamber movements to maintain their preferred temperature than others. For example, fish maintained at 28 °C moved more frequently to maintain their preferred temperature than 25 °C-maintained fish ($F_{3,43} = 3.11$, $P = 0.02$). During nighttime periods, fish moved more frequently to maintain their preferred temperatures than they did during the daytime periods ($F_{1,43} = 18.99$, $P < 0.001$). However, during daytime hours, fish selected a wider range of temperatures regardless of treatment temperature ($F_{1,43} = 22.50$, $P < 0.05$). There were no interactions between treatment, time period, preferred temperature, movements, and selected temperature ranges ($F_{3,43} = 0.91$, $P = 0.45$; $F_{3,43} = 2.67$, $P = 0.06$; $F_{3,43} = 0.90$, $P = 0.45$).

4. Discussion

Amphibious fishes may use behavioural strategies to minimise their exposure to unfavourable environmental temperatures. Maintaining barred mudskippers, *Periopthalmus argentilineatus*, for 5 weeks at constant temperatures that they naturally experience (22–32 °C) in the wild had no effect on their preferred water temperatures (26.7 °C). Fishes that had been maintained at 28 °C were also the most active compared to those maintained at either lower (22 and 25 °C) or higher water temperatures (32 °C). Despite the similarities in preferred water temperatures, fish that had been maintained at 32 °C emerged from the water at higher water temperatures, exhibited an increase in their resting oxygen uptake rates, and selected a wider range of water temperatures than their counterparts that had been maintained at cooler temperatures. Exposure to air prior to submersion provided the shortest recovery time of any of the exercise protocols (1.53 ± 0.41 h) likely due to their ability to uptake oxygen in air. The longest recovery time was required by those who were exercised for 3 min in water and allowed a 1 min air exposure (3.93 ± 0.71 h) followed by submersion. Although we recognize that the barred mudskipper occupies naturally thermally variable environments (12.40–39.50 °C), when they are maintained long-term at constant water temperatures closer to the upper limits of what they currently experience (32 °C), their oxygen uptake rates may increase and behaviour altered. Therefore, prolonged exposure to elevated temperatures (e.g., 32 °C) may force mudskipper species to either move or risk elevated energetic costs.

Mudskippers, similar to many intertidal species, have the ability to leave the water when water conditions become suboptimal, and several factors can alter this emergence temperature threshold. For example, *Favonigobius exquisitus* and *Pseudogobius* sp., two gobiid fish species common to the intertidal area of Moreton Bay, Australia, emerge from the water when exposed to high thermal stress (Ford et al., 2004). Similarly, *Kryptolebias marmoratus* emerge when exposed to elevated temperatures (Gibson et al., 2015). Local air temperatures experienced across daily and seasonal cycles, together with maximum daily water temperatures appear to play an important role in initiating emergence of amphibious fishes. Along the coast of central Chile, *Sicyases sanguineus* emerged at 17.8 °C when water temperatures mimicked maximum daily air temperatures (Ebeling et al., 1970). Similarly, barred mudskippers in this study maintained at cooler temperatures exhibited initial emergence at 32.63 °C reflecting average maximum daily air temperatures recorded at Magnetic Island (∼31.4 °C). Humidity, along with temperature, can also play an important role in emergence temperatures. Gibson et al. (2015) found that *K. marmoratus* individuals lost more body heat in low humidity environments than those in high humidity scenarios. As relative humidity increased, the amount of body heat lost by *K. marmoratus* decreased. Given that relative humidity in

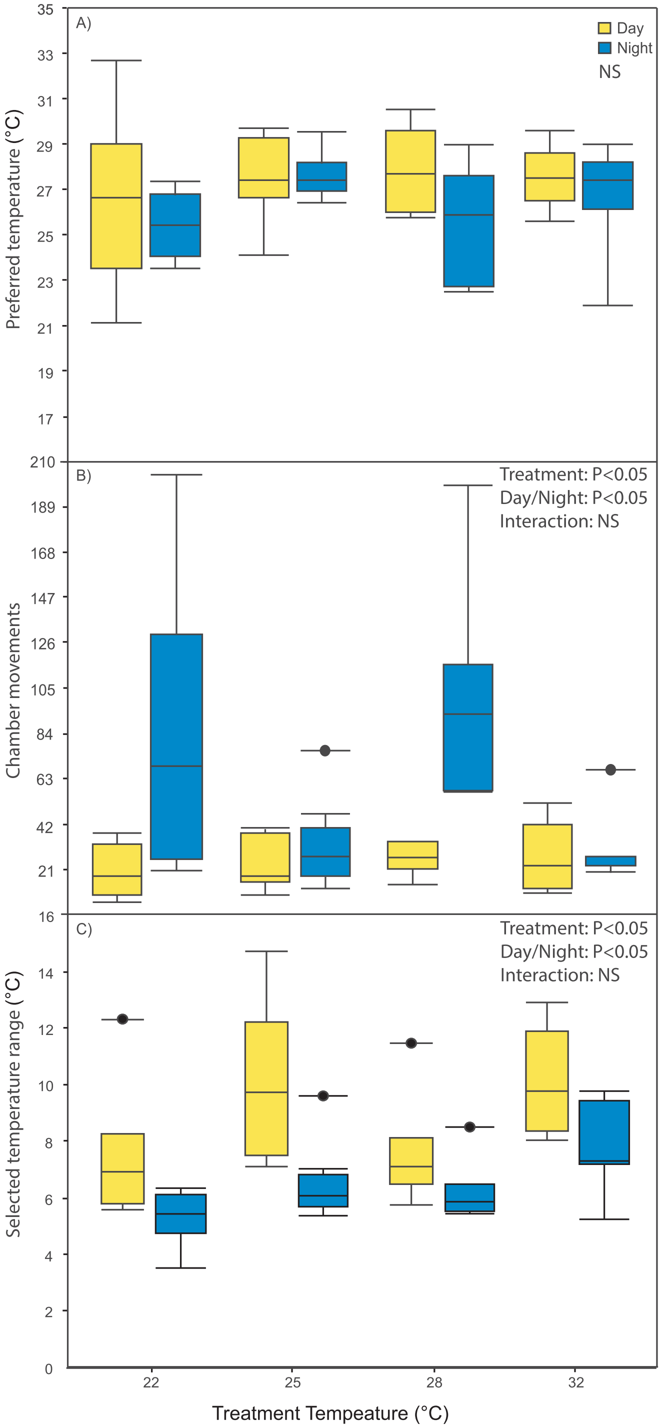

Fig. 4. A) Temperature preference (T_{pref}), B) chamber movements, and C) the selected temperature ranges of all treatment groups per 5-h trial (22 °C: n = 6, 25 °C: n = 7, 28 °C: n = 7, and 32 °C: n = 4). The boxes represent first and third quartiles, and the whiskers (errors) represent the minimum and maximum values outside of outliers. Outliers are solid lines with a closed circle. Within each box, median (solid line) values are included. Daytime values are in light (yellow) boxplots. Nighttime values are in dark (blue) boxplots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

this study was between 40% and 60%, emergence behaviours could have led to beneficial evaporative cooling mechanisms. Although we did not examine the specific physiological mechanisms underpinning emergence behaviours that occur at higher temperatures, previous studies have suggested potential mechanisms such as anaerobic metabolic pathways, antioxidative mechanisms, or heat-shock protein responses (Pörtner and Peck, 2010). These mechanisms may allow more time to be spent at elevated temperatures during a period of time where temperatures are increasing. A thorough investigation into these mechanisms is an avenue for future studies. Emergence behaviour can be a useful response for mudskipper species to escape extreme conditions; however, such behaviours come with associated trade-offs.

Under scenarios where emergence behaviours are too risky and/or energetically costly, it may be necessary to regulate body temperature while submerged. Barred mudskippers in this study showed a preference for relocating to thermal conditions similar to those experienced in Austral autumn months. The mangrove habitat on Magnetic Island experiences temperatures between 24.6 and 28.8 °C (i.e., the range of preferred temperatures determined for mudskippers in this study) for nearly 5 months of the year during both high and low tidal cycles. These temperatures are also suggested as the preferred temperature ranges for other amphibious species, although selected using alternative thermoregulatory behaviours such as body placement next to cooler tide pools (e.g., *Mnierpes microcephalus*; Graham, 1973). Furthermore, the temperature range throughout the year between low and high tides is much greater, fluctuating by 10–18 °C, than has been previously noted (e.g., 12 °C) in other intertidal habitats (Potts and Swart, 1984). Unlike fishes that are fully aquatic, amphibious fishes, such as mudskippers, may require more frequent movements to occupy preferred temperatures, given the dynamic nature of temperature within their habitats; however, such movements may expose these fish to new threats, including aerial or aquatic predation (Sayer and Davenport, 1991).

Ultimately using behaviours to regulate temperature can have several associated risks including but not limited to attracting predation, missing opportunities, and the energy trade-offs required for movement (Sayer and Davenport, 1991). For example, movement may draw the attention of predators and/or scare off potential prey (i.e., missed opportunity for foraging) (Angilletta, 2009). Furthermore, the energy conserved as part of moving to a preferred temperature needs to be greater than the energy required to relocate (Angilletta, 2009). Here, barred mudskippers increased movements during nocturnal periods, which may reflect a reduced predation risk at night. However, as temperatures approach the upper thermal limits of resident species, occupying thermally beneficial areas could become essential in order to reduce energetic costs associated with elevated temperatures.

Elevated temperatures, above an organism's natural range, can be associated with increased energetic costs, which in turn can affect a species' behavioural patterns. Many fully aquatic/marine fish species have exhibited elevated resting oxygen uptake rates at higher temperatures causing declines in traits such as aerobic scope and swimming performance (Johansen and Jones, 2011; Munday et al., 2008; Rummer et al., 2014). These declines can, although not always, coincide with declines in growth and reproductive output, as energy may be allocated to more essential life-sustaining processes (Lefevre, 2016; Pörtner and Peck, 2010). In contrast, recent studies have suggested that the decline in aerobic scope seen in some species may not be universal and may not align with other performance traits such as growth (Lefevre, 2016; Jutfelt et al., 2018). Aerobic scope in barred mudskippers in the present study did not decline as exposure temperatures were increased or decreased, which suggests that this metric may not align with all performance traits of this, and other amphibious fish, species. For example, unlike aerobic scope, emergence temperatures did follow the trend of increasing as exposure temperature increased. A previous study on *Blennius pholis* suggests that emergence depends on the oxygen demand of the fish (Davenport and Woolmington, 1981) and therefore, as oxygen demand increases upon exposure to elevated temperatures, emergence may be a valid strategy to alleviate negative effects of temperatures (e.g., evaporative cooling and/or oxygen uptake). Indeed, some mudskipper species are thought to have the ability to uptake enough oxygen in air to satisfy resting metabolic demands (Teal and

Carey, 1967; Gordon et al., 1968; Tamura et al., 1976; Kok et al., 1998; Takeda et al., 1999) and therefore could use air exposure as a valid strategy when recovering from aquatic exercise. Aquatic exercise in this study required a maximum 3.93 ± 0.71 h of recovery time before returning to resting levels. Similarly, Jew et al. (2013) suggested that 4.87 ± 2.19 h was required to recover from terrestrial exercise. Given mudskippers are uniquely able to utilize both aquatic and aerial environments, selecting preferred temperatures or emergence may allow these species to occupy thermally forgiving areas thus optimizing performance.

4.1. Conclusions

Maintaining barred mudskippers at constant elevated temperatures led to increases in their emergence temperatures and resting oxygen uptake rates, however had no detectable effect on their preferred water temperature of 27 °C. The shift in emergence temperature suggests that barred mudskippers may have the capacity to acclimate and shift their upper thermal limits with prolonged exposure to constant temperatures. Amphibious fishes, such as mudskippers, have the ability to occupy aerial and aquatic environment, and as such are exposed to changing temperatures in both environments. When exposed to treatment conditions in the aquatic environment, resting oxygen uptake increased with elevated treatment temperatures. This is consistent with the response of many strictly aquatic fishes (Nilsson et al., 2009; Rummer et al., 2014) and suggests as ocean warming continues, elevated temperatures may be energetically expensive for amphibious species. Understanding the responses to elevated temperatures as well as the potential avoidance behaviours (i.e., emergence and thermoregulation) used by amphibious fishes are key to understanding how these fish will cope with future climate change scenarios.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.09.005.

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